ISA 2022

XVIII. INTERNATIONAL SYMPOSIUM ON AMYLOIDOSIS

4TH - 8TH SEPTEMBER 2022
HEIDELBERG

QUO VADIS AMYLOIDOSIS?

ABSTRACT BOOK

AMYLOIDOSIS CENTER
Heidelberg University Hospital

CLÍNIC
BARCELONA
Hospital Universitari

European
Reference
Network
for rare or low prevalence
complex diseases

Network
Hematological
Diseases (ERN EuroBloodNet)

FOR
2969

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

Amyloidosis Research and Treatment Center
Foundation «IRCCS Policlinico San Matteo»
Department of Molecular Medicine
University of Pavia
Pavia, Italy

UNIVERSITÄT
HEIDELBERG
ZUKUNFT
SEIT 1386

UNIVERSITÄTS
KLINIKUM
HEIDELBERG

ISA 2022 Committees

Hosts

Stefan Schönland and Ute Hegenbart,
Amyloidosis Centre, Medical Clinic V,
Medical Faculty of Heidelberg University
Im Neuenheimer Feld 410
69120 Heidelberg

Co-Organizers

Carlos Fernández de Larrea and
Maria Teresa Cibeira, *Barcelona, Spain*
Giovanni Palladini, *Pavia, Italy*

Local Organizing Committee

Markus Weiler and Fabian Siepen
on behalf of the members of the Amyloidosis Center, Heidelberg, Germany

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Organizational Unit

Congress and Conference Management (UniKT)
Heidelberg University
Seminarstraße 2
69117 Heidelberg

Acknowledgements

We thank Marie Brumma (Amyloidosis Centre, Medical Clinic V) for her assistance in all organizational matters and especially for the intensive sponsor support in cooperation with UniKT.

We would like to thank our in-house agency UniKT, which brought the congress from conception to implementation with the tireless efforts of the entire team.

We are also grateful to our colleagues who will be available throughout the conference to help us manage the many tasks of a hybrid congress.

Dear all,

On behalf of the International Society of Amyloidosis we want to welcome you to the “XVIII. International Symposium on Amyloidosis” which will be held in the beautiful city center of Heidelberg. Many scientists, philosophers and poets from all over the world have a strong relationship to this unique place. We are proud that the meeting is hosted by the Ruperto Carola University, the one with the longest tradition in Germany, as it was already founded in 1386.

Stefan Schönland
President elect of ISA

Ute Hegenbart
Member of ISA Board

The motto of the XVIII. meeting is “Quo vadis, amyloidosis?” The scientific and clinical developments have been overwhelming in the last years. The general interest is growing as a number of very effective drugs became available in systemic amyloidoses. This is reflected by the scientific program which will feature distinguished keynote speakers, round tables with debates, and compelling presentations by junior scientists as well as established scientists from various fields of amyloidoses. Furthermore, we have created new items like a “best abstracts” session on Thursday. The conference will be held as a hybrid meeting with more than 1100 attendees after the fully virtual conference in 2020.

We heartily thank all the members of the ISA 2022 Committee, the ISA Board and the abstract reviewers for their support in addressing the many challenges of the Corona Pandemic era and the complex circumstances in which we live. We also would like to thank the sponsors for their generous financial support and the organization of industry symposia, as well as the Amyloidosis Foundation, which again provided a large number of grants for young scientists. The winners of the awards will be honored at the Gala Dinner on Wednesday evening in the “halle02”, a former building of the freight depot located in the newest district of Heidelberg named “Bahnstadt”.

We wish you a pleasant and stimulating time with us in Heidelberg, which encompasses academic excellence, cutting-edge medicine, modern urban development and, last but not least, tourist highlights and German Romanticism.

Stefan Schönland and Ute Hegenbart

Building opening hours: 8:00 AM – 9:30 PM	
4:30 PM – 5:00 PM Opening Ceremony	Neue Aula/Main Lecture Hall
5:00 PM – 7:00 PM Keynote Lecture & Opening Lectures	Neue Aula/ Main Lecture Hall
7:00 PM – 8:00 PM Welcome Reception	ground floor/2nd floor

Program is subject to changes

COLOR CODES

- SCIENTIFIC PROGRAM
- POSTER PRESENTATIONS
- SATELLITE SYMPOSIA
- EXHIBITION
- SOCIAL EVENTS
- BREAKS

Building opening hours: 7:00 AM – 9:30 PM

7:00 AM – 7:45 AM 2nd floor
Sponsored breakfast

7:15 AM – 8:30 AM Neue Aula/Main Lecture Hall
IKMG/ISA – Challenges in Monoclonal Gammopathy of Renal Significance

7:30 AM – 8:30 AM H14
Global Bridges: Building Diagnostic Capacity Worldwide

8:30 AM – 10:05 AM Neue Aula/Main Lecture Hall
Basic research – Light chains including cardiotoxicity
OP001 – 008

9:30 AM – 5:00 PM
ground floor/2nd floor
Exhibition on-site and virtual

10:05 AM – 10:30 AM Virtual Venue
Virtual Speaker Corner: Talk with industry partners

10:05 AM – 10:30 AM
New University, ground floor/2nd floor/courtyard
Coffee Break

10:30 AM – 12:05 PM Neue Aula/Main Lecture Hall
ATTRwt – Clinical aspects
OP009 – 016

12:05 PM – 1:20 PM Triplex Canteen/1st floor
Poster Presentation: AL and ATTR amyloidosis

12:05 PM – 1:20 PM
New University, ground floor/courtyard and Triplex Canteen
Lunch Break

1:20 PM – 3:00 PM Neue Aula/Main Lecture Hall
Imaging in amyloidosis OP017 – 024

3:05 PM – 4:10 PM Neue Aula/Main Lecture Hall
Preclinical models of systemic amyloidosis

4:10 PM – 4:30 PM
New University, ground floor/2nd floor/courtyard
Coffee Break

4:30 PM – 6:00 PM Neue Aula/Main Lecture Hall
Janssen: Navigating the patient journey in AL amyloidosis: a multidisciplinary approach

6:00 PM – 7:00 PM Neue Aula/Main Lecture Hall
Pfizer: How can one solution solve the multifaceted challenges of ATTR amyloidosis?

7:05 PM – 8:05 PM Alte Aula/Building "Alte Universität"
Merlini Award Ceremony

Building opening hours: 7:00 AM – 9:30 PM

7:00 AM – 7:30 AM | 2nd floor
Sponsored breakfast

7:30 AM – 8:30 AM | Neue Aula/Main Lecture Hall
Prothena: Addressing the Unmet Need in Advanced AL Amyloidosis: Key Insights from a Panel of Experts

8:30 AM – 10:05 AM | Neue Aula/Main Lecture Hall
AL – Clinical aspects OP025 – 032

10:05 AM – 10:30 AM
New University, ground floor/2nd floor/courtyard
Coffee Break

9:30 AM – 5:00 PM
ground floor/2nd floor
Exhibition on-site and virtual

10:30 AM – 12:05 PM | Neue Aula/Main Lecture Hall
Basic Research – New treatment targets and biomarkers OP033 – 040

12:05 PM – 1:15 PM
Triplex Canteen/1st floor
Poster Presentation: Basic science and imaging

12:05 PM – 1:15 PM
Virtual Venue
Virtual Speaker Corner: Talk with industry partners

12:05 PM – 1:15 PM
New University, ground floor/ courtyard and Triplex Canteen
Lunch Break

1:15 PM – 2:30 PM | Neue Aula/Main Lecture Hall
AA – Clinical aspects OP041 – 043

2:30 PM – 3:30 PM | Neue Aula/Main Lecture Hall
BridgeBio: Improving patient outcomes in ATTR-CM

3:30 PM – 5:00 PM | Neue Aula/Main Lecture Hall
Ionis/AstraZeneca: Optimizing Multidisciplinary Care in Patients With ATTR

5:00 PM – 7:00 PM | Heidelberg
Free Time/Explore Heidelberg

7:00 PM – 7:30 PM | Triplex Canteen/1st floor
Poster Presentation: Late breaking abstracts

7:30 PM – 8:15 PM | Triplex Canteen/Foyer
Challenging Cases

8:15 PM – 9:15 PM | Triplex Canteen/Foyer
Junior Meets Senior (Round Table)

7:00 PM – 10:00 PM | Triplex Canteen/Foyer
Get Together

Building opening hours: 7:00 AM – 9:30 PM

7:00 AM – 7:45 AM | 2nd floor
Sponsored coffee

7:45 AM – 8:30 AM | Neue Aula/Main Lecture Hall
Sobi™: Expert Discussion: Is Disease Remission in hATTR a Realistic Goal?

8:30 AM – 10:05 AM | Neue Aula/Main Lecture Hall
ATTRv – Clinical aspects OP044 – 051

9:00 AM – 10:00 AM | H14
EHA/ISA – Better understanding and targeting the clone in AL amyloidosis

9:30 AM – 5:00 PM
ground floor/2nd floor
Exhibition on-site and virtual

10:05 AM – 10:30 AM
New University, ground floor/2nd floor/courtyard
Coffee Break

10:30 AM – 12:05 PM | Neue Aula/Main Lecture Hall
Pathways to diagnosis OP052 – 059

12:05 PM – 1:20 PM | Triplex Canteen/1st floor
Poster Presentation: Pathway to diagnosis, Innovative drugs and non-AL / non-ATTR amyloidosis

12:05 PM – 1:20 PM
New University, ground floor/courtyard and Triplex Canteen
Lunch Break

1:20 PM – 3:00 PM | Neue Aula/Main Lecture Hall
Basic research – Amyloid fibril formation including proteolysis and tissue interactions OP060 – 067

3:00 PM – 4:30 PM | Neue Aula/Main Lecture Hall
Alexion: Beyond Survival: Unmet Medical Needs in AL Amyloidosis

4:30 PM – 5:00 PM | Virtual Venue
Virtual Speaker Corner: Talk with industry partners

4:30 PM – 5:00 PM
New University, ground floor/2nd floor/courtyard
Coffee Break

5:00 PM – 6:30 PM | Neue Aula/Main Lecture Hall
Alnylam: Meeting the needs of patients with hATTR amyloidosis: innovation in practice

7:00/7:30 PM – open end | "halle02" Heidelberg
Conference Dinner/ Award Ceremony

Building opening hours: 7:00 AM – 2:00 PM

7:00 AM – 7:45 AM — 2nd floor
Sponsored breakfast

7:45 AM – 8:30 AM — H14
Attralus: Unmet Need and Rationale for Anti-Amyloid Therapy in Systemic Amyloidosis

8:30 AM – 10:15 AM — Neue Aula/Main Lecture Hall
Best abstracts (including late breaking)
OP068 – 071
OPLB001 – 003

9:30 AM – 1:00 PM
ground floor/2nd floor
Exhibition on-site and virtual

10:15 AM – 10:45 AM
New University, ground floor/2nd floor/courtyard
Coffee Break

10:45 AM – 11:45 AM — Neue Aula/Main Lecture Hall
Translation – How to close the gap of basic and clinical research in systemic amyloidosis (Round Table)

11:45 AM – 12:30 PM — Neue Aula/Main Lecture Hall
Membership Meeting

12:30 PM – 1:00 PM — Neue Aula/Main Lecture Hall
Closing Ceremony

Lunch Boxes

SAVE THE DATE

The 2024 International Symposium
on Amyloidosis will be held at

Mayo Clinic in Rochester
Minnesota, USA
on June 9 – 13, 2024

Alnylam PHARMACEUTICALS
CHALLENGE ACCEPTED

For healthcare professionals only. The hATTR Matters platform is organized and funded by Alnylam® Pharmaceuticals. Users from certain countries will have access to content that includes data and products specific to Alnylam®.

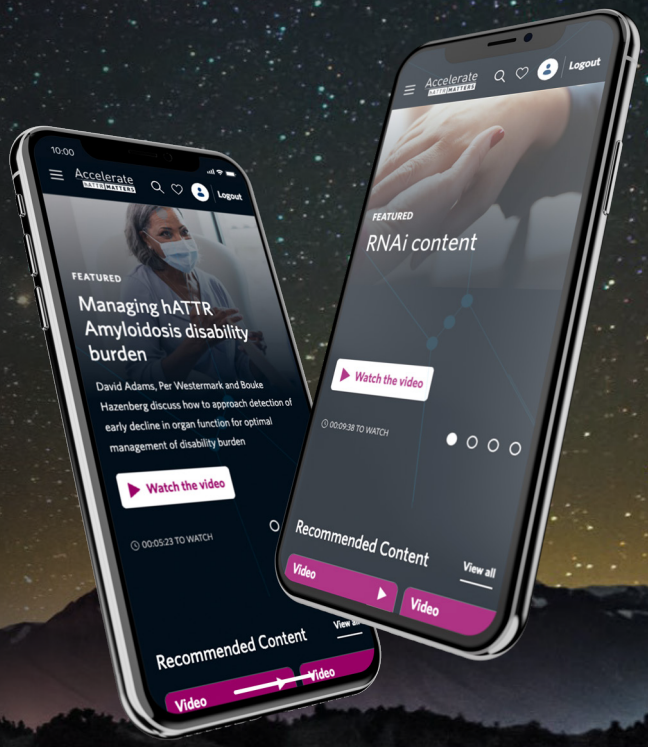
PRESENTING...

Accelerate hATTR MATTERS

The latest hATTR amyloidosis content is only one-click away.

This interactive platform offers dedicated **hATTR content**, produced by Alnylam® Pharmaceuticals, under its ACCELERATE initiative*.

Watch **exclusive live webinars**, **congress presentations** and **video content** created with a network of key amyloidosis experts.



Features:

ON-DEMAND

From 1 minute quick insights, to full length deep dives. On-demand content currently includes collections focusing on:

- Understanding the burden of hATTR amyloidosis
- Quick insights
- RNAi therapeutics - concept to medicine**
- Early intervention in hATTR amyloidosis
- Pathology of hATTR amyloidosis
- The multidisciplinary approach in hATTR amyloidosis
- Deep dives

With new material constantly being developed, **on-demand** collections will be updated regularly throughout the year.

LIVE

Immediate access to live content. Once you are registered on the Accelerate: hATTR Matters platform, you will have access to exclusive free to attend virtual events featuring insights from global amyloidosis experts. These will launch later in 2022.

All events are available via a simple one-click attendance confirmation.

To access the full range of content on offer register now at **www.hattrmatters.com** or use the QR code.

*The ACCELERATE initiative is dedicated to addressing unmet medical needs and improving the lives of people living with hATTR amyloidosis and those who care for them. **Coming soon.

ATTR, transthyretin amyloidosis; hATTR, hereditary transthyretin amyloidosis.

07.2022 NP-CEMEA-00255

Alnylam® PHARMACEUTICALS

Alnylam - Supporting medical education in ATTR amyloidosis*

Join us at a Satellite Symposium at ISA Congress 2022

Meeting the needs of patients with hATTR amyloidosis: innovation in practice

7 September 2022 | 17:00–18:30 CEST | Neue Universität – Heidelberg, Germany, & Online

Agenda

17:00–17:10 CEST
Welcome and introductions
Prof Thomas Skripuletz, Department of Neurology, Hannover Medical School, Hannover, Germany

17:10–17:40 CEST
Evolving experience with RNAi therapies in a multi-system disease:

A cardiologist's perspective on hATTR amyloidosis
Prof Marcus Anthony Urey, Division of Cardiology, Department of Medicine, University of California, San Diego, CA, USA

Experience of managing hATTR amyloidosis at a reference center
Prof Andoni Echaniz-Laguna
Department of Neurology, APHP, CHU de Bicêtre, Le Kremlin-Bicêtre, France

17:40–17:55 CEST
Neurofilament light chain (NfL) as a biomarker in hATTR amyloidosis
Dr Hans Nienhuis, Amyloidosis Centre of Expertise, University Medical Center Groningen, The Netherlands

17:55–18:15 CEST
Redefining standards of care
Dr Laura Obici, Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy

18:15–18:30 CEST
Panel discussion and closing remarks

THE ATTR ACADEMY

To facilitate continued learning, we're excited to introduce a **new e-learning course for nurses** in ATTR amyloidosis.

Scan the QR code to register for the ATTR Academy

For nurses and healthcare professionals only. This e-learning course is organized and funded by Alnylam® Pharmaceuticals. This is a disease awareness e-learning course for educational purposes. No information on medicines or Alnylam products will be given within the content of this e-learning course..

*For healthcare professionals only. This meeting is organized and funded by Alnylam® Pharmaceuticals and will contain information on Alnylam medicines. If viewing online, this symposium should only be viewed by healthcare professionals in a quiet area using headphones, and screens should not be visible to the public.
hATTR, hereditary transthyretin-mediated amyloidosis (hATTR or ATTRv; v for variant); RNAi, RNA interference; TTR, transthyretin.
07.2022 ONP-CEMEA-00060

ACTIVELY RECRUITING

Caelum Cardiac Amyloid Reaching for Extended Survival (CARES) Program

Caelum CARES 301[1]

A Phase 3, Double-Blind, Multicenter Study to Evaluate the Efficacy and Safety of CAEL-101† and Plasma Cell Dyscrasia Treatment Versus Placebo and Plasma Cell Dyscrasia Treatment in Plasma Cell Dyscrasia Treatment Naïve Patients With Mayo Stage IIIb AL Amyloidosis

NCT04504825
2019-004254-28*

Caelum CARES 302[2]

A Phase 3, Double-Blind, Multicenter Study to Evaluate the Efficacy and Safety of CAEL-101† and Plasma Cell Dyscrasia Treatment Versus Placebo and Plasma Cell Dyscrasia Treatment in Plasma Cell Dyscrasia Treatment Naïve Patients With Mayo Stage IIIa AL Amyloidosis

NCT04512235
2020-000713-32*

If you have patients who might meet the criteria for these studies, please stop in the **Alexion Booth**, contact us at **ClinicalTrials@alexion.com**, or visit **clinicaltrials.gov**

***These study identifiers correspond to the EudraCT trial number which can be found at https://www.clinicaltrialsregister.eu/.**
†CAEL-101 is not approved for use in this indication and is being studied for efficacy and safety.

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AstraZeneca Rare Disease

[1]A Study to Evaluate the Effectiveness and Safety of CAEL-101 in Patients With Mayo Stage IIIb AL Amyloidosis. Please access Clinicaltrials.gov by scanning the QR code. Published August 7, 2020. Updated March 24, 2022. Accessed June 28, 2022.

[2]A Study to Evaluate the Effectiveness and Safety of CAEL-101 in Patients With Mayo Stage IIIa AL Amyloidosis. Please access Clinicaltrials.gov by scanning the QR code. Published August 13, 2020. Updated March 28, 2022. Accessed June 28, 2022.

M/M/INT/UNB-AL/0018, 7/2022 | Intended for Healthcare Professionals only.

Please join us for

Beyond Survival:
Unmet Medical Needs in AL Amyloidosis

Wednesday 7 September 2022

3:00-4:30 PM Central European Time

Photo is for illustrative purposes only and not of actual patients.

INTERACTIVE PANEL DISCUSSION

Chairperson

Vaishali Sanchorawala, MD
Boston University
School of Medicine
Boston, Massachusetts, USA

Deborah Boedicker
Mackenzie's Mission
Arlington, Virginia, USA

Rodney H. Falk, MD
Brigham and
Women's Hospital
Boston, Massachusetts, USA

Julian Gillmore, MD
Royal Free Hospital London
London, United Kingdom

Isabelle Lousada
Amyloidosis Research
Consortium
Newton, Massachusetts, USA

Giovanni Palladini, MD
IRCCS San Matteo
Polyclinic Foundation
Pavia, Italy

Ashutosh Wechalekar, MD
National Amyloidosis Centre
University College London
London, United Kingdom

Don't miss insights from your physician and patient advocate colleagues.

Organized and funded by

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AstraZeneca Rare Disease

ISA 2022

Satellite Symposium at ISA 2022
Neue Aula, Floor 2
New University, Heidelberg, Germany

M/INT/UNB-AL/0015, 7/2022 | Intended for Healthcare Professionals only.

INTERNATIONAL SYMPOSIUM ON AMYLOIDOSIS 2022

IONIS AND ASTRAZENECA-SPONSORED SYMPOSIUM

OPTIMIZING MULTIDISCIPLINARY CARE IN PATIENTS WITH ATTR

Tuesday, 6 September 2022

3:30-5:00 PM • Neue Aula (New Auditorium)
Neue Universität, Heidelberg University, Heidelberg, Germany

Faculty

Pablo Garcia-Pavia, MD, PhD
Hospital Universitario Puerta de Hierro Majadahonda, CIBERCV
Madrid, Spain

Moderator

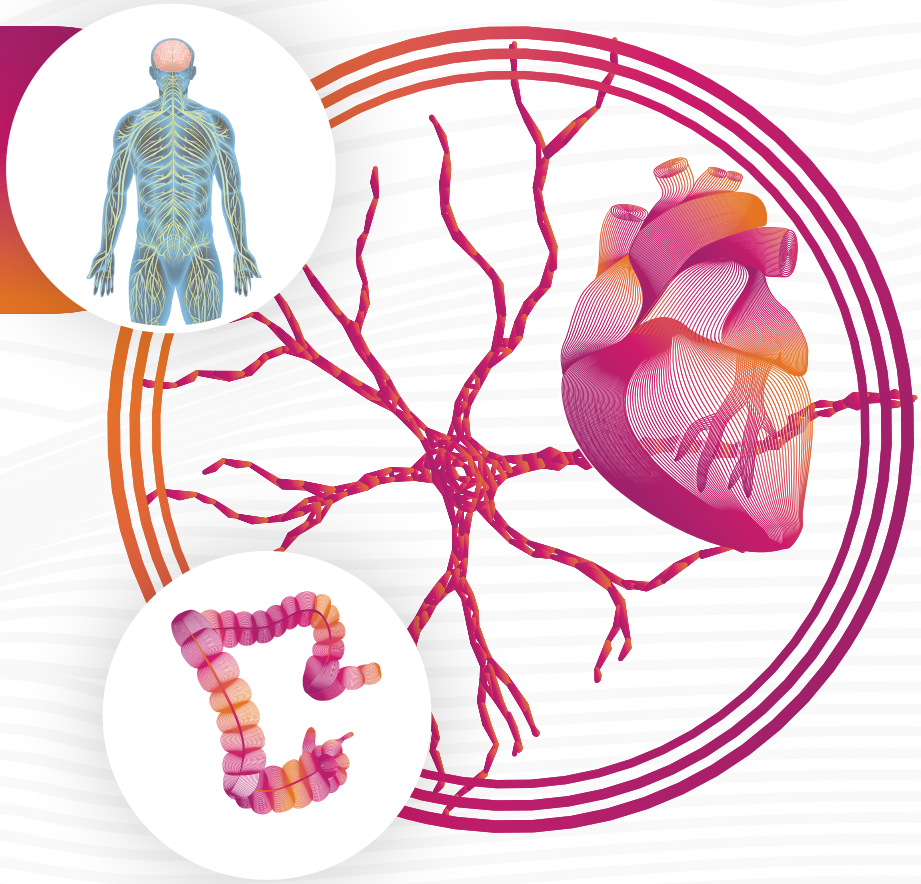
Arnt Kristen, MD
Heidelberg University
Heidelberg, Germany

Faculty

Teresa Coelho, MD
Centro Hospitalar Universitário do Porto – Hospital de Santo Antonio
Porto, Portugal

Please join Dr Arnt Kristen and our expert faculty for an in-depth discussion of the pathophysiology, diagnosis, and multidisciplinary management of patients with ATTR-PN, ATTR-CM, and mixed-phenotype ATTR.

The challenges in early patient identification and diagnosis, burden of disease, need for effective and timely treatment, monitoring of ATTR progression, and patient care coordination with other health care providers and amyloidosis centers of excellence will be discussed from a neurologist's and cardiologist's perspectives. Interactive case studies highlighting these topics will also be presented.



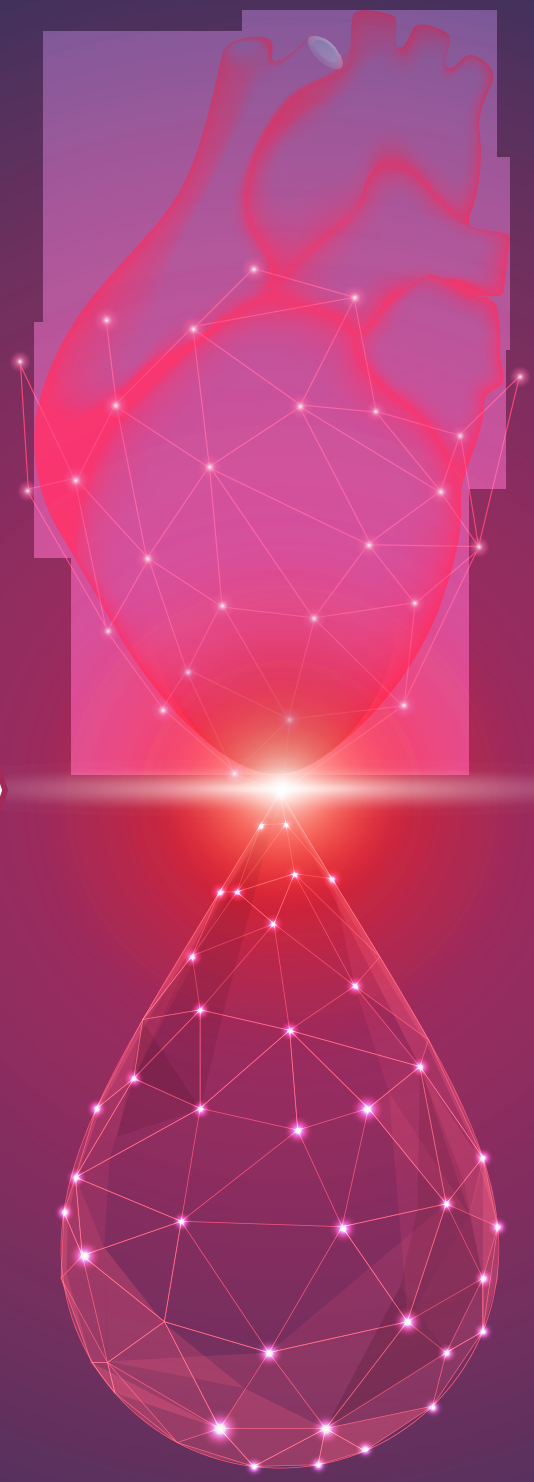
3:30 – 3:35 PM	**Welcome and Introductions** Arnt Kristen, MD
3:35 – 3:40 PM	**Patient Journey Video** Arnt Kristen, MD
3:40 – 3:50 PM	**ATTR: Disease Overview** Arnt Kristen, MD
3:50 – 4:05 PM	**Optimizing Patient Care: A Neurologist's Perspective** Teresa Coelho, MD
4:05 – 4:15 PM	**Interactive Case Studies: Neurology** Panel Discussion
4:15 – 4:30 PM	**Optimizing Patient Care: A Cardiologist's Perspective** Pablo Garcia-Pavia, MD, PhD
4:30 – 4:40 PM	**Interactive Case Studies: Cardiology** Panel Discussion
4:40 – 4:55 PM	**Q&A** Panel Discussion
4:55 – 5:00 PM	**Closing Remarks** Arnt Kristen, MD

Please visit our exhibit booths, #16 (ground floor) and #1 (second floor).

IONIS AstraZeneca

Z4-46941 Approved 07/22

IMPROVING PATIENT OUTCOMES IN **ATTR-CM**



BRIDGEBIO SPONSORED
SATELLITE SYMPOSIUM

06 September 2022
H 14:30 – 15:30
New Auditorium Hall

*at the **XVIII Meeting** of the*

INTERNATIONAL SOCIETY OF AMYLOIDOSIS

Heidelberg, Germany

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

bridgebio

IMPROVING PATIENT OUTCOMES IN ATTR-CM

Co-Chairs

JULIAN GILLMORE MD
University College London

MATHEW S. MAURER MD
Columbia University New York

Faculty Speakers

CLAUDIO RAPEZZI MD
University of Ferrara Ferrara

PABLO GARCIA-PAVIA MD
Hospital University Puerta de Hierro Madrid

TOPIC	SPEAKER
Welcome and introductions	Julian Gillmore MD Mathew S. Maurer MD
ATTR-CM consensus - what's new?	Claudio Rapezzi MD
Present and future in ATTR-CM	Pablo Garcia-Pavia MD
Q & A, closing remarks	Julian Gillmore MD Mathew S. Maurer MD

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BridgeBio International GmbH
Dammstrasse 19, 6300 Zug - Switzerland

HME Forum

Join us at HME-forum.com

The HME Forum is a resource for clinicians with experience in treating patients with a range of hematologic malignancies.

It combines expert interviews, congress coverage, and roundtable discussions that provide updates on key data to inform clinical practice. Interaction with the faculty is encouraged during the live webinar through real-time Q&A.

Myeloma Connect, Challenges in CLL, and Challenges in NHL are a series of live, interactive broadcasts that individually focus on a specific topic relevant to hematologists treating patients with multiple myeloma, chronic lymphocytic leukemia, and non-Hodgkin's lymphoma.

myeloma CONNECT

CHALLENGES IN CLL

CHALLENGES IN NHL

Please scan here to register for upcoming webinars

Organized and supported by Janssen Pharmaceutical Companies of Johnson & Johnson in EMEA

Janssen Pharmaceutica NV
Turnhoutseweg 30, 2340 Beerse, Belgium

EM-104245
Date of preparation: July 2022

Hematology Medical Education

Janssen
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HME

ISA 2022

JANSSEN SATELLITE SYMPOSIUM
INTERNATIONAL SYMPOSIUM ON AMYLOIDOSIS
4–8 SEPTEMBER 2022, HEIDELBERG, GERMANY

Live Symposium

Navigating the patient journey in AL amyloidosis: a multidisciplinary approach

Monday, 5 September 2022
16:30–18:00 CEST

SCIENTIFIC COMMITTEE

M Fontana
UK

G Palladini
Italy

FACULTY

F Bridoux
France

D Foard
UK

C Röcken
Germany

Program

Duration	Topic	Speaker
5 minutes	**Welcome and introduction**	G Palladini
20 minutes	**Diagnostic pitfalls and risk stratification in AL amyloidosis**	M Fontana
20 minutes	**Panel discussion**	All faculty
20 minutes	**Key considerations when selecting treatment for patients with AL amyloidosis**	G Palladini
20 minutes	**Panel discussion**	All faculty
5 minutes	**Summary and close**	G Palladini

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Janssen Pharmaceutica NV
Turnhoutseweg 30
2340 Beerse, Belgium

EM-104245
Date of preparation: July 2022

Hematology Medical Education

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A Pfizer-sponsored
XVIII International Symp

How can one solution solve the multifaceted challenges of ATTR amyloidosis?

September 5, 2022

18:00–19:00 CEST

Neue Aula, Neue Universität, Heidelberg, Germany

SPEAKERS

Markus Weiler

Department of Neurology, Heidelberg University Hospital, Germany

Arnt Kristen

Department of Cardiology, University of Heidelberg, Germany

Laura Obici

Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Italy

Rachele Bonfiglioli

Sant'Orsola Hospital, Bologna, Italy

ATTR amyloidosis, transthyretin amyloidosis; ATTR-PN, transthyretin amyloid polyneuropathy.

PP-VYN-DEU-2229; July 2022

l symposium at the posium on Amyloidosis

ISA 2022

AGENDA

18:00–18:05	**Welcome and introduction**	Markus Weiler (Co-Chair) Arnt Kristen (Co-Chair)
18:05–18:15	**Transthyretin in health and disease**	Laura Obici
18:15–18:25	**Improving patient outcomes in ATTR-PN**	Markus Weiler
18:25–18:40	**Addressing cardiac involvement in patients with ATTR amyloidosis**	Arnt Kristen
18:40–18:50	**Clinical clues to cardiac involvement in ATTR amyloidosis – how to identify patients early**	Rachele Bonfiglioli Arnt Kristen
18:50–19:00	**Panel discussion and Q&A**	All faculty

Pfizer

Making a Real Impact for Patients

Growing evidence suggests antibodies that optimally target misfolded proteins can have a real impact for patients. At Prothena, we are applying over 35 years of protein dysregulation experience to advance novel therapeutics to address rare peripheral amyloid and neurodegenerative diseases.

Learn more at
www.prothena.com

Prothena is the sponsor of AFFIRM-AL, an ongoing Phase 3 clinical research study evaluating the efficacy and safety of investigational birtamimab in Mayo Stage IV AL amyloidosis patients.

Visit **www.affirm-al.com** for more information.

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ORAL PRESENTATIONS

Next Generation Sequencing Identifies AL-related *IGLV* Genes in Patients with λ-isotype MGUS or Smoldering Multiple Myeloma

P Zhou[1], MM Mansukhani[2], D Toskic[1], S Scalia[1], LX Lee[3], SW Wong[4], SA Tuchman[5], J Hoffman[6], T Fogaren[1], C Varga[7], S Lentzsch[2], RL Comenzo[1]

[1]Tufts Medical Center, Boston, MA USA; [2]Columbia University Medical Center, NY, NY USA; [3]University of California, Irvine, CA USA; [4]University of California, San Francisco, CA USA; [5]University of North Carolina, Chapel Hill, NC USA; [6]University of Miami, FL USA; [7]Levine Cancer Institute, Rock Hill, SC USA.

Background: Eighty percent of AL patients harbor monoclonal free light chain (FLC) abnormalities for 10 years prior to diagnosis, consistent with a monoclonal gammopathy being a risk factor for AL. (*1-4*) Strategies for early identification of AL are critically needed; 75% of AL patients are λ-type and 9 of 33 immunoglobulin λ light-chain variable region (*IGLV*) genes on chr 22q11.2 account for over 85% of λ cases.(*5, 6*) Therefore, we have sought in two clinical studies to ascertain the risk of AL in λ MGUS and SMM patients by identifying their clonal *IGLV* genes by next generation sequencing (NGS) (NCT02741999, NCT04615572). We now report results of NGS of clonal *IGLV* genes.

Objective: Our primary objective is to develop methods that enable early diagnosis of AL or risk of AL. We sought to identify AL-related clonal λ *IGLV* genes in MGUS and SMM patients with a difference between involved and uninvolved FLC > 23mg/L, a κ-to-λ ratio below normal and no prior evidence of amyloid.(*1*) The 9 *IGLV* genes we employed as AL-related were *LV6-57*, *LV2-14*, *LV1-44*, *LV3-1*, *LV1-51*, *LV3-21*, *LV3-19*, *LV2-23* and *LV1-40*. Patients with one of these genes identified by NGS were evaluated for AL.

Materials & Methods: MGUS and SMM patients from multiple sites in the USA consented to participate and if eligible had blood or marrow aspirates shipped to a central lab and processed as previously described.(*7*) We performed RNA extraction on CD138-selected and on $2x10^6$ marrow MNC and synthesized cDNA. For NGS evaluation, 9 λ light-chain variable region and two λ light-chain constant region primers were used with the Q5 high-fidelity DNA polymerase; PCR products of 650-700bp were sheared by ultrasonication and, following end-repair and adapter ligation, sequenced on a MISeq (Illumina, San Diego CA). Approximately 500,000 reads were obtained per sample and Fastq files were converted to FASTA sequences and mapped using IGMT/HIV-Quest to obtain the numbers of CDR3 clonotypic reads linked to an *IGLV* gene. GraphPad PRISM V5 was used for statistical analyses.

Results: Thirty-seven patients (MGUS=7, SMM=30) enrolled and had blood (n=16) or marrow (n=21) shipped for NGS sequencing. NGS identified *IGLV* genes in CD138 samples,13/16 from blood and 21/21 from marrow; 17 marrows had paired MNC and CD138 samples used for NGS in duplicate runs with reads that were highly correlated with $r > 0.95$ and $P << 0.01$. In 14/17 (82%) MNC samples a single *IGLV* gene was identified. In 3 MNC/CD138 pairs 2 *IGLV* genes shared the same CDR3 clonotype (3-1/3-12; 2-23/5-52; paralog 1-44/1-36). In 4 MNC/CD138 pairs a single *IGLV* gene was identified in the MNC samples and confirmed in the CD138 samples, in 3 cases with 10X the reads of other *IGLV* genes in the CD138 samples and in 1 case with a paralog (1-44/1-36). AL-related genes were identified in 26/37 cases (70%) (2-14=6, 2-23=5, 1-44=5, 3-1=3, 3-21=5, 3-19=2) and amyloid was identified in 4/37 cases (11%; 2-14=2, 2-23=1, 3-1=1), 2 cardiac, 1 GI and 1 peripheral neuropathy. All 4 patients had SMM and had a single AL-related *IGLV* gene identified.

Summary & Conclusion: NGS can reliably identify clonal *IGLV* genes in cDNA from $2x10^6$ marrow MNC from λ MGUS and SMM patients who pass the FLC screen; 70% of the genes identified in this series were AL-related and 11% of patients were found to have AL. A screening study in λ MGUS and SMM patients who pass the FLC screen (NCT04615572) employing cDNA from $2x10^6$ marrow MNC continues to accrue.

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Elucidation of the cardiotoxicity of full-length light chains derived from patients with cardiac light chain amyloidosis in comparison to other plasma cell dyscrasias

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Background: Cardiac involvement in light chain (AL) amyloidosis (AL-CA) is a life-threatening complication and the major determinant of prognosis. The management of heart dysfunction and failure in AL is challenging: standard therapies for heart failure are poorly tolerated or ineffective, and rapid reduction of the amyloidogenic light chains (LCs) remains the only effective approach. Despite advances in our knowledge, the pathophysiological mechanisms of LCs cardiotoxicity in AL-CA is still poorly understood. Therefore, comparing the cardiac effects induced by amyloidogenic LCs to those from LCs derived from other plasma cell dyscrasias (PCDs) such as multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) could improve our understanding of the mechanisms of cardiac damage.
Objective: We aimed to 1) genetically identify and biotechnologically produce full-length LCs from patients with AL-CA, MM and MGUS or non-clonal LCs from healthy volunteers (HV), 2) identify if the LCs' impact on cardiomyocyte viability and 3) investigate the underlying mechanisms of cardiotoxicity *in vitro*.
Material & Methods: Bone marrow derived CD138+ cells from n=7 patients with AL-CA, n=2 patients with MM and n=2 with MGUS and peripheral blood mononuclear cells (PBMCs) from n=2 HV were isolated for RNA extraction and characterization of the LC gene family repertoire. At the protein level, LC expression was confirmed by immunoprecipitation in patients' serum followed by top-down proteomics. The overexpressed LC genes in each patient, encoding the full-length clonal LCs were cloned and produced in Shuffle E. coli cells. Three LCs genes from the HVs, were chosen based on their sequence similarity with the patients' LCs and the respective proteins were also biotechnologically produced. LCs folding, oligomerization and amyloidogenic potential were assessed via circular dichroism (CD), SDS page and electron microscopy respectively. Primary adult ventricular murine cardiomyocytes (pAVMCs) were isolated and exposed at various LC concentrations for evaluation of cell death and investigation of the cardiotoxicity mechanisms via gene and protein expression.
Results & Discussion: We identified the clonal LCs and isolated the respective proteins in all cases (7 AL-CA, 2 MM, 2 MGUS and 3 HV). Despite the similarity of the LCs in conformation as beta-sheets and oligomerization mainly as dimers, 5 out of 7 AL-CA derived LCs led to a significantly increased cardiotoxicity in pAVMCs and cardiomyocyte death, compared to non-clonal LCs derived from HV and the LCs derived from MM and MGUS, which did not alter cell viability. Interestingly, these 5 LCs had the highest amyloidogenic potency. The cardiotoxic LCs induced different molecular responses leading to cardiomyocyte death. κ-type AL-CA LCs induced apoptosis and overexpression of endoplasmic reticulum stress (ERS) markers while λ-type LCs increased unfolded protein response (UPR) markers and autophagy without inducing apoptosis. All LCs of κ-type, including the ones derived from MM and MGUS patients, led to increased proinflammatory cytokines indicating that this mechanism is independent of the observed cardiomyocyte death.
Summary & Conclusion: AL-CA derived LCs induce cardiotoxicity, which correlates to their amyloidogenic potential and cell death is primarily driven via ERS, UPR, autophagy and apoptosis. These pathways can be considered as potential targets for the development of novel cardioprotective strategies to support the failling myocardium on top of the existing anti-clonal therapies.

Support & Funding: The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the “HFRI PhD Fellowship grant” (Fellowship Number 195/2019) and under the “2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers” (Project Number:955)

Investigation of IGLV2-14 AL amyloidosis and multiple myeloma light chain sequences

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Background: Light chain amyloidosis (AL) is one of the most common forms of systemic amyloidosis and is caused by the misfolding and aggregation of immunoglobulin light chains (LC) to insoluble fibrils (1).

Objective: To adress the question why LC misfolding occurs in AL, we performed a comparison with the related disease multiple myeloma (MM). Patients of both diseases display an increased value of serum free LC (2, 3), however, the LCs of MM patients do not form fibrils. In a first analysis, IGLV2-14 LC sequences from patients with AL and dominant heart or kidney involvement were compared to the respective MM sequences and analyzed with respect to the presence or absence of a heavy chain (HC) in the serum immunofixation.

Material & Methods: The LC analysis of AL patients (n=13) was performed on cDNA using a multiplex primer set (4) and Sanger sequencing. For MM patients, LC sequences (n=8) were extracted from bulk RNA-Seq using MIXCR and validated (n=4) using Sanger sequencing.

Results: With an average of 7.6 mutations per immunoglobulin λ LC variable (IGLV)-segment and none mutation in the immunoglobulin λ LC constant (IGLC)-segment AL sequences were less frequently mutated than MM sequences (IGLV:10.5; IGLC:25%). In the stratification for the presence or absence of a HC, an increased IGLV-segment mutation count was detected for AL patients without detectable HC ($\bar{x}$ 8.8 vs. 6.9). In a detailed mutation analysis, the MM sequences displayed ten frequently mutated positions distributed throughout the complete IGLV-segment, whereas the mutation-hotspots of the AL sequences were restricted to two CDR-loci. Both cohorts shared the position 54S as mutation hotspot with a preferred AS exchange towards threonine (MM: 4/8; AL: 5/13) - all AL associated S54T mutations correspond to patients with dominant heart involvement. In contrast to the AL sequences (n=1) five MM sequences showed at least one additional charge in the CDR3. To evaluate the effect of the detected mutations on the theoretical stability of the LCs the isoelectric point (pI) was calculated. While the pI of the full-length LC of the AL (6.80±0.90) and MM (6.54±0.99) sequences did not significantly differ, a prominent difference could be noted when the presence of a HC was considered (Figure 1). For the full-length LC (7.45±0.74) and the IGLVJ segments (6.95±1.15) of AL patients without detectable HC a significant higher pI could be detected - which making them potentially more stable at the normal pH of human blood (pH ca. 7.4). In contrast, MM LC sequences and LC sequences of AL patients with HC showed a pI below 7 (MM HC IGLVJC: 6.60±1.04; AL HC IGLVJC: 6.40±0.75).

Summary & Conclusion: In a detailed sequence analysis, it was possible to detect a mutation in correlation with dominant heart involvement in AL, which, however, also occurs in the MM sequences. The co-occurrence of mutations suggests that there must be additional factors influencing amyloidogenicity. This correlate with the finding that the MM sequences display a higher overall mutation count and, in most cases, at least one mutation towards a charged AS in the CDR3. Another interesting finding is that the AL sequences corresponding to patients without detectable HC showed a significant increase in mutation count and pI. In summary, these findings lead to the hypothesis, that in the absence of a HC the expression and circulation of mutated free LCs with a pI near to the physiological pH of the blood occur, which exhibit a decreased solubility and increased aggregation tendency.

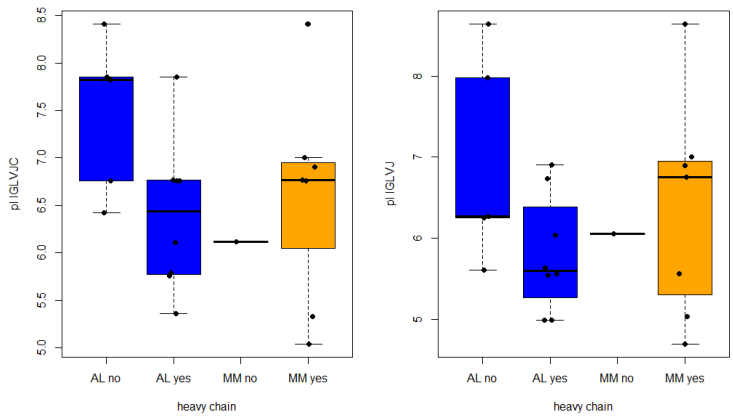

Figure 1.: Analysis regarding the isoelectric point of the IGLV2-14 subfamily and the presence of a heavy chain. AL = AL amyloidosis, MM = multiple myeloma, pI = isoelectric point.

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Support & Funding:

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Sequence diversity of the kappa light chains from patients with AL amyloidosis and multiple myeloma

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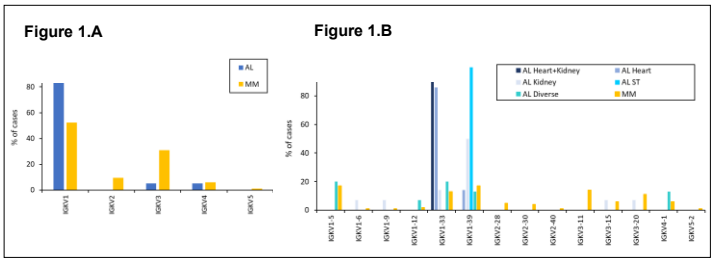
Background: AL amyloidosis (AL) is one of the most common forms of systemic amyloidosis in industrialized countries (1). The disease is caused by the misfolding and aggregation of immunoglobulin light chains (Ig LCs) segregated by monoclonal B-cells in underlying plasma-cell disease such as monoclonal gammopathy of clinical significance (MGCS) or multiple myeloma (MM) (2,3,4).

Objective: Only little is known about why and how the LCs of AL patients form amyloid deposits. Research into the variability of the LC sequences and their association to AL has usually been focused on the VJ-segments but ignored the C domain and the combination of the different V, J and C-gene segments. To answer the question why the LCs of AL patients form fibrils we analyzed the complete LC of patients with kappa AL and compared them to the LC of MM patients.

Material & Methods: In this study 38 patients with AL and 84 patients with MM were included. The median age at diagnosis in AL was 61 years (range: 34 to 81 years) and in MM 61 years (range: 42 to 70 years). The median of the difference between serum free LC (dFLC) in AL was 260 mg/l (range: 8 to 3784 mg/l) and in MM 358 mg/l (range: 5 to 30124 mg/l). For diagnostic purposes patient´s bone marrow was aspirated and CD138+ selected plasma cells were used to extract RNA. LCs from AL patients were analyzed by Sanger sequencing. Bulk RNA-sequencing was performed for the MM patients, the LC sequences were extracted using MIXCR and for mutation analysis the sequences were verified using Sanger sequencing.

Results: Our data indicate that members of the kappa 1 family are most commonly expressed in AL (90%) as well as in MM (52%) (Figure 1A). IGKV2 and IGKV5 could only be detected in MM (10%,1%). Investigations of the linkage of the variable and the joining regions identified that IGKV1 is most frequently associated to J2 in AL (34%) as also in MM (20%). With regard to the organ tropism in AL, differences in the IGKV1-subgroups could be determined. For instance, IGKV1-33 was primarily detected in patients associated with heart (86%, n=6) or heart and kidney (100%, n=1) involvement, whereas IGKV1-39 was found in patients with kidney (50%, n=7) and soft tissue (100%, n=1) involvement (Figure 1B). Mutational analysis of IGKV1-33, which is most commonly associated with J2, revealed hotspots in the complementarity determing regions (CDRs) 1 and 3 and framework region (FR) 3. In four out of seven AL cases and in two out of four MM cases a nonsynonymous substitutions was detected on position 70D (Figure 2). This exchange causes a loss of charge or a reversal of charge from negative to positive. One position (80P) accumulates nonsynonymous substitutions in more than 50% of the AL cases. At position 83I all AL cases associated with only heart or additionally kidney organ involvement showed nonsynonymous variants. In another 17 positions only the AL sequences were mutated, e.g. position 65S or 73F, three of the AL sequences were mutated, but none of the MM (Figure 2). No mutation could be detected in the C-region in AL and MM.

Summary & Conclusion: Kappa 1 is the most frequent IGKV family in AL and in MM. IGKV2 and IGKV5 were only found in the MM patients. Regarding organ tropism in AL, only differences in IGKV1-subgroups were detected, in contrast to lambda AL (5). Specific mutation patterns for IGKV1-33/J2 could be identified, especially for AL patients with only cardiac involvement or additional renal involvement.



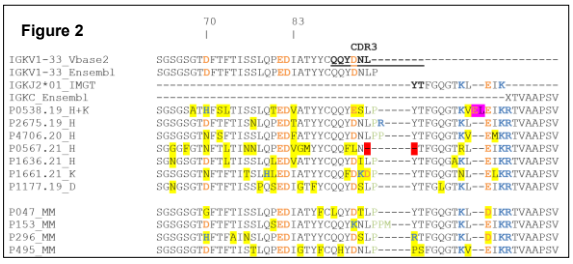


Figure 1.: Variable region gene usage in AL amyloidosis and in multiple myeloma patients. AL= AL amyloidosis, MM= multiple myeloma **A** IGKV family usage in AL and MM. AL (n=38), MM (n=84). **B** IGKV1-subgroups in AL (organtropism) and MM. Dominant kidney involvement (n=14), dominant heart involvement (n=7), diverse organ involvement (n=15), dominant heart and kidney involvement (n=1), Soft tissue (ST) involvement (n=1), multiple myeloma patients (MM) (n=84)

Figure 2.: Alignment section of IGKV1-33 and J2 in AL amyloidosis and multiple myeloma patients. AL= AL amyloidosis, MM= multiple myeloma. Highlighted in yellow are nonsynonymous substitutions. Orange negative charged and blue positive charged amino acids. Green Linker-region. Red deletions, purple insertions. The amino acid sequences were numbered based on the Vbase2 reference. CDR was aligned according to Kabat using abYsis.

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Single Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq): toward personalized medicine approaches in monoclonal gammopathies

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Background: In patients affected by monoclonal gammopathies, tumoral B cells or plasma cells secrete a monoclonal antibody (termed M protein), which can be used to track the presence of the tumor itself. Moreover, the M protein can directly cause potentially life-threatening organ damage, which is dictated by the specific, patient's unique clonal light and/or heavy chain, as in patients affected by immunoglobulin light chain (AL) amyloidosis. Yet, the current paradigm in the diagnosis and management of these conditions treats the M protein as a simple tumor biomarker to be identified/quantified. Patients' specific M protein sequences remain mostly undefined and molecular mechanisms underlying M-protein related clinical manifestations are largely obscure.

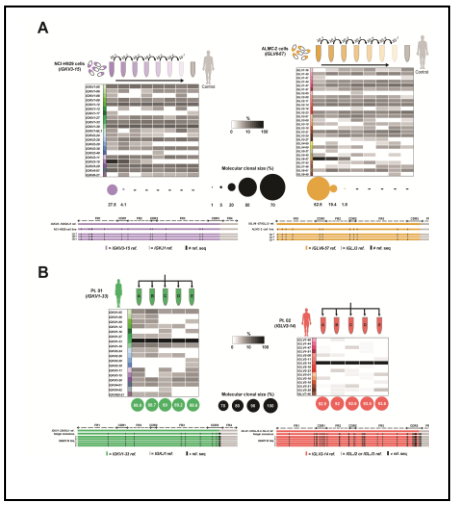
Objective: To establish and validate a high-throughput assay to reliably identify the entire variable region of clonal immunoglobulin (Ig) genes from a high number of biological samples in parallel.

Material & Methods: By combining the unbiased amplification of expressed Ig genes with long-read, single molecule real-time DNA sequencing and bioinformatics analyses, we have established a method to identify the full-length sequence of the variable region of expressed Ig genes and to rank the obtained sequences based on their relative abundance, thus enabling the identification of the full-length variable sequence of M protein genes from a high number of patients analysed in parallel.

Results: The assay, which we termed Single Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq), has undergone an extensive technical validation. Sequencing of contrived bone marrow samples generated through serial dilutions of plasma cell lines into control bone marrow, as well as sequencing of *bona fide* bone marrow samples from AL

patients and comparison with gold-standard techniques of Ig gene sequencing showed:
1) 100% sequence-accuracy at the individual base-pair level;
2) High repeatability in defining the molecular clonal size;
3) A high sensitivity in identifying clonal Ig sequences.
Noteworthy, SMaRT M Seq was applied to a cohort of 86 consecutive patients with AL amyloidosis (17 κ and 69 λ; median BMPC infiltration 9%, IQR 6-13%; median dFLC 176 mg/L, IQR 75-370 mg/L), including cases with undetectable M protein using conventional M protein studies. A full-length sequence of the variable region of the clonal light chain (LC) was obtained in all patients (median molecular clonal size of 88.3%, IQR: 70.7 – 93%). The most common κ germline genes were IGKV1-33 and IGKV4-01 (24% each of the 17 κ AL patients), and the most common λ germline genes were IGLV6-57 (26% of the 69 λ AL patients), IGLV2-14 (17%), IGLV3-01 (17%) and IGLV1-44 (10%). The most frequent λ and κ germline genes together (IGLV6-57, IGLV2-14, IGLV3-01, IGLV1-44, IGKV1-33 and IGKV4-01) accounted for 66% of all the clones. Germline gene usage correlated with selected clinical features.

Summary & Conclusion: We have established SMaRT M-Seq as a novel valuable assay to reliably identify the full-length variable sequence of M proteins. SMaRT M-Seq has undergone extensive technical validation, showing high accuracy, repeatability and scalable sensitivity.
Sequencing disease-associated M proteins from large cohorts of patients has the potential to uncover molecular mechanisms of M protein-related clinical manifestations which have remained largely unexplored so far, and could enable approaches of personalized medicine for the sensitive detection of patients' specific M proteins at diagnosis and after anti-clonal therapy.



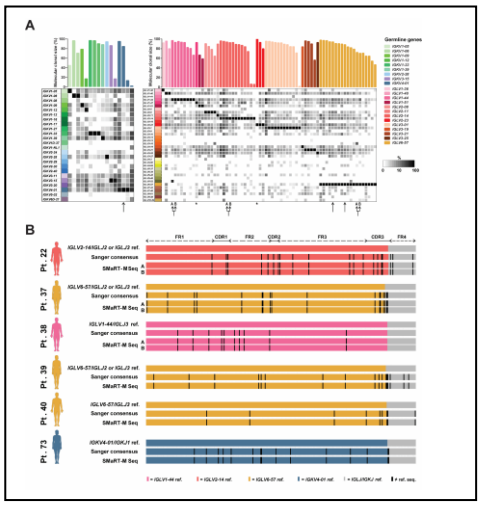


Figure 1. Technical validation of SMaRT M-Seq
A Expression levels (in shades of grey) of different *IGKV* (left) and *IGLV* (right) germline genes (each denoted by a distinctive colour) as assessed by SMaRT M-Seq, starting from serial Log_{10} dilutions of RNA from *IGKV3-15*-secreting NCI-H929 cells (left) or *IGLV6-57*-secreting ALMC-2 cells (right) into RNA of the bone marrow from a control subject (unspiked control sample in grey). **B** Expression levels of different *IGKV* (left) and *IGLV* (right) germline genes as assessed by SMaRT M-Seq, starting from five replicate bone marrow samples (A to E) from two patients (Pt. 01 and 02) affected by AL amyloidosis, with a plasma cell clone secreting an *IGKV1-33* (left) or an *IGLV2-14* (right) clonal light chain. In both **A** and **B**, scaled pie charts denote the molecular clonal size of the dominant clone identified in each tested sample (the corresponding *IGKV* or *IGLV* germline gene is indicated with the pie chart colour). Minus sign (-) indicates samples where no dominant clone could be identified with Vidjil. At the bottom, sequence alignments of the clonotypic variable region of the light chain secreted by NCI-H929 and ALMC-2 cells (**A**) or of the clonal light chain from patient 01 and patient 02 (**B**), as assessed by cloning and Sanger sequencing (Sanger) or by SMaRT M-Seq (denoted by the corresponding dilution, **A**, or replicate label, **B**), with the corresponding *IGKV*/*IGKJ* or *IGLV*/*IGLJ* germline genes (ref., with the J gene in grey and the V gene denoted by the corresponding colour). Black tick denotes sequence mismatches (≠ ref. seq.) in the clonotypic light chain with respect to the corresponding germline genes. FR: frame work region; CDR: complementarity determining region; Pt.: patient.

Figure 2. SMaRT M-Seq identifies the full-length variable sequence of clonal light chains in a cohort of AL patients
A Expression levels of different *IGKV* (left) and *IGLV* (right) germline genes (each denoted by a distinctive colour) as assessed by SMaRT M-Seq, starting from the bone marrow of 84 affected by AL amyloidosis analyzed in parallel in one sequencing round. Bar graphs indicate the molecular clonal size of the dominant clone identified by Vidjil analysis in each tested sample (the corresponding germline gene is indicated with the bar colour). In two patients (*) the dominant clone was identified by IMGT/HighV-Quest. Three patients were analyzed in duplicates (arrows). **B** Sequence alignments of the clonal light chain from six patients as assessed by cloning and Sanger sequencing (Sanger) or by SMaRT M-Seq (A-

B indicate technical duplicates), with the corresponding *IGKV*/*IGKJ* or *IGLV*/*IGLJ* germline genes (ref., with the J gene in grey and the V gene denoted by the corresponding colour). Black tick denotes sequence mismatches (≠ ref. seq.) in the clonotypic light chain with respect to the corresponding germline genes. FR: frame work region; CDR: complementarity determining region; Pt.: patient.

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An N-glycosylation hotspot in immunoglobulin κ light chains is associated with AL amyloidosis

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Background: Immunoglobulin light chain (AL) amyloidosis is caused by a small, minimally proliferating B cell/plasma cell clone secreting a patient-unique, aggregation-prone, toxic light chain (LC). The pathogenicity of LCs is encrypted in their sequence, yet molecular determinants of amyloidogenesis are poorly understood.
Higher rates of N-glycosylation among clonal κ LCs from patients with AL amyloidosis compared to other monoclonal gammopathies indicate that this post-translational modification is associated with a higher risk of developing AL amyloidosis.

Objective: To study sequence and spatial feature of N-glycosylation of immunoglobulin LCs associated with AL amyloidosis in comparison to control LCs from patients with other monoclonal gammopathies.

Material & Methods: We exploited LC sequence information from previously published amyloidogenic and control clonal LCs and from a series of 220 patients with AL amyloidosis or multiple myeloma followed at our Institutions and sequenced using SMaRT M-Seq to define sequence and spatial features of N-glycosylation, combining bioinformatics, biochemical, proteomics, structural and genetic analyses.

Results: Using sequence-based, *in silico* prediction of N-glycosylation, we found a peculiar pattern of N-glycosylation in amyloidogenic κ LCs, with ≈75% of the N-glycosylation sites laying in the framework region 3, particularly within the E strand, and consisting of the NFT sequon in ≈50% of cases, setting them apart with respect to non-amyloidogenic clonal LCs. Biochemical and proteomic analyses confirmed sequence-based N-glycosilation predictions in our cohort of patients.
Based on genomic and genetic analyses, the occurrence of mutations within selected regions of *IGKV* genes (progenitor glycosylation sites) during somatic hypermutation, rather than ultra-rare genomic variants, are likely to explain the N-glycosylation hotspot in amyloidogenic κ LCs.
Structural mapping showed that N-glycosylation sites lay on the solvent-exposed surface of the variable domain, thus not directly affecting the monomer-monomer interface in the context of the full dimeric protein.
Finally, based on currently available amyloidogenic and control, clonal LC sequences, we showed that using the presence of a putative N-glycosylation site specifically within the FR3 region (rather than at any position) improved the classification of a clonal κ LC as potentially amyloidogenic.

Summary & Conclusion: Our data further support a potential role of N-glycosylation in determining the pathogenic behavior of a subset of amyloidogenic LCs and may help refine current N-glycosylation-based prognostic assessments for patients with monoclonal gammopathies.

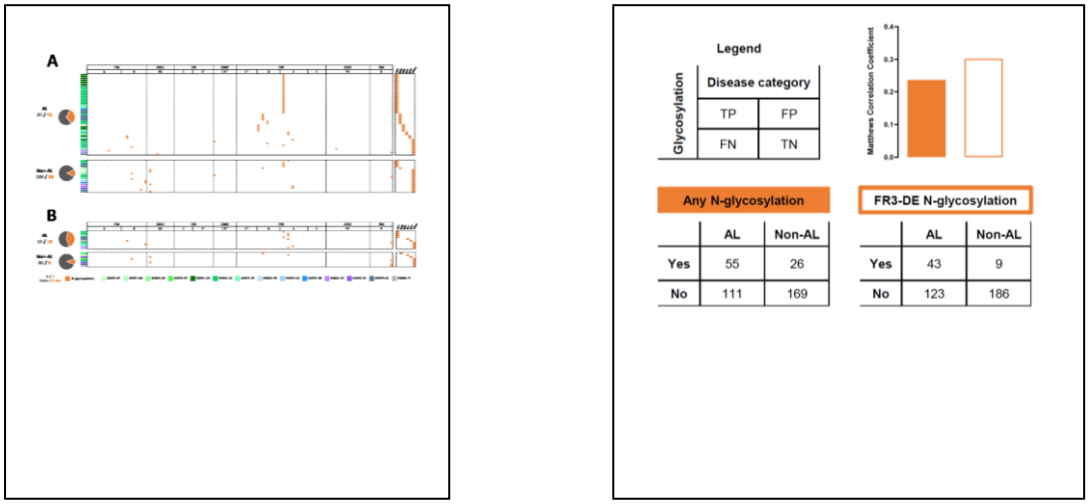

Figure 1. Sequence and spatial features of predicted N-glycosylation of amyloidogenic and non amyloidogenic clonal κ light chains: The pie chart shows the percentage of known amyloidogenic (AL) and non-amyloidogenic (Non-AL) clonal κ light chains predicted to be N-glycosylated by NetNGlyc (in A from published literature and in B from this study). Numbers (N) of unglycosylated (Unglyc) and N-glycosylated (N-glyc) sequences are indicated. The exact location of the N residue predicted to be glycosylated and the sequon type (NXS/T) are displayed in the heatmaps (each row denotes one sequence/patient). The corresponding germline gene is indicated with a color code. FR: framework region; CDR: complementarity determining region; J: J region.

Figure 2. Prognostic significance of N-glycosylation site mapping within κ light chains: Matthew Correlation Coefficient (MCC) considering the presence of N-glycosylation at any position (Any N-glycosylation) or the presence of an N-glycosylation site-specifically at the D or E strand of FR3 (FR3-DE N-Glycosylation) as a risk factor for amyloidogenicity of clonal κ light chains. N-glycosylation prediction is performed on known amyloidogenic (AL) and non-amyloidogenic (non-AL) clonal κ light chains based on NetNGlyc. True Positives (TP) were defined as AL κ sequences for which a N-glycosylation site was predicted (in any region or specifically in the FR3-DE region), False Positives (FP) as non-AL sequences with a redicted N-glycosylation site, True Negatives (TN) and False negatives (FN) as non-AL and AL sequences without redicted N-glycosylation site respectively.

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Support & Funding: This work was supported by grants from the Amyloidosis Foundation (MNu), the Italian Ministry of Health (Ricerca Finalizzata, grant #GR-2018-12368387) (MNu), the Italian Ministry of Research and Education (PRIN 20207XLJB2) (SR, GP), the CARIPLO Foundation (grant #2018-0257) (MNu), Fondazione ARISLA (project TDP- 43-STRUCT) (SR), Cancer Research UK [C355/A26819], FC AECC and AIRC under the Accelerator Award Program (BP, GM, GP, MNu). We would like to acknowledge the use of the Boston University AL-Base, supported by HL68705, in this work.

Amyloidogenesis by mesangial cells involves active participation of lysosomes

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Background: Previous studies have indicated that the formation of amyloid fibrils occurs in the cytoplasm of mesangial cells in the acidic mature lysosomal compartment. While there is literature supporting this(1-4), morphologic evidence that this is the case has been indirect.

Objective: The present study aimed at establishing the relationship between lysosomes and light chains to determine the specific role played by the lysosomal compartment in amyloid fibril formation.

Material & Methods: Human mesangial cells (HMCs) grown on Petri dishes were incubated with amyloidogenic light chains obtained and purified from the urine of a patient with AL (light chain-associated) amyloidosis for 48 hours. Controls included HMCs incubated with a myeloma cast nephropathy protein also isolated and purified from the urine and HMCs incubated without light chains. Protein concentration was 10 µg ml. Some of the samples were processed to obtain the lysosomal fraction by gradient technology. Specimens were processed for light and electron microscopy, stained with fluorescence for kappa and lambda and double ultrastructurally immunogold labeling for lambda, kappa light chains (with 13-15 nm gold particles) and lysosomal associated membrane protein (LAMP) (5-6 nm gold particles).

Results: Only HMCs incubated with AL-light chain were associated with the formation of amyloid fibrils. In the lysosomal gradient specimens, labeled lysosomes (with LAMP) were identified intimately associated with fibrils that were formed. Amyloid fibrils were clearly seen in the specimen routinely processed for light and electron microscopy in the extracellular space. Only those amyloid fibrils in the process of being generated by the lysosomes were identified in the lysosomal gradient specimens. The remainder of the fibrils were not in this gradient material. Double gold immunolabeling clearly identify the formation of fibrils in lysosomes.

Summary & Conclusion: This study clearly demonstrates that lysosomes represent the cellular machinery responsible for the fibril formation in the glomerular mesangium in AL-amyloidosis.

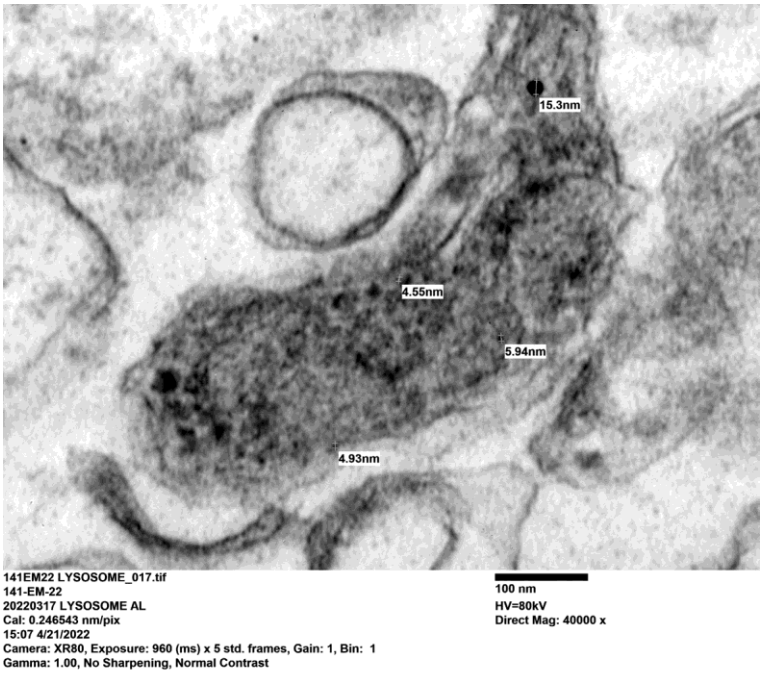

Figure 1.: Doble immunogold labelling of lysosomes isolated from human mesangial cells incubated with amyloidogenic lambda light chain. 14-15 nm gold particles marking lambda light chains and 5-6 nm gold particles marking lysosomal anti-membrane protein (LAMP).

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Mapping and modelling the molecular mechanisms that drive amyloidogenic light chain induced cardiotoxicity

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Background: Light chain (AL) amyloidosis is characterized by the deposition of immunoglobulin light chains (LCs) as amyloid fibrils in downstream target organs. The heart is the most commonly affected organ with patients experiencing life-threatening cardiac failure and poor tolerance to therapy. [1] Cardiac dysfunction is caused by architectural distortion from amyloid fibril deposition and directly by LCs. [2] Although late-stage processes such as oxidant stress, contractile dysfunction, and apoptosis of cardiomyocytes are thought to be involved, there is a limited understanding of the initial underlying molecular mechanisms driving LC-induced cardiotoxicity. [3,4]

Objective: We evaluated the transcriptomic changes occurring in cardiac cells upon *in vitro* exposure to cardiotropic, amyloidogenic LCs (c-AL LCs). We sought to characterize the earliest signatures of LC-induced cardiotoxicity and to validate our findings using a novel disease model for cardiac AL amyloidosis fueled by the use of induced pluripotent stem cell (iPSC)-derived cardiomyocytes.

Material & Methods: LC proteins were prepared from a patient with AL amyloidosis and severe cardiac involvement as previously described. [5] AC16 human ventricular cardiomyocytes as well as iPSC-derived cardiomyocytes were exposed to LCs for 48 hours and assayed for gross morphological changes via light microscopy. We also performed flow cytometry, and gene expression changes using mRNA sequencing with RT-PCR for hit validation. Functional changes were also assessed in iPSC-derived cardiomyocytes following exposure to LCs. Alternate stressors including heat shock, thapsigargin, and transthyretin amyloid (ATTR) proteins were included as controls to aid in the identification of LC-specific transcriptional signatures. (Figure 1A)

Results: As expected, and as controls for the integrity of our data set, AC16 cells exhibited upregulation of heat shock response genes (*HSPA6*, *HSPA7*, and *HSPA1B*) and genes associated with the UPR (*XBP1*, *ATF6*, and *PERK*) upon exposure to heat shock and global ER stressor thapsigargin, respectively. AC16 cells exposed to c-AL LCs displayed a higher number of significantly differentially expressed genes (7241) as compared to TTR proteins (TTR^{WT} 4103, TTR^{V122I} 2365, and TTR^{L55P} 396) highlighting the complex response of cardiac cells to c-AL LCs. Notably, we found no overlap in gene expression changes in AC16 cells exposed to AL LCs versus ATTR suggesting there is unique gene signature associated with LC-induced cardiotoxicity. (Figure 1B) RNASeq identified gene expression changes in hallmark pathways related to preservation of cardiac contractility (*XIRP1*, *PFKFB1*), myogenesis (myosin, actin, and troponin genes), early cardiac remodeling (proteoglycan, glycosaminoglycan, MMP, and TIMP genes), and adaptive immune response (*GPR183*, *IL1RL1*). Our findings were confirmed via RT-PCR analysis of select target genes. Independently, iPSC-derived cardiomyocytes were employed to further validate these findings.

Summary & Conclusion: Here, we reveal the possible molecular events occurring in cardiomyocytes after exposure to c-AL LCs and prior to marked cardiomyocyte damage. Upregulation of transcripts related to myogenesis and preservation of cardiac contractility, may provide evidence for the development of an adaptive hypertrophic phenotype which aims to reduce oxygen consumption, diminish cardiomyocyte stress, and preserve cardiac function in the initial phases of LC exposure. These findings could provide a rationale for the development of biomarkers and novel therapeutics.

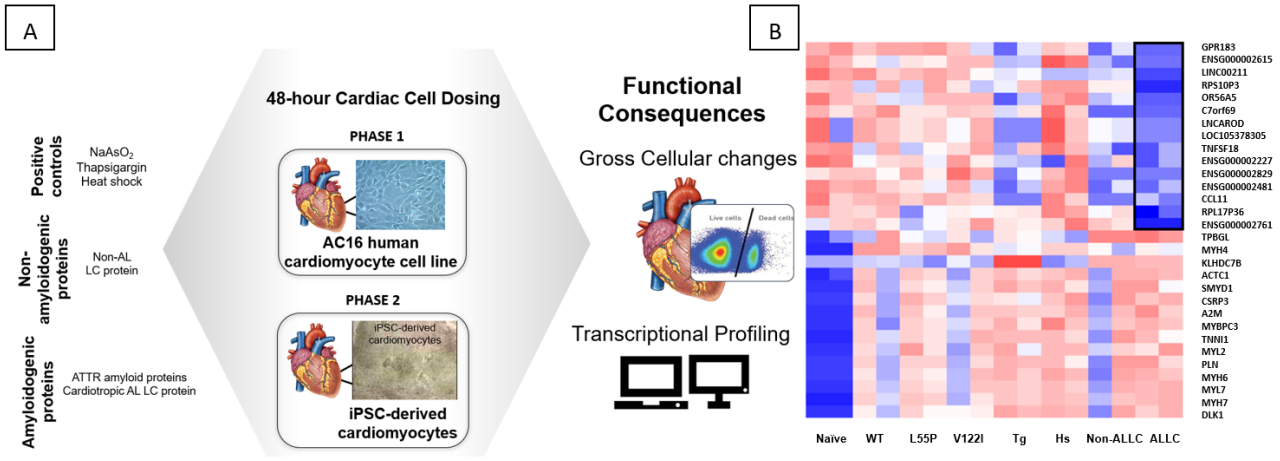

Figure 1.: A. Dosing Schema for AC16 cells and iPSC-derived cardiomyocytes. AC16 human ventricular cardiomyocytes

were dosed with heat shock proteins (global stressor), thapsigargin (endoplasmic reticulum stressor), recombinant amyloidogenic, cardiotropic kappa 1 light chains (0.02 mg/mL), recombinant non-amyloidogenic kappa 1 light chains (0.02 mg/mL), recombinant TTR proteins (0.02 mg/mL) for 48 hours. iPSC-derived cardiomyocytes were dosed with recombinant amyloidogenic, cardiotropic kappa 1 light chains (0.02 mg/mL) for 48 hours. **B.** The gene signature of AL amyloidosis is distinct from that of other stressors (thapsigargin, heat shock, other amyloidogenic proteins). RNA was isolated and bulk mRNA sequencing was performed. Heat map denotes the top differentially expressed genes in AC16 cells exposed to ALLCs with respect to each condition. WT – wild type transthyretin (TTR) amyloid protein, L55P – neuropathy-associated TTR amyloid protein, V122I – cardiomyopathy-associated TTR amyloid protein, Hs – Heat shock protein, Tg – Thapsigargin, Non-ALLC - non-amyloidogenic light chain, ALLC – amyloidogenic light chain.

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Long-term tafamidis treatment reduces the decline in quality of life among patients with transthyretin amyloid cardiomyopathy

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM) is associated with progressive heart failure and a declining quality of life.[1-3] In the Tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT), treatment with tafamidis for 30 months significantly reduced the decline in quality of life compared with placebo, as determined by Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score.[2] Placebo and tafamidis treated patients completing ATTR-ACT could go on to receive tafamidis in an ongoing long-term extension (LTE) study.[4,5]

Objective: Evaluate the effects of long-term tafamidis treatment on patient-reported quality of life in ATTR-ACT and its LTE study.

Material & Methods: Patients were randomised 2:1:2 to receive tafamidis meglumine (80 or 20 mg) or placebo for up to 30 months in ATTR-ACT. In the LTE study, patients who received tafamidis in ATTR-ACT continued this dose. Those who received placebo in ATTR-ACT were randomised 2:1 to tafamidis meglumine 80 mg or 20 mg. Following a protocol amendment, all patients in the LTE study transitioned to tafamidis free acid 61 mg, which is bioequivalent to tafamidis meglumine 80 mg. This analysis compared KCCQ-OS scores collected every 6 months for patients who received tafamidis meglumine 80 mg in ATTR-ACT and then tafamidis for a further 30 months in the LTE study, and patients who received placebo for 30 months in ATTR-ACT and then 30 months of tafamidis treatment in the LTE study (60 months overall treatment; unplanned interim analysis). A lower KCCQ-OS reflects poorer quality of life. The LS mean change from baseline analysis was based on a mixed models repeated measures model with an unstructured covariance matrix. Center and patient-within-center were included as random effects; treatment, visit, genotype, and visit-by-treatment interaction were included as fixed effects.

Results: In ATTR-ACT, 176 patients were randomized to tafamidis 80 mg and 177 to placebo. Patients who continuously received tafamidis in ATTR-ACT had a 6-point reduction in LS mean (SE) KCCQ-OS score after 30 months of treatment (-6.3 [1.53]), with no further decline over the next 30 months of tafamidis treatment in the LTE study (-5.9 [1.77] after 60 months; Figures 1 and 2). Patients receiving placebo in ATTR-ACT had a 20-point reduction in LS mean KCCQ-OS score after 30-months (-19.6 [1.94]). After switching to tafamidis in the LTE study, the rate of decline in KCCQ-OS score was slower (-24.7 [3.04] after 60 months; Figures 1 and 2).

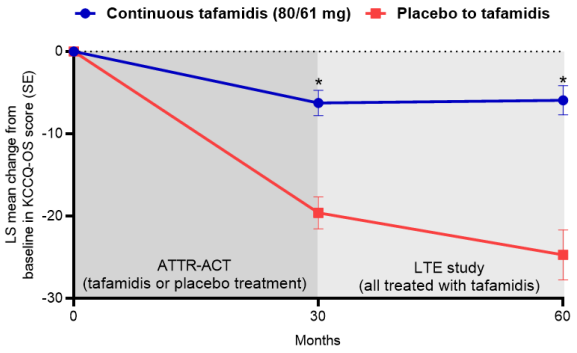
Summary & Conclusion: Treatment with tafamidis reduces the decline in quality of life among patients with ATTR-CM. Quality of life was stablised in patients continously treated with tafamidis for 60 months in ATTR-ACT and its LTE study. The rate of decline in quality of life was reduced in patients switching from placebo in ATTR-ACT to tafamidis in the LTE study. NCT01994889; NCT02791230

Figure 1: Table of KCCQ-OS scores in ATTR-ACT and its LTE study

	Continuous tafamidis 80/61 mg	Placebo to tafamidis
ATTR-ACT baseline		
KCCQ-OS score, mean (SD)	n = 176	n = 177
	67.12 (21.29)	65.90 (21.74)
After 30 months of treatment in ATTR-ACT		
	n = 110	n = 84
KCCQ-OS score, mean (SD)	68.76 (21.42)	53.83 (24.42)
Change from ATTR-ACT baseline, LS mean (SE)	-6.25 (1.53)	-19.60 (1.94)
Difference in change between groups, LS mean (95% CI)	13.38 (9.21, 17.54) P<0.001	
After 30 months of treatment in the LTE study (60 months total treatment duration)		
	n = 45	n = 22
KCCQ-OS score, mean (SD)	73.74 (19.19)	53.01 (22.91)
Change from ATTR-ACT baseline, LS mean (SE)	-5.92 (1.77)	-24.70 (3.04)
Difference in change between groups, LS mean (95% CI)	18.83 (12.64, 25.03) P<0.001	

ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; CI, confidence interval; KCCQ-OS, Kansas City Cardiomyopathy Questionnaire Overall Summary; LS, least-squares; LTE, long-term extension; SD, standard deviation; SE, standard error.

Figure 2: Change in KCCQ-OS in ATTR-ACT and its LTE study



*P<0.001 between groups. ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; KCCQ-OS, Kansas City Cardiomyopathy Questionnaire Overall Summary; LS, least squares; LTE, long-term extension.

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Atrial fibrillation as a prognostic factor for all-cause mortality in patients with transthyretin amyloid cardiomyopathy

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Background: Atrial fibrillation (afib) and flutter (aflutter) are among the most common manifestations of transthyretin amyloid cardiomyopathy (ATTR-CM).[1-3] Prior studies have suggested that afib does not influence mortality.[3-6] Tafamidis was approved for the treatment of ATTR-CM based on the landmark, placebo-controlled, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT), where all-cause mortality was a primary efficacy outcome.[7]

Objective: Evaluate whether afib/aflutter was prognostic for all-cause mortality in ATTR-ACT.

Material & Methods: ATTR-ACT was a multinational, Phase 3, randomized study of patients with ATTR-CM receiving tafamidis meglumine (80 or 20 mg) or placebo over 30 months. This analysis describes the characteristics of patients with and without current/historical afib/aflutter at the baseline of ATTR-ACT. Current/historical afib/aflutter was evaluated as an independent prognostic factor for all-cause mortality using Cox proportional hazards modelling.

Results: The 314/441 (71%) patients in ATTR-ACT with current/historical afib/aflutter were older, more commonly male, and more commonly White than those without (Table 1). A higher proportion of patients with current/historical afib/aflutter were NYHA class III and had a wild-type genotype than those without. Median 6MWT distance and the proportion of patients with preserved left ventricular ejection fraction (≥50%) were lower, GLS was less negative, and NT-proBNP and BUN were higher among those with current/historical afib/aflutter vs those without.

In ATTR-ACT, treatment, genotype, and NYHA class were included in the pre-specified Cox proportional hazards model assessing all-cause mortality. Once current/historical afib/aflutter was added to this model, all variables were found to be significant independent predictors of all-cause mortality ($P<0.05$; Table 2). The risk of mortality was 45% lower (hazard ratio = 0.55 [95% CI: 0.37, 0.82]) among patients without current/historical afib/aflutter than in those with. In an expanded stepwise model selection analysis including 23 baseline demographic and clinical covariates, current/historical afib/aflutter was not a significant independent predictor. This model showed BUN and NT-proBNP concentrations, 6MWT distance, genotype, treatment, and GLS to be significant independent prognostic factors for all-cause mortality ($P<0.01$; Table 2).

Summary & Conclusion: Patients in ATTR-ACT with ATTR-CM and current/historical afib/aflutter were older, more likely to be male, have a wild-type genotype, and more advanced heart failure than those without. A Cox model including treatment, genotype, and NYHA class, identified current/historical afib/aflutter as a significant independent predictor of all-cause mortality. In an expanded stepwise selection model, current/historical afib/aflutter remained important but was less prognostic compared to other covariates. Overall, these results demonstrate the importance of afib/aflutter in patients with ATTR-CM. NCT01994889

Figure 1. Baseline demographics and clinical characteristics for patients in ATTR-ACT with and without current or historical afib or aflutter at baseline

	Current or historical afib or aflutter at baseline?	
	Yes n = 314	No n = 127
Age, mean (SD) years	74.9 (6.8)	73.0 (7.3)
Sex, %		
Male	91.1	88.2
Female	8.9	11.8
Race, %		
White	86.0	68.5
Black	10.5	23.6
Asian	2.6	7.9
Other	1.0	0.0
Ethnicity, %	n = 312	n = 217
Not Hispanic or Latino	97.4	95.3
Hispanic or Latino	2.6	4.7
Genotype, %		
Wild-type	80.6	64.6
Variant	19.4	35.4
NYHA Class, %		
I or II	65.3	74.8
III	34.7	25.2
6MWT distance, median m (LQ, UQ)	335.0 (259.0, 435.0)	390.0 (303.0, 451.0)
NT-proBNP, median pg/ml (LQ, UQ)	3414.5 (2075.0, 5213.6)	2222.0 (1274.0, 3873.7)
Troponin I, mean ng/ml (LQ, UQ)	0.14 (0.08, 0.20) [n = 313]	0.14 (0.09, 0.19)
BUN, median mg/dl (LQ,UQ)	27.9 (21.8, 35.0)	24.0 (19.0, 30.0)
Echocardiographic measures		
LVEF	n = 310	n = 126
Median % (LQ, UQ)	49.1 (41.4, 55.5)	52.0 (43.0, 57.3)
≥50%, % patients	47.7	57.1
41-49%, % patients	28.7	23.8
≤40%, % patients	23.6	19.1
LVEDD, median mm	41.6 (37.0, 46.2) [n = 307]	42.2 (37.0, 46.6) [n = 124]
LV mass, median g	281.1 (230.4, 352.2) [n = 307]	282.1 (221.9, 343.4) [n = 123]
GLS, median %	-8.9 (-11.0, -6.9) [n = 307]	-9.8 (-11.9, -7.5) [n = 126]

6MWT, six-minute walk test; afib, atrial fibrillation; aflutter, atrial flutter; ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; BUN, blood urea nitrogen; GLS, global longitudinal strain; LQ, lower quartile; LV, left ventricular; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; SD, standard deviation; UQ, upper quartile.

Figure 2. Cox models for independent predictors of all-cause mortality

Covariate	Hazard ratio (95% CI)	P value
Initial model (replicating primary outcome in ATTR-ACT with addition of afib/aflutter)		
Treatment (tafamidis vs placebo)	0.72 (0.525, 0.991)	0.0438
Genotype (variant vs wild type)	2.27 (1.619, 3.192)	<0.0001
NYHA class (I/II vs III)	0.36 (0.261, 0.497)	<0.0001
Current/historical afib/aflutter (no vs yes)	0.55 (0.368, 0.821)	0.0034
Final expanded stepwise selection model		
Treatment (tafamidis vs placebo)	0.57 (0.413, 0.791)	0.0008
Genotype (variant vs wild type)	1.79 (1.277, 2.519)	0.0007
6MWT distance	1.00 (0.993, 0.996)	<0.0001
NT-proBNP concentration	1.52 (1.174, 1.963)	0.0015
BUN concentration	1.02 (1.012, 1.034)	<0.0001
GLS	1.08 (1.025, 1.138)	0.0042

6MWT, six-minute walk test; afib, atrial fibrillation; aflutter, atrial flutter; ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; BUN, blood urea nitrogen; CI, confidence interval; GLS, global longitudinal strain; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.

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Long-term safety and tolerability of acoramidis (AG10) in symptomatic transthyretin amyloid cardiomyopathy: Updated analysis from an ongoing phase 2 open-label extension study

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Background: A Phase 2 randomized, double-blind, placebo-controlled, 28-day trial evaluating the oral transthyretin (TTR) stabilizer acoramidis enrolled 49 individuals with symptomatic TTR amyloid cardiomyopathy (ATTR-CM).

Objective: Here we report an update on long-term outcomes in the open-label extension (OLE) study.

Material & Methods: Participants who completed the Phase 2 study[1] and enrolled in the OLE received oral acoramidis HCl 800 mg twice daily. Clinical and laboratory assessments were performed on days 1, 14, and 45 and at 3-month intervals thereafter.

Results: A total of 47 participants enrolled in the OLE. Based on available data through August 31, 2021, 31 participants remained in the study. The median exposure time was 35 months. Acoramidis was generally well tolerated; adverse events were consistent with disease severity, concurrent illness, and age. Change from baseline in N-terminal pro-brain type natriuretic peptide (NT-proBNP) at 30 months is shown in Fig. 1. Acoramidis demonstrated near-complete TTR stabilization. Serum TTR levels were sustainably increased from baseline, with mean concentration rising from 21.55 mg/dL at baseline to 30.06 mg/dL at Month 30 (+41%; reference range 20-40 mg/dL). Near-complete stabilization was verified using established ex-vivo assay with mean stabilization of 102.5 ± 8.9% at Month 30.

Summary & Conclusion: Long-term treatment with acoramidis was generally well tolerated and resulted in a median decline in NT-proBNP levels, normalization of serum TTR, and sustained stabilization of TTR in individuals with symptomatic ATTR-CM. Further evaluation in a Phase 3 trial is ongoing.

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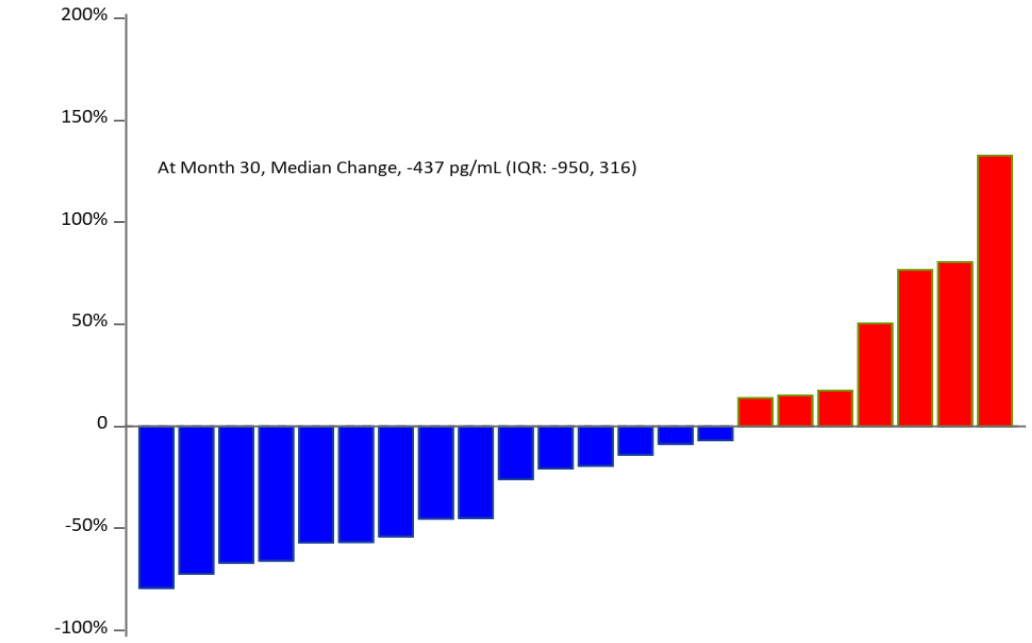
Support & Funding: Eidos Therapeutics, Inc.

Figure 1. Cumulative distribution of change from baseline in NT-proBNP at month 30



Note: Based on Study AG10-202 interim CSR data (Data snapshot: 28OCT2021, Data cutoff: 31AUG2021). There were 22 subjects with NT-proBNP assessments at month 30.

Chronic Intravenous Inotropic Therapy in Cardiac Amyloidosis

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Background
The feasibility of using chronic intravenous inotropic support (CIIS) in patients with advanced heart failure due to cardiac amyloidosis (CA) has not been evaluated. CIIS may present a viable option to improve the quality of life of CA patients despite the risk for complications.

Objective
We sought to evaluate the use of CIIS in CA patients as palliative and/or bridge therapy to heart transplantation (HT).

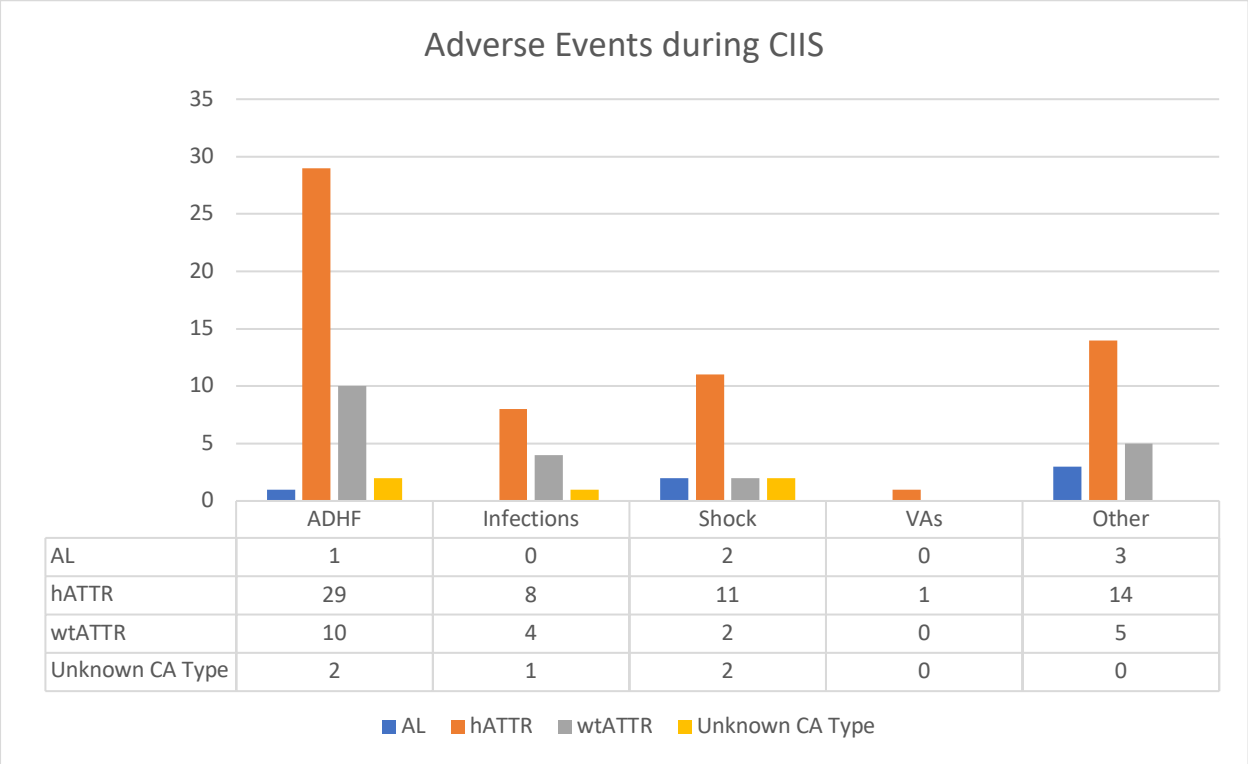
Methods
A single center retrospective cohort study of all CA patients placed on CIIS from 2011 to 2022 was conducted. Adverse Events including hospitalizations due to Ventricular Arrhythmias (VAs), Acute Decompensated Heart Failure (ADHF), and infections were reviewed and analyzed using descriptive statistics.

Results
The study group consisted of 44 patients of which 16% were subtyped as AL (n=7), 54% as hereditary ATTR (hATTR, n=24), and 23% as wild type ATTR (wtATTR, n=10). Demographic features included 64% men (n=28), 80% Black patients (n=35), with mean age 74 $\pm$ 7years. Three patients (7%) were identified as having CA but subtype was not noted. There was a total of 94 admissions post-CIIS. AL patients were on CIIS for 43 days on average (ranging from 10 to 142 days), with 86% (n=6) of them surviving less than 90 days. This same cohort experienced a total of 6 admissions post-CIIS with worsening cardiogenic shock (33%, 2 out of 6 admissions) as the leading cause of hospitalization. Hereditary ATTR patients fared better with a mean length of CIIS of 282 days and 33% (n=8) surviving >12 months. Amongst this group, most common causes of hospitalization were ADHF (47%, 29 out of 62 admissions), worsening cardiogenic shock, (18%, 11 out of 62) and infections (13%, 11 out of 62). Two hATTR patients were also successfully bridged to receive heart +/- liver transplantation. In the wtATTR subgroup, 30% (n=3) lived past 12 months, with an average length of 295 days on CIIS. ADHF (48%, 10 out of 21 admissions) was the leading cause of hospitalization. There were no hospital admissions for implantable cardioverter defibrillator (ICD) discharges though it is notable that 61% (n=27) of

CA patients had an ICD or wearable cardioverter/defibrillator device. There was a single case that noted VAs as the primary cause of hospitalization. Central line access malfunction, gastrointestinal bleeding and thromboembolic events were some of the noncardiac causes of hospital admissions. See Figure 1.

Conclusion

In a large single center cohort, CIIS is a feasible treatment for CA patients with ATTR patients demonstrating the capacity to tolerate this therapy for extended periods of time allowing for successful bridging to transplant in several instances. Future research should be focused on risk stratification for patient selection, quality of life assessment, and strategies to reduce hospitalizations of CA patients on CIIS.



	ADHF	Infections	Shock	VAs	Other
AL	1	0	2	0	3
hATTR	29	8	11	1	14
wtATTR	10	4	2	0	5
Unknown CA Type	2	1	2	0	0

Figure 1: Adverse Events during Chronic Intravenous Inotropic Therapy. Legend: CIIS= Chronic Intravenous Inotropic Support; AL=Amyloid Light Chain; hATTR=hereditary Transthyretin Amyloid; wtATTR=wild-type Transthyretin Amyloid; CA=Cardiac Amyloidosis; ADHF=Acute Decompensated Heart Failure; VAs=Ventricular Arrhythmias.

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Tolerability and side-effects of therapy in an open-label trial of inotersen for transthyretin amyloid cardiomyopathy (the INOCARD trial)

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Background: Inotersen is a second-generation antisense oligonucleotide targeted to reduce transthyretin (TTR) protein secreted by the liver. The FDA approved inotersen for patients with hereditary amyloid TTR (ATTR) polyneuropathy in October 2018, but the drug is not approved for ATTR cardiomyopathy. In the pivotal trial of inotersen for familial amyloid polyneuropathy (FAP), the mean age was 59 years and 22% of inotersen-allocated patients failed to complete the trial compared to 13% on placebo. Although cardiomyopathy was present in 63% of patients, those with more than mild heart failure were excluded (1). In contrast to FAP, the precursor protein in predominant cardiac TTR amyloidosis is most commonly wild-type and patients tend be in their 8th decade and above. We therefore designed an open-label study to determine the safety and efficacy of inotersen in patients with ATTR cardiomyopathy, either hereditary or wild-type.

Objective: The purpose of the study was to examine the (i) general wellbeing, (ii) functional and structural changes in the heart, (iii) cardiac biomarker levels, and (iv) tolerability of the drug in patients with ATTR cardiomyopathy treated with inotersen. Here we report the side-effect profile in this 24-month open-label study of inotersen for ATTR cardiomyopathy.

Material & Methods: All patients were treated with a fixed dose of 300 mg inotersen weekly, self-administered subcutaneously with pre-filled syringes in rotating sites of the abdomen. Safety labs analysing platelets, creatinine, and eGFR were drawn every other week, and patients were only permitted to self-inject once the PI had reviewed the labs and given approval to proceed. If platelets fell below 75 k/μL, or if the eGFR fell below 40 mL/min/1.73m^2 or decreased greater than 50% from baseline, study drug was stopped until further testing showed improvement. Subjects had a cardiac MRI, ECG, echocardiogram, 6-minute walk test, CPET, physical exam and comprehensive blood work every 6 months.

Results: Of 31 enrolled patients (mean age 72 ± 17yr, 28 wild-type, 30 males), 14 completed and 16 discontinued the trial prematurely with 1 sudden death during the study. Discontinuation was due to worsening renal function in 7 and worsening heart failure in 5 (of whom one subsequently died within a month after drug termination). Thrombocytopenia, anaphylaxis, hepatic dysfunction and hepatic failure led to drug discontinuation in 1 patient each. Among the 8 patients who discontinued prematurely due to renal impairment or thrombocytopenia, kidney and platelet count (figure) returned to normal or stabilized. Among the 14 patients who completed the 104 weeks of the study, only 4 were able to self-administer inotersen without a hold because of side-effects. Inotersen was held 1-3 times in 8 patients and ≥ 4 times in 2 patients.

Summary & Conclusion: Inotersen therapy for ATTR cardiomyopathy is poorly tolerated and often requires discontinuation for serious side effects. Even when tolerated, it requires frequent monitoring with occasional temporary holds due to platelet decrease or renal dysfunction.The discontinuation rate among this older population with predominant wild-type amyloid cardiomyopathy appears to be higher than in the pivotal trial of the drug for familial amyloid polyneuropathy. The next-generation antisense oligonucleotide, eplontersen, currently being studied in a multicenter randomized trial, appears in preliminary data to have a more favorable side-effect profile and holds promise for a safer therapy than inotersen.

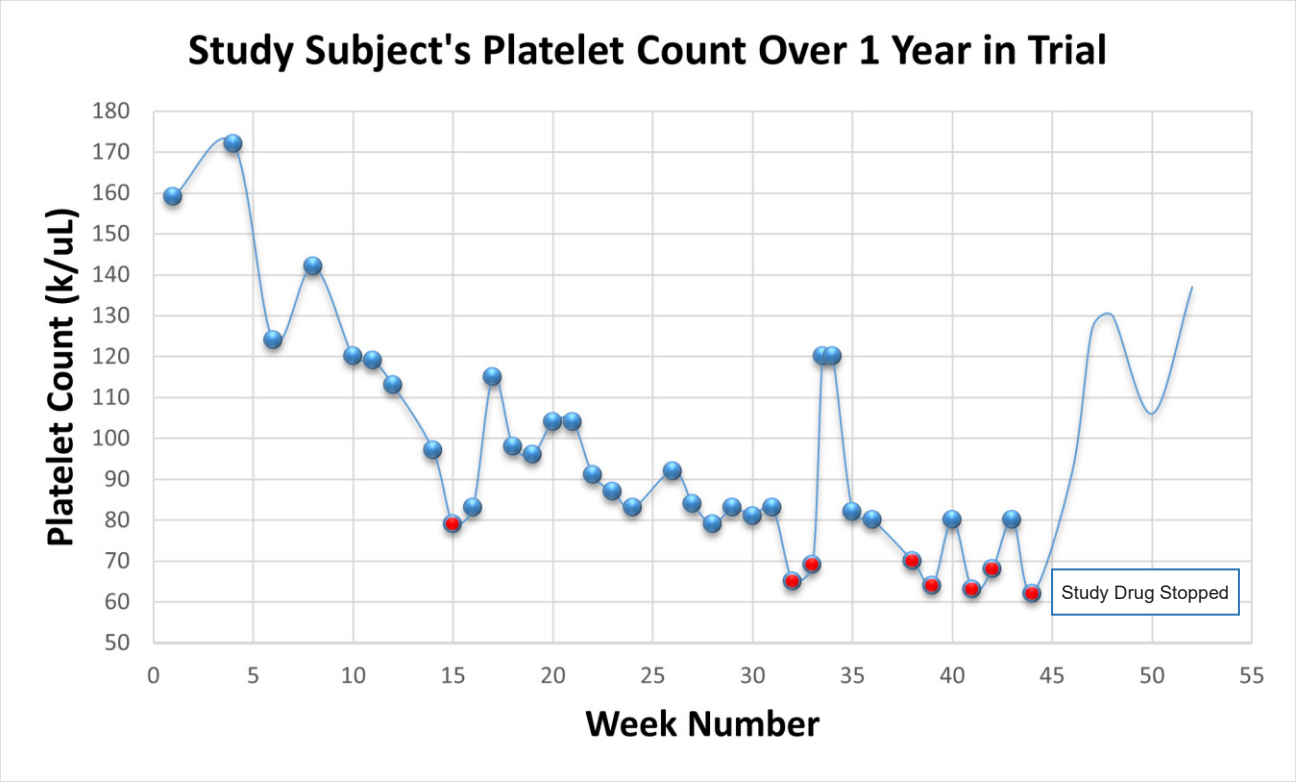

Figure 1: The platelet count of a study subject over the course of one year until trial discontinuation. Blue bubbles indicate study drug administration, while red bubbles indicate a hold on study drug injection. As study drug was injected weekly, platelets gradually dropped lower, but after study drug was halted, platelets rose up again.

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Support & Funding: The study was sponsored by Akcea Therapeutics. Study design, data collection and analysis were performed independently of the study sponsor.

Looking over your shoulder to catch amyloidosis earlier: shoulder pathologies are significantly more prevalent in patients with transthyretin cardiac amyloidosis

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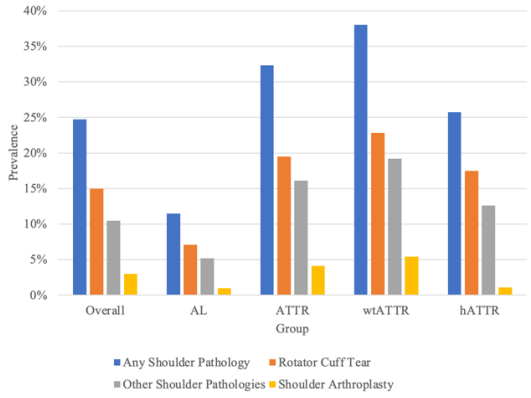
Background: Transthyretin cardiac amyloidosis (ATTR-CA) and light chain cardiac amyloidosis (AL-CA) are protein misfolding diseases that manifest in the heart often as underrecognized causes of restrictive cardiomyopathies in older adults.[1,2] ATTR-CA has been associated with multiple orthopaedic pathologies, such as, carpal tunnel syndrome (often bilateral), spinal stenosis, stenosing tenosynovitis (trigger finger), ruptured bicep tendons (RBT), rotator cuff tears (RCT), and lower extremity (hip and knee) arthroplasty years before it manifests in the heart.[3-7] There have been no studies on a wider range of shoulder pathologies in these patients.

Objective: The main purpose of this study was to report the prevalence of shoulder pathologies including shoulder replacement surgery (arthroplasty), shoulder arthroscopy, rotator cuff tears, labral tears, adhesive capsulitis (frozen shoulder), and shoulder instability in the CA population. An additional aim was to examine the prevalence of shoulder arthroplasty in the ATTR-CA and AL-CA population compared to an age-matched control group of the general population. The final aim was to describe the timing of rotator cuff tears and shoulder arthroplasty relevant to patients' diagnosis of CA.

Material & Methods: In this single-center, retrospective, case-control study, 1310 patients with cardiac amyloidosis, 830 with ATTR-CA, and 480 with AL-CA from our database were analyzed. Prevalence data for shoulder pathologies and other orthopaedic manifestations were collected. Odds ratios for CA patients who underwent shoulder arthroplasty compared against the age-matched published estimate of over 300 million patients in the general population were determined.[8] Years between a patients' first shoulder pathology (i.e., shoulder arthroplasty) and the year of their diagnosis with CA were determined using data from patients with a known date of surgery.

Results: Overall, 24.7% of CA patients presented with any form of shoulder pathology, which was more frequently found in patients with ATTR-CA compared to patients with AL-CA ($p<0.001$, Figure 1). Rotator cuff tears and other shoulder pathologies (i.e., shoulder arthroscopy) were most frequent, followed by shoulder arthroplasty. Significantly more ATTR-CA patients presented with rotator cuff tears ($p<0.001$), other shoulder pathologies ($p<0.001$), and shoulder arthroplasty ($p=0.003$) compared to AL-CA patients. The odds of ATTR-CA and AL-CA patients ages 60-years or older who underwent shoulder arthroplasty was 6.05 times greater (95% CI [4.26, 8.60]) and 1.63 times greater (95% CI [0.67, 3.94]), respectively, in comparison to age-matched controls (Figure 2). Patients with ATTR-CA also experienced their first rotator cuff tear a median of 7 years (IQR=2–13) and first shoulder replacement a median of 4.5 years (IQR=0–9) before diagnosis, which was earlier than those with AL-CA at 2 years (IQR=0–5.5) and 2 years (IQR=-0.5–4), respectively.

Summary & Conclusion: Shoulder pathologies are common in patients with ATTR-CA and may help to identify CA patients earlier in their disease progression. Thus, in addition to routinely asking patients about carpal tunnel syndrome, spinal stenosis, and biceps tendon rupture, clinicians should also routinely ask about a history of rotator cuff tear and other shoulder pathologies to strengthen suspicion of possible CA. The findings of this study may help clinicians suspect the disease earlier as well as potentially stimulate interest in ascertainment protocols for amyloid deposition during shoulder surgery in selected patients.



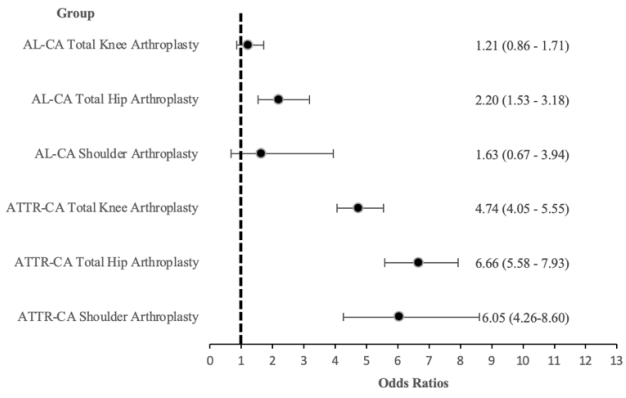


Figure 1. Prevalence of shoulder pathologies in the CA population**.**

Figure 2. Odds ratios of TKA, THA, and shoulder arthroplasty among patients with AL-CA and ATTR-CA compared to the age matched controls (60 years and over). Control data for TKA and THA are from Maradit et al. J Bone Joint Surg Am. 2015. Control data for shoulder arthroplasty are from Farley et al. J Bone Joint Surg Am. 2021.

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Support & Funding: CCF Term Chair for Amyloid Heart Disease

SARS-CoV-2 infection in systemic amyloidosis: the International Society of Amyloidosis' survey.

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Background: Systemic amyloidosis causes multi-organ dysfunction and patients can be at higher risk of complications and death due to SARS-CoV-2 infection. The International Society of Amyloidosis (ISA) Board called for an international data collection on patients with amyloidosis and COVID-19 in April 2020.
Objective: To report the data of the ISA survey on patients with systemic amyloidosis and COVID-19 from 4/2020 to 12/2021.
Materials & Methods: All members of the ISA were invited. All patients suffering from amyloidosis who experienced a SARS-CoV-2 infection were eligible. Data collection started before vaccines were available and therefore vaccine status was not collected. However, to account the effect of vaccination we compared data of patients enrolled before and after 12/2020. This might also reflect improvement in supportive care with the increasing experience in managing the infection. Sixteen institutions contributed to the project. At the data lock of December 1, 2021, 200 patients were collected. The distribution of diagnoses were systemic AL, 61%; transthyretin amyloidosis (ATTR), 31% [ATTRwt, 21%, and ATTRv, 10%]; localized 9 (4.5%); systemic AA 4 (2%); and Apolipoprotein A1 amyloidosis,3 (1.5%). We focused our analysis on patients with systemic AL and ATTR and main clinical data are reported in the Table.
Results: Among ATTR patients, 48% were hospitalized, 12% of whom required ventilation and an additional 19% were treated with supplementary oxygen. 54% of ATTR patients received pharmacological therapy. Among patients with AL amyloidosis, 44% were hospitalized, 12% required ventilation, and an additional 22% were treated with oxygen. Forty-two percent of AL patients received pharmacological therapy for COVID-19. Acute respiratory distress syndrome (ARDS) was reported in 11 (17%) patients with ATTR and 18 (15%) with AL. Twelve of the 18 AL patients with ARDS (66%) were on active chemotherapy. The infection was fatal in 25/184 (14%) cases: ATTR, 9 (14%); and AL,16 (13%). Recovery with sequelae rates were 3% for ATTR and 5% for AL. Overall, 15 patients with ARDS survived. All ATTR patients who died had heart involvement and at least one comorbidity. Seven of the 16 AL patients who died were on active chemotherapy at the time of SARS-CoV2 infection. Two patients with AL kidney involvement recovered from COVID-19 but developed subsequent worsening of renal function, requiring dialysis in one case. No differences were seen in the number of patients who had a severe presentation (pneumonia or ARDS) and in death rate between 2020 and 2021.
Summary and Conclusions: Participating centers are encouraged to report mild and asymptomatic patients, yet we cannot exclude a referral bias. Severity and outcome of COVID-19 in patients with systemic amyloidosis is comparable to that of other frail patients. For instance, mortality rate is lower than in multiple myeloma (33%)[1] and it is in the lower range of hematological malignancies in general (range from 13 to 39%)[2]. Being on active chemotherapy for AL amyloidosis was associated with worst presentation of the SARS-CoV2 infection.

Table. Characteristics & outcomes of 184 patients with systemic amyloidosis and SARS-CoV2 infection

Variable	**AL, n=121** **N (%) – median (IQR)**	**ATTR, n=63** **N (%) – median (IQR)**
Diagnosis year, 2020 /2021, n	75 / 46	46 / 17
Age, years	64 (62, 66)	75 (70, 79)
Male sex	68 (56)	52 (82)
Organ involvement Heart/kidney Liver/Soft tissue PNS/ANS/>2 organs	 73 (60) / 80 (66) 13 (11) / 20 (16) 11 (9) / 9 (7) / 62 (51)	 54 (85) / 6 (9) 1 (1) / 8 (12) 21 (33) / 8 (12) / 8 (12)
Comorbidities Hypertension / Ischemic heart disease Diabetes / COPD	 48 (39) / 13 (11) 11 (9) / 7 (6)	 30 (48) / 17 (27) 9 (14) / 4 (6)
On active treatment	48 (39)	35 (55)
Symptoms of COVID-19 Fever / Cough Anosmia / Ageusia Pneumonia / ARDS	 68 (56) / 59 (49) 19 (16)/19 (16) 55 (45) / 18 (15)	 38 (60) / 35 (55) 5 (8) / 6 (9) 38 (60) / 11 (17)
Outcomes		
Hospitalization	53 (44)	30 (48)
Invasive/non-invasive ventilation	7 (6) / 7 (6)	1 (1) / 7 (11)
Supplementary O2	27 (22)	12 (19)
Steroid therapy	30 (25)	16 (25)
Antiviral therapy	14 (11)	10 (16)
Anticoagulation therapy	18 (15)	9 (14)

PNS, peripheral nervous system; ANS, autonomic nervous system; COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome.

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Cardiac Transplantation in Transthyretin Amyloid Cardiomyopathy: Outcomes from Three Decades of Tertiary Centre Experience

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Background

Transthyretin cardiac amyloidosis (ATTR-CM) is a progressive and fatal cardiomyopathy. Treatment options in patients with advanced heart failure are limited to cardiac transplantation (CT). Despite small case series demonstrating comparable outcomes with CT between patients with ATTR-CM and non-amyloid cardiomyopathies, ATTR-CM is considered to be an absolute contraindication to CT in some centres, in part due to a perceived risk of amyloid recurrence in the cardiac allograft.

Objective

To assess outcomes in ATTR-CM patients following CT, in particular assessing for cardiac graft amyloid infiltration.

Methods

We retrospectively evaluated all ATTR-CM patients assessed at the UK National Amyloidosis Centre between 1990 and 2020 who underwent CT. Pre-transplantation disease and patient characteristics were determined and outcomes were compared with our large cohort of non-transplanted ATTR-CM patients. Censor date was 30th April 2022.

Results

Eleven (9 male, 2 female) patients with ATTR-CM underwent CT including 8 with wild-type ATTR-CM and 3 with variant ATTR-CM (ATTRv) – specifically the p.Val421Ile, p.Gly73eAla and Ser43Asn TTR gene variants. Median age at CT was 60.3 years and median follow up post-CT was 65.7 months. Pre-transplant, one patient was NYHA functional class IV, seven were functional class III and three were functional class II. Median (range) NT-proBNP concentration pre-transplant was 4478ng/L (1057-8778ng/L), median (range) left ventricular ejection fraction (LVEF) was 39% (27-56%) and mean (IQR) interventricular septal thickness (IVSd) was 18 mm (15.9-20.1 mm). At diagnosis, 6 patients had NAC ATTR stage II disease, 4 had stage I disease and the NAC ATTR stage was not evaluated in the remaining patient.

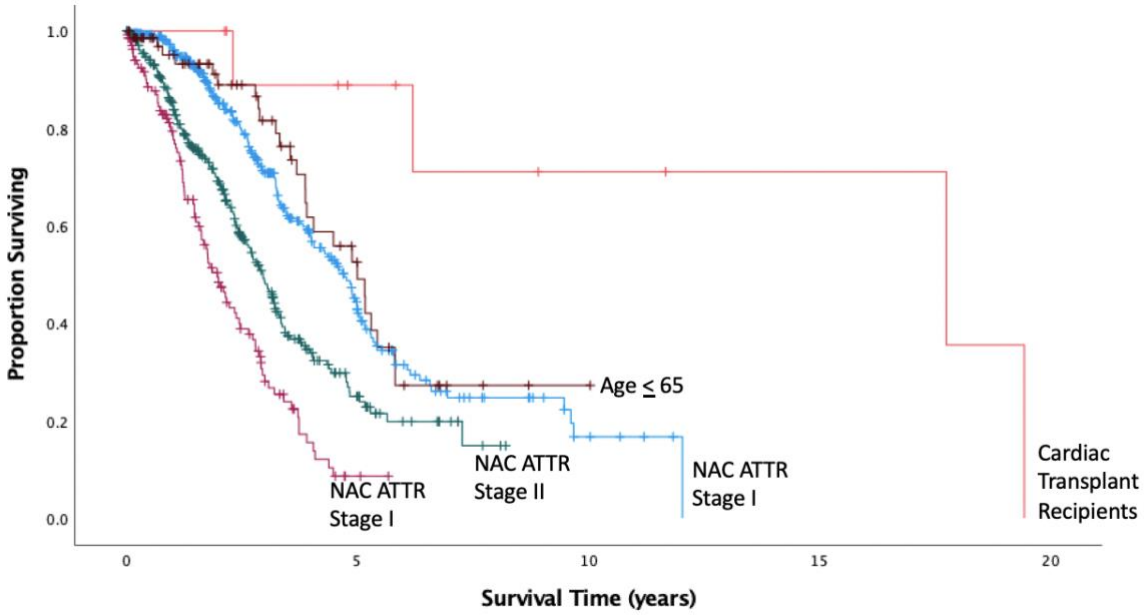
Post-CT renal impairment, including both acute kidney injury thought to be due to perioperative hypoperfusion, and progressive chronic kidney disease (CKD) was common and occurred in 8/11 CT recipients. Three patients required temporary post-operative haemodialysis with two recovering normal renal function, a further four were left with CKD. The final patient developed progressive CKD due to calcineurin inhibitor use. At time of censor no patients had recurrence of amyloid in the cardiac allograft as assessed by Tc-DPD scintigraphy and/or endomyocardial biopsy. All surviving patients were NYHA functional class I.

One, three, and five-year survival was 100%, 89% and 86%, respectively. Four patients died at 2.3, 6.2, 17.7 and 19.4 years post CT. The patient that died at 2.3 years died of complications related to p.Gly73Ala vATTR leptomeningeal amyloidosis. Survival among the cohort of patients who underwent CT was significantly prolonged compared to UK patients with ATTR-CM generally regardless of NAC ATTR disease stage ($p \leq 0.006$) and compared to patients diagnosed under 65 years of age ($p=0.028$). (Figure 1)

Conclusion

CT is well-tolerated, restores functional capacity and improves prognosis in ATTR-CM. The risk of amyloid recurrence in the cardiac allograft appears to be low. Careful consideration for CT should be given to patients with TTR variants associated with leptomeningeal disease.

Figure 1. Kaplan-Meier survival curves in 814 patients with ATTR-CM stratified by NAC Disease Stage and compared to cohort who underwent cardiac transplantation. Cardiac transplantation in this small cohort of selected patients was associated with a substantial prolongation of life expectancy compared to patients who did not undergo cardiac transplantation (Transplant vs NAC ATTR Stage I, p=0.005; Transplant vs NAC ATTR Stage II, p=0.002; Transplant vs NAC ATTR Stage III, p<0.001) including the cohort of 71 NAC patients who were diagnosed with ATTR-CM under age 65 years (p=0.028).



Results of the first-in-human PET/CT imaging study of the amyloid-reactive peptide ^{124}I-AT-01 (^{124}I-p5+14) for the detection of systemic amyloidosis

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Background: Systemic amyloidosis is a multi-organ disorder with variable presentation due to the heterogeneity of organ involvement in the diverse forms of amyloid diseases. Consequently, rapid and accurate diagnosis is challenging. Herein, we present the final results of the first-in-human study of the amyloid-reactive peptide ^{124}I-AT-01, (^{124}I-p5+14), for the detection of diverse forms of systemic amyloidosis in abdominothoracic organs, including the heart (NCT03678259). Peptide AT-01 is a synthetic, highly charged polypeptide capable of specifically binding two ubiquitous constituents of amyloid deposits – heparan sulfate glycosaminoglycans and amyloid fibrils (1,2).

Objective: In addition to monitoring safety and determining dosimetry, the single-site open-label Phase 1/2 trial was designed to evaluate the organ-specific radiotracer uptake positive percent agreement (PPA; sensitivity) in patients with diverse forms of amyloidosis and the organ-specific negative percent agreement (NPA; specificity) in healthy subjects. In addition, cardiac and renal retention of ^{124}I-AT-01 was quantified and compared to serum biomarkers associated with organ function.

Material & Methods: The study enrolled a total of *n*=57 subjects (>18 years of age). Fourty-eight subjects (*n*=48) had systemic amyloidosis (*n*=23 AL; *n*=20 ATTR; *n*=2 ALECT2; *n*=1 AGel; *n*=1 ALys; *n*=1 AApoa1), two (*n*=2) subjects had localized AL amyloidosis, two (*n*=2) subjects were asymptomatic ATTRv carriers, and a cohort of five healthy volunteers (*n*=5) were imaged. In addition to safety, the primary outcome measure was localization of ^{124}I-AT-01, by PET/CT imaging following a single dose via intravenous infusion, in organs known or suspected to contain amyloid based on evaluation of the medical record. Patients had a confirmed diagnosis of amyloidosis (based on biopsy, genotyping, or imaging studies). Blood samples were collected 24 h prior to adminstration of ~1.4 mg of ^{124}I-AT-01 (<2 mCi I-124) as a single IV bolus. PET/CT images were acquired 5-6 h post injection using a low dose CT (120 kVp, 50 effective mAs). PET/CT images were reviewed by a radiologist who was blinded to the clinical status of the subject, and organ-specific sensitivity was assessed in comparison to organ-associated amyloidosis based on observations made in the clinical record.

Results: Overall, ^{124}I-AT-01 was well tolerated, with no deaths or drug-related serious adverse events. The gender-averaged, whole-body effective dose for ^{124}I-AT-01, assessed in the first three subjects (*n*=3), was estimated to be 0.24 ± 0.02 mSv/MBq. ^{124}I-AT-01 was detected in one or more organs in >90% of patients with systemic amyloidosis, including uptake in the heart, kidney, liver, and spleen. The PPA between clinical evaluation and ^{124}I-AT-01 imaging in the heart and kidneys was 96.2% (95% CI: 80.4 – 99.9; *n*=26) and 78.6% (95% CI: 49.2 – 95.3; *n*=14), respectively. The NPA, assessed in healthy subjects, for the heart and kidneys was 100% and 80%, respectively (*n*=5). ^{124}I-AT-01 was seen above blood pool levels in the liver and kidney in one healthy subject (*n*=1; both organs were identified in one individual) but was later considered equivocal. Cardiac uptake of the radiotracer ($SUVR_{mean}$) correlated significantly with serum Nt-proBNP levels in patients with AL (r_s = 0.48, *p* = 0.018, *n*=24) but not in those with ATTR (r_s = 0.28, *p* = 0.241, *n*=20).

Summary & Conclusion: ^{124}I-AT-01 infusion was well tolerated. Amyloid deposits were detected in patients with diverse forms of amyloidosis, by PET/CT imaging of ^{124}I-AT-01, with high sensitivity, notably in the heart. The study results support the overall safety of ^{124}I-AT-01, the acceptable whole-body effective radiation dosimetry, and the potential utility of ^{124}I-AT-01 as a diagnostic imaging agent for the detection of cardiac amyloidosis, as well as amyloid deposits in other abdominothoracic organs.

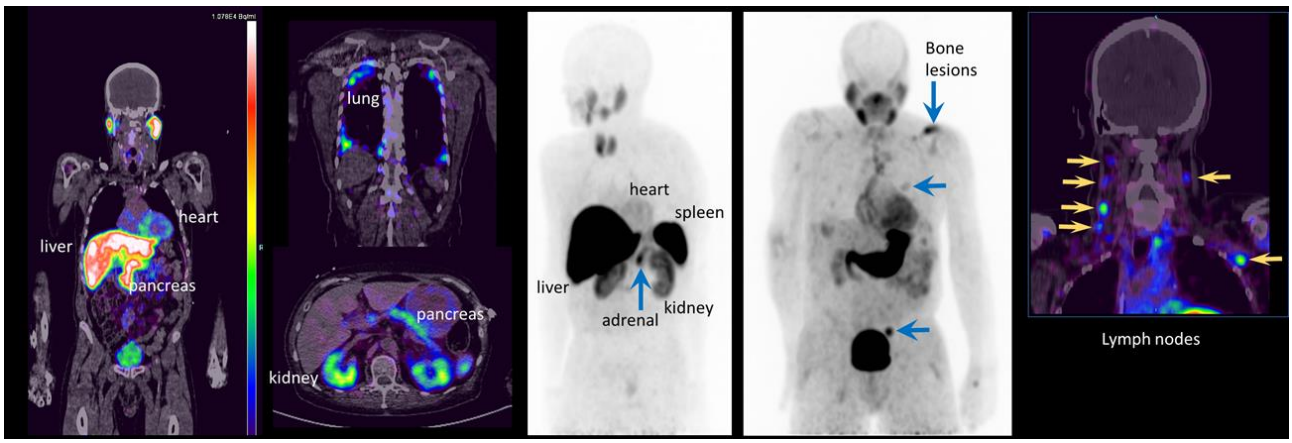

Figure 1.: Detection of amyloid in numerous anatomic sites imaged by PET/CT imaging of ^{124}I-AT-01.

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1. Wall, J.S. *et al.*, Molecules. 2015 Apr 27;20(5):7657-82. doi: 10.3390/molecules2005765. PMID: 2592351
2. Wall, J.S. *et al.*, Mol Imaging Biol. 2021 Nov 16. doi: 10.1007/s11307-021-01681-2. PMID: 3478666

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Pan-amyloid reactivity of radioiodinated peptide ^{124}I-AT-01 in patients with systemic amyloidosis demonstrated by PET/CT imaging

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Background: There are at least 17 different types of systemic amyloidosis (1), each defined by the precursor protein that misfolds and deposits as insoluble fibrils in organs and tissues. Systemic amyloidsoses are characterized by multi-organ involvement and diverse symptomology, which hinders accurate, early diagnosis and full appreciation of the extent of organ involvement with amyloid deposits. To address this, a pan-amyloid reactive peptide, AT-01 (p5+14), has been developed that can bind diverse types of amyloid through multivalent, electrostatic interactions with the ubiquitously present glycosaminoglycans and proteinaceous fibrils (2). When labeled with iodine-124, AT-01 can detect systemic amyloid by PET/CT imaging (3).

Objective: The imaging agent, ^{124}I-AT-01, has been evaluated in a Phase 1/2 PET/CT imaging study for the detection of amyloid pathology (NCT03678259). One goal of the trial was to investigate the uptake of the radiotracer in patients with diverse systemic amyloidoses, which was predicted in preclinical studies.

Material & Methods: Patients >18 years with an amyloidosis diagnosis were eligible. A total of 50 patients with light chain (ALκ and ALλ, *n*=25), transthyretin (ATTRv and ATTRwt, *n*=20), leukocyte chemotactic factor-2 (ALECT2, *n*=2), lysozyme (ALys, *n*=1), gelsolin (AGel, *n*=1), and apolipoprotein-A1 (AApoA1, *n*=1) completed the study. Five healthy subjects were imaged to assess the physiological distribution of the radiotracer. Herein, we report results from representative subjects enrolled in the trial who had diverse amyloid types. These patients received 1.4 ± 0.2 mg of ^{124}I-AT-01 (74.3 ± 1.1 MBq, with the exception of one ALECT2 patient who received 38.5 MBq) administered as a single IV bolus, and whole-body PET/CT images were acquired at 5 h post injection.

Results: Uptake of ^{124}I-AT-01 was observed in abdominothoracic organs, consistent with disease distribution described in the medical record and literature reports, in patients with all types of amyloidosis evaluated. The distribution of radiotracer in healthy subjects was consistent with renal catabolism and peptide dehalogenation, with subsequent redistribution and clearance of free radioiodide.

Summary & Conclusion: AT-01 is a pattern recognition peptide that binds electronegative motifs, with appropriate spacial distribution, on fibrils and highly sulfated heparan sulfate glycosaminoglycans in amyloid deposits. Thus, peptide binding is unbiased to the primary amino acid sequence of the amyloid precursor protein, and it does not require a fibril-specific neo-epitope. Consequently, ^{124}I-AT-01 can be used to visualize, by PET/CT imaging, many types of amyloid in numerous abdominothoracic organs, including the heart and kidneys.

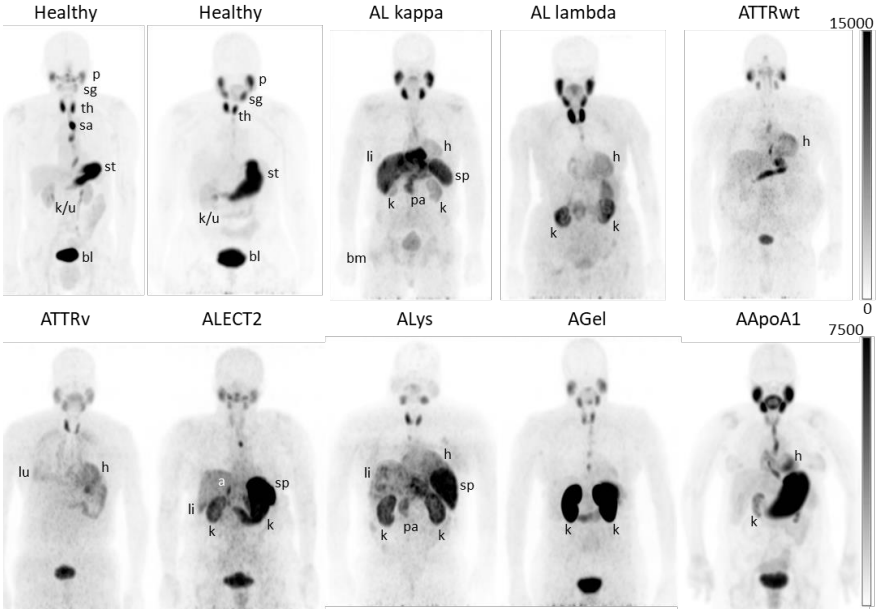

Figure 1.: Physiological and amyloid-associated uptake of ^{124}I-AT-01 in healthy subjects and patients with diverse forms of systemic amyloidosis. Maximum intensity projection PET images of representative healthy subjects and patients with

amyloidosis. All patients were administered 74 MBq (+/- 10%) I-124 and images were scaled to a minimum and maximum 0 – 15,000 Bq/cc, except ALECT2, who received 38.5 MBq and where the image is scaled to 7500, Bq/cc.

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Tracking multi-organ treatment response in systemic AL-amyloidosis with cardiac magnetic resonance derived extracellular volume mapping

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Background: Systemic light-chain (AL) amyloidosis commonly involves the liver, spleen and heart. Cardiac magnetic resonance (CMR) with extracellular volume (ECV) mapping has demonstrated accuracy in measuring hepatic, splenic and cardiac amyloid infiltration.

Objective: To assess: (1) multi-organ response to treatment using ECV mapping, (2) the association between baseline liver, spleen and myocardial ECV and prognosis, and (3) the association between changes in ECV and prognosis.

Material and methods: We identified 351 patients who underwent baseline serum amyloid P component (SAP) scintigraphy and CMR at diagnosis, of which 171 had follow-up imaging.

Results & Discussion: Liver and spleen ECV correlated with amyloid load assessed by SAP scintigraphy ($R=0.751, P<0.001$; $R=0.765, P<0.001$, respectively), with serial measurements accurately tracking treatment response. ECV regression was observed at 6-months in those with a good haematological response (liver=15%, spleen=15%, heart=5%). The remaining

patients with a good response had stable liver and spleen ECVs, but 20% had cardiac progression. By 12-months more patients with a good response demonstrated cardiac regression (liver=30%,spleen=36%, heart=32%), and this trend was maintained at 24-months.

Baseline liver and myocardial ECV were independent predictors of mortality when adjusting for liver, myocardial and spleen ECV (liver ECV: HR=1.03, 95%CI[1.01-1.05], P<0.001; myocardial ECV: HR=1.05, 95CI%[1.03-1.07], P<0.001). Baseline myocardial ECV also remained an independent predictor of mortality (HR=1.03, 95%CI[1.01-1.06], P=0.020) when adjusting for NT-pro-BNP, troponin-T and ejection fraction.

Multi-variable analysis adjusting for haematological response, change in myocardial, liver and spleen ECV demonstrated that haematological response, change in myocardial ECV (HR=1.11, 95%CI[1.02-1.19], P=0.011) and liver ECV (HR=1.06, 95%CI[1.01-1.11], P=0.015) remained independent predictors of prognosis at 6-months.

Dichotomous assessment at 12-months demonstrated that liver regression and a stable liver ECV, predicted survival compared to progression; and myocardial regression and a stable myocardial ECV, predicted survival compared to progression.

Summary & Conclusions: Multi-organ ECV quantification accurately tracks treatment response, and demonstrates different rates of organ regression, with the liver and spleen regressing more rapidly than the heart. A good haematological response alone is likely to induce visceral organ stabilisation/regression, but may not be sufficient to induce myocardial stabilisation/regression. Liver and myocardial ECV at diagnosis and increases in ECV at 6-months independently predict mortality. ECV mapping offers a multi-organ assessment of treatment response and accurate prognostication.

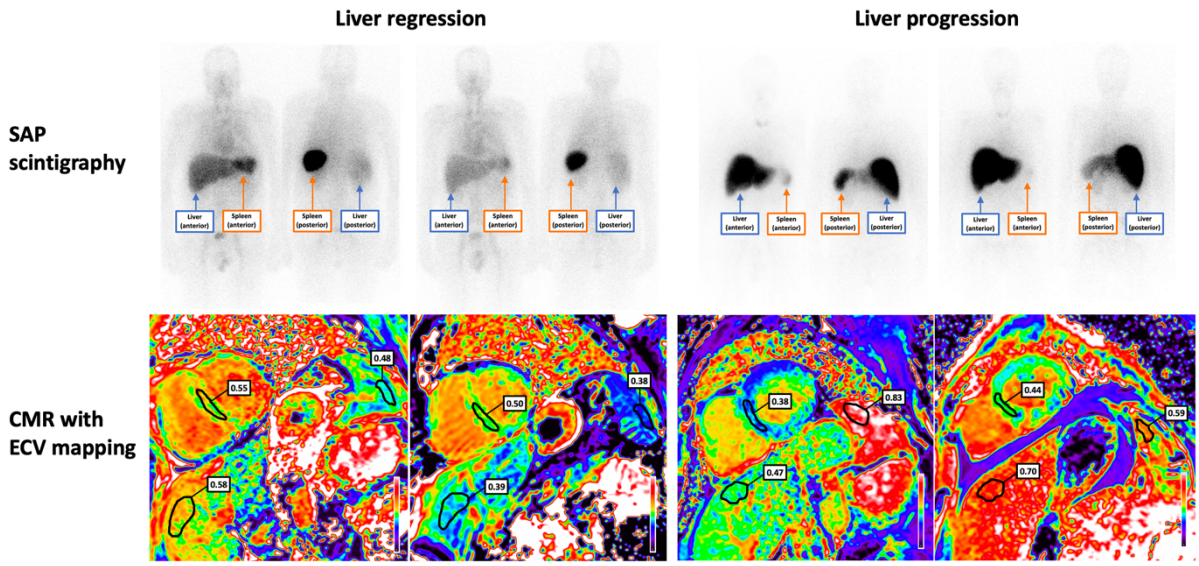

Figure 1. Left: SAP scintigraphy demonstrating liver and spleen regression. Corresponding CMR with ECV mapping demonstrating liver, spleen and cardiac regression.

Right: SAP scintigraphy demonstrating liver progression and spleen regression. Corresponding CMR with ECV mapping demonstrating liver and cardiac progression, and spleen regression.

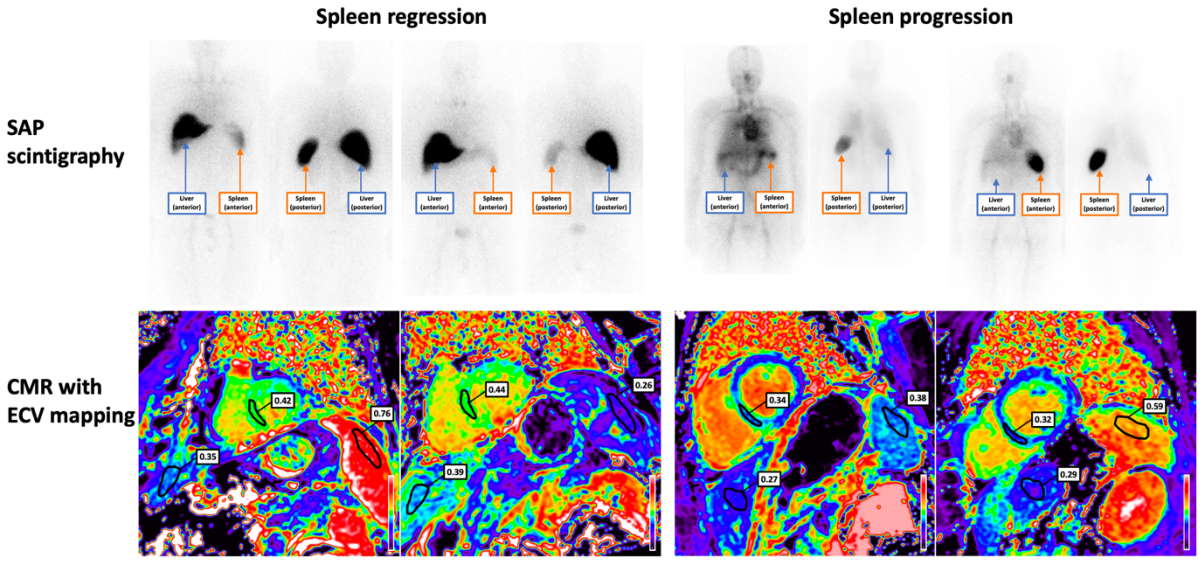

Figure 2. Left: SAP scintigraphy demonstrating spleen regression and a stable liver amyloid load. Corresponding CMR with ECV mapping demonstrating spleen regression, and a stable liver and myocardial ECV.

Right: SAP scintigraphy demonstrating spleen progression and no amyloid load in the liver. Corresponding CMR with ECV mapping demonstrating spleen progression, a normal and stable liver ECV, and a stable myocardial ECV.

Regression of Cardiac Bone-Tracer Uptake in Patients with Hereditary Transthyretin Amyloidosis after One Year Treatment with Patisiran. An early Marker of Treatment Response?

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Background: Hereditary transthyretin amyloidosis (ATTRv) is a progressive disease characterized by extracellular deposition of amyloid fibrils. Currently, several treatment options are available, one of the newest being gene silencing therapy with patisiran. There is a need for early markers of disease progression and treatment effect.

Objective: This study evaluated the potential utility of 99mTc-labeled hydroxydiphosphonate (HDP) bone scintigraphy in monitoring treatment effect of patisiran and compared bone scintigraphy to conventional follow-up parameters.

Material & Methods: In this retrospective cohort study, 23 ATTRv patients treated with patisiran and 15 ATTRv patients treated with a TTR-stabilizer were included. Data were collected from patient files and bone scintigraphies were evaluated using Perugini scoring and heart-to-contralateral ratio (H/CL). Cardiac tracer uptake on bone scintigraphy, and conventional parameters (left ventricular and inter septal wall thickness (LVPWt and IVSt) on echocardiography, amount of TTR in subcutaneous abdominal fat tissue aspirate (SAFTA) and serum NT-proBNP levels) were compared before and during treatment and changes in parameters were compared between the treatment groups.

Results&Discussion: Median treatment duration was 12 (interquartile range (IQR): 12 to 13) months in the patisiran group and 25 (IQR: 23 to 26) months in the TTR-stabilizer group. During patisiran treatment, median H/CL decreased 0.44 (p<.001) and Perugini score decreased one point in 6 patients (26% of patients; p=.014). Cardiac tracer uptake did not change significantly during TTR-stabilizer treatment. All conventional parameters did not change over time during patisiran treatment, but did increase significantly during TTR-stabilizer treatment. Between the groups, the median change in H/CL and in LVPWt was significantly lower in the patisiran group. Median change in H/CL was -0.29 (IQR: -0.66 to -0.04) in the patisiran group and +0.00 (IQR: -0.08 to +0.23) in the TTR-stabilizer group (p<.001). Median change in LVPWt was +0 mm (IQR: -2 to +1.5 mm) in the patisiran group and +1 mm (IQR: +0.5 to +3 mm) in the TTR-stabilizer group (p=.043). The changes in the other conventional parameters did not differ significantly between the treatment groups.

Summary & Conclusion: Our findings demonstrate that bone scintigraphy is the first modality to show therapy effect of patisiran in ATTRv patients after one year of treatment. Cardiac tracer uptake did not change in patients treated with a TTR-stabilizer. Conventional follow-up markers indicated progression of disease in patients treated with a TTR-stabilizer, whereas these markers did not change in patients treated with patisiran. Bone scintigraphy might be able to detect early treatment effect in ATTRv patients treated with patisiran.

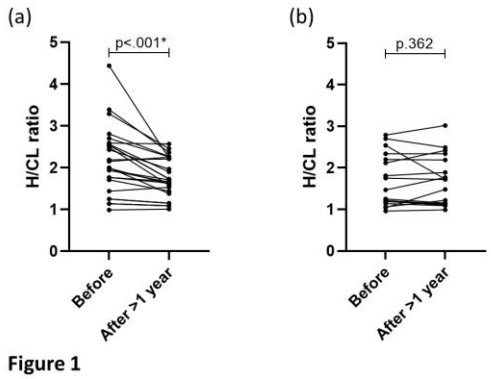

Figure 1

Figure 1.: Change in H/CL ratio on bone scintigraphy. A: during patisiran treatment. B: during TTR-stabilizer treatment.

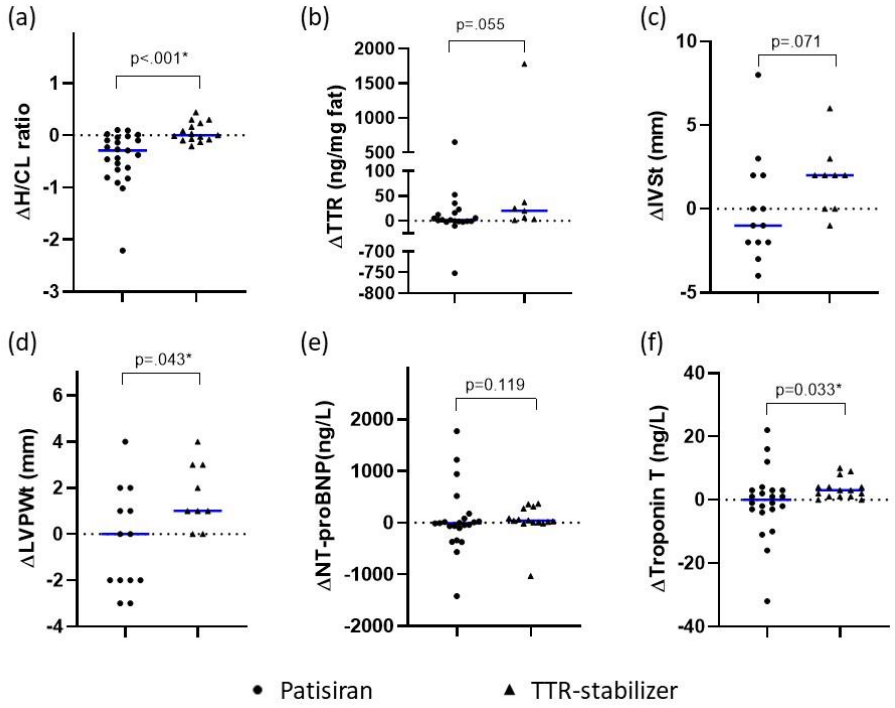

Figure 2.: Dot plots show the change in cardiac parameters during treatment with patisiran or a TTR-stabilizer. The solid horizontal lines represent group medians.

References: None

Support & Funding: None

Real World Experience of Tc-DPD scintigraphy as a diagnostic imaging tool in amyloidosis

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Background: ^{99m}Tc-labelled DPD and HMDP scintigraphy have been utilized as a diagnostic tool for amyloid cardiomyopathy for over a decade. Although endomyocardial biopsy remains the gold standard for determining presence and type of cardiac amyloid, the combined findings of a Perugini grade 2 or 3 ^{99m}Tc-DPD/HMDP scan and absence of biochemical evidence of a clonal dyscrasia have been widely adopted as non-biopsy diagnostic criteria for ATTR amyloid cardiomyopathy (ATTR-CM)[1].

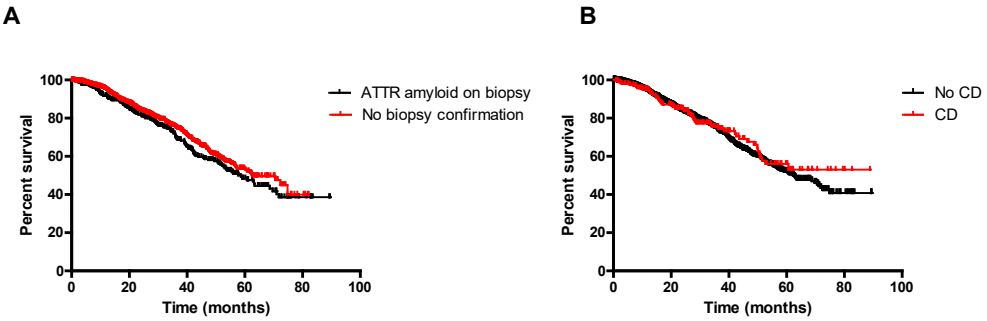
Objective: The primary objective was to evaluate the non-biopsy diagnostic criteria for ATTR-CM in a 'real world' setting whilst proposing cutoffs for kappa/lambda serum free light chain ratio in CKD which, in conjunction with absence of a serum or urine monoclonal band by immunofixation reliably exclude a clonal dyscrasia (CD), an essential component of the diagnostic algorithm. Secondary objectives included determining the wider role of DPD and HMDP scintigraphy in specialist amyloidosis centres.

Material & Methods: A multi-national retrospective study of 3354 patients with suspected or histologically proven cardiac amyloidosis referred to specialist amyloidosis centres between 2015 and 2021, all of whom underwent ^{99m}Tc-DPD/HMDP scintigraphy, serum and urine immunofixation, serum free light chain assay, measurement of MDRD eGFR and echocardiography. Cardiac magnetic resonance imaging (CMR) was used to supplement echocardiography and characterise cardiomyopathy in selected cases. Presence and type of amyloid were determined histologically by Congo red staining, immunohistochemistry and/or proteomic analysis and presence of amyloidogenic mutations were sought by sequencing of the *TTR* and *APOA1* genes where applicable.

Results: ^{99m}Tc-DPD/HMDP scans were Perugini grade 2 or 3 in 2080 patients, grade 0 in 1091 patients and grade 1 in 183 patients. Seventy-nine percent (1636/2080) of patients with a grade 2 or 3 ^{99m}Tc-DPD/HMDP scan had no biochemical evidence of a CD (i.e., fulfilled non-biopsy diagnostic criteria for ATTR-CM); amyloid was detected in 403 biopsies from these 1636 patients and in every case (100%) was ATTR type. There was no significant difference in survival between the 403 patients with histological presence of ATTR amyloid corroborating a non-biopsy diagnosis of ATTR-CM and the remaining 1233 patients who fulfilled non-biopsy diagnostic criteria for ATTR-CM without histological 'proof' of amyloid (Figure 1A, p=0.10). Presence of a CD in ATTR-CM did not influence prognosis (Figure 1B, p=0.53). Among 284/1091 (26%) patients with a grade 0 ^{99m}Tc-DPD/HMDP scan who did have cardiac amyloidosis, it was AL type in 276 (97%) cases, with 7/8 remaining cases being AApoAIV type. A CD was detected in 122/183 (67%) of patients with a grade 1 ^{99m}Tc-DPD/HMDP scan and 106/122 (87%) of these patients had cardiac AL amyloidosis. Sixty of 61 (98%) patients with a grade 1 ^{99m}Tc-DPD/HMDP scan and no CD had ATTR-CM.

Summary & Conclusion: The non-biopsy diagnostic criteria, with inclusion of novel serum free light chain ratio cutoffs according to eGFR for exclusion of a CD, remain highly specific for ATTR-CM in a real-world setting, provided they are carefully adhered to. Absence of cardiac uptake on ^{99m}Tc-DPD/HMDP scintigraphy all but excludes ATTR-CM, but occurs in a majority of patients with cardiac AL amyloidosis. In a patient with suspected cardiac amyloidosis, the finding of a grade 1 ^{99m}Tc-DPD/HMDP scan accompanied by a CD strongly suggests but does not prove AL type. The finding of a grade 1 ^{99m}Tc-DPD/HMDP scan in the absence of a CD indicates likely ATTR-CM.

Figure 1



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Assessing left ventricular strain in cardiac amyloidosis: the importance of accurate measurement technique.

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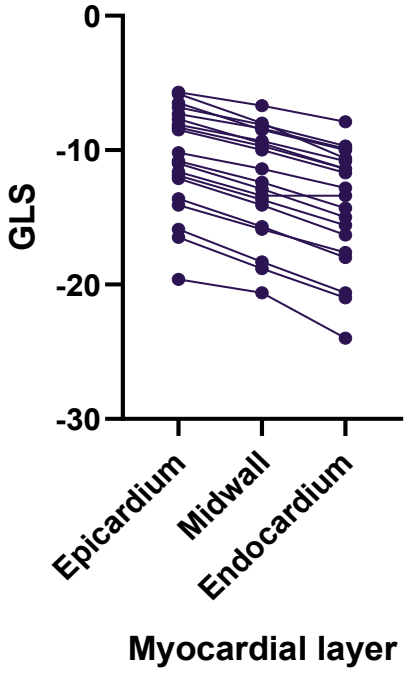
Background: Left ventricular (LV) speckle strain imaging (strain) measures the contraction of the heart along its long axis. In patents with cardiac amyloidosis, abnormaities of strain occur early, and regional differences usually occur with relative preservation of apical strain. Strain imaging is very reproducible, and changes in LV strain can thus detect changes in LV function before an alteration in ejection fraction is detected. Since strain represents LV shortening, it is expressed as a negative value, with normal values ≤ -19.0%. In clinical studies of AL amyloidosis, an absolute change in strain of ≥ -2% is considered a clinically significant value, representing a change in LV function and reflecting prognosis. In untreated cardiac TTR amyloidosis, median strain values deteriorate by an absolute -1.1% over 12 months, and tafamidis attenuates this deterioration (2). Thus it is critical that high-quality echocardiographic studies are performed if strain changes are to be used for prognostication or assessment of drug response. The LV wall in amyloidosis is usually thick due to infitration, and it is recognized that strain measurements close to the endocardium tend to be greater than towards the epicardium, representing an endocardial-epicardial gradient. Thus, if the region of interest (ROI) of strain measurent is not placed in the correct part of the myocardium, values could be falsely high or low depending on the placement site, thereby affecting reproducibility of serial measurements.

Objective: To determine the absolute differences in global longitudinal strain (GLS) in individual patients with amyloid heart disease when measurements are made in the endocardial, midwall and epicardial segments of the heart, in order to assess the clinical significance of inaccurate ultrasound positioning during echocardiography**.**

Material & Methods: All patients with amyloidosis undergoing echocardiography in our institution have the studies performed by 1 of 2 specially-trained echocardiograpy technicians in order to assure maximum quality and serial study reproducibility. All studies are performed on the same vendor's instrument (GE) and stored in raw-data format and all strain imaging is remeasured by 1 of 2 echocardiographer physicians, with careful ROI positioning to ensure complete coverage of the myocardium in the longitudinal views. Strain imaging results are calculated by proprietary GE software and reported as midwall strain, but the measurement technique allows assessment of epicardial and endocardial strain obtained on the same beats. 17 longitudinal segments are individually recorded as a bullseye plot and the segmental scores averaged to obtain GLS and plots can be obtained for each of the layers of the myocardium (figure). 20 patients with cardiac amyloidosis (8 AL and 12 TTR) were randomly chosen and the GLS compared between epicardial, midwall and endocardial measurements, analyzed by ANOVA**.**

Results: GLS in the individual 20 patients, measured in the midwall segment, ranged from -6.7% to –20.6% (mean -12.26%; 25-75% percentile -8.7% to -15.3%). In every patient, strain was least negative in the epicardial layer and greatest in the endocardial layer, with mean GLS for epicardium of -10.6%, mid- of -12.3% and epicardial of -14.1% ($p<0.0001$) (Figure 1). In many patients, the color-coded segmental pattern of strain differed considerably among the 3 layers, potentially leading to a subjective error in reporting the presence or absence of the well-described typical "bullseye" appearance typical of amyloidosis had the ROI been incorrectly placed (figure 2).

Summary & Conclusion: Although strain imaging is a very reproducible measurement for assessing changes in LV function in cardiac amyloidosis, failure to correctly position a region of interest at the time of image acquisition or raw-data (re)interpretation may lead to clinically significant overestimation (if measured in endocardium) or underestimation (if measured in epicardium) of the standard midwall strain reported and visualized by GE strain echocardiography software.



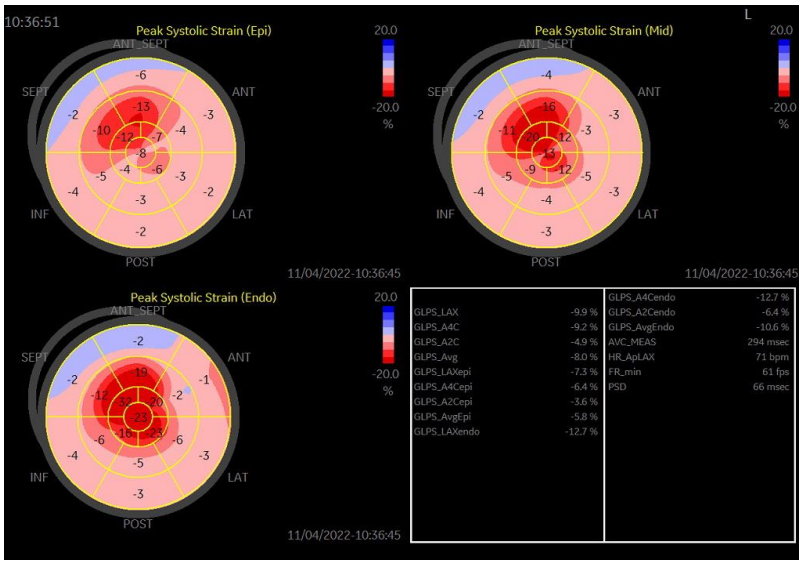


Figure1 (left). Individual values for GLS at each layer. A more negative value indicates greater strain.

Figure 2. Patient with wild-type TTR amyloidosis, showing typical differences between longitudinal strain in the epicardial (top left) layer (mean strain -5.8% with an apical segment of -8%), mid-ventricular (top right) layer (mean strain -8.0% , with an apical segment of -13%) and endocardial (bottom left) layer (mean strain, -10.6%, with an apical segment of -23%).

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Quantitative magnetic resonance neurography biomarkers: Cross-sectional results from a single center study in 80 subjects with symptomatic or asymptomatic hereditary transthyretin amyloidosis

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Background: Proton spin density (ρ) and T2-relaxation time (T2app) are microstructural markers of nerve tissue integrity *in vivo* and have been successfully applied to quantify nerve lesions in different diffuse neuropathies in recent years. Promising results were achieved in a small cohort of symptomatic and asymptomatic hereditary transthyretin (ATTRv) amyloidosis, but data is limited.[1]

Objective: To quantify peripheral nerve lesions in a large cohort of subjects with symptomatic or asymptomatic ATTRv amyloidosis by analyzing ρ and T2app in the tibial nerve, and to prove their validity as novel biomarkers for early diagnosis, estimation of disease severity, and macromolecular changes.

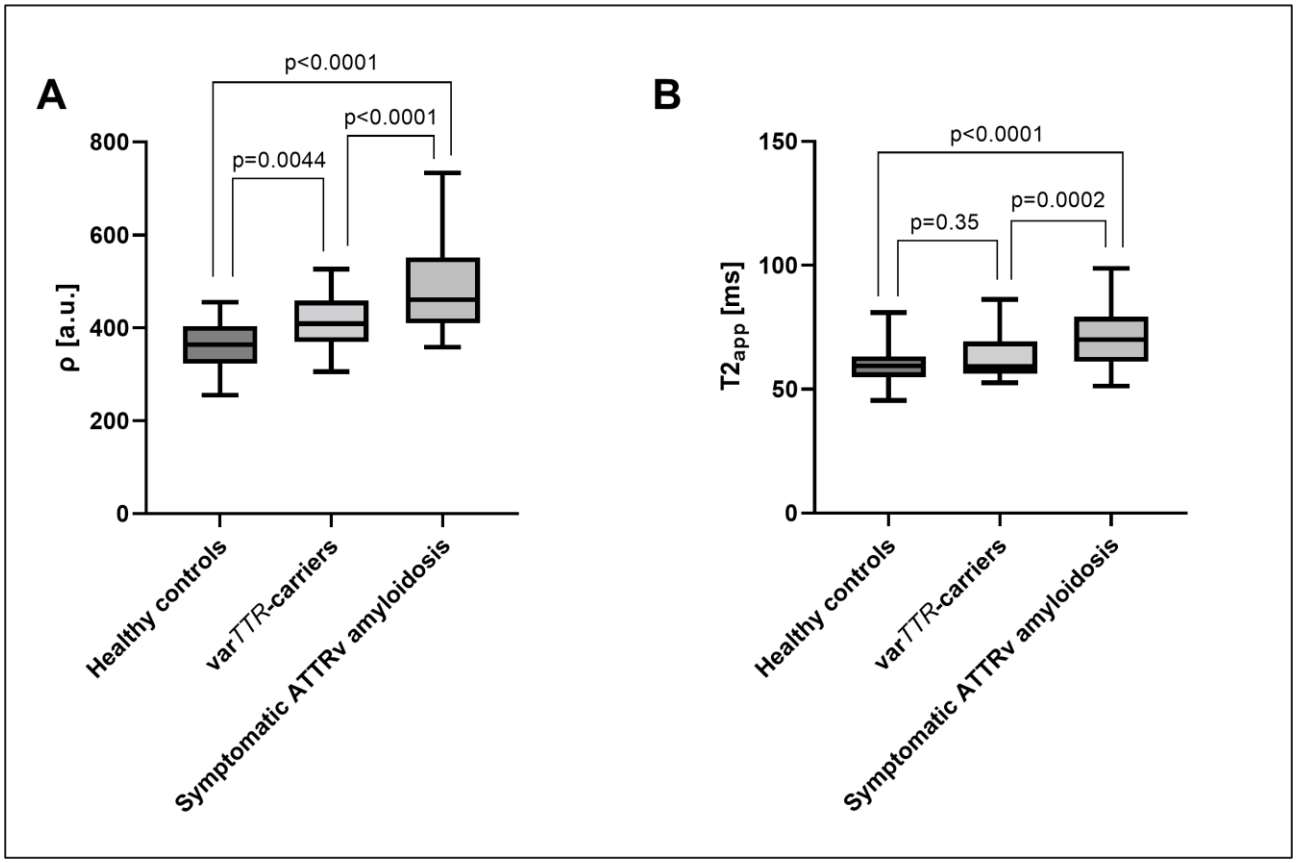
Material & Methods: 80 participants with genetically confirmed var*TTR* and 40 healthy volunteers prospectively underwent neurologic and electrophysiologic examinations including assessments for the Neuropathy Impairment Score-Lower Limb (NIS-LL). High-resoulution magnetic resonance neurography (MRN) was performed at the right mid to distal thigh by applying axial 2D dual-echo TSE sequences with spectral fat saturation for T2-relaxometry (TE_1=12ms, TE_2=73ms, repetition time 5210ms, acquisition time 7:30min). The tibial nerve was manually segmented on 10 consecutive central slices per participant and ρ and T2app were calculated.

Results: 40 participants (31 men, 9 women; mean age 58.3±2.0 years) were classified as having symptomatic ATTRv amyloidosis, while 40 participants were classified as asymptomatic carriers of the var*TTR* gene (var*TTR*-carriers; 17 men, 23 women; mean age 42.35±1.8 years). Controls were matched with var*TTR*-carriers (17 men, 23 women; mean age 42.35±2.3 years). Tibial nerve ρ differentiated well between symptomatic ATTRv amyloidosis, asymptomatic var*TTR*-carriers, and controls ($p<0.0001$, $F=30.86$). In detail, ρ was markedly higher in symptomatic ATTRv amyloidosis (484.2±14.8a.u.) vs. asymptomatic var*TTR*-carriers (413.1±9.4a.u.;$p<0.0001$) vs. controls (362.6±7.5a.u.; $p<0.0001$), and higher in asymptomatic var*TTR*-carriers vs. controls ($p=0.0044$). Tibial nerve T2app was increased in symptomatic ATTRv amyloidosis (70.6±1.8ms) vs. asymptomatic var*TTR*-carriers (62.3±1.3ms; $p=0.0002$) vs. controls (59.5±1.0ms; $p<0.0001$). Differences in T2app between asymptomatic var*TTR*-carriers and controls did not exist.
In asymptomatic var*TTR*-carriers ρ showed positive correlations with tibial nerve distal motor latencies (DML), and inverse correlations with tibial nerve conduction velocities (NCV) and compound muscle action potentials (CMAP) as well as with sural nerve NCV and sensory nerve action potentials (SNAP). In symptomatic patients, positive correlations were found between ρ and the NIS-LL. In symptomatic ATTRv amyloidosis T2app correlated positively with the NIS-LL and tibial nerve DML, and inversely with tibial nerve NCV and CMAP, peroneal nerve NCV, and sural nerve NCV. No correlations were found between T2app and demographic, clinical and electrophysiologic data in asymptomatic var*TTR*-carriers.

Summary & Conclusion: ρ best differentiates asymptomatic var*TTR*-carriers from symptomatic ATTRv amyloidosis and controls. In addition, ρ correlates well with electrophysiologic markers on a subclinical level in clinically and electrophysiologically completely asymptomatic var*TTR*-carriers, thus making it a useful biomarker for detecting nerve injury at the earliest. T2app is only increased in symptomatic ATTRv amyloidosis, and correlates well with clinical and electrophysiologic parameters in this group, suggesting it as a marker for estimating disease severity.

Figure 1: Quantitative MRN markers

Mean values of tibial nerve ρ (A) and T2app (B) were plotted for controls, asymptomatic var*TTR*-carriers, and symptomatic ATTRv amylodiosis. Tibial nerve ρ was lowest in healthy controls, increased significantly in asymptomatic var*TTR*-carriers, and was highest in symptomatic ATTRv amyloidosis. Tibial nerve T2app was only increased in symptomatic ATTRv amyloidosis versus asymptomatic var*TTR*-carriers, and versus controls.



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Assessment of the clinical value of whole-body MRI in untreated patients with systemic light chain amyloidosis

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Background: Whole-body (wb) MRI for assessment of bone marrow (BM) infiltration with monoclonal plasma cells is part of the recommended work-up in multiple myeloma (MM) [1]. Currently, wbMRI is not routinely performed in patients with systemic light chain (AL) amyloidosis.

Objective: The purpose of this pilot study was to assess the clinical value of wbMRI in patients with AL amyloidosis.

Material & Methods: We retrospectively assessed wbMRIs (coronal T1 and fat suppressed T2 weighted sequences) from the years 2006-2021 in all – at the time of MRI – untreated patients with AL amyloidosis and an underlying plasma cell dyscrasia (PCD, n=48). MRIs were examined for 5 BM infiltration patterns known from MM work-ups (normal, diffuse, focal, mixed [focal and diffuse] and salt-and-pepper) [2] or a novel pattern created for this study, namely T1/T2 hypointensity with the attempt to reflect amyloid deposits in MRI (Fig. 1). The background for this grouping is that an increase in plasma cell infiltration leads to a higher water content reflected by T1 hypointense/T2 hyperintense signal whereas amyloid deposits replace the fatty BM without increasing its water content. In addition, MRIs were assessed for number of focal lesions (FL), amyloid arthropathy (AA) (joint-associated FLs and soft tissue deposits), effusions, anasarca and organ size measurements. Besides, established clinical markers in serum and BM were recorded and led with standard radiographic features to the staging of the PCD in MGCS, SMM and MM. As our patient cohort was relatively small, descriptive analysis was performed without testing for statistical significance.

Results: Median time interval from first diagnosis to MRI was 1.5 months (0-41 months). Pericardial effusion was found in 1, pleural effusion in 9, ascites in 8 and anasarca in 10 patients. The highest median liver diameter was found in diffuse (18.0 cm), the lowest in normal pattern (13.6 cm). Median dFLC of all patients was quite high with 564 mg/l. FLC ratio was ≥ 100 in 9 of 10 patients with mixed pattern and in 6 of 7 with AA (Tbl. 1).
Patients with normal pattern in MRI had been diagnosed MGCS or SMM but not MM, 8 of 10 patients with mixed pattern had been diagnosed MM and none MGCS what indicates that these MRIs reflect the PCD burden well also in AL amyloidosis.
7 patients showed AA. 5 of these had a mixed pattern, 4 patients had ≥ 10 FL, 6 had a FLC ratio ≥ 100 and 6 had been diagnosed MM showing a strong correlation of AA with advanced PCD stage. 7 other patients showed T1/T2 hypointensity of the axial BM. Interestingly, amyloid was detected in 3 BM samples of 4 patients with T1/T2 hypointensity and histologic congo-red staining of the BM biopsy and in 3 BM samples of 4 patients with AA.

Summary & Conclusion: WbMRI in AL amyloidosis gives information about the extent and pattern of plasma cell infiltration of the BM and in addition diagnoses AA, effusions and anasarca. Patients with signs of AA in wbMRI tend to have an advanced PCD (MRI infiltration pattern, laboratory markers). WbMRI may help in verification of suspected AA and give an overview of the affected joints. WbMRI performed in MM showing AA may guide to a work-up for amyloidosis. T1/T2 hypointensity of the axial BM in MRI may give a clue to amyloid deposition whereas in routine clinical practice the diagnosis of amyloid deposits in BM is only made in the focal iliac bone biopsy. For evidence-based recommendations a further prospective study with wbMRIs and examination of BM samples for amyloid deposits in a larger patient cohort is needed.

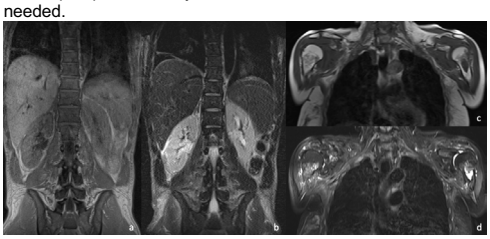

Fig. 1: a (T1) +b (T2): Part of the wbMRI of a patient showing T1/T2 hypointensity of the axial BM.
c (T1) + d (T2): Extensive juxta-articular soft tissue deposits in a patient with clinical "shoulder pad sign".

		Bone marrow infiltration patterns in MRI						MRI
	All patients	Normal	Diffuse	Focal	Mixed	Salt & pepper	T1/T2 hypointensity	Amyloid Arthropathy
Total number of patients	**48**	**17**	**4**	**5**	**10**	**5**	**7**	**7**
Diagnosis at time of MRI								
MGCS	9	4	1	1	0	1	2	0
SMM	26	13	2	3	2	2	4	1
MM	13	0	1	1	8	2	1	6
Affected light chain								
Kappa	13	2	1	3	3	3	1	3
Lambda	35	15	3	2	7	2	6	4
Amyloid in BM sample	11 (23)	4 (6)	0 (1)	1 (4)	3 (8)	0 (0)	3 (4)	3 (5)
Organ involvement								
Cardiac	30	11	4	3	6	2	4	3
Renal	22	10	2	2	3	3	2	1
Hepatic	3	1	1	0	0	1	0	0
Nerval	8	3	0	1	1	0	3	1
Gastrointestinal	9	2	2	1	3	0	2	2
Soft tissue	27	9	3	3	7	3	2	7
Laboratory markers								
dFLC [mg/l] median (range)	564 (24-19252)	147 (24-1544)	737 (515-1822)	465 (36-1079)	1448 (11-19252)	1833 (64-3749)	373 (35-2326)	1680 (778-19252)
FLC ratio ≥ 100	18	3	1	1	9	3	1	6
Plasma cell count (cyt) [%] median (range)	19 (3-90)	15 (3-28)	31 (14-74)	6 (4-31)	29 (7-90)	32 (5-50)	15 (10-20)	40 (13-74)
Plasma cell count ≥ 60%	2 (45)	0 (16)	1	0 (4)	1	0	0 (6)	1
t(11;14)	28 (45)	11 (14)	3	2	4	3	5	3

- *The group T1/T2 hypointensity comprises patients with or without additional focal lesions.*
- *In case of missing values, nr. of patients with available values is written in brackets.*

Tbl. 1: Clinical data separated into BM infiltration patterns and AA in wbMRI.

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Predictors of hematologic response and survival with stem cell transplantation in AL amyloidosis: a 25-year longitudinal study.

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Background: High-dose melphalan and autologous stem cell transplantation (HDM/SCT) is a standard of care for selected patients with systemic light chain (AL) amyloidosis. Factors predictive of hematologic complete response (CR) and prognostic of survival with HDM/SCT still need to be clarified. Additionally, the recent US FDA approval of daratumumab in combination with cyclophosphamide, bortezomib, and dexamethasone (Dara-VCd) has prompted re-evaluation of the role of HDM/SCT in transplant-eligible patients with AL amyloidosis.

Objective: To identify predictors of short- and long-term efficacy and safety in patients with AL amyloidosis treated with HDM/SCT.

Material & Methods: We collected data from a prospectively maintained database of patients with AL amyloidosis consecutively treated with HDM/SCT at our institution between July 1994 and September 2021. Hematologic responses were defined according to consensus opinion from the International Society of Amyloidosis.[1] For patients with a hematologic complete response (CR), duration of response (DOR-CR) was defined as the time between SCT (day 0) and hematologic relapse. Patients who died without evidence of hematologic relapse were censored for purpose of DOR-CR assessment. Event-free survival (EFS) was defined as time between day 0 and initiation of next line of therapy or death, whichever occurred first. Overall survival (OS) was defined as the time between day 0 and death from any cause or last follow-up. Treatment related mortality (TRM) was defined as death from any cause between day 0 and day +100. Cumulative incidence estimates for t-MDS/AML were computed by the Fine-Gray method to account for death as a competing risk. Univariable and multivariable logistic and hazard regression models were fitted for outcome measures. All calculations were performed with R software.

Results: Between 1994 and 2021, 648 patients with AL amyloidosis underwent HDM/SCT. Hematologic CR was achieved in 39% of patients. The median DOR-CR was 12.3 years, and 45% of patients with a hematologic CR had no evidence of a recurrent plasma cell dyscrasia at 15 years after HDM/SCT (**Figure 1A**). With a median follow-up of 8 years, the estimated median EFS and OS were 3.3 and 7.6 years, respectively (**Figures 1B-C**). Patients with a hematologic CR had a median OS of 15 years, and 30% of these patients survived >20 years. On multivariable analysis, difference between the involved and uninvolved free light chain (dFLC) >180 mg/L and bone marrow (BM) plasma cells >10% were independently associated with shorter EFS, whereas B-type natriuretic peptide (BNP) >81 pg/mL, troponin I >0.1 ng/mL, and serum creatinine >2.0 mg/dL were independently associated with shorter OS. We developed a prognostic scoring system for EFS, incorporating dFLC >180 mg/L and BM plasma cells >10% as adverse risk factors. Patients with low-risk (0 factors), intermediate-risk (1 factor), and high-risk (2 factors) disease had median EFS estimates of 5.3, 2.8, and 1.0 years, respectively ($p<0.001$; **Figure 1D**). The 100-day TRM was 3.3% in the latest era (2012-2021), and the 25-year risk of t-MDS/AML was 3.3%.

Summary & Conclusion: HDM/SCT induces durable hematologic responses and prolonged survival with improved safety in selected patients with AL amyloidosis. We propose a prognostic scoring system for EFS, which may help identify patients most likely to derive long-term benefit from HDM/SCT. Future randomized prospective trials are needed to determine the optimal role and timing of HDM/SCT in the context of novel treatment regimens.

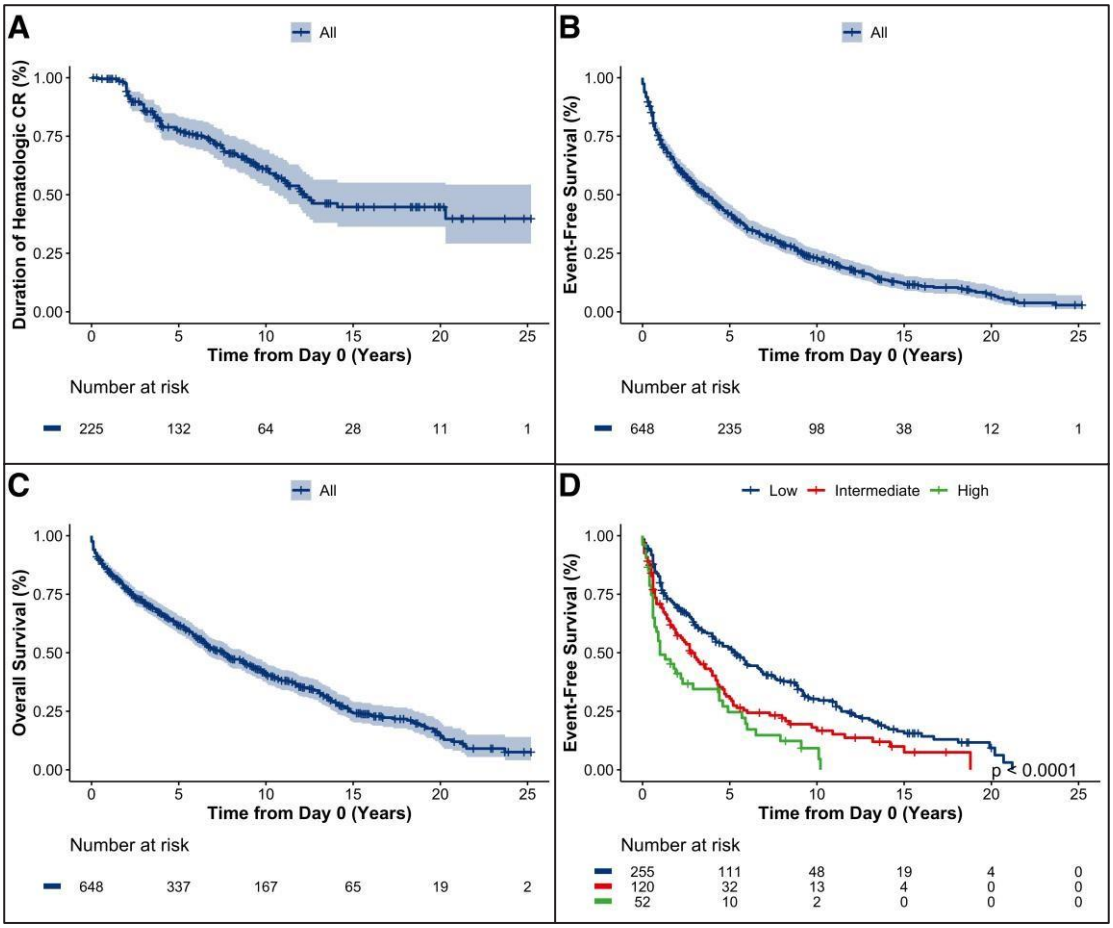

Figure 1. Survival outcomes following treatment with HDM/SCT. Kaplan-Meier curves for duration of response (DOR-CR) in patients who achieved a hematologic complete response (A); event-free survival (B); overall survival (C); and event-free survival (EFS) based on the proposed prognostic scoring system (D).

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The prognostic importance of flow cytometry-based measurable residual disease (MRD) in patients with systemic light chain amyloidosis

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Background: Most individuals with light chain (AL) amyloidosis are not cured after treatment and have some degree of plasma cell dyscrasia remaining in the bone marrow. Advances in flow cytometry-based techniques have enabled the detection of very low levels of disease. The clinical implications of measurable residual disease (MRD) in patients with AL amyloidosis remains under investigation.

Objective: In this prospective analysis, we sought to evaluate clinical outcomes based on MRD status among patients with hematologic complete response (hemCR) after treatment. Clinical endpoints included organ response rates; major organ deterioration–progression-free survival (MOD–PFS); treatment-free interval (TFI); and overall survival (OS).

Material & Methods: Patients with AL amyloidosis in hemCR underwent testing for MRD by multiparametric 10-color flow cytometry of bone marrow aspirates during post-treatment evaluations at the Boston University Amyloidosis Center. The sensitivity level upon which MRD status was established was 1 in 10^5 nucleated cells or higher [1]. We determined organ responses according to consensus guidelines [2] at the time of MRD assessment; those with end-stage organ failure before hemCR achievement were excluded from this analysis. MOD–PFS was defined as time from hemCR achievement to major organ deterioration (i.e., renal or cardiac failure), hematologic progression or death from any cause; whichever occurred first. TFI was defined as the time from last dose of treatment to the start of subsequent systemic therapy. OS was measured from hemCR achievement to death from any cause.

Results: Among 121 patients with a hemCR after therapy, 60 (50%) were MRD negative and 61 (50%) were MRD positive. Median time from hemCR achievement to MRD assessment was 26 months (IQR 13–73 months). Baseline characteristics were similar between groups. MRD positivity correlated with a higher level of involved free light chains (iFLC) in the serum at the time of MRD assessment (median 22.8 vs 17.7 mg/L, P=.018). The estimated clone size for those with MRD positivity was a median of 3.8×10^{-4} monotypic plasma cells (mPC)/ 10^6 cells (range 1.4×10^{-5} to 5.7×10^{-3}). HemCR was achieved by high-dose melphalan and stem cell transplantation in the majority of patients [**Table 1**]. Two or more lines of therapy were used for 52% and 32% of patients in the MRD negative and MRD positive groups, respectively (*P*=.083).

The MRD-negative group demonstrated a significantly higher rate of renal response (36/40 [90%] vs 28/44 [64%], P=.005) but a similar rate of cardiac response (19/24 [79%] vs 23/33 [70%], P=.423) in comparison to the MRD-positive group [**Figure 1**]. After a median follow-up of 56 months from hemCR achievement, patients with MRD negativity had a superior MOD–PFS (HR 0.29, P=.034) and TFI (HR 0.11, P=.012). Among those with MRD positivity, the estimated clone size was significantly higher for those who experienced an organ deterioration or hematologic progression event vs those who did not (median 8.4×10^{-4} vs 2.4×10^{-4} mPC/ 10^6 cells, P=.041). A total of 8 patients had died during follow-up, including 4 in each group. There was no difference in estimated OS between groups.

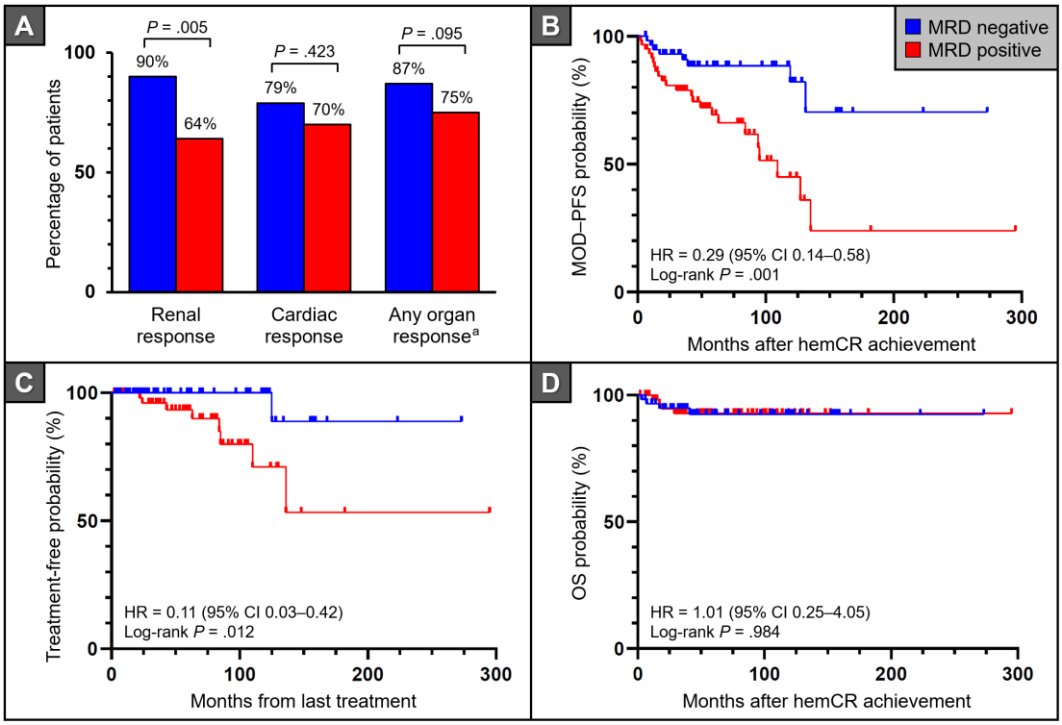
Summary & Conclusion: We evaluated the role flow cytometry-based MRD status in relation to clinical outcomes and found that MRD negativity is prognostic for improved renal response, MOD–PFS and TFI among patients with AL amyloidosis in hemCR. Longer prospective follow-up is needed to determine whether this translates into an OS benefit.

Table 1. Treatments by which hematologic complete response was achieved in each MRD group

	MRD negative	MRD positive	*P* value
High-dose melphalan and stem cell transplantation	24 (40)	25 (41)	.912
Bortezomib-based regimen	13 (22)	18 (30)	.323
Daratumumab	18 (30)	8 (13)	**.024**
CyBorD with daratumumab	3 (5)	4 (7)	.713
Immunomodulatory drug	2 (3)	6 (10)	.150

Abbreviation: CyBorD, cyclophosphamide, bortezomib and dexamethasone.

Figure 1. Clinical outcomes according to MRD status among 121 patients with AL amyloidosis in a hematologic complete response (hemCR), including **(A)** organ responses; **(B)** major organ deterioration–progression-free survival (MOD–PFS); **(C)** treatment-free interval; and **(D)** overall survival (OS). Blue signifies MRD negativity, whereas red signifies MRD positivity.



[a]*Kidney, heart and liver were included in the count for any organ response.*
Abbreviations: HR, hazard ratio; CI, confiedence interval.

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Prognostic Impact of Translocation t(11;14) and of other cytogenetic abnormalities in patients with AL amyloidosis in the era of contemporary therapies

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Background: translocation (11;14) is found in 40-60% of patients with AL amyloidosis compared to a much lower frequency in myeloma (less than 15%). This genetic aberration is associated with Cyclin D1, B-cell lymphoma 1 and 2 (bcl-1 and bcl-2) overexpression and apoptosis inhibition. Prognostically, t(11;14) has been associated with poorer prognosis in AL amyloidosis[1], probably related to lower sensitivity to bortezomib; however, new drugs such as daratumumab (an anti-CD38 monoclonal antibody) and venetoclax (a bcl-2 targeting drug) may offer new options for these patients.[2]
Objective: To assess the prognostic relevance of t(11;14) in newly diagnosed patients with systemic AL amyloidosis in the more recent era.
Material & Methods: We prospectively assessed t(11;14) status in 146 consecutive newly-diagnosed patients with systemic AL amyloidosis using fluorescence in situ hybridization, which were diagnosed and treated in the Department of Clinical Therapeutics, Athens, Greece from 2016 to 2021.
Results: 40% of patients were positive for t(11;14); the clinical characteristics of the cohort according to t(11;14) status are shown in table 1. In 24% of patients t(11;14) was the only cytogenetic aberration; other cytogenetic abnormalities were seen in 32% while 32% of the patients had two or more cytogenetic abnormalities. High risk (HR) aberrations [t(4;14), t(14;16), del17p or +1q21] were present in 31% of patients. Presence of t(11;14) was inversely associated with del13q (0.044), +1q21 (p<0.001) and HR cytogenetics (p<0.001). The baseline clinical profile, organ involvement patterns and treatments were similar among groups but t(11;14) was associated higher incidence of κ-LC amyloidosis (0.016). At 1, 3 and at 6-month landmarks hematologic response rates were numerically but not statistically higher in the non-t(11;14) group, however, patients with t(11;14) required more frequently second line treatment within 12 months since diagnosis (p=0.015). Accounting for the need of salvage therapy as an event, median event-free survival (EFS) was 11.3 months (95%CI 0.4-22.2) in t(11;14) positive patients vs 49 months (95%CI 44.3-53.5) for non-t(11;14) patients (p=0.015). Use of daratumumab in first line was associated with lower probability and longer time to 2nd line therapy (p<0.001). In multivariate analysis the adverse prognostic effect of t(11;14) on EFS was independent of renal or cardiac involvement, NTproBNP and Mayo stage and of 1st-line treatment (with or without daratumumab) (HR:2.1, p=0.014). EFS was not statistically different for patients with or without HR cytogenetics, although numerically longer for those with HR cytogenetics (48 vs 40 months, p=0.272). Early mortality (13% vs 10%) and median OS were similar among patients with and without t(11;14) (p=0.63) even after adjustment for primary treatment type, use of daratumumab at any line and Mayo stage (HR 1.03, p=0.9). The presence of other cytogenetics (either +1q21 or HR cytogenetics) was not associated with OS; when adjusting for t(11;14) there was still no prognostic effect for OS.
Summary & Conclusion: t(11;14) in AL amyloidosis is associated with a low frequency of concurrent aberrations in del13q and 1q21, more frequent need and shorter time to salvage therapy but, possibly due to use of effective salvage therapies, with neutral prognostic effect on OS. It remains to be evaluated whether bcl-2 inhibitors, at first or subsequent lines may offer an advantage in patients with t(11;14) translocation.

Clinical characteristics:	t(11;14) (+) n= 59	t(11:14) (-) n= 87	p-value
Age (Median) / %Male	66 / 61%	65 / 55%	0.5 / 0.56
dFLC (mg/L) / BM infiltration (%)	330 / 20%	208 15%	0.41 / 0.5
Organ involvement Renal/ Heart/ Liver/ NS	 58%/ 90%/ 22%/ 21%	 60%/ 79%/ 16%/ 31%	 All p>0.05
Mayo stage 1/2/3A/3B	10%/ 39%/ 30% /21%	15% / 35% / 24% /26%	0.7
Renal stage I / II / III	46% / 46% / 8%	50% / 44% / 6%	0.92
Diagnosis: κLC / λLC	35% / 64%	18% / 82%	**0.016**
Treatment type (1st line) Bortezomib-based Daratumumab-containing	 93% 27%	 95% 32%	 0.47
Dara @ any Tx line	47%	57%	0.22
Cardiac response 6m	52%	44%	0.55
Renal response 6m	40%	54%	0.37
Hematological response 1month 3month 6month	NR/ PR/ VGPR 29%/45%/26% 17%/28%/55% 17%/12%/71%	NR/ PR/ VGPR 19%/51%/30% 6%/19%/75% 10% /11% /79%	 0.5 0.12 0.7

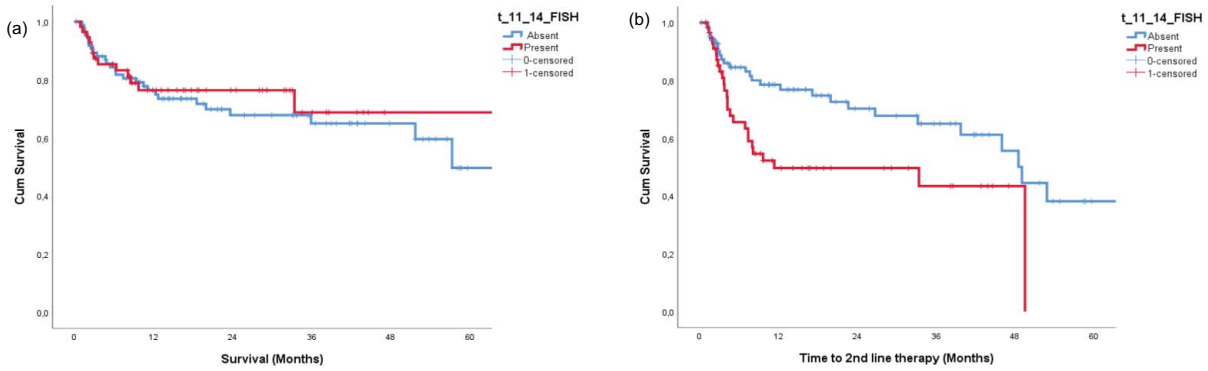

Table: Clinical characteristics of the cohort based on t(11;14) status

Figure: (a) Kaplan Mayer curve for overall survival (OS) based on t(11;14) status and (b) Time to second line therapy based on t(11:14) status

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Incidence and risk factors of sudden death in patients with cardiac amyloidosis.

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Background: Although sudden death (SD) is a potential complication in patients with cardiac amyloidosis (CA), its frequency and incidence are mostly unknown. Initial study reported a high incidence of SD whereas contemporary series have found lower event rates.

Objective: We sought to describe incidence of SD and evaluate its clinical predictors in patients with CA.

Material & Methods: All patients with CA (ATTR or AL) assessed at Sant'Orsola Hospital (Bologna, Italy) and Hospital Universitario Puerta de Hierro (Madrid, Spain) from 1986 to 2020 were retrospectively studied for SD events. Regression analysis was performed to identify risk factors for SD in univariate analysis. Those factors statistically significant were assessed through age-adjusted multivariate analysis.

Results: Analysis included 784 CA patients, 569 (72.6%) had ATTR and 215 (27.4%) had AL. ATTR patients were older (mean age 74.1±12.1 vs 64.5±10.8 years; P < 0.001) and had more implantable cardioverters implanted (2.1% vs 0.0%; P = 0.04). After a median follow-up of 1.7 years (IQR: 0.7-3.6), 26 patients (3.3%) experienced a SD event (15 AL and 11 ATTR). Patients with AL had significantly higher risk of SD compared with ATTR (HR 4.19; 95%CI: 1.92-9.13; P < 0.001). In patients with ATTR SD rate at 2 years was 1.8% (CI95% 0.9-3.9%). Previous pacemaker implantation (PPM) was the only variable that was associated with increased risk for SD and remained significant after age-adjusted analysis (HR 4.97; 95%CI: 1.39-17.7; P = 0.01). In patients with AL SD rate at 2 years was 8.0% (CI95% 4.7-13.2%). Previous history of cerebrovascular accident (CVA) and betablockers were both associated with an increased risk of SD. Both factors remained significant after age-adjusted multivariate: betablockers (HR 4.75; 95%CI: 1.57-14.4; P = 0.006) and previous history of CVA (HR 3.71; 95%CI: 1.15-11.9; P = 0.03).

Summary & Conclusion: SD is a frequent complication in patients with CA, particularly in those with AL. CVA and betablockers were both associated with SD in AL whereas implantation of PPM was the only predictor if SD found in ATTR. Our findings would be useful when designing strategies to prevent SD in CA patients.

	ATTR (569)	AL (215)
Age (years)	74.1 (12.1)	64.5 (10.8)
Male sex	469 (82.4%)	132 (60.9%)
ATTRv	155 (27.2%)	-
Comorbidities		
Atrial fibrillation	281 (50.0%)	32 (15.5%)
Stroke	42 (7.5%)	21 (10.2%)
CKD	11 (1.9%)	19 (8.8%)
Polyneuropathy	154 (30.0%)	42 (21.5%)
Previous syncope	54 (9.7%)	22 (10.7%)
Devices		
Pacemaker	69 (12.1%)	11 (5.1%)
ICD	12 (2.1%)	0 (0%)
ECG		
PR (ms)	200.7 (44.4)	181.3 (37.1)
Low voltages	129 (27.9%)	115 (55.8%)
AVB		
Normal	208 (57.1)	126 (68.5%)
1st degree AVB	151 (41.5%)	56 (30.4%)
2nd degree AVB	3 (0.8%)	1 (0.5%)
Complete AVB	2 (0.6%)	1 (0.5%)
Ventricular conduction abnormalities		
Absence	258 (46.5%)	132 (65.0%)
LBBB	53 (9.6%)	3 (1.5%)
RBBB	76 (13.7%)	16 (7.9%)

Incomplete BBB	103 (18.6%)	45 (22.2%)
Paced	48 (8.7%)	6 (3.0%)
RBBB + LAB	17 (3.1%)	1 (0.5%)
Echocardiography		
SWT (mm)	17.8 (3.3)	16.5 (2.9)
LVEF (%)	55.3 (12.1)	55.9 (12.8)
LA diameter (mm)	46.3 (6.9)	44.2 (6.8)
Pharmacological treatment (Prior to 1st visit)		
Betablockers	229 (41.9%)	57 (28.1%)
Calcium antagonist	28 (5.1%)	5 (2.5%)
Digoxin	13 (2.4%)	2 (1%)
Amiodarone	22 (4.0%)	9 (4.4%)

Figure 1: Table with baseline characteristics. AVB: Auriculoventricular block; ATTRv. Hereditary transthyretin amyloidosis, BBB: bundle branch block; CKD: Chronic kidney disease; ICD: Implantable cardioverter defibrillator; LA: Left atrium; LAB: Left anterior block; LBBB: Left bundle branch block; LVEF: Left ventricular ejection fraction; RBBB: Right bundle branch block; SWT: Septal wall thickness.

Figure 2: Kaplan-Meier curves for sudden death according to main predictors. A: SD in ATTR according to previous pacemaker implantation. B. SD in AL according to previous cardiovascular accident. C. SD in AL according to treatment with betablockers**.** BB: Betablockers; HR: Hazard ratio; PPM: Pacemaker; SD Sudden death;

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Clonal features affect survival of patients with non-cardiac light chain (AL) amyloidosis: a European study of 386 patients.

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Background: The presence and severity of heart involvement is the most important prognostic determinant in AL amyloidosis. However, in approximately 15% of patients the heart is not involved at diagnosis. Mayo Clinic investigators[1] classified these patients as Stage I, having NT-proBNP<332 ng/L and cardiac troponin (cTn) I <0.1 ng/mL or cTnT <0.035 ng/L. Researchers from the NAC-UK[2] observed that a NT-proBNP level within the reference rage (<152 ng/L) could identify stage I patients with a significantly better survival.

Objective: to identify variables predicting outcome in Stage I patients from two European centers.

Material & Methods: Pavia and Heidelberg prospectively maintained databases were searched for Stage I patient (diagnosed from 2004 to 2018). Patients with overt multiple myeloma and those who did not receive chemotherapy were excluded. Pavia patients were used as the testing set and Heidelberg as the validation set. Overall survival (OS) from diagnosis and hematologic event-free survival [HemEFS, defined as a hematologic progression[3], the switch to a different regimen due to insufficient treatment response and death were classified as event] were considered. Cut-offs of variables predicting survival were identified by ROC analyses based on death at 24 months.

Results: The Pavia cohort comprised 201 (15% of all patients with AL amyloidosis diagnosed in the study period) and the Heidelberg cohort 185 (10.4%). Clinical data are listed in the Table. Median OS was 11 years in both groups (with 93% of patients surviving 1 year and 72% surviving 5 years) in the Pavia cohort, and 92% of patients surviving 1 year and 74% surviving 5 years in Heidelberg patients (P=0.448). The median follow-up of living patients was 111 and 91 months, respectively. Median HemEFS was 24 months in the Pavia cohort (Hem-events N=153) and 38 months in the Heidelberg cohort (Hem-events N=137) (P=0.397).

The only variable consistently predicting OS in the two cohorts was dFLC (best cut-off 100 mg/L). A baseline dFLC of <100 mg/L was associated with better OS both in the Pavia (with 95% vs. 91% of patients surviving 1 year and 76% vs. 63% surviving 5 years, P=0.014) and in the German cohorts (with 93% vs. 89% of patients surviving 1 year and 80% vs. 72% surviving 5 years, P=0.031). The presence of liver involvement was a predictor of prognosis only in the Pavia series while in the Heidelberg group it was not. NT-proBNP values were not able to discriminate patients with a different outcome in both the groups.

Both BMPC >20% and dFLC >100 mg/L were able to predict a shorter Hem-EFS in both groups. In the overall population, receiving ASCT (n=54) was associated with a prolonged survival. Amongst patients who did not receive transplant, those who would have been eligible according to the ISA guidelines (n=301) also enjoyed a prolonged survival (with 93% vs 85% surviving at 12 months, P<0.001). In transplant eligible patients, actually receiving ASCT was not associated with a survival advantage (P=0.349) with the exception of the subgroup of patients with elevated involved ratio (n=41, with 95% vs 83% surviving at 12 months, P=0.005).

Summary & Conclusion: Stage I patients generally enjoyed a long survival with ¾ of subjects alive at 5 years. In our two patients groups, dFLC>100 at diagnosis was the only variable able to stratify survival and HemEFS. Eligibility to ASCT also favourably affect prognosis. Stem cell transplant grants OS advantage in eligible patients with involved/uninvolved ratio >100.

Table 1. Patients' characteristics.

Variable	Italy (N=201)	Germany (N=185)	P
Age, years	61 (54-69)	60 (53-67)	0.084
Male sex	113 (56)	118 (59)	0.131
Organ involvement			
Kidney	165 (82)	135 (72)	0.003
Liver	26 (13)	39 (21)	0.034
≥2 organs	52 (26)	110 (60)	<0.001
Renal Stage			0.024
I	76 (38)	91 (49)	
II	111 (55)	78 (42)	
III	14 (7)	16 (9)	
NT-proBNP, ng/L	151 (130-170)	156 (97-243)	0.452
eGFR, mL/min	75 (57-93)	84 (66-99)	<0.001
Proteinuria, g/24 hour	5.2 (1.4-8.1)	4.5 (1.0-8.6)	0.413
ALP, times u.r.l.	0.53 (0.41-0.73)	0.61 (0.49-0.91)	<0.001
dFLC, mg/L	64 (21-223)	85 (35-240)	0.079
dFLC >50 mg/L	116 (57)	121 (65)	0.122
dFLC >100 mg/L	78 (39)	78 (42)	0.504
Involved light-chain type: λ	157 (78)	139 (77)	0.492
Bone marrow plasma cells, %	10 (5-15)	9 (5-15)	0.937
Ratio involved/uninvolved FLC >100	14 (7)	29 (16)	0.006
Melphalan-dexamethasone	46 (23)	40 (22)	0.768
ASCT	15 (7)	49 (26)	<0.001
Bortezomib based	111 (55)	74 (40)	0.002
Others	29 (15)	22 (12)	0.468

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Light chain deposition disease: an international study in 523 patients.

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Background: Light chain deposition disease is a MGRS, defined by the presence of non-amyloid deposits of monoclonal light chain (mostly kappa) in the kidney and other organs. Different groups expolored the outcome of this disease[1,2,3]. However, specific staging systems and response criteria are lacking.

Objective: To define the natural history of LCDD, evaluate possible prognostic factors at diagnosis and search for specific response criteria in a large, unselected population.

Material & Methods: Patients with biopsy-proven LCDD from 13 centers were included. Response to 1st-line therapy was assessed 6 months after treatment initiation. Renal survival (RS) was defined as time from diagnosis to dialysis or last follow-up. Patients who died without requiring dialysis were censored at the time of death. Patients with end stage renal disease (ESRD) at diagnosis (estimated glomerular filtration rate, eGFR<15 mL/min) were excluded from the analysis of factors predicting RS. The cutoffs of baseline variables, as well as the cutoffs measured at response, best predicting RS at 24 months were identified by means of Receiver Operator Characteristics (ROC) analyses. Patients who survived 6 months were evaluable for response and were randomly divided in two groups (testing and validation cohorts) with 2/3 and 1/3 of patients (n=247 and n=124), respectively.

Results: 523 patients were included (diagnosed from 1992 to 2020). Baseline data are reported in Table 1. Median RS was not reached with 70 patients requiring dialysis, after a median follow-up of living patients of 4.5 years. Patients with ESRD at diagnosis were 206 (39%) [114 (22%) on dialysis and 92 (17%) with eGFR <15 mL/min] and concomitant overt multiple myeloma was reported in 157 (30%). The cutoffs best predictin RS were >2 g/24h for proteinuria [HR 1.44 (95%CI 1.07-1.93) P=0.013] and 30 mL/min for eGFR [HR 1.75 (95%CI 1.09-2.82) P=0.02]. The only other variable favourably predicting RS was receiving bortezomib based therapy [HR 0.49 (95% CI 0.28-0.83) P=0.009]. 139 patients died and the median OS was 13 years. By univariate analysis, concomitant MM and ESRD were associated with short OS [HR 1.70 (95% CI 1.19, 2.41), P=0.003 and HR 1.53 (95% CI 1.10, 2.14) P=0.011)] respectively]. Lastly, we analyzed candidate hematologic [percent changes and absolute value of dFLC and complete response (CR) per ISA criteria] and renal response criteria (percent changes in proteinuria) at 6 months. The cutoffs best predicting RS were 50% decrease in proteinuria from baseline and a absolute dFLC level <30 mg/L at 6 months or CR. Hematologic response was defined as either dFLC <30 mg/L or CR and renal response as a >50% decrease in proteinuria. Hematologic and renal response criteria consistently and signficantly predicted RS in the testing and validation cohorts (Fig. 1 A-D). Hematologic response also discriminated patients with better OS in both cohorts (Fig. 1 E-F). Hematologic response maintained its discriminated ability both in patients with and without MM. No difference in OS was noted between patients who obtained dFLC <30 mg/L and those who obtained CR according to ISA criteria (both in patients with or without MM).

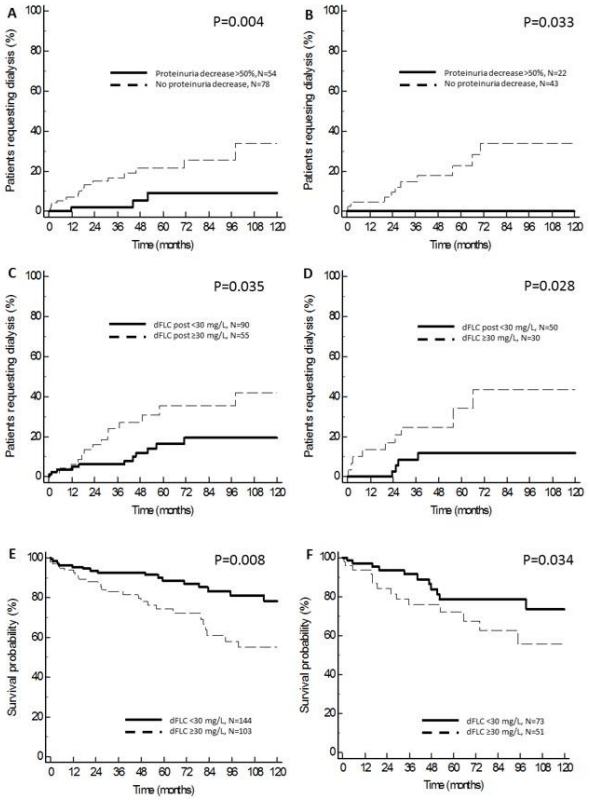
Summary & Conclusion: In this large international cohort, 39% of patients with LCDD presented with ESRD at diagnosis and this was associated with shorter OS indicating the need of early diagnosis. Approximately 1/3 had concomitant MM that was also associated with short survival. We propose criteria for hematologic and organ response that are able to predict renal and overall survival outcomes.

Table. Baseline data

Variables	N (%) – median (IQR)
Age, years	59 (49, 66)
Sex, male	336 (64)
Time of diagnosis: 1992-2000 / 2001-2004 2005-2008 / 2009-2012 2013-2016 / 2017-2020	 25 (4.8) / 50 (9.5) 100 (19.1) / 121 (23.1) 128 (24.5) / 99 (19)
Country: USA / Italy / UK France / Greece / Germany / Canada	 207 (40) / 85 (16) / 78 (15) 67 (13) / 37 (7) / 30 (5.5) / 19 (3.5)
Concomitant cast nephropathy	33 (6)
Organ involved: Kidney / Heart / Liver	 518 (99) / 76 (14) / 43 (8)
Proteinuria, g/24h	2.3 (0.7, 4.8)
eGFR, mL/min (N=317)	30 (21, 44)
CKD stages 1 / 2 / 3 4 / 5 / Dialysis at diagnosis	 13 (2.5) / 28 (5.5) / 118 (22.5) 158 (30) / 92 (17.5) / 93 (18)
dFLC, mg/L	276 (71, 915)
iFLC, mg/L	312 (97, 914)
Kappa:lambda	425 (77) : 98 (23)
Bone marrow plasma cells, %	12 (6, 25)
Treatment Type (1st line) Oral Melphalan / Bortezomib based IMIDs / ASCT / HD-DEX / VAD Rituximab-based / Oral cyclo	 44 (8) / 307 (58) 33 (6) / 27 (5) / 29 (5.5) / 17 (3) 23 (4) / 13 (2)

eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease, dFLC, difference between involved and uninvolved free light chains, iFLC, involved free light chains, MC, monoclonal component.

Figure 1: Renal survival according to decrease in proteinuria >50% from baseline (A and B), dFLC <30 mg/L at best response (C and D) (6 months landmark analysis). Overall survival calculated 6 months from diagnosis according to dFLC <30 mg/L at best response (E and F) (6 months landmark analysis). [Testing cohort: A, C and D; validation cohort: B, D and F]



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Elotuzumab in combination with IMIDS for AL amyloidosis patients with relapsed/refractory plasma cell dyscrasia and advanced organ involvement

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Background: Monoclonal antibodies evolve as promising option to clear malignant plasma cells in immunoglobulin light chain (AL) amyloidosis patients. The CD38-targeting IgG1-k antibody daratumumab showed an impressive hematologic response and a good safety profile in heavily pretreated AL amyloidosis patients[1–4]. The immunostimulatory monoclonal antibody elotuzumab, targeting a signaling lymphocytic activation molecule F7 (SLAMF7), has been approved for patients with relapsed/refractory multiple myeloma in combination with lenalidomide or pomalidomide (IMIDs) and dexamethasone. Apart from case reports, so far no data about the safety and effectiveness of elotuzumab in AL patients have been reported.

Objective: To evaluate the safety and effectiveness of elotuzumab administration in combination with pomalidomide or lenalidomide in AL patients with relapsed/refractory plasma cell dyscrasia.

Material & Methods: We retrospectively analyzed medical records of 26 AL patients with reported elotuzumab administrations that were regularly seen in our Amyloidosis centre and had sufficient follow-up data. All patients gave informed written consent for data analysis and approval was granted by the Ethics Committee of the University of Heidelberg. Our standard protocol for elotuzumab administration is 10 mg/kgBW weekly in the first 2 cycles followed by biweekly infusions afterwards. IMIDs were given from day 1 – 21 in a 28 day cycle. Dexamethasone was given once a week. Hematologic and organ response criteria were applied based on current consensus criteria and validated refinements[5–10].

Results: Patient baseline characteristics at initial diagnosis and at start of elotuzumab are summarized in Table 1. Patients received a median of 3 prior treatment lines, including proteasome inhibitors (92 %), IMIDs (89 %) and daratumumab (89 %). In the majoriy of cases, the last refractory therapy line was daratumumab (58 %). Elotuzumab administration was combined with pomalidomide in 20 cases (77 %) and lenalidomide in 6 cases. The median [range] dose of pomalidomide and lenalidomide were 3.0 [2.0 – 4.0] mg/day and 7.5 [2.5 – 15.0] mg/day, respectively. During a median follow-up of 19 months, a median of 5 [1 – 27] elotuzumab cycles were administered, with 3 patients continuing elotuzumab therapy at time of last follow-up. Elotuzumab administrations were discontinued in 23 patients due to insufficient hematologic response (13), infection (3), diarrhea (2), clinical deterioration (2), patient request (2) and death (1). The overall hematologic response rate was 29 % after 3 months (very good haematological response 17%) and 36 % after 6 months (very good haematological response 24%). The median overall survival was not reached and the median haematological event-free survival was 26 months (Figure 1). One patient became dialysis-dependent during the therapy period. Two patients showed organ response after 6 months, both of which achieved at least a VGPR.

Summary & Conclusion: To our knowledge, this is the first report about safety and effectiveness of elotuzumab in AL patients. The patient cohort was highly refractory to previous established anti-clonal therapies, including daratumumab, and showed advanced organ involvement. Nevertheless, elotuzumab therapy in combination with lenalidomide or pomalidomide was tolerated relatively well, showed reasonable response rates and even offered the chance for organ response to this challenging patient cohort.

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Table 1. Patient characteristics

Parameter	n= 26
At initial diagnosis	
Age [years]	54.50 [38.0, 77.0]
dFLC [mg/l] [m]	353.3 [10.5, 2498.2]
Gender female	13 (50.0)
LC isotype = lambda	19 (73.1)
HC isotype = A / D / G / not intact	5 (20.8) / 1 (4.2) / 11 (45.8) / 9 (34.6)
PC count, % [m]	20.0 [5.0, 45.0]
No organs involved	2 [1, 4]
Heart involved	16 (61.5)
Cardiac stage (I / II / IIIa / IIIb) * [m]	10 (50) / 8 (40) / 0 / 2 (10)
Kidney involved	15 (57.7)
Renal stage (I / II / III) †	6 (23.1) / 5 (19.2) / 4 (15.4)
Liver involved	3 (11.5)
At start of elotuzumab	
Age [years]	57.50 [42.0, 79.0]
dFLC [mg/l] [m]	120.0 [9.3, 670.8]
No organs involved	3 [1, 4]
NT-ProBNP [ng/l]	1206.50 [0.00, 23209.00]
NT-ProBNP >8500 ng/L [m]	7 (38.9)
eGFR [ml/min] [m]	45.0 [6.1, 108.1]
Proteinuria [mg/d] [m]	4.15 [0.00, 26.76]
Patients on dialysis	8 (30.8)
No previous chemotherapy lines	3 [2, 9]
Refractory to last therapy	24 (92.3)
PI exposed / refractory	24 (92.3) / 23 (88.5)
IMID exposed / refractory	23 (88.5) / 15 (57.7)
Daratumumab exposed / refractory	23 (88.5) / 22 (84.6)
Previous autologous transplant	9 (34.6)
Last refractory line daratumumab	15 (57.7)
Time from first therapy [months]	31.49 [3.48, 171.09]
Time from last therapy [months] [m]	1.02 [0.00, 70.04]

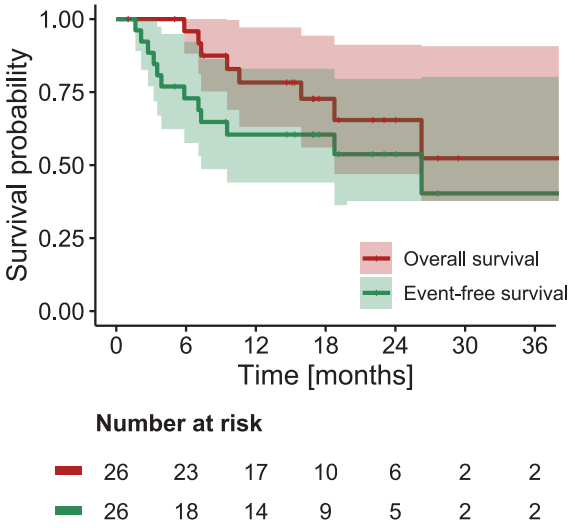


Figure 1. Kaplan-Meyer plots depicting the overall survival and hematological event-free survival for the entire cohort. Classification of event: death, therapy change or hematologic progression.

Categorical data is shown as count (% of respective total), continuous data is shown as median [range].
* "MAYO3B" staging system according to[11].
† According to[5]. Patients without kidney involvement are ignored for the calculation of fractions
[m] missing data (%): dFLC (3.8), PC count (19.2), Cardiac stage (23.1), NT-proBNP (30.8), eGFR (11.5), Proteinuria (30.8), Time from last therapy (3.8)

Background: Patients with stage IIIb AL amyloidosis (NT-pro-BNP>8,500 ng/l and troponin-T>35 ng/l) historically have dismal outcome, with a median overall survival (OS) of 4 months (Wechalekar *et al.* Blood. 2013). A study on 179 stage IIIb patients from the UK National Amyloidosis Center in the era of bortezomib and IMiD-based therapies demonstrated a 1-year OS of approximately 30% in all-comers and 70% in those achieving complete response (CR) or very good partial response (VGPR) (Manwani *et al.* Haematologica 2018). The introduction of anti-CD38 monoclonal antibody daratumumab with cyclophosphamide-bortezomib-dexamethasone (Dara-CyBorD) in the frontline setting has led to a dramatic increase in CR/VGPR rate, as seen in the ANDROMEDA-AL trial (Kastritis *et al.* N Eng J Med. 2021). However, a higher risk of grade 3/4 pneumonia and cardiac failure was seen in the daratumumab arm and the trial did not include stage IIIb patients. To our knowledge, there are no published data on clinical efficacy or safety of daratumumab-based frontline regimen in patients with newly diagnosed stage IIIb AL amyloidosis.

Objective: The objective of our study was to evaluate the safety and efficacy of daratumumab-based frontline therapy in patients with stage IIIb AL amyloidosis.

Material & Methods: We performed a retrospective cohort study on stage IIIb AL patients treated with Dara-CyBorD frontline therapy at New York Presbyterian-Columbia and Cornell hospitals.

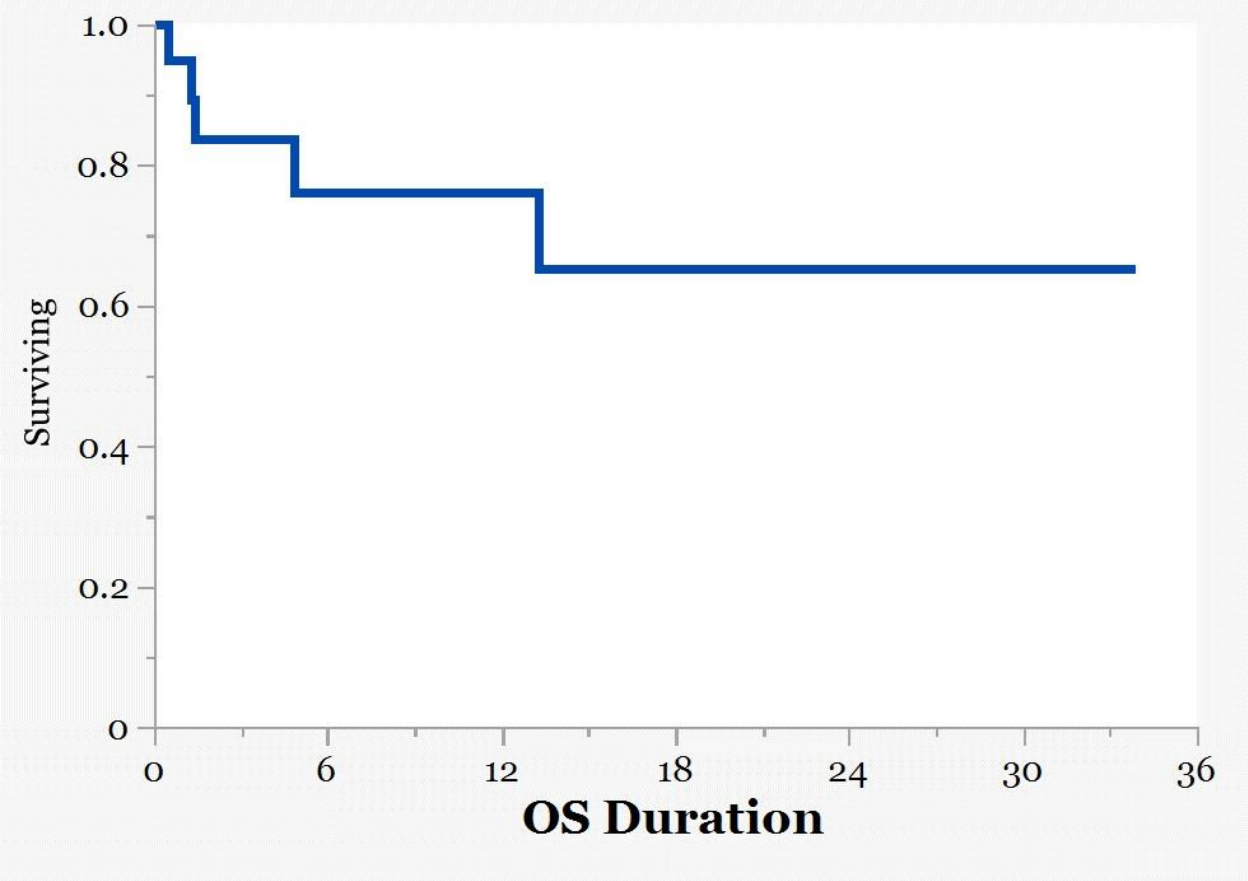
Results & Discussion: A total of 20 patients with stage IIIb AL amyloidosis were identified, among which, two were excluded due to being enrolled in a clinical trial of fibril-directed therapy. Among 18 remaining patients, important baseline characteristics were following: median age at diagnosis, 64 years (range, 40-81); 67% males; 78% lambda light chain isotype; median 24-hour urine protein, 0.78 g (range, 0.12-16.13); median dFLC, 59 mg/dl (range, 13-907); median NT-pro-BNP, 12,369 pg/ml (range, 8,565-70,000); median high-sensitivity troponin-T, 146 ng/l (range, 50-405); median interventricular septal thickness, 1.7 cm (range, 1.1-1.9); median systolic BP 103 mmHg (range, 71-136); median ejection fraction 48% (range, 23-65); and median of 2 organs involved (range, 2-4). Liver involvement was seen in 5/18, kidney in 13/18, and GI tract in 4/18 patients. None of the patients had received autologous transplant as frontline therapy.

The median follow-up of surviving patients is 10 months (range; 1-34). Regarding hematologic response, 12 have achieved CR, 2 VGPR, and 4 PR at latest follow-up, with a ≥VGPR rate of 78%. Organ response in heart was seen in 8/18 patients (44%), all of whom had ≥VGPR as hematologic response. Among 8 evaluable patients, only 1 achieved renal organ response. Five patients had died at most recent follow-up, with an estimated 12-month OS of 73% (95% CI, 45-90%) (Figure 1).

A total of 4/18 patients (22%) had ≥grade 3 infection, include VRE bacteremia (n=1), pneumonia (n=1), and CMV viremia (n=2). The cause of death in 5 patients were sudden cardiac death (n=2), multi-organ failure (n=2), and progressive cardiac deterioration (n=1), none of which were deemed treatment-related.

Summary & Conclusions: To our knowledge, this is the first study showing outcomes with Dara-CyBorD frontline therapy in stage IIIb AL patients. The estimated 1-year OS of 73% in our dataset compares favorably to historical controls (1-year OS of 30% in the UK dataset), likely due to increased depth of hematologic response. Close monitoring for infectious complications is warranted with daratumumab-based therapy.

Figures: Figure 1 attached (Kaplan-Meier curve for overall survival)



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Collagen associated with AL amyloid inhibits fibril phagocytosis - Collagen degradation renders amyloid sensitive to uptake by the innate immune system

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Background: Extracellular deposition of amyloid fibrils results in the destruction of organ architecture and compromised organ function. The current standard of care for light chain-associated (AL) amyloidosis is directed towards reducing synthesis of amyloidogenic precursor proteins and thereby preventing further amyloid deposition. In rare instances, amyloid deposits may resolve from organs in the context of a sustained complete hematologic remission. However, tissue amyloid is remarkably and incomprehensibly disregarded by cells of the innate immune system (phagocytes). Consequently, prognosis remains poor in patients with AL due to the persistence of pathologic amyloid deposits. We have hypothesized that certain of the non-fibrillar constituents of amyloid may hinder recognition of the amyloid fibrils and prevent their clearance by phagocytic macrophages.

Objective: The goal of this study was to interrogate the effect of non-fibrillar amyloid-associated proteins on macrophage phagocytosis of amyloid *in vitro* and subsequent clearance of the material in an AL amyloidoma mouse model.

Material & Methods: Synthetic rVλ6WIL fibrils were labeled with the pH-sensitive fluorophore pHrodo red to allow quantitation of phagocytosis by macrophages. Fibrils were co-incubated with albumin or amyloid accessory proteins - serum amyloid P component, apolipoprotein E or collagen, and fibril uptake by murine Raw264.7 or human THP-1 M0 cells was quantified by flow cytometry. Histological and immunohistochemical staining of collagen in AL and ATTR-containing tissue sections was used to assess the spatial relationship between collagen and amyloid deposits. Collagen degradation was performed using highly purified *Clostridium histolyticum* collagenase. The impact of collagen degradation on amyloid phagocytosis *in vitro* was determined by flow cytometry and *in vivo* by optical imaging following subcutaneous (SQ) injection of Dylight800/pHrodo red-labeled amyloidomas into NU/NU mice.

Results: Collagen, but not other proteins commonly associated with amyloid, i.e. serum amyloid P component or apolipoprotein E, inhibited immune cell recognition and subsequent phagocytosis of synthetic rVλ6WIL fibrils. Evaluation of patient-derived tissue samples indicated that tissue amyloid deposits contain abundant collagen. Collagenase treatment successfully overcame collagen-mediated inhibition of rVλ6WIL fibril phagocytosis. Analysis of 15 patient-derived AL and ATTR amyloid extracts revealed that targeted collagen degradation significantly enhanced amyloid phagocytosis by both human and murine macrophages *in vitro*. Furthermore, collagenase treatment of ALλ (CLA) amyloid extract significantly increased amyloid phagocytosis and clearance compared to untreated amyloid following SQ administration into NU/NU mice. The enhanced clearance of collagenase treated amyloid extract in mice coincided with an increased immune cell milieu in the amyloid lesion that included multinucleated giant cells and polymorphonuclear cells.

Summary & Conclusion: Collagen is one of several extracellular matrix components that is associated with amyloid deposits *in vivo*. The addition of collagen to AL amyloid-like fibrils inhibits their otherwise robust recognition and uptake by macrophages *in vitro*. Thus, amyloid-associated collagen may contribute to amyloids' ability to evade recognition by the innate immune system. We hypothesize that collagen may serve as a "don't eat me" signal that protects systemic amyloidosis from clearance. Treatment with collagenase reversed the protective effect of collagen and resulted in efficient uptake of human amyloid extracts by macrophages; thus, targeted collagen degradation in amyloid could facilitate the removal of tissue amyloid deposits in patients.

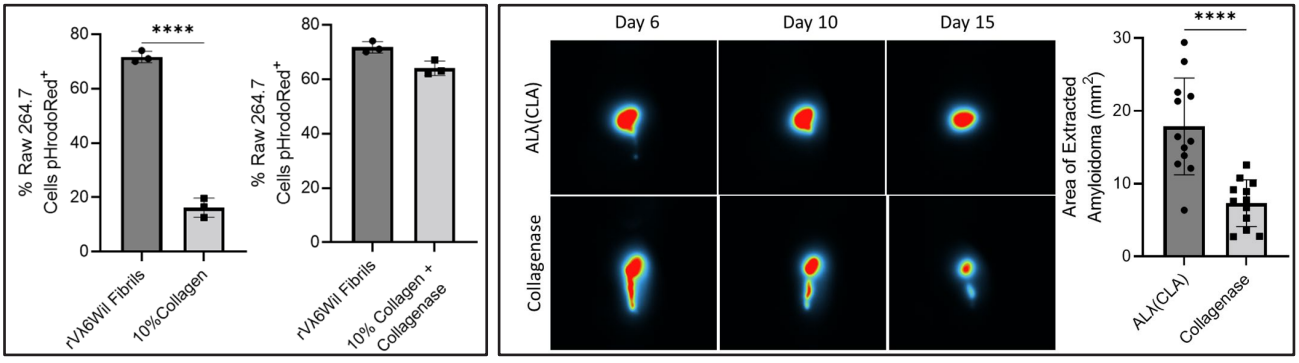

Figure 1.: Collagen (2 µg) was incubated with pHrodo red labeled rVλ6WIL fibrils (20 µg) for 1 hour, and Raw264.7 macrophage phagocytosis was determined by flow cytometry. Collagen-coated rVλ6WIL fibrils were treated with purified *Clostridium histolyticum* collagenase for 1 hour. Subsequent phagocytosis of treated fibrils was determined by flow cytometry. ****P<0.0001.

Figure 2.: Pretreatment of Dylight800 ALλ (CLA) with collagenase enhances the rate of amyloidoma clearance and fibril uptake *in vivo*. ALλ (CLA) was treated with either PBS or with purified collagenase overnight. Following incubation, samples were washed once with PBS and implanted into mice. Representative optical images from days 6, 10, and 13 of DyLight800 fluorescence from mice treated with either collagenase or vehicle control are shown. Amyloid extract area was determined using ImageJ. Data is representative of n=2 experiments, 12 animals per group per replicate. ****P<0.0001.

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In-vitro ultrasonic assay indicates importance of extracellular chaperon-like effect of serum albumin to protect dialysis patients from dialysis-related amyloidosis.

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Background: Dialysis-related amyloidosis (DRA) is caused by amyloid fibrils composed of β2-microglobulin (β2m) in long-term dialysis patients. Previous studies revealed that primary and secondary risk factors for the onset of DRA are aberrant high serum β2m concentration and long dialysis vintage, respectively. However, these two risk factors do not always cause DRA, indicating the existence of potential risk factors relating to the onset of DRA. In this study, since unveiling unknown risk factors contributes to the prevention of the onset of DRA, we investigated the potential risk factors by in-vitro biophysical assay to sera collected from dialysis patients using an originally developed ultrasonic instrument, HANABI-2000.

Objective: To suggest possible risk factors that control the onset of DRA.

Material & Methods: The human sera were collected from dialysis patients (n = 58) and non-dialysis controls (n = 30). Here, serum samples were collected on 28 dialysis patients immediately before and after a 5-hour maintenance dialysis treatment. The collected sera were mixed with recombinant β2m monomer solution. The mixed sample solutions were dispensed into a 96-well microplate, and then, the plate was set to the HANABI-2000 for the ultrasonic assay. During the assay, each sample solution was measured a time course of thioflavin-T (ThT) fluorescence intensity to monitor the kinetics of β2m amyloid fibril formation. The effects of sera on β2m fibril formation were evaluated in terms of the lag time and the maximum intensity of resultant ThT time-course curves, which correspond to formation rate of amyloid fibrils and amount of formed fibrils. To further discuss the results obtained, biophysical analyses, including transmission electron microscopic observation, circular dichroism spectroscopy, nuclear magnetic resonance spectroscopy, and quartz crystal microbalance measurement, were conducted.

Results: Experimental results showed that serum addition inhibits amyloid fibril formation of β2m in a dose-dependent manner. The degree of inhibition significantly varied among different patient groups; sera collected from non-dialysis controls showed stronger inhibition on amyloid fibril formation of β2m monomer than those collected from dialysis patients; within identical dialysis patients, sera collected immediately before maintenance dialysis treatment showed weaker inhibition than those collected after the treatment. Statistical analysis suggested that serum albumin is one of the important inhibition factors in the serum milieu, indicating that a decrease in the serum concentration of albumin can be a risk factor regarding the onset of DRA. Further biophysical analysis indicated that an extracellular chaperon-like effect of serum albumin inhibits amyloid fibril formation of β2m monomer in a serum milieu.

Summary & Conclusion: Our in-vitro ultrasonic assay indicated the importance of serum albumin as an inhibitor against amyloid fibril formation in a serum milieu. Because serum concentrations of serum albumin basically decrease in long-term dialysis patients, keeping higher serum albumin levels in dialysis patients will decrease the onset risk of DRA.

Support & Funding: The authors declare no other potential competing financial interests.

Dissecting FAM46C-dependent tuning of antibody secretion in systemic AL amyloidosis.

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Background: Plasma cells (PCs) are the terminal effector of B cell differentiation in charge of immunoglobulin (Ig) secretion. Such intensive effort requires rapid endoplasmic reticulum (ER) expansion and concerted expression of the machinery required for protein translocation, folding and trafficking. Moreover, the by-products of massive Ig production cause an exquisite dependence of PCs on pathways that maintain proteostasis with a fundamental clinical consequence: the extraordinary efficacy of proteasome inhibitors against multiple myeloma (MM) and systemic light chain amyloidosis (AL). Notably, a key player directly responsible for the proteomic reshaping of PC differentiation, is the non-canonical poly(A)polymerase FAM46C. Indeed, FAM46C is induced during PC differentiation to stabilize a number of mRNAs encoding Igs and ER-targeted proteins, thus sustaining humoral immunity [1,2]. Interestingly, FAM46C is mutated in up to 20% MM patients suggesting that MM may have a selective pressure in losing FAM46C to reduce Ig secretion to favor cell survival and growth [3,4]. In keeping with this, exogenous re-expression of FAM46C in mutated MM cells raised Ig secretory capacity beyond sustainability, causing ATP shortage, ROS accumulation and apoptosis [5].
Objective: Our project aims to discover the precise molecular circuits, centered of FAM46C, regulating PC secretory capacity in efficient coordination with degradative pathways, to discover new vulnerabilities of PCs and novel therapeutic opportunities against AL.
Material & Methods: To clarify the molecular mechanisms underlying FAM46C effects on Ig secretion we coupled protein biochemistry, imaging and unbiased proteomic assays in *in vitro* and *in vivo* models of AL.
Results: Thanks to the definition of its interactome, we disclosed that FAM46C specificity for the secretory apparatus resides in its localization at the ER membrane through its interaction with the FNDC3 proteins. As a result, FAM46C concertedly promotes the expression of ER and Golgi proteins, potently expanding the secretory apparatus and amyloidogenic light chain secretion in both *in vitro* and *in vivo* models. Moreover, we found that FAM46C role in PC goes beyond the mRNA stabilizing activity, indicating the existence of an integrated network coordinating ribosome biogenesis, protein translation and ER expansion. Indeed, among the few significantly FAM46C modulated proteins previously not involved in protein secretion, we found the methyltransferase WBSCR22 and its partner TRMT112, two highly conserved factors involved in ribosome and tRNA biogenesis and processing. Based on these data, we hypothesize that FAM46-dependent ER expansion is coupled with the induction of MTases promoting ribosome biogenesis and optimizing protein translation, in order to harmonize protein secretion with cellular homeostasis.
Summary & Conclusion: Altogether our data disclose a novel molecular circuit coordinating Ig synthesis and secretion with protein homeostasis that may be exploited to design new therapeutic strategies to reduce pathogenic light chain production, to decrease AL cell viability and to improve proteasome inhibitors efficacy.

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Development of novel human chimeric antigen receptor-macrophages (CAR-M) as a potential therapeutic for amyloid clearance

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Background: Amyloidosis is a protein misfolding disorder characterized by the abnormal deposition of protein fibrils in tissues and vital organs. Current treatment options rely on reducing new amyloid formation using anti-plasma cell therapy for AL or TTR silencing or stabilizing therapy for ATTR. In recent years, chimeric antigen receptor (CAR) technology has been developed for the ligand-specific activation of genetically-engineered immune cells, notably anti-tumor T-cells (CAR-T). However, CAR-engineered macrophages (CAR-M) are also being developed to target solid tumors (1). We therefore hypothesized that an amyloid-reactive CAR-M could be developed to facilitate the clearance of tissue amyloid by exploiting the pan-amyloid reactivity of peptides such as p5, which binds via multivalent electrostatic interactions to heparan sulfate glycosaminoglycans and fibrils ubiquitous to amyloid (2,3). Such a reagent would be capable of specifically binding amyloid and facilitating its clearance by CAR-stimulated phagocytosis.

Objective: The goal of this study was to generate CAR-M that were capable of recognizing and enhancing phagocytosis of amyloid. To facilitate this, the novel CAR-M have been developed using the pan-amyloid binding peptide p5 in addition to phagocytosis-activating cytoplasmic elements.

Material & Methods: Genes expressing the amyloid-binding, pro-phagocytosis CAR (Fig. 1), or negative control CAR, were cloned into a lentiviral gene expression vector, which was transfected into HEK-293T cells to package and generate lentiviral particles. Human THP-1 monocytes were transduced using the viral supernatant containing ~5-10 multiplicity of infection (MOI) lentivirus, with selection of stably transduced cells performed by sustained exposure to 1 µg/mL puromycin. The production of CAR by the THP-1 cells was assessed by immunofluorescent staining using 1 µg/mL goat anti-human IgG AlexaFluor 488 (Fcγ fragment-specific antibody) and analyzed by flow cytometry. Peptide p5 binds heparin, therefore, to assess surface expression of CAR, binding of 5-chloromethylfluorescein diacetate (CMFDA)-labelled CAR-M was performed using heparin-coated plates. Adhesion to the wells was measured by fluorescence micropscopy (Keyence BZ X800 V 1.3.1). Phagocytosis of pHrodo red-labelled synthetic amyloid-like rVλ6WIL fibrils and human amyloid extracts (ALκ, ALλ, ATTRwt and ATTRv) was studied using phorbol 12-myristate 13-acetate (PMA)-activated THP-1 CAR-M cells alone, or in combination with an anti-amyloid opsonin and human complement. Phagocytosis efficiency was quantified by measuring the increased fluorescence emission of pHrodo red as the substrates entered the acidified phagolysosome of the macrophage using fluorescence microscopy and image segmentation (Image Pro Premier V 9.0).

Results: THP-1 cells were successfully transduced with lentiviral particles carrying the amyloid-reactive CAR construct (Fig. 1). Pools of transduced cells and single cell clones were generated that exhibited positive cytoplasmic and cell surface staining of the human Fcγ, indicating expression and insertion of the CAR into the plasma membrane in the correct orientation (Fig. 2A and B). Heparin binding was shown to be greater for CAR-M THP-1 cells as compared to control CAR-M and native THP-1, confirming the plasma membrane insertion of the receptor and appropriate orientation in the membrane. PMA-activated CAR-M cells phagocytosed pHrodo-labelled synthetic rVλ6WIL fibrils and human AL and ATTR amyloid extracts significantly better than native THP-1 cells as evidenced by the enhanced pHrodo red fluorescence emission (Fig. 2C), with more than a two-fold increase in phagocytosis compared to control THP-1 cells for most substrates. This effect was further significantly enhanced by the presence of an amyloid-reactive opsonin (antibody-peptide fusion) and 20% human serum as a source of complement.

Summary & Conclusion: Human CAR-M cells can be generated with the pan-amyloid-reactive peptide, p5, as the target recognition element. Expression of these receptors results in appropriate plasma membrane insertion and enhanced phagocytosis of amyloid substrates as compared to native macrophages. This effect can be further enhanced by opsonizing the amyloid with an antibody and in the presence of human complement. This novel system may serve as an adjunct to anti-amyloid monoclonal antibodies for the clearance of tissue amyloid.

Figure 1

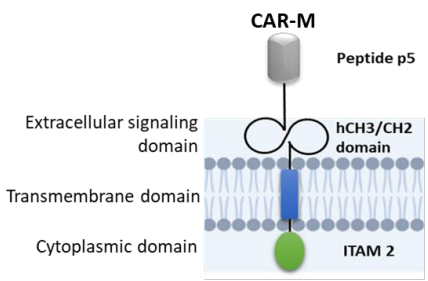

Figure 2

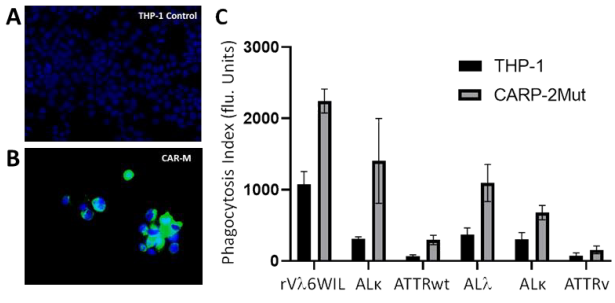

Figure 1.: Schematic showing the functional characteristics of the amyloid-binding CAR. Each construct was designed to include the peptide p5 as the target recognition element and hIgG CH3/CH2 region as an extracellular domain. The CD8 transmembrane region is followed at the C-terminal by two ITAM-containing intracellular signal transduction domains.

Figure 2.: Cell surface expression of CAR in human THP-1 enhanced phagocytosis of amyloid substrates. (A) Untransduced (control) THP-1 cells exhibit no binding of Alexafluor 488-conjugated anti-human Fcγ. (B) In contrast, CAR-M show intense fluorescent signal when immunostained with Alexafluor 488-conjugated anti-human Fcγ, indicating the presence of CAR in the plasma membrane. (C) Phagocytosis of pHrodo red-labelled rVλ6WIL fibrils and amyloid extracts by CAR-M was significantly greater as compared to native THP-1 cells.

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Preclinical characterization of AT-02, a pan-amyloid-binding immunoglobulin-peptide fusion protein capable of inducing enhanced phagocytosis of amyloid

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Background: Clearance of tissue amyloid deposits and the restoration of organ function, or hindering further functional deterioration, are critical clinical needs for patients with systemic amyloidosis. Recruiting and stimulating the innate immune system, notably phagocytic macrophages, is a promising strategy to effect amyloid removal. The binding of immunoglobulin (IgG1) to amyloid can induce complement fixation and macrophage-mediated dissolution of amyloid (1,2). However, in general, amyloid-specific antibodies are difficult to generate due to the high concentrations of circulating amyloid precursor protein or accessory proteins that serve as the target. Morever, they are often specific for a single type of amyloid. To address this, we have generated AT-02, a humanized IgG1 incorporating the pan amyoid-binding peptide, p5R (3), at the light chain C-terminus. This reagent binds diverse types of amyloid via the peptide interactions and retains the opsonizing capabilities of the IgG1. Thus, AT-02 can serve as an immunotherapeutic for the clearance of many forms of amyloid in patients with systemic amyloidosis.

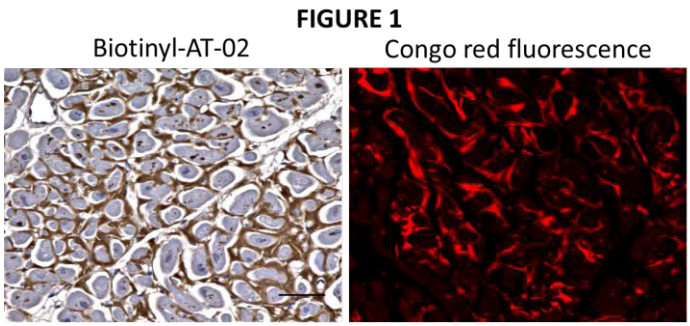
Objective: The goal of these studies was to investigate the pan-amyloid reactivity and therapeutic characteristics of AT-02. We have assessed the amyloid reactivity and the specificity of amyloid binding of AT-02 mediated by the pan-amyloid reactive peptide p5R. Additionally, we have studied the ability of the IgG1 Fc domain of AT-02 to induce phagocytosis of human amyloid extracts *in vitro* and *in vivo* .

Material & Methods: AT-02 was produced from a CHO pool using a perfusion cell culture production process, purified by Protein A, anion exchange, and cation exchange chromatographies, and characterized by mass spectrometry and SDS-capillary electrophoresis. The binding potency (EC_{50}) for amyloid-like rVλ6WIL fibrils and human AL and ATTR extracts was assessed by ELISA. Pan-amyloid reactivity was shown immunhistochemically using formalin-fixed tissues containing human AL, ATTR, and ALECT2 deposits. Phagocytosis of human AL and ATTR amyloid extracts, labeled with the pH-sensitive fluorophore pHrodo red, was studied *in vitro* using PMA-activated human THP-1 monocytes. Uptake of the amyloid extracts by activated THP-1 cells was quantified by segmentation of digital microscopy images. Induction of amyloid phagocytosis *in vivo* was performed by pretreatment of pHrodo red-labeled ALλ amyloid (2 mg) with 500 μg of AT-02 prior to subcutaneous implantation into NU/NU mice. Quantitative measurement of phagocytosis *in vivo* was performed using serial optical imaging of mice under isoflurane anesthesia.

Results: AT-02 was produced in a perfusion bioreactor to an accumulated titer of 15.5 g/L, and was purified with nearly complete retention of the p5R peptide. The potency (EC_{50}) of binding was subnanomolar for multiple types of amyloid. The EC_{50} was 0.15 nM for synthetic rVλ6WIL and Aβ(1-40) fibrils, 0.42 ± 0.12 nM (*n*=4) for ALλ and ALκ amyloid extracts, 0.18 nM for ATTRwt and 0.45 nM for ATTRv extracts. Immunohistochemical analyses revealed strong and specific reactivity with the amyloid desposits of AL, ATTR and ALECT2 in heart, kidney and spleen (Fig. 1). AT-02 co-localization was also observed with cardiac amyloid deposits in a novel murine model of AL amyloidosis. Incubation of pHrodo-red labeled ATTR and AL amyloid extracts with AT-02 up to 200 nM caused a significant dose-dependent increase in phagocytosis compared to an irrelevant hIgG1. Uptake of the amyloid by PMA-activated THP-1 cells was further significantly enhaced by the the addition of 20% (v/v) human serum as a source of complement. Pretreatment of pHrodo-red labeled ALλ amyloid extract with AT-02 resulted in significant enhancement of *in vivo* fluorescence emission associated with the uptake of the amyloid by murine macrophages (Fig. 2).

Summary & Conclusion: Clearance of amyloid is a significant clinical unmet need for patients with systemic amyloidosis. The humanized IgG1-peptide fusion, AT-02, specifically binds many forms of amyloid with subnanomolar affinity for human AL and ATTR amyloid extracts. High affinity binding of AT-02 to amyloid promotes phagocytosis of the material, which can be enhanced by the presence of human serum as a source of complement. Binding of AT-02 to amyloid can serve as a potent opsonin and induce macrophage-mediated phagocytosis, which is anticipated to lead to clearance

of tissue amyloid in patients with systemic amyloidosis.



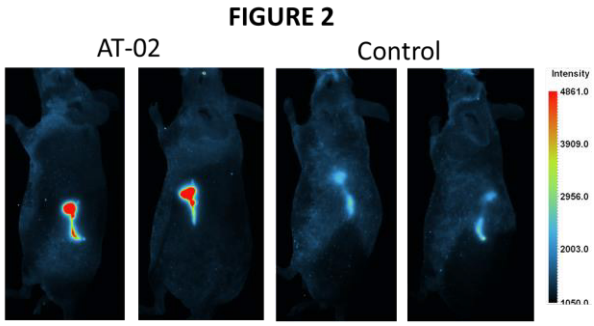


Figure 1.: Immunohistochemical staining of amyloid in the heart of a patient with ATTRv (T60A) amyloidosis. Biotinylated AT-02 intensely stained amyloid deposits (brown) around the cardiomyocytes. Amyloid was visualized in the tissue following alkaline Congo red staining and detection by fluorescent micropscopy. Bar = 50 μm (original objective mag. 40x).

Figure 2.: *In vivo* phagocytosis of pHrodo red-labeled human ALλ amyloid extract. At day 12 post injection, the fluorescence emission of acidified pHrodo red, associated with phagocytosis of the material, was significantly higher in mice following pretreatment (*n*=2 shown) of the amyloid with AT-02 as compared to control (untreated; *n*=2 shown) ALλ amyloid.

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Regulation of BCL2 family members by microRNA-9 and microRNA-181a in AL amyloidosis

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Background: Systemic light chain (AL) amyloidosis is a clonal plasma cell disorder characterized by deposition of misfolded immunoglobulin light chain products in vital organs, causing their dysfunction. MicroRNAs (miRNAs) are short, non-coding RNAs that regulate gene expression and have a role in cancer development and progression. They may be used as biomarkers to distinguish between cancer patients and healthy individuals. Moreover, miRNA-mRNA interactions may determine the molecular mechanism involved in AL amyloidosis pathogenesis and may suggest novel therapeutic approaches. To date, knowledge about miRNAs involvment in AL amyloidosis is lacking.

Objectives: to decipher specific miRNA expression profiles in AL amyloidosis compared to MM and healthy controls (HC) and to examine how miRNAs are involved in AL amyloidosis pathogenesis.

Material & Methods: miRNA and mRNA expression profiles were determined using the nCounter assay (NanoString technologies) and RNA-Seq, respectivly. Detection of potential miRNA targets, and enriched biological pathways was performed by the bioinformatics tool Ingunity Pathway Analysis (IPA). MiRNA and gene expression profiles were validated by qRT-PCR in 50 AL, 50 MM and 10 HC samples. The effect of aberrantly expressed miRNAs on potential molecular targets was analyzed in ALMC1 cells by transfecting the cells with miRNA mimic, following qRT-PCR, Western blot analysis mithochondrial depolarization assay and Annexin-PI staining.

Results: BM and plasma miRNAs were differentially expressed in AL amyloidosis compared to MM or HC. We found that the differentially expressed miRNAs and mRNA in AL patients regulates key signaling pathways related to cell cycle and anti-apoptosis mechanisms including mitochodrial dysfunction, cytokine signaling, NFkB signaling, activation of MAPK and PI3K/AKT pathways (Figure 1), all linked to cancer cell growth, proliferation and therapeutic resistance, therefore may be used as a therapeutic target. Specifically, genes related to the mitochondrial activity were upregulated in AL patients.particularly the anti-apoptotic BCL2 family genes (BCL2, MCL1, and BCL2L1).
miR-181a-5p and miR-9-5p, which regulate the above mentioned genes, were downregulated in BM samples from AL amyloidosis compared to MM patients, providing a mechanism for BCL2 family gene regulation (Figure 2).

Overexpression of these miRNAs in ALMC-1 cells led to downregulation of the BCL2 family anti-apoptotic genes and induced apoptosis by AnnexinV staining. This suggests a new mechanism for anti-apoptosis of aberrant plasma cells. Clinically, it proposes that BCL2 inhibitors, such as venetoclax, can reverse the anti-apoptosis effect of the downregulated miRNAs in AL patients. Treatment of ALMC-1 cells with venetocalx showed upregulation of those miRNAs, followed by downregulation on BCL2, MCL1 and BCL2L1 mRNA and protein levels. By performing ROC analysis, we further found that specific miRNAs may be used for the diagnosis of AL amyloidosis, and differentiate it from MM.

Summary and conclusions: We provide novel insights into the molecular mechanisms in AL amyloidosis mediated by miRNAs and the aberrant expression of oncogenic/tumor suppressor genes. The differential expression of miRNAs in AL amyloidosis may be used to understand disease pathogenesis and predict risk of progression to AL amyloidosis among patients with known plasma cell disorders. Additionally, signaling pathways involved in AL amyloidosis, mediated by miRNAs, may assist in tailoring more specific treatments.

Figure 1.

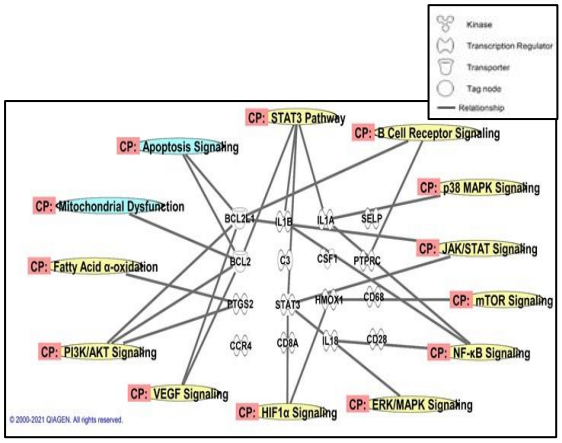

Figure 2.

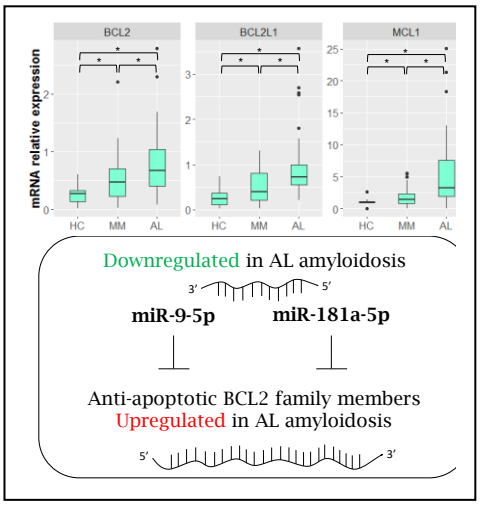

Figure 1.: Ingenuity pathway analysis showing the involvement of the key genes in regulating significant biological pathways.

Figure 2.: BCL2 family members are highly expressed in patients with AL amyloidosis and are targets of miR-9-5p and miR-181a-5p. BCL2, MCL1 and BCL2L1 expression levels measured by qRT-PCR in BM-derived CD138+ cell samples of at least 50 patients with AL amyloidosis, 50 patients with MM and 10 healthy controls (HC).

Support & Funding: This work was supported by the Israeli Society of Hematology (ISH).

Elevated fibrosis associated biomarkers in ATTR amyloidosis patients are associated with impaired cardiovascular outcome.

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Background: Amyloidosis comprises a group of diseases defined by extracellular deposition of misfolded proteins. In cardiac transthyretin amyloidosis variant (ATTRv) or wildtype (ATTRwt) transthyretin is deposited subendocardial or interstitial in the heart. The clinical course of ATTR cardiomyopathy (ATTR-CM) is highly variable from stable course with little symptoms over years to rapid progression and cardiac death within few months in other patients [1, 2]. Analysis of cardiac biopsies revealed fibrosis surrounding amyloid deposits in ATTR patients [3]. Thereby, the extent of fibrosis is associated with plasmal hsTNT and NTproBNP levels – the most distinctly established biomarkers for outcome in ATTR-CM [4].

Objective:
We therefore hypothesized that individual profibrotic responses towards the extramyocardial amyloid deposits might influence disease progression. Therefore, we extensively characterized fibrosis associated biomarkers in patients' plasma and correlate their expression with clinical outcome.

Material & Methods:
61 patients with hereditary ATTRv, 43 patients with ATTRwt and 21 healthy volunteers were included in the study. Fourteen fibrosis associated biomarkers (EN-RAGE/S110A12, IGFBP-1, 2, 3, 4 and 6, FGF-23, MMP-2, 7, 9 and 13, TIMP-2 and -4, RAGE-AGE) were analyzed using Luminex multiplex assays in patient plasma. Statistical analyses were performed using survival models and hierarchical cluster analysis to correlate biomarker levels with clinical presentation and outcome. Prespecified endpoints were cardiac decompensation (cDMP) or transplantation/death (HTX/D).

Results:
IGFBP-1, 2, 3, 4 and 6, FGF-23, MMP-2 and -7, TIMP-2 and -4 and RAGE-AGE plasma levels were significantly elevated in patients with cardiac ATTR compared to healthy controls or ATTR patients without cardiac affection ($p<0.001$). Univariate analysis revealed significant association of MMP-2, MMP-7, RAGE-AGE, IGFBP-1, IGFBP-2, FGF-23 and TIMP-2 for both endpoints, while IGFBP-3 and TIMP-4 revealed significant association with HTX/D but not cDMP. Cluster analysis identified two prognostically distinct groups of patients (high-risk HrC and low-risk cluster LrC; HR for cDMP: 11.7 [95% CI: 1.5-90.6, $p=0.019$], for HTX/D: 15.06 [95%CI: 1.99-114], $p=0.009$). Individuals assigned to HrC exhibited significantly increased myocardial wall thickness, hsTNT, and NTproBNP levels, patients were older had lower GFR, EDD and MAPSE.
Stepwise model selection of cluster membership and all fibrosis associated markers retained cluster assignment as significant feature for both endpoints.
Interestingly, interaction analysis revealed that FGF-23 and IGFBP-3 as well as RAGE/AGE and IGFBP-3 showed a positive correlation in HrC but negative correlation in LrC. FGF23 and hsTNT showed a marked

positive correlation in HrC, and only a weak positive association in LrC.

Summary & Conclusion:

Profibrotic biomarkers are elevated in ATTR patients and show strong association with outcome in ATTR-CM. Fibrosis marker derived profiles entail additional prognostic information. Further studies are needed to investigate the underlying mechanisms.

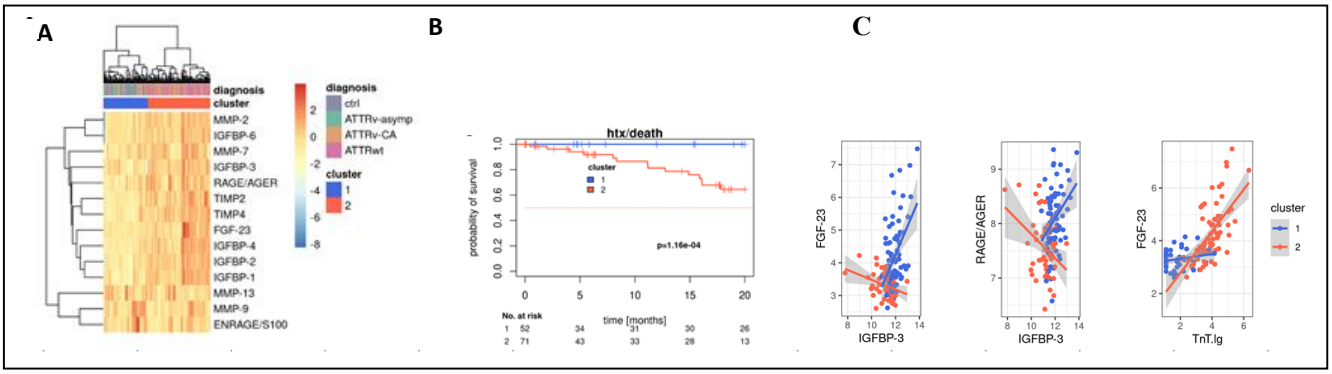

Figure 1.: A: Hierarchical cluster analysis of fibrosis associated markers quantified from patients plasma, identifying two main clusters (cluster 1, LrC, and cluster 2, HrC). Ward.D linkage, Euclidean distance, z-transformed values. B: Kaplan-Meier survival curves for endpoint heart transplantation/death and grouping from A, likelihood ratio test. C Markers showing a qualitatively different associations in pairwise tests between clusters (significant interaction, linear model analysis).

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Targeting Protein Secretion as a Novel Therapeutic Strategy in AL amyloidosis

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Background: AL amyloidosis (AL) is invariably related to deposition of misfolded free light chains (FLC) fibrils in target organs. SNARE proteins, which are the specific target of botulinum neurotoxin (BoNT), are involved in the docking and fusion of secretory vesicles.

Objective: Therapeutic strategies directly targeting FLC secretion are not available. Our hypothesis was that FLC are secreted through SNARE-coated vesicles. We further hypothesized that targeting specific SNARE proteins with the BoNT could lead to retention of FLC in the secretory pathway and cause a terminal UPR, leading to AL plasma cell (PC) apoptosis.

Methods: Gene expression profiling from IFM170 was used to interrogate SNAREs expression in malignant PC. We developed tetracycline inducible (Tet-On), bicistronic vectors expressing distinct BoNT serotypes (BoNT/A-F), T2A and GFP. Lentivirally transduced cells would express BoNT in a 1:1 stochiometric ratio with GFP, upon doxycycline (dox) administration. We transduced AL cell lines with Tet-On lentivirus expressing 7 distinct BoNTs. First, we performed time-course viability assays on polyclonally transduced cells and compared relative proportion of GFP+ cells over time. Then, we single-cell sorted transduced cells, triggered BoNT expression and assessed GFP kinetic and apoptosis at 24-48-72 hours post dox. SNAREs cleavage following induction of BoNT expression was evaluated via WB. To assess if BoNT cytotoxicity correlated with cessation of FLC secretion, we performed a secretion assay in monoclones expressing distinct BoNTs. We are generating a novel murine model of AL. We used tissue engineered bone (TEB) scaffolds coated with 250,000 GFP-labelled bone marrow stromal cells HS-5. After assessing cell attachment through fluorescence microscopy 24 hours later, we loaded $1x10^6$ mApple-labelled ALMC2. We performed fluorescence microcopy analysis, cell counting and IL-6 ELISA at Day5 after co-culture.

Results and Discussion: IFM170 GEP and AL/MM cell lines analysis showed VAMP2, VAMP3 and SNAP23 as the top expressed SNAREs. By using polyclonally transduced cells, we show that GFP+ (transduced) cells are rapidly depleted over time after dox, across all serotypes, except BoNT/B, consistent with cytotoxic effect. We noted an association between SNAP23 and VAMP3 cleavage and BoNT toxicity, suggesting that dual targeting of SNAP23/VAMP3 may be necessary to mediate BoNT cytotoxicity. We next show that only BoNTs causing early cytotoxicity significantly inhibited FLC secretion and induced UPR activation, presumably through FLC

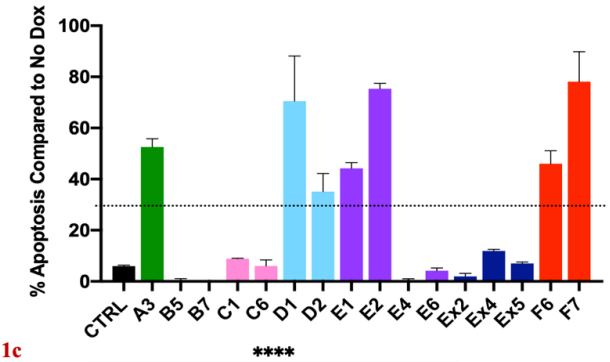
retention. Indeed, cytotoxic BoNTs, activated PERK pathway with eIF2a phosphorylation (p-eIF2a); ATF4, CHOP and GADD34 upregulation.

The preliminary data on our in vivo model suggest that HS-5 could potentially support AL amyloidosis cell lines engraftment in vivo, by creating a tumor-supportive microenvironment. The proliferation of ALMC2 cells, assessed by mApple fluorescence, is significantly higher in the presence of HS-5 stromal cells and abolished when cells are exposed to IL-6 blocking antibody.

Summary and Conclusions: We show that in all seven BoNT serotypes examined, except for BoNT/B, simultaneous cleavage of SNAP23 and VAMP3 predicts BoNT cytotoxicity and correlates with cessation of FLC secretion and terminal UPR. Overall, our preliminary data provide proof of concept that targeting FLC secretion is feasible and of therapeutic efficacy in preclinical AL models, suggesting potential clinical translatability of this innovative approach.

Support and Funding: This work was supported by the American Italian Cancer Foundation Research Fellowship (MM) and the Donald C. Brockman Memorial Research Grant from the Amyloidosis Foundation (MM).

Fig. 1a



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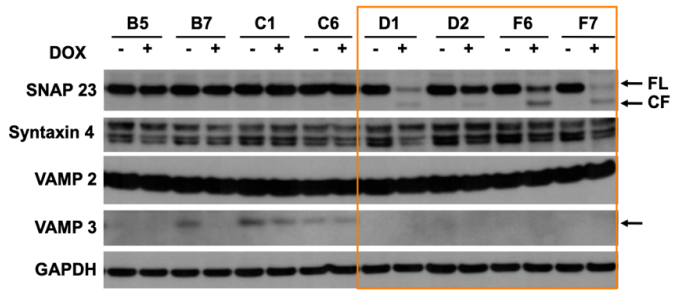

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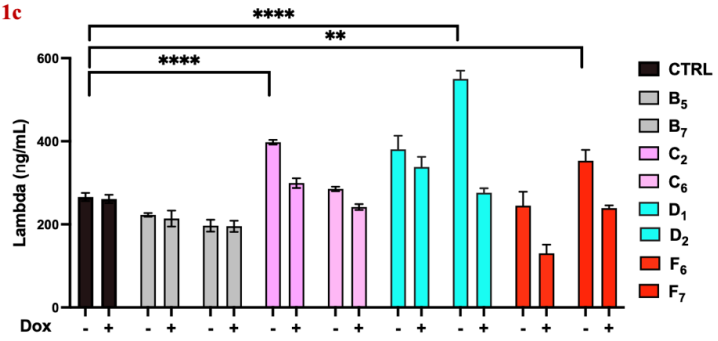

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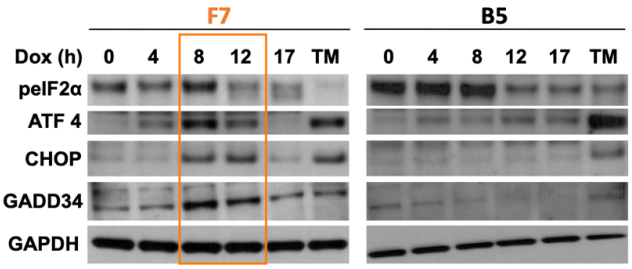


Fig. 1a Apoptosis in distinct monoclones expressing BoNT/A (green), B (grey), C (pink), D (light blue), E (purple), Ex (dark blue) or F (red) at 24; **1b** SNARE expression before or after 24 hours dox; **1c** ELISA assay assessing secreted lambda in distinct ALMC2 clones before/after dox. **1d** p-eIF2α, ATF4, CHOP and GADD34 upregulation 8 hours post dox in ALMC2 clones expressing BoNT/F but not BoNT/B (left and right panel, respectively).

Fig. 2a

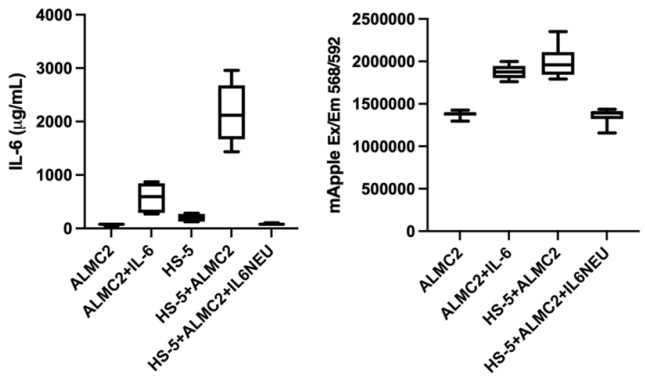


Fig. 2b

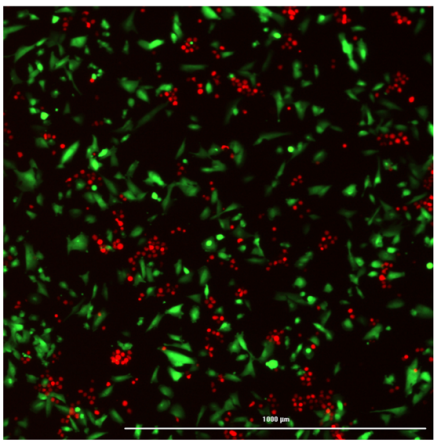


Fig. 2a ALMC2, HS-5 were cultured alone or as co-culture, with addition of IL-6 or IL-6 neutralizing antibody (IL6NEU) at Day 1 and Day 3. ELISA quantification of IL-6 secretion by HS-5 coated hydrogel scaffolds. Cell count was measured by fluorescence intensity: mApple for ALMC2 and GFP for HS-5. **2b** Fluorescence microscopy of GFP-labelled HS-5 stromal cells and mApple-labelled ALMC2.

Tocilizumab can prevent the progression of renal AA amyloidosis to end stage renal disease

Peter Kvacskay, Hanns-Martin Lorenz, Norbert Blank

Organisation: University Hospital Heidelberg, Germany

Background

AA-amyloidosis (AA) can be the consequence of any chronic inflammatory disease. AA can be associated with rheumatic diseases (rheu+AA), autoinflammatory syndromes (auto+AA) or AA of unknown origin or idiopathic AA (idio+AA). The major organ manifestation is renal AA that can progress to end stage renal disease (ESRD) and multiple organ failure. To our knowledge, there has not yet been any systematic study analysing and comparing different therapeutic options in preventing the progression of renal amyloidosis.

Objective

In this study, we analysed clinical and laboratory parameters of renal function and of inflammatory markers at baseline and the response of these parameters during follow-up in AA patients treated with different bDMARDs.

Material & Methods

This study is a monocentric retrospective analysis of the renal outcome of patients with rheu+AA, auto+AA and idio+AA who were treated with cytokine inhibiting biologic drugs (bDMARD). All patients had renal amyloidosis and the AA subtype was confirmed using immunohistochemistry. Serum creatinine, proteinuria, C-reactive protein (CRP), serum-amyloid-alpha (SAA) and other biomarkers were analysed during follow-up.

Results & Discussion

Eighty-three patients with renal AA were identified and followed for a mean observational period of 4.82 years with Tocilizumab (TOC) and other bDMARD therapy. The underlying diseases were rheu+AA (n=34), auto+AA (n=24) and idio+AA (n=25). The progression to ESRD was prevented in 60% (rheu+AA), 88% (auto+AA) and 81% (idio+AA) of patients treated with bDMARDS. After a treatment period of 18 months significant differences in the reduction of proteinuria or the progression to ESRD were detected. TOC showed a superior effectiveness and a significantly improved renal outcome compared to other bDMARDS in patients with rheu+AA and idio+AA. Furthermore, we showed that tocilizumab was able to prevent progression of AA amyloidosis to other organs including prevention of death in patients with CKD stage V at first visit.

Summary & Conclusions

In this study we analysed the time course and the effectiveness of different medical treatments on the renal outcome of patients with AA divided in three cohorts depending on the underlying disease. We found that immunosuppressive treatment was able to prevent progression of renal function to CKD stage V at least in 60.00 % in the rheu+AA group up to 87.50% in the auto+AA group. Our data showed furthermore that beginning progression to CKD stage V revealed after a period of 18 months until

which renal function remained stable in both patients with renal progression as in those with persisting stable renal function. As preferred treatment of renal AA amyloidosis, we found tocilizumab being significantly more effective in prevention of renal progression and progression of renal amyloidosis to other organs or death compared to other immunosuppressants in patients with rheu+AA and idio+AA.

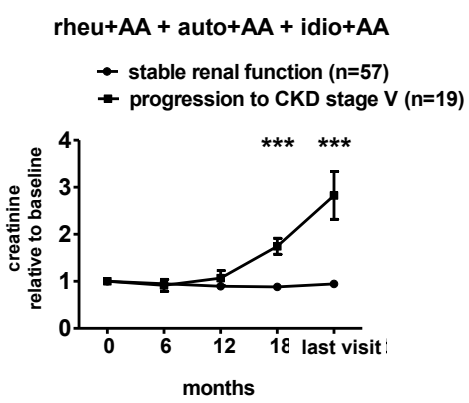
Figures

2 Figures.

Fig. 1 Time course of renal function with progression to CKD stage V/with stable renal function.

Fig. 2 Effectiveness of Tocilizumab vs. other treatments in prevention of ESRD

A


rheu+AA + auto+AA + idio+AA
stable renal function (n=57)
progression to CKD stage V (n=19)
creatinine relative to baseline
***
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0
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2
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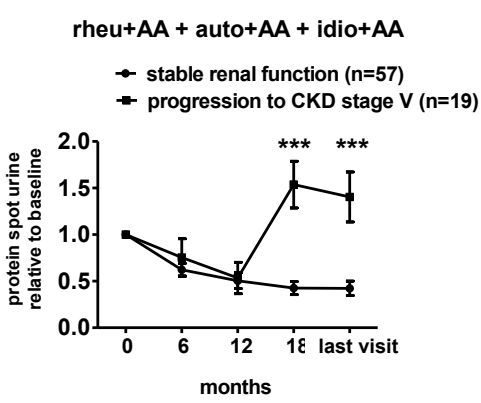
B


rheu+AA + auto+AA + idio+AA
stable renal function (n=57)
progression to CKD stage V (n=19)
protein spot urine relative to baseline
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0.0
0.5
1.0
1.5
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6
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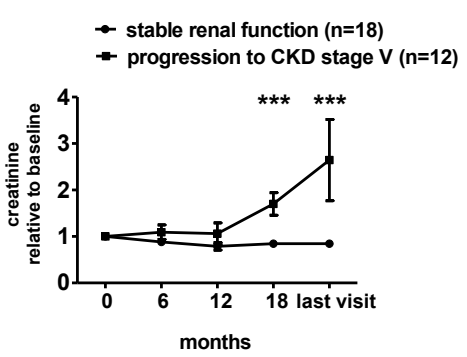
months

C


rheu+AA
stable renal function (n=18)
progression to CKD stage V (n=12)
creatinine relative to baseline
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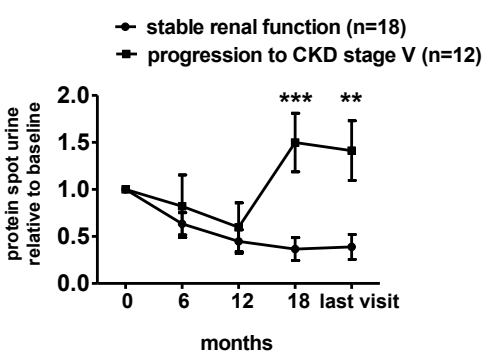
rheu+AA
stable renal function (n=18)
progression to CKD stage V (n=12)
protein spot urine relative to baseline
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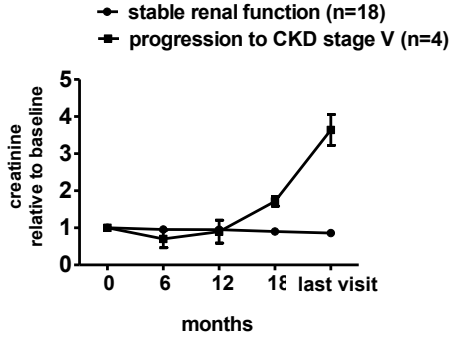
last visit
months

E


idio+AA
stable renal function (n=18)
progression to CKD stage V (n=4)
creatinine relative to baseline
0
1
2
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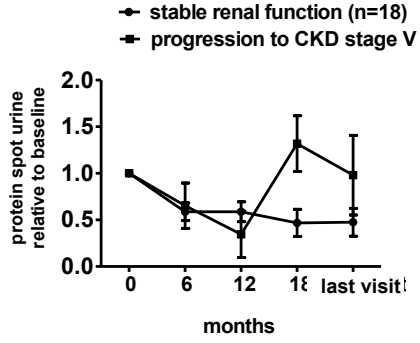
last visit
months

F


idio+AA
stable renal function (n=18)
progression to CKD stage V (n=4)
protein spot urine relative to baseline
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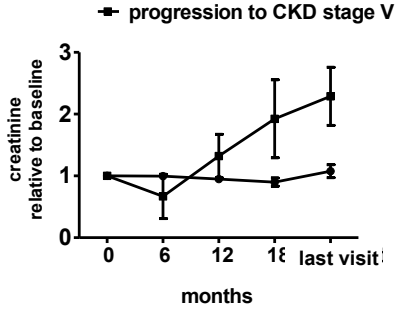
months

G


auto+AA
stable renal function (n=21)
progression to CKD stage V (n=3)
creatinine relative to baseline
0
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last visit
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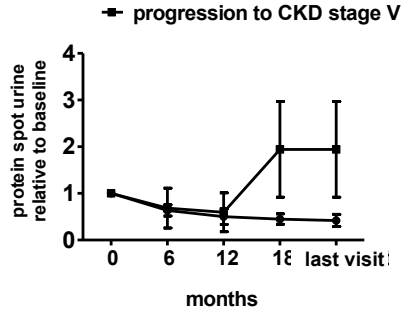
H


auto+AA
stable renal function (n=21)
progression to CKD stage V (n=3)
protein spot urine relative to baseline
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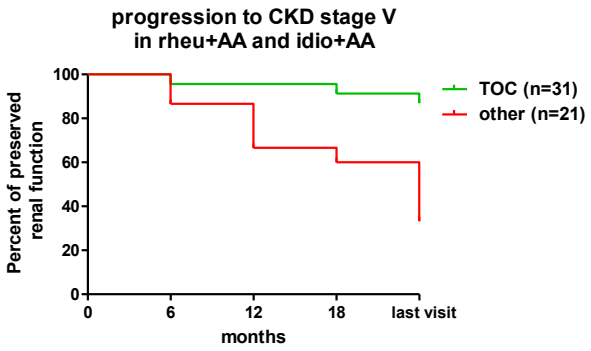
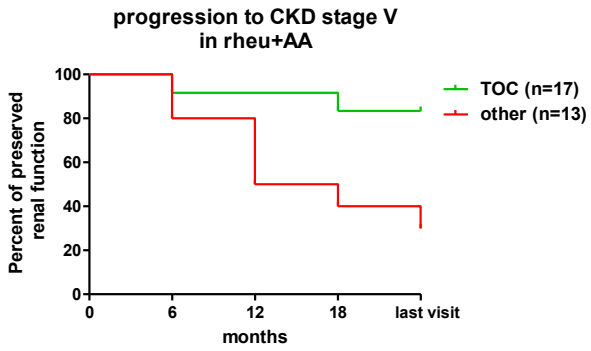
A



p = 0,0006 log-rank test

B



p = 0,0126 log-rank test

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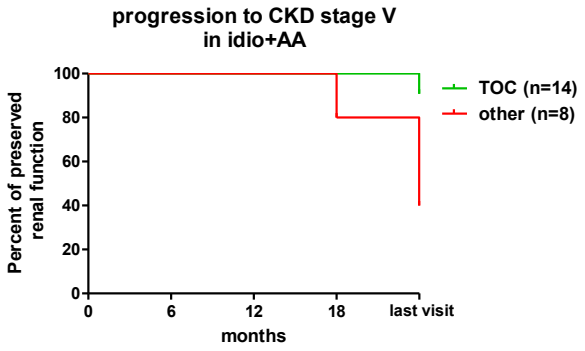

p = 0,0259 log-rank test

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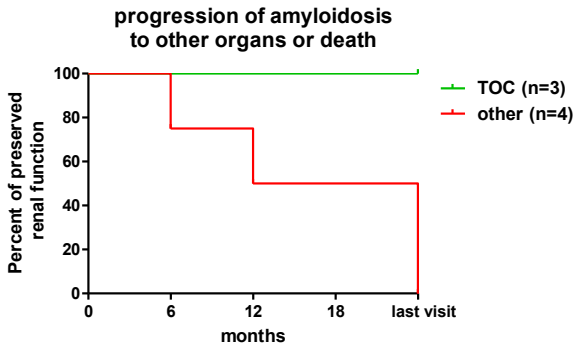

p = 0,0213 log-rank test

Natural history and risk stratification of AA amyloidosis based on a 40-year experience in the United States

JOSHI, TRACY[1], GUSTINE, JOSHUA[2], STARON, ANDREW[1], AKAR, HARUN[3], MENDELSON, LISA[1], LIBBEY, CARYN[1], HAVASI, ANDREA[1], SANCHORAWALA, VAISHALI[1]
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[2]Department of Medicine, Boston University School of Medicine & Boston Medical Center, Boston, MA, USA
[3] University of Health Sciences Turkey, İzmir Tepecik Education & Research Hospital, Clinics of Internal Medicine & Nephrology, İzmir, Turkey

Background: Systemic AA amyloidosis is a rare complication of chronic inflammatory disorders, caused by the extracellular deposition of insoluble serum amyloid A [1, 2]. There is a paucity of data regarding the natural history, prognostic markers, and risk stratification of patients with AA amyloidosis. A study by the UK NAC group represents the largest cohort to date of patients with AA amyloidosis[3].

Objectives: To describe the natural history of AA amyloidosis and identify prognostic markers for survival in a large cohort of patients from the United States.

Methods: We conducted a retrospective review of all individuals with AA amyloidosis evaluated at the Boston University Amyloidosis Center between 1980 and 2020. Pertinent clinical and laboratory data were collected from the medical records of consented patients. All patients had clinicopathological evidence of systemic AA amyloidosis, with positive Congo red staining of a biopsy specimen that was typed by immunohistochemistry, immunogold electron microscopy, or mass spectrometry, as appropriate. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation. ESRD was defined as the initiation of renal replacement therapy or renal transplantation, whichever occurred first. Overall survival (OS) was defined as the time from diagnosis of AA amyloidosis to death from any cause or last follow-up. Time-to-event outcomes were calculated using the Kaplan-Meier method. The Cox-proportional hazard regression method was used to fit a multivariable model for OS. All calculations were obtained with the R software (R Foundation for Statistical Computing, Vienna, Austria).

Results: The study cohort was comprised of 169 patients with AA amyloidosis. The underlying chronic inflammatory conditions considered etiologic for AA amyloidosis for the entire cohort and stratified by epoch of diagnosis are shown in **Figure 1**. suppurativa. Twenty-six patients (20%) did not have an underlying inflammatory condition identified despite extensive testing and were considered to be idiopathic.

Among all patients, the distribution of affected organs was: kidneys (91%), gastrointestinal tract (22%), liver (14%), heart (8%), soft tissues (6%), autonomic nervous system (4%), and other organ systems (21%). Sixty-one patients (36%) progressed to ESRD. The median time from diagnosis of AA amyloidosis to ESRD was 5.9 years (95% CI 3.7-9.6).

With a median follow-up of 3.4 years (95% CI 2.4-4.6 years), 96 patients (57%) have died. The median OS was 6.3 years (95% CI 2.4-8.6 years), and the estimated 10-year OS was 37% (95% CI 29-48%). In the multivariate analysis, a baseline erythrocyte sedimentation rate (ESR) ≥80mm/hr and eGFR ≤25 mL/min/m^2 were independently associated with an increased risk of death. We subsequently developed a prognostic scoring system for OS incorporating both ESR and eGFR (**Table 1**). A total of 101 patients (60%) received disease-modifying treatment for the underlying inflammatory condition.

Conclusions: We present a 40-year longitudinal study describing the natural history of AA amyloidosis, which represents the largest patient series from the United States. We developed a prognostic scoring system for OS incorporating baseline ESR and eGFR that is readily adaptable to clinical practice.

Figure 1. Underlying disorders causing AA amyloidosis over a four-decade period

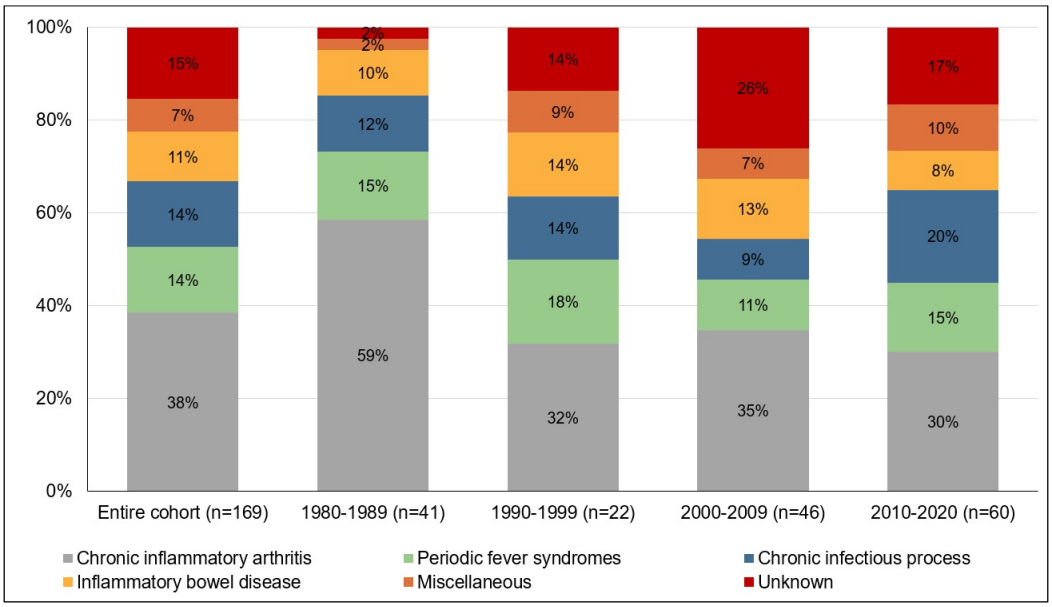

Table 1. Prognostic scoring system for survival in AA amyloidosis.

Risk group	Score	No. of patients (%)	Median OS (95% CI), years	HR for death (95% CI)	P-value
Low	0	47 (31)	11.9 (6.8–17.9)	Ref	Ref
Intermediate	1	72 (47)	6.0 (4.7–8.6)	1.98 (1.18–3.32)	0.020
High	2	33 (22)	4.8 (1.9–8.2)	2.89 (1.40–5.99)	0.001

Patients were scored as follows: ESR (<80 mm/hr: 0 points; ≥80 mm/hr: 1 point) and eGFR (>25 mLmin/m2: 0 points; ≤25 mL/min/m2: 1 point). A total of 152 patients with complete data were included in this analysis.
Abbreviations: OS, event-free survival; HR, hazard ratio; CI, confidence interval; Ref, reference group.

References

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3. Lachmann, H., Goodman, H., Gilbertson, J., Gallimore, J. R., Sabin, C., Gillmore, J., & Hawkins, P., *Natural history and outcome in systemic AA amyloidosis.* The New England Journal of Medicine 2007. **356**: p. 2361-71.

Support and funding: The Amyloidosis Center database is supported by the Amyloid Research Fund of Boston University School of Medicine and the Wildflower Foundation Research Grant.

Long-term transplant outcomes in recipients with renal amyloidosis

WIEN, TALE N.[1], VASSTRAND, HILDE J.[1], RAKI, MELINDA[2], GUDMUNDSDOTTIR, HELGA[3], REISÆTER, ANNA V.[3,4], HARTMANN, ANDERS[4], ÅSBERG, ANDERS[4]

[1]Dept. of Internal Medicine and Dept. of Medical Research, Bærum Hospital, Vestre Viken HF, Norway, [2]Department of Pathology, [3]Dept. Nephrology and [4]Dept. of Transplantation Medicine, Oslo University Hospital, Norway

Background: Since the first successful kidney transplantation (KTX) for systemic amyloidosis was performed in 1968, transplant outcomes have improved. Still a concern for poor allograft and patient outcome with recurrent amyloid deposition and extra renal amyloid deposits challenge organ allocation to this patient group (1-4).

Objective: We aimed to study long-term outcomes after KTX in recipients with amyloidosis, identify causes of graft loss, and to compare with post-transplant outcomes in recipients with other causes of renal failure.

Material & Methods: We report outcomes from the Norwegian Renal Registry (NNR) of all patients with amyloidosis in Norway receiving a first kidney (only) transplant from 1988 through 2017 (AMY, n=128) with follow-up until March 31st 2022. Amyloid type was AA in 109 of 128 AMY KTX recipients. The 19 non-AA recipients included 13 with AL, 4 of undetermined type, 1 ALECT2 and 1 AFib. Data were supplemented with manual review of patient records. Norwegian KTX recipients in the same time period with diabetic nephropathy (DIA, n=509) and autosomal dominant polycystic kidney disease (ADPKD, n=759) served as controls. We calculated patient-, graft- and death-censored graft survival using the Kaplan-Meier method.

Results: At time of censoring 44/128 AMY KTX recipients had lost their graft, 63 were deceased and 21 alive with a functioning graft. KTX recipients comprised 31% of all AA patients and 15% of all AL patients in renal replacement therapy in Norway during the study period. Mean age (SD) years at KTX was 50.0 (13.9) for AA, 61.1 (10.9) for non-AA, 55.1 (12.5) for DIA and 55.2 (11.0) for ADPKD. Patient and death censored graft survival curves are shown in figures 1 and 2. 10-year patient survival (95% CI) was 44% (35.7-54.9) in AA, 23% (0.10-54.5) in non-AA, 47% (42.3-51.0) in DIA and 74% (70.3-76.9) in ADPDK. Patient survival (Figure 1) was significantly different between groups and a subgroup analysis showed a significantly higher survival in AA compared with non-AA.
10-year death-censored graft survival (95% CI) was 71% (61.2-82.7) in AA, 52% (30.0-91.6) in non-AA, 78.9% (74.7-83.4) in DIA and 87.8% (85.3-90.4) in ADPDK. Death-censored graft survival (Figure 2) was significantly different between groups and significantly different between AA and non-AA in a sub-analysis.
Recurrent amyloid caused 18 AA graft losses after a median of 10.8 (IQR 7.2-15.1) years post transplantation. Death-censored graft survival was poorer in non-AA group, where 7 grafts were lost; 1 non-viable graft and 3 rejections in the first month post transplant, and 3 recurrent (AL) amyloid graft losses after 3.3, 4.1 and 6.1 years respectively. Rejection was cause of graft loss after a median (IQR) years of 6.1 (1.6-9.9) in 17/109 AA, 5.7 (2.0-10.0) in 82/509 DIA and 9.1 (4.5-12.8) in 105/759 ADPKD recipients.

Summary & Conclusions: Our study demonstrates improved patient and graft survival for AA amyloidosis in the Norwegian KTX AMY cohort since our previous study 1974-1989 (1). Like a recent study from United Kingdom (2), we demonstrate a comparable patient survival to DIA in the AA amyloidosis group. Our non-AA KTX group is small, dominated by AL, and shows a significantly poorer survival compared to AA. Recurrent amyloidosis in the kidney graft is a major cause of graft loss in amyloidosis patients that needs attention in post transplant care, but overall, transplantation appears an acceptable treatment option in selected patients.

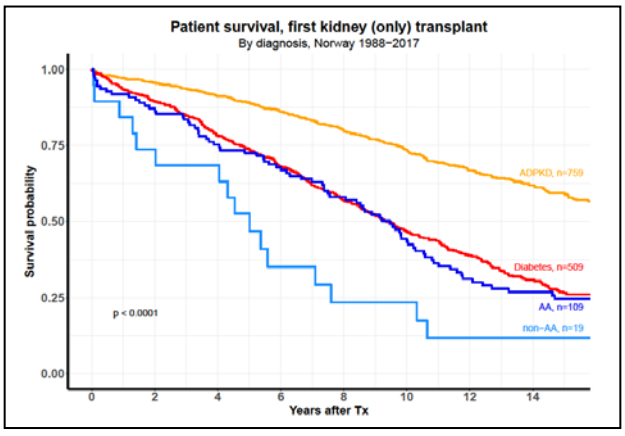

Figure 1 : Patient survival after KTX.

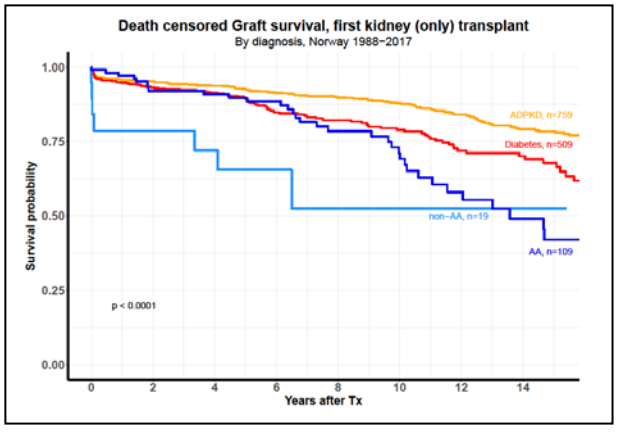

Figure 2 : Death censored graft survival after KTX.

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2. Law S. et al, Nephrol Dial Transplant, 36:355-65, 2021.
3. Angel-Korman A. et al, Transplantation, 104:2035-47, 2020.
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Support & Funding: The project is funded by grants from Vestre Viken Hospital Trust and from South-Eastern Norway Regional Health Authority.

Patisiran Global Open-label Extension Study at 36 Months: Effect of Long-term Treatment on Mortality and Ambulatory Function in Patients with hATTR Amyloidosis with Polyneuropathy

WIXNER, JONAS[1]; UEDA, MITSUHARU[2]; MARQUES JUNIOR, WILSON[3]; DALIA, SAMIR[4]; ARUM, SETH[5]; HALE, CECILIA[5]; JAY, PATRICK[5]; CAPOCELLI, KELLEY[5]; ADAMS, DAVID[6]

[1]*Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden*

[2]*Kumamoto University Hospital, Kumamoto, Japan*

[3]*Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, Brazil*

[4]*Mercy Clinic Joplin - Oncology and Hematology, Joplin, MO, USA*

[5]*Alnylam Pharmaceuticals, Cambridge, MA, USA*

[6]*APHP, National reference center for FAP (NNERF), CHU Bicetre, INSERM U1195, Université Paris Saclay, Le Kremlin Bicêtre Cedex, France*

Background:

Hereditary transthyretin-mediated (hATTR) amyloidosis, also known as ATTRv amyloidosis, is a progressive and fatal disease. Without treatment, patients experience debilitating polyneuropathy with loss of ambulatory function and a median survival of 4.7 years from diagnosis. The effects of long-term treatment with patisiran, an RNAi therapeutic approved for the treatment of hATTR amyloidosis with polyneuropathy, are being assessed in the ongoing Global Open-Label Extension (OLE) study (NCT02510261).

Objective:

To describe the interim 36-month mortality and ambulatory function data in patients with hATTR amyloidosis with polyneuropathy from the ongoing Global OLE study.

Material & Methods:

Patients in the Global OLE study were analyzed in three groups based on their enrollment in the parent studies: APOLLO-placebo, (n=49), APOLLO-patisiran, (n=137), and Phase 2 OLE patisiran (n=25). In the Global OLE, all patients received patisiran 0.3 mg/kg once every three weeks for up to 5 years. Mortality was analyzed from parent study enrollment in all patients who received ≥1 dose of patisiran in the Global OLE (n=224) (Figure 1).

Results & Discussion:

At Global OLE baseline, the APOLLO-placebo group had more severe disease than the APOLLO-patisiran or Phase 2 OLE groups, including a lower proportion of patients able to walk unaided (familial amyloid polyneuropathy [FAP] stage 1), reflecting disease progression while on placebo in the parent study. At data cut-off (Jan 27, 2021), the maximum duration of patisiran treatment varied by group (APOLLO-placebo, 36 months; APOLLO-patisiran, 54 months; Phase 2 OLE, 60 months), and median survival from start of parent study was not reached in any group by Month 36 of the Global OLE. Mortality was lower in patients who initiated treatment earlier in their disease course (APOLLO-patisiran, 13.5%; Phase 2 OLE, 11.1%) when compared with the APOLLO-placebo group (34.7%). In a multivariate analysis of 6 variables that were individually predictive of mortality, *N*-terminal pro-brain natriuretic peptide >3000 ng/L, New York Heart Association Class >1, and placebo assignment in the parent study remained significant risk factors for mortality, whereas FAP Stage >2, non-V30M genotype, and mean left ventricular wall thickness ≥1.3 cm were not significant. At Month 36, most patients remained ambulatory (polyneuropathy disability score [PND] <IV), and greater proportions of the APOLLO-patisiran and Phase 2 OLE groups (55.5% and 80.0%, respectively) showed stabilized or improved ambulation (assessed by PND score) than in the APOLLO-placebo group (42.9%). The majority of patients enrolled in the Global OLE have received patisiran for at least 54 months, including parent study exposure, with some having received patisiran for up to 7 years. 70.1% of patients enrolled in the Global OLE were still receiving patisiran at data cut-off.

Summary & Conclusions:

At Month 36 in the ongoing 5-year Global OLE, survival was greater in patients who received patisiran treatment earlier. The therapeutic benefit of patisiran on ambulatory function, first demonstrated in APOLLO, was sustained and was greatest in groups that initiated patisiran treatment earlier with a lower disease burden. These results highlight the substantial impact of earlier diagnosis and treatment in patients with hATTR amyloidosis with polyneuropathy.

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Progression and distribution pattern of cerebral amyloid angiopathy in hereditary ATTR amyloidosis patients visualized by ^{11}C-PiB-PET imaging

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Background: Disease modifying therapies (i.e., liver transplantation, TTR tetramer stabilizers, and nucleic acid therapeutics) markedly improved survival in hereditary ATTR (ATTRv) amyloidosis patients. Ironically, however, the prolonged disease duration induced *de novo* CNS amyloidosis, ATTR-type cerebral amyloid angiopathy (CAA) as choroid plexus continues to produce variant TTR[1-3]. Under such situations, it is highly necessary to elucidate characteristics of ATTR-type CAA and develop useful biomarkers to monitor ATTR-type CAA.

Objective: To investigate the clinical characteristics, and distribution and progression pattern of ATTR-type CAA by using Pittsburgh compound B (PiB)-positron emission tomography (PET) imaging.

Material & Methods: We enrolled 31 (12 females) ATTRv amyloidosis patients with V30M (p.V50M) variant (ATTRV30M) and performed ^{11}C-PiB-PET in all patients. Mean age of onset (± SD) and age at study inclusion, were 34.4 ± 8.6 and 50.1 ± 11.7 years, respectively. Twenty patients received liver transplantation and 13 patients were treated with disease-modifying pharmacological therapies (4 patients received both liver transplantation and pharmacological therapies). Two patients were treatment naïve at the time of PiB-PET. Follow-up PET was performed in 15 patients.

Results: A total of 8 patients (6 females) developed CNS symptoms due to CAA. Duration of illness from onset of ATTR amyloidosis to CNS symptom onset ranged from 12 to 21 years (mean ± SD, 17.0 ± 3.1). Seven patients developed transient focal neurologic episodes, 2 patients developed multiple cerebellar hemorrhages, and 2 patients developed cognitive decline. The amount of ^{11}C-PiB accumulation increased as a function of disease duration in all ATTRv amyloidosis patients (correlation coefficient = 0.67, P = 0.000007). Annual increase rate of SUVR in female patients (0.013/year) was significantly greater as compared with that in male patients (0.005/year, P = 0.046, Figure 1). SUVR was significantly increased at the follow-up PET analysis (0.69 ± 0.06) as compared with the first PET analysis (0.66 ± 0.06, P = 0.002). The 3D-SSP analysis of PiB-PET demonstrated that CNS amyloid deposition started in the upper middle surface of cerebellar cortex around 10 years after onset, and then spread out into entire surface of cerebellum, Sylvian fissure, and anterior part of longitudinal fissure of cerebrum around 15 years after onset (Figure 2A). After 20 years from onset, ATTR amyloid deposition expanded to entire cerebral surface (Figure 2B).

Summary & Conclusion: PiB-PET is a useful biomarker in early detection and treatment evaluation in early-onset ATTRV30M patients. Female gender is associated with rapid progression of ATTR-type CAA.

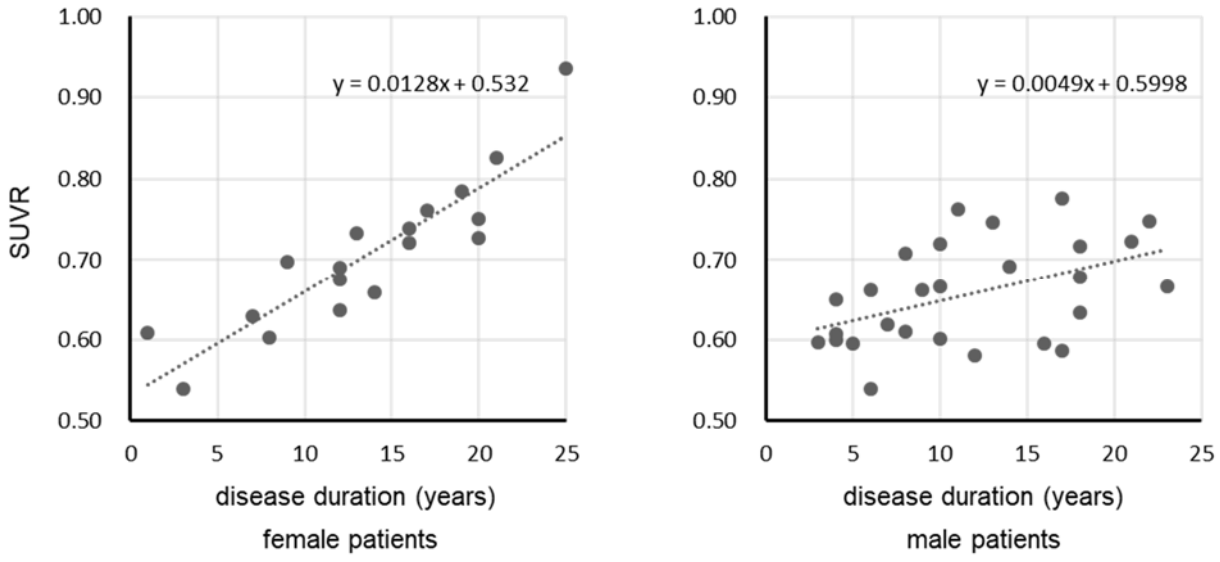

Figure 1.: Quantitative analysis of CNS ATTR amyloid deposition as a function of disease duration.

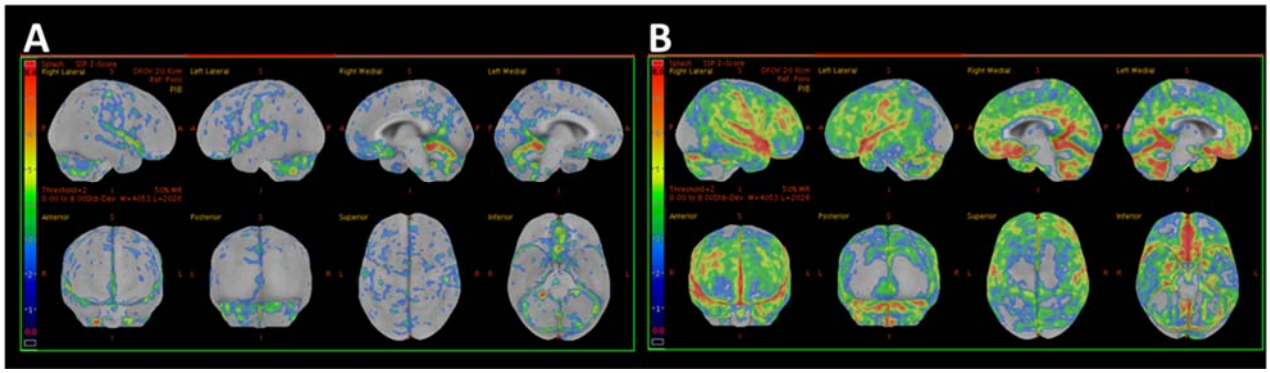

Figure 2.: Distribution and progression of CNS ATTR deposition visualized by 3D-SSP analysis of ^{11}C-PiB-PET imaging. (A) 54-year-old ATTR V30M male patient with CNS symptoms (13 years after onset). (B) 53-year-old ATTR V30M female patient with CNS symptoms (25 years after onset).

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An international Delphi survey for the definition of a multidisciplinary holistic approach to the care of hereditary ATTR amyloidosis

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Background: Hereditary transthyretin amyloidosis (hATTR or ATTRv) is a progressive and potentially life-limiting rare disease. There are no universal standards for the holistic care of patients and their families (including psychological, social and economic support). Clinical practice varies between countries, as do the resources to manage patients. In 2019, the Amyloidosis Alliance called for an expansion of services, acknowledging the need for a broader and more holistic approach to care so patients' quality of life can be improved.

Objective: The aim of this international Delphi survey is to evolve expert-patient-led practical guidance to inspire and encourage a holistic approach to care – which is managed by specialists in a multidisciplinary setting and supported by allied healthcare professionals (HCPs). This presentation outlines the scope of the guidance and the progress to establish a consensus among an International Voting Panel representing the clinical community and patient advocacy groups.

Material & Methods: Delphi is an iterative process using rounds of online voting leading to a convergence of experts' judgements and opinions.[1] In this study, input was sought (at every stage) from a panel of patient advocacy group representatives as well as members of the multidisciplinary care team (including two neurologists, a cardiologist, a physiotherapist, a psychologist, a nutritionist and a nurse specialist) to ensure the guidance reflected the priorities of patients as well as the clinical expertise of the multidisciplinary team. The joint 14-member patient-HCP Primary Panel was convened to identify the key concepts for consideration to improve standards of care, and to develop the recommendations for the Delphi voting. Expert insights from the patient-HCP Primary Panel were enriched by the findings from a systematic literature review (prepared in advance of the first Primary Panel meeting). Over 180 healthcare professionals from >12 countries (identified based on their experience in this field) and patient advocates were invited to be part of an International Voting Panel. The threshold for consensus was defined *a priori* as at least 75% group agreement for each recommendation (using a 5-point Likert scale).

Results: The recommendations were informed by findings from a systematic literature review of 844 papers on the management of hATTR amyloidosis and additional research to identify expert guidance from other chronic neurologic diseases. Seven themes were explored in detail and guidance evolved on: 1: The route to early diagnosis and treatment; 2: Integrated care for monitoring and management; 3: Allied healthcare professional support, 4: Family-centered care and caregiver support; 5: Patient-Doctor dialogue and shared decision-making; 6: Access to social support and 7. Social networking and spiritual support. The validity and

feasibility in practice of the guidance, proposed by the Primary Panel are now being tested and ranked in the anonymized online Delphi voting rounds. The results of the international vote and final iteration of guidance will be presented at the meeting.

Summary & Conclusion: It is hoped that this evolving international consensus, engaging both patients and patient advocates, as well as healthcare professionals, will inspire specialist centers to develop services and processes to ensure that people living with hATTR amyloidosis have access to holistic care.

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Support & Funding: Preparation of the report and its recommendations was funded by Alnylam Pharmaceuticals.

Eplontersen in ATTR-polyneuropathy: results from the 35-week interim analysis of NEURO-TTRansform

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Background: Hereditary transthyretin (TTR) amyloidosis (ATTRv) is a rare, severe, progressive, debilitating, and ultimately fatal disease caused by systemic accumulation of TTR amyloid fibrils in multiple organ systems.[1] Eplontersen (ION-682884) is an investigational ligand-conjugated antisense drug designed to degrade hepatic *TTR* mRNA and inhibit TTR protein synthesis.[2] Eplontersen has the same sequence as inotersen and is conjugated to a triantennary *N*-acetyl galactosamine ligand to support receptor-mediated hepatocyte uptake.

Objective: To evaluate effects of eplontersen at the Week 35 interim time point in patients with ATTRv polyneuropathy (ATTRv-PN) enrolled in the phase 3, international, multicenter, open-label, randomized NEURO-TTRansform study (NCT04136184).

Material & Methods: Adult patients aged 18–82 years diagnosed with ATTRv-PN as defined by Familial Amyloid Polyneuropathy (FAP or Coutinho) Stage 1–2, a documented TTR sequence variant, and signs/symptoms consistent with polyneuropathy (Neuropathy Impairment Score [NIS] ≥10 and ≤130) were enrolled in the study. Patients were randomized 6:1 to receive 45-mg subcutaneous eplontersen every 4 weeks, or 300-mg subcutaneous inotersen weekly for 34 weeks and then eplontersen, until end of treatment at Week 81. The inotersen reference group was intended to ensure that no gross differences in patient population and response existed between the NEURO-TTRansform and NEURO-TTR (NCT01737398) studies.[3] Patients enrolled in the eplontersen group were compared with a historical placebo group (placebo arm in the NEURO-TTR study[3]). The Week 35 interim analysis includes the coprimary endpoints of percentage change from baseline in serum TTR concentration and change from baseline in modified NIS plus 7 (mNIS+7), the secondary efficacy endpoint of change from baseline in the Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN) score, and safety. Efficacy outcomes will be evaluated in the full analysis set, which includes all randomized patients who received at least 1 dose of eplontersen or inotersen and who have a baseline and at least 1 postbaseline efficacy assessment for mNIS+7 score or Norfolk QOL-DN questionnaire total score.

Results: The NEURO-TTransform study enrolled 168 patients across 15 countries, with 144 randomized to the eplontersen arm and 24 to the inotersen reference arm. At baseline, 115 patients (79.9%) in the eplontersen arm and 18 patients (75.0%) in the inotersen arm had FAP Stage 1 disease, whereas 29 patients (20.1%) in the eplontersen arm and 6 patients (25.0%) in the inotersen arm had FAP Stage 2 disease. The most common TTR variant was the V30M sequence variant, occurring in 85 patients (59.0%) in the eplontersen arm and 16 patients (66.7%) in the inotersen arm. Efficacy and safety results from the interim analysis will be presented.

Summary & Conclusion: The Week 35 results from the NEURO-TTRansform study will provide the first readout on the efficacy and safety profile of eplontersen in patients with ATTRv-PN. The results from this study will provide important information on clinical and health-related quality of life outcomes to better inform future choices for patients with ATTRv-PN.

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Diflunisal treatment for hereditary transthyretin amyloidosis – the Swedish DFNS-02 trial

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Background: Diflunisal is a non-steroidal anti-inflammatory drug that stabilizes the transthyretin (TTR) tetramer and thereby prevent amyloid fibril formation and reduces neurological deterioration in patients with hereditary transthyretin amyloidosis (ATTRv).

Objective: The Swedish DFNS02 was an open study, started to monitor the effect of diflunisal in patients with ATTRv amyloidosis.

Material & Methods: DFNS02 was an open-label observational study designed to monitor the effect of diflunisal 500 mg daily on neurological impairment, cardiac involvement and nutritional status in patients with ATTRv amyloidosis and was open for enrollment from September 2015 to 2021. The primary outcome measures were changes in the Kumamoto scale and cardiac impairment measured by echocardiographic assessment of global systolic strain (is not fully analyzed yet). The secondary outcome measures were changes of the nutritional status (modified body mass index, mBMI), cardiac function (septal thickness and pro-BNP), Karnofsky performance scale and safety follow-up blood tests (hemoglobin, platelets, creatinine, and liver enzymes). Evaluations were performed at 12 and 24 months, respectively.

Results: Thirty-three patients were included in the study, 22 males and 11 females with mean age of 69 years (33-84). Of those, 12 (37,5%) had received diflunisal prior to their inclusion in DFNS02, and 19 (58%) completed the 24-month study follow-up. The main reasons for early termination were drug licensing issues (21%), anticoagulation (14%), side effects from the study drug (7%) and COVID pandemic (7%). For those who completed the study protocol, total Kumamoto score remained stable over time (median score 12 vs. 11 vs. 12, p = 0.12), as did the sub-scores for sensory neuropathy, motor neuropathy, autonomic neuropathy and organ dysfunction. No significant change was noted in nutritional status (median mBMI 957 vs. 926 vs. 904, p =0.9). Over the observational period, no significant changes were found in pro-BNP levels (median value 978 vs. 1217 vs. 605 ng/l, p = 0.40), nor in cardiac septum thickness (median thickness 13.8 vs. 13.7 vs 13.1 mm, p = 0.7). No significant changes were found in the safety follow-up blood tests.

Summary & Conclusion: The DFNS02 trial supports the efficacy and safety of diflunisal for ATTRv amyloidosis in patients with polyneuropathy as well as in patients with cardiomyopathy with results in line with the previous placebo-controlled trial. Total Kumamoto scores, nutritional status septum thickness, ProBNP and blood tests remained stable over time. No significant deterioration could be seen over the study period. The high drop-out rates were mainly due to regulatory issues making diflunisal unavailable to Swedish patients. Diflunisal is shown to be effective in stabilising and halt the disease progression in patients with ATTRv.

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Kidney phenotype and immune activation in hereditary ATTR amyloidosis during inotersen therapy

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Background: Hereditary transthyretin amyloidosis (ATTRv) is a systemic disorder characterized by predominant peripheral neuropathy and cardiomyopathy. Kidney involvement vary from the absence of clinical disease to renal replacement therapy. Inotersen, an antisense oligonucleotide (ASO), ameliorate the course of ATTRv neuropathy through reduction of the plasma TTR concentration. Glomerulonephritis was previously documented under inotersen therapy (NEURO-TTR trial). Accurate follow-up is desirable, towards a preventive strategy to avoid superimposed kidney damage.

Objective: Systematic evaluation of ATTRv patients under inotersen (standard dosage, 300 mg, once-weekly, subcutaneous, commercial use), aiming early detection of *de novo* kidney disease, including immune surveillance; definition of a kidney phenotype if suitable.

Material & Methods: Patients evaluation under a nephrological perspective and standard practice. Parameters - hemogram; serum - creatinine, urea, cystatin C (CysC), Igs; C3, C4, ANAs, ANCAs, Anti-MBG; anti-histones, C1q if clinical relevance; spot urine: albumin/creatinine (mg/g), protein/creatinine (g/g) sediment; eGFR based on CKD-EPI creatinine-CysC 2012 formula.

Results: 43 patients were observed (25M, 18F), all ATTR Val30Met (*p.Val50Met*) variant; 2 treatment *naïve*, 6 after NEURO-TTR trial, and 35 after a tafamidis course. The mean age of neuropathy was 38.2 yrs, at 1st inotersen administration 46.3 yrs; mean follow up 21 months (2-104); 15 patients (9M, 6F) dropped out of treatment regarding safety issues (mean 24.3 months); eGFR was, on average, 102.0/87.2/86.5 ml/min/1.73 m^2 at enrollment/12 and 18 months after inotersen. The mean urine albumin/creatinine was 11.8 at baseline, 15.9/26.2/25.8 at 3/6/12 months.
Two females developed PR3-ANCA positivity (peak 451 QL): one with anti-histones positivity and C4 consumption; one with decreased eGFR (44 mL/min); in both, biopsies showed glomerular amyloid deposits, no evidence according PR3-ANCA vasculitis or granulomas; 2 patients presented atypical ANCA patterns (P and C), one with C4 decrease, other with isolated CysC elevation. In one male persistent undetectable C4 was observed.
Two males presented kidney events – one, after 7 years of inotersen, showed a progressive renal dysfunction, albuminuria, low C3 and C1q levels; biopsy showed scarce amyloid deposits, interstitial fibrosis, inflammatory lymphocytic infiltrate; tubular atrophy in 90% of the cortical; <10 IgG4 positive plasma cells/HPF; he also presented uveitis (TINU). The second patient, 4 months after therapy showed nephrotic syndrome, rapidly progressive renal failure, low C3 and C4, eosinophilia, and abnormal urinary sediment; histopathology evidenced acute interstitial nephritis, crescentic glomerulonephritis and glomerular C3 deposition. Corticosteroid therapy was instituted, ASO suspended. When, for any event, ASO was interrupted, patients with previous C3 or C4 low levels, recuperate to normal values.

Summary & Conclusion: eGFR decreased during the first year of treatment, however, *de novo* proteinuria/albuminuria was rare. Complement activation, PR3-ANCA and X-ANCA suggest that ATTRv patients, Val30Met (*p.Val50Met*) mutation, under inotersen therapy have predisposition to autoimmune manifestations. Kidney phenotype is wider than the previously described and appeared combined. PR3-ANCA was not associated to kidney vasculitis, and crescentic lesions were present in the absence of autoantibodies. Identification of immune/kidney manifestations allowed timely inotersen suspension and recover.

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Association of hereditary V122I amyloidogenic transthyretin variant with heart failure: A systematic review and meta-analysis

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Background: Hereditary valine substitution with isoleucine at position 122 (V122I) amyloidogenic transthyretin variant is an autosomal-dominant condition known to place carriers of the V122I at risk of deposits of misfolded transthyretin that causes heart diseases including heart failure in elderly individuals of African origin.

Objective: To assess the susceptibility of people with V122I amyloidogenic transthyretin variant to heart failure and risk of death.

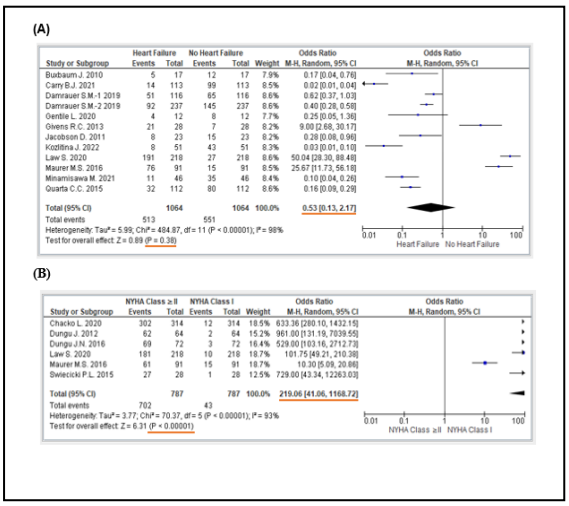
Material & Methods: A comprehensive search of the PubMed database for studies that investigated genetic V122I amyloidogenic transthyretin variant and heart failure (HF) together with associated heart diseases in March 2017 or earlier was carried out. Pooled odd ratios (OR) for a comparison of only V122I patients with or without HF, HF in V122I and wild-type transthyretin amyloidosis (wtATTR), and HF in V122I and non-amyloid control on the one hand, and hazard ratios (HR) for risk of death in V122I patients and wtATTR patients at 95% confidence intervals were estimated and summarized using fixed-effect (FE) and random-effect (RE) Mantel-Haenszel model and Generic Inverse Variance Model in Review Manager software version 5.4.

Results & Discussion: Sixteen studies with 17 datasets of 1553 V122I patients, 1787 wtATTR patients, and 148889 non-amyloid participants were included in the meta-analysis of HF and prognostic significance of V122I and wtATTR in patients. Out of the 17 data sets, 5 reported that all study subjects had HF for 478 V122I patients and the remaining 12 datasets showed reports of V122I with or without heart failure for 1064 patients, of which 513 had HF and 551 were without HF, 6 reported NYHA Class I-IV for 787 V122I patients of which 43 had NYHA Class I and 702 had NYHA Class ≥II. Of the 12 datasets that reported V122I patients with or without HF, 5 reported HF in V122I and wtATTR, of which 301 of 377 V122I had HF and 955 of 1787 wtATTR controls had HF while 6 datasets from 5 studies showed 208 of 675 V122I and 15366 of 148889 non-amyloid controls had HF. Three datasets with HR for 320 V122I patients and wtATTR 934 patients at risk of death were analyzed.

The odds ratio of a 95% confidence interval for different groups of analysis is as follows: insignificantly lesser V122I association with HF than those without HF (OR=0.53, 95%CI:0.13-2.17, P_{OR}=0.38); much greater and statistically significant V122I association with NYHA Class ≥II than NYHA Class I (OR=219.06.32, 95%CI:41.06.62-1168.72, P_{OR}<0.00001); significant HF association with V122I than wtATTR controls (OR=1.54, 95%CI:1.12-2.13, P_{OR}=0.008), and more significant HF association with V122I than non-amyloid controls (OR=1.62, 95%CI:1.36-1.92, P_{OR}<0.00001).

In addition, compared to wtATTR control, V122I was shown to significantly predict reduced overall survival of V122I patients yielding an overall prognostic effect analysis (HR=2.45, 95%CI:1.11-5.44, P_{HR}=0.03).

Summary & Conclusion: This study showed hereditary V122I patients are susceptible to HF with a more significant association with NYHA Class ≥II. A comparison of the HF susceptibility of hereditary V122I with wtATTR and NAC controls indicates V122I has significantly greater susceptibility and a significant prognostic effect than controls. In conclusion, the V122I variant is significantly associated with HF and relatively high risk of death.



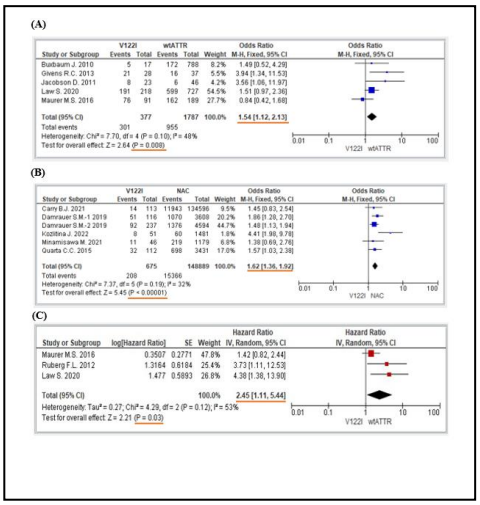


Figure 1.: Meta-analysis of the association of V122I cases with HF; OR of HF versus no HF **(A)**, and NYHA Class ≥II versus NYHA Class I **(B)**.

Figure 2.: Meta-analysis of the association of V122I, wtATTR and NAC controls with HF and risk of death from V122I carrier status; OR of V122I versus wtATTR **(A)**, OR of V122I versus NAC **(B)**, and HR of V122I versus wtATTR **(C)**.

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ISA Abstract 2022

ISA meeting location and dates: Heidelberg from 04-08 September 2022.

Abstract Submission deadline: Saturday April 23, 12 noon CET (Central European Time)

Abstract character limit, title: Maximum 200 characters

Abstract character limit, text: ≤3600 characters (including spaces), excluding title, author list, and figures/tables

Current draft character count: 3028 characters

Topic: ATTRv: clinical management – clinical – ATTR type

Keywords (1-5 required): tafamidis, transthyretin amyloid polyneuropathy, early treatment, neuropathy, small fiber

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Tafamidis reduces skin denervation and amyloid in skin biopsies of very early symptomatic hATTRv patients after one year of treatment.

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Background.
Progressive sensorimotor and autonomic symptoms characterize hereditary ATTR amyloid polyneuropathy. Neuropathic pain is usually the first clinical manifestation caused by disease of the Aβ, Aδ, and C small nerve fibers innervating the skin. Disease-modifying therapy focuses on motor abnormalities, underestimating subjective small fiber-associated nerve function. Tafamidis, a selective TTR stabilizer, is indicated at a dose of 20mg QD to halt neuropathy progression. Challenges in the early recognition of disease onset are significant offsets to initiating early treatment.

Objective.
To evaluate the histopathological onset of disease in patients with ATTR amyloidosis with neuropathic pain and the progression of denervation and amyloid deposition after one year of treatment with tafamidis (T+) compared to patients naïve to tafamidis treatment (T-).

Material and Methods.
Forty patients with ATTRv amyloidosis and sensory neuropathic symptoms underwent evaluations for early small fiber neuropathy (UENS scores) and large fiber neuropathy (NIS score), quantitative sensory testing (QST), neuropathic questionnaires, and skin biopsies for IENFD and amyloid deposition assessments. Inclusion criteria included a pathologic TTR variant, neuropathic symptoms, and abnormal QST (cold detection thresholds ≥4 degrees from the mean age normative values). Intraepidermal nerve fiber density (IENFD) and amyloid deposition index (ADI) estimations measured in the skin biopsies of the distal thigh and distal leg were compared at baseline and after one year of treatment. Tafamidis pharmacokinetics was performed in T+patients.

Results.
30 T+patients and 10 T-patients were included. Baseline characteristics were similar, with a mean age of 37 years (17% females). Gly47Ala mutation was found in 13%, Ser50Arg in 66%, Ser52Pro in 3%, and Y136H in 11%. All patients had reduced IENFD in the distal leg and distal thigh. UENS and NIS scores correlated inversely to IENFD measurements (p=0.00). After one year, IENFD was equal to or better in 6(20%) T+p and decreased in all T-p. In T+patients with denervation, IENFD reduction was significantly less than in T-p [Table 1]. Amyloid deposition decreased in 9(30%) T+patients after one year and increased in all T-patients. Amyloid deposition in the skin was also significantly greater in T-p[Table 1]. Plasma tafamidis concentration was higher in patients with IENFD improvement or equal to the previous

year (8.9 vs. 7.3, p=0.00) and in patients with less amyloid deposition than the last year (9.4µM vs. 6.1µM, p=0.00).

Summary and Conclusions:
Neuropathic pain, UENS, and QST were reliable indicators of skin denervation in ATTR polyneuropathy. Tafamidis decreased the progression of skin denervation and amyloid deposition in ATTRv amyloid patients with very early sensory neuropathic symptoms. Higher plasma concentrations of tafamidis were associated with better outcomes, suggesting that increasing the dose could achieve better plasma concentrations and better response rates.

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Table:
Table 2. Mean differences between patients

	Baseline	Year 1	Mean difference	95% CI		P
IENFD DT (fiber/mm)						
Tafamidis	19.3±10.5	17.1±9.8	-2.3±3.1	-3.57	-1.19	p=0.00
No Tafamidis	24.3±9.6	16.1±7.9	-8.2±2.0	-9.91	-6.53	
IENFD DL (fiber/mm)						
Tafamidis	12.3±8.5	8.3±6.4	-3.97±4.8	-5.8	-2.14	p=0.00
No Tafamidis	13.6±7.1	8.0±6.2	-5.60±1.2	-6.6	-4.56	
ADI (10^2) DT						
Tafamidis	16.5±20	19.9±20	3.3±6.8	0.6	5.9	P=0.01
No Tafamidis	8.1±10	24.2±20	16.1±13	4.5	27.6	
ADI (10^2) DL						
Tafamidis	16±20	19±20	3.3±1.2	0.05	5.9	p=0.01
No Tafamidis	8±1.0	24±20	16.1±4.8	4.5	27.6	

IENFD: intraepidermal nerve fiber density; DT: distal thigh, DL: distal leg, ADI: amyloid deposition index;

Automated cardiac amyloidosis risk detection on whole body bone scintigraphy using deep-learning approach

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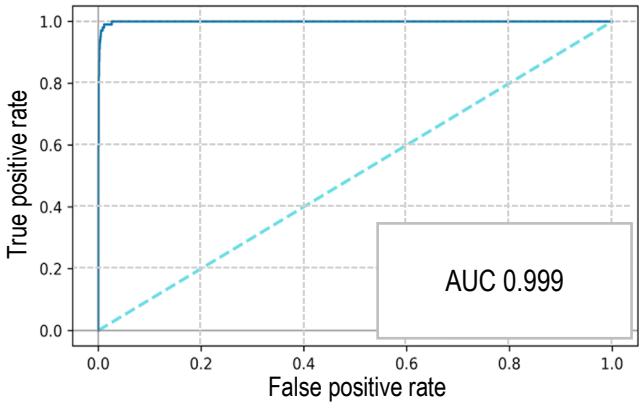
Background: Cardiac uptake on ^{99m}Tc whole-body bone scintigraphy (WBS) is almost pathognomonic of Transthyretin cardiac amyloidosis (TTR-CA)[1]. However, this scintigraphic feature remains largely unknown, leading to misdiagnosis despite characteristic images. Moreover, physician who prescribe the most WBS are often less aware of the disease. Retrospective review of all WBS in hospital database to detect those with cardiac uptake may allow the identification of undiagnosed patients[2]. Nevertheless, the process of reviewing the tens of thousands of WBS contained in hospital databases is time consuming and difficult to implement on a large scale.

Objective: To develop and validate the first deep learning-based model that automatically detects significant cardiac uptake (≥ Perugini grade 2) on WBS from hospital databases, in order to quickly review WBS databases and retrieve undiagnosed patients.

Methods: The model is based on a convolutional neural network with image-level labels. All included images were manually reviewed to validate the Perugini score initially assigned. Then, images were automatically preprocessed in order to standardize their format. The performance evaluation was performed with C-statistics using a 5-fold cross-validation scheme stratified, so that the proportion of positives and negatives WBS remained constant across folds, and using an external validation dataset. In order to further diagnose our model, we used an occlusion-based method to display the areas of the image that contribute the most to the positive predicted score.

Results: The training dataset consisted in 3048 images: 281 positives (≥ Perugini 2) and 2767 negatives (Perugini 0 and 1). The external validation dataset consisted in 1633 images: 102 positives and 1531 negatives. Performance for the 5-fold cross-validation and external validation were respectively of 98.9% (±1.0) and 96.1% for sensitivity, 99.5% (±0.4) and 99.5% for specificity, 0.999 (SD. 0.000) and 0.999 for AUC of ROC curves (**Figure 1**). Occlusion-based heatmap clearly highlights that the heart area contributes the most to positive prediction (**Figure 2**).

Conclusions: Our detection model is effective at identifying patients with significant cardiac uptake on WBS and may help to review large WBS databases to retrieve patients with cardiac amyloidosis, in order to refer them to the appropriate healthcare pathway.



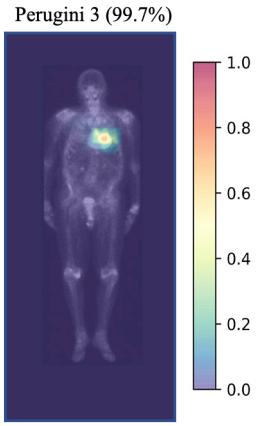


Figure 1 : ROC curve of the external validation dataset.

Figure 2 : Heatmap of the activation zones on the external validation dataset (L-UH): Highlighted area contributes the most to the positive prediction in the convolution layers. The heart area is clearly highlighted. Image predicted probability to be positive is indicated at the top of the image. At the right: probability scale.

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Support & Funding: The initiative for this study and its design were independent. It was funded by a research grant from Pfizer France.

Establishment of the nation-wide pathology consultation system for the typing diagnosis of amyloidosis in Japan: Steep increase in the number of transthyretin-positive cardiac biopsy cases

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Background: On March 26, 2019, the Ministry of Health, Labour and Welfare (MHLW) of Japan approved tafamidis for transthyretin (TTR)-related (ATTR) cardiomyopathy (CM). By referring to the inclusion criteria of the TTR amyloidosis CM clinical trial (ATTR-ACT), MHLW also announced the patient criteria to officially cover the cost of tafamidis therapy, which included the pathological confirmation of amyloid deposits on biopsy specimens and, in patients without *TTR* mutation, the immunohistochemical confirmation of TTR in amyloid deposits (1). Back in April 2018, the MHLW-funded group for surveys and research of amyloidosis in Japan (GSRA-J) started the nation-wide pathology consultation of amyloidosis (2).

Objective: To reveal the frequency of each amyloid subtype (AA, ALκ, ALλ, ATTR, Aβ2M, others) in Japan and to confirm that the approval of tafamidis for ATTR-CM has elicited interest in this disease among Japanese cardiologists, which increased the number of cardiac biopsy cases for the definite diagnosis of this disease.

Material & Methods: Nine institutes attended the consultation and shared the panel of anti-κ chain$_{116-133}$, anti-λ chain$_{118-134}$, anti-TTR$_{115-124}$ (rabbit polyclonal, see ref. 2), anti-AA (Dako) and anti-β2-microglobulin (Dako) antibodies. When the typing diagnosis was unavailable by immunohistochemical analysis, we performed proteomic analysis (LMD-LC-MS/MS) at Shinshu University, Kumamoto University, or at Nippon Medical School.

Results: From April 2018 to November 2021, we received 4085 consultation cases, of which 3428 cases were Congo-red positive (83.9%). We could determine the type of amyloidosis by immunohistochemistry in 3198 cases of Congo-red positive cases (93.3%). Of these 3198 cases, the incidences of AA, ALκ, ALλ, ATTR, Aβ2M and others were 119 (3.7%), 376 (11.8%), 960 (30.0%), 1669 (52.2%), 21 (0.7%), and 53 (1.7%), respectively. We performed proteomic analysis in 154 cases and could determine the type of amyloidosis in 108 cases. Totally, we could determine the type of amyloidosis in 3306 Congo-red positive cases (96.4%, 902 cases/year). From April 2018 to November 2021, we received 1641 cardiac biopsy cases, of which 1091 cases were ATTR positive (66.5%). As shown in Figure 1, the mean numbers of total and ATTR-positive cases per month were 17.2 and 10.0, respectively from April 2018 to March 2019, but dramatically increased to 64.8 (3.8-fold) and 43.8 (4.4-fold), respectively from December 2020 to November 2021.
These data indicate that the approval of tafamidis in Japan has elicited interest in ATTR-CM among Japanese cardiologists, which has increased the number of cardiac biopsy cases for the definite diagnosis of ATTR-CM. In March 2022, the Japanese Circulation Society summed up the number of tafamidis-prescribed ATTR-CM patients to be 1450 (ATTRwt 1348, ATTRv 102). Including the cases diagnosed by the biopsies of other organs/tissues (e.g., abdominal fatty tissue), our consultation system may diagnose most of these patients.

Summary & Conclusion: During the last 4 years, the incidence of ATTR amyloidosis cases exceeded 50% of all the consultation cases in Japan, mainly because the approval of tafamidis in Japan has elicited interest in ATTR-CM among Japanese cardiologists, which has increased the number of cardiac biopsy cases for the definite diagnosis of ATTR-CM. Our consultation system may diagnose most of the ATTR-CM patients treated with

tafamidis in Japan.

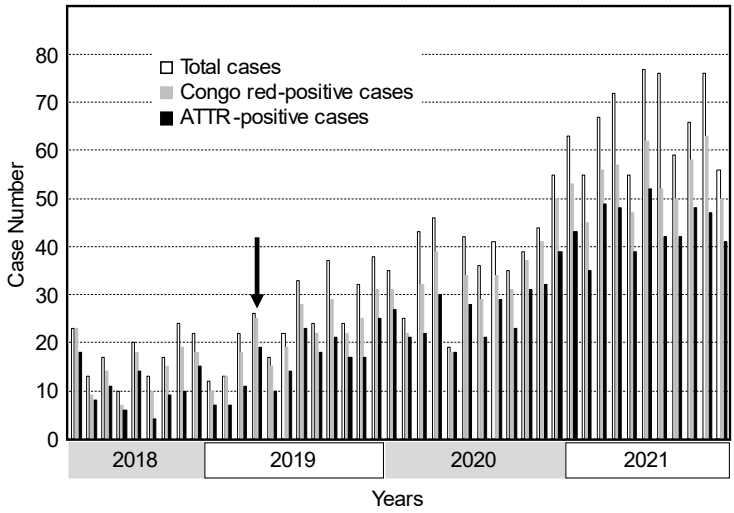

Figure 1.: The record of cardiac biopsy consultation by the group for surveys and research of amyloidosis in Japan from April 2018 to November 2021. Arrow: Approval of tafamidis for ATTR cardiomyopathy in Japan.

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Support & Funding: This work was supported in part by the Grant for surveys and research of intractable diseases from the Ministry of Health, Labour and Welfare, Japan (2017-2022, P.I. Naiki Hironobu).

The amyloid proteome: a two-way approach for a protein classification system

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Background: It is of utmost importance to determine the misfolded protein in amyloidosis to provide a proper diagnosis and treatment. Misfolded proteins can be identified by either immunohistochemistry or mass spectrometry techniques. While a listing on known amyloid proteins is consecutively updated [1], reports on disease-specific, amyloid-associated compounds are more scattered. Despite that, amyloid-associated compounds might be the key to understand disease mechanisms of a complex protein misfolding disease. Therefore, we need to take the amyloid "environment" and the deposits' composition into focus. This could enable researchers and clinicians to find new biomarkers for earlier diagnosis and putative druggable targets.

Objective: Several research groups have already reported a number of amyloid-associated proteins. Now, we want to systematically explore the amyloid composition, categorize proteins found in amyloid deposits including known amyloid-associated compounds, and point to previously underestimated players in the pathology of amyloid. Therefore, we used a two step bioinformatical and experimental process to classify the amyloid proteome.

Material & Methods: First, we performed a comprehensive literature research on published liquid chromatography and tandem mass spectrometry (LC-MS/MS) data that were obtained for diagnostic purposes [2]. This literature research used data on the relative abundance of proteins found in various amyloidosis- and tissue-types. Protein data was collected, sorted, and explored using R, and finally classified into amyloid protein categories (APCs). Second, we analyzed formalin-fixed and paraffin-embedded carpal tunnel tissue samples containing ATTR from patients with carpal tunnel syndrome. Consecutive tissue sections were divided into amyloid-enriched and -depleted samples and forwarded to bottom-up LC-MS/MS [3]. Label-free intensity profiling (LFIP) identified proteins correlating to the ATTR abundance. GO enrichment analysis provided information on potentially active pathways in ATTR of carpal tunnel tissue.

Results: Our literature review proposes an amyloid categorization into four APCs: 1) the fibril-forming protein found in the patient; 2) potential fibril-forming proteins found in other types of amyloidosis; 3) amyloid-associated proteins, e.g., signature proteins and pathologically relevant proteins; 4) tissue constituents or locally accumulated proteins due to amyloid formation. Using LFIP on LC-MS/MS data has shown to be a sophisticated experimental approach to classify amyloid components. This technique depicted, which components were linked to the ATTR amount, including low abundant molecules that might have been overlooked. Interestingly, many components of the complement system, an innate immune defense mechanism, correlated significantly to the amount of ATTR, as well as many apolipoproteins. GO-enrichment analysis confirmed enrichment of high density lipoproteins, complement-associated proteins, and others.

Summary & Conclusion: Both, our bioinformatical and experimental approach, demonstrate that amyloid deposits present a complex mixture of many interconnected components that surely play an important role in amyloid pathogenesis. We can observe distinctive and repeating protein patterns depending on the amyloid- and tissue-type. Their interconnection still remains to be unraveled, therefore, these results hopefully encourage further research into amyloid-associated compounds to advance diagnosics as well as patient management by discovering novel, putatively druggable targets.

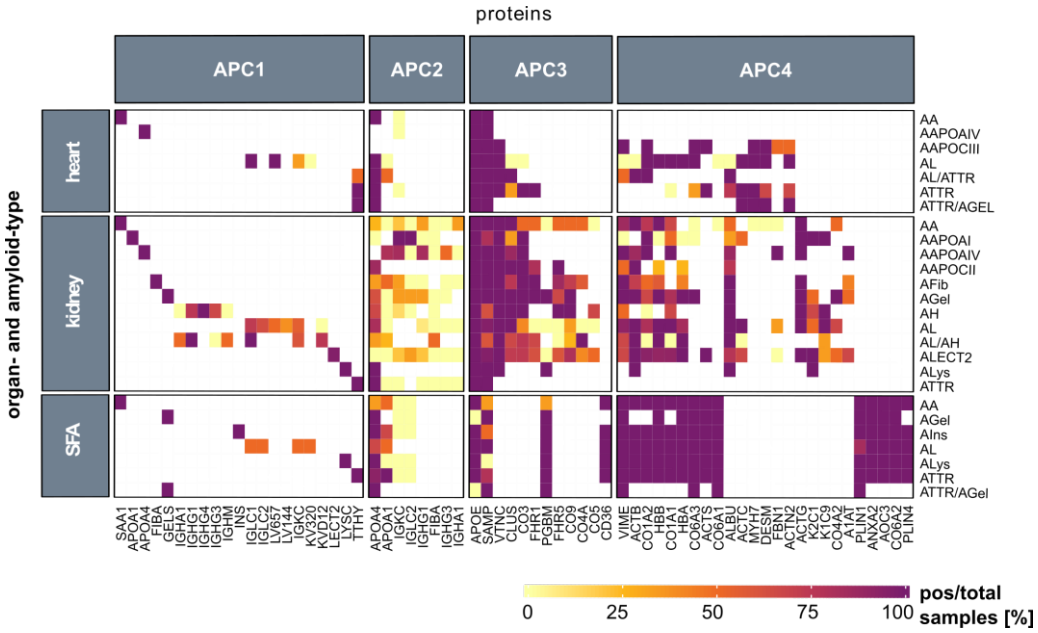

Figure: Protein distribution among heart and kidney tissues and SFA samples. Proteins are categorized into amyloid protein categories (APCs) according to their occurrence in one, two, or three of the respective tissue types. Protein names refer to the UniProt entry name, and only human proteins are listed. Each heatmap cell is colored based on the ratio comparing the number of positive samples to the total number of samples tested per protein (bottom) for the corresponding organ- (left) and amyloid-type (right). White cells correspond to missing values. Proteins are arranged according to the protein with the highest ratio for all amyloid types and organs analyzed (adapted from [2]).

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Support & Funding: This study was supported by the Germany Research Foundation (FOR 2969).

Fat aspiration as a screening tool for symptomatic systemic amyloidosis - sensitivity and specificity analysis with better sensitivity results for females with all major subtypes

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Background: The diagnosis of systemic amyloidosis (SA) requires the positive staining of a tissue specimen with Congo red. For subcutaneous (abdominal) fat aspiration substantial variability for sensitivity and specificity has been reported. Furthermore, sex specific differences in amyloid load have been described (1). We introduced the technique at the University Hospital Heidelberg in May 2013 with the support from the Amyloidosis Centre Groningen.

Objective: To evaluate sensitivity and specificity for amyloidosis screening with fat aspiration. Compare gender-specific differences for subtypes of SA.

Materials & Methods: For sensitivity and specificity analysis results from consecutive fat aspirates performed for amyloidosis screening between September 2013 and June 2020 were evaluated. Fat aspirates were graded according to (2). For sensitivity analysis patients with symptomatic SA proven by either histopathology or scintigraphy and for specificity analysis patients with clinical and/or histopathological exclusion of SA were chosen. Clinical data were extracted from our database. For simplification, deposits were classified as substantial when semi-quantitatively graded as 3+ or 4+ (>10% of analyzed tissue stains positive). All patients gave written informed consent in accordance with the Declaration of Helsinki. For analysis of differences in sensitivity and age, Pearson's chi squared test and the Kruskal-Wallis test were utilized. P-values <0.05 were classified as significant.

Results: Probes from 2830 patients were analyzed during the time frame and graded as positive in 1320 cases, while deposits were classified as substantial 686 times.
An overall sensitivity of 70% was achieved for patients with proven SA. For systemic light-chain (AL) 90.3%, for transthyretin wild-type (ATTRwt) 18.8%, for AA 72.3% and for transthyretin variant (ATTRv) amyloidosis a 71% sensitivity rate was achieved (see Table 1).
Sensitivity for detection of amyloid overall and in AL, ATTRwt, AA and ATTRv was significantly better in females. Furthermore, substantial amyloid deposits were significantly less common in males overall and in AL. Differences were also noted between AL subtypes as sensitivity for lambda was significantly better compared to kappa ($p<0.00001$).
The specificity group compromised 617 probes amongst which four were graded as positive for a specificity of at least 99.4%. Minimal deposits were found in a man with localized AL skin, in a man with localized AL GI tract with insulin dependent diabetes and in an elderly man without cardiomyopathy or neuropathy. Substantial deposits were once detected in an elderly man and not found on follow-up most likely related to probe mislabeling.
We detected amyloid in the group with clinically certain SA without further histopathological proof in 110/114 and within the group with clinically possible SA in 16/398 cases.
Overall, the male SA cohort was significantly older compared to the female cohort, resulting from the ten-fold larger male ATTRwt cohort (median age 68 vs. 64,3 years, $p<0.0001$). Amongst subgroups median age for females within AA (51,7 vs. 60,2 years, $p=0.00024$) and ATTRwt (76,2 vs. 80 years, $p=0.015$) was significantly higher.

Summary & Conclusion: Fat aspiration for symptomatic SA yielded excellent specificity and sensitivity results especially in AL, ATTRv and AA. Sensitivity was even better in females. We hypothesize that women might deposit more amyloid in their fat tissue or are able to tolerate more amyloid (AL), rarely become symptomatic (ATTRwt) or only at a higher age (AA, ATTRwt).

Table 1: Percentages with numbers in brackets for detection of amyloid and substantial deposits for all patients, male and female sex for groups and subgroups. p-values for differences between male and female sex for groups/subgroups regarding detection rate and substantial deposition rate. Significant differences in bold.

	Amyloid detected						p-Value	
	ALL	**Substantial**	**MALE**	**Substantial**	**FEMALE**	**Substantial**	**detection**	**substantial**
Systemic amyloidosis	70.0 (1190/1701)	36.2 (616)	**62.1 (719/1158)**	**31.1 (360)**	**86.7 (473/543)**	**47.1 (256)**	**<0.00001**	**<0.00001**
Systemic AL amyloidosis	90.3 (922/1023)	55.5 (568)	**88.0 (558/634)**	**52.4 (332)**	**93.6 (364/389)**	**60.7 (236)**	**0.0038**	**0.0095**
lambda subtype	92.9 (729/785)	59.4 (466)	**91.5 (452/494)**	**56.5 (279)**	**95.2 (277/291)**	**64.3 (187)**	0.052	**0.032**
kappa subtype	81.8 (189/231)	44.2 (102)	**77.0 (104/135)**	**39.3 (53)**	**89 (85/96)**	**51 (49)**	**0.025**	0.076
treatment naive	91.4 (724/792)	58.0 (459)	**89.3 (441/494)**	**54.3 (268)**	**95.0 (281/297)**	**64.1 (191)**	**0.0056**	**0.0066**
lambda subtype	94.1 (571/607)	61.0 (370)	**92.4 (355/384)**	**58.1 (223)**	**96.9 (216/223)**	**65.9 (147)**	**0.026**	0.056
kappa subtype	80.7 (146/181)	49.2 (89)	**75.9 (82/108)**	**41.7 (45)**	**88 (64/73)**	**60 (44)**	**0.0497**	**0.014**
Systemic ATTR wt amyloidosis	18.8 (69/368)	0.8 (3)	**16.8 (56/334)**	0.6 (2)	**38 (13/34)**	3 (1)	**0.0022**	0.18
Systemic ATTRv amyloidosis	71 (63/89)	29.2 (26)	**66 (46/70)**	26 (18)	**90 (17/19)**	42 (8)	**0.043**	0.16
Systemic AA amyloidosis	72.3 (107/148)	11.5 (17)	**60 (41/68)**	10 (7)	**83 (66/80)**	13 (10)	**0.0026**	n.s.
treatment naive	85 (53/62)	21 (13)	**73 (16/22)**	23 (5)	**93 (37/40)**	20 (8)	**0.034**	n.s.
Systemic amyloidosis clinically	96.5 (110/114)	60.5 (69)	97 (59/61)	61 (37)	96 (51/53)	60 (32)	n.s.	n.s.
clinically systemic AL type	100 (71/71)	76 (54)	100 (32/32)	84 (27)	100 (39/39)	69 (27)	n.a.	0.14
clinically systemic ATTRv	90 (26/29)	41 (12)	90 (18/20)	35 (7)	89 (8/9)	56 (5)	n.s.	n.s.
Specificity group	0.6 (4/617)	0.2 (1)	1.2 (4/343)	0.3 (1)	0 (0/274)	0 (0)	n.s.	n.s.
Systemic amyloidosis possible	4.0 (16/398)	0 (0)	3.8 (7/247)	0 (0)	6.0 (9/151)	0 (0)	0.12	n.a.

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Sequence of diagnostic testing in cardiac amyloidosis patients: early monoclonal protein study is associated with better outcomes in AL amyloidosis.

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Background: Isolated cardiac amyloidosis (CA) is a complex diagnostic scenario, highlighting the need for physician awareness in differential diagnosis. Among the different types of amyloidosis, nearly all cases of CA are caused by light chain (AL) and transthyretin amyloidosis (ATTR). Patients with suspect CA without monoclonal components (MC) in both serum and urine and normal free light chain (FLC) ratio can have a non-biopsy diagnosis of ATTR amyloidosis with bone scintigraphy (1). However, in all suspected CA with a MC (approximately 20% of patients with ATTR amyloidosis), amyloid typing is mandatory. Thus, the diagnostic pathway of CA diverges based on MC-studies.
Objective: To assess if different sequence of diagnostic tests can affect outcomes in patients with cardiac AL amyloidosis.
Material & Methods: Pavia Amyloidosis prospectively maintained database was searched for patients with isolated cardiac AL amyloidosis referred to our Centre from January 2016 to December 2020. Patients with a known monoclonal gammopathy (MG) and with multiple organ involvement were excluded.
We searched for the date of symptom onset and first suspect of CA (i.e. recognition of clinical, imaging or laboratory signs of CA). In addition, we recorded the date of the different diagnostic tests performed: i.e. echocardiogram; serum and urine immunofixation and FLC measurement (MC-study); bone scintigraphy and cardiac magnetic resonance (defined as advanced cardiac imaging). We calculated the interval between those time-points and the final diagnosis: (a) from symptom onset to diagnosis, (b) from first suspect to final diagnosis, (c) from first suspect to MC-study and (d) to advanced cardiac imaging tests. We then searched for possible intervals of time amongst those, that were able to predict death at 3 months, by means of a Receiver Operating Characteristic (ROC) analysis.
Results: A total of 94 patients were included in the analysis (25% of all patients with cardiac AL amyloidosis diagnosed in the study period). Clinical data are listed in Table 1. Six (6%) patients died <1 month from diagnosis, and 27 (29%) died <3 months. Median overall survival (OS) of the whole cohort was 8 months, and the median follow-up of living patients was 39 months (range 16-73). The median time from symptom onset to diagnosis was 9 months (range 1-44) and the median time from the first suspect to diagnosis was 2 months (range 0-9). An interval from the first suspect to MC-study ≥6 weeks was the only predictor of death at 3 months. None of the other tested periods were associated with a significant ability to predict survival. A delay in MC-study ≥6 weeks identified patients with more advanced cardiac stage (50% vs. 25% were in stage IIIb, P=0.02) and was associated with a significantly worse outcome (median survival 13 months vs. 4 months, P=0.012, Figure 1). In the whole cohort, a total of 76 (81%) patients underwent at least one advanced imaging examination. Amongst those, 37 (49%) performed the imaging tests before MC-study with a higher percentage of patients who had a delay in MC-studies evaluation (69% vs. 27%; P<0.001).
Summary & Conclusion: In patients who present with isolated cardiac AL amyloidosis with previously unknown MG, a relatively short delay in identifying the amyloid MC results in a considerable reduction of survival. A delay in MC-study was associated with more advanced cardiac stage. MC studies should be the first step in the work-up of patients with suspected CA to guide biopsy vs. non-biopsy diagnostic approach.

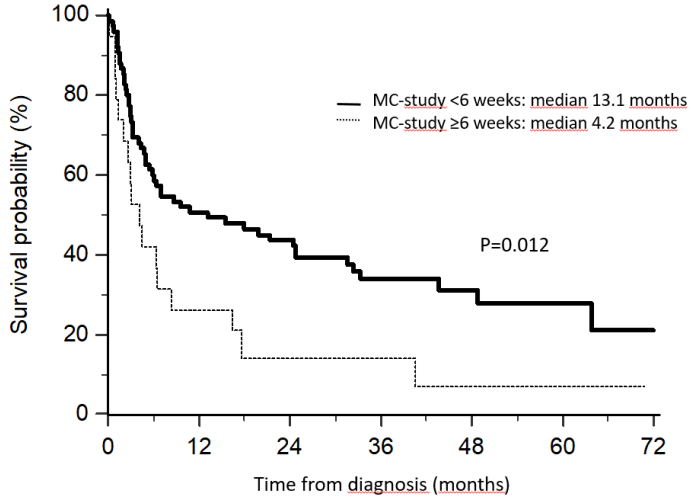
Table 1. Patients' characteristics at diagnosis.

Variable	Total (N=94)	MC-study<6 weeks (N=68)	MC-study≥6 weeks (N=26)	P
Age at diagnosis, years	65 (57-72)	65 (59-73)	64 (57-72)	0.91
Male/female - n. (%)	67/28 (70/30)	47/21 (70/30)	19/7 (73/27)	0.87
NYHA class				0.91
I	5 (5)	2 (3)	3 (10)	
II	54 (57)	42 (62)	46 (58)	
III	35 (38)	24 (35)	11 (42)	
IV	0 (0)	0 (0)		
Systolic blood pressure – mmHg	117 (109-129)	117 (109-129)	118 (97-138)	0.74
cTnI – ng/mL	0.113 (0,055-0,248)	0.105 (0,052-0,278)	0.159 (0,06-0,241)	0.42
NT-proBNP – pg/mL	7475 (4545-13551)	5953 (4255-11547)	10720 (5448-23100)	0.03
eGFR, mL/min	64 (42-88)	69 (42-69)	60 (45-83)	0.44
dFLC (mg/L)	339 (178-562)	292 (178-586)	353 (176-551)	0.99

Cardiac Stage				0.04
II	44 (47)	35 (52)	9 (35)	
IIIa	20 (21)	16 (23)	4 (15)	
IIIb	30 (32)	17 (25)	13 (50)	
IVS – mm	16.1 (14-18)	15.3 (14.9-18.8)	16.1 (14-18)	0.65
PWT – mm	15 (13-16.6)	15 (13.6-16.6)	15 (12.7-16.6)	0.45
LVEF - %	55 (48-60)	54 (45-60)	55 (48-60)	0.29
Interval between - months				
Symptom onset and diagnosis	9 (6-14)	8 (5-13.75)	10 (7-22.5)	0.07
Symptom onset and first suspect	5 (2-13)	5 (2-12)	7 (3.5-22)	0.18
First suspect and diagnosis	2 (1-4)	2 (1-3)	3 (2-4)	0.52

NYHA, New York Heart Association, cTNI, cardiac Troponin I, NT-proBNP, N-terminal fragment of brain-type natriuretic peptide, eGFR, estimated Glomerular Filtration Rate, dFLC, difference between involved minus uninvolved serum free light chains, IVSd, interventricular septum, PWT, posterior wall thickness, LVEF, left ventricular ejection fraction. Data expressed as median (range) or as number of patients (%).

Figure 1.: OS stratified by MC-study assay <6 weeks or ≥6weeks



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Artificial intelligence modeling for earlier identification of cardiac amyloidosis.

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Background: Cardiac amyloidosis (CA) is a life-threatening infiltrative cardiomyopathy including 3 main types: immunoglobulin light chain (AL), wild-type transthyretin (ATTRwt), and hereditary transthyretin (ATTRv). Diagnosing CA is challenging as clinical signs and symptoms are non-specific and the disease is rare. Our group has developed an artificial intelligence (AI) model using a standard 12-lead electrocardiogram (ECG) to raise suspicion of CA to facilitate earlier diagnosis of CA but the impact of models using transthoracic echocardiogram (ECHO), a preferred modality for diagnosing CA, is unknown (1).

Objective: To develop an ECHO-based model to diagnose CA and fuse it with the AI-ECG model to improve the overall predictive performance of the models.

Material & Methods: A total of 2,346 patients with CA and 11,732 age- and sex-matched controls were identified from the Mayo Clinic institutional amyloid database from January 1, 2000 to May 31, 2019 (1). ECHO variables associated with the diagnosis of CA were selected a priori and then retrospectively collected for patients with known CA. Variables with more than 25% missing data were excluded. Thirty-five ECHO variables were included and the entire data set was split into 50:50 as training:validation datasets (i.e., “extended” model). We used the training dataset to make random forest and regression tree-based “ensemble” models to distinguish between ATTR vs. controls and AL vs. controls. Using the most impactful ECHO variables to predict CA from controls, “simplified” models were created, trained and then validated on the validation set (**Figure 1**). Next, we combined the simplified ECHO with the AI-ECG score sets (ECHO+ECG) and trained and validated these models. Receiver operating characteristic area under the curve (AUC) were determined for each model.

Results: Overall, 2,346 CA cases (1,666 AL and 684 ATTR patients) and 11,732 controls were included and divided equally into training and validation datasets. The ensemble models had high performance in detecting AL and ATTR cases from controls on the validation set (**Table 1**). The simplified ECHO AL and ATTR models had improved performance in distinguishing cases from controls on the validation set (**Figure 1**). When AI-ECG score was added to the simplified variable ECHO models, the performance of both AL and ATTR models improved (0.9249 to 0.9455 and 0.9601 to 0.977, respectively) (**Table 1**). When compared to ATTR AI-ECG model, the performance of the combined ATTR ECHO+ECG model was significantly better (0.9503 vs 0.977, p<0.001). Similarly, AL ECHO+ECG model performed significantly better than AL AI-ECG only model (0.9348 to 0.9455, p<0.001).

Summary & Conclusion: ECHO variables had very high predictive value for recognizing CA cases from controls, as evident from high AUC of these models. The addition of AI-ECG score to ECHO models further improved the performance for both AL and ATTR CA. This has important implications for early identification and treatment of patients with CA using AI-enhanced algorithms.

Figure 1. Ensemble-based models for “simplified” ECHO variable validation cohort. AL vs controls (left); ATTR versus controls (right).

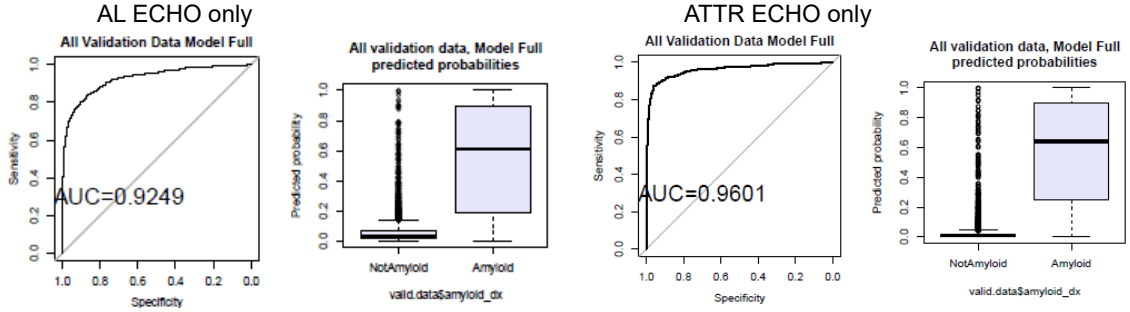

Table 1: AI algorithm performance by cardiac amyloidosis subtype comparing extended and simplified AI-ECHO variable models validation cohort.

	Ensemble ECHO Model Performance	Algorithm Performance for Simplified AI-ECHO		
	ECHO Only AUC	ECHO Only AUC	ECG Only AUC	ECHO + ECG AUC
AL	0.8952	0.9249	0.9348	0.9455
ATTR	0.9428	0.9601	0.9503	0.977

Notes: AL – Immunoglobulin light chain amyloidosis; ATTR – transthyretin amyloidoisis; Receiver operating characteristic area under the curve (AUC). N=833 AL, 342 ATTR and 5,866 control patients.

Simplified variable set for AL: relative wall thickness (RWT), pericardial effusion, medial E/e' velocity, interventricular septum thickness (IVS), posterior wall thickness (PW), aortic valve peak continuous wave velocity (Avpeak), cardiac index (CI), systolic blood pressure (SBP) and weight. *Simplified variable set for ATTR:* RWT, IVS, PW, medial E/e' velocity, Avpeak, CI, SBP, sex and weight.
ECHO + ECG models used the same variables with addition of AI-ECG amyloid score.

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Drs Grogan, Lopez-Jimenez, Dispenzieri, Attia, Abou Ezzeddine, Friedman, and Murphree and Mayo Clinic have licensed the algorithm described in this work to Anumana and may benefit from its commercialization.
The remaining authors have nothing to disclose.

A novel mass spectrometry-based method for the identification of subtype specific amyloidogenic proteins from fat aspirates

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Background: Mass spectrometry-based proteomics (MS) is a powerful technology for large-scale analysis of proteins and has become the method of choice for the detection of amyloidogenic protein in a quantitative manner in organ biopsies and fat aspirates, and has become the preferred method for amyloid subtyping at several amyloidosis centres. MS does not only identify the subtype-specific protein but also measures an amyloid protein signature that is shared across all amyloidosis subtypes in various tissues. While organ biopsies primarily are used for the subtyping of localized amyloidosis, the examination of fat aspirates using Congo Red staining and mass spectrometry-based assays has proven to be a practical and sensitive method for the diagnosis of systemic amyloidosis. Where sampling of the fat aspiration is quite simple and fast, the downstream sample preparation process before the mass spectrometric analysis is complicated and labour-intensive, and includes many labour intensive steps, such as several de-lipidation steps and overnight dialysis[1-2] before the further downstream processing steps that include protein isolation, reduction, methylation and cleavage using trypsin. This is not compatible with the workflows and response times required in a clinical chemistry laboratory.

Objective: In present work, we therefore developed a fast, simple and robust sample preparation procedure and combined it with a novel MS acquisition method for the fast and highly precise detection of amyloidogenic proteins in fat aspirates.

Materials & Methods: Fat aspirates from 110 patients with suspected systemic amyloidosis were Congo Red (CR) stained, whereof 47 were CR positive. The 63 patients with CR negative fat aspirates serving as controls in present study were ruled out by further analysis. Fat aspirate samples were prepared for MS analysis using a novel microwave-assisted 2-step digestion procedure employing lysozyme C and trypsin. Samples were analysed by mass spectrometry using a novel combined data dependent acquisition (DDA) method and a parallel reaction monitoring (PRM) method by exploiting the unique features of a Thermo Eclipse Tribrid mass spectrometer. DDA data were analysed using Proteome Discover 2.4 (Thermo Scientific), whereas PRM data were analysed using Skyline (https://skyline.ms/wiki/home/software/Skyline/page.view?name=default). Statistical analysis of the data from the MS analysis was done in R statistical software. Identification of amyloid-associated signature proteins was done by the Student's t-test ($p<0.05$) and the Boruta feature selection method whereas proteins for data normalization were identified using a non-parametric test. Peptides for PRM analyses were selected after manual inspection of DDA data.

Results: In this study, we developed a sample preparation method to analyse fat aspirate samples from patients with systemic amyloidosis. Protein digestion prior to mass spectrometry analysis was reduced to less than 60 minutes using a microwave-assisted digestion protocol, and total analysis time – from sample receipt, LC-MSMS analysis and database search to diagnosis - was reduced from days to less than four hours. Using this protocol we retrospectively analysed 110 fat aspirates collected from patients suspected with systemic amyloidosis, whereof 47 were cases of systemic amyloidosis that were well-typed according to clinical diagnostic standards, that also included immune electron microscopy (IEM). A rare systemic amyloidosis case – insulin-derived amyloidosis – was also identified. Moreover, besides the well-known amyloid associated proteins ApoA4, ApoE, SAP, Clusterin and Vitronectin, several novel proteins not previously associated with systemic amyloidosis were also identified.

Summary & Conclusion: A novel microwave-assisted sample preparation method for amyloid subtyping with mass spectrometry was developed. Using this method, time from sample receipt to diagnosis could be reduced from days to less than four hours. We successfully subtyped 42 out of 47 cases included a rare InsA case. Moreover, several novel amyloid-associated proteins were also identified.

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Pre-symptomatic diagnosis of systemic AL amyloidosis by biomarker based screening in patients with MGUS.

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Background: Although increase circulating FLC precede the onset of AL amyloidosis symptoms by 4 years or more[1], in approximately 40% of AL amyloidosis patients the diagnosis is made more than 1 year after the onset of symptoms[2, 3]. Biomarkers [(N terminal pro natriuretic peptide (NT-proBNP) for the heart, albuminuria for the kidney, and alkaline phosphatase (ALP) for the liver] can detect asymptomatic organ involvement thus allowing early diagnosis. Thus, we advocated the screening with biomarkers of amyloid organ involvement of all patients with monoclonal gammopathy of undetermined significance (MGUS) and abnormal FLC ratio.[4,5]

Objective: to present the clinical features and outcome of patients with MGUS diagnosed with AL amyloidosis while asymptomatic during biomarker-based screening.

Material & Methods: In 2012, we started a biomarker-based screening program in all patients evaluated at the Division of Hematology of Pavia for MGUS. Patients with at least one abnormal biomarker were evaluated at the Amyloidosis Center for a possible diagnosis of amyloidosis.

Results: Twenty-two patients were identified between January 2012 and December 2020. This representing 1.6% of the patients followed for MGUS in the study period corresponding to an incidence of 2.5/1000 person-year. A positive biopsy for AL amyloid deposits was detected: abdominal fat pad aspirate in 18 (82%), minor salivary gland biopsy and organ biopsy (kidney) in 2 (9%) patients respectively. Eight were males and their median age was 67 years (range 38-79 years). The median time from the identification of MGUS to the diagnosis of AL amyloidosis was 3.6 years (range 0.8-22 years). The monoclonal components were IgAλ in 5 patients, IgDλ in 1, IgGλ in 10, and IgMλ in 3 and light-chain only in 3. The median dFLC value was 67 mg/L (range 21-283 mg/L). The biomarker found abnormal during follow-up was proteinuria (median 1.14 g/24h, range 0.8-2.5 g/24h, predominantly albumin) in 12 patients, NT-proBNP in 10 (median 789 ng/L, range 350-2935 ng/L), and ALP in 1 (509 U/L, upper reference limit 279 U/L). Six patients had more than 1 elevated biomarker. Cardiac involvement patients were stage II (n=7) and stage IIIa (n=3). Median left ventricular wall thickness was 13.2 mm (range 10.0-14.8 mm) and median ejection fraction was 62% (range 50-70%) in patients with elevated NT-proBNP. Estimated glomerular filtration rate was >50 mL/min in all patients with proteinuria, resulting in renal stage I in all. One patient with cardiac amyloidosis died of ischemic heart disease 1 year after diagnosis, 1 patient died due to stroke and 1 for bowel rupture due to diverticulitis (no amyloid deposits were found by histological evaluation). All other patients are alive and none required dialysis after a median follow-up of 36 months. Eight patients received cyclophosphamide/bortezomib/dexamethasone (CyBorD), 4 bortezomib/melphalan/dexamethasone, 4 MDex, and 2 bortezomib/rituximab/dexamethasone and bendamustine and rituximab in 1 case. 14 patients obtained organ response associated with complete or very good partial and 2 obtained PR. The remaining 6 subjects responded to second-line therapy.

Summary & Conclusion: In this patient population, the rate of progression from MGUS to AL amyloidosis was 2.3/1000 person-year and biomarker based screening allowed recognition of the disease at a pre-symptomatic stage in all cases. This allows achieving a satisfactory response at first or second line therapy in all patients and only 1 patient died with heart failure also had concomitant ischemic heart disease.

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Kinetics of the aggregation process of the human λ-III immunoglobulin light chain FOR005 involved in AL amyloidosis at atomic resolution

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Background: Light chain amyloidosis (AL) disease is caused by abnormal proliferation of monoclonal plasma cells resulting in high concentration of aggregation prone light chains in serum. These light chains get deposited in various organs of the body, particularly in the heart, kidney, spleen etc., leading to organ failure and eventually death.

Objective: Characterization of the molecular mechanism of misfolding of the VL (variable light chain) fragment of the AL amyloidosis patient FOR005, and molecular understanding of the role of mutations in the native monomeric and fibrillar state.

Material & Methods: We recombinantly expressed isotopically labeled native VL protein coding for the patient as well as of the closest germline sequence. We obtained fibrils employing a seeding protocol involving ex-vivo material. We characterized these proteins using solution-state as well as MAS (Magic Angle Spinning) solid-state NMR. Other biophysical methods like ThT fluorescence, CD, TEM and DLS are used to get complementary information.

Results: Using solution-state NMR, we have structurally characterized early aggregation intermediates in the misfolding of the light chain variable domain of the patient FOR005 implicated in AL-amyloidosis. For the first time, we are able to understand the individual steps involved in protein misfolding at atomic resolution. We show how the natively folded protein first partially unfolds, before it converts into a high molecular weight random coil structure that is stabilized by electrostatic interactions. The oligomer facilitates high local concentrations of aggregation prone regions which are subsequently able to convert into amyloid fibrils. From MAS solid state NMR, we find that residues 11-42 and 69-102 adopt β-sheet conformation in patient VL fibrils [1]. Fibrils from the GL protein and from the patient protein harboring the single point mutation R49G can be both heterologously seeded using patient ex-vivo fibrils. Seeded R49G fibrils show an increased heterogeneity in the C-terminal residues 80-102, which is reflected by the disappearance of all resonances of these residues [2].

Summary & Conclusion: Misfolding occurs via unfolding of the natively folded state follwed by oligomerization. The mutation R49G induces a conformational heterogeneity at the C-terminus in the fibril state, whereas the overall fibril topology is retained.

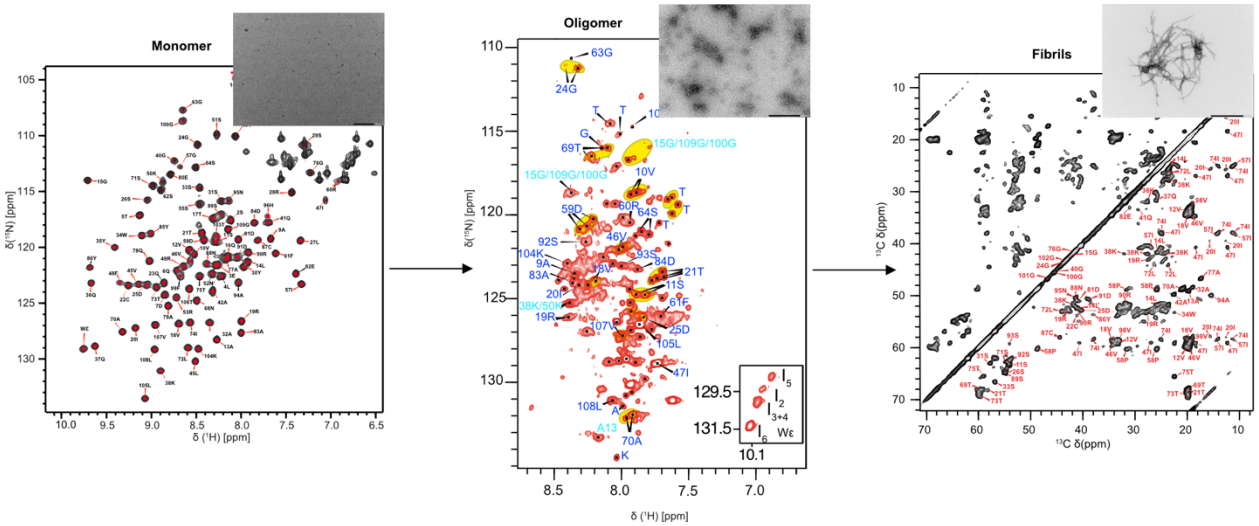

Figure 1: Characterization of misfolding of FOR005 using solution-state and MAS solid-state NMR. The monomeric natively folded protein (left) is converted into oligomers (middle), and subsequently into fibrils (right).

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The *ex vivo* cryo-EM structure of AA amyloid from a domestic short hair cat

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Background: In systemic AA amyloidosis, misfolded serum amyloid A protein (SAA) accumulates in deposits of cross-β amyloid in multiple organs of humans and animals [1]. Amyloids occur at high SAA serum concentrations during chronic inflammation. Prion-like transmission may lead to extreme disease prevalence in captive animals, e.g. 70% in cheetah kept in zoos [2]. In a recent clinical-histopathological study, we have reported an amyloidosis prevalence of 60% among 80 domestic short hair (DSH) cats kept in three independent shelters in Northern Italy.

Objective: To determine the molecular structure of AA amyloid extracted from the kidney of a DSH cat deceased with renal failure.

Material & Methods: We applied histological and immuno-histochemical staining as well as LC-MS/MS to identify AA amyloid in spleen, kidney and liver tissue of a DSH cat. Amyoid was extracted from the kidney and blotted on grids in liquid ethane using a Vitrobot. Single particle cryo-EM data were collected on a Talos Arctica 200 kV. Helical reconstruction[3] yielded a map with a nominal resolution of 3.3 Å. The model was built *de novo* starting from an unusual backbone bulge identified as $P_{66}GGAW_{70}$ in the LC-MS/MS-identified amino acid sequence.

Results: The fibril is composed of two identical 76-residue long proto-filament chains, each harboring an unusual backbone bulge with a *cis*-Proline. Two twisted proto-filaments with parallel in-register sheets are stabilized through staggered ionic lock and hydrophobic cluster interactions. Each chain adopts an extended hairpin structure with a central β-arch that orients two ~25 residue long, non-planar meandering tails to stick together via side chain contacts. Although >70% sequence homologus to human and mouse SAA[4], the cat's amyloid fold seems unrelated, exhibiting weak type-2 polymorphism. Its unique eight-residue insert contributes to an extended inter-protomer interface, rendering the cat's AA amyloid the most stable assembly. Shared disease profiles and almost identical AA amyloid sequences in cat and cheetah suggest a similar amyloid fold, which may be associated with increased prion capacity.

Summary & Conclusion: The presented cryo EM structure is unique in representing the first *ex vivo* structure of spontaneously occurring amyloid obtained from an animal kept in the stressful environment of a man-made habitat. Based on shared disease profiles and almost identical fibril sequences, we hypothesize that cat and cheetah may accumulate AA amyloid of similar structure and increased prion capacity, revealing itself in crowded shelter and zoo populations.

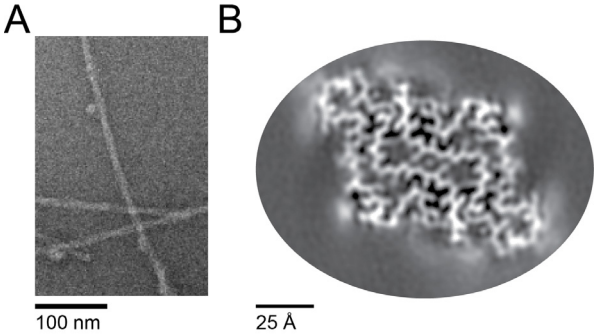

Figure 1.:
3.3 Å resolution cryo-EM structure of AA amyloid from a cat's kidney
(A) Micrograph of negative-stained fibril extracted from the kidney.
(B) Cross-sectional slice through the reconstructed map as obtained from Relion. Two identical chains are related by C2 symmetry.

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The interplay between protein dynamics and proteolysis in LC amyloid aggregation

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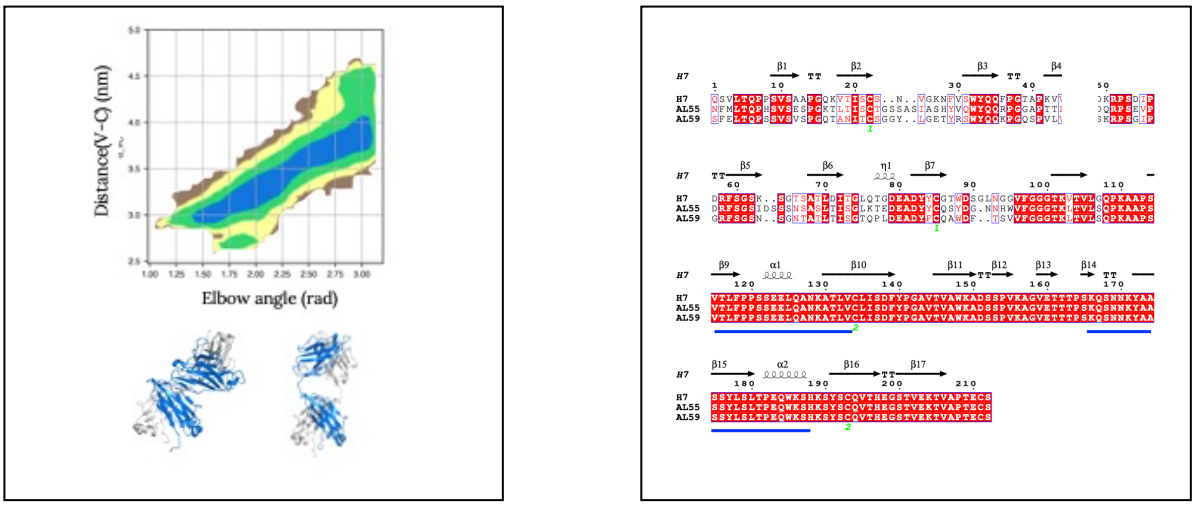
Background: Light chain amyloidosis (AL) is caused by sustained overproduction of amyloidogenic light chains (LCs) by bone marrow B-cells. The resulting high LC concentration results in the formation of amyloid deposits in heart and other target organs. Protein dynamics has been reported to positively correlate with amyloidogenicity: typically amyllidogenic LCs are more dynamics compared with non-amyloidgenic ones[1]. Amyloid deposits ex vivo are heavily proteolysed[2,3].

Objective: Here we aim to shed light on the molecular properties and molecular events necessary for the aggregation of amyloidogenic LCs, particularly focusing on protein dynamics and proteolysis.

Material & Methods: Protein dynamics of clinically characterised LCs has been performed *in vitro* by limited proteolysis of purified LCs and *in silico* by molecular dynamics simulations combined with Small Angle X-ray Scattering (SAXS). Proteolytic pattern of amyloid deposits extracted from cardiac tissue of AL patients has been analysed by mass spectrometry and compard with available Cryo-EM structures of ex vivo amyloid firbils.

Results: The kinetics of proteolytic digestion by model proteases such as tripsin or protease K depend on protein rigidity, being the most flexible proteins the fastest to be digested. In this way we and others showed that amyloidgenic LCs typically display an increased flexibility compared to control LCs [1,4,5]. Recently we collected SAXS data of homodimeric LCs and such data were fed into molecular dynamics simulations describing the overall dynamics of amyloidogenic and non-amyloidogenic LCs. While short range fluctuations are comparable in all LCs, long range flexibility is increased in amyloidogenic LCs.
Amyloid deposits have been extracted from several patients and multiple organs of the same patient, then they have been compared with their native homodimeric structures and with the Cryo-EM structures of their fibrillar assembly. In all cases full length LCs have been observed and proteolytic hot spots in the constant domain are reproducibly identified.

Summary & Conclusion:
Our data indicate that flexibility is an important trait of amyloidogenic LCs. Even if variable domains are the LCs regions which are changing between amyloidogenic and non-amyloidigenic LC variants, the most prominent differences in LC dynamics are in the long range interactions and not in the intradomain dynamics. The analysis of LCs proteolytic pattern in ex vivo deposits shows that proteolytic cleavage occurs all along the LC sequence; regions folded in the fibrillar core are often protected.



Figure

Figure 1.: (Above) Conformational ensembles displaying interdomain dynamics for an amyloidogenic LC; (below) two conformation of an homodimeric LC.

Figure 2.: Multialignment of three independent amyloidogenic LCs whose deposits have been extracted from cardiac tissue of AL patients and their proteolytic pattern was studied. On top secondary structure elements for native LCs are shown; blue lines indicate proteolysis hot spots identified in the constant domains in all analyzed deposits. Proteolytic pattern observed in variable domains strongly varies from patient to patient.

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Time-resolved nano-spectroscopy with single-molecule sensitivity for blood-based non-immune diagnosis of amyloid diseases

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Background: Misfolding and self-assembly of proteins and peptides into oligomers, their subsequent transformation into fibrilar aggregates enriched in β-sheet secondary structure, so-called amyloid aggregates, and sedimentation in the diseased organ as they grow large enough and fold so that their density becomes higher than that of the surrounding medium, is a hallmark of amyloid diseases. There are over 40 diseases, such as Alzheimer's disease, Parkinson's disease, type II diabetes, systemic amyloidodis *etc.*, which are associated with amyloid formation. While the protein that forms the amyloid material is different in different amyloid diseases, the β-sheet enriched secondary structure of the amyloid fibrils is similar in all of them. We have invented a method that can detect these structured amyloid aggregates enriched in β-sheet secondary structure, which we call nano-plaques, in biological fluids, most notably in blood serum, but also in cerebrospinal fluid (CSF). Our method has the ultimate, single-molecule sensitivity, and can simultaneously measure the concentration and size of nano-plaques [Tiiman et al 2015, Tiiman et al 2019].

Material & Methods: A new method for ultra sensitive detection of very low levels of amyloid nanoplaques in biological fluids is developed. The method detects fluorescence intensity bursts from the amyloid-sensitive fluorescence dye Thioflavin T (ThT) bound to nano-plaques using a time-resolved detector with single-photon sensitivity (Fig. 1). Individual structured nano-plaques are detected with single-molecule sensitivity (Fig. 1) and the number of peaks, which is a proxy for aggregate concentration, is counted using an automate routine [Tiiman et al 2019]. Temporal autocorrelation analysis of timeseries, as done in Fluorescence Correlation Spectroscopy (FCS), is performed to determine the nano-plaque size.

Results: Using our method, we have observed that nanoplaque levels are significantly increased both in the blood [Tiiman et al 2019] as well as in the CSF of patients with clinical AD [Aksnes et al 2020]. In addition, nanoplaque concentrations in the CSF were negatively associated with the concentration of Aβ42, but not related to total tau or phosphorylated tau measures [Aksnes et al 2021]. Finally, we have also observed that nanoplaque levels were negatively associated with several cytokines, in line with recent findings suggesting that the upregulation of some cytokine markers has a protective role and is negatively associated with Alzheimer's disease progression [Aksnes et al 2022].

Summary & Conclusion: Our method, which neither relies on the use of immune-based probes or radiotracers, nor on the use of signal-amplification or protein separation techniques, provides a minimally invasive test for a fast and cost-effective early determination of structurally modified peptides/proteins in the peripheral blood as well as in other biological fluids.

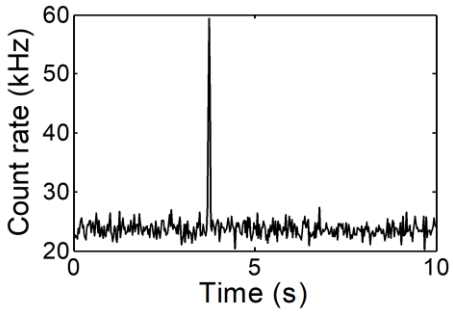

Figure 1. Time-resolved fluctuations in ThT fluorescence recorded in blood serum. The clearly identifiable peak in fluorescence intensity reflects the rare passage of a bright ThT-reactive structured amyloidogenic oligomer, i.e. a nanoplaque.

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In vitro and *in vivo* effects of SerpinA1 on the modulation of transthyretin proteolysis

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Background: Transthyretin (TTR) proteolysis has been recognized as a mechanism contributing to transthyretin-related amyloidosis (ATTR amyloidosis). Accordingly, amyloid deposits can be composed mainly of full-length TTR or contain a mixture of both cleaved and full-length TTR, particularly in the heart [1]. The fragmentation pattern at Lys48 suggests the involvement of a serine protease, such as plasmin [2]. Moreover, the most common TTR variant, TTR V30M, is susceptible to plasmin-mediated proteolysis, and the presence of TTR fragments facilitates TTR amyloidogenesis [3]. In addition, recent studies revealed that the serine protease inhibitor, SerpinA1, was differentially ex-pressed in hepatocyte-like cells (HLCs) from ATTR patients [4].

Objective: To evaluate the effects of SerpinA1 on *in vitro* and *in vivo* modulation of TTR V30M proteolysis, aggregation, and deposition.

Material & Methods: *In vitro* plasmin-derived TTR proteolysis experiments were performed by incubating TTR with plasmin and/or SerpinA1 at 37 °C for 24 h, under stagnant conditions. TTR fragments were detected by Western blotting and identified through N-terminal sequencing, whereas TTR aggregation was evaluated by thioflavin T assays. For the *In vivo* studies, SerpinA1 downregulation was achieved through subcutaneous administration of specific antisense oligonucleotides (ASO) targeting SerpinA1 to transgenic mice carrying human TTR V30M mutation. TTR deposition on tissues was assessed by immunohistochemistry. Serine protease activity and plasmin activity was determined using commercial kits.

Results: We found that plasmin-mediated TTR proteolysis and aggregation are partially inhibited by SerpinA1 *in vitro*. Furthermore, *in vivo* downregulation of SerpinA1 increased TTR levels in mice plasma and deposition in the cardiac tissue of older animals. In addition, following SerpinA1 knockdown, the presence of TTR fragments was observed in the heart of HM30 mice but not in other tissues. Increased proteolytic activity, particularly plasmin activity, was detected in mice plasmas.

Summary and Conclusion: Our results indicate that SerpinA1 modulates TTR proteolysis and aggregation *in vitro* and *in vivo*. Particularly, the *in vivo* data suggest that TTR proteolysis occurs before TTR deposition and fibril formation. Overall, these findings might contribute for the development of more targeted and effective therapies for the treatment of ATTR amyloidosis.

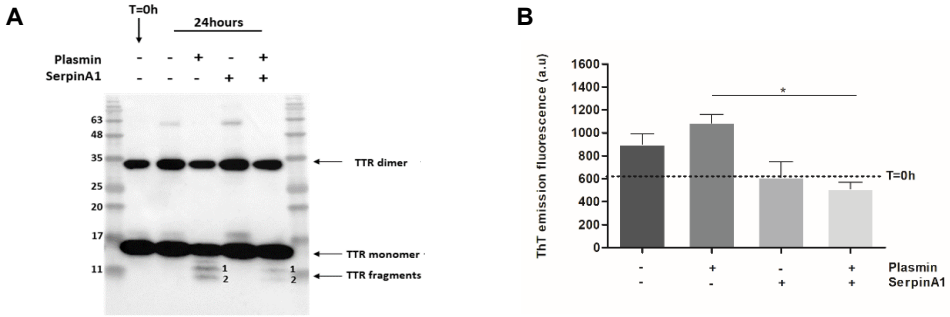

Figure 1.: TTR fragments were detected by Western blotting **(A)** and TTR V30M aggregation was followed by thioflavin T assay **(B)**.

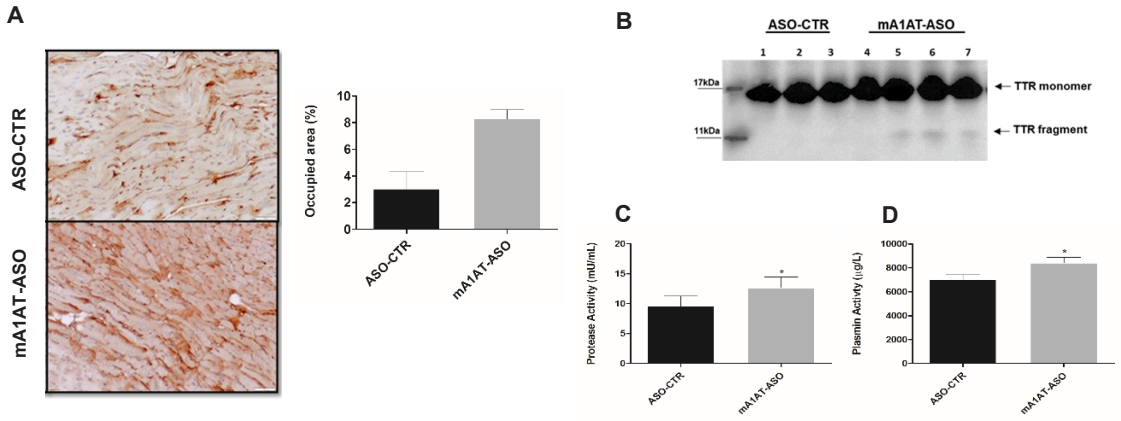

Figure 2.: Immunohistochemical analysis of mouse heart upon ASO treatment targeting SerpinA1 to old animals **(A)**. Western blotting analysis revealed the presence of TTR fragments upon SerpinA1 downregulation in the heart **(B)**. Serine protease activity **(C)** and plasmin activity **(D)** measured in plasma samples of HM30 mice.

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AA amyloid-containing diet potentiates Aβ42 induced effects in transgenic *Drosophila melanogaster*

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Background: An amyloid deposit usually consists of a single fibrillated protein, and only a few studies report on the presence of multiple proteins in a deposit. However, in a commonly used mouse model, AA amyloid formation is not only triggered by homologous fibril seeds but also by a plethora of proteins with fibril structures, including curli, Sup 35, and ATTR (1). AA amyloidosis develops in cows and can be detected in animals used for human consumption. There is a risk that ingestion of bovine AA amyloid-contaminated food results in the seeding of amyloid formed by the corresponding human protein or cross seeding of a different amyloid protein. Recently we used the ThT assay and showed that bovine AA extracts could potentiate the seeding of amyloid Aβ in vitro (2).

Objective: Investigate if feeding with human and bovine amyloid AA affects the phenotype of Aβ transgenic flies.

Material & Methods: The study was performed on Drosophila melanogaster expressing two copies of Aβ 42 (Alz3/Alz8). Wild-type flies crossed with the driver line were used as controls. The transgene expression was driven by the pan-neuronal driver elavC155, and the survival was monitored. The pdf driver was used to direct expression to the 16 NLv neurons, and the effect on the circadian rhythm was determined using the Drosophila activity monitoring system (DAMS). Amyloid was extracted from human and bovine tissue containing confirmed AA amyloid, or normal tissue. The amyloid extracts were applied on top of the standard food, and tubes were changed every other day. Flies were kept at a 12-hour light and dark cycle at 20˚C in 50% humidity.

Results: The survival of wild-type flies was not affected by adding any of the amyloid extracts to the food, and the median survival was 57 days (range 56-58 days) (Figure 1 A and B). The expression of Aβ42 is toxic, and the median survival in Alz3/Alz8 flies shortened to 34 days. However, a further reduction was observed in flies fed a diet supplemented with human AA amyloid extracts 1 and 2, where the median survival was 27 and 29 days, respectively. The addition of bovine amyloid to the food had no effect on survival.

The DAMS allows for monitoring activity over time. The flies were hatched and fed the diets for 10 days, and activity was monitored for 7 days. Aβ42 transgenic flies on standard food had lower activity than wild-type flies on the same diet, but the circadian rhythm was conserved. In Aβ42 transgenic flies fed a bovine AA extract was, the circadian rhythm lost, and the flies exhibited increased activity.

Summary & Conclusions: Obtained data is in line with our recent published result supporting cross-seeding between protein AA and Aβ42.

Figure 1.

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Dichotomy of Circulating Non-native TTR (NNTTR) Levels in Polyneuropathy and Cardiomyopathy Patients Provides a Glimpse to ATTR Tissue Specificity

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Background: More than 130 genetic variants in transthyretin (TTR) are linked to autosomal-dominant and progressive systemic Transthyretin Amyloidosis (ATTR). Systemic ATTR primarily manifests as familial amyloid polyneuropathy (FAP) and familial or senile systemic forms of cardiomyopathies (FAC and SSA). Tissue specificity appears to be linked to specific TTR variants, although most FAP patients will eventually develop cardiomyopathy, and vice versa. In systemic ATTR deposition the synthetic source of the precursor is the liver rather than the target tissue. However, the origin of the apparent tissue hierarchy, while generally related to a specific mutation, is not clear. Given the lack of full understanding of the structure-toxicity-tissue specificity relationships in ATTR, we hypothesized that either different misfolding intermediates are in play for polyneuropathy and cardiomyopathy or, that the processes that result in deposition and eventual tissue compromise, differ in the two target tissues.
We previously generated custom monoclonal antibodies that are specific for cryptic epitopes on non-native TTR (NNTTR). Immunoassays based on these NNTTR antibodies are being developed to detect misfolded conformations of TTR in biological samples. Our studies to date indicate that the circulating NNTTR detected by the NNTTR-ELISA assay seems to be a pathophysiologic biomarker related to FAP disease progression and response to therapy.

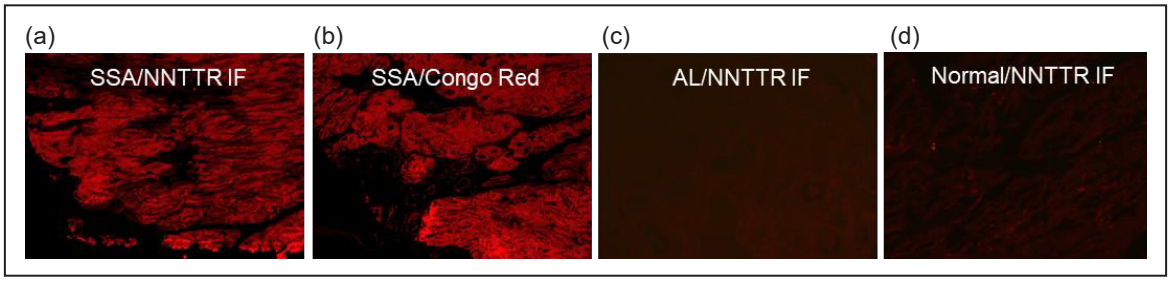
Objective: Examine the presence (and concentrations when appropriate) of circulating and tissue bound NNTTR epitopes in samples from ATTR patients with polyneuropathy and cardiomyopathy. Determine whether the same NNTTR antigenic determinants detected in the plasma are present in the tissue TTR deposits.

Material & Methods: Previously described monoclonal NNTTR antibodies specific for misfolded conformations of TTR were used. 50 Plasma samples and 10 tissue samples from ATTR patients and carriers were collected according to IRB approvals. Sandwich ELISA was used to quantify plasma NNTTR levels in plasma samples. Immunohistochemistry using the same antibody was performed on salivary gland and cardiac tissues from ATTR patients using standard protocols.

Results: The NNTTR-ELISA assay detects circulating plasma NNTTR in >98% of the FAP patients. However, little to no signal above background was seen in FAC or SSA patient plasma samples. In contrast, the NNTTR antibodies clearly detect misfolded forms of TTR in tissue samples (heart, labial salivary gland biopsies) from ATTR cardiomyopathy patients.

Summary & Conclusion: Using antibodies specific for NNTTR, we demonstrate that the specific conformers of misfolded TTR that are circulating in FAP patients are not detectable in patients with clinically dominant FAC and SSA. However, using the same antibodies, the misfolded TTR conformers are clearly detectable in cardiac tissue deposits in a pattern consistent with their Congo Red staining. While it is possible that the differences in circulating NNTTR are only quantitative they may reflect the fact that ATTR variant-dependent tissue specificity may result from the differing capacities of various tissues to either bind or generate the TTR conformations (or both) ultimately responsible for tissue compromise.

Figure 1.: Immunofluorescence of myocardial biopsy tissues from (a) SSA stained with mixed NNTTR antibodies; (b) SSA stained with CongoRed; (c) AL and (d) healthy normal contro stained with NNTTR antibodies, respectively.



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Support & Funding: Protego Biopharma, Inc.; NIH SBIR grant R43TR002436 and U44NS114151.

Heterotypic amyloid interactions and their effect on amyloid assembly

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Background: Heterotypic amyloid interactions between related protein sequences have been observed in functional and disease amyloids. While sequence homology seems to favour heterotypic amyloid interactions, we have no systematic understanding of the structural rules determining such interactions nor whether they inhibit or facilitate amyloid assembly.

Objective: Using structure-based thermodynamic calculations and extensive experimental validation, we performed a comprehensive exploration of the defining role of sequence promiscuity in amyloid interactions with a goal of defining their impact on amyloid susceptibility and polymorphism.

Material & Methods: We have employed computational modelling using the FoldX force field, the Cordax[1] amyloid core prediction algorithm and the patient-derived amyloid fibril cryoEM structures. Experimentally, we have studied amyloid aggregation kinetics of tau and Abeta in vitro, in the presence of homologous peptide fragments, we studied interaction patterns of peptide microarrays, we have performed morphological analysis of the fibrils using intrinsic and dye-based techniques. We have also used biosensor lines for tau and Abeta seeded aggregation using recombinant as well as ex-vivo seeds. Finally, we have reanalysed proteomics data of amyloid plaque composition and have detected an enrichement of heterotypic amyloid interactions.

Results: Using both Ab and tau as a model system we demonstrate that proteins with local sequence homology can modify fibril nucleation, morphology and spreading of aggregates in cultured cells[2,3]. Depending on the type of mutation such interactions inhibit or promote aggregation in a manner that can be predicted from structure. We find that these heterotypic amyloid interactions can result in the subcellular mislocalisation of these proteins. Moreover, equilibrium studies indicate that the critical concentration of aggregation is altered by heterotypic interactions.

Summary & Conclusion: Our findings suggest a structural mechanism by which the proteomic background can modulate the aggregation propensity of amyloidogenic proteins and we discuss how such sequence specific proteostatic perturbations could contribute to the selective cellular susceptibility of amyloid disease progression.

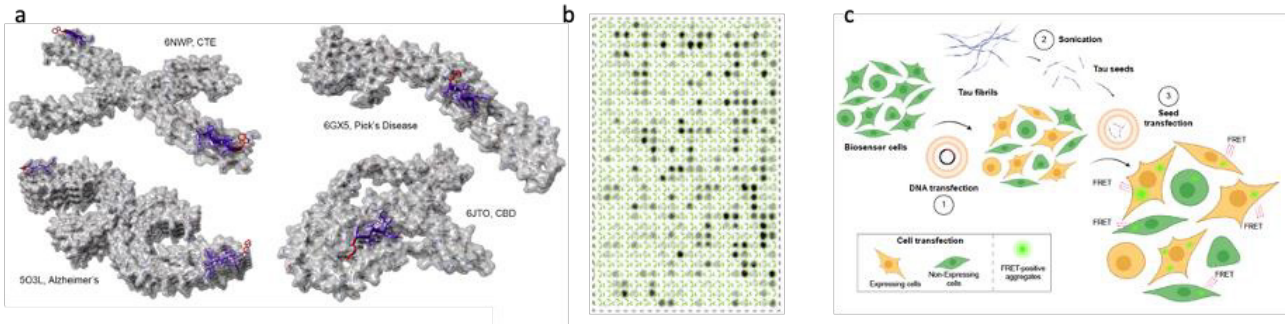

Figure 1.: (a) Computational modelling for heterotypic amyloid interactions using atomic structures. **(b)** Peptide microarrays showing the interaction between the Abeta peptide and sequence segments from other proteins that share homology to the aggregation prone regon of Abeta. **(c)** Workflow of studying heterotypic amyloid interactions in biosensor cell lines using recombinant or patient-derived seeds.

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Support & Funding: This work was supported by the Flanders institute for Biotechnology (VIB); KU Leuven; the Fund for Scientific Research Flanders (FWO); the Stichting Alzheimer Onderzoek.

BEST ABSTRACTS

BEST ABSTRACT

Feasibility of a novel academic BCMA-CART (HBI0101) for the treatment of relapsed and refractory AL amyloidosis

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Background: AL amyloidosis (AL) treatments are generally based on those employed for multiple myeloma (MM). Anti B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR)-T cell therapy, already approved for MM, may be too toxic for AL patients. Significant adverse events, especially cytokine release syndrome (CRS), limit this approach, rendering it suitable for the more resilient patients, but very challenging for the frail. Such are AL patients. Moreover, AL- plasma cells (PCs) showed significantly attenuated expression of BCMA, as compared to MM-PCs implying that other targets, may be preferred for designing future AL-directed CART therapy.

Objectives: Here we describe the ex-vivo applicability of a novel in-house, academic anti-BCMA CAR construct (HBI0101) on AL primary cells, as well as the safety and efficacy in four patients with relapsed/refractory (RR) primary AL, treated in a phase I clinical trial (NCT04720313).

Material & Methods: Bone marrow (BM) aspirates were collected from patients with MM and AL and analyzed using Navios flow cytometer. In-vitro PCs elimination was assessed after co-culture with CAR t cells.
The clinical trial was designed to evaluate HBI0101 safety and efficacy in MM patients and additional plasma cell dyscrasias, including AL. Patients enrolled had to be refractory to at least three lines of treatment and to have no other available registered therapy. The phase I first part of the trial consisted of the administration of HBI0101-transduced T cells, at escalating cell doses of 150-, 450- and 800x10^6 CAR+ cells. The study was authorized by the ethics committees. One patient was treated on a compassionate basis due to a concomitant active malignancy (myelodysplastic syndrome).

Results: We found that BCMA is sufficiently expressed on AL plasma cells (Figure 1), and describe the in-vitro activity of an in-house, novel academic anti-BCMA CAR construct (Figure 2a), showing marked efficacy and specificity for AL patients' BM derived primary plasma cells.
We report, for the first time, the safety and efficacy of BCMA CART treatment in four AL patients with relapsed and refractory (R/R) disease, as part of the phase I clinical trial. Three patients had MAYO stage IIIa cardiac involvement. proBNP ranging from 2000- 7500 pg/ml. The treatment proved relatively safe, with a short and manageable grade 3 cytokine release syndrome (CRS) evident in two patients and no neurotoxicity in any. Two of three cardiac patients had de-compensation of their diastolic heart failure, yet in one it was short (2 days) and in the other it was ongoing for 10 days, both easily managed. One had decompensation of hepatic cirrhosis, which stabilized after 4 months with concomitant reduction in alkaline phosphatase levels.
In these heavily pretreated patients, responses were remarkable, showing a fast and efficient complete hematologic remission, normalization of dFLC (Figure 2b) and minimal residual disease (MRD) negativity achieved in all four. These results translated into clinical improvement and subsequent organ responses in all patients. HBI0101 cell in-vivo expansion was noted in all four infused patients (Figure 2c).
Although the follow up duration reported here is short, responses are still ongoing. Within a median follow-up period of 5.2 (2.5-9.5) months all four patients maintain their responses.

Summary & Conclusion: BCMA-CART cells provide a first proof-of-concept that this therapy is safe enough and highly efficacious for the treatment of advanced, RR AL patients.

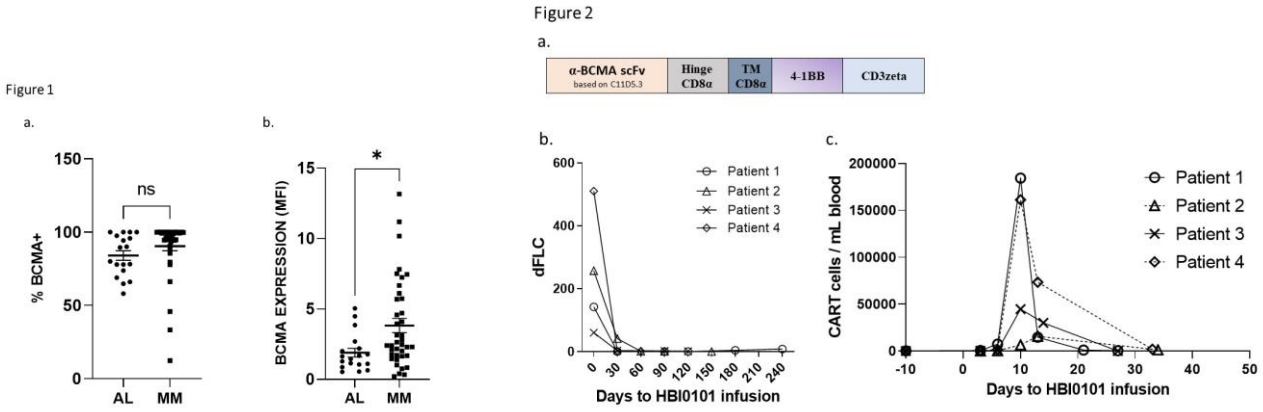

Figure 1: BCMA levels in AL amyloidosis (AL, n=18) and multiple myeloma (MM, n=39) patients. BCMA % of expression **(a.)** and MFI **(b.)** were determined by flow cytometry. Samples were gated on CD38++CD138++ cells.

Figure 2: (a.) Schematic figure of the HBI0101 CAR construct. C11D5.3-CD8-4-1BBZ encoding the anti-BCMA CAR is depicted. BCMA-CAR consists of a C11D5.3 anti-BCMA single chain variable fragment (scFv), CD8α hinge and transmembrane regions, the cytoplasmic portion of the 4-1BB costimulatory molecule, and the CD3ζ T-cell activation domain. **(b.)** Difference between involved (iFLC) and uninvolved FLC (dFLC) of four AL patients was assessed at baseline and post HBI0101 CART infusion. **(c.)** The number of HBI0101 CART per 1mL blood was determined by quantification of CAR transgene levels by qRT-PCR method following CART infusion at the indicated times, and further adjusted to the percent of transduction at the day of CART infusion.

Support & Funding: This work is supported by generous support from Steinfeld and Cuniff family and by the Amyloidosis Patient Association of Israel.

BEST ABSTRACT

A Proteomic Atlas of Renal Amyloid Plaques Provides Insights Into Disease Pathogenesis

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[1]Division of Hematology, [2]Department of Quantitative Health Sciences,[3]Department of Laboratory Medicine and Pathology,[4] Department of Biochemistry and Molecular Biology, Mayo Clinic, MN, USA

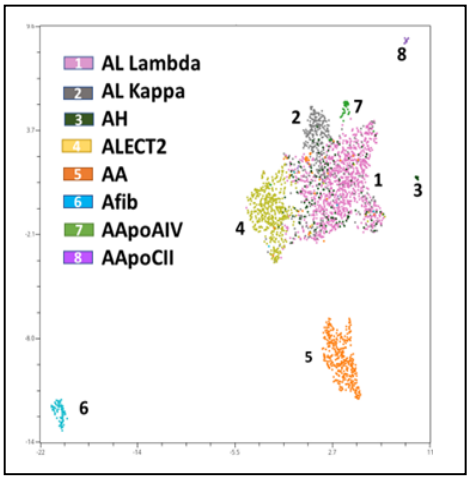
Background: The mechanisms of tissue damage in renal amyloidosis are not known and are important in identifying novel therapeutic approaches for renal recovery in this disease. The amyloid plaque proteome can be obtained using laser capture microdissection and mass spectroscopy (LMD-MS) of clinical samples obtained for amyloid typing.

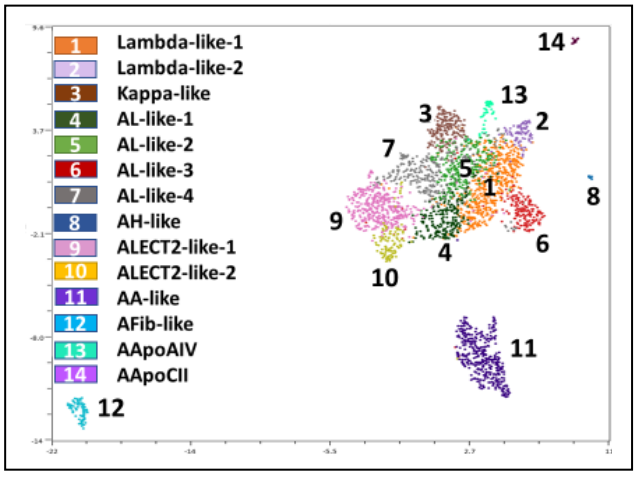
Objective: To characterize the plaque proteome of renal amyloidosis and its association with clinical parameters.

Material & Methods: We used LMD/MS to identify all the proteins deposited in amyloid plaques (*expanded proteome*) and proteins over-expressed in plaques compared to controls (*plaque-specific proteome*). We included: 1487 AL,131 AH, 474 ALECT2, 418 AA, 80 AFib, 38 AApoAIV 22 AApoCII, and 14 controls (9 membranous nephropathy and 5 normal kidneys). We had detailed clinical data on 228 newly diagnosed AL patients that were seen at our institution. Uniform Manifold Approximation and Projection (UMAP) was used for dimensionality reduction and phenograph and hierarchical clustering for clustering of patients. Wegstalt overrepresentation analyses were used for biologic pathway identification.

Results: We identified 498 distinct proteins across all amyloid types. UMAP analyses (**figure, left**) suggest that AFib, AA, and AApoCII have very distinct proteomes compared to other types. Pathway analyses showed that complement pathway proteins were increased in these 3 amyloid types compared to the rest. Interestingly ALECT2 appeared proteomically closer to AL in the high dimensional space compared to other types, suggesting more similar pathophysiology. Pathway analyses again revealed complement pathway enrichment in ALECT2 versus AL. Clustering of cases based on the expanded proteome identified 2 ALECT2 subtypes and 7 AL subtypes (**figure, right**). The main differences within the AL and ALECT2 subtypes were driven by increased expression of complement proteins and, for AL only, decreased expression of 14-3-3 family proteins, which are widely implicated in renal tissue dysfunction. The total proteomic count was higher for all non-AL/AH cases except AApoAIV. The renal AL plaque specific proteome consisted of 24 proteins, including amyloid signature proteins (AApoE, AApoAIV, vitronectin, clusterin) and some well characterized markers of renal damage (14-3-3B, A1AT, HSPB1, MOES, S100A6, TAGL, TIMP3, TYB4). Hierarchical clustering of cases based on their plaque-specific proteome identified 4 clusters. Of these, cluster 1 was associated with improved renal survival (not independently of renal stage) and was characterized by higher overall proteomic content but lower levels of light chains, collagen, and most signature proteins.

Conclusion: This is the first comprehensive analysis of the renal amyloid plaque proteome. We find increased complement deposition in AA, AFib, AApoCII and ALECT2 in comparison to AL. This is in agreement with our cardiac data for ATTR versus AL suggesting that complement proteins are expressed in more indolent amyloid types[1]. We also found significant heterogeneity within the same amyloid type-driven predominantly by complement proteins. More indolent (non-AL) amyloid types have a higher proteomic content compared to AL, similar to our cardiac data. AL cases with improved renal survival have a higher proteomic content but lower collagen and amyloidogenic protein deposition. This suggests that protein burden does not explain amyloid toxicity in tissues and could serve as a surrogate of slower disease physiology.





Figure,left.: UMAP-based dimensionality reduction of most common types suggests that AFib, AA, and AApoCII have distinct proteomes to all other types
Figure,right.: Phenograph clustering identifies overlap between different amyloid types and distinct patterns within the same amyloid subtype.

Support & Funding: This work was supported by the Paul Calabresi K12 Career Development Award (CA90628-21) and the Mayo Clinic Myeloma SPORE

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BEST ABSTRACT

Cryo-EM structural study of cardiac ATTR fibrils and structure-based development of detection probes and anti-seeding inhibitors.

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Background: The current treatments for cardiac ATTR amyloidosis (ATTR-CA) show limited efficacy when administered at late stages, and patients are often diagnosed late, thereby hindering intervention and treatment. Our previous results suggest that the progression of ATTR-CA at late stages is mostly driven by amyloid seeding rather than de-novo nucleation (1,2). We hypothesize that the structures of ATTR fibrils can be used for the design of detection probes for early diagnosis and anti-seeding inhibitors for the treatment at late stages.

Objective: To obtain structural information from *in-vitro* and *ex-vivo* ATTR fibrils, and to use this information to develop diagnostic and therapeutic tools.

Material & Methods: ATTR fibril structures were determined by x-ray crystallography and cryo-electron microscopy (cryo-EM). Peptide conception was performed by rational design and computational optimization using the Rosetta-based algorithm ZipperDB. The *in-vitro* effectiveness of the anti-seeding peptides was assessed by thioflavin-T seeding assays, negative staining electron microscopy, and immuno-dot blot (1,2). Their *in-vivo* effectiveness was evaluated in two *Drosophila* models of ATTR (3). The specificity and avidity of our detection probes were analyzed by immunoblots and ELISAs (not published).

Results & Discussion: Our structural studies of cardiac ATTR fibrils by x-ray crystallography and cryo-EM depict heterogeneous structural features in various patients with hereditary ATTR (Figure 1). These differences may be explained by the destabilization of a polar interface by the introduction of a hydrophobic residue. Using structural information, we engineered structure-specific peptides (Figure 2). We designed and modified them to serve either as anti-seeding inhibitors or as detection probes (Figure 2A). We found that anti-seeding inhibitors halt amyloid seeding of ATTR fibrils extracted from multiple organs of ATTR-CA patients at substoichiometric ratios (Figure 2B). Our inhibitors also delay disease phenotype in two *Drosophila* models of ATTR (3). Our peptide probes can detect ATTR aggregates in tissues of ATTR-CA patients that are not present in AL patients and control samples at picomolar concentrations (Figure 2C).

Summary & Conclusion: ATTR mutations can originate structural imprints that result in the formation of distinct assemblies. The structural information obtained from ATTR fibrils can be capitalized into the development of diagnostic and therapeutic tools for early detection or late treatment of ATTR-CA.

Figure 1

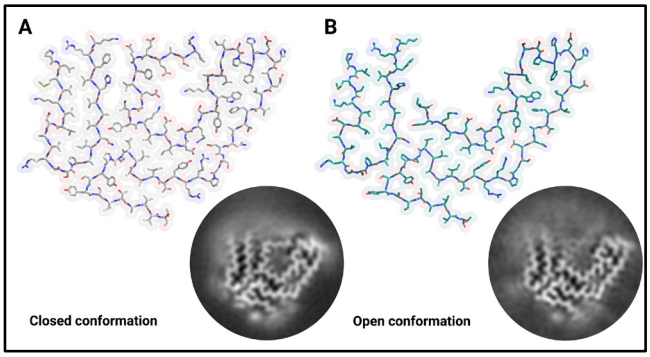

Figure 2

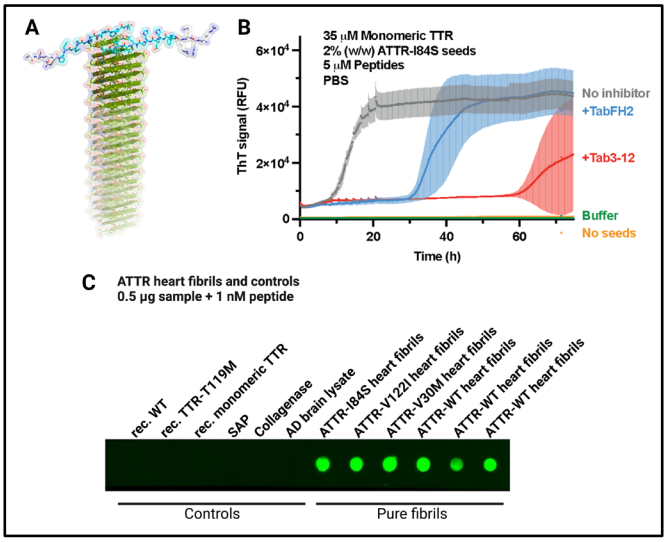

Figure 1. Cryo-EM structure determination of cardiac ATTR fibrils extracted from patients. We found two distinct conformations. The closed conformation (**A**) was first described by Schmidt et al. (4) in two ATTR-V30M patients. The open conformation (**B**) has not been described until now.

Figure 2. Structure-based development of anti-seeding inhibitors and detection probes **(A)**. We modified these peptides in different ways so they can act as anti-seeding inhibitors **(B)** or detection probes **(C)**.

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BEST ABSTRACT

An European collaborative study on 476 patients with AA amyloidosis: identification and validation of survival and renal staging systems

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Background: the availability of accurate diagnostic techniques, recognition of the importance of achieving low SAA concentrations, and accessibility of novel effective treatments for the underlying disease has greatly affected the management of AA amyloidosis. However, differently from other types of systemic amyloidosis, staging systems allowing prognostic stratification of survival and organ damage have not been established in AA amyloidosis.
Objective: identification and validation of prognostic staging systems for overall survival (OS) and renal survival (RS) in AA amyloidosis
Material & Methods: the prospectively maintained databases of the Amyloidosis Centers of Pavia and Heidelberg were searched for patients with AA amyloidosis. Multivariable analysis was performed to identify prognostic factors for OS and RS in the Pavia cohort and cut-offs of continuous variables were identified by ROC analysis predicting death or dialysis at 24 months. Since the cut-offs of NT-proBNP and BNP were low, we elected using their upper reference limits. The proposed survival and renal staging systems were tested in the Pavia cohort and the Heidelberg cohort served as validation.
Results: 476 patients with AA amyloidosis were included in the study (233 diagnosed in Pavia between 1991-2020 and 243 diagnosed in Heidelberg 1975-2020). Patients' characteristics are reported in Table 1. Patients evaluated in Heidelberg were younger (median age 54 vs. 59 years-old; P<0.024), and more frequently had an autoinflammatory disease/recurrent fever as underlying inflammatory disease (33% vs. 7%; P<0.001). In Pavia there were more patients with recurrent infections (11% vs. 5%; P=0.028) or with Idiopathic AA (36% vs. 23%; P=0.003). After a median follow-up of living patients of 3.8 years, 109 patients died with a median OS of 13 years. Serum albumin <3.0 g/dL, eGFR <45 mL/min x 1.73 m^2 and elevated NT-proBNP/BNP (i.e., NT-proBNP >332 ng/L or BNP >100 ng/L) were identified as risk factors for OS at multivariable analysis (Table 2). We proposed a 3 stages survival staging system (stage I: 0 risk factors, stage II: 1-2 risk factors; stage III: 3 risk factors) that discriminated between patients with significantly different OS both in the testing and the validation cohorts (Figure 1AB). One hundred and twenty patients progressed to end-stage renal failure and median RS was 9.4 years. Multivariable analysis identified eGFR <35 mL/min x 1.73 m^2 and 24h-proteinuria >3 g/24h as risk factors for RS (Table 2). Renal staging system was built assigning patients with 0 risk factors to stage I, 1 risk factor to stage II and 2 risk factors to stage III and discriminated between patients with a higher risk of dialysis both in the testing and the validation cohorts (Figure 1CD).
Summary & Conclusion: this study for the first time establishes and validates a powerful biomarker-based staging systems for OS and RS in AA amyloidosis. Early stages patients have a remarkably good survival and low rate of progression to dialysis. On the other hand, advanced stage patients have a median survival of only 2-3 years and progress to dialysis after a median time of approximately 2 years. This emphasizes the need of early diagnosis and intervention in AA amyloidosis, similarly to AL and ATTR amyloidosis.

Table 1. Patients' characteristics

Variables	Overall population 476 pts. N (%) – median (IQR)	Pavia cohort 233 pts. N (%) – median (IQR)	Heidelberg cohort 243 pts. N (%) – median (IQR)	P
Age, years	56 (46-77)	59 (48-68)	54 (44-65)	0.024
Sex, male	197 (41)	93 (40)	104 (43)	0.585
Underlying inflammatory disease				
Rheumatologic disease	156 (33)	70 (30)	86 (35)	0.252
Inflammatory bowel disease	45 (9)	28 (12)	17 (7)	0.086
Autoinflammatory/recurrent fevers	87 (18)	17 (7)	80 (33)	<0.001
Recurrent infections	37 (8)	25 (11)	12 (5)	0.028
Castleman disease	12 (3)	10 (4)	2 (1)	0.030
Unknown (Idiopathic AA)	139 (29)	83 (36)	56 (23)	0.003
Organ involvement Kidney / Heart	456 (96) / 64 (13)	219 (94) / 29 (12)	237 (96) / 35 (14)	0.860 / 0.624
SAA, mg/L	35.0 (12.3-101.0)	30.5 (10.9-87.9)	40.5 (15-115)	0.059
C reactive protein, mg/L	21.3 (8.4-49.5)	22.0 (6.2-46.0)	20.8 (9.7-51.8)	0.464
Serum albumin, g/dL	3.4 (2.8-4.0)	3.1 (2.6)	3.6 (3.8)	<0.001
Urine protein loss, g/24h	3.70 (1.00-7.44)	3.47 (1.06-6.29)	4.00 (0.94-7.97)	0.822
eGFR, mL/min x 1.73 m^2	31 (17-60)	31 (18-54)	30 (15-62)	0.887
Dialysis before diagnosis, yes	80 (17)	33 (14)	47 (19)	0.165

AA, reactive amyloidosis; eGFR, estimated glomerular filtration rate; SAA, serum amyloid A

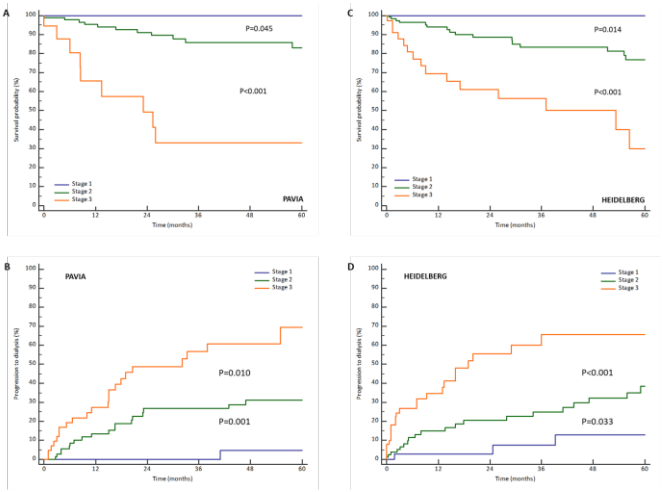
Table 2. Multivariable analysis of factors predicting OS and RS in the Pavia cohort

Multivariable analysis for OS			
Variables	OS		
	HR	95% CI	P
SAA >40 mg/L	1.37	0.60-3.11	0.456
eGFR <45 mL/min x 1.73 m^2	3.47	1.02-11.85	0.047
Serum albumin <3.0 g/dL	2.81	1.21-6.53	0.017
Elevated NT-proBNP/BNP, yes*	10.19	2.94-35.29	<0.001
Multivariable analysis for RS			
SAA >25 mg/L	1.28	0.71-2.29	0.566
eGFR <35 mL/min x 1.73 m^2	3.69	1.97-6.91	<0.001
Proteinuria >3.0 g/24h	2.36	1.28-4.32	0.006

AA, reactive amyloidosis; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; NT-proBNP, amino terminal fragment of type-B natriuretic peptide; OS, overall survival; RS, renal survival SAA, serum amyloid A

* NT-proBNP >332 ng/L and BNP >100 ng/L

Figure 1. Survival and renal staging system in AA amyloidosis



Risk factors for survival staging: Serum albumin <3.0 g/dL, eGFR <45 mL/min x 1.73 m^2 and elevated NT-proBNP/BNP. Risk factors for renal staging: eGFR <35 mL/min x 1.73 m^2 and proteinuria >3 g/24h. Survival staging system in the Pavia (testing cohort): cumulative proportion of living patients was 100% at 5 years in stage I (16 patients), 91% at 2 years and 83% at 5 years in stage II (98 patients) and 49% and 33% in stage III (18 patients) (A). Survival staging system in the Heidelberg (validation cohort): cumulative proportion of living patients was 100% at 5 years in stage I (34 patients), 88% at 2 years and 77% at 5 years in stage II (125 patients) and 61% and 30% in stage III (35 patients) (B). Renal staging system in the Pavia (testing cohort): cumulative proportion of patients requiring dialysis was 0% at 2 years and 4% at 5 years in stage I (43 patients), 27% at 2 years and 31% at 5 years in stage II (94 patients) and 47% and 69% in stage III (51 patients) (C). Renal staging system in the Heidelberg (validation cohort): cumulative proportion of patients requiring dialysis was 3% at 2 years and 13% at 5 years in stage I (43 patients), 20% at 2 years and 38% at 5 years in stage II (94 patients) and 55% and 66% in stage III (51 patients) (D)

BEST ABSTRACT

Primary Results From APOLLO-B, A Phase 3 Study Of Patisiran In Patients With Transthyretin-Mediated Amyloidosis With Cardiomyopathy

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Background:
Transthyretin-mediated (ATTR) amyloidosis is a rapidly progressive, debilitating, and fatal disease caused by misfolded TTR accumulating in multiple organs and tissues. Patients with hereditary (hATTR) amyloidosis (also known as ATTRv amyloidosis) or wild-type (wtATTR) amyloidosis with cardiomyopathy exhibit decreased survival. Patisiran, an IV RNAi therapeutic that inhibits synthesis of wt and variant TTR, was approved for the treatment of hATTR amyloidosis with polyneuropathy. Exploratory analyses of a predefined cardiac subpopulation in the APOLLO study demonstrated the potential for patisiran to improve the manifestations of cardiomyopathy in patients with hATTR amyloidosis with polyneuropathy.

Objective:
To investigate the safety and efficacy of patisiran in patients with ATTR amyloidosis with cardiomyopathy in the ongoing APOLLO-B study (NCT03997383).

Material & Methods:
Patients were 18–85 years of age with evidence of cardiac amyloidosis by echocardiography (end-diastolic interventricular wall thickness >12 mm), and either ATTR amyloid deposition on tissue biopsy or fulfilling nonbiopsy diagnostic criteria for ATTR amyloidosis with cardiomyopathy. Patients had a medical history of heart failure (HF) due to ATTR amyloidosis with at least 1 prior hospitalization for HF, or current clinical evidence of HF. Patients were randomized (1:1) to receive patisiran IV 0.3 mg/kg or placebo Q3W for 12 months. After the double-blind, placebo-controlled period, all patients may receive patisiran in an open-label extension period for up to 36 months. The primary endpoint was change from baseline in 6-MWT at Month 12 with patisiran treatment, compared with

placebo. Secondary endpoints evaluated the effect of patisiran vs placebo at Month 12 on QOL (KCCQ-OS score) as well as two sets of composite endpoints: 1) All-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT; and 2) All-cause mortality and frequency of all-cause hospitalizations and urgent HF visits. The pharmacodynamic effect of patisiran on serum TTR level (change from baseline through Month 12) was also assessed.

Results & Discussion:
Enrollment completed (359 patients) in May 2021. About 80% of patients have wtATTR amyloidosis. Primary and secondary endpoint data will be presented, including change in 6-MWT and KCCQ-OS score at Month 12, in addition to safety data.

Summary & Conclusion:
The primary results of the APOLLO-B study, which will be presented for the first time, will inform the potential of patisiran as a treatment for the cardiomyopathy of ATTR amyloidosis. APOLLO-B will continue to investigate the efficacy and safety of patisiran on ATTR amyloidosis in an open-label extension period.

Support & Funding:
This study was funded by Alnylam Pharmaceuticals. Editorial assistance in the development of the abstract was provided by Adelphi Communications Ltd, UK, funded by Alnylam Pharmaceuticals in accordance with Good Publication Practice (GPP3) guidelines.

BEST ABSTRACT

Venetoclax targeted therapy in t(11;14) AL amyloidosis patients: a retrospective analysis from the French Amyloidosis Network.

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Topic 9. AL 2: upfront therapy, progression, rescue therapy – clinical – AL type

Background: BCL-2 inhibition represents a promising therapeutic approach in multiple myeloma (MM). Venetoclax (VEN) is an oral BCL-2-selective BH3 mimetic and is currently approved in CLL. VEN has shown activity in MM cell lines with t(11;14), in part because there may be greater BCL-2 co-dependence in such clones. VEN is safe in MM patients (pts) with encouraging clinical activity. VEN should provide a novel targeted therapy in AL amyloidosis as most of the pts harbored a t(11;14). Various retrospective cohorts are now reported with VEN alone or in combination with bortezomib and/or daratumumab, with rapid and deep response.
Objective: To report efficacy of VEN based regimen in AL amyloidosis pts in a real-life setting.
Material & Methods: This retrospective study included 52 pts with AL amyloidosis, from the French Amyloidosis Network, who received VEN as part of their regimen. Individual data were prospectively and retrospectively collected in the French amyloidosis database. Pts should have received at least 1 cycle of VEN-containing regimen to be analyzed. Responses were reviewed using current criteria for CR (serum and urine negative immunofixation and normal iFLC) and for VGPR (dFLC<40 mg/l).
Results: Forty-five pts (86%) with a complete dataset for diagnosis and response were analyzed in that study. Twenty-nine pts had ≥2 organs involved with heart in 71% and renal in 58%, MAYO stage 3 in 21 pts and median NT-proBNP and troponin levels of 1621 ng/l (IQR: 299-3958), 54 (15,5-87,8)/ 0,07 (0,03-0,15), respectively. Thirty-five pts were FLC only with a median dFLC for the whole cohort of 288 mg/l (IQR:109-610). Nineteen pts had a dFLC>180 at diagnosis. Baseline characteristics are described in table 1. All except 3 pts had a t(11;14). Median time from diagnosis to VEN therapy was 30 months (12-65). VEN (400-800 mg/day) was given alone (n=20), or in association with dexamethasone/DEXA (n=5), bortezomib/BOR (n=12), daratumumab/DARA (n=2), or all (n=6). Eight pts received frontline VEN to deepen or restore response, and 37 received VEN at relapse with a median of 2 prior lines of therapy (min/max: 1–6); 31 pts were DARA exposed and 8 were DARA “refractory”. Median time on therapy was 6 months. At data cut-off (06/22), 21 pts had discontinued therapy because of disease progression (n=6), or death while in CR (n=1). The hematologic response rate was 84.5%; 62% of pts achieved CR, 16% VGPR, and 6.5% PR. Only 1/3 pt with no t(11.14) achieved VGPR. Responses were fast and median time to VGPR (n=30) and CR (n=25) was 1 month (1-3), and 2,5 months (1-3), respectively. For VEN monotherapy, 55% of pts reached CR; with the addition of DEXA, BOR, DARA or both, CR was 60, 58, 100, and 83%, respectively. In frontline pts, all except 1 achieved CR with the addition of VEN. In relapsed pts, 21/37 (57%) achieved CR and 7 (19%) achieved VGPR. Considering DARA exposed relapsed pts, 11/23 (48%) achieved CR and 6 (26%) VGPR; considering the 6 R/R pts, only 1 reached CR and 2 VGPR. VEN was well tolerated with no

unexpected adverse events (AEs). Twelve pts reported AEs, mainly diarrhea/nausea (50%), neutropenia (30%), and infections (50%). Univariate and multivariate analyses to predict response will be provided.
Summary & Conclusion: Venetoclax in AL amyloidosis is a promising targeted therapy. With CR of >50% with VEN alone or VEN-based regimen, these results confirm the efficacy of VEN in pts with t(11;14) AL and support randomized clinical trials, especially with daratumumab and/or bortezomib, even in exposed pts (and somehow refractory).

Characteristics	All patients (n=45)
Age, years, median (IQR)	63 (54-70)
Male, n (%)	29 (64.5)
Lambda light chain: n (%)	30 (67.0)
Light chain only: n (%)	35 (78.0)
Plasma cells, %, median (IQR)	8.0 (4.5-14.0)
<5/>5/>10/>20,n	13/11/13/6
MGUS/SMM, n	24/19
t(11.14), n (%)	42 (93.5)
dFLC baseline, mg/l, median (IQR)	287.50 (108.95-610.45)
Baseline NT-proBNP, ng/l, median (IQR)	1621.00 (294.25-4275.25)
Baseline creatinine, microM, median (IQR)	93.5 (71.5-148.25)
ECOG performance status: n(%)	
0	5 (11.0)
1	16 (35.5)
2/3	17 (37.5)
Mayo Clinic cardiac stage: n (%)	
I	15 (33.5)
II	9 (20.0)
IIIA/IIIB	21 (46.5)
Involved organs, n, median (IQR)	2 (1-3)
Kidney, n (%)	26 (58)
Heart	32 (71)
Nerve	7 (15.5)
Gastrointestinal tract	6 (13.5)
Liver	6 (13.5)
Soft tissue	10 (22.5)
Time from diagnosis: months, median (IQR)	30 (12-65)
Number of previous lines: n, median (IQR)	2 (1.25-3)
Never reach VGPR: n (%)	11/32 (34)
Relapsed: n (%)	37 (82)
Previous therapy: n (%)	
Bortezomib	41 (91.0)
IMiDs	24 (53.5)
Daratumumab	31 (69.0)
DARA Refractory : n (%)	8 (18.0)

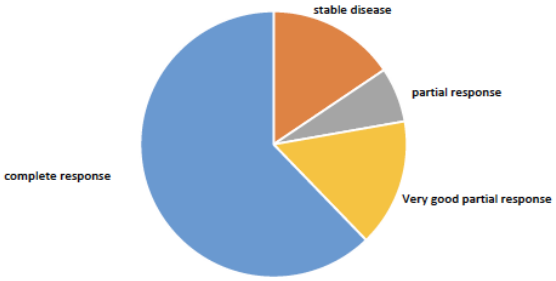

Table 1: Baseline characteristics and previous therapies
Figure 1: Best response to VEN

BEST ABSTRACT

Amyloidosis-related orthopedic events, low plasma transthyretin, and risk of cardiac events

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Background: At diagnosis, patients with cardiac amyloid transthyretin amyloidosis (ATTR-CA) have a high prevalence of certain orthopedic events (spinal stenosis, carpal tunnel syndrome, and biceps tendon rupture).[1] We tested the hypothesis that these orthopedic events precede cardiac events consistent with ATTR-CA, that individuals with orthopedic events at baseline and a low plasma transthyretin have a higher risk than those with normal transthyretin, and that the mechanistic link between orthopedic and cardiac events is through transthyretin tetramer destabilization.

Objective: First, to test whether a composite endpoint consisting of surgical treatment for carpal tunnel syndrome, spinal stenosis, or biceps tendon rupture was associated with incident cardiac events consistent with amyloidosis in a large study of the Danish general population followed for a median 9.3 years. Second, to test whether individuals with orthopedic events at baseline and plasma transthyretin concentration <20 mg/dL, corresponding to the lower limit of the 95% reference interval, versus ≥20 mg/dL had an even higher risk of cardiac events. Third, to test whether genetic variants in the transthyretin gene (*TTR*) associated with increasing tetramer destabilization, were associated with both incident orthopedic and cardiac events.

Material & Methods: In observational analysis in the Copenhagen General Population Study (CGPS; n=93,637), we first tested whether amyloidosis-related orthopedic events at baseline were associated with incident amyloidosis and cardiac events consistent with ATTR-CA (heart failure, atrial fibrillation, myocardial infarction, or death), and whether a low plasma transthyretin concentration was associated with an even higher risk. In genetic analysis, in CGPS and the Copenhagen City Heart Study combined (n=102,496), we tested whether *TTR* genotypes associated with stepwise lower transthyretin tetramer stability, marked by lower plasma transthyretin, were associated with both incident orthopedic and cardiac events.

Results: In individuals with versus without orthopedic events at baseline, hazard ratios (HRs) were 10.7 (95% confidence interval: 3.9-29.3) for amyloidosis, and 1.3 (1.1-1.4) for cardiac events (Figure 1). Furthermore, in individuals with orthopedic events at baseline, HRs for cardiac events were 3.8 (1.9-7.6) in those with transthyretin <20 mg/dL versus ≥20 mg/dL (Figure 2) and 1.4 (1.2-1.6) in those without orthopedic events. Finally, HRs as a function of *TTR* genotype increased with increasing transthyretin tetramer destabilization and lower plasma transthyretin up to 3.0 (1.4-6.6) for orthopedic events and 1.6 (95% CI: 1.0-2.6) for cardiac events, implying a common mechanistic background for these endpoints through transthyretin tetramer destabilization.

Summary & Conclusion: Amyloidosis-related orthopedic events are associated with increased risk of incident amyloidosis and cardiac events consistent with ATTR-CA, with the highest risk in those with low plasma transthyretin <20 mg/dL. Furthermore, orthopedic and cardiac events are mechanistically linked through transthyretin tetramer destabilization.

FIGURE 1.

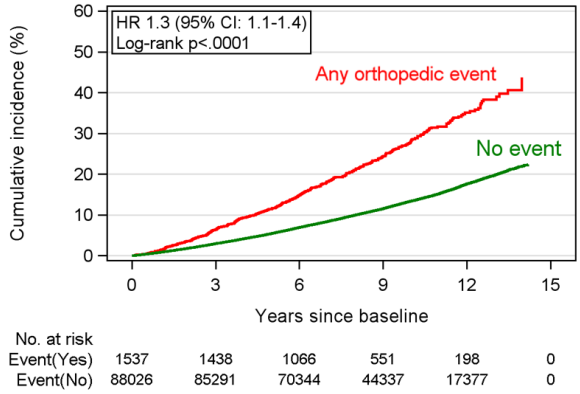

FIGURE 2.

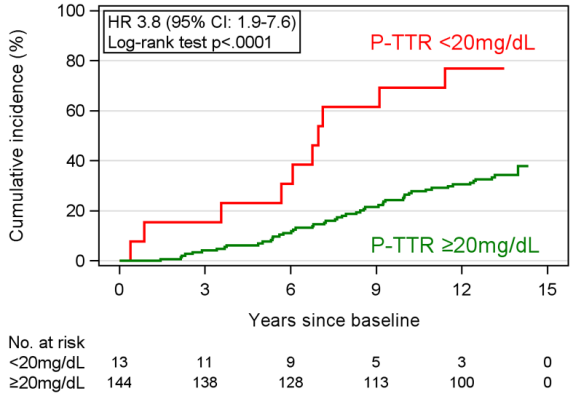

Figure 1.: Cumulative incidence of cardiac events consistent with ATTR-CA (heart failure, atrial fibrillation, myocardial infarction, or death) by baseline status for any amyloidosis-related orthopedic event (surgical treatment for carpal tunnel syndrome, spinal stenosis, or biceps tendon rupture) in 93,637 individuals from the Copenhagen General Population Study.

Figure 2.: Cumulative incidence of cardiac events consistent with ATTR-CA in individuals with any amyloidosis-related orthopedic event and plasma transthyretin concentration <20 mg/dL versus ≥20 mg/dL at baseline in 12,495 consecutive individuals from the Copenhagen General Population Study with ascertained plasma transthyretin.

References

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POSTER PRESENTATIONS MONDAY, 5TH

Title: Morbidity and mortality measured through "Days Alive and Out of Hospital" (DAOH) in patients with AL amyloidosis according to cardiac involvement and specific treatment.

Authors: María Lourdes Posadas Martinez, Mariana Vaena, Teo Epstein, Maria Sol Osorno, Erika Bárbara Brulc, Marcelina Carretero, María Soledad Sáez, Patricia Beatriz Sorroche, Javier Pollan, María Adela Aguirre, Elsa Nucifora.

Background: Cardiac amyloidosis is the main cause of morbidity and mortality in patients with systemic amyloidosis. Days alive and out of hospital (DAOH) is a novel patient-centered outcome that assesses the burden of disease, through the measurement of morbidity and mortality.

Objectives: To describe DAOH in patients with AL amyloidosis by cardiac compromise and autologous stem cell transplantation (ASCT).

Materials & Methods: Prospective cohort of consecutive patients diagnosed as AL from the Institutional Registry of Amyloidosis (RIA) from the Health Maintenance Organizations (HMO) of the Italian Hospital of Buenos Aires between 01/01/2010 and 31/08/2021. Patients with follow-up in another institution or less than 365 days were excluded. Quantitative variables were described with their median and interquartile range, and categorical variables with absolute and relative frequencies. We calculated DAOH at 1, 3, and 5 years, subtracting days hospitalized and days dead from the potential follow-up time. Only patients with a follow-up equal to or greater than the potential follow-up time were included in each group. We use Kruskal Wallis to compare DAOH between cardiac and non-cardiac compromise and ASCT and non-ASCT.

Results & Discussion: 78 patients were eligible, of whom 48 were included. Of these, the total completed the follow-up for DAOH at 1 year, 32 for DAOH at 3 years and 22 for DAOH at 5 years (Figure 1). The median age was 64 years, 54% were women and the median Charlson score was 5 (IR 3-7). The most frequently affected organs were the kidney (60%) and heart (56%). Eighty-seven percent of patients received specific pharmacological treatment, 35% received ASCT and 15% heart transplantation. Ninety-two percent had at least one episode of hospitalization during the follow-up time. The median number of hospitalizations was 3 (IR 1-4). The median DAOH was 346 (IR 274-365), 1042 (IR 231-1083), and 1739 days (IR 352-1793) at 1, 3, and 5 years, respectively. At 1 year, the median of DAOH was 347 (IR 333-363) and 362 days (IR 313-365) in those with and without ASCT, respectively (p 0.33). At 5 years, the median of DAOH was 1787 (IR 1759-1804) and 1558 days (IR 595-1793) in those with and without ASCT, respectively (p 0.04). Mortality was 12% vs 56% in those with and without ASCT, respectively. At 5 years, the median of

DAOH was 1042 (IR 431-1783) and 1768 days (IR 1627-1820) in those with and without cardiac involvement, respectively (p 0.04)(Figure 2).

In the present study we found that DAOH differs significantly between patients with cardiac involvement and those who received ASCT.

Regarding cardiac involvement, our results coincide with the multiple studies that evaluate cardiac involvement as a key prognostic factor in traditional overall survival studies [1,2].

We consider that the ASCT is an excellent evolutionary parameter regarding the patient's journey. Despite requiring a longer hospital stay during and after, reducing DAOH at 1 year, it has a long-term benefit, demonstrated by greater DAOH at 5 years in patients who received ASCT.

Summary & Conclusion: Days alive and out of hospital is a measure of patient-centered outcomes that describes the patient's disease journey. This is the first paper that describes DAOH in amyloidosis AL, but this outcome may become a relevant measure for chronic disease follow-up.

Keywords: Inmunoglobulin light chains; cardiac amyloidosis; autologous stem cell transplantation; patient-centered outcome; prognosis

Figure 1. Flowchart. Patients with immunoglobulin light chain amyloidosis of the Institutional Registry of Amyloidosis belonging to the Health Maintenance Organizations of the Italian Hospital of Buenos Aires. Period 2010-2021.

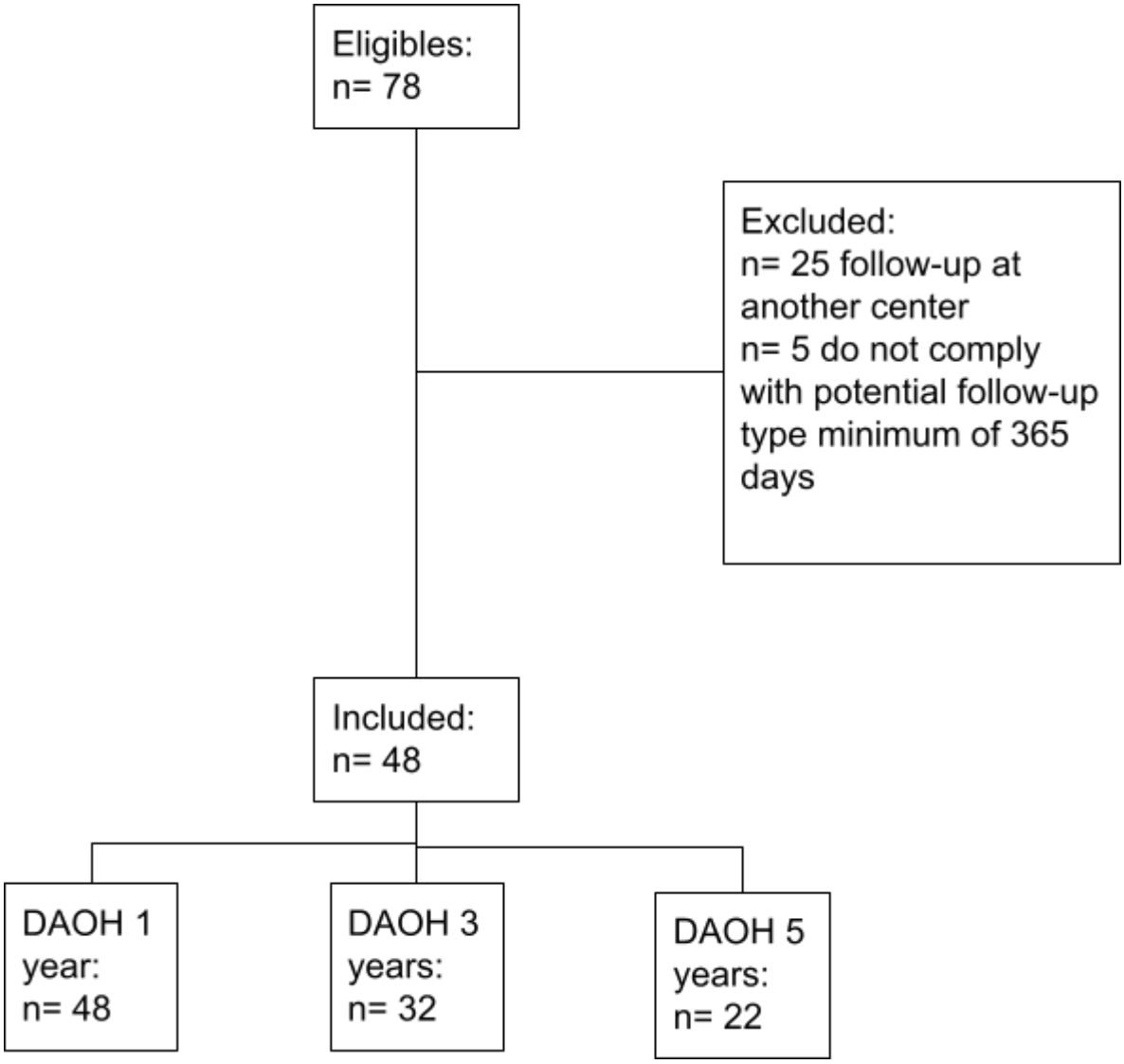
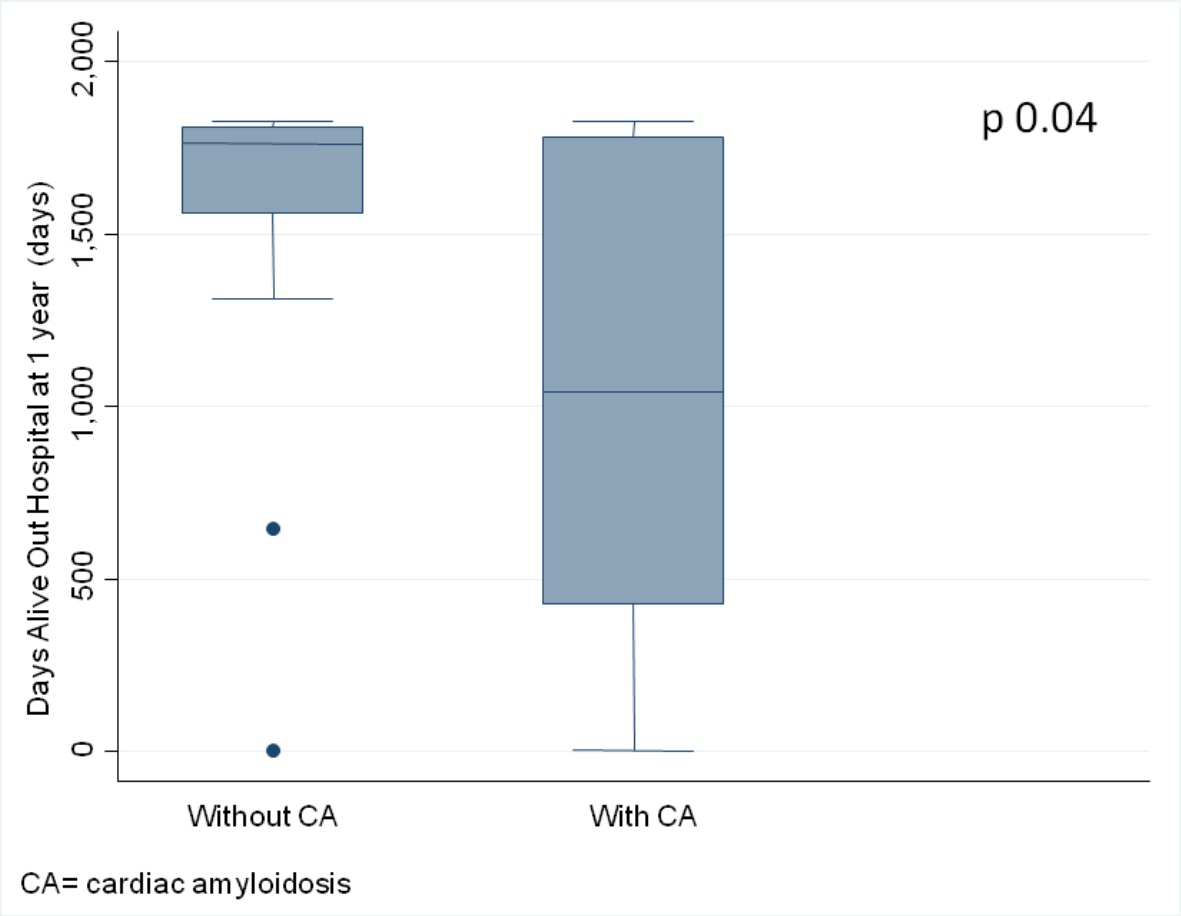


Figure 2. Box plot. Days Alive and Out of Hospital at 5 years in patients with amyloidosis AL with and without cardiac involvement.



Selection of appropriate quality of life instrument to measure patient-reported outcomes in systemic light chain (AL) amyloidosis

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Background: Light chain (AL) amyloidosis and its treatment can severely impact health-related quality of life (HRQL). Numerous tools exist to capture patient-reported outcomes (PROs) to measure symptom burden and HRQL in this disease, but little guidance exists for clinical researchers to be able select the best PRO tool.

Objective: We sought to study discriminations between severity of domain and HRQL deficit between AL amyloidosis patient groups in a cross-sectional study using 3 health measurement instruments.

Material & Methods: People with AL amyloidosis who were members of the Amyloidosis Support Groups, Inc. were invited to respond to an IRB-approved survey. In addition to baseline sociodemograhic information, individuals provided information on their amyloidosis subtype, type and number of organs involved, and cardiac biomarkers. Participants completed PROMIS, SF-36, and those with cardiac involvement additionally completed the KCCQ-12. The following scores were considered based on prior work as most significantly affected in AL amyloidosis[1,2]: total score of physical health (PROMIS GPSS, SF-36 PCS, KCCQ-12 Total), physical health domain, fatigue, and social roles. Internal consistency was compared using Cronbach's omega (0.7 is considered acceptable for psychometric scales), convergent/divergent validity was compared between domains using Pearson's correlation coefficient (correlation considered medium if 0.3-0.49 and strong at ≥0.5). Known groups validity was assessed by Cohen's *d* to discriminate between the 2004 Mayo stage groups (effect size ≤0.20- small, 0.21-0.49- moderate, ≥0.5- large).

Results: Of 297 survey participants, the median age at diagnosis was 60 years (23-82), 52% female, 90% white, AL (lambda) in 69%, with 39% reporting 3 or more organ involvement (58% cardiac, 58% renal, 30% neurological, 11% hepatic AL). The 2004 Mayo stage was calculated in a subset of participants who reported their cardiac biomarkers at diagnosis (N=106) and at last check closest to the survey (n=101). Therapy included prior chemotherapy in 88% and stem cell transplant in 52%, with 50% on current active therapy. Internal consistency was >0.7 across domains of interest in all 3 instruments. Figure 1 shows the correlation coefficients and figure 2 shows Cohen's d (green color in both tables show strong correlation or large effect size, yellow- medium correlation and moderate effect size, red- small effect size).

Summary & Conclusion: Multiple quality instruments can measure PROs in AL amyloidosis and the choice of the most appropriate item should be based on the context of use and concept of interest in an individual study. By including a multidimensional and flexible set of measures to choose from, PROMIS is an excellent choice for measuring physical health, fatigue, and social roles domains in AL amyloidosis.

Measure	PROMIS				SF-36				KCCQ-12		
	GPSS	PF	FT	SR	PCS	PF	VT	SF	Total	PL	SL
PROMIS											
Global Physical Summary Score (GPSS)	1										
Physical function (PF)	0.82	1									
Fatigue (FT)	0.81	0.69	1								
Social Roles (SR)	0.82	0.81	0.74	1							
SF-36											
Physical Componsent Score (PCS)	0.9	0.79	0.78	0.85	1						
Phyiscal Function (PF)	0.78	0.78	0.65	0.75	0.82	1					
Vitality (VT)	0.8	0.8	0.85	0.75	0.82	0.65	1				
Social Functioning (SF)	0.72	0.72	0.68	0.77	0.81	0.63	0.69	1			
KCCQ-12											
Total	0.77	0.75	0.66	0.8	0.79	0.77	0.68	0.7	1		
Physical Limitation (PL)	0.62	0.69	0.49	0.65	0.63	0.63	0.51	0.5	0.8	1	
Symptom Limitation (SL)	0.69	0.72	0.58	0.76	0.72	0.72	0.6	0.67	0.91	0.71	1

Figure 1.: Convergent validity across domains

Measure	2004 AL amyloidosis stage			Cohen's d, stage 3 versus 1 (95% confidence interval)	p-value
	Stage 1	Stage 2	Stage 3		
PROMIS					
GPSS	47.4 (10.3)	43.2 (7.8)	41.6 (9.0)	**-0.64 (-1.15; -0.13)**	0.034
Physical Function	46.7 (8.3)	43.2 (8.5)	40.4 (8.2)	**-0.76 (-1.26; -0.25)**	0.009
Fatigue	50.7 (10.1)	53.4 (8.6)	56.8 (10.8)	**0.61 (0.11; 1.12)**	0.040
Social Roles	51.0 (9.8)	47.2 (7.6)	44.9 (9.6)	**-0.68 (-1.19; -0.18)**	0.051
SF-36					
PCS	50.1 (9.8)	45.4 (8.1)	44.6 (8.7)	**-0.62 (-1.14; -0.11)**	0.056
Physical Function	49.3 (9.9)	46.5 (8.3)	42.2 (9.5)	**-0.76 (-1.28; -0.25)**	0.016
Vitality	48.8 (11.9)	46.4 (10.7)	44.4 (10.6)	**-0.40 (-0.90; 0.11)**	0.29
Social Functioning	49.7 (9.0)	47.7 (8.4)	44.6 (10.6)	**-0.55 (-1.06; -0.04)**	0.11
KCCQ-12					
Total	73.3 (20.0)	67.2 (22.4)	64.6 (22.0)	**-0.40 (-1.08; 0.28)**	0.54
Physical Limitation	72.6 (26.2)	66.1 (24.3)	68.4 (17.3)	**-0.19 (-0.87; 0.50)**	0.57
Social Limitation	76.2 (20.1)	64.4 (27.9)	62.0 (28.1)	**-0.54 (-1.22; 0.15)**	0.23

Figure 2: Known groups validity across AL amyloidosis stage

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Symptom burden and quality of life in AL amyloidosis patients among recently diagnosed and long-term survivors

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Background: Light chain (AL) amyloidosis and its treatment can produce significant symptoms and impaired health-related quality of life (HRQL).

Objective: We sought to understand how patient-reported outcomes and symptom burden differs among recently diagnosed AL amyloidosis patients and those who are long-term survivors in a cross-sectional study.

Material & Methods: Members of the Amyloidosis Support Groups, Inc. with AL amyloidosis were invited to complete this IRB-approved survey. In addition **to** baseline sociodemographic information, individuals provided information on their amyloidosis subtype, time from diagnosis, type and number of organs involved, and cardiac biomarker**s**. Patients were staged using the Mayo 2004 staging system. HRQL was measured using PROMIS and PRO-CTCAE questionnaires. PROMIS was scored with norm-based scoring that yield standardized distributions with a mean of 50 and standard deviation of 10 for a nationally representative sample of US adults. Higher score measures more of the domain it scores (e.g., Physical function- score >50 denotes better physical function than US average, Fatigue- score >50 denotes greater fatigue than US average).[1] The composite PRO-CTCAE grade was calculated based on frequency, severity, and interference as 0, 1, 2 or 3.[2]

Results: Of 297 participants who completed the survey, the median age at diagnosis was 60 years (23-82), 52% female, 90% white, AL (lambda) in 69%, with 39% reporting 3 or more organ involvement (58% cardiac, 58% renal, 30% neurological, 11% hepatic AL). The 2004 Mayo stage was calculated in a subset of participants who reported their biomarkers at diagnosis (N=106) and at last check (n=101). Stage improved in 43%, was unchanged in 49%, and worsened in 8%. Time from diagnosis to survey was < 2 years in 64 (22%), 2-5 years in 105 (36%), > 5 years in 126 (43%), and unknown in 2 participants. Therapy included prior chemotherapy in 88% and stem cell transplant in 52%, with 50% on current active therapy. Table 1 shows HRQL summary and domain scores in the current cohort with reference populations and prior study of newly diagnosed AL patients. Table 2 shows HRQL scores and symptom burden by time from diagnosis to survey completion.

Summary & Conclusion: While symptom burden is highest among patients who are within 2 years from diagnosis, long-term survivors of AL amyloidosis continue to report persistent detriment in HRQL. Two-year survivors have better global physical function, fatigue, social roles, anxiety, and depression scores compared to those <2 years from diagnosis. This study provides insight into the significant and persistent symptom burden of AL amyloidosis and its treatment. Symptoms should be routinely measured and used to provide best supportive care to all AL amyloidosis patients, including long-term survivors and those not on active therapy.

PROs	U.S. General Population (N=14,128)	US Healthy Population (N=2,161)	U.S. Cancer Patient norms (N=5,284)	Newly diagnosed AL (N=59)[3]	Current Cohort (N=297)	p-value*
GPSS	50 (10)	-	-	42.5 (12.1)	44.9 (9.7)	<0.001
GMSS	50 (10)	-	-	48.5 (9.4)	48.5 (10.1)	<0.001
Physical function	50 (10)	56.1 (6.7)	44.8 (0.2)	39.8 (10.8)	43.7 (9.0)	<0.001
Fatigue	50 (10)	45.3 (8.3)	52.2 (0.2)	55.6 (12.2)	53.4 (10.3)	<0.001
Social Roles	50 (10)	53.3 (7.9)	50.3 (0.2)	47.1 (10.9)	48.1 (9.4)	<0.001
Pain interference	50 (10)	45.5 (6.5)	52.4 (0.2)	51.2 (10.9)	50.7 (9.6)	0.2
Sleep disturbance	50 (10)	-	50.6 (0.2)	51.8 (9.9)	50.1 (9.6)	0.92
Anxiety	50 (10)	47.3 (7.7)	49.2 (0.2)	55.5 (8.7)	50.4 (8.8)	0.45
Depression	50 (10)	47.4 (7.8)	48.5 (0.2)	53.4 (9.2)	48.7 (7.8)	0.006
Cognitive Function	50 (10)	-	52.1 (0.2)	-	52.7 (7.3)	<0.001

Table 1.: Change in HRQL and symptom burden by time from AL amyloidosis diagnosis.

* p-value compares scores of current cohort with U.S. General Population.

For GPSS, GMSS, Physical Function, Social Roles and Cognitive function, higher score is better function, for Fatigue, Pain, Sleep, Anxiety and Depression, higher score is worse function.

	Overall Cohort (N=297[1])	≤2 years (N=64)	2-5 years (N=105)	>5 years (N=126)	p-value
PROMIS Summary Scores and Symptom Domains. T-scores shown with standard deviation					
GPSS, N=280	44.9 (9.7)	42(9)	45(9)	46(11)	0.037
GMSS, N=280	48.5 (10.1)	46(10)	48(9)	50(11)	0.10
Physical function, N=290	43.7 (9.0)	42(9)	44(8)	44(9)	0.11
Fatigue, N=286	53.4 (10.3)	56(9)	53(10)	52(11)	0.047
Social Roles, N=283	48.1 (9.4)	46(9)	48(9)	49(10)	0.048
Pain interference, N=281	50.7 (9.6)	52(10)	51(10)	50(9)	0.26
Sleep disturbance, N=283	50.1 (9.6)	51(9)	50(10)	49(9)	0.56
Anxiety, N=287	50.4 (8.8)	53(9)	50(9)	49(8)	0.011
Depression, N=287	48.7 (7.8)	51(9)	49(8)	47(7)	0.004
Cognition, N=281	52.7 (7.3)	52(7)	53(8)	52(7)	0.64
Individual symptoms using PRO-CTCAE (number and percent with composite grade ≥1)					
Total severity, N=271, Mean (SD)	5.7(4.2)	6.2(4.3)	5.9(4.0)	5.3(4.3)	0.35
Number of symptoms, N=271, Mean (SD)	5.0(2.4)	5.3(2.2)	5.3(2.2)	4.5(2.7)	0.05
Difficulty in swallowing, N=278	84 (30%)	19 (33%)	34 (34%)	31 (26%)	0.38
Decreased appetite, N=278	92 (33%)	20 (34%)	41 41%)	31 (26%)	0.055
Nausea, N=277	85 (31%)	20 (34%)	34 (34%)	31 (26%)	0.31
Vomiting, N=273	19 (7%)	4 (7%)	9 (10%)	6 (5%)	0.45
Constipation , N=275	114 (42%)	25 (44%)	42 (43%)	47 (40%)	0.85
Diarrhea, N=274	132 (48%)	28 (48%)	47 (48%)	57 (48%)	>0.99
Shortness of breath, N=274	166 (61%)	39 (67%)	58 (60%)	69 (57%)	0.49
Arm or Leg swelling, N=275	135 (49%)	29 (50%)	55 (56%)	51 (44%)	0.14
Numbness or tingling in hands or feet, N=275	175 (64%)	42 (73%)	60 (63%)	73 (61%)	0.33
Dizziness, N=275	103 (38%)	27 (46%)	33 (34%)	43 (36%)	0.29

Table 2. Symptom burden comparison by time from diagnosis.

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Performance of a survival staging system incorporating sST2 in patients with light chain amyloidosis

sST2 Predicts Mortality in Amyloidosis Beyond NT-proBNP and Troponin T

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ABSTRACT

Background: Cardiac involvement is the major determinant of prognosis in patients with light chain (AL) amyloidosis. Soluble suppression of tumorigenesis-2 (sST2) is a biomarker related to inflammation and fibrosis. Unlike NT-proBNP, sST2 is not affected by renal function.

Objective: We sought to investigate performance of sST2-incorporated stating system in comparison with current stating system based on NT-proBNP and analyzed if sST2-based staging system was superior to revised Mayo staging system (NT-proBNP-based system) in advanced chronic kidney patients (CKD).

Methods: We identified 277 patients who were enrolled in prospective amyloid registry at Samsung medical center from November, 2015 to November 2019. All patients underwent laboratory test and imaging studies including echocardiography at the time of diagnosis. Advanced CKD was defined as eGFR < 30 mL/min/1.73 m^2. Among our cohorts, 27 (11.8%) were advanced CKD. The cut-off value of sST2 (30ng/mL) was used from previous studies(1,2).

Results: Both sST2 and NT-proBNP-based system were effective in adjudicating cardiac involvement, as suggested by statistically significant AUC (Figure 1). There was a strong agreement (kappa=0.80) between NT-proBNP-based system and sST2-based staging (Figure 2). For predicting survival, both sST2 and NT-proBNP showed satisfactory for predicting survival ($p<0.001$), however, in advanced CKD patients NT-proBNP were not effective for predicting survival (Figure3).

Summary and Conclusions sST2-based staging system showed strong agreement with current revised Mayo staging system (NT-proBNP based). sST2 based staging system showed superior predictive performance for survival in advanced CKD patients compared to current NT-proBNP based stating system.

Figure 1. ROC curves based on cardiac involvement

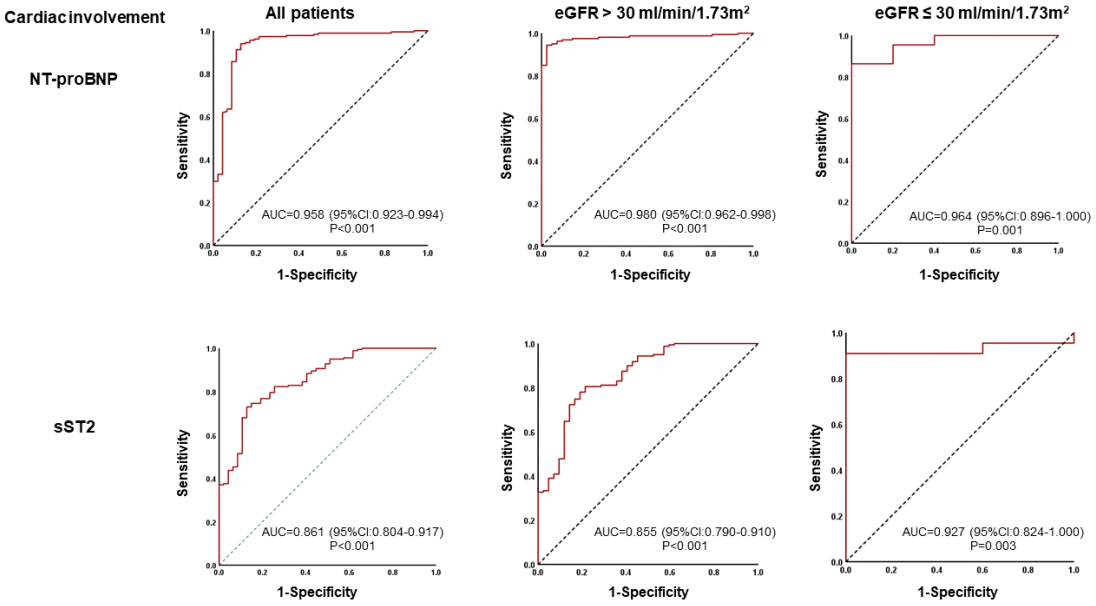
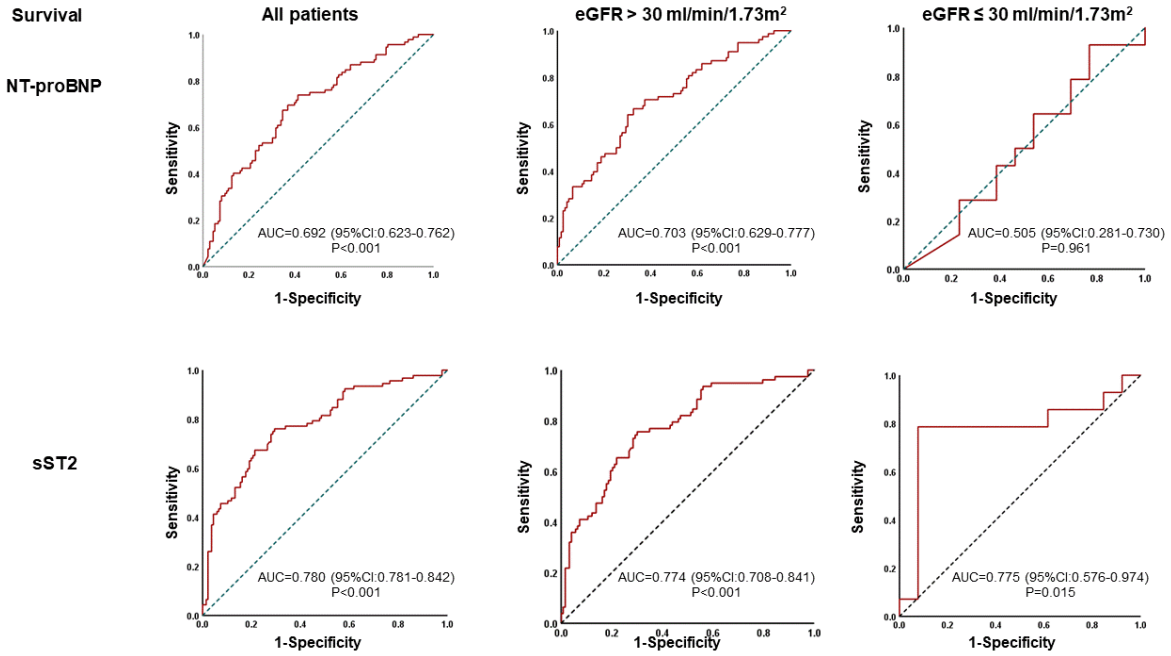


Figure 2. Concordance between revised Mayo and sST2-based stage (kappa=0.80)

	Revised Mayo I	Revised Mayo II	Revised Mayo III	Revised Mayo IV
sST2 Stage I	5	3	0	0
sST2 Stage II	2	43	16	0
sST2 stage III	0	11	38	25
sST2 stage IV	0	0	12	73

Figure 3. ROC curves based on survival.



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Evaluation of the Patterns Leading to Diagnosis in Patients With Amyloid Light-Chain Amyloidosis Using the Komodo Database

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Background: Light-chain amyloidosis (AL) is a rare, progressive, systemic disease and causes significant morbidity and mortality. Nonspecific symptoms may be confused with those of other cardiovascular (CV) diseases and delay diagnosis.

Objective: The goal of this analysis was to describe a current AL population and diagnostic journey with real-world data.

Material & Methods: Retrospective cohort study of adults (age ≥ 18 years) with AL was conducted using US claims data including closed medical (inpatient and outpatient) and outpatient-pharmacy-dispensed claims between January 1, 2015 and January 1, 2021, from the Komodo Health payer-complete dataset. Continuously-enrolled patients were determined by insurance eligibility periods and had undergone insurance adjudication. Patients with ≥24 months continuous coverage from first claim of any condition to first AL diagnosis (index date; ICD-10: E85.81) and follow-up ≥6 months post-diagnosis were included. Results were reported using descriptive statistics.

Results: 2,621 AL patients were identified with mean age of 65.6 years and 55.6% male (Table 1). AL patients visited 2, 5, 8 and 11 specialists on average on index date and within 1, 6 and 12 months before or on index date, respectively. AL diagnosis was most frequently made by internal medicine doctors. Within 12 months before or on index date, internal medicine was the most often visited specialist, followed by diagnostic radiology and cardiology (74.1%, 72.7% and 64.0%, respectively. Table 2). Prior to diagnosis, CV symptoms were reported most (91.1%, table 2) and were more common than renal (64.4%) or gastrointestinal (53.7%) symptoms. Mean time from first symptom to diagnosis was longer for those with CV symptoms (31.4 months, table 2) than with renal (20.0 months) or gastrointestinal (24.8 months).

Summary & Conclusion: Many AL patients have CV symptoms before diagnosis yet have longest time to diagnosis likely due to nonspecific symptoms and overlap with CV conditions common in advanced aged patients. Greater awareness of presenting symptoms by cardiologists is needed to improve early AL diagnosis.

Table 1.: Patient baseline charactersistics and diagnostics on index date

Total Patients	**N = 2,621**	**100.0%**
Age, years		
Mean (Std Error)		65.6 (11.4)
Median (min-max)		66 (19-88)
Age Group, years		
18 to 44	106	4.0%
45 to 64	1,098	41.9%
65+	1,417	54.1%
Sex		
Male	1,456	55.6%
Female	1,165	44.4%
Primary Diagnosis of AL		
Yes	1,365	52.1%
No	1,256	47.9%
Multiple Myeloma Diagnosis		
Yes	502	19.2%
No	2,119	80.8%

Table 2.: Specialists visited and pre-index CV symptoms

Top 5 Specialists Visited	On Index Date		Within 12 Months Before or on Index Date	
Unique Patients	**N = 2166**	**100.0%**	**N = 2575**	**100.0%**
Internal Medicine	588	27.1%	1,907	74.1%
Diagnostic Radiology	-	-	1,873	72.7%
Hematology & Oncology	434	20.0%	-	-
Pathology	427	19.7%	1,535	59.6%
Cardiology	380	17.5%	1,648	64.0%
Medical Oncology	362	16.7%	-	-
Emergency Medicine	-	-	1,219	47.3%
Pre-index CV Symptoms	**Proportion with CV Symptoms**		**Time from Earliest CV Symptoms to Index Date, months**	
	N (N=2,621)	**%**	**Mean (SD)**	**Median (Min-Max)**
CV Symptoms	2388	91.1%	31.4 (17.9)	32 (0-70)
Dyspnea	1,786	68.1%	24.4 (17.7)	23 (0-70)
Edema	1,339	51.1%	20.5 (17.2)	17 (0-69)
Arrythmias	1,374	52.4%	25.0 (18.3)	24 (0-70)
Fatigue	1,192	45.5%	26.9 (18.5)	26 (0-70)
Unspecified Heart Failure	997	38.0%	15.7 (14.9)	11 (0-67)
Left ventricular hypertrophy	876	33.4%	18.2 (16.2)	14 (0-68)
Hypotension	683	26.1%	15.5 (15.6)	10 (0-67)
Fainting/syncopal episodes	538	20.5%	20.2 (17.0)	16 (0-65)
Cardiomyopathy	390	14.9%	17.1 (15.6)	12 (0-65)

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Healthcare resource utilization in patients with light chain amyloidosis in Europe

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Background: Systemic light chain (AL) amyloidosis is a rare plasma cell disease characterized by the accumulation of amyloid fibrils in tissues and organs throughout the body, leading to multiorgan dysfunction. Beyond anti-clonal therapy, this disease treatment requires multidisciplinary teams and resources to manage its multiorgan complications. However, the burden of the disease for health care system and health care resource use (HCRU) remain underexplored.

Objective: To assess the HCRU in patients (pts) with AL amyloidosis across several European countries.

Material & Methods: EMN23, a retrospective, multicenter study, investigated the demographic and clinical characteristics, the treatment pathways, and the HCRU of adult pts with AL amyloidosis who initiated treatment in 2004–2018 from 13 sites across 10 European countries. The HCRU analysis on the 2011–2018 period included patient data from 7 countries (Greece, France, the Netherlands, Portugal, the Czech Republic, Austria, and Spain [only dialysis information available]). Patients who participated in clinical trials at any line were excluded. HCRU measures included hospitalizations, imaging examinations, adverse events of special interest (AESIs; intensive care unit (ICU) admission, heart failure, need for pacemaker, infections requiring hospitalization, peripheral neuropathy), and dialysis information.

Results: In 2011–2018, HCRU data and safety information were available for 462 pts (531 pts. with dialysis data). Most (63.4%) pts were males ≥65 years old (52.4%). At baseline, 41.6% (192/462) pts had 1 organ involved, 36.1% (167/462), had 2 and 20.8% (96/462) pts had ≥3 organs involved. The proportions of pts per the Eastern Cooperative Oncology Group (ECOG) status of 0, 1, 2, and 3 were 20.8% (96/462), 24.9% (115/462), 19.0% (88/462), and 7.8% (36/472), respectively; (not reported for 26.4% (122/462) pts). At diagnosis, Mayo 2004/European cardiac stages I, II, IIIa, and IIIb distribution was 75 (16.2%), 126 (27.3%), 127 (27.5%), and 59 (12.8%), respectively (Table) and was unknown for 75 (16.2%) pts. The number of pts with ≥1 hospitalization was 280 (60.6%). Duration of hospitalization was longer for patients at stage IIIa and IIIb compared to lower stages (Table). Ultrasound and X-rays were the most common imaging exams across most cardiac stages (Stage I: 45.3%; Stage II: 42.1%, Stage IIIa: 47.2%; Stage IIIb: 39.0%), but MRIs and scintigraphy were also used commonly (see Table). At first-line, the proportions of pts treated with immunomodulatory (IMiD)-based regimens, bortezomib-based regimens, chemotherapy, and autologous stem cell transplantation were 2.2% (10/462), 62.3% (288/462), 12.6% (58/426) and 11.5% (53/462), respectively. The proportions of pts with ≥1 SAE and ≥1 AESI were 49.4% (228/462) and 28.8% (133/462), respectively. Infectious complications and heart failure were common reasons for

hospitalization, with half of the AESI records for cardiac stage IIIb patients being cardiac failure and 1/10 AESI records being ICU admissions. A median number of 70.5–139.6 dialyses per pt per year were received by 70 (13.2%) pts, across cardiac stages.

Summary & Conclusion: The current analysis shows that in the recent years (post-2010), the burden of HCRU for pts with AL Amyloidosis was considerable, across all Mayo2004/European cardiac stages. The main burdensome components were hospitalizations and dialysis. A cost-of-illness study from the healthcare perspective will be conducted, to reveal the cost burden on the healthcare system of pts with AL amyloidosis in Europe.

Support & Funding: The study was sponsored by the European Myeloma Network and funded by Janssen.

Reduction of cardiac AL amyloid deposition after complete response visualized by PiB-PET imaging

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Background: There has been recent progress in research on cardiac amyloidosis using ^{11}C-Labeled Pittsburgh compound B (PiB) [1] and other amyloid PET diagnostic agents [2]. Previous studies reported that amyloid tracer uptake differed according to amyloidosis subtype [2, 3], and in immunoglobulin light chain (AL) amyloidosis, the degree of cardiac PiB uptake was correlated with both the histological amyloid burden in the myocardium and unfavorable disease prognosis [4]. Although a small number of AL amyloidosis patients who underwent repeated amyloid PET before and after treatment have been reported, significant reduction of cardiac tracer uptake has not yet been demonstrated [5].

Objective: To investigate changes in myocardial amyloid burden and clinical parameters before and after treatment using PiB-PET in patients with systemic AL amyloidosis who achieved hematological complete response (CR) by chemotherapy.

Patients & Material & Methods: Two to six PiB-PET scans were performed at baseline and 6 – 59 months after achievement of CR in 7 AL patients. Using PET/CT images, the standardized uptake value (SUV) peak of the heart ($SUV_{myocardium}$) was measured by placing the volume of interest (VOI) on the region of left ventricular myocardium with maximal intensity. Next, a VOI was placed in the left atrium and the SUV mean of blood pool accumulation ($SUV_{background}$) was measured. Then, myocardium/background ratio (MBR = $SUV_{myocardium}/SUV_{background}$) and its reduction rate (MBR reduction rate) = (MBR baseline-MBR latest point)/MBR baseline × 100 were determined. The correlations of the N-terminal of the prohormone brain natriuretic peptide (NT-proBNP), troponin T (TnT), plasmin-α2–antiplasmin inhibitor complex (PIC), and interventricular septal diameter (IVS) as clinical cardiac parameters with MBR were investigated.

Results: Statistical analysis showed significant reduction of MBR from 4.23 ± 1.46 to 3.33 ± 1.34 after CR (Mean ± SE, P = 0.028, Wilcoxon's signed-rank test). Changes in parameters in a typical case (73 years old, Female) are shown in Fig. 1 and 2. Sequential PiB-PET scans clearly demonstrated regression of radiological cardiac amyloid burden over a period of 5 years (Fig. 1), which correlated with cardiac markers (Fig. 2). Statistical analysis showed significant correlations between rate of MBR reduction and those of NT-proBNP (P = 0.0025) and TnT (P = 0.014), whereas MBR showed no correlations with PIC or IVS (Spearman's rank correlation coefficient).

Summary & Conclusion: This study first demonstrated that PiB-PET can visualize significant reduction of cardiac AL amyloid deposition after CR. Amyloid reduction was closely correlated with serum cardiac biomarkers (i.e., NT-proBNP and TnT). On the other hand, IVS did not change, suggesting that histological myocardial remodeling would take a much longer time. In conclusion, PiB-PET is a promising tool for noninvasive monitoring of the amount of cardiac amyloid deposition.

Figure 1

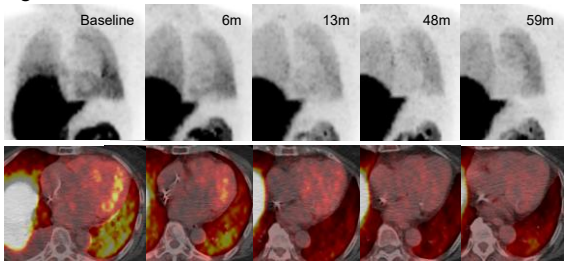

Figure 2

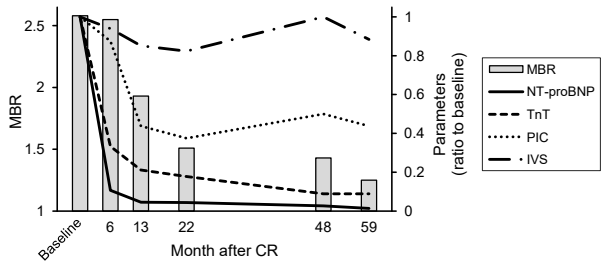

Figure 1.: Sequential changes in maximum intensity projection (MIP) images of PiB-PET (upper line) and fused PET/CT axial images (lower line) of a typical case (73 years old, Female). Radiological cardiac amyloid burden disappeared gradually 5 years after CR.

Figure 2.: Changes in radiological (MBR), serum (NT-proBNP, TnT, PIC), and echocardiography (IVS) parameters after achieving CR in the same typical case as Fig. 1. Gray bars indicate MBR values (left axis). Changes in parameter values (solid or dotted lines) other than MBR are indicated as ratios relative to baseline (right axis).

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The impact of longitudinal strain (LS) response in patients with advanced cardiac AL amyloidosis

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Background: The burden of cardiac involvement is the main determinant of prognosis in systemic AL amyloidosis. We recently reported longitudinal strain (LS) at diagnosis and changes in LS after treatment as independent prognostic markers and additive to the current cardiac biomarkers based staging for cardiac AL. We report the prognostic utility of LS and changes following treatment focusing on patients with high risk cardiac AL amyloidosis (European modification of Mayo 2004: stages IIIa and IIIb).

Objective: The primary objective was to assess the impact of LS at diagnosis on outcomes and changes in LS following treatment specifically in high risk patients. The secondary objectives were to assess the impact of a change in LS through the prism of a haematological response including those with a dFLC <10mg/l or iFLC <20mg/l. Finally, we assessed the impact of a sustained change in LS following first line therapy on prognosis.

Material & Methods: All patients from a prospective observational study of newly diagnosed AL amyloidosis (ALCHEMY) with confirmed cardiac amyloidosis and Mayo stage 3a or 3b were included. In addition to intention to treat baseline analysis of overall survival, landmark analyses were undertaken at 6 and 12 months post treatment. LS was performed and calculated as previously published (1) including an improvement in overall LS of >2.0%, as the minimum value to account for inter-observer variability, (2) termed an 'LS response (LS-R).
We subdivided LS-R into 4 categories: an LS sustained response (LS-SR) defined as patients maintaining an LS-R ≥-2.0% compared to baseline at each and every landmark analysis; a LS gained response (LS-GR) as an improvement in LS of ≥-2.0% compared to a previous time point of no LS-R; a longitudinal strain lost response (LS-LR) as an initial LS-R which then reverted to within <-2.0% of baseline and an LS no response (LS-NR) for patients not achieving an improvement in LS at any point.

Results: 412 patients were identified and median LS was for stage 3a and 3b was -11.25% and -9.1% (p=<0.0001) respectively. Median OS for the entire cohort based on previously identified baseline LS group (≤−16.2%, between −16.1% and −12.2%, between −12.1%, and −9.1% and ≥−9.0%) was 46 months, 30 months, 20 months and 5 months respectively (p=<0.001). We noted an LS-R in 46/194 (23.7%) by 12 months.159 patients were assessed at both 6 and 12 month with LS-SR in 14(8.8%), LS-GR in 21(13.2%), LS-LR in 17(10.7%) and LS-NR in 107(67.3%)
The overall survival (OS) was significantly better for patients with an LS-R at 12 month assessment for stage 3a (median OS not reached (NR) vs. 54 mo (p=<0.001) and in patients with LS-R plus complete haematological response (CR) vs. those who just achieved a CR but no LS-R (median OS NR vs 57 mo (p=0.006)) as well as patients with LS-R plus dFLC <10mg/l vs. a dFLC<10mg/l without LS-R (median OS NR vs 71 mo (p=0.01)).
Patients with LS-SR between 6 and 12 month or LS-GR at 12 months had a significatly better OS (median NR) compared to those with LS-NR or LS-LR (median 59 mo and 70 mo, respectively (p=0.054)). At 3 yrs, the OS was: for LS-SR 86%, LS-GR 79%, LS-LR 71% and LS-NR 65% and at 5 years LS-SR 75%, LS-GR 63%, LS-LR 35% and LS-NR 25%.

Summary & Conclusion: LS at baseline and LS-R remain informative markers of outcome even in patients with high-risk cardiac AL amyloidosis beyond conventional cardiac and haematological serological markers. Crucially, patients who

achieve and sustain an LS response have a better prognosis even in the context of a deep haematological response.

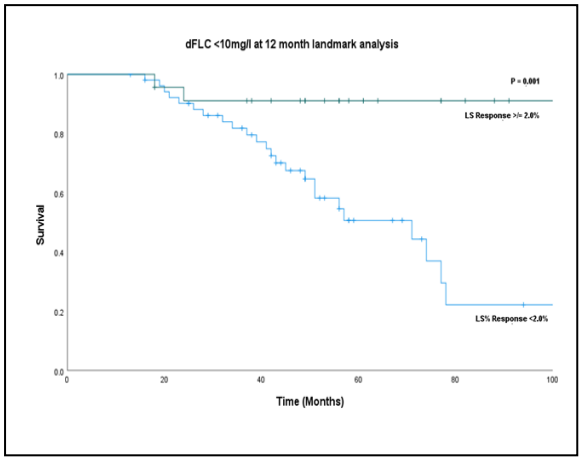

Figure 1.: Overall survival at the 12 month landmark analysis for stage 3a and 3b patients who had achieved a deep haematological response (dFLC <10 mg/L) showing significantly superior survival for patients who achieved an LS response compared to those who did not achieve an LS response even when a dFLC of <10mg/l was achieved following therapy.

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CMR with T1 mapping in systemic light-chain (AL) amyloidosis: from cardiac amyloid regression to refining treatment response

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Objectives: To assess the ability of cardiovascular magnetic resonance (CMR) to: 1) measure changes in response to chemotherapy; 2) assess the correlation between haematological response (HMR) and changes in extracellular volume (ECV); 3) assess the association between changes in ECV and prognosis over and above existing predictors.

Material & Methods: In total, 176 patients with cardiac AL amyloidosis were assessed using serial NT-proBNP, echocardiography, free light chains and CMR with T1 and ECV mapping at diagnosis and subsequently 6, 12 and 24 months after starting chemotherapy. HMR was graded as complete response (CR), very good partial response (VGPR), partial response (PR) or no response (NR). CMR response was graded by changes in ECV as progression (≥0.05 increase), stable (<0.05 change) or regression (≥0.05 decrease).

Results & Discussion: At 6 months, CMR regression was observed in 3% (all CR/VGPR) and CMR progression in 32% (61% in PR/NR; 39% CR/VGPR). After 1 year, 22% had regression (all CR/VGPR) and 22% had progression (63% in PR/NR; 37% CR/VGPR). At 2 years, 38% had regression (all CR/VGPR) and 14% had progression (80% in PR/NR; 20% CR/VGPR). Thirty-six (25%) patients died during follow-up (40±15 months); CMR response at 6 months

predicted death (progression HR 3.821; 95% CI 1.950-7.487; p<0.001) and remained prognostic after adjusting for HMR, NT-proBNP and longitudinal strain (p<0.01) Figure 1..

Summary & Conclusions: CMR with ECV measurements can track changes in patients with AL cardiac amyloid deposits over time, which most likely represent changes in the cardiac amyloid burden. ECV can track not just progression with unsuccessful chemotherapy, but also demonstrates reduction, which is most likely to represent amyloid regression when free light chain precursors are removed by effective chemotherapy (Figure 2). Changes in ECV is independently associated with prognosis, supporting the unique role of CMR in assessing treatment response.

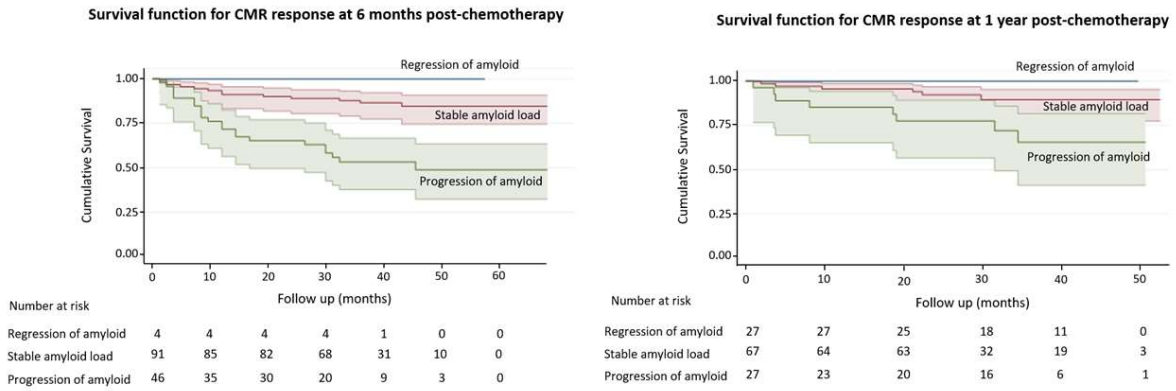

Figure 1. Kaplan-Meier survival curves, with shaded 95% confidence regions, displaying survival in all patients according to change in amyloid burden (measured by the change in ECV on follow-up CMR) after 6-months (left panel) and 1 year of chemotherapy (right panel).

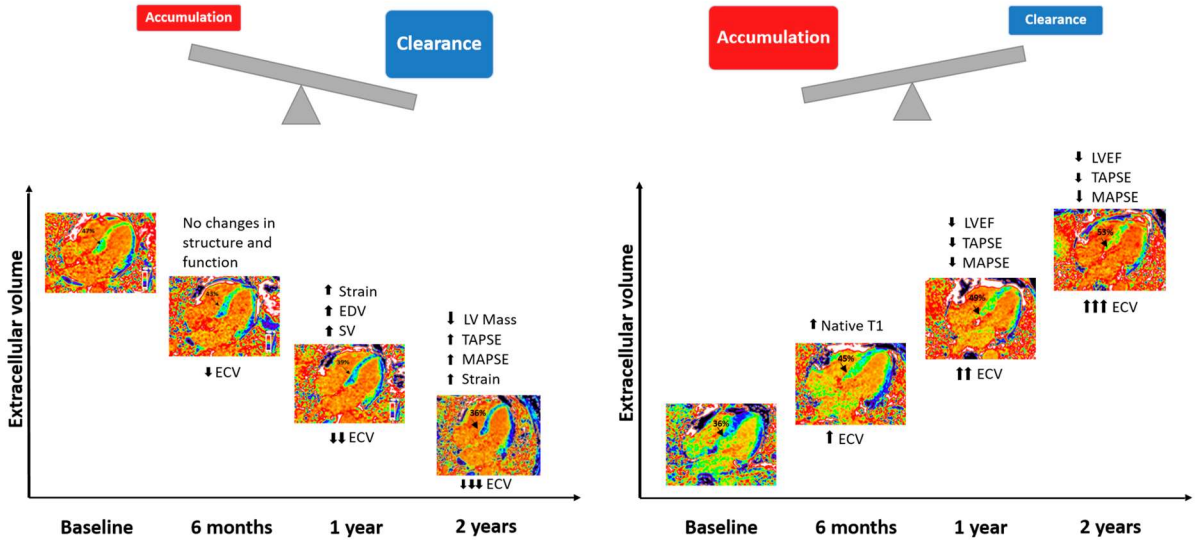

Figure 2. Hypothesized cardiac amyloid regression (left panel) and progression (right panel) across time after chemotherapy

Outcomes for patients with systemic light chain (AL) amyloidosis and Mayo stage 3B disease

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Background: Cardiac involvement is the leading factor that defines prognosis in primary systemic light chain (AL) amyloidosis. Based on cardiobiomarkers an ultra-high risk population has been identified, those with Mayo stage 3b disease defined as stage 3 with NTproBNP >8500 ng/L. The introduction of novel immunotherapies, like daratumumab, has improved outcomes for patients with AL amyloidosis, but the management of those with stage 3B disease remains an unmet need while such patients are usually excluded from clinical trials.

Objective: To report the clinical characteristics and treatment outcomes of patients with Mayo stage 3b AL amyloidosis from a referral center in Athens, Greece.

Material & Methods: We analyzed the data of 80 prospectively registered patients with Mayo stage 3b AL amyloidosis defined as either NTproBNP > 8500 ng/L or BNP > 700 ng/L and cTnT >0.035μg/L or cTnI > 0.1 μg/L or hsTnT > 54 ng/L.

Results: The median age was 68 years (range 38-87), all had cardiac and 38 (47.5%) patients had also renal, 17 (21.3%) liver, 26 (32.5%) nerve and 27 (33.8%) soft tissue involvement ;16 (20%) patients had only heart involvement. The median level of NTproBNP was 14998 ng/L (range 8500–75000 ng/L) and median dFLC was 405 (8-7353) mg/L; 35 (44%) had SBP <100 mmHg, 15 (19%) had SBP < 90 mmHg and distribution at NYHA stages was 31%, 32.5%, 24% & 12.5% for class 2, 3A, 3B & 4, respectively. Diuretics were the most commonly used drug for heart failure management (median dose of furosemide was 40 mg/day, range 0-500). During the disease course, 59 (74%) patients required hospitalization, mostly for congestive heart failure and fluid management (43%), acute renal failure (13%), infections (6.5%) and syncope (4%) and 17% required ICU admission.

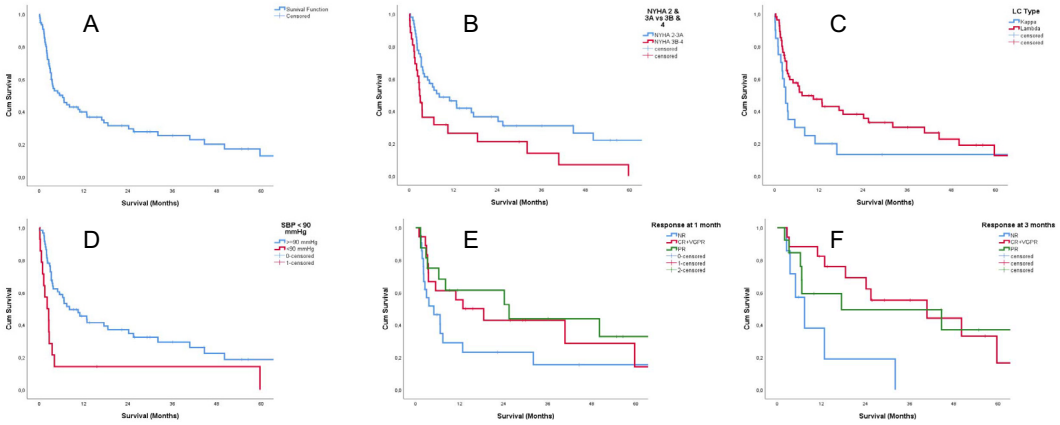
Primary treatment was VCd in 61%, in 21% treatment included also daratumumab, 7.5% received lenalidomide-based regimens and 5% melphalan/dexamethasone while 3(4%) patients died prior to treatment initiation. Eight patients (10%) died during the first cycle of therapy.

Overall, 48 (60%) patients were evaluable for response during 1st line therapy; on intention to treat best response was CR+VGPR in 25% and PR in 15% (ORR: 40%). At 1 month landmark, hem-response (≥PR) was associated with better OS (24.1 vs 4.9 months, p=0.027) and at 3 months, at least hemVGPR was associated with a median OS of 40.7 months vs 17 months for hemPR and 7.4 months for those without hematologic response (p=0.028). On intention to treat, 3-month cardiac response rate was 10% and at 6 months was 14%. The median OS for the whole cohort was 6.3 months. On univariate analysis, κ-light chain (median OS 2.9 vs 7.4 months, p=0.028), nerve involvement (3.4 vs 10.45 months, p=0.024), SBP <90 mmHg (2 vs 8 months, p=0.002) and NYHA class (2.7 months for NYHA 3B-4 vs 8 for NYHA 2-3A, p=0.02) were associated with poorer median OS. Factors associated with early mortality were orthostasis (p=0.019), purpura (p=0.049) and NYHA stage 3B-4 (p=0.004). Twenty patients (25%) received salvage

therapy; median time to next treatment for these patients was 3.1 months and the median OS from the start of 2nd line therapy was 10 months.

Summary & Conclusions: Patients with stage 3b AL amyloidosis benefit from early hematologic response, but early mortality remains unacceptably high and cardiac response rates low. Only a minority of these patients may have a second chance which is still associated with poor prognosis. There is an urgent need for new treatment strategies for this vulnerable patient population.

Figure 1: A. Overall Survival (OS). B. OS according to NYHA stage C. light chain type (κ vs λ) D. SBP < 90 mmHg E. according to response at 1-month landmark and at F. 3-months landmark



Finding a needle in a haystack: oligomer detection via urinary extracellular vesicles in AL light chain amyloidosis

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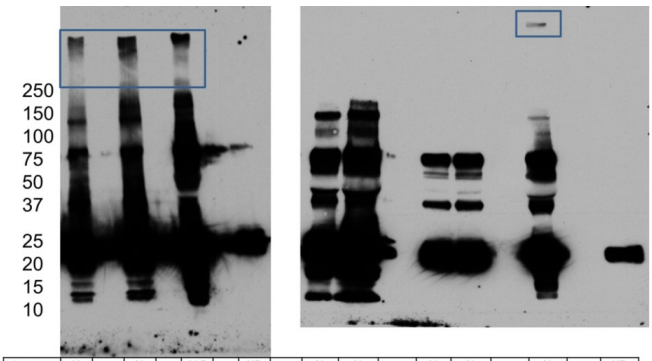
Background: Light Chain (AL) Amyloidosis stems from the overproduction of monoclonal, amyloidogenic immunoglobulin light chains (LC) by plasma cells with subsequent deposition and progressive dysfunction of the heart and kidneys, predominantly. Urinary extracellular vesicles (uEVs) are produced by renal epithelial cells throughout the nephron. Our previous work has shown that uEVs from active AL amyloidosis patients contain high molecular weight LC oligomers. The oligomers are large (>250kDa), resistant to heat and chemical denaturation, but low abundance. Renal dysfunction in AL results in high urine protein, compounding technical challenges to use uEVs as analytical tools.

Objective: Develop a reliable methodology to detect low abundance light chain protein targets within complex urinary EV samples without interference from antibodies used or normal repertoire immunoglobulins. In addition, we would like to develop a method to standardize samples so we can compare between patients and between samples from the same patient taken at different times.

Material & Methods: uEVs were isolated via ultracentrifugation from 24 hour Urine Collections from Plasma Dyscrasia Clinic Patients and Healthy Donors as previously reported (1, 2). We have reported the methods of our study through the Nature protocol exchange (3) and EV track (4).

Results: In this study, uEVs were isolated from AL amyloidosis, non-amyloid Monoclonal Gammopathy of Undetermined Significance, (MGUS), Proliferative Glomerulonephritis, and healthy donors. Total protein concentration was assessed by Bradford and HPLC, particle concentration and size distribution by Nanoparticle Tracking Analysis (NTA), oligomer presence by Western blot, and general morphology by transmission electron microscopy. Results suggest that uEV protein, urine volume, and particle concentrations are not directly correlated. Multiple strategies for overcoming non-specific antibody binding in uEV samples were validated. Sensitivity for pre-clinical testing was improved with a urine sample requirement algorithm. Critically, sample standardization was assay dependent rather than simply equal protein or urine volume; a valuable consideration for the EV field.

Summary & Conclusion: Our results show that antibody selection is crucial for the success of our experiments. Non-specific antibodies can cause serious reproducibility issues. Even pre-adsorbed secondary antibodies are unreliable at high sample concentrations. Standardization of uEV biomarker detection offers applications for other EV research projects. Urine is an extremely variable biofluid and uEV isolation is labor intensive, so ensuring adequate and appropriate study material is critical to generate reliable results.



Patient sample	AL 263	AL 263 TP	ALD 64-G	HD 101-B	AL 250	AL 250	AL D64-F	AL D64-F	AL D64-F	HD 101-B
Total Protein	17.2 µg	28.4 µg	33.3 µg	13.2 µg	21.7 µg	65.1 µg	4.6 µg	7.2 µg	32.6 µg	6.6 µg
Non-Albumin uEV	15 µg	24.7 µg	15 µg		3.7 µg	11 µg	2.1 µg	3.3 µg	15 µg	
	ANTI-Kappa Free Light Chain				ANTI-Lambda Free Light Chain					

Figure 1.: Kappa (left panel) and lambda (right panel) free light chain oligomers are detected (blue boxes showing high molecular weight bands above 250 kDa species) utilizing a pre-incubation of primary and secondary antibody to reduce potential non-specific binding. ALD64-G was used to normalize AL263TP (Total Protein). 15µg of non-uEV protein is required to detect oligomers in active disease. Healthy control samples are loaded as maximum amount the well will hold because the urine inherently contains less protein.

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Support & Funding: This study was supported by Mayo Clinic Department of laboratory medicine and pathology small grants (MRA, NL), the generous support from Dr. Fernando Cosio (MRA), the Obaid fund (CAS), the Mayo Foundation and the generosity of Amyloidosis patients and their families.

Longitudinal testing for measurable residual disease (MRD) using multiparametric flow cytometry in patients with systemic light chain amyloidosis

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Background: Flow-based evaluation of measurable residual disease (MRD) can provide an index of robust disease control in hematologic disorders, particularly if MRD negativity is sustained over time. The role of longitudinal MRD testing as part of disease tracking in light chain (AL) amyloidosis has not yet been established. Understanding the temporal kinetics of MRD in relation to clinical outcomes could help define optimal frequency and duration of MRD testing.

Objective: To investigate the depth and dynamics of MRD during post-treatment disease surveillance of patients with AL amyloidosis in hematologic complete response (hemCR).

Material & Methods: We ascertained MRD status for a cohort of patients at the Boston University Amyloidosis Center using multiparametric 10-color flow cytometry of bone marrow aspirates (sensitivity level of $\leq 10^{-5}$). Sequential MRD testing was performed at ≥12-month intervals for patients returning for follow-up, until hematologic relapse or start of subsequent therapy. Sustained MRD negativity was defined as MRD negativity on 2 occasions ≥12 months apart. Hematologic progression was established by serum free light chain levels and serum/urine immunofixation electorphoresis studies according to consensus guidelines [1]. Start of subsequent therapy due to organ deterioration in the absence of hematologic relapse was considered clinical progression.

Results: Among 121 patients in hemCR after therapy who underwent 1st MRD assessment (median 26 months from hemCR achievement, IQR 13–73 months), 60 were eligible for longitudinal MRD testing. The remainder were ineligible due to end of prospective follow-up, disease progression or death.

Of the 60 patients who underwent 2nd MRD assessment (median 12 months after 1st MRD assessment, IQR 12–24 months), 28 (47%) had sustained MRD negativity; 24 (40%) had persistent MRD positivity; and 8 (13%) had MRD status conversion [**Figure 1**]. Thirteen patients underwent a 3rd MRD assessment, each maintaining the same MRD status as seen on 2nd MRD assessment. All patients with sustained MRD negativity remained free of hematologic or clinical progression during the follow-up period. Of the 5 patients who experienced loss of MRD negativity (which occurred 12–114 months after last treatment), 2 had subsequent hematologic progression. MRD relapse preceded hematologic progression by 23 months in both patients; neither experienced organ deterioration requiring initiation of subsequent therapy. Among those with persistent MRD positivity, 15/24 (63%) displayed durable hemCR and organ stability; moreover, 6/16 (38%) and 3/15 (20%) of these patients attained even deeper renal and cardiac responses, resepctively, in between sequential MRD testing.

While larger MRD clone size on 1st assessment correlated with subsequent hematologic progression, clone growth was not apparent on longitudinal MRD testing preceding hematologic progression [**Figure 2**]. In fact, clone progression (defined as >1-log growth in MRD clone size) only occurred in 2/24 (8%) patients with persistent MRD positivity, none of whom demonstrated hematologic or clinical relapse.

Summary & Conclusion: We studied longitudinal MRD monitoring in AL amyloidosis and observed sustained MRD negativity in 47% of patients with a hemCR after therapy. Although limited by small sample size, our study suggests that loss of MRD negativity could be an early marker of progressive disease. However, there seemed to be a disconnect between MRD clone dynamics and clinical outcomes, possibly due to bone marrow sampling and spatial heterogenity of disease.

Figure 1. MRD evolution patterns in relation to clinical outcomes. Flow cytometry-based longitudinal MRD assessments were performed ≥12 months apart. Clinical progression was defined as the start of subsequent treatment due to organ deterioration in the absence of hematologic progression. Circle cap lines signify end of prospective follow-up.

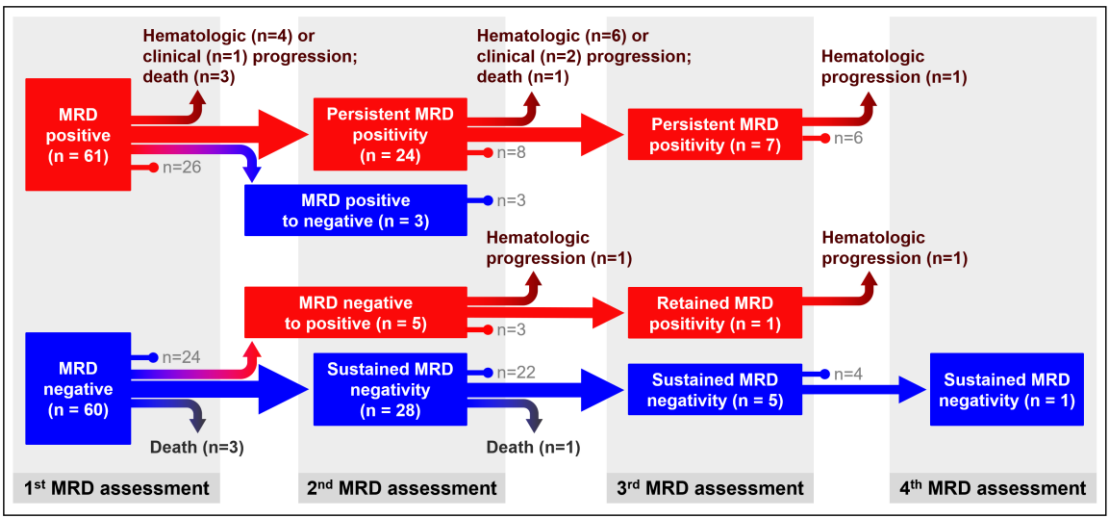
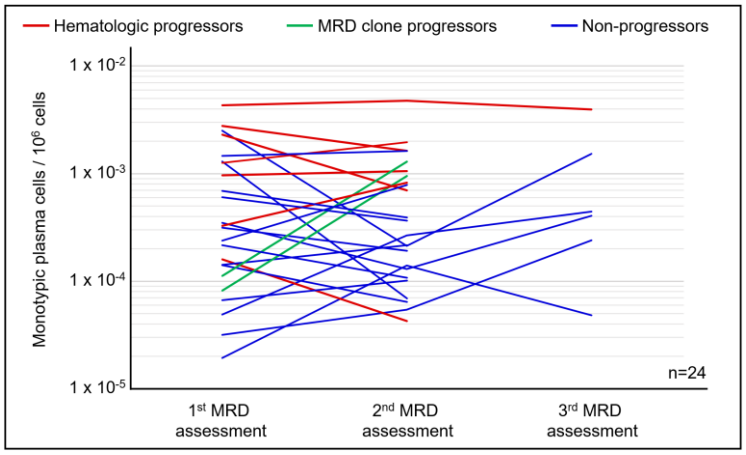


Figure 2. Depth and dynamics of MRD clones on sequential assessments for patients with persistent MRD positivity, stratified by hematologic progressors (red) and non-progressors (blue). Two patients (green) displayed MRD clone progression (>1-log growth) without experiencing hematologic or clinical progression during the follow-up period.



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Racial differences in the cytogenetic underpinnings of light chain amyloidosis

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Background: We previously found that Black individuals with systemic AL amyloidosis have an earlier age at diagnosis and a 24% higher hazard for death after age-adjustment, compared to Whites [1]. While these differences seem to be explained at least in part by sociodemographic factors and under-utilization of stem cell transplantation (HDM/SCT) among Black patients, there may also be an underlying biologic predilection according to ancestry.

Objective: To provide insight into the cytogenetic underpinnings of racial disparities in AL amyloidosis.

Material & Methods: We examined the frequencies of cytogenetic abnormalities (i.e., IgH translocations, chromosomal deletions, gains and hyperdiploidy) in newly diagnosed patients with systemic AL amyloidosis seen at the Boston University Amyloidosis Center, who had undergone analysis with interphase fluorescence in situ hybridization (FISH) of baseline bone marrow specimens. Data accrual from consented patients was approved by the Institutional Review Board. Comparisons were made between self-identified racial groups (Black vs. White). Statistical differences were estimated by χ^2 and one-way ANOVA tests.

Results: Of 328 patients (42 [13%] Black; 286 [87%] White) with systemic AL amyloidosis, 255 (78%) had at least one FISH cytogenetic alteration [**Table 1**]. The aberrancy with greatest difference between racial groups was t(11;14), present in 26 (62%) Blacks vs. 131 (46%) Whites (P=.051). Presence of any IgH translocation was significantly higher in Blacks, compared to Whites (76% vs. 55%, P=.009). There was a trend toward greater prevalence of 1q21 gain among Whites (21% vs. 11%, P=.170).

Baseline characteristics were generally similar between racial groups, although the median age at diagnosis tended to be younger among Blacks (61 vs. 63 years, P=.077) and more Whites had stage ≥III cardiac involvement (32% vs. 24%, P=.294). In the frontline setting, 13 (31%) Blacks and 70 (24%) Whites received HDM/SCT; meanwhile, 22 (52%) Blacks and 149 (52%) Whites received bortezomib-based regimens.

After adjusting for age, cardiac stage and use of HDM/SCT in a Cox proportional hazards regression model, the hazard of death was estimated to be higher for Blacks vs. Whites, although non-significant (HR 1.51, 95% CI 0.85–2.69, P=.162). This disparity was not diminished after further adjusting for t(11;14) status (HR 1.62, 95% CI 0.90–2.92, P=.107). In an exploratory analysis of hematologic responses (i.e., very good partial response or better) to front-line treatments between racial groups, response to HDM/SCT tended to be higher among Blacks (85% vs. 65%, P=.158), whereas response to bortezomib-based regimens tended to be higher among Whites (66% vs. 47%, P=.130).

Summary & Conclusion: While this analysis was limited in detecting statistical differences due to the low number of Black patients included, the findings indicate potential differences in the cytogenetic signature of AL amyloidosis between Black and White patients. We observed that IgH translocations occur more frequently among Blacks with this disease. Nonetheless, higher frequency of t(11;14) among Blacks did not account for the survival disparity observed in our cohort. There was a seeming divergence in treatment success between racial groups. This may in part be explained by differences in cytogenetic underpinnings, taking into consideration prior investigations showing t(11;14) to be associated with inferior responsiveness to bortezomib-based regimens and superior responsiveness to HDM/SCT.

Table 1. Baseline characteristics and chromosomal abnormalities in AL amyloidosis by self-identified racial group.

	Black patients (n = 42)	White patients (n = 286)	*P*
Baseline characteristics			
Median age, years (IQR)	61 (52–69)	63 (57–69)	0.077
λ amyloidogenic light chain, n (%)	34 (81)	232 (81)	0.980
Median dFLC, mg/L (IQR)	128 (48–262)	120 (62–289)	0.750
Heart involvement, n (%)	29 (69)	176 (62)	0.348
BNP-based cardiac stage, n (%)			0.572
stage I	13 (31)	76 (27)	
stage II	19 (45)	119 (42)	
stage III	3 (7)	43 (15)	
stage IIIb	7 (17)	48 (17)	
Kidney involvement, n (%)	28 (67)	202 (71)	0.600
Median proteinuria, g/day (IQR)	3.2 (0.3–6.6)	2.9 (0.2–7.4)	0.382
Cytogenetic abnormalities, n (%)			
Any cytogenetic aberrancy detected	36 (86)	219 (77)	0.184
IgH translocations:			
t(11;14)	26 (62)	131 (46)	0.051
t(4;14)	0 (0)	7 (2)	0.305
t(14;16)	2 (5)	2 (<1)	0.025
t(14;20)	1 (2)	3 (1)	0.463
Unknown partner	4 (10)	18 (6)	0.435
Deletions:			
13q14	14 (33)	90 (32)	0.808
17p13	1 (2)	5 (2)	0.775
1p	1 (2)	1 (<1)	0.114
Gains:			
1q21	4/35 (11)	48/224 (21)	0.170
Any trisomy	13 (31)	97 (34)	0.704
Hyperdiploidy	3 (7)	37 (13)	0.284

Abbreviations: IQR, interquartile range; dFLC, difference in the involved and uninvolved light chains; BNP, B-type natriuretic peptide; HDM/SCT, high-dose melphalan and autologous stem cell transplantation; IgH, immunoglobulin heavy chain.

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Support & Funding: The Amyloidosis Center database is supported by the Amyloid Research Fund of Boston University School of Medicine.

Evaluation of NT-proBNP as surrogate endpoint in AL amyloidosis: development of a platform for federated, multi-institution meta-analysis of randomized trials

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Background: NT-proBNP would be attractive as a surrogate endpoint for drug evaluation in AL amyloidosis as it would enable faster trials and reduce the need for accrual of organ damage and mortality events during follow-up. NT-proBNP has shown strong prognostic associations with survival in AL amyloidosis in multiple studies, but such associations are insufficient for establishing surrogacy. Further evidence, from multiple randomized trials, is needed to evaluate whether *treatment effects* on NT-proBNP are predictive of *treatment effects* on survival. Data from multiple trials in AL amyloidosis are available but reside across different institutions and countries and cannot be readily pooled for research.

Objective: As a step towards validating NT-proBNP surrogacy, we collaboratively implemented a system for federated analysis of multiple randomized trials in AL amyloidosis, i.e., a system in which (1) patient-level data are analyzed locally, without need for cross-institution data transfers, and (2) results of local analyses are pooled centrally to produce final evidence. As a first analysis using this system, we are replicating prognostic associations between NT-proBNP and overall survival and evaluating duration of NT-proBNP response.

Material & Methods: Analyses of four randomized trials are currently underway in this multi-institution collaborative study: ANDROMEDA (NCT03201965), TOURMALINE-AL1 (NCT01659658), EMN-03 (NCT01277016), and NJCT-0703 (NCT01998503). A hub-and-spoke platform for federated analytics was established, with the 'hub' including a central research team, common data model (CDM), statistical analysis plan, and standardized analytical programs, all centrally developed with collaborator input. Researchers at each institution ('spokes') are implementing the CDM, running analytical programs, reviewing output, and sharing aggregate results with the hub. The primary candidate for surrogacy is NT-proBNP response at 6 months, defined as a decrease in NT-proBNP of >30% and >300 ng/L relative to baseline among evaluable patients with baseline NT-proBNP ⩾650 ng/L. Associations between NT-proBNP response and survival are being assessed in landmark analyses using Kaplan-Meier curves, log-rank tests, and Cox proportional hazard models. Time to loss of response status after month 6 is also being analyzed. Sensitivity analyses will consider response at months 3, 9, and 12, and NT-proBNP progression-free survival as exploratory surrogates. A random-effects meta-analysis will be conducted to assess pooled associations between NT-proBNP outcomes and survival across all trials.

Results: The four trials comprise 759 patients with AL amyloidosis, both newly diagnosed and relapsed/refractory. Analysis has been conducted for two trials and results are consistent with prior research. In TOURMALINE-AL1, for example, 82 out of 168 randomized patients were evaluable for response and 16 of 82 were classified as responders at month 6. Responders at month 6 had longer subsequent survival vs. non-responders (75% vs. 50% alive at month 30; p=0.04). 50% of responders at month 6 lost response status (relative to baseline) within the subsequent 6 months.

Summary & Conclusion: A platform for federated analytics in AL amyloidosis is technically feasible and can accelerate collaborative learning from clinical trials. In preliminary findings, NT-proBNP response was associated with subsequent survival. The collaborative platform will augment these results with findings from additional trials and will ultimately be used for surrogate validation.

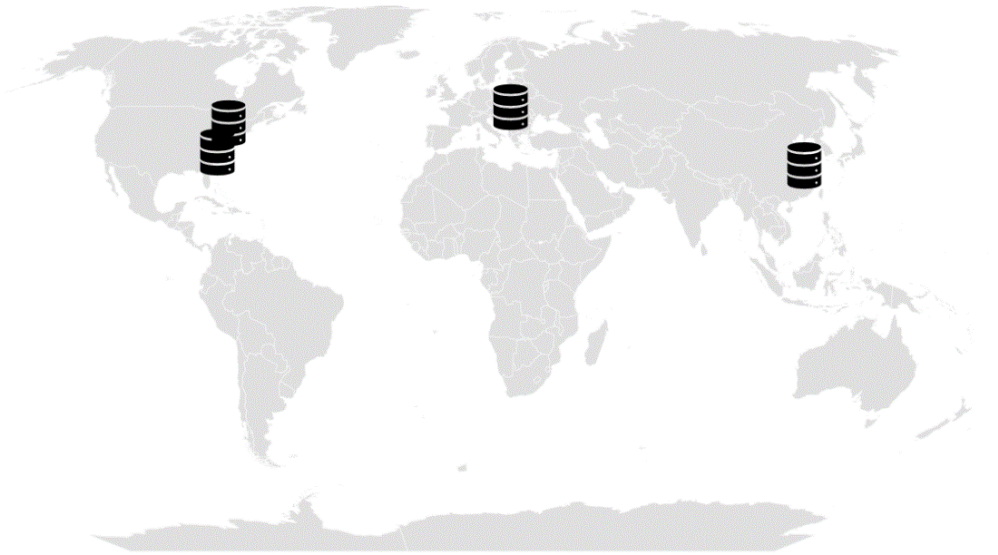

Figure 1.: Locations of clinical trial data currently included in the federated analytics platform.

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Patterns of target organ amyloid deposition in patients with AL amyloidosis; role for diagnosis and prognosis

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Background: Diagnosis of immunoglobulin light chain (AL) amyloidosis requires histological confirmation of amyloid deposits either in the involved organs (renal, cardiac, liver etc) or in a surrogate site such as abdominal fat (AFA) or salivary gland etc.[1] [2] Up to 90% of patients will have evidence of amyloid deposits in the AFA and/or the bone marrow (BM) and this can be used to avoid logistically more demanding target organ biopsies Whether the presence of amyloid deposits in these tissues has also prognostic impact needs to be defined.

Objective: To assess patterns of amyloid deposition in target organs and surrogate tissues and their prognostic role

Material & Methods: We assessed tissue biopsy positivity for amyloid deposition in 362 consecutive newly-diagnosed patients with AL amyloidosis diagnosed and treated in the Department of Clinical Therapeutics (Athens, Greece), with at least one tissue biopsy available for evaluation.

Results: The characteristics of the cohort are shown in table 1. Diagnosis was established based on amyloid detection in AFA in 42%, kidney in 39%, heart in 2%, salivary gland in 3%, liver in 3%, GI in 5% and other tissue in 6% of patients. Diagnostic sensitivity of AFA was 84% and of BM 60% and combined sensitivity was 91%, however, 9% of patients required target organ biopsy to set the diagnosis following negative AFA and BM biopsy. In 24% the diagnosis was made following target organ biopsy only. We then evaluated patterns of tissue biopsy positivity for amyloid: the concordance between AFA and BM was 61%, between kidney and AFA 53%, and between BM and kidney 37%. Notably, 65% of patients with positive kidney biopsy had negative peripheral tissue biopsies, and among them 38% had isolated renal involvement. Positive AFA was associated with advanced Mayo stage (p=0.001), cardiac involvement (p<0.001) and soft tissue (p=0.022) but not renal or nerve involvement. BM amyloid deposition was associated with nerve (p=0.019) and liver involvement (p=0.003) and BM plasma cell (BMPC) infiltration (p=0.041). Double positivity in AFA and BM (versus no positivity or single positivity) was associated with higher NTproBNP (p=0.003), higher BMPC infiltration (p=0.009), higher baseline dFLC (p=0.008) and amount of proteinuria (p=0.003). Median OS for patients with BM amyloid deposits was 41 (95%CI 32-51) vs 78 months (95%CI 62-94) for patients with no detectable BM deposits (p=0.022). Double positivity in BM/AFA was associated with a worse median OS: 41 (95%CI 26-55) vs 75 months (95%CI 55-94) for patients with single/no positivity in either tissue (p=0.009). Double positivity in the kidney/AFA was seen in 31% and in BM/kidney in 78% but neither combination had adverse prognostic impact. Amyloid positive BM was an independent adverse prognostic factor for median OS (HR:1.47, p=0.022) when adjusting for both BMPC and dFLC. Double positivity for amyloid in AFA/BM also retained its negative prognostic power on median OS (HR:1.7, p=0.011) when adjusting for BMPC and dFLC in the multivariate model.

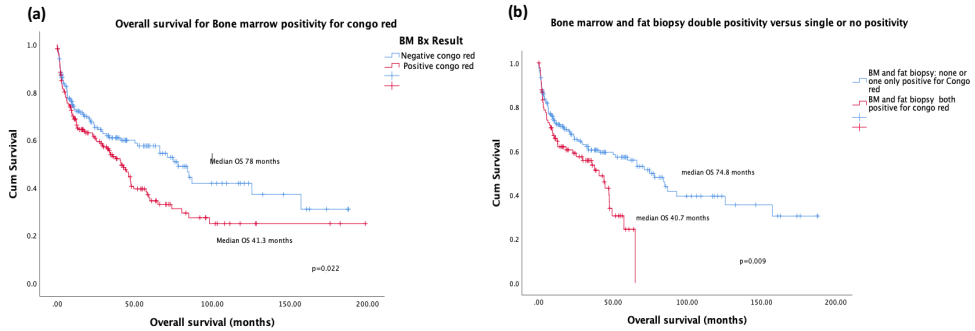
Summary & Conclusion: Surrogate site biopsies are a safe option for the diagnosis of most patients with AL amyloidosis with a relatively high sensitivity. Beyond diagnostic value, the presence of detectable amyloid deposits in the BM and AFA may have a prognostic importance, perhaps as a crude measure of amyloid burden and of advanced disease. In a significant proportion of patients, however, peripheral tissue biopsy lacks the necessary sensitivity to set the diagnosis, which requires targeted organ biopsy.

Table: Clinical characteristics of 312 newly diagnosed AL amyloidosis patients.

Clinical characteristics:	(n=362)	Clinical characteristics	(n=362)
Age (Median)	65.5	Male/Female	56%/ 44%
dFLC (median) (mg/L)	228	BM infiltration (median)	15%
Number of involved organs		Performance status	
1 /2 /3/ 4	33%/43%/20%/4%	0 / 1/ 2/ 3 /4	19%/41%/23%/11%/6%
Organ involvement			
Renal / Heart/ Liver/ NS	65%/ 77%/ 19%/ 25%	Mayo stage 1/2/3A/3B	15%/42%/23%/20%
Treatment type		Hematological response at	NR/ PR/ VGPR
Bortezomib based	80%	1 month	31%/43%/26%
Lenalidomide-based	8%	3 months	21%/56%/23%
Dara- monotherapy	2%	6 months	16%/68%/16%
Mdex	10%	12 months	14%/70%/16%
Tissue biopsy	N biopsies (%positive)		
AFA	209 (84%)	GI	37 (65%)
BM	351 (51%)	Salivary gland	19 (58%)
Renal	133 (97%)	Liver	15 (80%
Cardiac	13 (54%)	Other	18 (100%)

Figure (a) Kaplan Mayer curve for overall survival (OS) based on status of amyloid positivity in the Bone Marrow (BM)

biopsy. (b) Kaplan Mayer curve for OS in patients with double tissue positivity (BM/AFA) versus patients with no or single tissue positivity in either BM or AFA.



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Diagnostic Hospitalization and Associated Costs in Patients with Amyloid Light-Chain Amyloidosis

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Background: Light-chain (AL) amyloidosis is a rare, fatal disease due to extracellular deposition of misfolded immunoglobulin light chains. Clinical experience suggests some patients are first diagnosed with AL amyloidosis during an acute admission for organ dysfunction and undergo a diagnostic work-up.

Objective: The study's aim was to estimate the rate of such diagnostic events among hospitalized patients and measure associated healthcare utilization and costs.

Material & Methods: This retrospective analysis used 2017-2020 data from the Premier® Healthcare Database to identify hospitalized patients aged ≥18 years with ≥1 inpatient claim for AL amyloidosis (ICD-10-CM code E85.81) in any diagnosis field during the study period (10/1/2017-12/31/2020). Patients with a diagnosis for other amyloidosis types (E85.0x-E85.3x) or certain chronic inflammatory disease were excluded. Patients were stratified into diagnostic and other hospitalization. Diagnostic hospitalization was defined where the patient had a diagnostic biopsy (bone marrow, kidney, liver, abdominal fat pad, salivary gland, gingival, or endomyocardial) and did not have a solid organ or hematopoietic stem cell transplant. Study outcomes included hospitalization costs (in 2020 USD) and length of stay (LOS).

Results: Of 1,341 hospital admissions, 234 (17.6%) were diagnostic admissions. Diagnostic admissions did not differ from other hospitalizations regarding characteristics such as patient demographics, payer type, and hospital location. However, diagnostic hospitalizations were characterized by longer LOS (14.5 vs. 8.4 days, $P<.001$), higher cost ($40,052 vs. $24,360, $P<.001$) and higher total charges ($161,526 vs. $104,129, $P<.001$) than non-diagnostic ones.

Summary & Conclusion: Healthcare utilization and costs are high among patients hospitalized with AL amyloidosis and particularly high for those who have not been diagnosed prior to being admitted for an acute event. From a cost perspective, it may be desirable to have a diagnostic work-up performed in the outpatient setting when possible.

Atypical neurological presentation of immunoglobulin light-chain amyloidosis.

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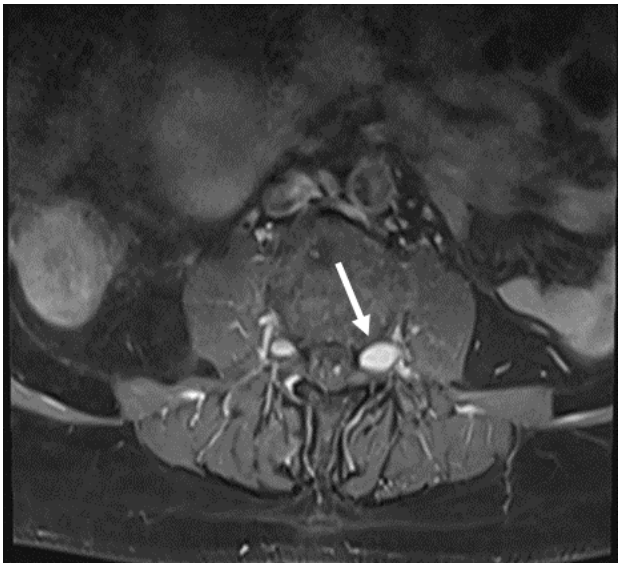
Background: AL amyloidosis is a rare condition that can involve peripheral nervous system with various clinical presentations[1]. Amyloid multiple mononeuropathy has been rarely reported[2]. Early recognition of AL amyloidosis with peripheral neuropathy as an initial symptom is very important to initiate treatment promptly and improve functional recovery and to detect possible life-threatening organ involvement (cardiac)[3]. We report here a clinical case of asymetric multiple mononeuropathy in a 70-year-old woman who presented with progressive onset of neurological symptoms with various cranial nerves (CN) and a left lumbar plexus involvement. The diagnosis of neurological AL amyloidosis was proven by neuromuscular biopsy.

Case Report: Since 6 months, a 70-year-old woman experienced a progressive left face anaesthesia and painful paresthesias of the left foot with muscle weakness of the left lower limb. She further developed dysphonia, dysphagia without agueusia and binocular diplopia. She was finally referred to our hospital: her neurological examination revealed a multiple mononeuropathy with an involvement of the left CN X (paralysis of the soft palate and paralysis of the left vocal cord), of the CN VI bilaterally (binocular diplopia in the distant view) and C2-C3 root sensory involvement. She had also sensory and motor symptoms and signs suggestive od a left lumbosacral plexus involvement. Brain and cervical imaging as well as cerebrospinal fluid analyses were normal. Nerve conduction study showed an axonal sensory and motor neuropathy of the left lumbosacral plexus. Lumbar MRI revealed left L3 root thickening in the proximal foraminal and extra-foraminal region with intense and homogeneous contrast enhancement, compatible with neuritis (Figure 1). Biological analysis did not demonstrate diabetes, drug toxicity or vitamin deficiency, infection, inflammatory/immune vasculitis or paraneoplastic syndrome. There was an increase in lambda free light-chains and kappa/lambda ratio was 0.07 (lambda:187.12 mg/L, kappa:8.84, dFLC:179 mg/L) without M-spike. Serum VEGF level was normal. Bone marrow examination showed a plasma cell dyscrasia with lambda light-chain restriction. Fat pad and salivary glands biopsies were negative. Assessment of systemic extension was negative. Finally, biopsy of the left sural nerve confirmed amyloidosis with Congo red stain (Figure 2). Immunohistochemistry did not confirme the AL lambda subtype but AA and TTR testing were negatives. Screening for TTR mutation was also negative. After 3 cycles of daratumumab-melphalan-dexamethasone, the patient improved her left leg tenderness but achieved only a partial hematologic response (HR). Reduced doses of bortezomib were added at cycle 4 and translated into a complete HR.

Summary & Conclusion: The typical pattern of amyloid neuropathy is a painful symmetrical, both motor and sensory, length-dependent, lower limb-predominant neuropathy[1]. AL amyloidosis multiple mononeuropathy is rare[2] and the differential diagnosis is wide[3-4]. Nerve biopsy may be negative in 30% of reported cases[3-4]. Treatment should target the underlying clone to acheived a rapid and deep reponse to improve prognosis and functional recovery. In neurological disease without other organ involvement, alkylating agent combined with dexamethasone provides a hematological response rate of up to 65%[5]. However, for severe motor deficits and/or systemic disease, the addition of daratumumab and/or bortezomib with a special attention to nerve toxicity, may allow most of patients to achieve complete response[6].



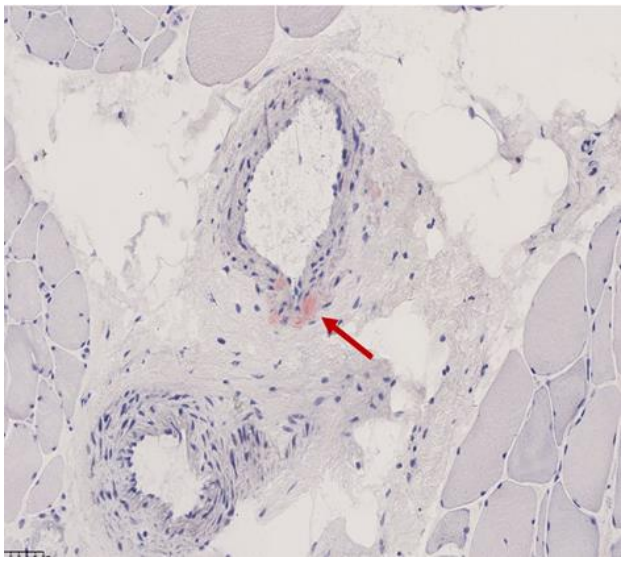


Figure 1: Lumbar MRI revealed left L3 root thickening in the proximal foraminal and extra-foraminal region with intense and homogeneous contrast, compatible withneuritis.

Figure 2: Left sural nerve biopsy of the patient: Endoneurial perivascular deposition of amyloid (Congo red, original magnification, ×200).

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Characterization and outcome of patients with systemic AL amyloidosis requiring dialysis prior to initial therapy

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Background:

Light chain (AL) amyloidosis is a rare protein misfolding disease. It is characterized by the deposition of amyloid fibrils composed of monoclonal light chains in tissues. In more than 90% of patients the underlying cause is a plasma cell dyscrasia which produces amyloidogenic light chains. The deposits may be confined to a single organ or systemically affect the entire body, while the kidneys are affected in about 60% of patients.

Material and Methods:

We retrospectively analyzed all AL patients who underwent dialysis prior to initial therapy and were diagnosed at our center between 2010 and 2022. The aim of this study is to characterize these patients and analyze their outcomes such as remission, hematologic progression-free and overall survival as there are still only limited data available for this specific subgroup.

Results:

We identified 32 patients with AL amyloidosis. Remarkably, 24 of the 32 patients (75%) had kappa-type light chain. The underlying clonal disease was monoclonal gammopathy in 17 patients (53%) and smoldering myeloma in 15 patients (47%). The dFLC values ranged from 5.5 to 8781 mg/l with a median of 161.3 mg/l. Most patients had renal involvement (94%), followed in second place by cardiac involvement (56%). 2 patients were on dialysis because of other conditions: 1 patient had an underlying IgA nephropathy and was on dialysis 3 years before the diagnosis of AL amyloidosis. The other patient was diagnosed with membranoproliferative glomerulonephritis and required dialysis 7 years before the diagnosis of AL amyloidosis. At the time of diagnosis, only 5 patients were anuric (16%). The median NT-pro BNP level was 7931.5 ng/l and higher in patients with cardiac involvement (14611 vs 3375 ng/l, p=0.022).

Most patients received bortezomib and dexamethasone (VD) or bortezomib, dexamethasone, and cyclophosphamide (VCD) as first-line treatment (63%). One of the patients received high dose melphalan followed by autologous stem cell transplantation after 3 cycles of VD. Daratumumab was used in combination with VD or VCD in 5 patients (19%). 2 patients (6%) received melphalan in combination with dexamethasone (MD). At 3 and 6 months, only 2 patients achieved complete remission (CR). We identified 8 patients (25%) who showed a very good partial response (VGPR) at 3 months and 7 patients (22%) with a VGPR at 6 months. A partial response (PR) was seen in 6 patients (19%) at 3 months and in 4 patients (13%) at 6 months. In 4 patients, the response was better at 6 months than at 3 months after initiation of treatment. Only 1 patient experienced hematologic progression 3 months after treatment initiation. Interestingly, 2 patients (6%) who were found to have a VGPR were able to discontinue dialysis after an initial treatment of 2-4 cycles of VCD/VD.

Median progression-free survival (PFS) was 24 and the median overall survival (OS) was 45.5 months after a median follow-up of 19.5 months.

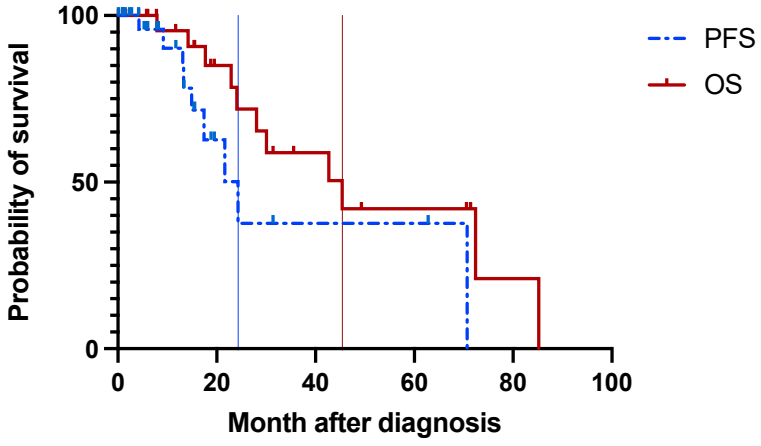
Summary & Conclusions:

We present the characteristics of 32 patients with AL amyloidosis who received dialysis before initial therapy. In summary, only a moderate response rate to initial chemotherapy could be achieved. It is possible that responses are underestimated as renal failure increases dFLC. However, median PFS of 2 years and OS of nearly 4 years are quite encouraging. Further encouraging is that a rapid response to therapy may avert long-term dialysis in some patients.

Table: Characterization of patients with systemic amyloidosis requiring dialysis prior to initial therapy.

Variable	Amyloidosis patients on dialysis (before treatment) N=32
Sex, male, n(%)	**25** (83)
Age, years, median (range)	**61** (41-78)
Monoclonal heavy chain, n(%)	**17** (53)
Light chain isotype, n (%)	**14** (44)
Missing data, n(%)	1 (3)
Light chain type, n(%)	
Kappa	**24** (75)
Lambda	**8** (25)
Missing data, n(%)	0 (0)
Underlying clonal disease, n(%)	
MGCS	**17** (53)
SMM	**15** (47)
MM	0 (0)
dFLC, mg/l, median (range)	**161, 3** (5,5 – 8781)
Missing data, n(%)	1 (3)
BMC infiltration (%), median (range)	**10** (0 – 50)
Missing data, n(%)	3 (10)
Organ involvement, n(%)	
Heart	**18** (56)
Kidney	**30** (94)
Liver	**8** (25)
Soft tissues	**12** (38)
PNS	**3** (9)
ANS	**7** (22)
GI	**10** (31)
Other	**5** (16)
Number of involved organs, n(%)	
1	**3** (9)
2	**12** (38)
>= 3	**17** (53)
Residual excretion ml/d, median (range)	**700** (0 - 2700)
Missing data, n (%)	10 (31)
Anuria, n(%)	**5** (16)
Oliguria n(%)	**4** (13)
Normal excretion, n(%)	**11** (34)
Missing data, n(%)	10 (31)
s.creat (mg/dL), median (range)	**5,1** (0,9 - 13,16)
missing data, n(%)	1 (3)
eGFR before treatment,	**12,2** (4 - 79)
missing data, n(%)	2 (6)
u.prot (g/24h),	**5,7** (0,33 - 18,8)
missing data, n(%)	10 (31)
u.alb (g/24h)	**1,8** (0,015 - 12,3)
missing data, n(%)	20 (63)
TnI (µg/l), median (range)	**0,04** (0,01 – 1)
Missing data, n(%)	13 (41)
hsTnT (pg/ml), median (range)	**87** (18 – 268)
Missing data, n(%)	15 (47)
Nt-proBNP (ng/l), median (range)	**7931,5** (63 - 79983)
Missing data, n(%)	6 (19)

Figure: PFS and OS in 32 patients with AL amyloidosis requiring dialysis at first diagnosis



Beta-2-microglobulin and lactate dehydrogenase as prognostic parameters in light-chain amyloidosis

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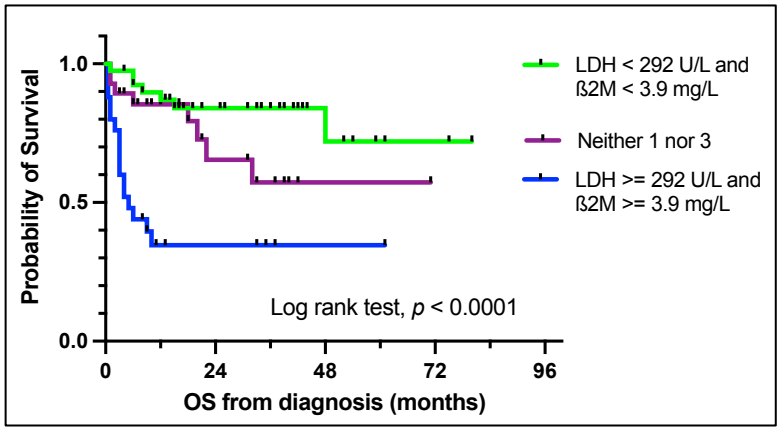
Background: Systemic light chain amyloidosis is a protein misfolding disease caused by a clonal plasma cell in the bone marrow, which results in organ failure, due to deposition of misfolded light chains in affected organs. Prognostic risk scores for patients with cardiac or renal involvement are well established (Mayo 2004, 2012 and Palladini stages 2014). However, no staging systems predicting survival of AL amyloidosis patients, independent of the pattern of organ involvement, are used in routine clinical practice. Beta-2-microglobulin (ß2M) and lactate dehydrogenase (LDH) are parameters known to be prognostic in plasma cell dyscrasias and lymphoproliferative diseases and have been shown to predict survival in AL amyloidosis (Gertz et al., 1990; Muchtar et al., 2017).

Objective: To study the impact of pretreatment levels of ß2M and LDH on survival in AL amyloidosis.

Material & Methods: Data were collected from 120 patients that presented between 2015 and 2021 in our department of Hematology and Stem Cell Transplantation. We assessed cut-off values for ß2M and LDH in 93 patients, using simple logistic regression and ROC-analysis after exclusion of patients with multiple myeloma. Eight patients with diagnosed multiple myeloma only based on free light chain ratio were included. Kaplan-Meyer-Survival analysis and Cox regression hazard model were applied to assess the hazard ratios. The used statistical software was GraphPad PRISM, version 9.3.1.

Results:
The study included 93 patients with histologically confirmed diagnosis of AL amyloidosis. Of these, 77% had cardiac involvement and 71% renal involvement. Patients were divided into 3 groups after assessing cut-off values for LDH and ß2M, which significantly negatively affected survival (group 1: $n = 40$, LDH < 292 U/L, ß2M < 3.9 mg/L; group 3: $n = 25$, LDH ≥ 292 U/L, ß2M ≥ 3.9 mg/L and group 2: $n = 28$, neither 1 nor 3). Our data show a significantly negative outcome with increasing levels of LDH and ß2M (Log-rank test, $p < 0.0001$) and a median overall survival of 5 months in group 3. Performing a univariate cox regression model showed that the risk of death in group 3 is increased 6.9 times compared to group 1 (HR = 6.9, 95%CI 2.92 to 18.23, $p < 0.0001$) and 3.4 times compared to group 2 (HR = 3.4, 95%CI 1.448 to 8.427, $p = 0.005$), whereas no significant increase in risk of death was found in group 2 compared to group 1 (HR = 2.039, 95%CI 0.72 to 5.84, $p = 0.17$). In patients with cardiac involvement, 46% of patients in group 1 had Mayo stage II, 46% in group 2 had Mayo stage IIIa and 68% of patients in group 3 had Mayo stage IIIb. Palladini stage III in patients with renal involvement was not recorded in group 1, whereas 14% and 45% of patients in groups 2 and 3 had Palladini stage 3. At the time of diagnosis, 2 patients in group 2 and 3 in group 3 were on dialysis. Regardless of initiated treatment regimen, patients in group 3 achieved poorer hematological response rates (42% vs. 62% in groups 1 and 2) and 32% died within 2 months after treatment, compared to 14% in group 2 and 6% in group 1. Before initiating treatment, 4 patients in group 3 died. In contrast, no patients in groups 1 or 2 died before treatment.

Summary & Conclusion: ß2M and LDH are suitable prognostic parameters in AL amyloidosis, regardless of the pattern of organ involvement. Further studies with other cohorts are needed to confirm these findings.



Kaplan Meyer survival analysis showing the prognostic role of ß2M/LDH in the different groups in AL

	Group 1 (*n* = 40) LDH < 292 U/L ß2M < 3.9 mg/L	**Group 2 (*n* = 28)** LDH < 292 U/L, ß2M ≥ 3.9 mg/L or LDH ≥ 292 U/L, ß2M < 3.9 mg/L	**Group 3 (*n* = 25)** LDH ≥ 292 U/L ß2M ≥ 3.9 mg/L
Mayo stages 2004	(*n* = 26)	(*n* = 24)	(*n* = 22)
Stage I	1 (4%)	1 (4%)	0
Stage II	12 (46%)	4 (17%)	1 (5%)
Stage IIIa	11 (42%)	11 (46%)	6 (27%)
Stage IIIb	2 (8%)	8 (33%)	15 (68%)
Palladini stages 2014	(*n* = 25)	(*n* = 21)	(*n* = 20)
Stage 1	12 (48%)	5 (24%)	2 (10%)
Stage 2	13 (52%)	12 (57%)	9 (45%)
Stage 3	0	4 (14%)	9 (45%)

Mayo 2004 and Palladini 2014 stages in the three different groups.

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Diagnostic value of liver stiffness as marker of hepatic amyloid deposition in systemic AL amyloidosis

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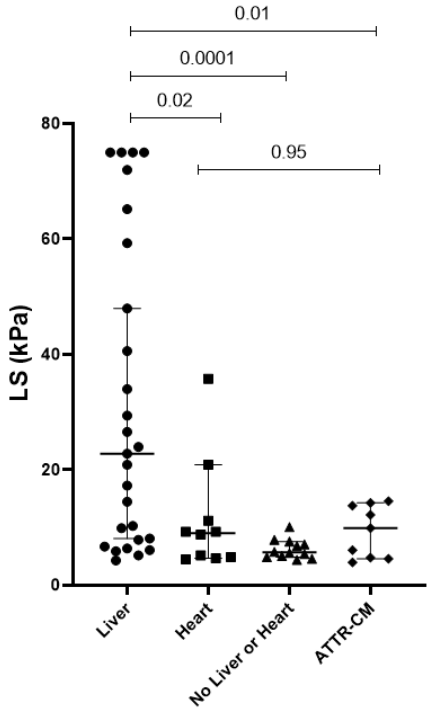
Background: Hepatic involvement in AL amyloidosis is often asymptomatic but does affect prognosis and should be taken into account during follow-up. An increased plasma level of alkaline phosphatase (ALP) or increased liver span are part of the conventional diagnostic criteria for establishing hepatic involvement[1], however these markers are nonspecific. ^{123}I-labeled serum amyloid P component (SAP) scintigraphy is a specific and sensitive method to establish hepatic involvement but is not widely available. Liver stiffness measured by transient elastography is increased in AL amyloidosis patients with hepatic involvement and could be useful in establishing liver involvement and monitoring treatment response in AL amyloidosis.

Objective: To assess the diagnostic performance of liver stiffness for liver involvement in AL amyloidosis using SAP scintigraphy as golden standard and its utility for monitoring liver disease during follow-up.

Material & Methods: Liver stiffness was measured prospectively in 49 treatment naïve patients with systemic AL amyloidosis and 9 patients with transthyretin amyloidosis with cardiomyopathy (ATTR-CM). Nineteen AL amyloidosis patients were monitored during follow-up. Serum amyloid P component (SAP) scintigraphy was used as gold standard for liver involvement. SAP scintigraphy, liver span, and ALP were performed in all patients.

Results: Of the 49 patients, 27 patients had liver involvement (of whom 24 also had heart involvement), 10 patients had heart involvement, 12 patients had no heart or liver involvement. Median liver stiffness was significantly higher in AL amyloidosis patients with liver involvement (22.8 kPa, range 4.3 - 75), than in AL amyloidosis patients without liver involvement (6.3 kPa, range 4.4 - 35.8) (p = 0.000). Also a significant difference was seen between AL amyloidosis patients with liver involvement (22.8.9, range 4.3 - 75) versus, heart involvement (9.0, range 4.5 - 35.8) (p = 0.02), no liver or heart involvement (5.7, range 4.4 - 10.1) (p = 0.0001), and ATTR-CM patients (9.9 kPa, range 4.0 - 14.6) (p = 0.01) (figure 1). Furthermore, liver stiffness values seemed to significantly decrease over time in AL amyloidosis patients with liver involvement with a good hematologic response to treatment (figure 2).

Summary & Conclusion: Liver stiffness is a non-invasive tool which seems to be useful in clinical practice to establish liver involvement in patients with AL amyloidosis. In addition, it is a promising marker in the follow-up of AL amyloidosis patients for establishing hepatic response over time.



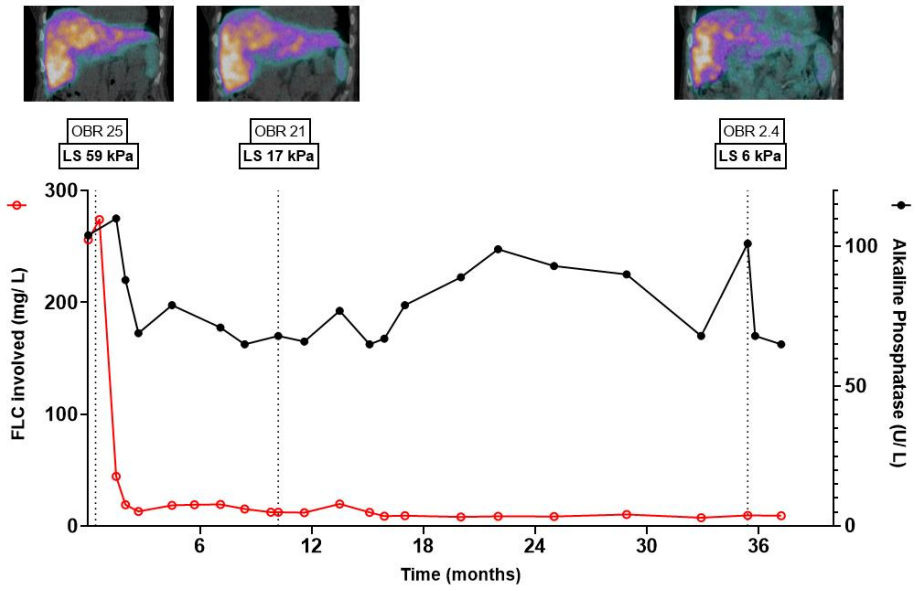


Figure 1: Dot plot showing the distribution of liver stiffness in patient with AL amyloidosis with liver involvement (N=27) (pure liver involvement & liver and heart involvement together), AL amyloidosis with pure heart involvement (N=10), AL amyloidosis with no liver or heart involvement (N=12), and ATTR-CM controls (N=9).
kPa: kilopascal; LS: liver stiffness; ATTR-CM: wild type transthyretin amyloidosis with cardiomyopathy

Figure 2: Longitudinal data of a patients were changes in liver stiffness were followed over time.

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The Pattern of Organ Responses Varies in Patients with Systemic Light-chain Amyloidosis (AL) and Heart or Kidney or Heart and Kidney Involvement Who Achieve Deep Hematologic Responses

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Background: In AL misfolded clonal free immunoglobulin light chains (FLC) deposit in vital organs causing severe dysfunction.(*1*) Anti-plasma cell therapy that reduces or eliminates the involved FLC (iFLC) underlies organ responses.(*2-4*) We asked whether the timing of individual organ responses may be influenced by the number of organs involved at diagnosis and response to therapy. We evaluated the pattern of responses in patients with the two most commonly involved organs (heart, kidney) who achieved deep hematologic responses to therapy (CR=complete response, VGPR=very good partial response).(*5*)

Methods: We performed a retrospective analysis of AL patients diagnosed by tissue biopsy between 2007-2019 who had heart and/or kidney involvement at diagnosis and achieved hematologic CR/VGPR with treatment. Mann-Whitney was used to compare rates of organ responses and Log-rank tests were applied to compare time to organ response among the subgroups as well as overall survival (OS). Results were considered to be significant if two-sided *P*-value was less than or equal to 0.05.

Results: We identified 111 patients with a median age of 62.5 years (range, 40-80) who met these criteria, 65 of whom (59%) were male. Cardiac involvement only was present in 34 (30.6%), renal involvement only in 31 (28.0%), and both cardiac and renal involvement in 46 (41.4%). The median OS for the entire cohort was 112 months (95% CI 100-NA). The overall cardiac response rate was 62.5%, with a median time to response of 8 months (range, 1-73 months). Overall renal response rate was 67.1% with a median time to response of 10 months (1-57). Log-rank analysis showed a significant difference in the OS based on post treatment iFLC levels (<10 vs. 10-20 vs. >20 mg/L) as we have previously described.(*6*) Patients with kidney involvement only had significantly improved OS compared to those with cardiac involvement only (p=0.05) as expected. However, there was no difference in the OS of patients with cardiac only vs. cardiac and renal involvement (p=0.58), while there was a trend towards shorter OS in patients with cardiac and renal vs renal (p=0.09). The lower iFLC levels achieved post-treatment influenced cardiac response rate (p=0.07), and significantly impacted renal response rate (p<0.01). For patients with cardiac involvement, iFLC responses did not have a significant impact on time to cardiac response, whereas for patients with renal involvement, faster responses were noted in those achieving lower iFLC levels (p=0.017) (Figure 1). There was no significant difference in time to cardiac response between patients with cardiac only vs. cardiac and renal involvement (p=0.93) whereas patients with renal only vs cardiac and renal involvement had a faster time to renal response (medians 14 (10-29) vs 43 (13-not reached) months, p=0.018) (Figure 2).

Summary & Conclusions: In AL patients with renal involvement who achieve CR/VGPR with treatment, post-treatment iFLC levels and co-presence of cardiac involvement play significant roles in the timing of renal responses. In AL patients with cardiac involvement who achieve CR/VGPR, post-treatment iFLC levels but not the co-presence of renal involvement influences the rate of cardiac response but neither influences the timing. These differences may be due to organ-specific factors such as proteomic adaptations or relative iFLC toxicity or complex cardio-renal hormonal interactions. Further hypothesis-driven study of these differences is warranted in this era of new and effective anti-plasma cell therapies.

Figure 1. Post-therapy iFLC and Renal Responses

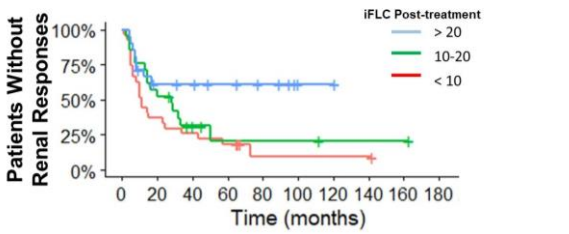
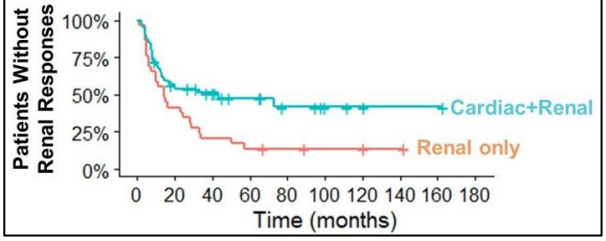


Figure 2. Renal Only vs Cardiac+Renal



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Outcomes of patients with AL amyloidosis and end-stage renal disease requiring dialysis

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Background: Renal involvement in AL amyloidosis is associated with a high risk of progression to end stage renal disease and dialysis, even among patients achieving a deep hematologic response. Although prognosis is mainly affected by cardiac involvement, severe renal dysfunction is associated with limitations in treatment options and poor quality of life.

Objective: To report the characteristics and outcomes of patients with AL amyloidosis requiring dialysis.

Material & Methods: We analyzed data of 265 prospectively registered patients with renal involvement or patients that progressed to dialysis even without confirmed renal involvement.

Results: Patients' characteristics are shown in Table 1: 86 required dialysis; 11 (4.2%) at diagnosis and 75 (28.4%) during their disease course; 81 patients had renal involvement but 5 patients did not meet renal involvement criteria at the time of initial diagnosis. Among those with renal involvement, 1-, 2- & 5-year dialysis rate was 16%, 21% & 34%; 5 patients who progressed to dialysis without confirmed renal involvement had CHF and required dialysis mainly in order to manage fluid overload; one patient underwent peritoneal dialysis. Among patients on dialysis (N=86), 64.6% had also cardiac, 23% liver, 21.3% nerve and 11.3% soft tissue involvement. At diagnosis, distribution at renal stages was 7.4% / 54.3% / 38.3% for stages 1, 2 & 3 and for Mayo stage was 16% / 48% / 36% for stages 1, 2 & 3. Initial treatment for patients that progressed to dialysis was bortezomib-based in 48 (55%), lenalidomide-based in 16(18.4%), melphalan-based in 15(17%), daratumumab in 2(2.4%) and ASCT in 2(2.4%). At the time of initiation of dialysis, hematologic response status was CR/VGPR in 23(31%), PR in 14(19%) and 25(33.3%) patients were either at hematologic relapse or had not achieved a hematologic response. Thirty-four patients required treatment after the initiation of dialysis: 12/34(35%) received bortezomib, 12 (35%) lenalidomide, 9(26%) melphalan or cyclophosphamide, 5(14%) daratumumab, 1(3%) received pomalidomide and 1(3%) patient ixazomib. At least hemVGPR at 1- or 3-months from start of initial therapy was associated with longer renal survival (median time to dialysis 130 vs 74 months, p=0.032 and not reached vs 64.5 months, p<0.001, respectively); the median time to initiation of dialysis for those that developed ESRD was 12.7 months (range 0.2-214); 19% required dialysis >5 years from the start of therapy. Median OS for the whole cohort (N=265), from the start of 1st line therapy was 66 months. Median OS for those who required dialysis was 59.6 months vs 98 months for those that did not (p=0.43). Median OS post dialysis initiation was 28 months (but 1.7 months for the 5 patients that did not meet criteria for renal involvement). Time to dialysis did not significantly affect survival post dialysis. Cardiac involvement remained a poor prognostic factor for patients undergoing dialysis (median OS 8 vs 79 months, p=0.008). Median OS post dialysis for patients with CR/VGPR at time of initiation of dialysis was 34.7 months vs 10 months for patients with PR/NR. Causes of death for patients on dialysis were sudden cardiac death (n=18), sepsis (n=9), amyloidosis progression (n=5), progressive cardiac failure (n=3), 2nd malignancy (n=2), stroke (n=1) and unknown (n=17).

Summary & Conclusion: Dialysis in patients with AL is associated with poorer quality of life but these patients can still receive active therapy, improve their hematologic response and survival and be candidates for renal transplantation.

Table 1. Baseline characteristics

	Baseline (N=264)	Patients progressing to or requiring dialysis (N=86)
Age in years	65 (38-84)	65 (38-84)
Male / Female (%)	54.3 / 45.7	65.1 / 34.9
Organ involvement (%) Cardiac	64.9	64.6
Liver	19.2	22.9
Soft tissue	17.7	11.3
Nerve	22.3	21.3
Number of organs involved	2 (1-4)	2 (1-4)
eGFR mL/min/m^2	63.05 (2.88-199.5)	37.44 (2.88-149.89)
Proteinuria gr/24h	6286 (140-39100)	7500 (140-36120)
NTproBNP ng/L	2192 (20.9-75000)	2482 (42.1-75000)
Renal stage (%) I / II / III	21.1 / 54.7 / 19.2	7.4 / 54.3 / 38.3
Mayo stage (%) I / II / IIIa / IIIb	18.9 / 41.5 / 16.6 / 15.1	16 / 48 / 20/ 16.1
Serum albumin gr/dL	3 (1-4.9)	2.9 (1.5-4.9)
BMPC %	15 (0-80)	15 (0-80)
iFLC mg/L / dFLC mg/L	174(9-9000) /150(0.6-8987.8)	164(16-5990)/129.5(7.3-5970)
dFLC > 180 (%)	42.6	41.3
SBP < 100 mmHg (%)	25.3	26.7
Treatment (%) Bortezomib based	63.4	59.3
Lenalidomide based	10.9	19.8
Melphalan based	2.5	18.5
Daratumumab based	9.8	2.5

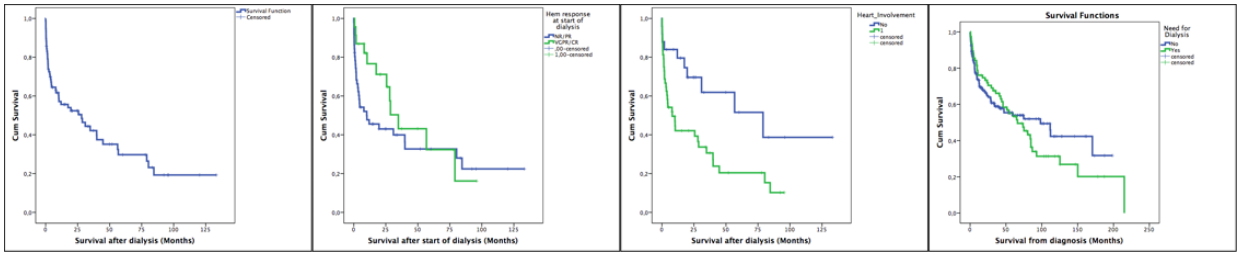

Figure 1: Survival after start of dialysis for all patients starting dialysis (A), according to the depth of hem response at start of dialysis (B) those with and without heart involvement (C) and overall survival since diagnosis for those who required dialysis and those who did not (D)

Identifying symptoms of AL amyloidosis in electronic health records using natural language processing, ICD codes, and manually abstracted registry data

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Background: The clinical presentation of light chain (AL) amyloidosis varies widely depending on the involved organs[1], making it difficult to diagnose[2]. One third of AL amyloidosis patients see 5+ physicians before diagnosis[3], and the median time from symptom onset to diagnosis is 2.7 years[4]. New approaches for comprehensive identification of symptoms could help facilitate timely diagnosis and thereby mitigate organ progression and improve clinical outcomes.

Objective: To investigate the occurrence of AL amyloidosis symptoms from (1) unstructured clinical notes abstracted by a natural language processing (NLP) algorithm, (2) ICD codes, and (3) a human-abstracted research registry.

Material & Methods: The study population consists of 1,223 patients with a biopsy-confirmed AL amyloidosis diagnosis between January 1, 2010 and August 31, 2019 at Mayo Clinic Rochester, MN. We had access to these patients' Mayo Clinic electronic health records (EHRs) and a research registry that was manually abstracted from their EHRs by a trained medical abstractor. We selected 15 symptoms characteristic of AL amyloidosis and available in the registry and curated lists of synonyms and ICD codes to accurately represent them. We extracted the 15 symptoms from the notes and diagnostic codes portions of the EHRs, considering records 1 year before to 90 days after diagnosis and excluding records occurring after the start of treatment. For the registry, we considered records any time before diagnosis. To determine whether a mention of a symptom in the note was an affirmative diagnosis, we applied an NLP ("augmented curation") phenotype-sentiment classification model. The algorithm was developed by fine-tuning a SciBERT model on a set of 18,490 manually annotated sentences, and it achieves an out-of-sample accuracy of 93.6% with recall and precision values above 95%[5]. We manually reviewed the notes of a sample of symptom cases identified through augmented curation alone and evaluated the accuracy of all three methods for identifying proteinuria by comparing to a "gold standard" derived from structured lab data.

Results: More cases of AL amyloidosis symptoms were identified by augmented curation versus ICD codes or registry data. Prevalent symptoms, including edema (n=876), fatigue (n=786), dyspnea (n=770), and proteinuria (n=712), showed high concordance across augmented curation, ICD codes, and registry data (Figure 1). In cases picked up through augmented curation alone, a manual review of notes revealed that the symptom was present but usually not clearly attributed to AL amyloidosis in the note. Augmented curation and the registry identified similar numbers of lab-confirmed proteinuria cases (423 cases by augmented curation and 434 by the registry out of 578 lab-defined cases), and had similar specificity (67.2% vs. 66.4%) and sensitivity (73.2% vs. 75.1%) values.

Summary & Conclusion: ICD codes miss a substantial number of symptoms compared to augmented and manual curation of clinical notes. Human abstraction is prone to misclassification of symptoms when the note is written before the AL amyloidosis diagnosis, while augmented curation picks up such cases and illuminates the false negatives. These results suggest that an NLP-based approach is a valuable tool for comprehensive capture of symptoms in real time. In future work, it will be interesting to explore augmented curation of clinical notes as a first step for screening for AL amyloidosis and other rare diseases that are misdiagnosed or slow to diagnose.

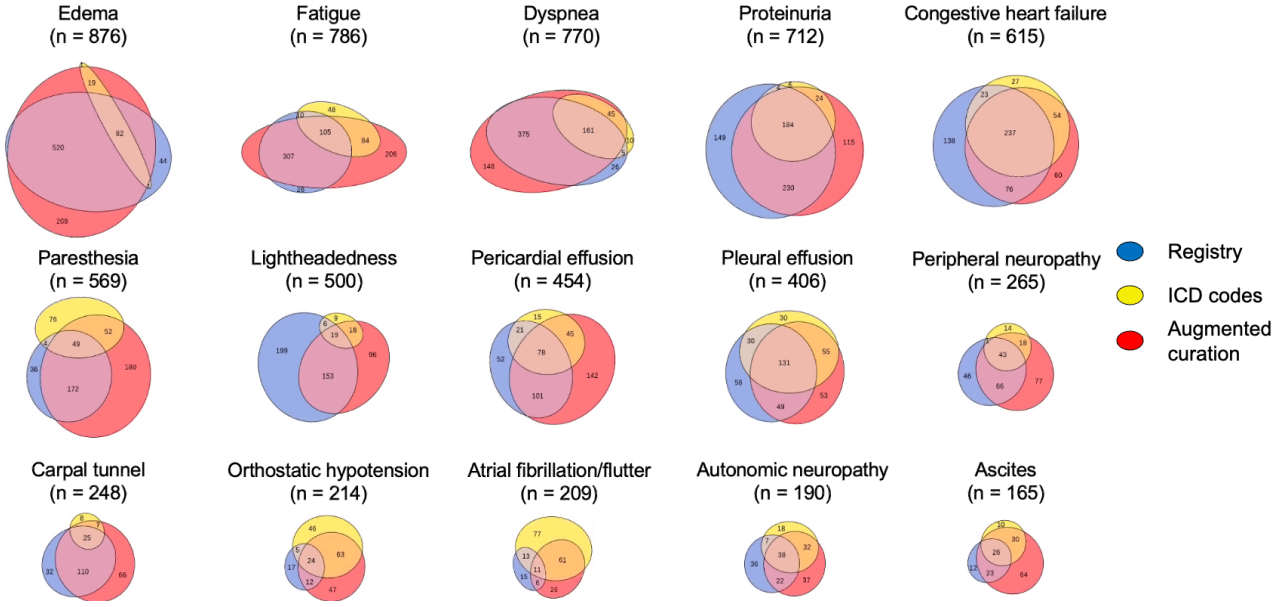

Figure 1.: Euler diagrams showing the intersections between the three data sources for all symptom categories. The number of patients with the symptom according to the data source(s) is given in each section of each Euler diagram. Diagrams are sorted by, and their areas are proportional to, the number of cases provided by the union of the data sources.

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A revised renal staging system for long-term renal survival in patients with AL amyloidosis with renal involvement

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Background: AL amyloidosis is characterized by frequent renal involvement (60-70% of patients), with an important risk of progression to end-stage kidney disease (ESKD) and a significant impact on quality of life. eGFR reduction (<50 mL/min /1.73 m^2) and severe proteinuria (> 5g/24 hours) at diagnosis have recently been validated as important predictors of renal outcome [1-2], allowing the stratification of risk of progression to ESKD into three different prognostic groups.

Objective: Our purpose was to validate the staging system for renal outcome proposed by Palladini et al. [1] in our cohort of patients. Subsequently we tried to identify the difference in terms of long-term renal survival between the population with severe proteinuira and preserved eGFR and the population with eGFR reduction and proteinuria above the cutoff in order to allow a further stratification of risk of progression to ESKD.

Material & Methods: We retrospectively selected patients with diagnosis of AL amyloidosis and evidence of renal involvement referring to three different third-level centers (Careggi University Hospital, Florence, Fatebenefratelli Hospital, Rome and S.Orsola-Malpighi Hospital, Bologna) between January 2007 and July 2021. Renal involvement was defined as a proteinuria > 0.5g/24h according to the 2005 ISA criteria [3]. Patients with history of CKD, with different renal disease or on dialysis at the time of diagnosis were excluded. Observation period was defined as the time between diagnosis and last visit. The end point of renal survival was defined as the time from diagnosis to dialysis initiation. Renal survival curves were plotted according to Kaplan-Mayer and log-rank comparisons. Statistical significance was established at P <0.05.

Results: The study included 139 patients with renal involvement with a median follow-up duration of 45 months. Among them, 33 patients (23.7%) developed ESKD at last follow-up. Applying the staging system proposed by Palladini et al. we stratified our population into 3 different renal stages with a statistically significant difference in terms of progression to ESKD between the stages (Fig. 1A; log-rank 16.76; p<0.0001). In particular 49 patients were classified in stage I; 67 in stage II and 23 in stage III. At last follow up, 2 patients in stage I (4%), 20 patients in stage II (29,8%) and 11 patients in stage III (47,8%) developed ESKD. After confirming the prognostic value of renal staging system proposed by Palladini et al. we've further divided the Stage 2 patients into 2 different categories distinguishing the patients with proteinuria above the cutoff (> 5g/24 hours) and conserved eGFR from patients with reduced eGFR and proteinuiria under cutoff, respectively Stage IIA and Stage IIB. The proposed renal staging system was able to detect significant differences in renal survival among renal stage IIA and stage IIB subjects with 19% and 43.3% of patients becoming dialysis dependent at last follow up, respectively.

Summary & Conclusion: eGFR reduction (<50 mL/min/1.73 m^2) and severe proteinuria (>5 g/24 hours) at diagnosis can stratify patients with AL amyloidosis and renal involvement into 4 distinct renal survival prognostic groups (Fig. 2B; log-rank 19.17 p<0.00001): low risk of progression to ESKD (conserved eGFR and proteinuria under the cutoff); intermediate-low risk (conserved eGFR and proteinuria above the cutoff); intermediate-high risk (reduced eGFR and proteinuria under the cutoff) and high risk (reduced eGFR and proteinuria above the cutoff).

Figure 1

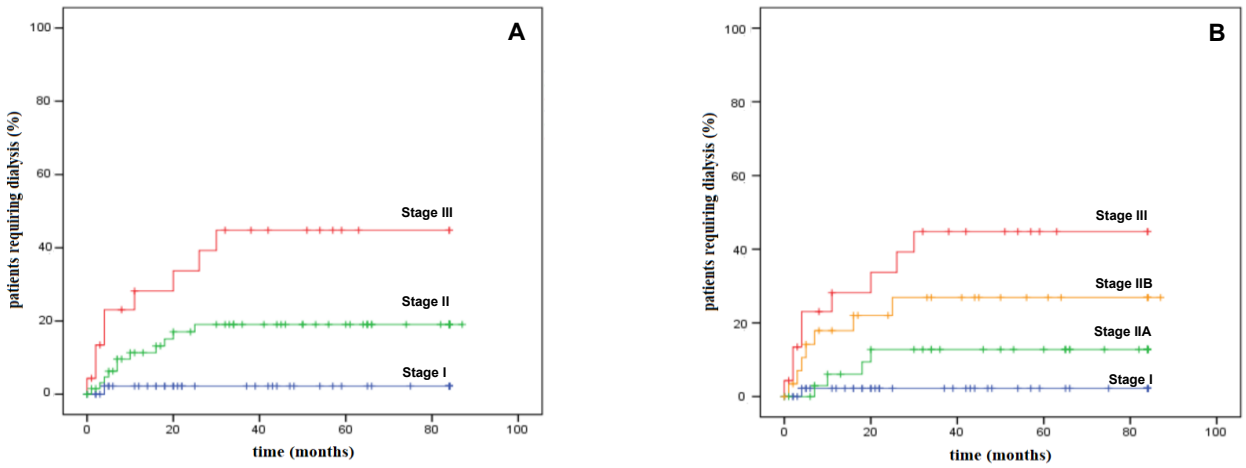

Figure 1:
A) Progression to dialysis according to staging system proposed by Palaldini et al.: Stage I: eGFR > 50 mL/min/1.73 m^2 and proteinuria < 5g/24 h; Stage II: eGFR < 50 mL/min/1.73 m^2 or proteinuria > 5g/24 h; Stage III: eGFR < 50 mL/min/1.73 m^2 and proteinuria > 5g/24 h.
B) Progression to dialysis according to revised staging system. Stage I: eGFR > 50 mL/min/1.73 m^2 and proteinuria < 5g/24 h; Stage IIA: eGFR > 50 mL/min/1.73 m^2 and proteinuria > 5g/24 h; Stage IIB: eGFR < 50 mL/min/1.73 m^2 and proteinuria < 5g/24 h; Stage III: eGFR < 50 mL/min/1.73 m^2 and proteinuria > 5g/24 h

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Role of subcutaneous abdominal fat tissue aspiration in the diagnosis of systemic immunoglobulin light-chain amyloidosis

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Background: Immunoglobulin light-chain (AL) amyloidosis is a rare plasma cell disorder characterized by the extracellular deposition of monoclonal light chain-derived amyloid fibrils. The organ affinity of amyloid is patient-dependent, leading to a highly variable clinical presentation of the disease. This fact favors common delays in the diagnosis resulting in progression of organ damage. Therefore, an early diagnosis is crucial, particularly when the heart is significantly involved. In this sense, subcutaneous abdominal fat aspirate (SAFA) is a minimally-invasive and extensively available diagnostic method. However, the variable sensitivity of this procedure among centers has made its use controversial.

Objective: This study aimed to analyze the effectiveness of SAFA to demonstrate amyloid deposition as well as to allow amyloid typing in clinical practice.

Material & Methods: We retrospectively analyzed a series of patients with AL amyloidosis diagnosed and treated at a single institution, who underwent a SAFA between 1982 and 2021 as part of clinical practice. From July 2013, after receiving a specific training in Pavia (Italy), the SAFA was performed by two hematologists from the Amyloidosis and Myeloma Unit, while rheumatologists were in charge of it previously. The objective of this procedure was to obtain at least four smeared slides and one cell block paraffin-embedded preparation for histological examination. Congo red-stained slides were assessed by two observers and, if positive, typing by immunohistochemistry was performed on the cell block preparation.

Results: Two hundred and thirty-eight patients were consecutively diagnosed with AL amyloidosis at our institution during the study period. Among them, 167 (70%) patients underwent a SAFA and were included in the analysis. The main reason for not having this test was a previous positive histologic diagnosis based on a different tissue. Congo red stain confirmed the presence of amyloid in fat tissue in 125 out of 167 patients (74.8%). From July 2013, 85 procedures (50.8%) were performed by hematologists and the proportion of positive SAFA did not change (77% versus 72%, p=0.4). Other clinical and laboratory features such as light chain isotype, bone marrow plasma cell infiltration, and type or number of involved organs were not associated with Congo red positivity. Amyloid typing by immunohistochemistry was undertaken in 78 out of the 125 patients with positive fat aspirate and amyloid type was identified in 43 of them (55%; Table 1). Immunohistochemistry was not performed in 47 (37.6%) patients, half of them because it had already been done in another tissue.

Conclusions: In our experience, aspirate of subcutaneous abdominal fat shows a high effectiveness to detect amyloid deposits in patients with AL amyloidosis, with a sensitivity of 75% in clinical practice. This data, together with its minimally-invasive nature, the fast results and extensive availability, make this procedure a very convenient screening diagnostic test. Although the scarcity of tissue sample may be a common inconvenience of this method, amyloid typing by immunohistochemistry was feasible in 55% of patients in whom it was performed, avoiding biopsies of other organs that have a higher risk of complications and allowing an earlier diagnosis of the disease.

Table1. Amyloid typing performance in 78 samples by immunohistochemistry

Performance	N (%)
Amyloid type evaluable AL lambda AL kappa Negative for all tested protein precursors	44 (56.4) 31 (70.4) 12 (27.3) 1 (2.3)
Non- evaluable *	34 (43.6)
Total	78 (100)

* Due to scarce deposits or insufficient tissue sample for immunohistochemistry study

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Functional Status and Heart Failure Quality of Life Provide Incremental Prognostic Value in Light Chain Amyloidosis

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Background: Prognostic staging in systemic AL amyloidosis is performed using the Mayo stage, based on NT-proBNP, troponin, and free light chains. In AL amyloidosis, quality of life (QOL) was associated with worse outcomes. Whether functional status and heart failure QOL provide prognostic value beyond the Mayo stage is unknown.

Objective: This study aimed to assess the prognostic value of functional status and heart failure QOL over Mayo stage in patients with systemic AL amyloidosis.

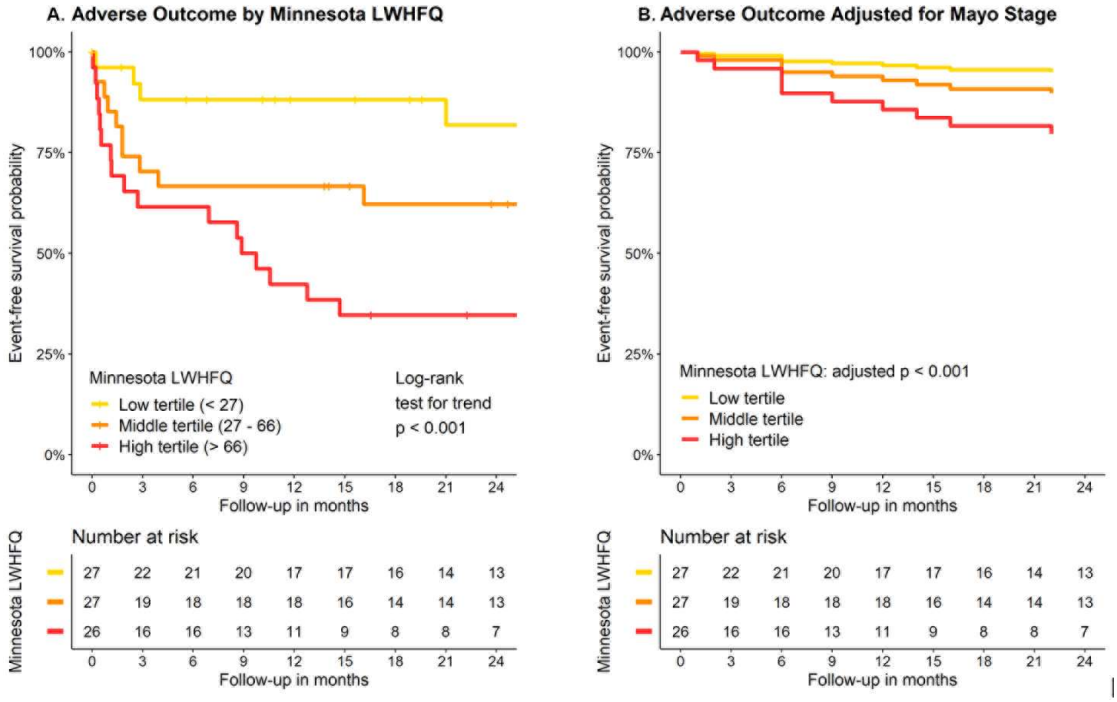
Material & Methods: Participants with recently diagnosed, biopsy-proven systemic AL amyloidosis were enrolled in a prospective study. Functional assessment was performed using the Karnofsky Performance Status (KPS, 0-100, higher is better), and categorized as tertiles of ≤70, 80, and ≥90. Heart failure QOL was assessed with the Minnesota Living with Heart Failure Questionnaire (MLWHFQ, 21 questions, 0-105 points, lower is better), and categorized as tertiles of <27, 27-66, and >66. Mayo stages I – IV were based on NT-proBNP ≥1800 pg/mL, troponin T ≥0.025 ng/mL, and difference in free light chains ≥180 mg/L. Adverse outcomes were all-cause death or heart failure hospitalization. Survival analysis was performed using Kaplan-Meier analysis, log-rank test, Cox regression with hazard ratios (HR) and 95% confidence intervals (95% CI), and Akaike's information criterion (AIC, lower is better). Optimal cut points for adverse outcome prediction were calculated using log-rank maximization.

Results: This study included 81 participants with a median age of 61 years, 57% males, and 75% cardiac amyloidosis. At baseline, median KPS was 80 (IQR 70–90) and median MLWHFQ was 50 (18–73). During a median follow-up of 16 months (IQR 3–37), 18 participants died (22%) and 24 were hospitalized for heart failure (30%), resulting in 36 participants with adverse outcomes (44%).

KPS: The incidence of adverse outcomes increased across KPS tertiles from 28% to 59% (log-rank p=0.017). Event rates increased across KPS tertiles from 0.23 (95% CI 0.13–0.38) to 0.52 (95% CI 0.35–0.75) events/patient-year. But in multivariable Cox models adjusted for Mayo stage, KPS did not independently predict adverse outcomes (HR 0.98, 95% CI 0.96–1.01, p=0.23). The optimal KPS cut point for prediction was 70.

MLWHFQ: The incidence of adverse outcomes increased across MLWHFQ tertiles from 19% to 73% (log-rank p<0.001, Figure 1A). Event rates increased across MLWHFQ tertiles from 0.14 (95% CI 0.07–0.27) to 0.71 (0.50–0.96) events/patient-year (Figure 1A). In multivariable Cox models adjusted for Mayo stage, MLWHFQ independently predicted adverse outcomes (HR 1.02, 95% CI 1.01–1.04, p<0.001, Figure 1B). Combining Mayo stage and MLWHFQ into the same model improved the AIC for prediction of adverse outcomes: Mayo stage 223.2, MLWHFQ 221.9, both together 215.8. The optimal MLWHFQ cut point for prediction was 45.

Summary & Conclusion: Patients with recently diagnosed systemic AL amyloidosis had a high incidence of death and heart failure hospitalization. Functional status and heart failure QOL, measured by KPS and MLWHFQ, predicted such adverse outcomes, and MLWHFQ predicted events independently of Mayo stage. Combining Mayo stage and MLWHFQ further improved the prediction of adverse outcomes. This demonstrates predictive validity for the MLWHFQ in AL amyloidosis, but further content and psychometric validations are needed. Therefore, we propose that after thorough validation, MLWHFQ should be considered for inclusion in future prognostic staging models for systemic AL amyloidosis.



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Title: Heart and autologous stem cells transplantation in AL amyloidosis.

Authors: María Adela Aguirre, Marcelina Carretero, Franco Faelo, Eugenia Villanueva, Erika Bárbara Brulc, María Soledad Sáez, Patricia Beatriz Sorroche, Cesar Belziti, Diego Perez de Arenaza, Ricardo Marenchino, María Loures Posadas Martínez, Elsa Mercedes Nucifora

Background: Seventy percent of AL amyloidosis patients develop cardiac involvement, which is a key prognostic factor. Heart transplantation and subsequent induction chemotherapy and autologous stem cell transplantation (ASCT) are treatment possibilities for selected patients.

Objective: To describe the evolution of serum light chain between heart transplantation and autologous stem cell transplantation.

Materials & Methods: Case series of consecutive patients diagnosed as AL systemic amyloidosis who underwent a heart and autologous transplantation from the Institutional Amyloidosis Registry (RIA) of the Italian Hospital of Buenos Aires, between January 2010 and November 2021. The quantitative variables were described with their median and interquartile range and the categorical variables as absolute and relative frequencies. Overall and disease-free survival was estimated using Kaplan-Meier.

Results & Discussion: Among 106 patients with AL amyloidosis, 18 had a heart transplantat and 6 had a heart trasplant followed by chemotherapy induction and ASCT (Figure 1). All patients with ASCT were younger than 70 years, with a serum creatinine value < 1.5 mg/ml, ejection fraction > 45%, with normal respiratory function tests and fewer than two organs involved. Five patients had light chain data available. In the period between transplants, the light chain involved decreased in 2 patients and remained stable in 3 (Table 1). The median time between heart transplantation and ASCT was 344 days (IR 317-582 days). All patients received low-dose CyBorD regimen from disease diagnosis to heart transplantation and then resumed the same full-dose regimen three months after heart transplantation and until the ASCT. Tacrolimus and mycophenolate mofetil were the immunosuppressive regimen used after heart transplantation. The 2-year overall survival rate and progression-free survival rate were 100%. At the end of follow-up, none patient relapsed in the solid organ transplantation.

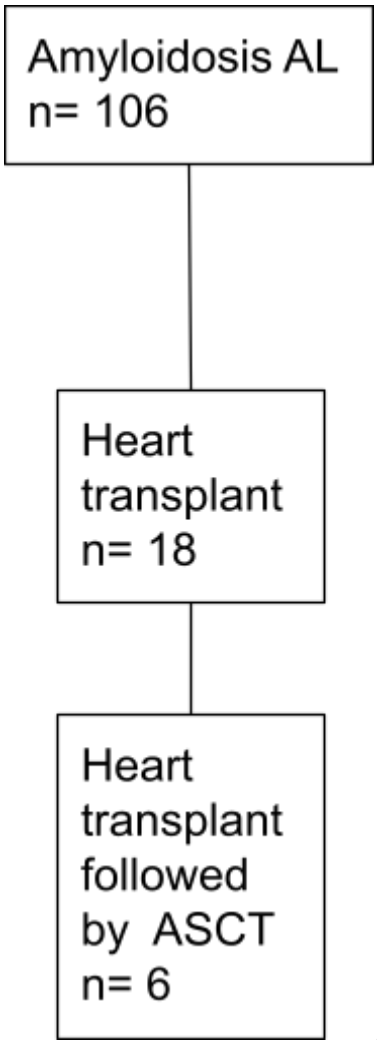
In the present study, we found that no patient showed disease progression in the period between transplants. Our results are similar to the study by Renteria et al., [1] in which only 1 patient out of the 12 analyzed showed disease progression in the period between transplants, with a median time between transplants of 6 months, and without chemotherapy between transplants. However, our patients had a median between transplants of 11 months, with induction chemotherapy established between these periods, which supports our results.

We want to highlight that two of the patients had a delay in receiving the ASCT due to difficulties in accessing the care center as a consequence of the preventive and mandatory social isolation imposed in our country by the SaRs-CoV 2 pandemic. This could explain the greater time between transplants when compared to the study by Ranteri et al.

Summary & Conclusions: Light chains decreased or remained stable in all cases. Immunosuppressive therapy after heart transplantation may have some effects on clonal plasma cells, but the effect is unclear. This work may lead to future studies on the effects of immunosuppressants such as tacrolimus and mycophenolate mofetil on plasma cells.

Key words: Immunoglobulin Light chain Amyloidosis; Treatment; Organ Transplantation; Stem Cell Transplantations

Figure 1. Flowchart. Patients with immunoglobulin light chain amyloidosis of the Institutional Registry of Amyloidosis of the Italian Hospital of Buenos Aires. Period 2010-2021.



ASCT: Autologous Stem Cell Trasplantation

Table 1. Serum free light chains, prior to heart transplantation and in the period between heart transplantation and ASCT. n=5.

Case	Serum free light chains previous to heart transplantation	Serum free light chains between heart transplantation and ASCT
Case 1	Kappa 22,9 mg/L Lambda 22,9 mg/L Ratio kappa/lambda 0.97	Kappa 27.1 mg/L Lambda 36 mg/L Ratio kappa/lambda 0,6.
Case 2	Kappa < 5,7 mg/L Lambda 143 mg/L Ratio kappa/lambda 0,97	Kappa 4,74 mg/L Lambda 42,7 mg/L Ratio kappa/lambda 0,11.
Case 3	Kappa 8,58 mg/L Lambda 599,8 mg/L Ratio kappa/lambda 0,01	Kappa 11,84 mg/L Lambda 534,09 mg/L Ratio kappa/lambda 0,02.
Case 4	Kappa 447,94 mg/L Lambda 5,25 mg/L Ratio kappa/lambda 85,32	Kappa 190,06 mg/L Lambda 6,98 mg/L Ratio kappa/lambda 27,23
Case 5	Kappa 12,14 mg/L Lambda 382,75 mg/L Ratio kappa/lambda 0,03	Kappa 18,34 mg/L Lambda 371,29 mg/L Ratio kappa/lambda 0,05.

ASCT: Autologous Stem Cell Transplantation

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Droxidopa for Treatment of Refractory Orthostatic Hypotension in Patients with AL Amyloidosis: A Case Series

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Background: Orthostatic hypotension (OH) due to autonomic dysfunction is a well-known complication of light chain (AL) amyloidosis, which can become progressively debilitating and difficult to manage. Treatment of the underlying plasma cell dyscrasia will decrease subsequent amyloid deposition. Management of OH secondary to AL amyloidosis improves quality of life and facilitates delivery of plasma cell therapy. Pharmacologic interventions include fludrocortisone, sympathomimetic agents such as midodrine, droxidopa, the acetylcholinesterase inhibitor pyridostigmine, or the norepinephrine transporter (NET) inhibitor atomoxetine. Fludrocortisone is often poorly tolerated in patients with amyloidosis as it may exacerbate edema. Droxidopa is a synthetic amino acid analog that is directly metabolized to norepinephrine by dopa-decarboxylase, which increases blood pressure (BP) by inducing peripheral arterial and venous vasoconstriction.

Objective: Assess the effectiveness of droxidopa in AL amyloidosis patients with severe OH refractory to midodrine and to describe the effective dose, duration of therapy, adverse effects and reasons for discontinuation.

Material & Methods: A regional retrospective study was done in patients with AL amyloidosis with severe, refractory OH who received droxidopa. Retrospective data was reviewed from 2018 to 2021 at a single academic center in the United States.

Results & Discussion: Of the five patients that were included in this study, three patients had AL amyloidosis and two patients had MM-associated AL amyloidosis. All five patients had cardiac, renal, autonomic nervous system and peripheral nervous system involvement. Two patients (40%) also had gastrointestinal involvement. No patients were eligible for high-dose melphalan with autologous peripheral blood stem cell transplantation (HDM/SCT) due to poor performance status and advanced organ involvement, and thus were treated with cyclophosphamide, bortezomib and dexamethasone (CyBorD). All patients achieved very good partial response or complete hematologic response. The main findings are summarized in table 1. All patients had severe, symptomatic OH that was objectively confirmed in the clinic. Initial treatment for all patients included midodrine, ranging from 5 to 30mg TID based on individual tolerance. Three of the patients also were initially treated with fludrocortisone 0.05 to 0.2mg daily. One patient was treated with pyridostigmine 30mg TID (case 5). Given persistence of symptoms despite therapy, droxidopa was started at 100mg TID in all patients, and the dose was titrated as tolerated. None required the maximal approved dose of 600mg TID. After initiation of droxidopa, four patients (80%) reported improvement in symptoms in addition to improved measurements of orthostatic blood pressure values. By the end of this study, three patients (60%) continued treatment with droxidopa (cases 1-3); one was weaned-off after resolution of symptoms (case 5) and one was discontinued due to supine hypertension (case 4).

Summary & Conclusions: Data shows that droxidopa is an effective treatment of OH refractory to midodrine in patients with AL amyloidosis with overall good tolerance. Slow titration may be important to minimize rapid changes in blood pressure. Studies are warranted to assess the use of droxidopa as first-line therapy in OH.

Figures:

Table 1: Patient characteristics and treatment outcome

Case	Age (years)	Diagnosis	Treatment regimen	Organ Involvement	Max Midodrine Dose (Other therapies)	Max Droxidopa Dose	Resolution of symptoms (Time in months)*	Side Effects
1	69	Lambda clonal plasma cell dyscrasia	CyBorD followed by daratumumab + pomalidomide followed by daratumumab	Cardiac Renal ANS PNS GI	30mg TID (FC)	100mg TID	Yes (12)	None
2	50	Lambda clonal plasma cell dyscrasia	CyBorD followed by bortezomib switched to ixazomib switched to daratumumab	Cardiac Renal ANS PNS GI	15mg TID (FC, HC)	200mg TID	Yes (24)	None
3	64	Kappa-LC restricted MM	CyBorD followed by pomalidomide	Cardiac Renal ANS PNS	10mg TID (None)	300mg TID	Yes (1)	None
4	66	Lambda clonal plasma cell dyscrasia	CyBorD followed by daratumumab followed by venetoclax + dexamethasone	Cardiac Renal ANS PNS	15mg TID (FC)	100mg TID**	No (2)	Supine HTN
5	54	Kappa- LC restricted MM	CyBorD + daratumumab	Cardiac Renal ANS PNS	30mg TID (PB)	400mg TID	Yes (8)	None

ANS: autonomic nervous system, CyBorD: Cyclophosphamide, Bortezomib, Dexamethasone, FC: fludrocortisone acetate, GI: gastrointestinal, HC: hydrocortisone, HTN: hypertension, LC: light chain, MM: multiple myeloma, PB: pyridostigmine bromide, PNS: peripheral nervous system, TID: three times a day

*By the end of this study, three patients continued treatment with droxidopa (cases 1-3); one was weaned-off after resolution of symptoms (case 5) and one was discontinued for supine HTN (case 4).
** Prescribed 100mg TID but took 500mg TID.

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AL Amyloidosis – a reason to transplant MGUS phenotype?

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Background: AL amyloidosis (AL) is a deposition disease in which antibody light chains or parts of it cause disruption of organ function. The underlying cause is monoclonal gammopathy, mostly due to monoclonal gammopathies of unknown significance (MGUS), smoldering myeloma or multiple myeloma (MM). High-dose chemotherapy with melphalan (HD) with subsequent autologous stem cell transplantation was derived from myeloma therapy and is considered the most effective therapy for AL, if tolerable [1]. However, only half of AL cases classify as myeloma with bone marrow plasma cells (BMPC) >10% and (SLiM-)CRAB criteria, the other half presents with stable BMPC <10% [2]. It is known that higher bone marrow infiltration is a risk factor for survival [3]. While MGUS can also be the underlying cause for AL, it is biologically and genetically distinct from MM [4].

Objective: Therefore, the benign phenotype of MGUS may not warrant the need for high dose chemotherapy. We decided to assess the retrospective data on patients with systemic AL and MGUS criteria. This is an analysis wheter there was a benefit in progression free survival (PFS) in patients with BMPC <10% and absent (SLiM-)CRAB criteria that were treated with HD at our center.

Methods: We retrospectively analyzed 149 patients with biopsy-proven systemic AL amyloidosis diagnosed and treated between January 1, 2006 and December 31, 2021. We excluded patients with >10% BMPC and/or positive (SLiM-)CRAB criteria. Patients previously treated for AL or MM and AL cases due to lymphoproliferative disorders were excluded. We also excluded patients that did not receive a follow-up or had non-measurable disease. We considered the highest estimate from aspirate or biopsy as true BMPC infiltration. A line of therapy was considered the same therapy regardless of subsequent dose reductions or escalations. All patients that were treated with HD had a cumulative melphalan dose of at least 140 mg/m². Non-HD therapies consisted of proteasome inhibitor monotherapy or in combination or Daratumumab mono or in combination. We evaluated hematological and organ progression according to common consensus criteria [5-7]. High risk cytogenetics were assessed according to current IMWG criteria [8]. For survival analysis, we used the Kaplan-Meier estimator. For hypothesis testing, we used the log rank test. P values < 0.05 were considered statistically significant. Our ethics commitee approved the retrospective analysis.

Results: Overall, we identified 50 patients with MGUS phenotype that were available for evaluation. 27 (54 %) received HD and 23 (46 %) were treated without ever receiving HD. Medium BMPC infiltration was 7.3 and 7.4%, respectively. Both groups mainly exhibited lambda phenotype (78 and 75%). FISH analysis was available in 21 patients. t(11;14) and del13q14 were the most common (41/44% and 33/22%, respectively). We found 5 high-risk phenotypes (del17p and gain1q), all treated with HD. ECOG status at diagnosis was significantly lower in the HD population compared to no HD (0.67 vs. 1.06, respectively). PFS did not differ significantly between the two groups (p=0,43).

Discussion: AL amyloidosis can present in various forms. Predictive markers such as cytogenetics and organ involvement are routinely used for treatment selection [9]. However, this is not yet true for BMPC infiltration. It is known that an infiltration of >20% is an independent predictor for response, most likely due to a more aggressive phenotype, associated with higher immunoglobulin levels, aggressive cytogenetics and positive (SLiM-)CRAB criteria [10]. HD, as many other AL therapies, are borrowed from myeloma therapies, tailored for an aggressive biology. The great success that translates into AL [11] is at the cost of significant toxiciy [12] and only 20% of diagnosed AL patients are fit for HD [9]. There are several ongoing trials for MGUS treatment, none featuring HD [13].
Here, we analyzed PFS of HD-based treatment vs. no HD in a low risk population with <10% BMPC. We could not find a statistical benefit for HD. The retrospective data from this set analyzes the clinical decisions made upon entirely different criteria, high risk cytogenetics being one of them. Accordingly, we found high-risk markers only in the HD group. However, after adjusting for high risk cytogenetics, we still found no significant difference (p=0,71). Still, we cannot adjust for all clinical criteria that may have played a role in decisionmaking. 6 patients in the HD group received upfront HD without induction, a sequence today known to be inferior [14]. However, in a subgroup analysis of the study, BMPC <10% was not associated with in improved response with induction. Interestingly, performance status, likely a factor for therapy decision against HD, did not negatively influence PFS despite a high rate of therapy interruptions.
Melphalan is known to significantly alter expression profiles of plasma cells, possibly contributing to the transition to a more malignant phenotype [15], mitigating the effective antiplasmatic therapy. We know that therapy duration is an important factor for myeloma patients and quality of life is greater in patients in therapy-free intervals. This correlates with HD in myeloma patients [16]. However in this population, we did not find a need for maintenance or prolonged therapy in the no HD group. Notably, the number of Daratumumab-based therapies in this population is still low (n=6). So far, the impact of novel therapies remains unclear. This also applies to other parameters such as depth of response and duration of therapy.

Summary & Conclusion: This dataset may provide a first rationale for less aggressive therapy and questions the

significance of HD in a biologically low risk AL population. This falls in line with the mSMART 2020 guidelines that give the option to observe even HD fit patients with good response after induction therapy . Prospective trials are needed to confirm these retrospective, single-center results.

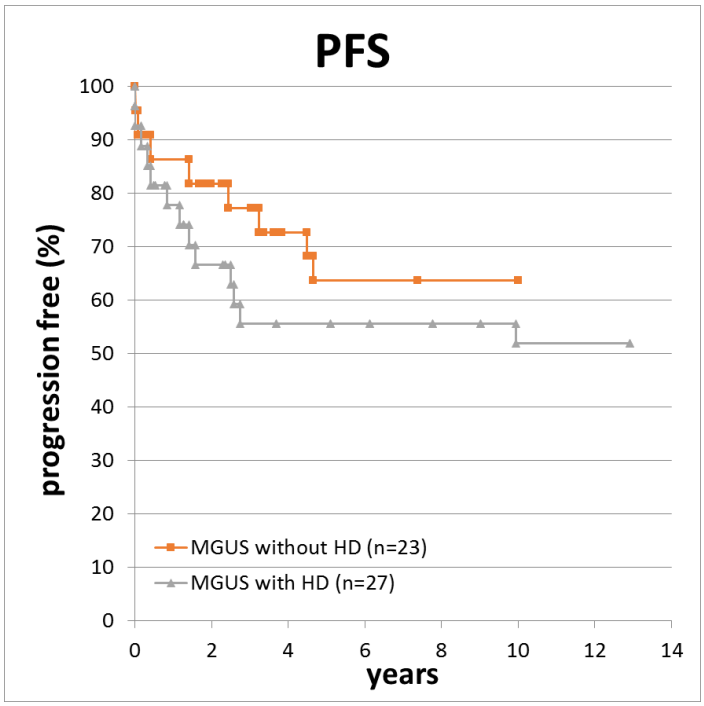

Figure 1: PFS of patients with AL amyloidosis and MGUS phenotype by therapy.

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Daratumumab for the treatment of Relapsed/Refractory AL amyloidosis: Experience from the Amyloidosis Program of Calgary (APC)

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Background: Systemic immunoglobulin light chain (AL) amyloidosis is a disorder characterized by the production of clonal serum free light chain that misfold, aggregate, and deposit in vital organs.[1] First-line therapies are well established, but in the relapsed refractory setting, there are many options including proteasome inhibitors, alkylating agents, immunomodulatory drugs, and monoclonal antibodies.[2] Daratumumab is a first in class human IgG1k monoclonal antibody that binds with high affinity to a unique epitope of CD38.[3] In the relapsed/refractory setting, use of daratumumab monotherapy or as part of combination regimen gives very promising results.[4]

Objective: The primary objective of this study was to assess the role of Daratumumab-based regimens (DBR) in the treatment of relapsed/refractory AL amyloidosis patients at the Amyloidosis Program of Calgary.

Material & Methods: All consecutive relapsed/refractory AL amyloidosis patients treated with DBR assessed at the Amyloidosis Program of Calgary from 01/2018 to 03/2022 were evaluated. The diagnosis of AL amyloidosis and assessment of hematological and organ response was performed based on the consensus criteria published in 2005 and modified in 2012.[4,5] Hematological and organ response was assessed as per standard guidelines. Patients with at least 1 cycle of treatment were included.

Results: Nineteen consecutive patients with AL amyloidosis treated with DBR at the Amyloidosis Program of Calgary from 01/2018 to 03/22 were identified. Clinical characteristics are seen in **Table 1.** Median age at diagnosis was 64 (range 46-82). According to the Mayo Clinic staging criteria (2012)[3]: 3 patients were classified as stage I (15.8%), 4 Stage II (21.1%), 6 Stage III (31.6%), and 5 Stage IV (26.3%). Of these, 16 (84.2%) received lenalidomide, dexamethasone, and daratumumab (LDD). Most patients received DBR as second line therapy (n=14; 73.6%). Overall response rate was achieved in 94.7% of cases, with 15.8% in CR, 13 (68.4%) in VGPR, and 5 (26.3%) in PR. Organ response was noted in 11 cases (57.9%). Of those, 8/14 (57%) evaluable cases achieved cardiac response at a median of 4.5 months and 4/11 (36%) achieved renal response at a median of 6 months. At the time of analysis, 14 patients (73.7%) remain alive and progression-free with a median follow up of 10.1 months. Median OS and PFS have not been reached. Estimated median MOD PFS was 23 months. In total, 11 events defining MOD-PFS were reported in the group.

Summary & Conclusion: We report here that daratumumab produced rapid and deep hematologic and organ responses in a cohort of AL patients with at least 1 prior line of therapy in a real-world setting. Daratumumab was well tolerated, even among patients with advanced cardiac AL involvement.

Table 1. Clinical characteristics for patients with AL amyloidosis treated at the Amyloidosis Program of Calgary from Jan/2018 to March/2022

Clinical characteristic	**N=19**
Age, median (range)	64 (46-82)
Gender Male Female	 13 (68.4%) 6 (31.6%)
Creatinine (µmol/L)	98
LDH (IU/L)	209
Troponin T (ng/L)	33.5
NTproBNP (ng/L)	896
Mayo Clinic Stage at diagnosis Stage I Stage II Stage III Stage IV Unknown **Organ involvement:** Heart Kidney Nerve GI tract Soft Tissue	 3 (15.8%) 4 (21.1%) 6 (31.6%) 5 (26.3%) 1 (5.3%) 13 (68.4%) 16 (84.2%) 5 (26.3%) 4 (21.1%) 11 (57.9%)
Prior lines of therapy, median (range)	1 (1-3)
DBR Daratumumab, Lenalidomide and Dexamethasone Bortezomib, Daratumumab and Dexamethasone Ixazomib, Daratumumab and Dexamethasone	 16 (84.2%) 2 (10.5%) 1 (5.3%)
Overall Response rate Complete Response VGPR/CR Very Good Partial Response Partial Response **Organ Response** Cardiac Response Renal Response	18 (94.7%) 3 (15.8%) 13 (68.4%) 10 (52.6%) 5 (26.3%) 11 (57.9%) 8/14 (57%) 4/11 (36%)
Time to first Hematological Response, median Time to best Hematological Response, median Time to Cardiac Response, median Time to Renal Response, median	4 weeks 8 weeks 4.5 months 6 months

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Efficacy and safety of daratumumab monotherapy in newly diagnosed patients with stage 3b light chain amyloidosis: a phase 2 study by the European Myeloma Network

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Background: Cardiac involvement and severity of cardiac dysfunction in light chain (AL) amyloidosis is a critical prognostic factor[1]. Patients (pts) at Mayo cardiac stage 3b have a poor prognosis with a median overall survival (OS) of just 4 months and high rates of early death with current therapies; thus, there is a need for novel, non-toxic, effective treatments for these pts. Daratumumab (DARA), a human anti-CD38 antibody, has shown efficacy & tolerability in pts with AL amyloidosis[2].

Objective: To evaluate the efficacy & safety of DARA monotherapy used off-label in newly diagnosed pts with stage 3b AL amyloidosis.

Material & Methods: The ongoing EMN22 phase 2, multinational, open-label study (NCT04131309) aims to enroll 40 newly diagnosed pts with stage 3b AL amyloidosis. Eligible adult pts have high-sensitivity troponin T (hsTnT) >54 pg/mL and N-terminal pro-brain natriuretic peptide (NT-proBNP) ≥8,500 pg/mL. DARA monotherapy, 16 mg/mL by intravenous infusion (09/2019–01/2020) and 1,800 mg by subcutaneous injection (01/2020 and thereafter), is administered weekly during cycles (C)1 & 2, every 2 weeks for C3–6, and every 4 weeks thereafter. Pts not achieving a hematological very good partial response (VGPR) or better by the end of C3 can receive additional weekly bortezomib & low dose dexamethasone (Vd). Treatment continues up to 2 years from initiation or until disease progression or initiation of a new therapy. Primary endpoint is OS rate at 6 months. This descriptive analysis included pts initiating treatment ≥6 months before the cut-off date (14/01/2022); the median (95% confidence interval [CI]) OS was obtained by Kaplan-Meier analysis.

Results: Of 27 pts included, 8 (30%) continued study treatment by the cut-off date and 19 (70%) had discontinued. The pts median age was 68 (range 45–84) years, and most were male (16, 59%). At screening, 10 (37%) and 17 (63%) pts had New York Heart Association class II & IIIA symptoms, respectively; the median NT-proBNP was 15,512 pg/mL (range 8,816–72,522), hsTnT was 133 pg/mL (range 60–692), and the difference of involved to uninvolved free light chains was 406 mg/l (range 24–3,377). Beyond the heart, the median number of other organs involved was 2 (range 0–5), most commonly kidneys (14 pts, 52%) and peripheral nerves (11 pts, 41%). The median duration of DARA therapy was 7 months (range <1–24); seven (26%) pts received additional Vd. At a median observation time of 8 months (range <1–11), the overall response rate (ORR) was 67% (18 pts; complete response [CR]:19.0% [5 pts], VGPR:37% [10 pts], partial response:11% [3 pts]). The ORRs at 1, 2, & 3 months were 59% (16 pts), 63% (17 pts), and 63% (17 pts), respectively. Median time to first response was 7 days (range 6–114), and to VGPR or better 54 days (range 6–219). Median OS was 9 months (95% CI, 3–not reached). The 6- & 12-month median (95% CI) OS rates were 63% (42–78) and 49% (28–67), respectively. Twenty-five (93%) pts had ≥1 non-serious adverse event. Twenty (74%) pts had ≥1 serious adverse event (SAE), comprising 15 (56%) pts with ≥1 cardiac-related SAE and 11 (41%) pts with a fatal SAE. Six SAEs were treatment-related: 2 with DARA (grade 3 pneumonia, grade 5 sepsis); 3 with bortezomib (grade 2 fatigue, grade 2 fall, and grade 3 troponin I increase); and 1 with dexamethasone (grade 3 cardiac failure).

Summary & Conclusion: Among pts with Mayo stage 3b AL amyloidosis, a subgroup with poor prognosis, DARA monotherapy induced rapid & deep hematological responses and no new safety signals; the median OS surpassed that reported previously.

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Role of Doxycycline in the treatment of patients with AL amyloidosis receiving Bortezomib-containing regimens in the frontline setting: Experience from the Amyloidosis Program of Calgary

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Background: Light chain (AL) amyloidosis, the most common type of systemic amyloidosis, occurs when the free light chains normally associated with immunoglobulins are produced in excess by clonal or frankly malignant plasma cells.[1] Pre-clinical and retrospective data suggests that Doxycycline added to treatment regimens has benefit in AL amyloidosis.[2,3] However, a recent multicenter, open-label, randomized controlled trial comprised of Mayo 2004 stage II to III AL amyloidosis patients treated with CyBorD did not demonstrate a progression free survival (PFS) or cardiac PFS benefit with added Doxycycline.[4]

Objective: The main objective of this study is to explore the role of Doxycycline combined with Bortezomib-containing regimens (BCR) for newly diagnosed AL amyloidosis patients with cardiac involvement and to compare with a cohort of consecutive patients treated with BCR only.

Material & Methods: All consecutive newly diagnosed AL amyloidosis patients treated with BCR at the Amyloidosis Program of Calgary (APC) from 01/2012 to 03/2022 were evaluated. The diagnosis of AL amyloidosis and assessment of hematological and organ response was performed based on the consensus criteria published in 2005 and modified in 2012.[5,6] Patients with at least 1 cycle of treatment were included. Doxycycline was given at 100 mg twice daily from the time of diagnosis until progression or toxicity. Survival curves were constructed according to the Kaplan-Meier method and compared using the log rank test. All statistical analyses were performed using the SPSS 24.0 software.

Results: Sixty-four consecutive patients with AL amyloidosis treated with BCR at the APC from 01/2012 to 03/22 were identified. Thirty-nine patients received Doxycycline in addition to BCR (BCR-D) for a median of 8 months. Five patients discontinued Doxycycline due to GI (n=3; 7.6%) and skin toxicity (n=2; 5.1%). Clinical characteristics are seen in **Table 1.** Median age at diagnosis was 68 years for the BCR-D group and 64 years for BCR alone (p=0.3). The rest of clinical characteristics were similar among the two groups. Patients treated with BCR-D received mainly CyBorD and CyBorMe (51.2% and 48.7%), compared to 52% and 8% in the BCR alone (p=0.001). Overall response rate was similar among the groups (BCR-D=89.7% vs BCR=84%; p=0.4). No significant differences on VGPR/CR, dFLC at 1-month, time to first response, time to best response, or organ response were noted between the BCR alone and BCR-D groups (**Table 2**). At the time of analysis, 16 (64%) BCR-D and 18 (46.1%) BCR patients were alive and 13 (52%) BCR-D and 13 (33.3%) BCR patients had progressed (p=0.4 and 0.1, respectively). Median overall survival (OS) has not been reached for the BCR group compared to a median of 25.6 months for the BCR-D group (p=0.07). Further, median progression free survival (PFS) was similar for BCR and BCR-D (p=0.8). Survival at 1-year was 84% in BCR versus 70% in the BCR-D group (p=0.2).

Summary & Conclusion: Our retrospective study demonstrated that Doxycycline combined with BCR failed to prolong OS, PFS, or cardiac responses compared with BCR alone in patients with cardiac AL amyloidosis. Since this is a small, retrospective, non-paired study, more data is needed to confirm these findings.

Table 1. Clinical Characteristics of patients with AL amyloidosis receiving BCR according to the use of concomitant Doxycycline

Characteristic	BCR + Doxycycline, N=39	BCR alone N=25	P value
Age (median)	68	64	0.3
Gender			0.4
Male	21 (53.8%)	16 (64%)	
Female	18 (46.1%)	9 (36%)	
Hb (g/L)	124	122	0.4
Creatinine (µmol/L)	96	80	0.5
B2microglobulin (µmol/L)	3.0	3.24	0.3
Albumin (g/L)	29	32	0.08
Stage I	2 (5.1%)	0 (0%)	0.2
Stage II	4 (10.2%)	4 (16%)	
Stage III	14 (35.8%)	4 (16%)	
Stage IV	17 (43.5%)	14 (56%)	
Unknown	2 (5.1%)	3 (12%)	
LDH (IU/L)	226	232	0.4
BMPC (%)	12	10	0.3
NTproBNP ng/L	2642	3246	0.4
Troponin T ng/L	53	65	0.4
Light chain:			0.6
Kappa	8 (20.5%)	8 (32%)	
Lambda	30 (76.9%)	17 (68%)	
Biclonal	1 (2.5%)	0	
Organ Involvement			
Cardiac involvement	39 (100%)	25 (100%)	NS
Kidney involvement	26 (66.6%)	19 (76%)	0.4
Liver involvement	7 (17.9%)	2 (8%)	0.2
Nerve involvement	4 (10.2%)	5 (20%)	0.2
GI involvement	8 (20.5%)	5 (20%)	0.9
Lung involvement	1 (2.5%)	2 (8%)	0.3

Table 2. Treatment regimens and response rates for patients with AL amyloidosis receiving BCR or BCR-D at the Amyloidosis Program of Calgary from 2012 to 2022

Characteristic	BCR-D , N=39	BCR, N=25	P value
Bortezomib-Containing Regimens			0.001
CyBorD	20 (51.2%)	13 (52%)	
CyBorMe	19 (48.7%)	2 (8%)	
CyBord plus clinical trial drug	0 (0%)	9 (36%)	
Other	0	1 (4%)	
Median number of cycles of the BCR part	4	6	0.6
Hematological Response			
Overall Response Rate	35 (89.7%)	21 (84%)	0.4
VGPR/CR	19 (49%)	15 (60%)	0.3
Complete Response	10 (25.6%)	5 (20%)	0.3
dFLC at 1-month (median)	69	50.5	0.5
Time to first response (median)	4 weeks	4 weeks	0.4
Time to Best response (median)	12 weeks	8 weeks	0.2
Organ Response			
Overall Organ Response	16 (41%)	12 (48%)	0.5
Cardiac Response	15/39 (38.4%)	14/25 (56%)	0.1
Renal Response	9/26 (34.6%)	9/19 (47%)	0.3

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Effect of the presence of t(11;14) for patients with AL amyloidosis treated with Bortezomib-containing regimens: Experiences from the Amyloidosis Program of Calgary

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Background: Systemic immunoglobulin light chain (AL) amyloidosis is a protein misfolding disease caused by the conversion of immunoglobulin light chains from their soluble functional states into highly organized amyloid fibrillary aggregates, leading to organ dysfunction.[1] The proteasome inhibitor, Bortezomib, has become a backbone in the first-line treatment of patients with AL amyloidosis who are not eligible for high-dose Melphalan and stem cell transplantation. The presence of t(11;14), seen in up to 40–60% of patients with AL amyloidosis, may be associated with poorer response when treated with Bortezomib based regimens.[2]

Objective: The main objective of this study was to explore the role of t(11;14) in clinical outcomes for patients with AL amyloidosis treated with upfront Bortezomib-containing regimens (BCR) at the Amyloidosis Program of Calgary (APC) from 01/2017 to 03/2022.

Material & Methods: All consecutive newly diagnosed AL amyloidosis patients treated with BCR at the APC from 01/2017 to 03/2022 were evaluated. We choose the year 2017 as a cut off for assessment as testing for t(11;14) via FISH cytogenetics was implemented in 2017 at our center, in addition to t(14;16), t(4;14) and TP53 deletion. The diagnosis of AL amyloidosis and assessment of hematological and organ response was performed based on the consensus criteria published in 2005 and modified in 2012.[3,4] Hematological and organ response was assessed as per standard guidelines. Patients with at least 1 cycle of treatment were included.

Results: Fourty-seven consecutive patients with AL amyloidosis treated with BCR at the APC from 01/2017 to 03/22 were identified. Clinical characteristics are seen in **Table 1**. Twenty-two cases were positive for t(11;14) and 25 were not. Median age at diagnosis was 67 and 64 years, respectively (p=0.3). Patients with t(11;14) translocation were compared to patients without t(11;14) translocation using the Mayo Clinic staging criteria (2012).[3] Within the t(11;14) cohort, 4 (18%) were stage I; 2 (9%) were stage II; 4 (18%) were stage III; and 11 (50%) were stage IV. In the No t(11;14) group, 3 (12%) were stage I; 4 (16%) were stage II; 7 (28%) were stage III; and 9 (30%) were stage 4 (p=0.06). A trend towards higher monoclonal plasma cells was noted in the No t(11;14) group (12% vs 10%; p=0.03). The rest of clinical characteristics were similar among the two groups. Patients with t(11;14) exhibited a trend towards lower Overall Response Rate (ORR) compared to the No t(11;14) group (81% vs 92%; p=0.2). Further, deeper responses were noted for the No t(11;14) group, with a rate of Very Good Partial Response (VGPR) or better in 76% vs 40.9% (p=0.01), and a Complete Response (CR) rate of 40% vs 9% (p=0.04). No changes in median Overall Survival (OS) was noted among the two groups; however, a trend towards shorter Progression-Free Survival (PFS) was described in the t(11;14) group (estimated of 34 vs 54 months) (p=0.06).

Summary & Conclusion: In conclusion, patients with AL amyloidosis and the presence of t(11;14) have inferior clinical outcomes with respect to hematologic and organ responses, when treated with BCR as first-line therapy. Longer follow-up is required to assess the impact of t(11;14) over OS and PFS.

Table 1. Clinical Characteristics of patients with AL amyloidosis receiving BCR according to presence of t(11;14)

Characteristic	t(11;14) AL , N=22	No (t11;14) AL, N=25	P value
Age (median)	67	64	0.3
Gender Male Female	 13 (59%) 9 (41%)	 12 (48%) 13 (52%)	0.4
Hb (g/L)	124	124.5	0.2
Creatinine (µmol/L)	97	77.5	0.6
B2microglobulin (µmol/L)	2.46	2.9	0.4
Albumin (g/L)	27	29	0.6
Stage I Stage II Stage III Stage IV Unknown	4 (18%) 2 (9%) 4 (18%) 11 (50%) 1 (4.5%)	3 (12%) 4 (16%) 7 (28%) 9 (36%) 2 (8%)	0.6
LDH (IU/L)	226	231	0.5
BMPC (%)	10	12	0.03
NTproBNP ng/L	2288	1984	0.4
Light chain: Kappa Lambda Biclonal	 6 (27%) 16 (72%) 0	 7 (28%) 17 (68%) 1 (4%)	0.6
Organ Involvement Cardiac involvement Kidney involvement Liver involvement Nerve involvement GI involvement Lung involvement	 18 (81%) 17 (72%) 2 (9%) 2 (9%) 4 (18%) 1 (4.5%)	 20 (80%) 18 (72%) 2 (8%) 4 (16%) 3 (12%) 1 (4%)	 0.5 0.7 0.8 0.4 0.5 0.9

Table 2. Response rates for patients with AL amyloidosis receiving BCR at the Amyloidosis Program of Calgary according to the presence of t(11;14)

Characteristic	t(11;14) AL , N=22	No (t11;14) AL, N=25	P value
Bortezomib-Containing Regimens			0.2
CyBorD	4 (18%)	7 (28%)	
CyBorMe	13 (59%)	15 (60%)	
CyBord plus clinical trial drug	5 (22%)	3 (12%)	
Hematological Response			
Overall Response Rate	81%	92%	0.2
VGPR/CR	9 (40.9%)	19 (76%)	0.01
Complete Response	2 (9%)	10 (40%)	0.03
VGPR	7 (31.8%)	9 (36%)	0.7
PR	9 (40.9%)	4 (16%)	0.04
No response	4 (18%)	2 (8%)	0.04
Organ Response			
Overall Organ Response	6 (27%)	14 (56%)	0.04
Cardiac Response	5/18 (27%)	12/20 (60%)	0.03
Renal Response	7/17 (41%)	9/18 (50%)	0.6

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Cyclophosphamide, Bortezomib and Methylprednisolone (CyBorMe) for the treatment of AL amyloidosis: Updated report from the Amyloid Program of Calgary (APC)

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Background: Cyclophosphamide, Bortezomib, and Dexamethasone (CyBorD) is an effective and well-tolerated treatment regimen for patients with light chain (AL) amyloidosis. [1] CyBorD produces rapid and deep hematological responses (HR); however, it remains inadequate to enhance outcomes in patients with advanced cardiac disease.[2] Recently, we reported on the use of Methylprednisolone combined with Cyclophophamide, and Bortezomib (CyBorMe) for the treatment of AL amyloidosis instead of Dexamethasone. Methylprednisolone has a shorter half-life and more rapid onset when compared to Dexamethasone, [3] highlighting its potential to minimize steroid related toxicity.

Objective: The primary objective of the study was to assess degree of hematological, organ, rapidity of response, and tolerability of CyBorMe treatment and compare with a historic cohort of cases treated with CyBorD at our institution.

Material & Methods: All consecutive newly diagnosed AL amyloidosis patients treated with CyBorD and CyBorMe from 01/2012 to 03/2022 were evaluated. The diagnosis of AL amyloidosis and assessment of hematological and organ response was performed based on the consensus criteria published in 2005 and modified in 2012.[4,5] CyBorD was given as previously reported,[1] and CyBorMe used Methylprednisolone at 500 mg IV q7 days for 3-4 weeks instead of Dexamethasone. Approval for CyBorMe was attained at our center in June of 2019, after which, the majority of patients diagnosed were treated with CyBorMe and comparison was made to a historic cohort of CyBorD treated patients. Patients with at least 1 cycle of treatment were included**.**

Results: Fifty patients were treated with CyBorD and 33 with CyBorMe. After a median of 4.5 cycles of CyBorD and 3 cycles of CyBorMe, HR was seen in 88% and 94% of cases, including CR in 30% and 36%, VGPR in 32% and 33%, and PR in 30% and 24% for CyBorD and CyBorMe, respectively (p=0.18). Time to first response was faster with CyBorMe compared to CyBorD (4 vs 6 weeks; p=0.003). Similar proportions of advanced cardiac stages (stage III and IV) were see between groups and cardiac response was observed in 50% and 36% of patients treated with CyBorMe and CyBorD, respectively (p=0.3). Discontinuation due to intolerance was higher in the CyBorD group at 26% compared to the CyBorMe group at 9% (p=0.04).

Summary & Conclusion: CyBorMe appeared to be efficacious and well-tolerated in patients with AL amyloidosis. Prospective studies using CyBorMe in patients with stage III/IV AL amyloid are warranted aiming to minimize toxicity.

Table 1. Clinical Characteristics of patients with AL amyloidosis treated with CyBorMe and CyBorD at Tom Baker Cancer Centre

Characteristic	N=50 (CyBorD)	N=33 (CyBorMe)	P value
Age (median)	64	69	0.17
Gender			
Male	30 (60%)	18 (60%)	0.6
Female	20 (40%)	15 (40%)	
Hb (g/L)	127	119	0.6
Creatinine (µmol/L)	92	127	0.2
B2microglobulin (µmol/L)	2.91	2.86	0.4
Albumin (g/L)	29	29	0.9
Stage I	8 (16%)	10 (30%)	0.6
Stage II	10 (20%)	7 (21%)	
Stage III	11 (22%)	7 (21%)	
Stage IV	16 (32%)	9 (27%)	
Unknown	5 (10%)	0	
LDH (IU/L)	205	218	0.2
BMPC (%)	7%	12%	0.1
NTproBNP ng/L	1810	1048	0.4
Light chain:			0.10
Kappa	10 (20%)	13(39%)	
Lambda	39 (78%)	19 (57%)	
Organ Involvement			
Cardiac involvement	35 (70%)	21 (63%)	0.5
Kidney involvement	36 (72%)	24 (72%)	0.9
Liver involvement	5 (10%)	3 (9)%	0.8
Nerve involvement	8 (16%)	4 (12%)	0.6
GI involvement	9 (18%)	3 (9%)	0.2
Lung involvement	2 (4%)	0%	0.2

Table 2. Hematological and organ response for patients with AL amyloidosis treated with CyBorD and CyBorMe at the Tom Baker Cancer Centre

Characteristic	CyBorD group	CyBorMe group	P value
Overall Response Rate (ORR)	88%	94%	0.18
Complete Response (CR)	28.5%	36%	
Very Good Partial Response (VGPR)	33.3%	36%	
Partial Response (PR)	30.9%	27%	
Time to first response (weeks, median)	6	4	0.003
dFLC at 1 month (median)	44	33	0.3
Time to Best Response (weeks)	12 weeks	6weeks	0.001
Median number of cycles	4.5	3	0.9
Discontinuation	26%	9%	0.04
Organ Response	44%	48%	0.6
Cardiac Response	36% (11/30)	50% (9/18)	0.3

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Safety and Efficacy of Propylene Glycol-Free Melphalan in Patients with AL Amyloidosis Undergoing Autologous Stem Cell Transplantation: Results of a phase II study

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Background: High dose melphalan and autologous stem cell transplantation (HDM/SCT) is an effective treatment for appropriately selected patients with light-chain (AL) amyloidosis. Despite rigorous patient selection, up to 29% of patients with AL amyloidosis who receive HDM/SCT develop acute renal injury[1,2] and 13% develop cardiac arrhythmias in the peri-transplant period[3]. This toxicity is potentially exacerbated by the co-solvent propylene glycol (PG) found in melphalan hydrochloride. This clinical trial investigated the safety and efficacy of PG-free melphalan during HDM/SCT in AL amyloidosis (NCT02994784).

Objective: Primary objectives were evaluation for renal dysfunction (creatinine increase ≥1 mg/dL or creatinine doubling to ≥1.5 mg/dL for ≥2 days), new cardiac arrhythmias, and autonomic dysfunction manifested as hypotension (drop in systolic blood pressure ≥20 mmHg from baseline). Secondary objectives included time to neutrophil and platelet engraftment, treatment related mortality, overall hematologic response, organ response, and number of peri-transplant hospitalizations.

Materials & Methods: Enrollment began in April 2018. Eligibility criteria included: histologic diagnosis of systemic AL amyloidosis, involvement of ≥1 vital organ, eGFR ≥30 mL/min/m^2, DLCO ≥50%, LVEF ≥40%, ECOG <3, NYHA class <3, and no prior HDM/SCT. Patients underwent SCT per institutional guidelines, receiving the conditioning regimen of either 140 or 200 mg/m^2 IV PG-free melphalan on days -3 and -2 in 2 equally divided doses. Stem cell infusion occurred on day 0.

Results: 28 patients enrolled and 27 underwent HDM/SCT. Baseline characteristics are shown in Table 1. Patients received either 140 mg/m^2 (n = 3) or 200 mg/m^2 (n = 24) PG-free melphalan. Median time to neutrophil engraftment was 10 days (range, 8-14), and median time to platelet engraftment was 17 days (range, 14-27). Peri-transplant hospitalization occurred in 23 patients (85%). The most common causes for hospitalization were diarrhea (30%) and febrile neutropenia (44%). The most common non-hematologic adverse events included nausea (74%), fatigue (82%), and diarrhea (93%). Treatment-related mortality on day 100 was 0. Two patients (7%) developed renal dysfunction, 4 (15%) experienced new cardiac arrhythmias, and 3 (11%) developed orthostatic hypotension. Of the 27 patients with 6-month follow-up, the hematologic responses were: 12 CR (44%), 6 VGPR (22%), 4 PR (15%), 2 stable disease (7%), and 3 patients (11%) had started salvage therapy due to inadequate hematologic response. Of the 12 patients with cardiac involvement, 11 had evaluable disease[4], of which 4 (36%) had response at 6 months. Of the 23 patients with renal involvement, 12 (52%) patients had response at 6 months. At a median follow up of 18 months, the hematologic event-free survival was not reached.

Summary & Conclusion: PG-free melphalan had comparable rates of cardiac toxicity, and lower rates of renal toxicity compared to historical data for patients treated with melphalan hydrochloride. All patients achieved neutrophil and platelet engraftment, and there were no treatment-related deaths. At 6 months following HDM/SCT, the overall hematologic response rate (CR + VGPR + PR) was 81%, and 58% of patients achieved an organ response. These results suggest that PG-free melphalan is safe and efficacious as a high-dose conditioning regimen for SCT in patients with AL amyloidosis.

Table 1: Baseline patient characteristics

	Total patients (n = 27)
Males, n (%)	18 (67%)
Median age, years (range)	60 (44-76)
Median organ systems involved (range)	2 (1-5)
Median NT-proBNP, pg/mL (range)	482 (0-6220)
Median albuminuria, g/24 hours (range)	3.3 (0-22.9)
Median serum creatinine, mg/L (range)	0.89 (0.59-2.07)
Median dFLC, mg/L (range)	46.4 (2.8-700)

dFLC, difference in involved to uninvolved free light chain

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Treatment outcomes according to salvage chemotherapy modalities for relapsed/refractory AL amyloidosis

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Background: Systemic AL amyloidosis is an affliction featured by the recurrent accumulation of misfolded and aggregated light chains in vital organs[1]. The standard of treatment has been fundamental with bortezomib-based induction chemotherapy followed by autologous stem cell transplantation (ASCT) [2, 3]. However, it remains challenging to develop salvage therapeutic modalities due to the rarity, even though the survival outcomes of relapsed/refractory(RR) AL amyloidosis have been miserable [4, 5].

Objective: Thus, this study was conducted to evaluate the optimal salvage treatments for RR AL amyloidosis according to a comparison of subsequent treatment outcomes.

Methods: We collected medical records of 61 AL amyloidosis patients who received lenalidomide, pomalidomide, and daratumumab monotherapy as salvage treatments. Consensus guidelines demonstrated the organ and the hematologic response regarding chemotherapy. Moreover, survival outcomes and safety issues were analyzed. This study was approved by the Institutional Review Board of Samsung Medical Center (IRB 2012-08-059).

Results: The median age was 63 years (range 43-84 years), and 40 (65.6%) patients were younger than 65 years. Forty-six patients (75.4%) presented cardiac amyloidosis, and 30 patients (49.2%) had kidney amyloidosis. Forty-one patients (67.3%) showed AL amyloidosis with a bone marrow plasma cell (PC) burden of ≥ 10%. Among 51 patients available to estimate the Mayo 2012 criteria, 34 patients (66.7%) were classified as stage VI. Among 58 patients who could assess renal stage, only three presented stage III. As the front line option, 78.7% of patients (n=48) received bortezomib-based chemotherapy, and 15 patients received ASCT.
As the salvage therapy, 61 patients (100%) received lenalidomide, 13 patients (21.3%) received pomalidomide, and 12 patients (19.6%) received daratumumab. The median number of previous chemotherapies was 1(1-5) in lenalidomide, 2 (2-4) in pomalidomide, and 3 (3-5) in daratumumab. The heart response rate was 2.4% in lenalidomide, 0.0% in pomalidomide, and 40.0% in daratumumab. The hematologic response was estimated at 63.6% in lenalidomide, 41.7% in pomalidomide and 63.6% in daratumumab. The median follow-up duration was 55.2 months (95% CI 40.3-70.2). Moreover, the median progression-free survival (PFS) was 13.4 months (95% CI 8.0-18.9) in lenalidomide, 6.3 months (95% CI 2.8-9.8) in pomalidomide, and not reached in daratumumab. The median overall survival (OS) of all patients was not reached in the median value. In terms of safety, the patients who received pomalidomide showed somewhat frequently hematologic toxicities compared to others.

Conclusion: AL amyloidosis patients resistant to the first-line chemotherapy has no clear

subsequent treatment options due to combined poor general condition, organ dysfunction, and comorbidities. In this study, we reported the salvage treatment outcomes of 61 patients who were treated with different salvage chemotherapies. However, it is not available to evaluate these chemotherapies equally because therapeutic efficacy was compared under different situations. Although over 50% of patients combined with known poor prognostic factors, such as over 10% of bone marrow PC burden and advanced MAYO 2012 stage, daratumumab, and lenalidomide showed a reasonable organ and hematologic response rates with manageable toxicities. Thus, lenalidomide and daratumumab showed slightly better effective treatment results, but further research is needed to standardize the salvage treatment for RR AL amyloidosis patients.

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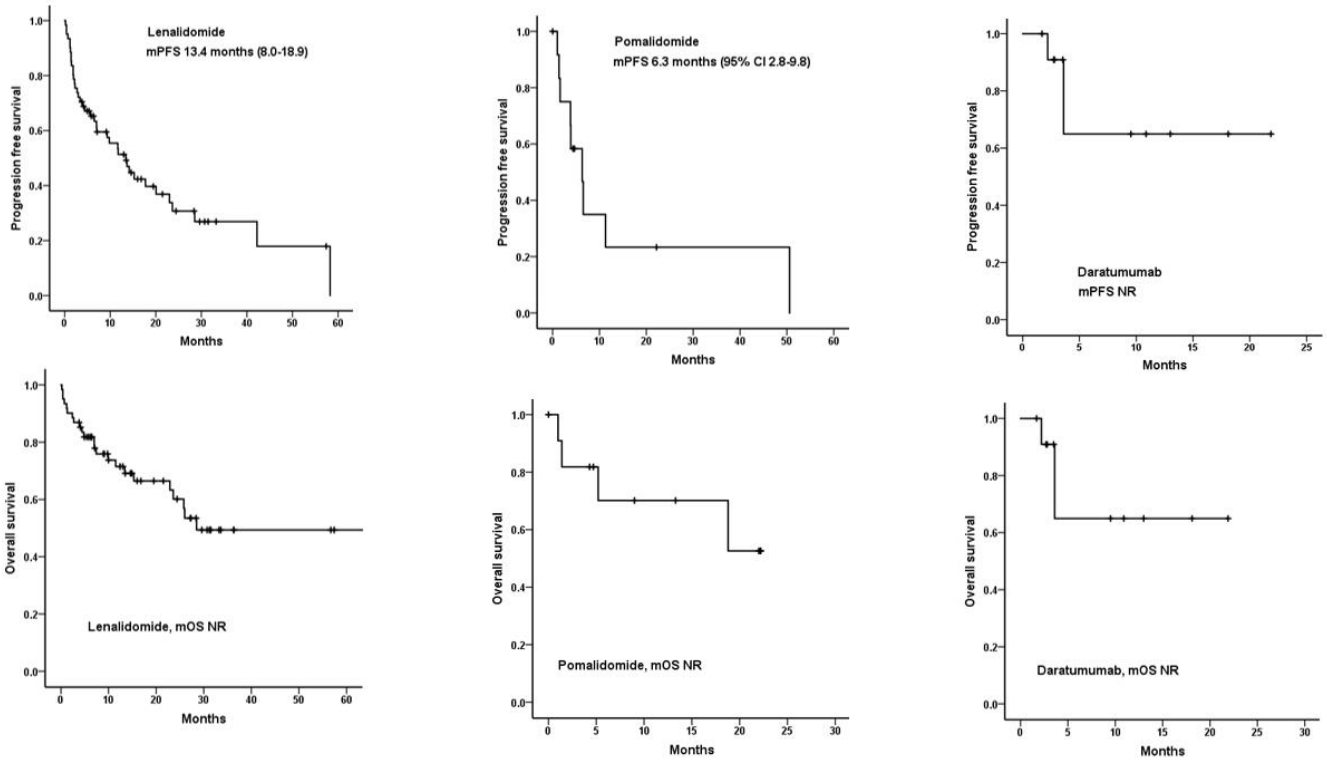
Figure. PFS, and OS according to chemotherapies



Autologous stem cell transplantation in primary amyloidosis: a single centre experience

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Background: Treatment approach to AL amyloidosis is similar to MM. Average survival rate of AL patients without treatment is known to be between 10-14 months. Autologous stem cell transplantation (ASCT) is an effective consolidation therapy. However, these patients are generally diagnosed in advanced stages where intense therapy options can no longer be applicable. Moreover, some entities like capillary leak syndrome and bleeding diathesis may complicate ASCT during mobilisation, myelosuppression, or engraftment.

Objective: Experience and information on ASCT in AL amyloidosis is rather scarce and limited. In our centre,in recent years, increased awareness of AL amyloidosis has led to early diagnosis and increased use of ASCT for AL amyloidosis patients. We aimed to share our experience of usage of ASCT for AL amyloidosis.

Material & Methods: Relevant data of AL patients between 2014-2021 were retrospectively analysed using archive files and digital hospital sources. Involvement sites at diagnosis stage , light chain levels, treatments, response, mobilisation and conditioning regimens, supportive therapies, progression - survival and morbidity/mortality outcomes were enlisted and descriptive statistics analyses were conducted.

Results: Demographic characteristics of 14 patients are summarised in Table 1. Mean interval between the diagnosis and transplantation was 14 months (9-48). Soft tissue, cardiac and renal involvement were the most common presentation. 6 of 14 patients (42.8%) met the criteria for MM. According to R-Mayo criteria, 8 patients were at Stage 2. Mean Pro-BNP before transplantation was 449,5 pg/ml. G-CSF and dexamethasone were used for mobilisation. In all patients mobilisation was successful and there were none with grade 3/4/5 adverse events. Melphalan was used as conditioning. In three patients with renal amyloidosis, advanced heart failure and multiple organ involvement,respectively, melphalan dose was reduced.During engraftment three patients needed steroids and two needed diuretics. One patient died due to sepsis duringASCT (Table 2). Routine administration of G-CSF was avoided, two patients with febrile neutropenia were given G-CSF. Mean follow-up time after ASCT was 20,5 months. No CMV reactivation was observed. Mean Pro-BNP post-transplant was 330 pg/ml. Three patients needed therapy due to progression.

Summary & Conclusion: At a median of 20.5 (5-81 months) months follow-up after ASCT, median overall and progression free survival rates were 92.8% and 71.4%, respectively, which favored ASCT as a safe, effective treatment modality providing long-time disease-free state. Patients fullfilling MM criteria (43%) were more common than in the previous reports (8-20%). This may be attributed to the slow and sneaky course of isolated AL, causing late diagnosis of the disease and after major and severe organ involvement making ASCT less feasible ,whereas patients with MM like disease are recognized and treated earlier, enabling ASCT consolidation. 4 patients in our study who died or had an early progression after ASCT, had increased plasma cells at diagnosis, supporting previous literature stating increased bone marrow plasma cells as a bad prognostic factor. Increased occurrence of capillary leak syndrome during mobilisation and engraftment was not observed in this cohort; which can be explained by the low patient counts and/or with the increased awareness and early intervention and also with the us dexamethasone prophylaxis during mobilisation and routine G-CSF avoidance after ASCT.

Table 1:

Male/female	6/8	**Transplant Preparation Regime**	
Mean age*	55,14	140 mg/m2 i.v. melfalan	5/14
Treatment		200 mg/m2 i.v. melfalan	9/14
VCD	12/14	**Hematological response (pre-ASCT)**	
VAD	2/14	CR (complete remission)	5/14
Other	2/14	VGPR (very good partial remission)	4/14

Organ Involvement		PR (partial remission)	4/14
Soft tissue	7/14	**Serum**	
Kidney	7/14	Light chain Kappa/Lambda	6/8
Heart	7/14	IgG	5/14
Neurological	5/14	IgA	2/14
Gastrointestinal	3/14	**Engraftment time***	
Bone marrow	4/14	Neutophil	13 gün
Liver	4/14	Thrombocyte	9 gün
Plasma cells >%10	8/14	**Mobilisation Regime**	
Revised Mayo Stage		G-CSF	13/14
Evre 1	0/14	G-CSF + PLERİKSAFOR	1/14
Evre 2	8/14	**Total follow up**	51 ay
Evre 3	3/14	**Follow-up time after ASCT**	20,5 ay
Evre 4	3/14	**Hematological response (3rd month)**	
Mayo Stage		CR (complete remission)	6/14
Evre 1	2/14	VGPR (very good partial remission)	4/14
Evre 2	7/14	PR (partial remission)	3/14
Evre 3	5/14	**Progression**	3/14
Stem Cell Count (10^6/kg CD34+)*	3,6	**Progression time after ASCT**	10,3 ay

Table 2:

Patient	Soft tissue	Kidney	Heart	Liver	Neurological	Gastro-intestinal	Bone Marrow	Transplant Date	Follow-up
1	+	-	-	-	-	-	-	03/2019	Alive, no progression
2	-	+	-	-	+	+	-	02/2019	Alive, need dialysis
3	+	-	+	-	-	-	-	03/2015	Sağ, relapse at 16th month
4	-	+	-	-	-	-	-	04/2014	Dead, 35th day
5	+	-	+	-	-	-	+	02/2018	Alive, relapse at 6th month
6	+	+	-	+	-	-	-	11/2016	Alive, responsive
7	-	+	-	+	-	-	+	03/2015	Sağ, 9. ayda nüks
8	-	-	+	-	-	-	+	02/2020	Alive, increased EF, diminished need of duretics
9	+	+	+	-	+	-	-	06/2020	Alive, no progression
10	-	-	+	-	-	+	+	06/2020	Alive, no progression
11	-	+	-	-	+	-	-	11/2020	Alive, no progression
12	+	+	+	+	+	-	+	01/2021	Alive, no progression
13	-	-	+	-	+	-	-	05/2021	Alive, increased EF, diminished need of diuretics
14	+	-	-	-	-	-	-	08/2021	Alive, no progression

Table 1. Patient Demographics and characteristics

Table 2: Organ Involvement Before ASCT and follow up results

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Support & Funding: none

Bortezomib-based induction therapy is associated with superior hematologic responses and survival after stem cell transplantation in patients with AL amyloidosis

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Background: The question remains as to whether bortezomib-based induction therapy prior to high-dose melphalan and autologous stem cell transplantation (HDM/SCT) has a role in the treatment of systemic light chain (AL) amyloidosis. Early single-arm studies demonstrated a high risk of patient attrition (14–30%) with the use of induction therapy before HDM/SCT, due to organ deterioration or complications during the induction phase. However, in a recent trandomized trial evaluating HDM/SCT with or without bortezomib and dexametheasone (BD) induction in patients with renal involvement, all patients successfully proceeded to transplant, and superior hematologic responses and survival were shown with induction.[1]

Objective: To evaluate the impact of bortezomib-based induction therapy on treatment outcomes following HDM/SCT in patients with systemic AL amyloidosis.

Material & Methods: We collected data from a prospectively maintained database of patients with AL amyloidosis consecutively treated with HDM/SCT at our institution between January 2008 and September 2021. Bortezomib-based induction therapy for 2–4 cycles was considered for patients who had a bone marrow plasma cell percentage (BMPC%) >10%, or at the discretion of the treating physician. Hematologic responses were defined according to consensus guidelines.[2] Event-free survival (EFS) was defined as time between day 0 and initiation of next line of therapy or death, whichever occurred first. Overall survival (OS) was defined as the time between day 0 and death from any cause or censored at last follow-up. Univariable and multivariable logistic and hazard regression models were fitted for outcome measures. All calculations were performed with R software.

Results: Between 2008 and 2021, 277 patients with AL amyloidosis underwent HDM/SCT. Seventy-two patients (32%) received bortezomib-based induction therapy. Compared to patients treated with HDM/SCT alone, patients who received bortezomib-based induction had a signficiantly higher hematologic complete response (CR) rate (54% vs. 35%; OR 2.17, 95% CI 1.18-4.03; p=0.009); and longer EFS (6.5 vs. 2.7 years; HR 0.56, 95% CI 0.38-0.821; p=0.002; **Figure 1A**) and OS (not reached vs. 11.9 years; HR 0.51, 95% CI 0.29-0.91; p=0.019; **Figure 1B**). The estimated 10-year OS for patients with and without bortezomib induction was 68% and 52%, respectively. In a multivariate analysis, hematologic CR, EFS, and OS were significantly higher in patients treated with bortezomib-based induction after adjusting for age, sex, lambda light chain isotype, prior treatment, BMPC%, difference between involved and uninvolved free light chains (dFLC), serum creatinine, proteinuria, alkaline phosphatase, B-type natriuretic peptide (BNP), troponin I, and melphalan dose ($p<0.05$ for all comparisons). The clinical benefit of bortezomib-based induction therapy on hematologic CR, EFS, and OS also remained present when we focused our analysis only to the subset of patients with a BMPC% >10% ($p<0.05$ for all comparisons; **Figures 1C-D**).

Summary & Conclusion: This retrospective analysis demonstrates that bortezomib-based induction therapy prior to HDM/SCT is associated with superior rates of hematologic CR, EFS, and OS in patients with AL amyloidosis. Moreover, these findings support the current expert consensus guidelines that recommend the consideration of bortezomib-based induction therapy for patients presenting with a BMPC% >10%.[3] Limitiations of this study include its lack of intent-to-treat or randomized design.

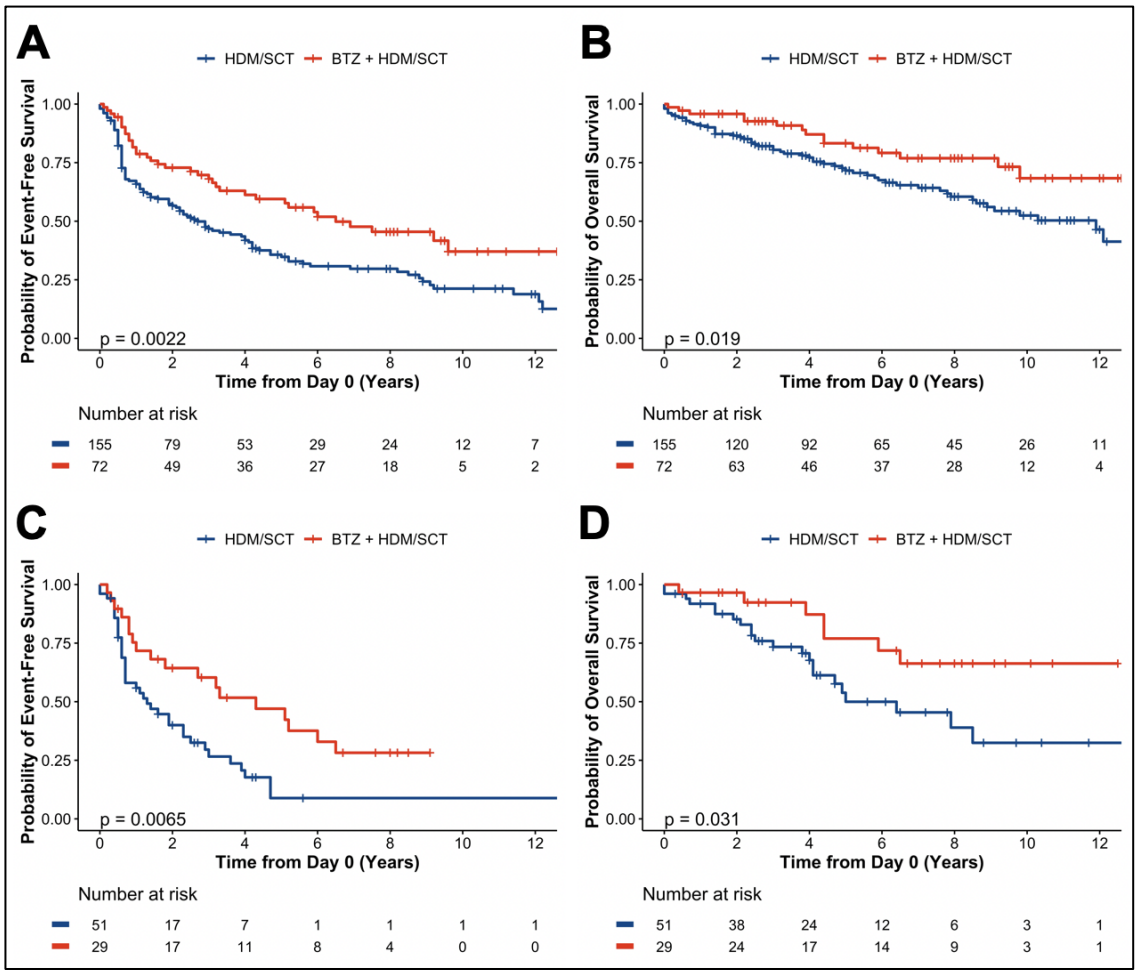

Figure 1. Impact of bortezomib-based induction therapy on survival outcomes. Kaplan-Meier curves for event-free survival (EFS) and overall survival (OS) following HDM/SCT stratified by receipt of bortezomib (BTZ)-based induction therapy for all patients (A-B) and among patients with a BMPC% >10% (C-D), respectively.

References

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Venetoclax in Relapsed or Refractory AL Amyloidosis with t(11;14) and BCL2 overexpression.

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Background: The oral BCL2 inhibitor venetoclax demonstrates activity in patients with multiple myeloma, preferentially in plasmacell clones harbouring t(11;14). Therefore, targeted therapy with venetoclax is a promising treatment option for patients with systemic light chain (AL) amyloidosis, where t(11;14) is a common finding. Data from prospective clinical trials with BCL2 inhibitors are lacking in AL amyloidosis.

Objective: To report on the feasibility, safety and efficacy of venetoclax in relapsed/refractory AL amyloidosis with t(11:14) and BCL2 overexpression**.**

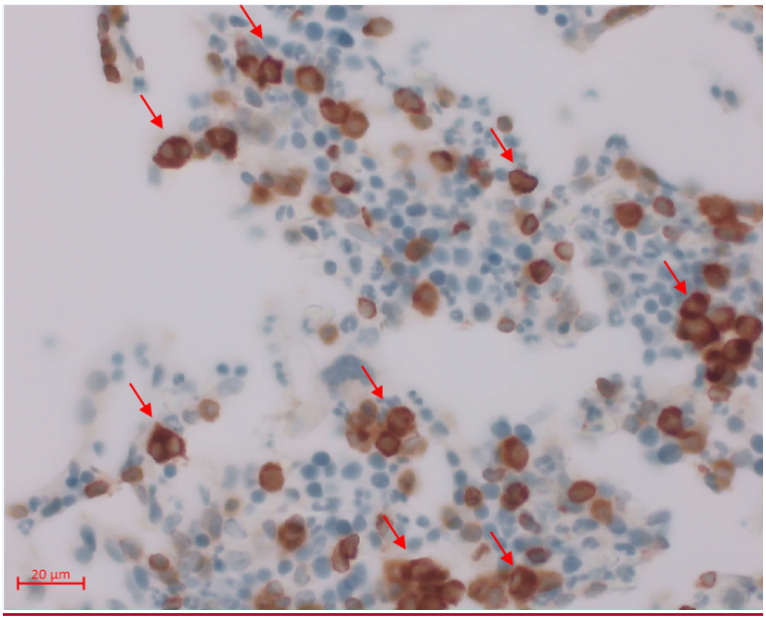
Material & Methods: Members of the Swiss Amyloidosis Network were contacted in order to identify relapsed/refractory AL amyloidosis patients treated with venetoclax. We performed a retrospective national multicenter study (data cut-off on March 31st 2022). Immunohistochemistry and iFISH analysis were performed locally.

Results: We identified 9 patients in 3 centers fulfilling the inclusion criteria.The median age at diagnosis was 66.6 years, 5/9 patients were female. Cardiac involvement was present in 8/9 patients, and 5/9 patients had renal involvement. According to the revised Mayo risk model, 4/9 patients had stage IV disease, and 4/9 patients stage III disease, one patient had no troponin T measurement at baseline. Median bone marrow (BM) plasmacell infiltration was 10% (range 10%-20%). All samples displayed BCL2 overexpression in >50% of the plasmacells, and all patients had t(11;14) by iFISH with a median positivity of 39% (range 33%-59%) in CD38 selected cells. The difference between involved and non-involved free light-chains (dFLC) was 223 mg/L (range 88-678 mg/L), and the NT-proBNP levels 3625 pg/ml (range 652-10352 pg/ml). Patients had a median of two prior lines of therapy (range 1-4), including high-dose melphalan and autologous stem cell transplantation (2/9), daratumumab (9/9), bortezomib (6/9), and lenalidomide (5/9). Venetoclax was administered in combination with bortezomib and dexamethasone (4/9), as monotherapy (3/9), with dexamethasone (1/9), or with daratumumab (1/9). The final venetoclax dose was 400mg (7/9), 600mg (1/9) and 800mg (1/9). All patients had an initial ramp-up phase (mostly over three days), and received antiinfective prophylaxis with valacyclovir and trimethoprim/sulfomethoxazole. Intravenous immunoglobulins were regularly substituted in 5/9 patients. The first hematological response (HR), and the best HR were observed after 26 days (range 11-125), and after 106 days (range 35-281), respectively. The overall HR was 100% (6/9 CR, 2/9 VGPR and 1/9 PR), with a dFLC of ≤ 10 mg/L in 7/9 patients. Adherence to therapy was reduced in two patients (one VGPR, and one PR). A cardiac response was observed in 7/8 patients and a renal response in 3/5 patients. Venetoclax-related adverse events (AE) ≤ grade 2 occurred in 9/9 patients, mostly mild infectious complications, peripheral neuropathy, constipation, anemia and neutropenia. Grade 3 AE or higher occurred in 3/9 patients (anemia and neutropenia), no deaths were reported. No event of tumor lysis syndrome or acute renal failure was observed. Median therapy duration was 369 days. Treatment is ongoing in 7/9 patients.

Summary & Conclusion: Venetoclax is an effective and manageable treatment option for patients with relapsed/refractory AL amyloidosis with t(11;14) and BCL2 overexpression. Venetoclax leads to a deep hematologic response, and to improvement of organ function. Further studies with BCL2 inhibitors are

warranted in this patient population.

Figure 1. BCL2 expression in BM plasma cells (red arrows) by IHC.



Support & Funding: No funding source involved

Birtamimab in Patients with Mayo Stage IV AL Amyloidosis: Rationale for Confirmatory AFFIRM-AL Phase 3 Study

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Background: Amyloid light chain (AL) amyloidosis--a progressive disorder caused by misfolded light chains produced by plasma cells--is associated with high mortality, poor quality of life, and increased healthcare costs, particularly in newly diagnosed patients with advanced disease (Mayo 2012 Stage IV, median overall survival <6 months). Birtamimab is a monoclonal antibody designed to neutralize circulating soluble and deplete deposited insoluble amyloid, by promoting phagocytic clearance. In 2018, the Phase 3 VITAL study in newly diagnosed, treatment-naive patients was terminated based on a futility analysis of the primary endpoint (time to all-cause mortality [ACM] or time to cardiac hospitalization >90 days after first study drug infusion); the final hazard ratio (HR) numerically favored birtamimab + standard of care (SOC) over placebo + SOC (0.835 [95% CI: 0.5799, 1.2011]; p=0.330). Post hoc analysis of ACM over 9 months revealed a substantial survival benefit (HR=0.413 [95% CI: 0.191, 0.895]; p=0.025) in patients at high risk for early death (Mayo 2012 Stage IV), for which no approved treatments exist. Post hoc analyses of secondary endpoints in this subgroup indicated meaningful improvements in health-related quality of life (assessed with 36-Item Short Form Health Survey version 2; SF-36v2) and 6-minute walk test (6MWT) distance with birtamimab + SOC (p<0.05) at 9 months. In the overall study, the most commonly reported treatment-emergent adverse events (fatigue, nausea, peripheral edema, constipation and diarrhea) were similar in both treatment groups.

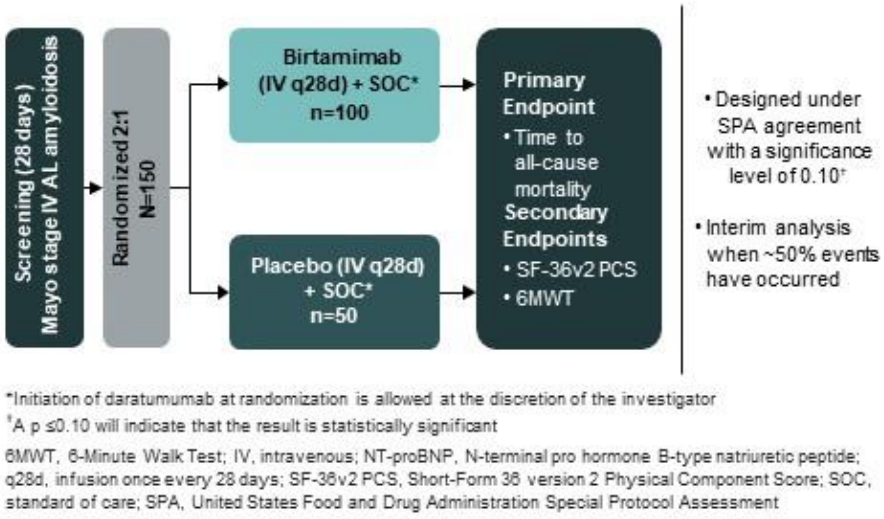
Objective: The Phase 3, double-blind, placebo-controlled AFFIRM-AL study (NCT04973137) will enroll up to 150 Mayo Stage IV patients with newly diagnosed, untreated AL amyloidosis and is designed to confirm the survival benefit observed in the VITAL study in patients with Mayo 2012 Stage IV AL amyloidosis.

Material & Methods: Patients will be randomized (2:1) to 24 mg/kg intravenous birtamimab or placebo every 28 days. Both arms will receive concomitant SOC chemotherapy with a first-line bortezomib-containing regimen; at the discretion of the investigator, initiation of daratumumab (D) at randomization is allowed (Figure 1). Patients will be stratified at randomization based on their 6MWT distance (<300 vs ≥300 meters) and initiation of D. The primary efficacy endpoint is time to ACM. Secondary endpoints are change from baseline to month 9 in the physical component summary of the SF-36v2 and 6MWT distance. Given the >50% reduction in the risk of ACM observed in the post hoc analysis of VITAL for patients with Mayo Stage IV disease, the AFFIRM-AL study is designed to confirm this effect of birtamimab, at a significance level of 0.10 under a Special Protocol Assessment (SPA) with the U.S. Food and Drug Administration (FDA).

Results: Approximately 130 global sites are planned; site initiation and patient randomization are ongoing.

Summary & Conclusion: Treatments that improve survival in AL amyloidosis are needed for patients with advanced cardiac involvement, as median overall survival for patients with Mayo 2012 Stage IV disease is <6 months. The AFFIRM-AL study is designed to confirm the >50% reduction in the risk of ACM observed in the VITAL study in patients with Mayo 2012 Stage IV AL amyloidosis.

Figure 1. AFFIRM-AL Global Study Design



*Initiation of daratumumab at randomization is allowed at the discretion of the investigator

†A p ≤0.10 will indicate that the result is statistically significant

6MWT, 6-Minute Walk Test; IV, intravenous; NT-proBNP, N-terminal pro hormone B-type natriuretic peptide; q28d, infusion once every 28 days; SF-36v2 PCS, Short-Form 36 version 2 Physical Component Score; SOC, standard of care; SPA, United States Food and Drug Administration Special Protocol Assessment

Support & Funding: This study is sponsored by Prothena Biosciences Ltd, Dublin, Ireland, a member of the Prothena Corporation plc group.

Congress: XVIII International Symposium on Amyloidosis; September 4–8, 2022; Heidelberg, Germany; https://www.isaheidelberg2022.org/

Submission deadline: April 30, 2022

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Abstract topics: Innovative drugs/new therapies – basic or clinical – all types

Assessing clinical outcomes in patients with AL amyloidosis across different criteria for hematologic complete response: Results from ANDROMEDA

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Background: In 2012, the International Society of Amyloidosis (ISA) defined hematologic complete response (hemCR) to treatment in AL amyloidosis as negative serum and urine immunofixation and

normal free light chain ratio (FLCr).[1] A recent clarification to the ISA criteria stated that "abnormal FLCr does not preclude the achievement of CR when the uninvolved (uFLC) concentration is greater than that of the iFLC."[2]The ANDROMEDA trial (NCT03201965), which evaluated the efficacy of daratumumab, bortezomib, cyclophosphamide, and dexamethasone (D-VCd) vs VCd alone in patients with AL amyloidosis, defined hemCR as negative serum and urine immunofixation, normal FLCr, normal free light chain (FLC) levels; normal uFLC level and normal FLCr were not required if the involved FLC level was < upper limit of normal. Confirmation at a subsequent visit during or after study treatment was also required.[3]

Objective: To demonstrate the consistency of results from ANDROMEDA using the ISA criteria and ANDROMEDA study criteria.

Materials & Methods: Patients were randomized to receive D-VCd or VCd. Hematologic (heme) response was assessed using the clarified ISA criteria and ANDROMEDA study criteria at 3 and 6 months (mo). The requirement for confirmation was removed for these analyses. Concordance of the two criteria was measured using Cohen's Kappa coefficient (κ) and its 95% CI. Landmark analysis of major organ deterioration progression-free survival (PFS) and major organ deterioration event-free survival (EFS) were presented by heme response at 3 and 6 mo using the Kaplan-Meier method. Hazard ratios (HR) and 95% CI were estimated using a Cox proportional hazard model with heme response as the sole explanatory variable. Results were compared using Harrell's concordance (C) statistic.

Results: Patients were randomized to D-VCd (n=195) or VCd (n=193). At a median follow-up of 11.4 mo, heme responses at 3 and 6 mo were similar between the two criteria (**Figure**). Rates of hemCR and very good partial response (VGPR) or better were higher with D-VCd than VCd at 3 and 6 mo, irrespective of which criteria were applied (**Figure**). The κ for heme response at 3 mo was 0.91 (95% CI 0.88–0.94) and at 6 mo was 0.89 (95% CI 0.86–0.93), indicating a strong concordance between the two criteria. Achieving a deep heme response was associated with prolonged major organ deterioration-PFS at 3 mo using the ISA criteria (<CR vs CR: HR, 2.82; 95% CI 1.08–7.36; p=0.0342 and C=0.58 and <VGPR vs ≥VGPR, HR, 4.49; 95% CI 2.16–9.33; p<0.0001 and C=0.68) and the ANDROMEDA study criteria (<CR vs CR: HR, 4.16; 95% CI 1.45–11.96; p=0.0082, C=0.60 and <VGPR vs ≥VGPR, HR, 5.13; 95% CI 2.42–10.88; p<0.0001 and C=0.68). Deep heme response was also associated with prolonged major organ deterioration-EFS at 3 mo with the ISA criteria (<CR vs CR: HR, 5.30; 95% CI 2.44–11.51; p<0.0001 and C=0.62 and <VGPR vs ≥VGPR, HR, 5.65; 95% CI 3.51–9.07; p<0.0001 and C=0.72) and the ANDROMEDA study criteria (<CR vs CR: HR, 8.66; 95% CI 3.50–21.42; p<0.0001 and C=0.64) and <VGPR vs ≥VGPR, HR, 6.64; 95% CI 4.08–10.81; p<0.0001 and C=0.73). Results were consistent at 6 mo. Harrell's C-statistics suggested consistency between criteria.

Summary and Conclusions: Heme response rates in ANDROMEDA were consistent between the established and clarified ISA criteria and ANDROMEDA study criteria, with higher rates of deep heme responses in patients treated with D-VCd than VCd. Regardless of the criteria used, deep heme response was a predictor for prolonged major organ deterioration-PFS and -EFS.

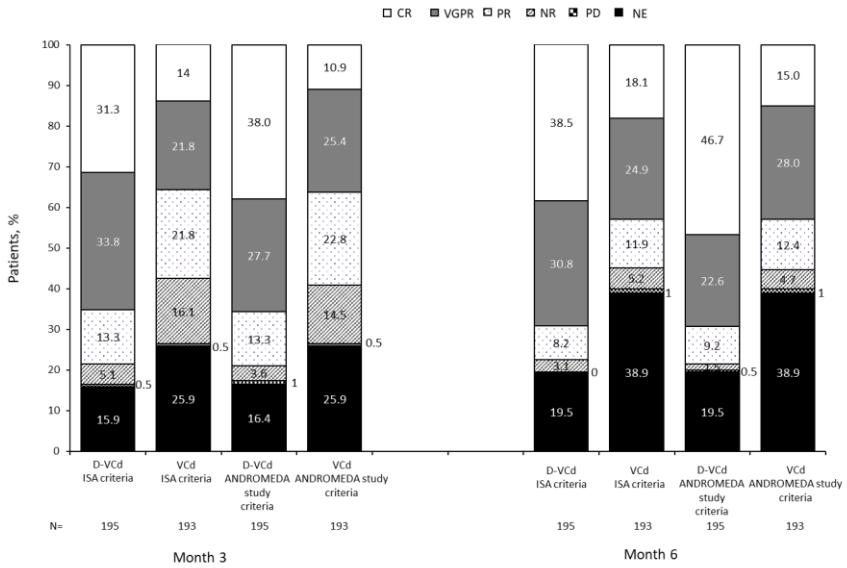

Figure. Hematologic response in the ITT population

CR, complete response; NE, not evaluable, NR, no response; PR, partial response; PD, progressive disease; VGPR, very good partial response

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Efficacy of bortezomib based regimens in elderly patients with newly diagnosed AL amyloidosis and heart failure. Grupo de Estudio Latino Americano de Mieloma Múltiple.

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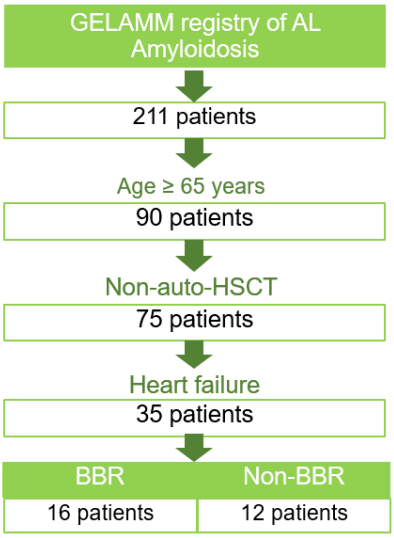
Background: Immunoglobulin light chain amyloidosis (AL amyloidosis) is a monoclonal gammopathy characterized by the systemic deposition of amyloid fibrils derived from the immunoglobulin light chains in peripheral tissues, affecting predominantly kidneys and heart [1]. The use of Bortezomib during induction therapy followed by consolidation with high dose melphalan and autologous hematopoietic stem cell transplantation (auto-HSCT) has become the standard therapy in fit younger patients fulfilling strict eligibility criteria [2]. The goal of the AL amyloidosis treatment is to eradicate the amyloidogenic clone, obtain organ function recovery, and in elderly patients the thorough control of adverse events related to therapy is essential [3]. In this sense, the use of triplet regimens with bortezomib backbone may appear to be efficacious and well tolerated [4].

Objective: Elderly patients represent a significant proportion of patients with AL amyloidosis. Considering that heart failure (HF) is a negative prognostic factor, we aimed to present the Latin American experience using bortezomib based regimenes (BBR) in patients with age ≥65 years with newly diagnosed AL amyloidosis (NDAL) and HF.

Material & Methods: Retrospective cohort study including patients aged ≥65 years with NDAL not receiving auto-HSCT as consolidation, and presenting HF at diagnosis,. The analysis was based on the Grupo de Estudio Latinoamericano de Mieloma Múltiple (GELAMM) registry, from January 2009 to December 2019 in centers from Uruguay, Chile, Argentina, Cuba, Colombia, México, and Paraguay. The primary outcomes evaluated included 7-year overall survival (OS), and overall response rate (ORR). Proportions were compared with Fisher exact test. Survival analysis was performed using the Kaplan-Meier method and Log-Rank test.

Results: thirty five patients with amyloid-related HF at diagnosis (Figure 1A) were included. Median age was 73 years (IQR 8.0), and 68.6% were males (24/35). The frequency of lambda chain AL amyloidosis was 75% (24/32). At diagnosis, nephrotic syndrome was registered in 53.1% (17/32), macroglossia in 50% (16/32), pupuric lesions in 65% (13/20), hepatomegaly in 38.2% (13/34), asthenia in 75% (15/20), weight loss in 47.1% (16/34), polyneuropathy in 46.9% (15/32), disauthonomy in 61.8% (21/34), gastrointestinal symptoms in 44.1% (15/34).
The majority of patients received BBR (57.1%, 16/28), with VCD being the most frequent regimen (15/16). Non-BBR included Thalidomide and Melphalan based protocols in 6/12 and 3/12, respectively. The median number of cycles in BBR vs Non BBR, were four for both groups. The ORR with BBR was 83.3% (10/12), and 33.3% with non-BBR (3/9)(p=0.03). BBR achieved very good partial response or better in 41.2% (5/12).
The 7-year OS for the whole cohort was 13.6% (95%CI, 5%-41%) (Figure 1B). In patients receiving BBR the 7-year OS was 23.4% (95%CI 8%-61.9%) wheras in non-BBR it was 14.3% (95%CI, 3%-76.4%)(Figure 1C). The median survival in the BBR group was 26 months, and 13 months in non-BBR (Log-Rank, p=0.46). Comparing NT-proBNP ≥8500ng/L with <8500ng/L, the 5-year OS were 26.7% (95%CI, 8.9%-80.3%) and 0%, respectively (Log-Rank, p=0.65).

Summary & Conclusion: In elderly patients with NDAL and HF, the use of BBR during induction tend to achieve a higher ORR, median survivall and survival rate at 7 years. However, the reduced number of patients included, and lack of data in some cases, does not allow us to establish stronger conclusions.



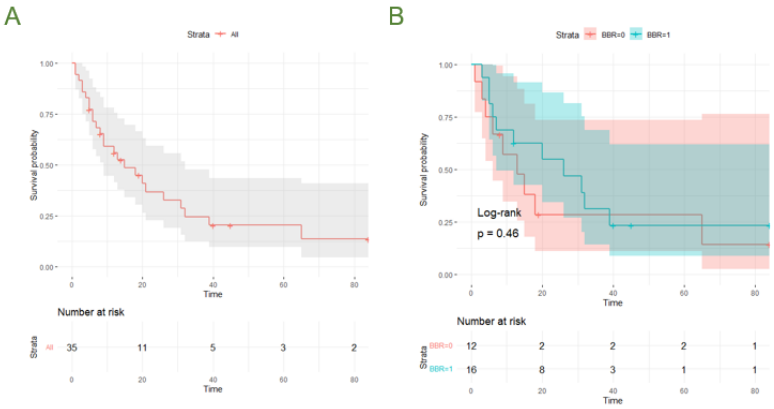


Figure 1.: Selection of patients included in the analysis.
Figure 2.: A. Kaplan-Meier curve of patients aged ≥65 years with newly diagnosed AL amyloidosis and heart failure not receiving auto-HSCT as consolidation therapy. B. Kaplan-Meier curve of patients aged ≥65 years with newly diagnosed AL amyloidosis and heart failure not receiving auto-HSCT as consolidation therapy, classified by the use of bortezomib based regimenes as induction therapy; BBR, bortezomib based regimenes (Receiving BBR=1, no receiving BBR=0).

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Time to next treatment (TtNT) is an independent prognostic marker for outcome in newly diagnosed patients with AL amyloidosis

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Background: Early and deep hematological responses are critical for the outcome of patients with AL amyloidosis and should be the target of initial therapy[1]. Delays to achieve this goal or target organ deterioration often trigger treatment modifications and thus time to next treatment line (TtNT) reflects a number of different parameters in the treatment decision algorithm, being an indication of either suboptimal response, disease (hematologic or organ) progression or excessive toxicity.
Objective: To assess the importance of TtNT as an independent prognostic factor for overall survival in newly-diagnosed patients with AL amyloidosis.
Material & Methods: We assessed TtNT in consecutive patients with AL amyloidosis, diagnosed in the Department of Clinical Therapeutics, Athens, Greece. We used the 6-month (TtNT≤6) and the 12-month (TtNT≤12) landmarks to identify patients who either started second line treatment (due to any cause) within 6 and 12 months respectively.
Results: The baseline clinical and treatment characteristics are shown in Table 1. At 6-months (n=292), 21% of patients had started 2nd-line treatment and 27% at 12-months (n=244). TtNT≤6 and TtNT≤12 were both associated with higher BMPC infiltration (p<0.001) and TtNT≤12 with higher dFLC (p=0.002). There was no association with Mayo or renal stage. Hematologic response rates in patients with TtNT≤6 vs TtNT>6 at 1-month were NR/PD in 55% vs 24%, PR in 28.4% vs 25% and VGPR in 16% vs 51% (p<0.001) and at 3-months 46% vs 13%, 24% vs 22% and 31% vs 65% respectively (p<0.001). Response rates at 1-month for patients with TtNT≤12 vsTtNT>12 were NR/PD in 55% vs 18%, PR in 31% vs 22% and VGPR in 15% vs 60% (p<0.001) and 42% vs 7%, 31% vs 16% and 27% vs 77% at 3-months (p<0.001). Inversely, median TtNT based on 3-month hematologic response was 14 months for PR and not-reached for ≥VGPR (p<0.001) and based on 6-month hematologic response 14 months for PR vs 107 months for ≥VGPR. There was no difference in renal response or PD rates at 3 months between those with and without TtNT≤6, but renal PD at 6 months was more frequent for TtNT≤6 (50% vs 29%, p=0.033). Patients with TtNT≤12 had more often renal PD at 6 months (46% vs 28%, p=0.027) although there was no difference in renal response rates. Both TtNT≤6 and ≤12 were associated with shorter time to dialysis (p<0.001). Patients with TtNT≤6 had lower cardiac response rates at 3-months (15% vs 32%, p=0.06) and at 6-months (19% vs 45%, p=0.028). Similarly, TtNT≤12 was associated with lower cardiac response rates at 3- (16.3% vs 37%, p=0.013), 6- (26% vs 50%, p=0.012) and 12-month (13.5% vs 40.4%, p=0.001) landmarks. Primary therapy was associated with TtNT≤6 (18% for Bortezomib-based, 15% for daratumumab-containing and 33% for other, p=0.014). TtNT ≤6 was 27% before 2010, 15% between 2010-2018 and 26% after 2018 (p=0.069), reflecting novel agent availability at first and second treatment lines and the evolution of treatment strategies requiring a more rapid and deeper hematologic response. Median OS was 60 vs 93 months for TtNT≤6 vs TtNT>6 (p=0.028) and 84 vs 112 months for TtNT≤12 vs TtNT>12 (p=0.559). TtNT≤6 (p=0.011, HR 1.8) remained an independent prognostic factor when adjusting for renal and Mayo stage, dFLC and BMPC infiltration.
Summary & Conclusion: A short time-to-next-treatment (<6 months), as a reflection of the complexity and multiparametric nature of treatment decision making in AL amyloidosis is an independent prognostic marker and potential surrogate for long term outcomes in patients with AL amyloidosis.

Clinical characteristics:	**TtNT ≤ 6 months n= 62**	**TtNT > 6 months N= 230**	**p-value**
Age (years, Median)	64	65	0.90
Male/Female (%)	52%/ 48%	56%/44%	0.57
dFLC (mg/L)	192	167	0.33
BM infiltration (median)	15%	20%	0.006
Number of involved organs 1 /2 /3/ 4	43% /38% /17%/2%	43% /39% /15% /3%	0.99
Performance status 0 / 1/ 2/ 3 /4	20% /47% /26% /5% /2%	26% /48% /19% /6% /1%	0.71
Organ involvement			
Renal	64%	70%	0.38
Heart	71%	67%	0.59
Liver	17%	22%	0.38
PNS/ANS	25%	19%	0.29
Mayo stage 1/2/3A/3B	20% /45% / 23% /12%	22% /49% /19% /10%	0.83
eGFR_MDRD (median)	86	74	0.25

Treatment type			
Bortezomib based	53%	67%	0.014
Daratumumab-containing	8%	12%	
Other	39%	21	

Table: Baseline and clinical treatment characteristics of patients based on Time-to-next-Treatment (TtNT) ≤ 6 months versus > 6 months

Figure: Kaplan Mayer curve for overall survival (OS) based on time-to-next treatment, ≤ 6 months versus > 6 months.

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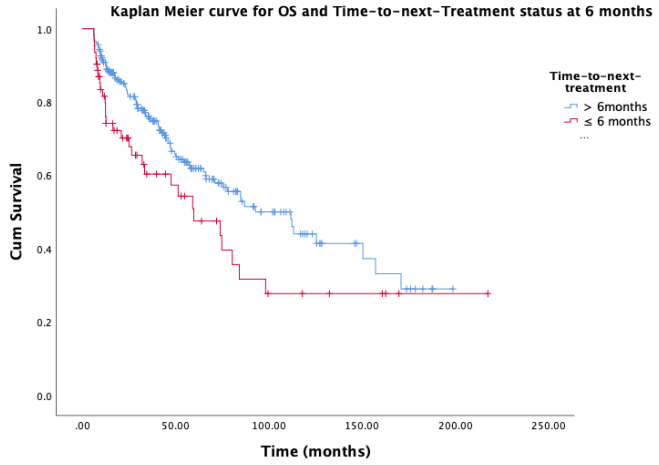

Second autologous stem cell transplantation as salvage therapy in selected patients with relapsed/progressed light-chain (AL) amyloidosis

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Background: High-dose melphalan (HDM) followed by autologous stem cell transplantation (ASCT) is an effective therapy in patients with immunoglobuline light-chain (AL) amyloidosis. Long-term experience from large specialized transplant centers has shown its potential to obtain deep and durable hematologic and organ responses as well as prolonged survival. Moreover, second ASCT has been suggested as a safe and effective rescue therapy in patients with AL amyloidosis at first relapse or progression after durable responses to first ASCT.

Objective: The aim of this study was to analyze our experience with second ASCT as salvage therapy in AL amyloidosis.

Material & Methods: We retrospectively analyzed a series of patients who received a second ASCT for relapsed or progressed AL amyloidosis at our institution from November 1997 to December 2020. For this purpose, characteristics of patients, transplant procedures, as well as outcomes, were collected. Eligibility criteria used for second ASCT were the same used for first ASCT in addition to a good quality and durable hematologic and organ response after the first procedure.

Results: Five patients with relapsed or progressed AL amyloidosis received a second ASCT at our institution in the study period, which means a 7% of all patients (N=72) who had undergone a first transplant. The median age of the series was 62 years (range, 38 to 66) and 4 patients (80%) were males. Light-chain isotype was lambda in 2 and kappa in 3 patients. Serum free light-chain (sFLC) values were only available at diagnosis in 2 patients and the involved sFLC was <100 mg/L in both. Bone marrow plasma cell infiltration ranged from 1 to 10% in 4 patients and it was 40% in the remaining. Involvement of heart, kidney and liver were present in 3 patients each, while single organ involvement was documented in 60% of patients. Cardiac disease was severe only in a 59 year-old female patient who underwent a heart transplant followed by HDM six months later. The median time between the first and the second ASCT was 8.4 years (range, 4.2-10.5). In all cases, the second transplant was the second line of therapy at relapse or progression after a long-lasting previous hematologic and organ response. Two patients received induction therapy before the second procedure, both bortezomib-based, and one obtained a very good partial response. All 5 patients required stem cell mobilization with G-CSF prior to second ASCT. Conditioning consisted in full-dose melphalan (200 mg/m^2) in all except one patient who received an intermediate dose (140 mg/m^2) due to impaired renal function (eGFR 45 ml/min). Patients were infused with a median of 3 x10^6 CD34+/kg (range, 2.2 to 6.8 x10^6 CD34+/kg). Two patients died within one month after stem cell infusion due to opportunistic pulmonary infections, including the patient with a previous heart transplant. The three surviving patients achieved hematologic responses of 39, 42 and 135-months duration, with the last patient still being alive in complete response.

Summary & Conclusions: We describe a small series of patients who received a second ASCT as salvage therapy for relapsed or progressed AL amyloidosis at our institution. Patients were selected according to previous prolonged hematologic and organ response after first ASCT (over 4 years in all cases) and standard eligibility criteria for HDM in this disease. Despite our high transplant-related mortality (2 out of 5 patients), we still consider this option in very selected patients, but excluding those with previous solid organ transplant.

Table1. Patients' characteristics, second ASCT and outcomes.

Gender / age[1] (years)	Light-chain isotype	BMPC[2] (%)	Organs involved	Previous HR / duration (months)	Induction therapy prior to 2nd ASCT	Melphalan dose (mg/m^2)	TRM	HR to 2nd ASCT / durantion (months)
M/38	kappa	1	Heart Kidney liver	PR/48	No	200	No	PR/39
M/66	Kappa	40	Heart Kidney liver	CR/117	VD with VGPR	140	No	CR/135+
M/62	Lambda	8	Liver	PR/112	No	200	Yes[3]	-
F/59	Lambda	1	Heart	PR/96	CyBorD with no response	200	Yes[4]	-
M/62	Kappa	10	Kidney	CR/57	No	200	No	CR/42

[1]Age at second ASCT. [2]Bone marrow plasma cell infiltration at diagnosis. [3]Pulmonary infection (parainfluenza virus-4 and probable aspergillosis). [4]Pulmonary infection (parainfluenza virus-3 and aspergillus fumigatus). HR: hematologic response; PR: partial response; CR: complete response; VD: bortezomib/dexamethasone; VGPR: very good PR; CyBorD: cyclophosphamide, bortezomib and dexamethasone; TRM: transplant-related mortality.

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IsAMYP: A Phase 2 single-arm study to evaluate the efficacy of isatuximab, pomalidomide and dexamethasone, in patients with AL amyloidosis not in VGPR or better after any previous therapy.

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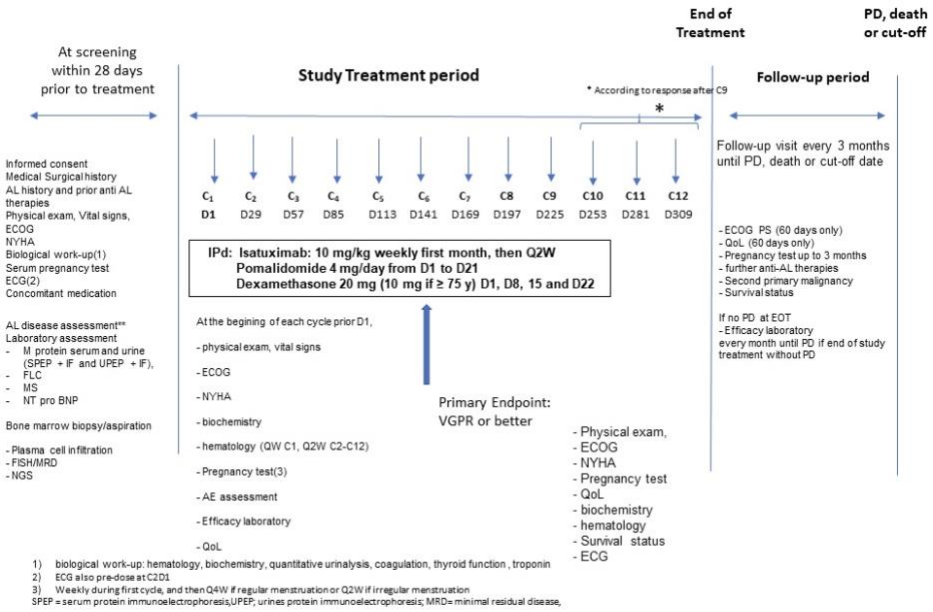
Topic
AL 2: upfront therapy, progression, rescue therapy – clinical – AL type

Background: Systemic AL amyloidosis is caused by the deposition of misfolded monoclonal immunoglobulin free light chains (sFLC) in various organs. Treatment of AL amyloidosis relies mainly on chemotherapy aimed at suppressing the underlying secreting plasma cell clone. Organ responses and survival are greatly influenced by the degree of hematological (hem) response evaluated by the decrease in sFLC. Over the last 5 years, anti CD38 monoclonal antibodies (mAb), such as daratumumab (DARA), have emerged as breakthrough targeted therapies for pts with multiple myeloma (MM). CD38, is a transmembrane glycoprotein that functions both as a signal-transducing receptor and a multifunctional ectoenzyme. Its expression is increased in MM and AL amyloidosis plasma cells. DARA received approval in combination with CyBorD in frontline AL amyloidosis[1]. In the relapse setting, DARA demonstrated a good efficacy and safety profile[2,3] but its activity could be enhanced with IMiD®, since it could increase CD38 levels on plasma cells. In AL amyloidosis, various groups demonstrated that pomalidomide (POM) is very effective and better tolerated than lenalidomide, especially in pts with renal insufficiency[4,5] with no dose modification (4 mg). Combining an anti CD38 mAb to POM could therefore be an attractive regimen for relapsed pts. Isatuximab (ISA) is another anti CD38 mAb that binds selectively to a unique epitope on the CD38 receptor and has been approved in RRMM in combination with POM or carfilzomib plus dexamethasone (DEX)[6,7] with a good safety profile. **Objective:** The aim is to evaluate the efficacy and safety of the combination of ISA, POM, and DEX in pts with AL amyloidosis who did not reach at least very good partial response (VGPR) and/or relapsed. **Material & Methods:** In this multicenter, single-arm, phase 2 study, we planned to include 46 previously treated pts. Main inclusion and non-inclusion criteria comprise: ≥1 previous line of therapy without VGPR or better at time of inclusion; measurable hematologic disease: dFLC > 50 mg/L; symptomatic organ involvement; adequate bone marrow and organ functions; no dialysis; no overt MM, no cardiac stage IIIb pts; ECOG>2; no previous anti CD38 or POM therapy (if refractory to POM). Pts eligible to enter the study will receive 28-days cycles of ISA (10 mg/kg, IV, weekly for 1st cycle then every-other-week), POM 4 mg (days 1-21) and DEX (10-20 mg, weekly). The treatment period will be 12 months, unless complete response at the completion of 9 cycles, disease progression or unacceptable toxicity occurs. The primary endpoint will be rates of VGPR or better at the completion of 6 cycles. Secondary endpoints will be: overall hem. response rates at various time points; progression-free survival; organ response rates at 1 year; overall survival; time to hem. and organ responses; safety and tolerability; and quality of life (EQ-5D-3L). Exploratory endpoints will comprise: impact of t(11.14) on responses; evaluation of minimal residual disease by NGS and by mass spectrometry. **Results:** This trial started in February 2022 and, as of April 28th, 5 patients were screened: 2 are receiving therapy, 2 are in

screening and 1 is screen fail. To date, no unexpected toxicities were reported. DSMB meetings will be called every 6 months or whenever indicated. **Conclusion:** This is the first prospective study of ISA in combination with POM and DEX in AL amyloidosis. Patients characteristics will be updated and preliminary results plus safety data will be reported during the ISA symposium.

Figure 1: study design and flow chart



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Epidemiology of light-chain amyloidosis in Latin America: a retrospective analysis of 212 patients. Grupo Latinoamericano de Estudio del Mieloma Múltiple (GELAMM).

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Background: Light-chain amyloidosis (AL) is the most common and severe subtype (70%) of amyloidosis. Very limited data have been published on this topic in Latin America (LATAM).

Objective: to describe the epidemiology and frontline therapy of patients diagnosed with AL amyloidosis in the last ten years in LATAM.

Material & Methods: a retrospective cohort of all consecutive patients with newly diagnosed AL amyloidosis based on the Latin American Multiple Myeloma Study Group (GELAMM) registry, from January 2009 to December 2019 in centers from Argentina, Chile, Colombia, Cuba, México, Paraguay and Uruguay. Categorical variables were described as percentages and absolute frequency. Quantitative variables were described as the median and interquartile range (IQR).

Results: Two hundred and eleven patients were included. The majority (55%) were diagnosed after 2014. Median age at diagnosis was 62 years (IQR 17) and 52% were male. Median bone plasma cell infiltration was 10% (IQR 15), 67% were lambda chain AL. Initial histological site of positive biopsy was detailed in 181 cases, as shown in Table 1. A second site was biopsied in 47 cases, mostly bone marrow (49%). Cytogenetic analyses (conventional and/or FISH for t(4;14); t(14;16)and del17p) were reported in 60 cases being abnormal in 4, without any predominance. All individuals were symptomatic at diagnosis. Asthenia, heart failure, nephrotic syndrome, and weight loss were the most frequent symptoms, present in 56%, 49%, 46% and 43%, respectively. Other symptoms include periorbital purpura (38%), dysautonomia (36%), peripheral neuropathy (34%), macroglossia (33%), and liver involvement (26%). Mass spectrometry was performed in 9 patients, in all confirming the diagnosis and subtype of Ig. Serum free light-chains (sFLC) were reported in 119 and cardiac biomarkers in 114 patients. Frontline chemotherapy was proteasome inhibitors-based in 44% (VCD 34%), and non-bortezomib based in 56% (including melphalan-based in 33% and other in 23). Thirty three (18%) patients received autologous stem cell transplantation, in 32 as frontline consolidation and in 1 as the only treatment. Eighteen patients (9%) received cardiac transplants. No data regarding renal transplants

were obtained. Overall response rate (≥PR) to frontline treatment was 44%. The best hematologic response was CR in 36% patients, VGPR in 10% patients, PR in 20% patients and NR in 16 % patients.

The main limitations of this study are due to its retrospective design and the insufficient data to assess prognosis and response, considering sFLC were not available in 44% of cases. Similarly to international reports, ASCT is done in < 20% of patients. Cardiac and renal involvement were lower than reported, probably reflecting the limitations of a multicenter registry and a suboptimal organ involvement evaluation. Overall, diagnosis and prognostic evaluation are suboptimal due to the limited availability of cardiac biomarkers and adequate typing techniques.

Summary & Conclusion:
This study provides real-world data of AL amyloidosis in the last 10 years in LATAM countries. Bortezomib-based frontline therapies are the most commonly used. ASCT is done in a minority of patients. We need to improve the quality of diagnosis and stratification and increase the availability of novel therapies.

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Timeline change of AL amyloidosis treatment response and clinical outcomes; a single-center experience from Turkey

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Organisation(s): Istanbul University Istanbul Faculty of Medicine, Turkey

Background: In AL amyloidosis major issues which affect patients' clinical outcomes are discrimination from other forms of amyloidosis and early diagnosis before persistent progressive organ damage. The treatment targets especially the underlying plasma cell clone and the daratumumab + bortezomib combination is emerging as a novel standard of care. Among anti-amyloid effects doxycycline combined with bortezomib-based protocol looking for a location on AL amyloidosis management.

Objective: We here summarised our experience with AL amyloidosis treatment from past to present to find out the factors affecting clinical outcomes.

Material & Methods: AL amyloidosis patients who have been diagnosed from January 2001 to December 2021 were included. Melphalan with high dose dexamethasone was the initially used induction which was replaced by bortezomib-based regimens combined with cyclophosphamide and dexamethasone and in last years with daratumumab as reimbursement was started with written health authority permission. The data was collected retrospectively electronically from the institutional database.

Results: Sixty-eight patients were included in the study. The median age was 58.5 years (range, 30–88 years) with a male: female ratio of 1:1. The median number of involved organs at diagnosis was 2 (range, 0 – 4). The most frequently affected organ was the kidney (54%) which was followed by the heart (52%) and gastrointestinal system (25%). Most (80%) of the patients had received a bortezomib-based regimen as induction. Only 16% of the patients could proceed to autologous stem cell transplantation. The median follow-up duration is 28.4 months (range, 1-142 months). The death rate by the end of follow-up was 36.75%. The cause of death is mostly cardiac involvement (44%) which was followed by infection (32%). A previous history of ischemic heart disease was found to be significantly associated with high mortality (p=0.002). ECOG≥ 2 was associated with mortality (p<0.001) and shorter OS (p<0.0001); 24-month OS rate was 37.17% (95% CI 0.1764-0.5687) in ECOG≥2 group compared to 89.63% (95% CI 0.7455-0.96) in the ECOG<2 group. Higher mortality was associated with dysrhythmia p=0.007; the survival rate of 24-month was 33.85% (95% CI 0.0865-0.6190) vs 78.79% (95% CI 0.6485-0.8771), p=0.0285. Mayo 2012 score (based on troponin and pro-BNP levels) had a very strong correlation with mortality and 24-month survival (p:0.001 and p:0.0002) Mortality was significantly higher in patients with interventricular septum diameter ≥1.5 cm (p=0.029); at 24-month survival, the difference was not statistically significant. IgA and lambda clonality is associated with higher mortality (p=0.024 and 0.03 respectively) there is also a significant association in 24-month survival (p=0.021 and 0.046 respectively). Patients with baseline hemoglobin <11.2gr/dl had higher mortality and worse 24-month survival rate (p:0.01 and 0.006 respectively).

There was no significant relationship observed between serum light chain ratio, bone marrow plasma cell ratio, proteinuria, glomerular filtration rate, albumin levels, with mortality, and 24-month survival.

Discussion: The cardiac involvement proved to be the major predictor of poor outcomes in AL amyloidosis. It is early to document the daratumumab based regimen effect on this outcome.

Summary & Conclusion: Beyond the new treatment strategies with daratumumab-bortezomib-based induction in combination with probable anti-amyloid treatment the organ response especially heart involvement outcome needs to be documented regarding survival impact.

Autologous Stem Cell Transplantation in AL amyloidosis in two centers from Latin America

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Background: in Light-chain amyloidosis (AL) the depth of the hematologic response is a predictive marker of survival. Autologous hematopoietic stem cell transplantation (ASCT) is a useful strategy for disease control. Only 25% of patients with newly diagnosed AL are candidates for ASCT. However, in those who are candidates, deeper and more durable responses with improved OS and PFS have been reported after ASCT.

Objective: To describe the characteristics of patients with AL Amyloidosis who received an ASCT in centers of Latin America, evaluate the pre- and post-transplant response, overall survival (OS) and progression-free survival (PFS).

Material & Methods: this is a retrospective cohort of all consecutive patients with newly diagnosed AL amyloidosis based on the Latin American Multiple Myeloma Study Group (GELAMM) registry, from January 2004 to March 2021. Categorical variables were described as percentages and absolute frequency. Quantitative variables were described as the median and interquartile range (IQR).

Results: Thirty-three patients from the entire registry (n=212, 15.5%) received ASCT, 6 were excluded from the present analysis due to insufficient data. Patients' characteristics are described in Table 1. The median age at ASCT was 59 (IQR 50-65) and the main organs involved were kidney (51%) and heart (48%). Thirteen patients had cardiac involvement at diagnosis, 4 were in NYHA functional class III, of whom 2 received a heart transplantation prior to chemotherapy, and another 2 received chemotherapy treatment with improvement in cardiac function, which made the subsequent ASCT possible. One patient required dialysis prior to transplantation, subsequently receiving a kidney transplant.
The median number of treatment lines before ASCT was one, (IQR 1-1) being CYBORD the most used chemotherapy regimen (70%). Only one patient received ASCT as the only frontline therapy. Most patients were in ≥ PR (22/24, 3 CR) at the time of transplant. Conditioning regimen was Melphalan 200mg/m2 in 19 and 140 mg/m2 in 8 patients. The median time to engraftment was 12 days (IQR 11-14), and the median time to hospital discharge was 22 days (IQR 18-29). Three patients required intensive care admission, and one patient died in the first 100 days due to sepsis. Sixteen patients deepened the response post-transplantation, 13 of them achieving complete remission. No patient underwent subsequent maintenance.
The median follow-up of the entire cohort was 60 months (IQR 21-90). Nine patients relapsed, the median time to relapse was 86 months (73-124 months) and the incidence rate was 0.6/100 patients-months. Relapse-free survival at 12 months was 100%, at 24 months was 95% (69-99), and at 120 months was 29% (5-58) (Fig 1a). Median progression free survival (PFS) was 59 months (22-86). PFS at 12 months was 96% (75-99), at 24 months 68% (46-81), and at 120 months 8% (2.24) (Figure 1b). The incidence rate of PFS was 1.6/100 patients-months. The median overall survival (OS) was 65 months (22-101). OS at 12 months 96% (IC

76-99), at 24 68% (IC 46-82), at 48 60% (38-76) and at 120 months 23% (IC 8-45%) (Figure 1c). There were 8 deaths, 5 due to disease progression.

Summary & Conclusion: ASCT in AL amyloidosis allows deepening responses and prolonging OS and PFS, with a low rate of complications if done in adequately selected patients[1-3]. In this cohort, patients receiving upfront ASCT had long survival, even without maintenance. ASCT achieved substantial improvement in the depth of responses, with a low mortality rate.

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Support & Funding: none.

Fig 1. Survival curves

Fig 1a. Kaplan-Meier survival estimate in AL patients with ASCT

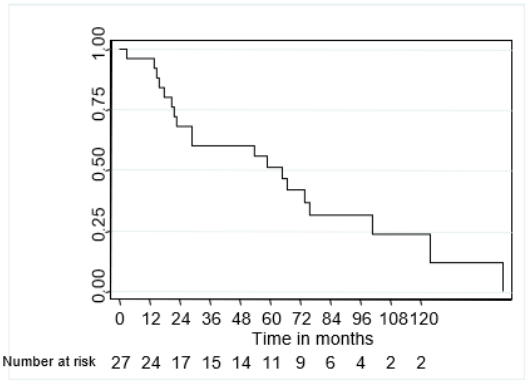

Fig 1b. Kaplan-Meier relapse estimate

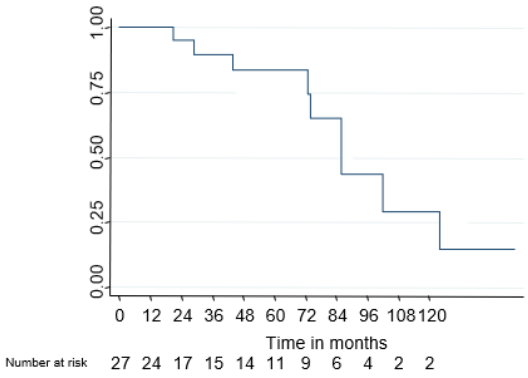

Fig 1c. Kaplan-Meier survival death or relapse estimate

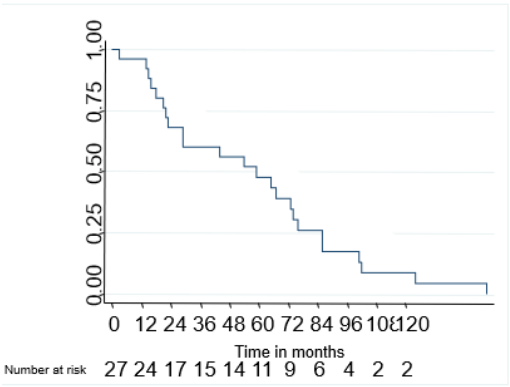

Table 1. Characteristics of patients (n=27)			
Age (median, IQR)			59 (50-65)
Sex (M/F)			16/11
AL kappa/lambda			6/21
Charlson score (median, IQR)			3 (2-5)
P. Status (ECOG) (median, IQR)			1 (1-2)
Bone Marrow infiltration %, (median, IQR)			11% (5-20)
N. Organs involved (median, IQR)			2 (1-2)
Organ involvement			
- Heart			13 (48%)
NYHA class (III/IV)			4/16
MAYO Score			
1			2
2			9
3			2
4			3
- Kidney			14 (51%)
- Renal insuficiency (creatinine > 2 mg/dl)			3 (11%)
-Liver			2 (7%)
- Gastrointestinal			5(18%)
- Peripheral Neuropathy			9 (33%)
- Dysautonomia			10 (37%)
Frontline therapy (pre-ASCT)			
CyBorD			19
Len/dex			1
Daratumumab/Bortezomib			2
Other			3
None			1
Number of lines before transplant (median)			1 (RIC 1-1)
Pre-ASCT response (N=24)		**Post ASCT response (N=25)**	
PR	12	PR	1
VGPR	7	VGPR	8
CR	3	CR	16
SD	2	SD	0

Real-world data on safety and efficacy of upfront daratumumab-based therapy in patients with light chain (AL) amyloidosis and high plasma cell burden evaluated at 3 months

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Background: The anti-CD38 monoclonal antibody daratumumab is a powerful anti-plasma cell agent. Since it has been approved in the frontline setting for newly diagnosed multiple myeloma patients, it became accessible to AL amyloidosis patients with high plasma cell burden. The phase III clinical trial ANDROMEDA shown that the addiction of daratumumab to the upfront therapy with cyclophosphamide, bortezomib and dexamethasone (D-CyBorD) improves both hematologic and organ response rate in AL amyloidosis and led to the first ever-approved regimen for AL amyloidosis by FDA and EMA[1].

Objective: To evaluate the efficacy and safety of daratumumab combinations as upfront treatment in AL amyloidosis patients in a real-world setting.

Material & Methods: Our maintained dataset was searched for newly diagnosed AL amyloidosis patients treated with daratumumab-based therapy in 2021. Hematologic and organ responses were assessed according to the International Society of Amyloidosis criteria 3 months after treatment initiation.

Results: Fifty-six consecutive patients were included in the study, table 1. Nineteen (34%) patients were treated with daratumumab in combination with lenalidomide (D-RD), 31 (56%) with bortezomib [21 (38%) associated with melphalan and dexamethasone and 10 (18%), with off label use of D-CyBorD], six patients (11%) received daratumumab monotherapy due to their frailty (stage IIIb). Grade ≥3 adverse events occurred in 5 (9%) patients: anaemia, thrombocytopenia, pneumonia, deep vein thrombosis and bradycardia. Median follow-up of living patients was 9 months (range: 6-13 months), the median number of cycles administered was 2 (range: 2-6) and the median time to response was 2 months (range: 1-3 months). Nine (16%) patients died due to progressive disease. Treatment is still ongoing in 37 (66%). The overall hematologic response rate was 78% (CR 14%, VGPR 46%, PR 18%). Cardiac and renal response were observed in 9 (19%) and 7 (19%) patients respectively. Comparing patients who would not have been eligible for ANDROMEDA clinical trial (36%) with the eligible ones, the hematological response rate was lower but not significantly different (ORR 75% vs 81%; ≥VGPR 60% vs 61%).

Summary & Conclusion: Treatment with daratumumab-based regimens in patients with AL amyloidosis and high plasma cell burden is effective and feasible. Early deep haematological responses are frequent and organ responses are seen in 20% patients at three months.

Table 1.: Clinical and demographic characteristics of the study population.

Patients characteristics (n=56)	Median (IQR) n (%)		Median (IQR) n (%)
Median age	64 (56 – 72)	**Mayo Cardiac stage**	
Male sex	32 (57)	I	1 (2)
Involved light-chain type		II	27 (48)
K : λ	12 (22) : 44 (79)	IIIa	9 (16)
dFLC (mg/L)	301 (100 – 520)	IIIb	14 (25)
Bone marrow plasma cells	19 (13 – 28)		
Involved/uninvolved≥ 100	5 (9)	NYHA class≥ 3	11 (20)
CRAB	8 (14)	PS-ECOG≥ 2	15 (27)
Organ involvement			
Heart	50 (91)	**Renal stage**	
Kidney	38 (68)	I	17 (30)
Liver	7 (13)	II	18 (32)
PNS	7 (13)	III	3 (5)
ANS	9 (16)	Dialysis at diagnosis	1 (2)
>2 organs	16 (29)		

References

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Comparison of bortesomib-based induction regimens with other treatment modalities in patients with newly diagnosed systemic light chain amyloidosis.

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Background: Rapid suppression of amyloid production is the main goal of therapy in light chain (AL) amyloidosis. CyBorD regimen (bortezomib, cyclophosphamide and dexamethasone) as the standard of care for newly diagnosed patients. In our study, we analyzed the response to induction therapy in 105 newly diagnosed patients with systemic AL amyloidosis ineligible for autologous stem cell transplantation (ASCT).

Objective: To evaluate the efficacy of proteasome inhibitors in the treatment AL-amyloidosis patients.

Material & Methods: The median age was 63 years (31-81). At the time of diagnosis, 62% of patients had three or more organs involved. In the patients with established Mayo stage, the following distribution was observed: stage I, 22% (n=20); stage II, 52% (n=47); stage III, 26% (n=24), 15 patients had IIIb stage (NT-proBNP >8500 ng/l). Renal involvement was documented in 94 patients: stage I, 23% (n=22); stage II, 48% (n=45); stage III, 29% (n=27). All consecutive patients were divided into 3 groups: group 1 was treated with CyBorD 26% (n=28); group 2 with other bortezomib-based regimens (bortezomib/dexamethasone (VD), bortezomib/ melphalan/dexamethasone (BMDex)), 59% (n=62); group 3 included bortezomib-free regimens (melphalan/dexamethasone (MDex), cyclophosphamide/prednisolone, corticosteroids), 14% (n=15). The median number of courses in all groups was 4.

Results: The 3-year overall survival (OS) was 70.3%, the median follow-up was 27.8 months (22 days to 11 years). Unfavorable factors for OS were as follows: age over 70 years (p=0.007); male gender (p=0.015), Mayo stage IIIb (p=0.07); and renal stage III (p<0.001). OS in patients who achieved hematological response (HR) was higher (93% vs 40%) than in patients without it (p<0.001). (Figure 1B). The percentage of HR was higher in the CyBoRD group, 94% vs 80% and 44% in groups 2 and 3, respectively (p=0.033). (Figure 2A). Median time to HR was 10.3 (9-14.6) months. In multivariate analysis, the use of any regimen other than CyBorD had a negative impact on OS (p=0.012). The 3-year PFS on the CyBorD regimen was 82% vs 52% and 61% in groups 2 and 3 (p=0.28). The percentage of patients with organs response (OR) was also higher in the CyBorD group. Cardiac response was achieved in 78% vs 53% and 16% on other regimens (p=0.0003) (Figure 2B). Renal response was recorded in 90% vs 87% and 50% (p=0.0021) (Figure 2C). The median time to cardiac response was 19.3 (12.8-70) months (13 months on CyBorD therapy), renal 12 (9.5-18.3) months (7 months on CyBorD therapy), liver 25.5 (13.3-70.7) months (Figure 1A).

Summary CyBorD is an effective upfront option for the patients with systemic AL amyloidosis, however, the presence of a progressive heart damage remains a predictor of early mortality in these patients.

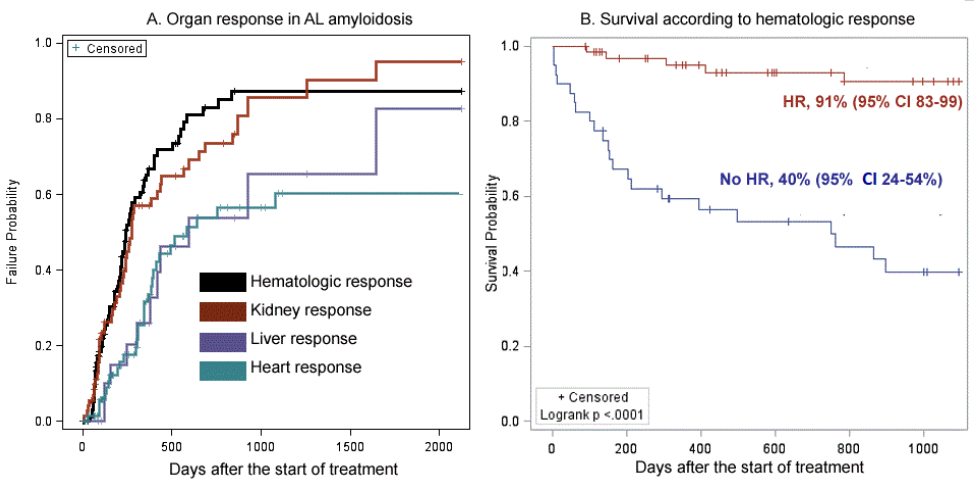

Figure 1.: Kinetics of hematologic and organ responses in the study group (A). Impact of hematologic response on overall survival (B)

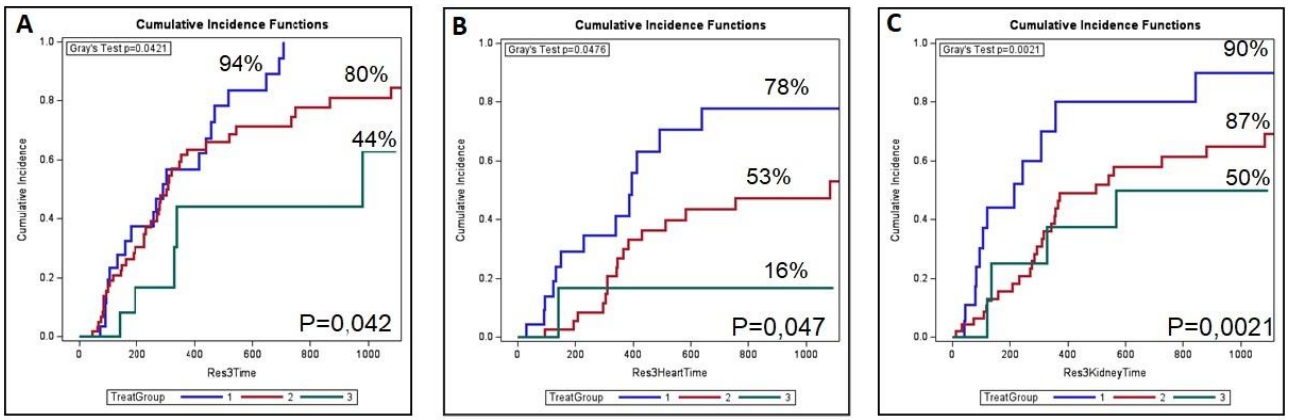

Figure 2.: The percentage of HR (A), cardiac (B) and renal (C) responses according to the treatment groups.

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Cellular Therapy and Transplantation (CTT). Vol. 10, No. 3-4, 2021 doi: 10.18620/ctt-1866-8836-2021-10-3-4-38-45

Autologous stem cell transplantation (ASCT) remains effective therapy for systemic immunoglobulin light chain amyloidosis (AL): Experience from a single Australian Amyloidosis Service

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Background: ASCT has been used as treatment for AL for over 20 years. However, due to treatment-related toxicity, its use is restricted to patients with good performance status and non-severe organ involvement, thus, only approximately 20% of AL patients receive such therapy. With increasing availability of effective, well-tolerated novel therapies such as daratumumab in AL, the role of ASCT has been brought into question.

Objective: We sought to analyse the outcomes of patients undergoing ASCT for AL at a single amyloidosis service in Australia.

Material and Methods: Using the Electronic Medical Records system, we identified all patients who had undergone ASCT for AL at the Victorian and Tasmanian Amyloidosis Service in Australian since its foundation in 2014. Patient characteristics and outcomes were analysed.

Results and Discussion: ASCT was administered in 25 patients between September 2014 – March 2022. Median age was 64 years at time of transplant (range 48-72). Males accounted for 84%. Median Revised Mayo Stage was 2 and the median ECOG was 1. Median numbers of organs involved was 1, consisting of cardiac in 15, renal in 12, and peripheral nerve in 5.

All patients received pre-transplant therapy; two patients had an IgM clone and received rituximab with cyclophosphamide and dexamethasone (CD), two patients with severe neuropathy at diagnosis received CD only, while the remainder received bortezomib with CD. Melphalan conditioning was split dose in all; 200mg/m2 in 22 patients and 140mg/m2 in three. Median engraftment time was 12 days (range 11-20). Six patients (24%) required ICU admission, and one (4%) died during ASCT of Campylobacter septicaemia leading to multi-organ failure.

Haematologic very good partial responses (VGPR) or greater were achieved in 22 patients (88%), with the majority achieving an accompanying organ response. With median follow up was 24.8 months (IQR 16.7 – 45.0), progression-free survival at 12 months was 79%. Four patients have died, but only one from disease progression. Median overall survival has not been reached.

Summary & Conclusions: Consistent with other studies, ASCT was effective therapy for AL in our patient cohort with high haematologic and organ response rates, and durable remissions. Careful patient selection meant transplant-related mortality was below 5%. With the long-term efficacy of novel agents such as daratumumab still to be determined, ASCT remains an important treatment option.

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Daratumumab, pomalidomide and dexamethasone (DPd) in relapsed/ refractory light chain amyloidosis previously exposed to daratumumab (NCT04270175): Interim results

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Background: We hypothesized that daratumumab (Dara), pomalidomide and dexamethasone (DPd) in relapsed/refractory amyloidosis (RRAL) patients (pts) with previous Dara exposure will yield deeper hematologic (hem) responses (i.e. complete response/CR) compared to pomalidomide (Pom)/dexamethasone (dex).

Objective: Primary objective is to determine hem best response within first 12 months of treatment with DPd. Secondary objectives include hem overall and very good partial response rates (ORR and VGPR, resp), stringent dFLC response, minimal residual disease (MRD) negative CR/VGPR rates, peripheral blood M-protein detection rates by serum mass spectrometry, time to first and best hem response, time to next therapy, median hem PFS/OS, organ response rates/duration and time to organ response.

Material & Methods: Multicenter single-arm phase 2 study in which 21 pts receive DPd until either PD or unacceptable toxicity. Eligibility criteria include >18 years, RRAL without concurrent MM and ≥1 prior lines of which one includes Dara. Pts receive DPd (12 cycles) with optional continuation beyond C12 if ≥VGPR achieved. Treatment regimen is Dara 1800mg subcutaneous wkly x 8 (C1-2), q2wks (C3-6) and q4wks (C7-12+), Pom 4mg PO D1-21 of 28d cycle in C1-12, and dex 20mg IV as premed D1 and D8, 20mg PO D2 and D9 and 40 mg PO D15 and D22. Dex 40mg PO is continued wkly (C2-6) and decreased to 20mg IV on D1 and 20mg PO on D8,15 and 22 (C7-12+). Dex reduction is allowed a priori as clinically appropriate. All pts gave written informed consent as approved by Institutional Review Boards.

Results & Discussion: Between 3/2021 and 1/2022, 5 pts enrolled across 3 of the 4 participating institutions (Table 1). Among the 5 pts, 2 completed 12 cycles and 2 on active treatment have completed 3 and 8 cycles, each. One pt discontinued treatment after C2 for worsening pre-existing peripheral neuropathy. Hem ORR is 60% including 2 CRs (both stringent dFLC responses) occurring after 3 and 4 cycles, 1 PR and 2 NRs (in pt who completed 3 cycles to date and pt discontinuing study treatment after C2). Median time to first and best hematologic response were 1 and 4 mos, resp. 2/5 pts (40%) and 5/5 (100%) are evaluable for cardiac and renal responses, resp. One of the 2 evaluable cardiac pts achieved a cardiac response after 2 cycles. Three pts (60%) achieved renal response in a median of 2 cycles. No cardiac or renal progression occurred and all pts remain alive.

DPd was generally well tolerated with expected adverse events (AEs), most commonly manageable cytopenias. Two pts experienced gr 4 neutropenia, one suspected of having underlying benign ethnic neutropenia. Both pts were able to continue on study with Pom reduced from 4mg to 2mg. One pt discontinued treatment due to a treatment-emergent gr 3 peripheral neuropathy with history of known prior neurotoxicity from other chemotherapies.

The FDA approval of Dara in the upfront setting has changed the treatment paradigm in RRAL, and the feasibility of Dara re-exposure combined with effective partners other than proteasome inhibitors in relapsed disease and after ASCT remains unknown. We show early evidence of safety and efficacy of DPd in RRAL in previously Dara treated patients.

Summary & Conclusions: This interim report of the first 5 enrolled RRAL pts with previous Dara exposure treated with DPd shows early and deep hem response rates (≥CR 40%) and a high renal response rate. Updated outcomes including MRD analyses (both by flow cytometry and clonoSEQ from bone marrow) will be presented at the meeting.

Table 1. Baseline Patient Characteristics

Characteristic	**n (%)**
Age, y, median (range)	56 (49- 73)
Sex	
Male	2 (40)
Race	
White	3 (60)
Black	1 (20)
Hispanic	1 (20)
ECOG performance status	
0	0 (0)
1	5 (100)
2	0 (0)
Time from diagnosis (months), median (range)	40 (10- 98)
Monoclonal light chain and IgH isotype	
Lambda	**4 (80)**
Lambda light chain only	1 (20)
IgG lambda	2 (40)
IgA lambda	1 (20)
Kappa	**1 (20)**
Kappa light chain only	1 (20)
dFLC, mg/L, median (range)	44 (38- 101)
Organ involvement at screening, median (range)	1 (1- 2)
Multiorgan involvement (≥2 organs)	2 (40)
Involved organ[a]	
Cardiac	2 (40)
Renal	5 (100)
Soft tissue/ Neuropathy	2 (40)
GI (Hepatic or Non-hepatic)	0 (0)
Mayo 2004/EU cardiac stage[1-2]	
I	3 (60)
II	2 (40)
IIIa	0 (0)
Evaluable for cardiac response[b]	2 (40)

NYHA class I II	 4 (80) 1 (20)
NT-proBNP, pg/mL, median (range)	245 (50- 1997)
Renal stage[4] I II III Evaluable for renal response[c]	 3 (60) 2 (40) 0 (0) 5 (100)
Median baseline eGFR > 50 mL/minute per 1.73m^2 ≤ 50 mL/minute per 1.73m^2	 4 (80) 1 (20)
24-hr urine protein, mg/24h, median (range)	3634 (920- 5052)
Time from last Dara (months) median (range)	9 (3- 27.5)
Duration of prior Dara (months) median (range)	22 (2.5- 49)
Hematologic Response to prior Dara **CR** **VGPR** **PR** **SD**	 1 (20) 1 (20) 2 (40) 1 (20)

Data are n (%) unless noted. dFLC= difference in the involved and uninvolved free light chains; ECOG= Eastern Cooperative Oncology Group; eGFR= estimated glomerular filtration rate; GI= gastrointestinal; NYHA= New York Heart Association.

[a] n (%) includes all patients with ≥1 organ involved.

[b] Based on the Palladini 2012 response criteria (i.e., baseline NT-proBNP ≥ 650 ng/L required for cardiac response determination).[3]

[c] Includes patients who met International Society of Amyloidosis (ISA) 2005 criteria for renal involvement (i.e., proteinuria >0.5 g/24h, predominantly albumin).[4] Renal response based on the Palladini 2014 renal response criteria.[5]

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Functional Status and Heart Failure Quality of Life Improve Following Therapy in Light Chain Amyloid Cardiomyopathy

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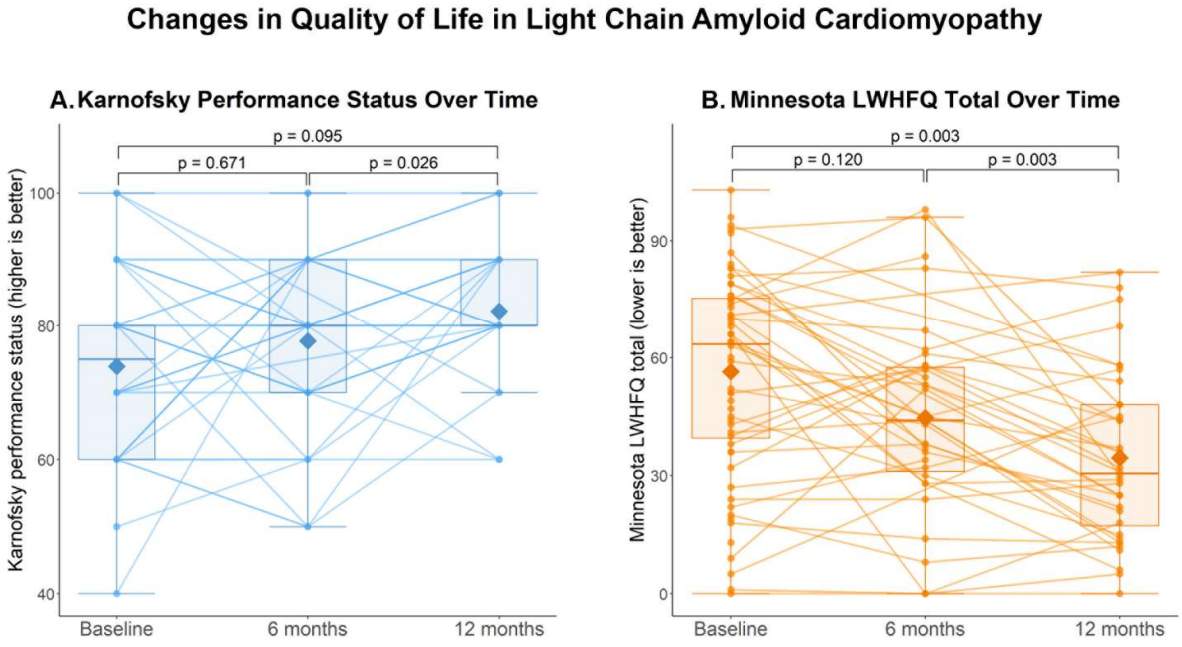
Background: In systemic light chain (AL) amyloidosis, quality of Life (QOL) is severely impaired across multiple domains. With appropriate therapy, some QOL metrics improve in the first 6 months. However, it is unclear how functional status and specific heart failure QOL evolve with therapy in patients with vs. without AL cardiomyopathy (AL-CMP).

Objective: This study aimed to assess changes in functional status and heart failure related QOL at 6 and 12 months in patients with recently diagnosed systemic AL amyloidosis with or without cardiomyopathy.

Material & Methods: Participants with recently diagnosed, biopsy-proven systemic AL amyloidosis were enrolled in a prospective study. Participants were categorized into study groups of AL-CMP (abnormal cardiac serum biomarkers) or AL-non-CMP (normal cardiac serum biomarkers and normal LV wall thickness). Functional assessment was performed with the Karnofsky Performance Status (KPS, 0–100, higher is better). Heart failure QOL was assessed with the Minnesota Living with Heart Failure Questionnaire (MLWHFQ, 21 questions, 0–105 points, lower is better). Functional status and heart failure QOL were evaluated at baseline, 6 months and 12 months for AL-CMP, and at baseline and 6 months for AL-non-CMP. Data were compared using Wilcoxon rank-sum test (unpaired) and Wilcoxon signed-rank test (paired). P-values were adjusted for multiple testing using the sequential Holm procedure.

Results: This study included 81 participants: 61 AL-CMP (75%) and 20 AL-non-CMP (25%). Median age was 61 years (interquartile range [IQR] 57–67 years) and 46 were males (57%). Among participants, 41 AL-CMP (67%) and 18 AL-non-CMP (90%) underwent ≥ 1 follow-up visit. In the AL-CMP cohort, 13 (21%) died within 12 months. Using personalized treatment regimens, mostly with combinations of cyclophosphamide, bortezomib, dexamethasone, and/or daratumumab, 33 AL-CMP (92% of 36) and 13 AL-non-CMP (76% of 18) participants reached very good partial or complete response at 6 months.
At baseline, KPS and MLWHFQ were significantly worse in active CA than in active non-CA (all $p<0.001$): median KPS in AL-CMP was 75 (IQR 60–80) and in AL-non-CMP 90 (80–92), while median MLWHFQ in AL-CMP was 64 (IQR 40–75) and in AL-non-CMP 4 (0–19). On follow-up visits in AL-CMP, scores significantly improved at 12 months: median KPS at 6 months was 80 (IQR 70–90, $p=0.67$ vs. baseline), and at 12 months 80 (80–90, $p=0.026$ vs. 6 months, Figure A), while median MLWHFQ at 6 months was 44 (31–58, $p=0.12$ vs. baseline), and at 12 months 30 (17–48, $p=0.003$ vs. 6 months, Figure B). In AL-non-CMP, QOL did not significantly change at 6 months: median KPS was 90 (IQR 90–09), and median MLWHFQ 4 (IQR 0–14).

Summary & Conclusion: Functional status and heart failure QOL were better in patients with systemic AL-non-CMP compared to AL-CMP at baseline, with no significant improvement in 6 months. By contrast, systemic AL-CMP patients had worse functional status and heart failure QOL at baseline, but in survivors who returned for follow-up evaluations, functional status and heart failure QOL improved at 12 months. These results suggest that therapy for systemic AL amyloidosis improves QOL during the first year in patients with cardiomyopathy.



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Atrial Fibrillation Does Not Influence the Occurrence of Cerebrovascular Accidents Among Patients with Amyloidosis

Ramtej Atluri, Saurabh Malhotra

Abstract

Background:

Patients with cardiac amyloidosis are known to have a higher prevalence of atrial fibrillation (AF) than the general population. The impact of AF on the occurrence of cerebrovascular accidents (CVA) among patients with amyloidosis is not known.

Methods:

Weighted analysis of National Inpatient Sample from 2017 was conducted. Hospitalizations with the diagnosis of amyloidosis were identified using ICD-10 codes, and this population was further stratified by the presence of AF. Occurrence of CVA was determined and its independent predictors were identified on multivariable regression analysis.

Results:

A total of 18,505 hospitalizations for amyloidosis were identified, of which 6,340 had AF. Rates of AF and CVA (2.4% vs 0.4%, $p<0.001$) were both significantly greater among those with amyloidosis than those without (*Figure & Table 1*). However, among those with amyloidosis there was no difference in the occurrence of CVA regardless of AF (2.11% vs 2.61%). When adjusted for patient demographics, comorbidities, and hospital characteristics, presence of amyloidosis was an independent predictor of both of AF (aOR= 1.79, 95% CI 1.65-1.95, $p<0.001$) and CVA (aOR= 2.67, 95% CI 2.16-3.3, $p<0.001$).

Conclusion:

Patients with amyloidosis have a 3-fold greater rate for AF and a 5-fold greater rate for CVA. However, in patients with amyloidosis, occurrence of CVA is not influenced by a diagnosis of AF. Patients with amyloidosis may require aggressive assessment of occult paroxysmal AF via ambulatory rhythm monitoring.

Table 1: Cerebrovascular accident rates in those with and without Amyloidosis stratified by the presence of Atrial Fibrillation.

Outcome	Atrial Fibrillation	No Atrial Fibrillation	*P*-value
Amyloidosis (n=18,505)	n= 6,384 (34.5%)	n= 12,121 (65.5%)	
Cerebral Infarction	2.11%	2.6%	0.339
No Amyloidosis (n=35,779,984)	n= 4,071,762 (11.38%)	n= 31,701,065 (88.6%)	
Cerebral Infarction	1.1%	0.4%	<0.001

Figure 1

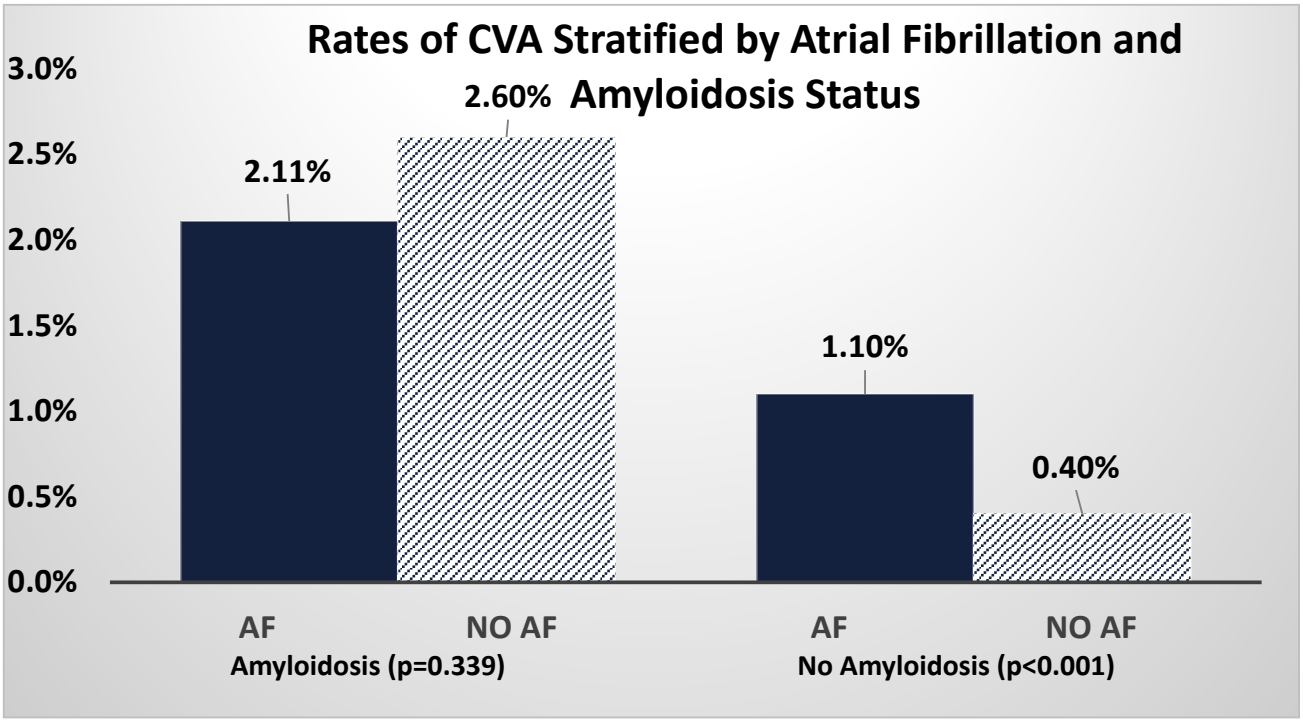


Table 2: Rates and odds of Cerebrovascular accidents and Atrial Fibrillation in those with Amyloidosis.

Outcome	Amyloidosis	No Amyloidosis	*P*-value
(N=35,798,453)	n=18,505 (0.05%)	n=35,779,984	
Atrial Fibrillation	34.5%	11.38%	<0.001
Cerebral Infarction	2.4%	0.4%	<0.001
Amyloidosis	**Adjusted Odds Ratio***	**95% Confidence Interval**	***P*-value**
Atrial Fibrillation	1.79	1.65-1.95	<0.001
Cerebral Infarction	2.67	2.16-3.29	<0.001
***Adjusted for age, charlson comorbidity index, coronary artery disease, diabetes, hypertension, chronic kidney disease, heart failure, gender, hospital location and teaching status, hospital bed size, median household income and black ethnicity.**			

Title: Morbidity and mortality measured through "Days Alive and Out of Hospital"(DAOH) in patients with amyloidosis.

Authors: María Lourdes Posadas Martinez, Teo Epstein, Mariana Vaena, Maria Sol Osorno, Erika Bárbara Brulc, Marcelina Carretero, María Soledad Sáez, Patricia Beatriz Sorroche, Javier Pollan, María Adela Aguirre, Elsa Nucifora.

Background: Several studies evaluate overall survival in different types of amyloidosis, but the impact on morbidity is less well known. Days alive and out of hospital (DAOH) is a novel patient-centered outcome that assesses the burden of disease, through the measurement of morbidity and mortality.

Objectives: To describe DAOH in patients with AL, ATTR and AA amyloidosis in an Institutional Registry of Amyloidosis.

Material and Methods: Prospective cohort of consecutive patients diagnosed as AL or ATTR or AA, from the Institutional Registry of Amyloidosis (RIA) belonging to the Health Maintenance Organizations of the Italian Hospital of Buenos Aires between 01/01/2010 and 31/08/2021. Patients with follow-up in another institution or less than 365 days were excluded. Quantitative variables were described with their median and interquartile range, and categorical variables with absolute and relative frequencies. We calculated DAOH at 1, 3, and 5 years, subtracting days hospitalized and days dead from the potential follow-up time. Only patients with a follow-up equal to or greater than the potential follow-up time were included in each group. We use Kruskal Wallis to compare DAOH between types of amyloidosis.

Results and Discussion: 177 patients were eligible, of whom 60 were excluded and 117 included for analysis. Of these, the total completed the follow-up for DAOH at one year, 71 for DAOH at 3 years and 51 for DAOH at 5 years (Fig.1). Of the 117 patients included, 36% were women, the median age was 73 years, and the Charlson score was 4 (IR 3-6). The most frequently affected organ was the heart (66%), followed by the kidney (37%). AL and ATTRwt amyloidosis were the most frequent types. The 77% had at least one episode of hospitalization during the total follow-up time. The median number of hospitalizations was 2 (IR 1-3). The median of DAOH was 360 (IR 323-365), 1055 (IR 712-1094) and 1712 days (IR 712-1798) at 1, 3 and 5 years, respectively. The median of DAOH at 1 year was 346 (275-365), 365 (IR 353-365), and 355 days (207-365) for AL, ATTR, and AA, respectively (p 0.01)(Fig.2). The median of DAOH at 5 years was 1739 (IR 352-1793), 882 (IR 804-1762) and 1791 days (IR 1627-1824) for AL, ATTR and AA, respectively (p 0.36).

In the present study we found that DAOH varies between types of amyloidosis. The AL type presented lower DAOH at 1 year, compared to ATTR and AA. This may be due to the fact

that at the time of diagnosis, patients with AL are more seriously ill, requiring early hospitalizations, either in order to establish support or specific treatments such as heart transplantation, autologous stem cells, and oncospecific therapy.

The small sample size in DAOH at 5 years could explain the lack of power to detect differences between the types of amyloidosis. However, ATTR shows a median of around 900 days shorter compared to AL and AA. This may be because, unlike the faster-progressing of AL, ATTR is a slower-progressing disease, which occurs mainly in the elderly and frail population, which increases mortality and the number of hospitalizations for all causes over time.

Regarding type AA, it seems to be the group with the lowest morbidity and mortality. This could be due to the fact that it is a chronic disease, with mainly renal involvement, which does not put the patient's life at risk in the short term.

Summary & Conclusion: DAOH is a patient-centered outcome measure that describes the patient's disease journey. This is the first work that describes DAOH in the 3 types of systemic amyloidosis. This result can become a relevant measure for monitoring chronic diseases.

Keywords: Inmunoglobulin light chains; transthyretin amyloidosis; amyloidosis AA; patient-centered outcome; prognosis; morbidity; mortality

Figure 1. Flowchart. Patients with systemic amyloidosis of the Institutional Registry of Amyloidosis belonging to the Health Maintenance Organizations of the Italian Hospital of Buenos Aires. Period 2010-2021.

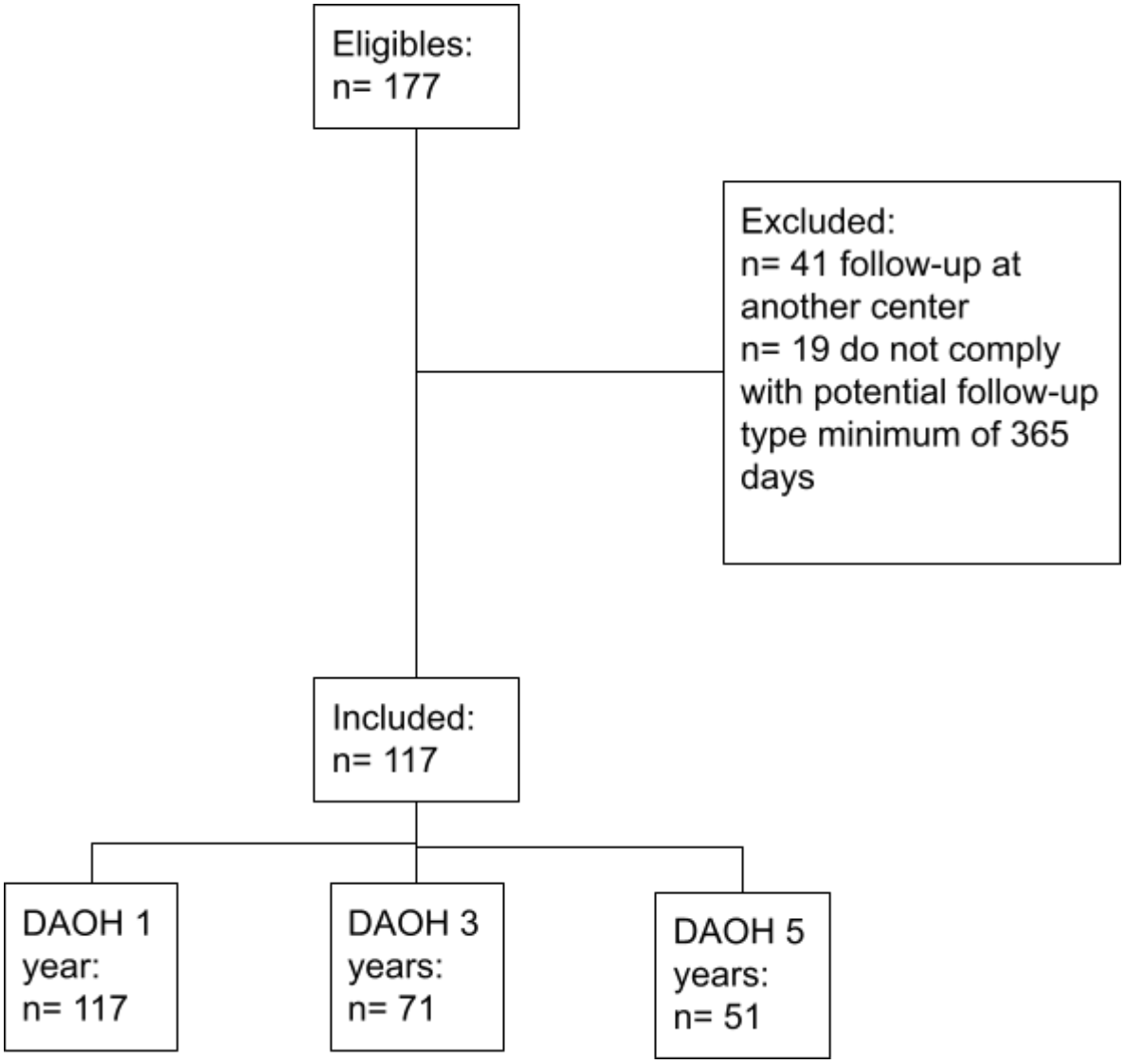
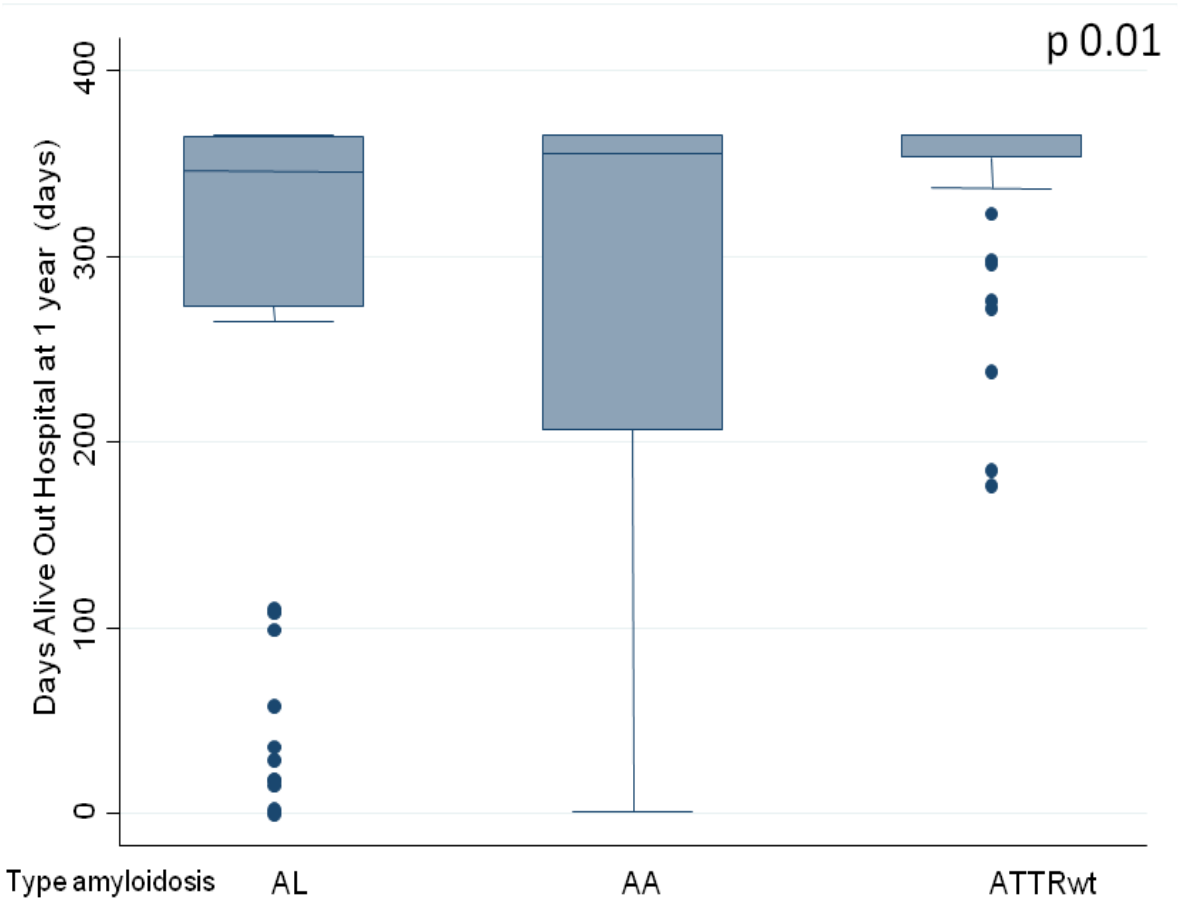


Figure 2.Box plot. Days Alive and Out of Hospital at 1 year according to type of amyloidosis.



Factors associated with morbidity and mortality, measured through "Days Alive and Out of Hospital"(DAOH) in patients with AL and ATTR amyloidosis.

Authors: María Lourdes Posadas Martinez, Maria Sol Osorno, Mariana Vaena, Teo Epstein, Erika Bárbara Brulc, Marcelina Carretero, María Soledad Sáez, Patricia Beatriz Sorroche, Javier Pollan, María Adela Aguirre, Elsa Nucifora.

Background: Cardiac involvement and age are known prognostic factors for overall survival in systemic amyloidosis. However, the impact of these on morbidity is less well known. Days alive and out of hospital (DAOH) is a novel patient-centered outcome that assesses the burden of disease, through the measurement of morbidity and mortality.

Objectives: To evaluate factors associated with morbidity and mortality assessed through DAOH.

Materials & Methods: Prospective cohort of consecutive patients diagnosed as AL or ATTRwt from the Institutional Registry of Amyloidosis (RIA) belonging to the Health Maintenance Organizations of the Italian Hospital of Buenos Aires between 01/01/2010 and 31 /08/2021. Patients with follow-up in another institution or less than 365 days were excluded. Quantitative variables were described with their median and interquartile range, and categorical variables with absolute and relative frequencies. We calculated DAOH at 1 year, subtracting days hospitalized and days dead from the potential follow-up time. Only patients with a follow-up equal to or greater than the potential follow-up time were included. The factors associated with DAOH were evaluated with a multiple linear regression model.

Results & Discussion: 149 patients were eligible, of whom 53 were excluded and 96 were included (Figure 1). The median age was 76 years and 34% were women. The most frequently affected organ was the heart (78%) and renal (31%). Seventy-five percent of patients had at least one episode of hospitalization during follow-up at one year, with a median number of hospitalizations of 2 (IR 0.5-3).

The median of DAOH at 1 year was 360 (IR 327-365). The median of DAOH was 346 (IR 274-365) and 365 days (IR 353-365) for AL and ATTRwt, respectively. Multivariate regression analysis showed that ATTRwt was associated with higher DAOH compared to AL whilst cardiac involvement and age were associated with lower DAOH and, respectively (Table 1).

In the present study we found that the morbidity and mortality associated with amyloidosis can be affected by different factors. Age and the presence of cardiac involvement at the time of diagnosis were associated with less DAOH at one year and therefore higher morbidity and mortality. These results coincide with studies that evaluate cardiac involvement as a prognostic factor using traditional methods [1,2]. On the other hand, when comparing patients with AL amyloidosis and ATTRwt, it was observed that the ATTRwt was associated

with lower morbidity and mortality and higher DAOH at one year, similar to the results observed by the Rubin et al [3].

The present study uses DAOH as a new measure of morbidity and mortality and, unlike "time to event" studies, it takes into account not only the first hospitalization, but all of them, in number and duration, and prioritizes the time to death. At the moment, there are no other published studies that have evaluated factors associated with morbidity and mortality using DAOH.

Summary & Discussion: Days alive and out of hospital is a measure of patient-centered outcomes that describes patients' journey with disease. This is the first paper that demonstrates DAOH can be affected by subtype of amyloidosis, age and cardiac involvement, which strengthens the use of this measure in disease follow-up.

Keywords: Immunoglobulin light chains; transthyretin amyloidosis; patient-centered outcome; prognosis; morbidity; mortality

Figure 1. Flowchart. Patients with AL or ATTRwt of the Institutional Registry of Amyloidosis

belonging to the Health Maintenance Organizations of the Italian Hospital of Buenos Aires. Period 2010-2021.

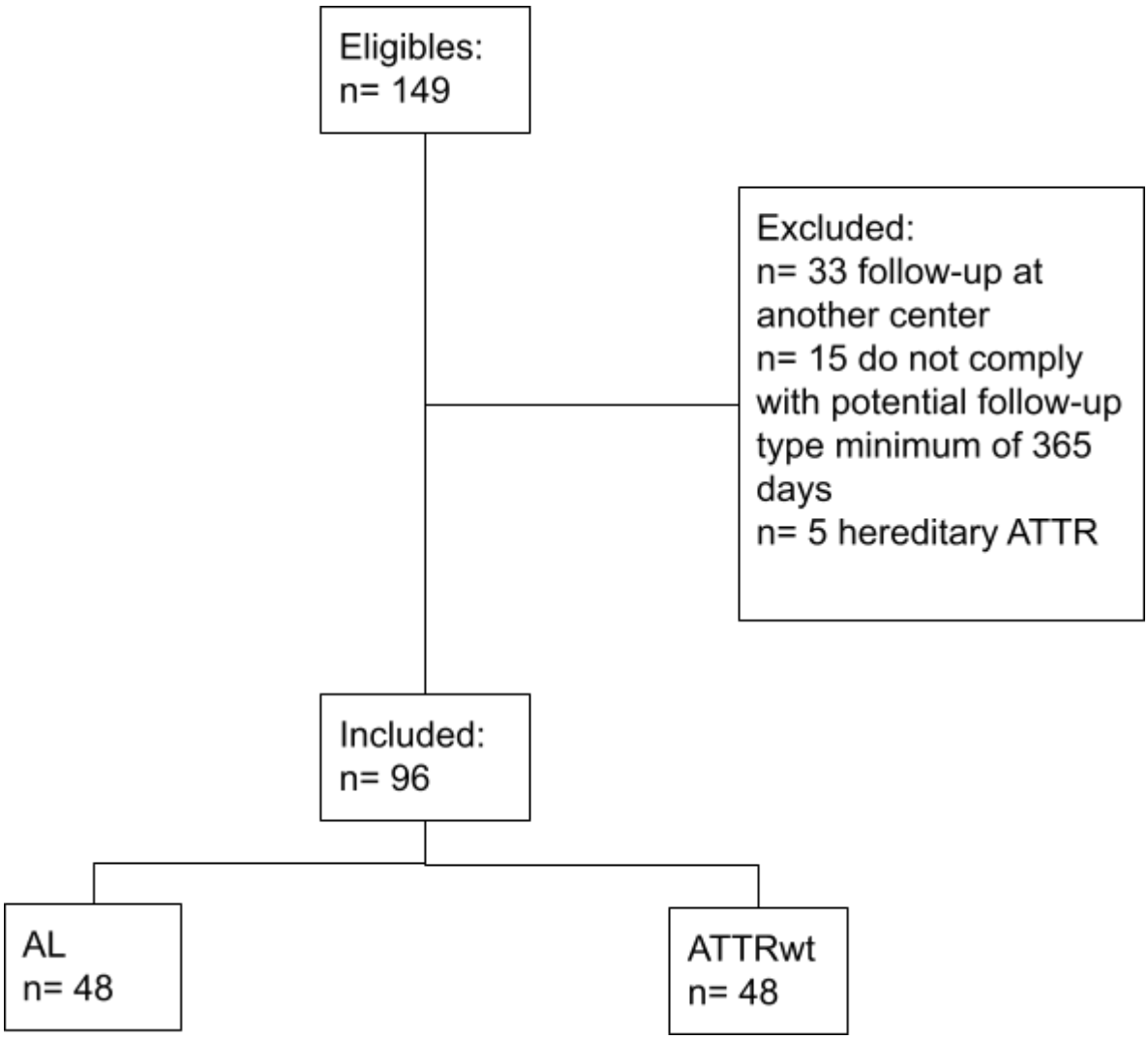


Table 1. Multiple linear regression model showing adjustment for variables: type of amyloidosis, cardiac involvement, and age. Patients with AL or ATTRwt of the Institutional Registry of Amyloidosis belonging to the Health Maintenance Organizations of the Italian Hospital of Buenos Aires. Period 2010-2021.

DAOH 1 year	Coef. b	p value	CI95%
AL vs ATTRwt	126	< 0.001	74 to 177
Cardiac involvement	-74	0.005	-127 to -23
Age	-2.05	0.041	-4 to -0.08

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Significant tricuspid regurgitation is associated with adverse outcomes in patients with transthyretin amyloidosis.

Introduction
Patients with transthyretin amyloidosis (ATTR) have poor outcomes due to the development of heart failure. Tricuspid regurgitation (TR) is associated with adverse outcomes in patients with heart failure.

Purpose
The purpose of this study was to evaluate if the presence of significantTR is associated with adverse cardiac outcomes (death or hospitalization for heart failure) in patients with ATTR and cardiac involvement.

Methods
This was a retrospective cohort of patients with amyloidosis ATTR included in the Institutional Registry of Amyloidosis (ClinicalTrials.gov NCT01347047). All patients have cardiac ATTR involvement in the echocardiogram and evaluation of right ventricle chamber for tricuspid regurgitation. Cardiac involvement was defined as uptake on pyrophosphate scintigraphy or typical involvement on cardiac magnetic resonance.
Significant TR was defined as moderate or severe regurgitation according to qualitative and quantitative measurements.
A two years follow up was performed in this cohort to assess the incidence of the composite outcome of death and hospitalization for heart failure.

Results
Overall, 93 ATTR patients were included in the study. 5 were variant transthyretin (ATTRv). The mean age at diagnosis was 82,5 years [IQR 75 -86]. 86 % were male, and the mean left ventricular ejection fraction was 52 % [IQR 43 - 60]. 32,2 % (n=30) had significant TR. Patients with significant TR had similar age (79,9 vs 81,6 p=0,3), higher pro-BNP values (6327 vs 3711 p=0,004), and lower left ventricular ejection fraction compared to patients without significant regurgitation. In the univariate analysis, the incidence of composite death or heart failure was higher in patients with significant TR compared to patients without significant TR. In a Cox regression multivariate analysis, only pro-BNP (HR 1.00, 95% CI 1.00005- 1.0002, p=0.001), and significant TR (HR 2.23, 95% CI 1.12- 4.42, p=0.021), were associated with the composite death or hospitalization for heart failure.

Conclusion
Patients with ATTR and significant TR have worse outcomes compared to patients without significant TR. These findings, might support to explore further research for interventions that reduce severity of the tricuspid regurgitation in patients with ATTR and heart failure.

N=93	Non-significant TR (n=63)	Significant TR (n=30)	P value
Age (years)	79,9 [77,9 - 81,8]	81,6 [79 - 84,1]	0,3
Male (%)	85 (n=54)	86 (n=26)	0,9
History of hypertension (%)	84 (n=53)	80 (n=23)	0,6
History of Diabetes (%)	23,8 (n=15)	26,6 (n=8)	0,7
Chronic renal failure(%)			0,003
CrCl 30-60	36,5 (n=23)	56,6 (n=17)	
CrCl <30	3,1 (n=2)	16,6 (n=5)	
Carpal tunnel (%)	38 (n=24)	30 (n=5)	0,4
Neuropathy (%)	22,2 (n=14)	23,3 (n=9)	0,3
Prior CAD (%)	26,9 (n=17)	33,3 (n=10)	0,5
Prior Atrial Fibrillation (%)	44,4 (n=28)	86,6 (n=26)	< 0,001
Nt ProBnp (ng/dl)	3711 [2703 - 4719]	6327 [4758 - 7895]	0,004
LVEF (%)	55,8 [53,2 - 58,4]	46,1 [42,2 - 49,9]	< 0,001
Septal Thickness (mm)	17,4 [16,3 - 18,5]	16,9 [14,8 - 18,9]	0,6
Severe AS (%)	9,5 (n=6)	13,3 (n=4)	0,5

Table 1. COHORT BASAL CHARACTERISTICS.

TR: tricuspid Regurgitation; CrCl: Creatinine Clearance; CAD: coronary artery disease. TR: Tricuspid Regurgitation; LVEF: left ventricular ejection fraction; EDVD: end diastolic ventricular diameter; ESVD: end systolic ventricular diameter; AS: aortic stenosis

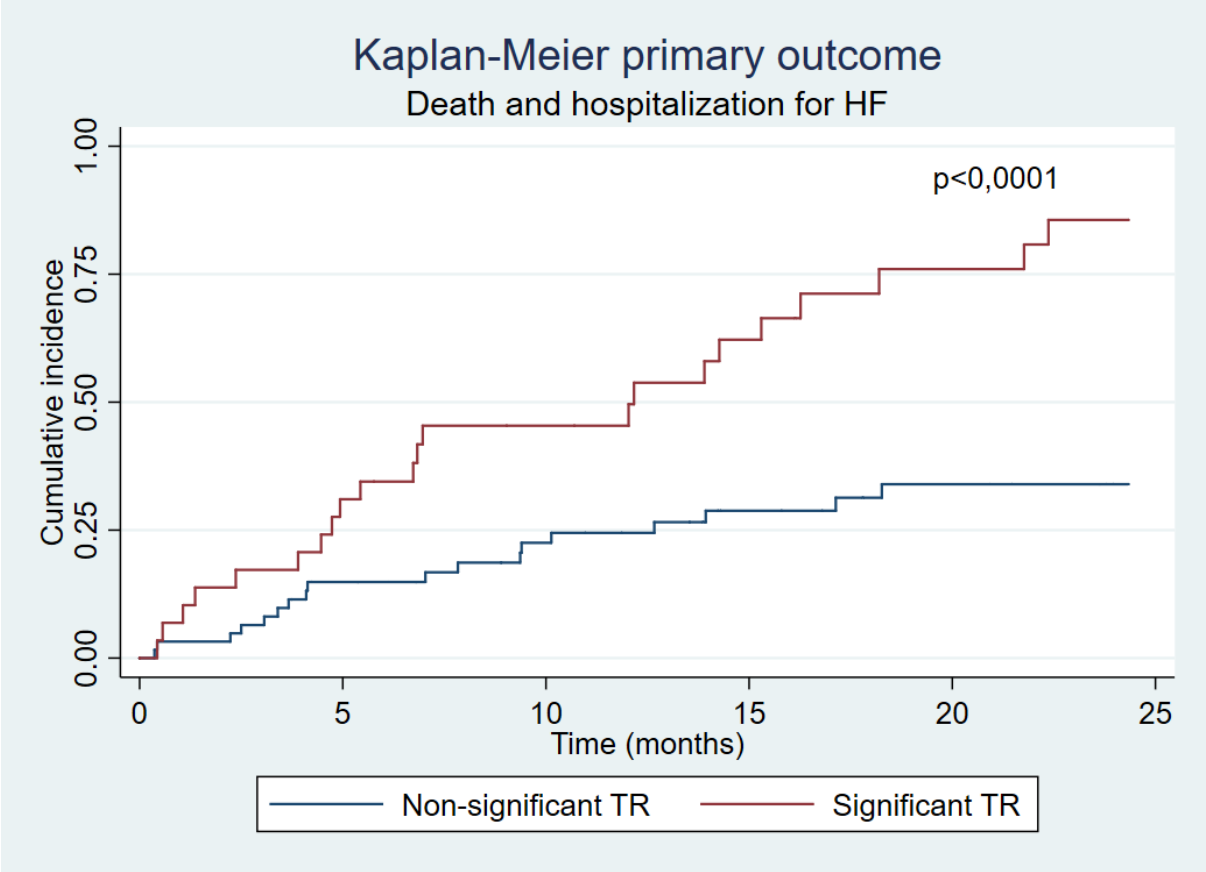

Figure 1. Kaplan Meier curves for the combined outcome of Death-HF Hospitalizations in patients with and without significant regurgitation.

Prevalence of hereditary transthyretin amyloidosis among elderly patients with transthyretin cardiomyopathy

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM) is a progressive heart disease caused by the extracellular deposition of transthyretin (TTR) amyloid fibrils, either in its hereditary (ATTRv) or wild-type (ATTRwt) form. ATTR-CM is increasingly recognized as a cause of heart failure in the elderly.
Although wild-type ATTR-CM is the most frequent form of ATTR-CM found, hereditary ATTR-CM (ATTRv) can also occur, despite there is limited information about its frequency. Unfortunately, ATTR-CM is frequently assumed to be of wild-type origin and TTR genetic testing is not performed when ATTR-CM is diagnosed in elderly individuals.
Diagnosis of the hereditary nature of ATTR-CM allows genetic counseling, permits familial screening (facilitating early diagnosis of relatives) and, if polyneuropathy is present, enables treatment with certain TTR-specific drugs.

Objective: We sought to determine the prevalence of ATTRv among elderly ATTR-CM patients, identify predictors of ATTRv and evaluate the clinical consequences of ATTRv diagnosis in this population.

Material & Methods: This was a retrospective study comprising all consecutive ATTR-CM patients evaluated at a university hospital in Spain from December 2008 to November 2021. Patients in whom genetic testing had already been performed before ATTR-CM diagnosis because of neurological disease or following ATTRv diagnosis in a relative were excluded. Clinical data were extracted from medical records and age ≥70 years was used to define the elderly cohort.
Continuous variables were reported as mean±standard deviation, or median and interquartile range. Categorical variables were reported as number and percentage.

Results: A total of 300 ATTR-CM patients were included (median age 78 years at diagnosis, 82% were ≥70 years, 16% females, 99% Caucasian). Eighty-two patients from the 203 referred patients (40.4%) did not have TTR genetic testing performed at the referring center, and this proportion increased with age. ATTRv was diagnosed in 35 (12%; 95%CI: 3.1-8.8) and 13 (5.3%; 95%CI: 5.6-26.7) patients in the overall cohort and in those ≥70 years, respectively. Prevalence of ATTRv among elderly female patients with ATTR-CM was 13% (95%CI: 2.1-23.5). Diagnosis of ATTRv in elderly ATTR-CM patients allowed initiation of TTR specific treatment in 5 individuals, genetic screening in 33 relatives from 13 families, and identification of 9 ATTRv asymptomatic carriers.

Summary & Conclusion: ATTRv is present in a substantial number of ATTR-CM patients ≥70 years (5.3%).
Female sex and polyneuropathy are independently associated with ATTRv in this population. Identification of ATTRv in elderly patients with ATTR-CM has clinical meaningful therapeutic and diagnostic implications both for patients and their families. These results support routine genetic testing in patients with ATTR-CM regardless of age.

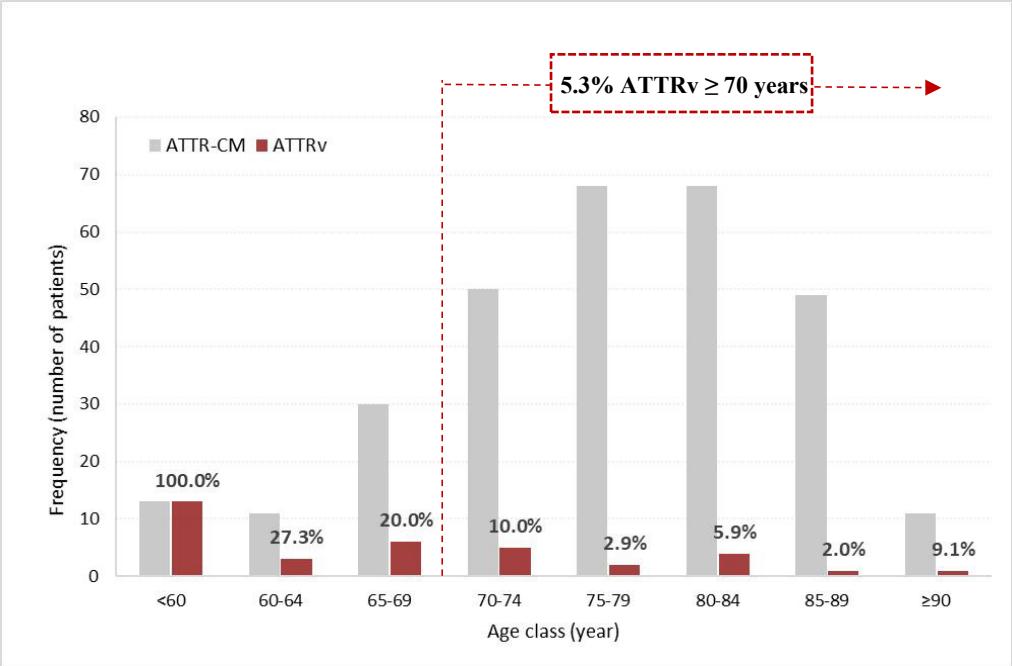

Figure 1.: Prevalence of ATTRv in patients with ATTR-CM according to age.

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Clinical findings and comorbidities in wtATTR patients with suspected amyloid neuropathy

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Background

Wild-type transthyretin amyloidosis (wtATTR), previously known as “senile” amyloidosis primarily causes cardiomyopathy but multisystemic involvement and deposition of amyloid in other tissues has been increasingly recognized, including peripheral neuropathy. Clinical involvement is typically dominated by cardiomyopathy, and peripheral neuropathy is milder than seen with variants of hereditary ATTR.

Objective

To describe clinical features of wtATTR with suspected peripheral neuropathy

Material & Methods

Retrospective review of medical records of wtATTR patients who were evaluated clinically in neuromuscular clinic at University of Pittsburgh Medical Center and underwent electrodiagnostic testing for suspected neuropathy between 2017 and 2022. Diagnosis of wtATTR was confirmed by imaging and/or tissue evidence of transthyretin amyloid deposition and negative genetic testing for TTR mutations.

Results & Discussion

Our cohort included 18 patients with wild-type transthyretin amyloidosis who were evaluated for peripheral neuropathy. Our subjects were predominantly men (95%) with a mean age of 72.2 years at the time of diagnosis (range 65-85) and were followed for an average of 2.8 years (range 1-6). Neuromuscular comorbidities included inclusion body myositis, primary lateral sclerosis and polyneuropathy associated with IgM gammopathy and anti-MAG antibodies in one subject each (5%).

All patients underwent nerve conduction studies and needle electromyography, and 4 patients underwent autonomic testing. Electrodiagnostic testing showed large fiber sensorimotor polyneuropathy in 10 subjects (56%), distal sensory polyneuropathy in 1 subject (5%), lumbosacral radiculopathies in 7 subjects (39%) and cervical radiculopathies in 1 subject and did not show large fiber sensorimotor polyneuropathy in 7 subjects (39%). Four subjects underwent autonomic testing which was normal in 3 and showed length-dependent sudomotor loss in 1 subject suggestive of small fiber neuropathy without evidence of generalized dysautonomia (5%). Bilateral carpal tunnel syndrome was found in 15 subjects (83%), two (11%) had unilateral carpal tunnel syndrome, and 1 (5%) had no history of carpal tunnel syndrome.

Cardiomyopathy was found in 17 subjects (94%), and 13 also had atrial fibrillation (72%). In one subject, wtATTR was an incidental finding on liver biopsy, but there was no evidence of wtATTR cardiomyopathy on diagnostic testing. One subject had a history of stroke and another had a history of myocardial infarction (5% each).

There were 7 patients with renal insufficiency (mean eGFR 42.7, range 25-52). None of subjects required hemodialysis. There was no evidence of renal amyloidosis in any of the subjects. Fifteen subjects had

hypertension (83%). Six subjects (33%) had history of lumbar spine surgeries, six subjects (33%) had knee replacements and 1 had hip replacement (5%)

Summary & Conclusions.

Our study showed large fiber polyneuropathy only in 61% of subjects with wtATTR and symptoms suggestive of neuropathy that could have been related to spinal stenosis and radiculopathies. It also confirms high prevalence of bilateral carpal tunnel syndrome in patients with wtATTR. We did not find evidence of generalized dysautonomia, but only 4 subjects (22%) underwent autonomic testing. Given the advanced age, high prevalence of various comorbidities is expected and some of symptoms may have multifactorial etiology. Large longitudinal studies are needed to characterize clinical features and multisystemic involvement in wtATTR.

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Identification of Wild-type Transthyretin Cardiac Amyloidosis in Patients with Recent Carpal Tunnel Syndrome – The CACTuS Study

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Background: Wild-type transthyretin cardiac amyloidosis (ATTRwt) is an infiltrative cardiomyopathy with a poor prognosis. The condition typically affects males above the age of 65. The condition is associated with carpal tunnel syndrome (CTS) which often precedes the ATTRwt diagnosis by several years. Potentially, screening for ATTRwt in patients with recent CTS surgery can identify patients in an earlier disease stage than those diagnosed through clinical practice. Previous screening studies have been conducted but have failed to enroll patients of the optimal demographic.

Objective: The aim of the study was 1) to screen patients operated for CTS within the last 5 years for ATTRwt using red flags associated with the condition, 2) to determine whether patients with screened ATTRwt had less advanced disease compared to patients with ATTRwt diagnosed through clinical practice.

Material & Methods: Patients aged ≥ 60 years at the time of CTS surgery from 2016 to 2020 were invited for screening. The study sought to enroll 90 % males. Red flags were defined as elevated biomarker levels of NT-proBNP or cardiac troponin, an electrocardiogram patterns of low voltage, pseudoinfarction, or atrioventricular conduction disorders, left ventricular hypertrophy (LVH), and relative apical sparring on longitudinal strain analysis. All patients with a red flag were referred for a diagnostic scintigraphy. Patients with ATTRwt diagnosed by screening were compared to patients with clinical ATTRwt (n = 51) matched by age, gender, and CTS surgery.

Results and Discussion: The study enrolled 120 participants (age 74.5 ± 6.1 years, 90 % male) and the suspicion of ATTR was raised in 67 (55.8 %). Ten participants (8.3 %) were diagnosed with ATTRwt. Patients identified with ATTRwt were predominantly asymptomatic, had mildly elevated NT-proBNP, mildly increased LVH, preserved left ventricular ejection fraction and systolic longitudinal function which differed significantly from clinical ATTRwt controls ($p<0.001$).

Summary & Conclusion: The study found an ATTRwt prevalence of 8.3 % in a population of age and gender-selected patients with a recent history of CTS. The patients with ATTRwt diagnosed through screening had less structural and functional cardiac involvement than patients diagnosed with ATTRwt through clinical practice.

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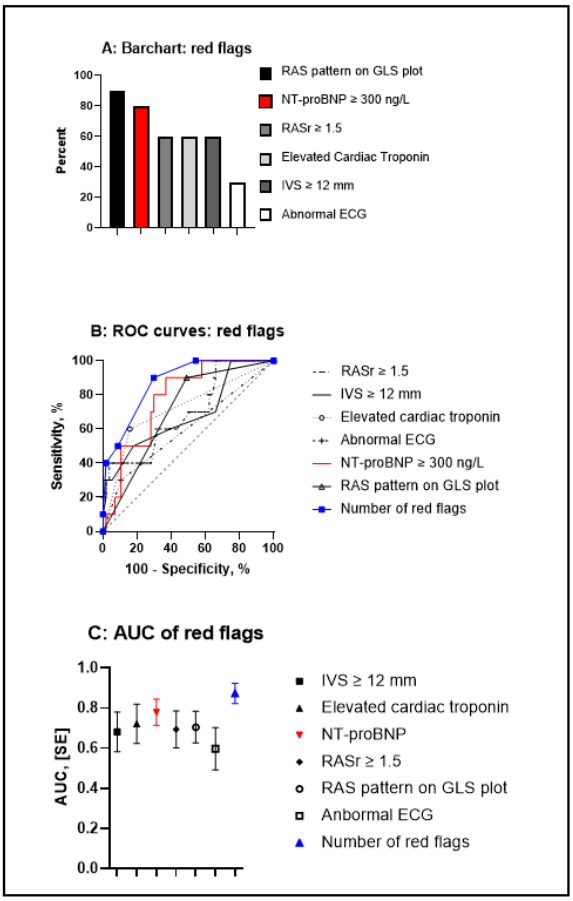
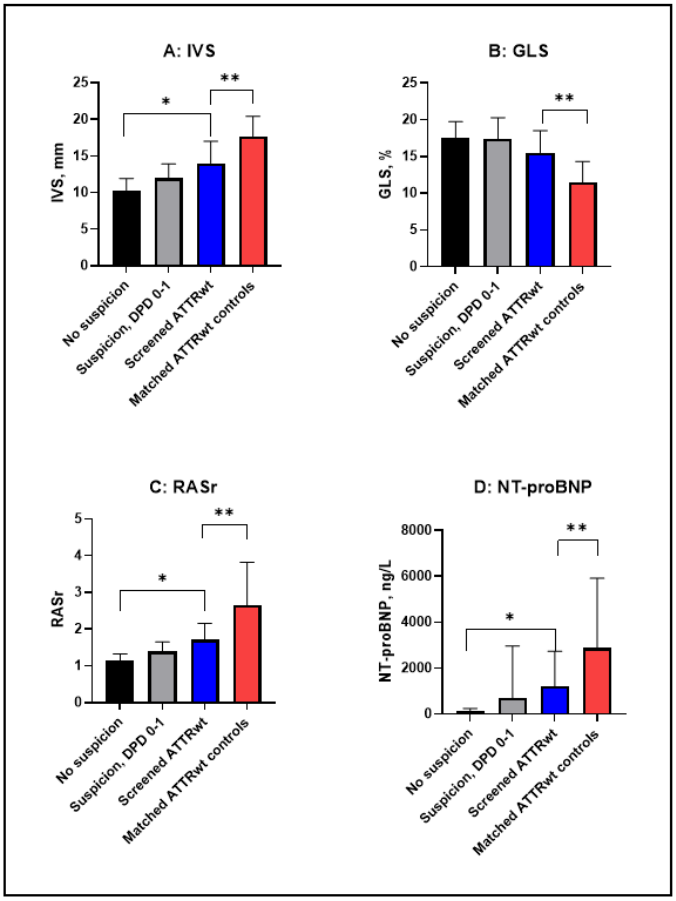
Figures


A: Barchart: red flags
Percent
RAS pattern on GLS plot
NT-proBNP ≥ 300 ng/L
RASr ≥ 1.5
Elevated Cardiac Troponin
IVS ≥ 12 mm
Abnormal ECG
B: ROC curves: red flags
Sensitivity, %
100 - Specificity, %
RASr ≥ 1.5
IVS ≥ 12 mm
Elevated cardiac troponin
Abnormal ECG
NT-proBNP ≥ 300 ng/L
RAS pattern on GLS plot
Number of red flags
C: AUC of red flags
AUC, [SE]
IVS ≥ 12 mm
Elevated cardiac troponin
NT-proBNP
RASr ≥ 1.5
RAS pattern on GLS plot
Anbormal ECG
Number of red flags


A: IVS
IVS, mm
B: GLS
GLS, %
C: RASr
RASr
D: NT-proBNP
NT-proBNP, ng/L
No suspicion
Suspicion, DPD 0-1
Screened ATTRwt
Matched ATTRwt controls

Evolution of demographics of patients with transthyretin amyloid cardiomyopathy over time: implications for disease awareness strategies and future trial design

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM), is a fatal disease which leads to heart failure due to deposition of transthyretin amyloid fibrils. Although underdiagnosed, recognition of ATTR-CM has increased in recent years, likely due to improved disease awareness, development of non-invasive diagnostic techniques and disease-modifying therapies. The Phase 3 Tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT; NCT01994889) demonstrated reduced all-cause mortality and cardiovascular-related hospitalizations in patients with ATTR-CM (excluding New York Heart Association [NYHA] Class IV) treated with tafamidis vs placebo. A subsequent, ongoing long-term extension study (LTE; NCT02791230) enrolled those who completed ATTR-ACT and an additional cohort of patients several years later (including those with NYHA Class IV) to provide early access to tafamidis until local availability by prescription for ATTR-CM.

Objective: To investigate any changes in the baseline population demographics and clinical characteristics of ATTR-CM patients over time.

Materials & Methods: ATTR-ACT enrolled patients from 2013-2015. Following the ATTR-ACT read out, the early access cohort enrolled from 2018-2021. Patient demographics and clinical characteristics between all ATTR-ACT patients and the early access cohort (unplanned interim analysis) were compared.

Results: ATTR-ACT enrolled 441 patients. The early access cohort enrolled 1478 patients, 19 of whom were NYHA Class IV. Most patient baseline demographics and clinical characteristics were similar between the cohorts, including age, region, NYHA Class and Kansas City Cardiomyopathy Questionnaire Overall Summary score (**Table**). In the early access cohort, there was a higher percentage of ATTR wild-type (ATTRwt) and lower baseline levels of cardiac biomarkers (NT-proBNP, Troponin I) vs ATTR-ACT.

Summary & Conclusions: This analysis from ATTR-ACT and an early access cohort allows comparison of two temporally distinct cohorts of patients with ATTR-CM. The data illustrate that in general, baseline demographics of patients have remained similar over time, even with increasing disease awareness, non-invasive diagnostics, and disease-modifying therapy now available. There was a larger proportion of ATTRwt patients, and lower baseline levels of biomarkers of heart failure (NT-proBNP, Troponin I) reported in the more recent cohort, which also had 19 NYHA Class IV patients enrolled. Since ATTR-ACT specifically sought to include a certain percentage of patients with a variant genotype, the relative differences in proportions between cohorts may be artificial. Examining the baseline demographics of future trials would allow investigation into whether the growing awareness of ATTR-CM leads to earlier diagnosis and improvements in patient outcomes.

Table

Baseline characteristic	ATTR-ACT All ITT patients: 2013-2015 (N=441)	Early access cohort Tafamidis: 2018-2021 (N=1478)
Age, mean (SD), years	74.3 (7.01)	76.5 (7.75)
Gender, n (%)		
Male	398 (90.3)	1312 (88.8)
Female	43 (9.8)	166 (11.2)
Region, n (%)		
North and South Americas	282 (64.0)	884 (59.8)
Europe	142 (32.2)	467 (31.6)
Asia	17 (3.9)	49 (3.3)
Australia	0	78 (5.3)
NYHA Classification, n (%)*		
Class I/II	300 (68.0)	1002 (67.8)
Class III	141 (32.0)	456 (30.9)
Class IV	0	19 (1.3)
TTR Genotype, n (%)		
Variant	106 (24.0)	212 (14.3)
Wild-type	335 (76.0)	1266 (85.7)
NT-proBNP, n	441	122
Median pg/mL (range)**	3022.0 (298.0, 22020.6)	1670.0 (50.8, 28059.4)
Troponin I, n	440	123
Median ng/mL (range)**	0.14 (0.03, 12.2)	0.09 (0.03, 1.77)
KCCQ-OS Score, mean (SD)†	66.7 (21.5)	67.7 (23.4)

*1 patient was missing NYHA Classification in the early access cohort;
**Not available for full early access cohort, as added in a later protocol amendment from 09 August 2019;
†16 patients were missing KCCQ-OS score in the early access cohort.

ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; ITT, intend to treat; KCCQ-OS, Kansas City Cardiomyopathy Questionnaire Overall Summary; NYHA, New York Heart Association; SD, standard deviation; TTR, transthyretin.

Table: Baseline demographics and clinical characteristics of patients in ATTR-ACT and an associated LTE early access cohort.

Support & Funding: This study was sponsored by Pfizer. Medical writing support was provided by Caitlin Watson, PhD, of Engage Scientific Solutions, and funded by Pfizer.

Descriptive analysis of women with transthyretin amyloid cardiomyopathy: examining the patient demographics of a growing patient population

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM) is an underdiagnosed, fatal disease where deposition of transthyretin amyloid fibrils in the myocardium leads to heart failure. Historically recognized predominantly in men, increasing awareness of ATTR-CM has the potential to lead to the diagnosis of more women. The Phase 3 Tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT; NCT01994889) demonstrated reduced all-cause mortality and cardiovascular-related hospitalizations in patients with ATTR-CM treated with tafamidis. A subsequent, ongoing, long-term extension study (LTE; NCT02791230) enrolled those completing ATTR-ACT and an additional cohort of patients, to provide early access to tafamidis until local availability (prescription for ATTR-CM).

Objective: To describe the demographics and clinical characteristics of women with ATTR-CM enrolled in ATTR-ACT and an associated LTE early access cohort.

Materials & Methods: Patients in ATTR-ACT were enrolled from 2013-2015. Following the read-out of ATTR-ACT, the early access cohort enrolled patients from 2018-2021. Pooled patient baseline demographics of women in ATTR-ACT and the early access cohort (unplanned interim analysis) were compared with those of men from these two groups. NT-proBNP and troponin I values were not available for the full early access cohort (added in a protocol amendment, 09 August 2019).

Results: ATTR-ACT and the early access cohort enrolled 209 women and 1710 men. At baseline, mean age (76.5 vs 76.0 years), median NT-proBNP (2828.0 vs 2746.2 pg/mL) and median Troponin I (0.10 vs 0.13 ng/mL) were similar in women vs men, respectively. Higher proportions of Asian (20.0%) and Black or African American (27.2%) patients were female vs White (8.3%). A higher proportion of women vs men (40.7% vs 30.0%) were New York Heart Association (NYHA) Class III. The proportion of variant genotype was higher among female patients (45.5%) vs men (13.0%; **Table**).

Summary & Conclusions: This analysis from ATTR-ACT and an early access cohort examined the demographics and clinical characteristics in the growing population of women with ATTR-CM. While characteristics were generally similar between women and men, some differences emerged. A higher proportion of Asian and Black or African American patients were female vs White. A higher proportion of women were NYHA Class III vs male patients and more women had a variant genotype. As ATTR-ACT was designed to include a certain proportion of patients with a variant genotype, relative differences in the proportions of women and men may be artificial. The availability of non-invasive diagnostics, disease-modifying therapy and a general increase in disease awareness may lead to diagnosis of more women with ATTR-CM over time. Future studies have the potential to provide more robust data on the specific characteristics of women with ATTR-CM, ultimately leading to earlier diagnosis and improving patient outcomes.

Table

Baseline characteristic	Women (N=209)		Men (N=1710)		Total (N=1919)
	n	% of group	n	% of group	n
Race					
White	133	63.6	1477	86.4	1610
% of characteristic	8.3		91.7		
Black or African American	55	26.3	147	8.6	202
% of characteristic	27.2		72.8		
Asian	17	8.1	68	4.0	85
% of characteristic	20.0		80.0		
Other	4	1.9	18	1.1	22
% of characteristic	18.2		81.8		
NYHA Classification*					
Class I	25	12.0	232	13.6	257
% of characteristic	9.7		90.3		
Class II	94	45.0	951	55.7	1045
% of characteristic	9.0		91.0		
Class III	85	40.7	512	30.0	597
% of characteristic	14.2		85.8		
Class IV	5	2.4	14	0.8	19
% of characteristic	26.3		73.7		
TTR genotype					
Variant	95	45.5	223	13.0	318
% of characteristic	29.9		70.1		
Wild-type	114	54.6	1487	87.0	1601
% of characteristic	7.1		92.9		

*1 male patient was missing NYHA Classification.

NYHA, New York Heart Association; TTR, transthyretin.

Table: Baseline demographics and clinical characteristics of women and men with ATTR-CM enrolled in ATTR-ACT and an associated LTE early access cohort

Support & Funding: This study was sponsored by Pfizer. Medical writing support was provided by Caitlin Watson, PhD, of Engage Scientific Solutions, and funded by Pfizer.

Title: Assessment and Management of Older Patients with Transthyretin Amyloidosis Cardiomyopathy

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Background: Transthyretin amyloidosis cardiomyopathy (ATTR-CM) is caused by the deposition of misfolded transthyretin (TTR) proteins as amyloid fibrils in the myocardial extracellular space.[1] TTR is a predominantly hepatic derived transport protein responsible for transporting thyroxin and retinal binding protein (hence the name 'trans-thy-retin') through the circulation and is also commonly referred to as prealbumin.[2] ATTR-CM is subdivided into two types; hereditary (ATTRh, also variably referred to in the literature as mutant or variant type), which is caused by a mutation of the TTR gene, and wild-type ATTR (ATTRwt, previously referred to as senile systemic amyloidosis), which is an age-related disorder more commonly affecting men and occurring in the absence of a TTR gene mutation.[3,4] ATTR-CM is predominantly a disease of older adults. While the true prevalence of ATTR-CM is uncertain, it is commonly considered to be an under-recognized cause of heart failure in the community.[4] Improvements in diagnostic approaches, in particular significant advances in noninvasive cardiac imaging assessment, and disease awareness, along with an aging population, have resulted in increasing incidence rates of ATTR-CM.5 This, coupled with the improving availability of approved novel disease modifying therapies that improve survival for ATTR-CM patients are likely to contribute to a steady rise in prevalence in the future as patients live longer.[5] As a result of these significant advancements, optimizing the care of older ATTR-CM patients has become increasingly important. This task has implications for both the assessment and management of older ATTR-CM patients and presents a number of unique challenges for clinicians caring for this population. This review will examine several current aspects of the management of older ATTR-CM patients, including shared care with multiple medical specialists, the emerging importance of frailty assessment and other considerations for using ATTR therapies.

Objective(s):
Multidisciplinary care inclusive of collaboration with geriatric and elder care medicine specialists, and others such as neurology, orthopedics, electrophysiology and transcatheter aortic valve replacement clinics, is now an important component of ATTR-CM management. This review examines current aspects of the management of older ATTR-CM patients, including shared care with multiple medical specialists, the emerging importance of frailty assessment and other considerations for using ATTR therapies

Study methods: Literature review

Results: The predominant clinical manifestation of ATTR-CM is heart failure, while other cardiovascular manifestations include arrhythmia and aortic stenosis. Given their older age at diagnosis, patients often present with multiple age-related comorbidities, some of which can be exacerbated by ATTR, including neurologic, musculoskeletal, and gastrointestinal problems. Considerations related to older patient care, such as frailty, cognitive decline, polypharmacy, falls/mobility, functional capacity, caregiver support, living environment, quality of life and establishing goals of care are particularly important for many patients with ATTR-CM.

Conclusion(s): The prevalence of ATTR-CM is likely to continue to rise with improvements in disease awareness and diagnostic approaches in the context of an aging population. Advancements in disease modifying therapy will improve survival. With a greater number of older ATTR-CM patients being diagnosed and living longer, the need for a patient-centered multidisciplinary care approach rises for this complex patient population. Frailty assessment is anticipated to become increasingly important for this population, however further research is needed to determine how best to assess frailty and incorporate it into clinical care.

Funding Sources: None

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Preservation of Left Ventricle Stroke Volume in Patients with ATTRwt Cardiac Amyloidosis Treated with Selective TTR Stabiliser Tafamidis.

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Background: ATTR wild type (wt) cardiac amyloidosis is the commonest form of infiltrative cardiac disease caused by the extracellular deposition of wild type transthyretin (TTR). With time a progressive increase in LV wall mass leads to the reduction in LV cavity size but LV ejection fraction remains preserved until later stage of the disease. Echocardiography enables the estimation of LV wall thickness, LV volumes and LV strain and is the preferable imaging modality in initial diagnosis and follow up of this condition. Tafamidis is the only approved selective TTR stabiliser used to treat patients with this condition. Its protein stabilising action is responsible for favourable effect of the drug on major cardiovascular outcomes (including mortality and hospitalisation) found in ATTR-ACT study but its direct effect on myocardial function has not been yet fully elucidated.

Objective & Methods: We set out to examine the value of SV index in follow up of patients with ATTRwt cardiac amyloidosis. The study included patients diagnosed at the Queensland Amyloidosis Centre in Brisbane between 2010 and 2021. Comprehensive echocardiography was performed at Princess Alexandra Hospital Echocardiography Laboratory. Measurements of diastolic LV wall thickness, 2D and 3D LV ejection fraction, LV mass, 3D end systolic and end diastolic LV volumes and Doppler evaluation of diastolic function were performed. Stroke volume was measured using standard formula SV= π^2 (LVOT/2) ^{2} x LVOT VTI and was indexed by BMS in m2. LVOT VTI was measured by an experienced cardiac sonographer. 3 LVOT samples were recorded and an average VTI was calculated. The measurements were validated by an independent clinician to assess an inter-observer variability.

Results: Total of 164 patients was analysed (Fig 1). These patients had routine follow up and completed echocardiographic assessments at baseline and every 12 months. 93% of them (152 out of 164 patients) were males with median age of 80 years (66-91). The median NT-proBNP level was 1692pg/l, median hsTroponin I 46.5ng/l and median eGFR 55.9 mls/min/m2. The median SVi was 35mls/m2. 83 patients (51%) were in Gilmore stage 1, 54 patients (33%) were in stage 2 and 26 patients (15%) were in stage 3. Total of 31 patients received selective TTR stabiliser Tafamidis and their 12 monthly follow up data was available for analysis. In patients receiving Tafamidis we observed a preservation of SVi at 12 months of therapy whereas patients on no treatment showed progressive decline in SVi values (median change in SVi 0.16 vs. -1.94, $p<0.01$, Fig 2).

Summary & Conclusion: In ATTRwt cardiac amyloidosis a progressive extracellular amyloid deposition increases myocardial mass and wall stiffness. This directly leads to an upward and leftward shift in the end-diastolic pressure–volume relationship and results in observed reduction in stroke volume. Reduced stroke volume index and low LV strain have been included amongst others within the echocardiographic phenotype associated with worse prognosis. Recent publication showed an improvement in LV strain in patients treated with TTR stabiliser Tafamidis. Our study suggests protective effect of the drug on LV stroke volume which may explain the direct effect of the drug on myocardial function. As a result, we propose the addition of SVi to LV strain analysis to offer an additional measurable marker of response to the treatment.

Characteristic	Frequency
Sex - Males	93%
Age (years)	80 (66-91)
Clinical Stage (Gilmore)	
Stage 1	51% (83/164)
Stage 2	33% (54/164)
Stage 3	16% (26/164)
On TTR stabiliser (Tafamidis)	19% (31/164)
Echo/Laboratory parameters	**Median (SD)**
IVS (cm)	1.6 (0.35)
LVOT VTI (cm)	15 (4)
SVi (mls/m2)	35 (9)
GLS average (-%)	11.9 (7)
NT-proBNP (ng/l)	1692(3023)
hsTnI (ng/l)	46.5 (80)
eGFR (mls/min/m2)	55.9 (17)

Figure 1: Characteristics of 164 patients with ATTR cardiac amyloidosis included in this analysis

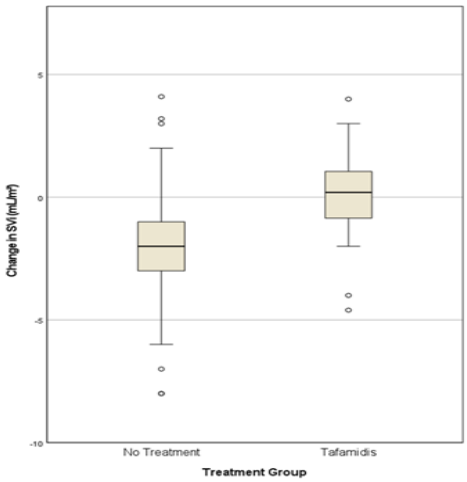

Figure 2: Change in SVi at baseline and 12 months in patients with ATTRwt cardiac amyloidosis according to treatment assignment: no therapy (left bar) vs. on therapy (right bar), median change 0.16 vs. -1.94, p<0.01.

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Implementation of a machine learning model to assess transthyretin amyloid cardiomyopathy risk in an external platform

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Background: Wild-type transthyretin amyloid cardiomyopathy (ATTRwt-CM) is an under-recognized cause of heart failure (HF) characterized by amyloid fibril deposits in the heart.[1] A machine learning (ML) model was developed to assess a patient's risk of ATTRwt-CM and performed well in identifying ATTRwt-CM versus non-amyloid HF.[2] We expand on this work by presenting a container-based platform implementation to improve third-party accessibility to the ATTRwt-CM ML model.

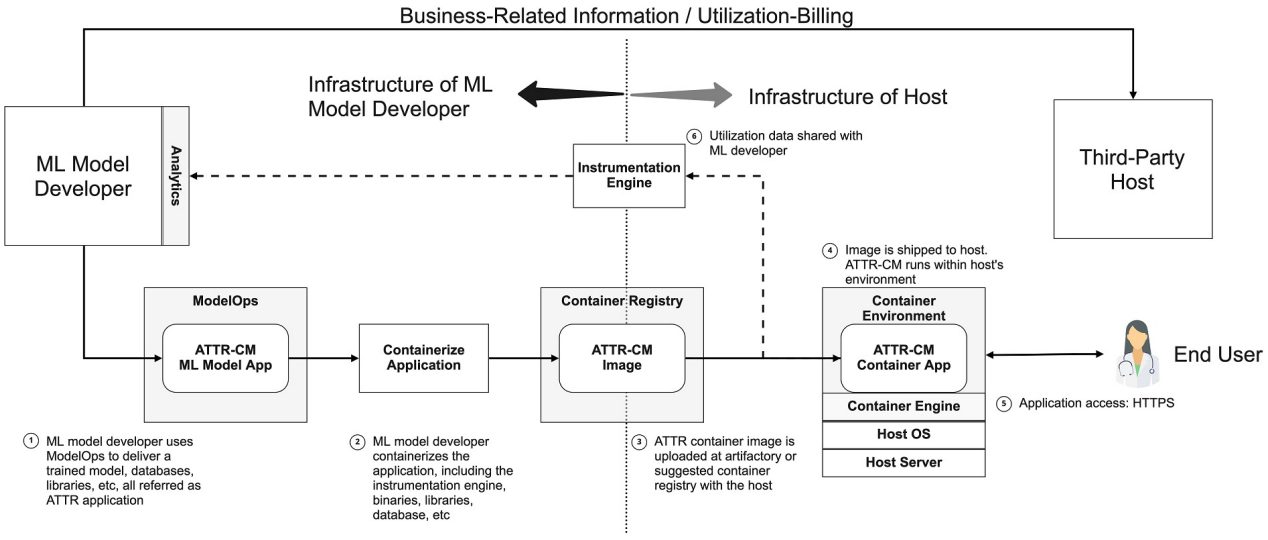
Objective: To describe the implementation of the ATTRwt-CM ML model in a third-party platform for use by healthcare providers (HCPs) to identify patients with high ATTRwt-CM risk and facilitate earlier diagnosis.

Material & Methods: Several factors were considered in choosing the best approach to deliver the model to a third-party platform. The application (app) needs to be trusted by HCPs, easily managed and scalable, and assume low/moderate technical knowledge of the host. The app should be able to track utilization, recall, billing, and other services, and to grow and adapt to meet the demands needed for ML model iterations and adjustments learned through model use, including Federated Learning opportunities. Lastly, patient data should remain within the host's platform (per General Data Protection Regulation rules), and the model's intellectual property (IP) should be protected. Technical approaches and architectural designs considered included shipping as a standalone container image (i.e., a static file with executable code to create a container in a computing system); shipping as media; creating a SMART on FHIR app; sharing open source; running a web app through a browser; and creating an executable app.

Results: The team opted to ship the app as a standalone container image to a third-party host used by a variety of hospitals to test, verify, and validate the feasibility of this approach for sharing this and other ML models (Figure). Objectives of this pilot included defining the architectural design and technical parameters for sharing the container image; capturing metrics from running the container image in a test environment; creating a scalable framework to manage multiple apps hosted on numerous third-party platforms; exploring security and IP issues when running the app on a third-party platform; and understanding the effort and validity of container image integration by the third-party platform. The container will take in patient data, analyze it, and return the results to the HCP. The ML model developer and host will agree on user interface visualizations and interaction with the model results, which will be tested by HCPs. The developer will not have access to patient data but will have access to metrics such as app engagement and performance data.

Summary & Conclusion: Implementation of the ATTRwt-CM ML model in a third-party platform may enable earlier identification and treatment of this fatal disease. Learnings from this pilot may also inform delivery of other related ML models to HCPs.

Figure. Shipment of container image to host.



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Support & Funding: This study was sponsored by Pfizer.

Clinical and Socioeconomic Differences Among Patients with Transthyretin Cardiac Amyloidosis Belonging to North and South of Chicago.

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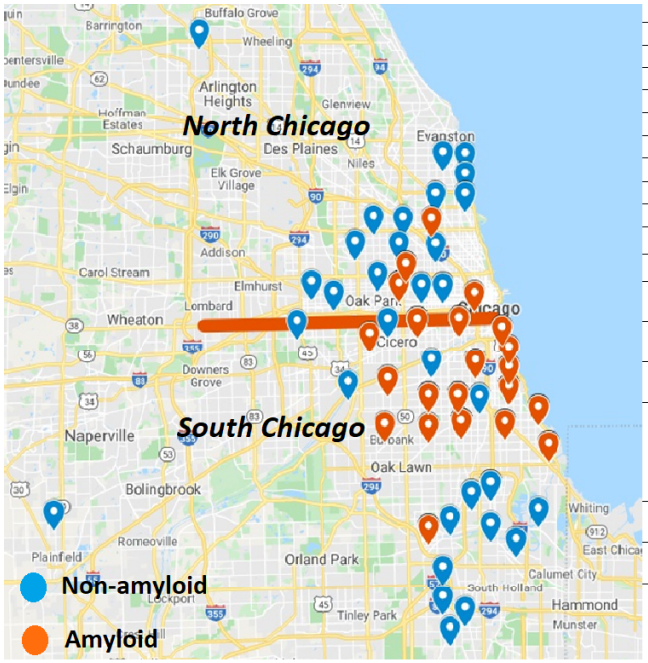
Background: Disparities exist within the city of Chicago and are known to be influenced by the location of residence. Similar to the entire US, there is an increasing prevalence of transthyretin cardiac amyloidosis (ATTR-CA) in Chicago.

Objective: We aimed to determine local clinical and socioeconomic differences among ATTR-CA patients within Chicago.

Material & Methods: A retrospective analysis of all data from patients undergoing Technetium Pyrophosphate (PYP) scintigraphy at Cook County Health was performed. Diagnosis of ATTR-CA was based on the results of PYP scan and assessment of paraproteinemia. Demographic and clinical data were obtained from electronic medical records. Socioeconomic data from zip code of the patient's residence was obtained from publicly available 2010 census summary data. Interstate-290 was used to demarcate north and south Chicago (Figure).

Results: Overall, 161 patients (47 north vs. 114 south Chicago) were included in this analysis. Of these, 45 (14 [32%] north vs. 31[68%] south) had ATTR-CA. Among those with ATTR-CA, there was a greater proportion of black patients from south Chicago and these patients had a lower mean LVEF (Figure). ATTR-CA patients from south Chicago belonged to zip codes with lower annual income, higher unemployment, a higher number of uninsured individuals, and fewer social security beneficiaries (Figure).

Summary & Conclusion: Local clinical and socioeconomic differences exist among ATTR-CA patients within Chicago. These differences could influence the understanding of the disease, access to medical therapy and outcomes from ATTR-CA.



	South [n=31(68.8%)]	North [n=14(32.2%)]	P value
Age	77 (73.8-80.1)	74.8 (68.8-80.8)	0.64
Female	12 (38.7%)	5 (35.7%)	0.84
African American	27(87%)	10(71.4%)	0.44
CKD stage ≥3	22(71%)	12(86%)	0.28
CHF	30(97%)	12(86%)	0.16
HTN	16(51%)	6(43%)	0.58
DM	9(29%)	2(14%)	0.37
CAD	1(3.2%)	2(14%)	0.16
Atrial fibrillation	10(32%)	5(35%)	0.66
EF	38.1 (31.9-44.2)	44.6 (35.2-54)	0.29
Died	5(16%)	4(28%)	0.21
Socioeconomic status, mean (SD)			
Population	56536 (44766-68306)	67606 (54418-80795)	0.178
Male	46.5(45.6-47.4)	52.3(49.8-54.8)	<0.001
Racial distribution:			
European white	21.2(12.2-30.2)	37(23.7-50.2)	0.02
African American	66.9(53.4-80.4)	41.8(21.5-62.1)	0.017
Hispanic	19.9(1.2-29.7)	41.7(27.8-55.5)	0.011
Asian	2.7(0.16-5.6)	5(1.5-8.5)	0.055
Income	37391 (32946-41835)	46184 (34373-57996)	0.169
Social security beneficiaries	15.6(14.3-16.8)	12.2(10-14.3)	0.006
Insurance:			
Private	42.3(38.6-46)	44.8(35.3-54.3)	0.95
Public	46.8(43.5-50.2)	37.5(29.1-46)	0.15
None	9.6(8.6-10.6)	13.7(11.2-16.2)	0.0008

Figure 1.: Showing the map of amyloid and non-amyloid patients distributed between North and South Chicago. Orange line showing demarcation between North and South Chicago. Table showing baseline clinical characteristics and zip-code level socioeconomic characteristics of patients.

Support & Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Relationship of tafamidis binding site occupancy, transthyretin stabilization, and disease modification in tafamidis treated transthyretin amyloid cardiomyopathy patients

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Background: Tafamidis inhibits progression of transthyretin (TTR) amyloid cardiomyopathy (ATTR-CM) by binding TTR tetramer and inhibiting its dissociation to monomers which can denature and result in amyloid fibril formation and deposition in cardiac tissue.

Objective: While the phase 3 ATTR-ACT clinical data clearly demonstrated efficacy, quantification of TTR stabilization in patients and the degree to which the approved dose captures the full potential of the mechanism has yet to be assessed.

Material & Methods: Isothermal titration calorimetry and subunit fraction exchange were used to determine affinities to the two binding sites on TTR. These values were used to develop a model of tafamidis binding to TTR in plasma that was applied to individual patient data to calculate TTR binding site occupancy and the change in total TTR levels induced by TTR stabilization. Population pharmacodynamic (PD) models were developed for three measures of disease progression, plasma NT-proBNP levels, Kansas City Cardiomyopathy Questionnaire – Overall Score (KCCQ-OS), and six-minute walk test (6MWT) distance, to evaluate patient response with the degree of TTR occupancy.

Results: In vitro binding data of wild-type TTR confirmed tafamidis binds two sites of TTR with negative cooperativity and provided precise estimates of the binding affinity to TTR and albumin. Modeling individual patient data of tafamidis exposure and increased TTR plasma levels using the in vitro derived binding affinity values confirms single site binding is consistent with complete tetramer stabilization. Patients given 80 mg tafamidis meglumine, the clinically approved dose for ATTR-CM, had a 92% reduction in unbound, unstabilized TTR, which correlated with a 53% decrease in NT-proBNP elevation, a 56% decrease in KCCQ-OS worsening and a 49% reduced decline in the 6MWT. For 100% receptor occupancy and stabilization, the expected effects on these measures are 58%, 61%, and 54% for NT-proBNP, KCCQ-OS, and 6MWT, respectively.

Summary & Conclusion: These results demonstrate a quantitative relationship between TTR stabilization, the mechanism of action of tafamidis, and accepted laboratory and patient-based outcomes in ATTR-CM. These results also support the value of TTR stabilization as a clinically beneficial treatment option which maintains the protein in its physiologically active form within the body.

Support & Funding: This study was sponsored by Pfizer.

Diagnostic Path, Clinical Characteristics and Outcomes of Patients With ATTR Cardiomyopathy in Greece

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Background: Transthyretin (TTR)-related cardiomyopathy (CM) is caused by deposition of TTR amyloid fibrils in the heart but also in various other tissues. Wild type (wt) ATTR primarily affects the heart causing restrictive CM while TTR variants may also be associated with a predominantly cardiac rather than neuropathic phenotype. ATTR-related CM is a common cause of heart failure among older individuals and the characteristics of this vulnerable population may differ significantly in different series and different countries.

Objectives: to describe the characteristics and outcomes of patients with ATTR CM in a real-world setting from Greece.

Material & Methods: this is an analysis from a prospectively maintained database of consecutive patients with ATTR CM from a single center (Department of Clinical Therapeutics).

Results: The analysis included 90 patients with ATTR CM diagnosed between 2014 and 2021. Median age at diagnosis was 81 years (range 52-92) and 85% were males. At the time of diagnosis, approximately 50% of patients were classified as New York Heart Association (NYHA) II; median NTproBNP was 2761 pg/ml and median hsTnT was 55 ng/l. All patients had positive Tc99m-PYP bone scans and median H/CL ratio was 1.7 (range 1.5-2.4). Fat aspirates were positive for Congo red in 26% of tested patients. Concomitant paraproteinemia was found in 10% of patients while genetic testing revealed *TTR* mutations in 9%. The clinical presentation varied: peripheral edema was present in 57%, 23% had received a pacemaker and 52% had a history of atrial fibrillation while aortic stenosis was found in 14% of patients. In cardiac echo, median IVS and posterior wall thickness were both 15mm, median LV ejection fraction was 50% and median GLS was -10%. A cardiac MRI was conducted in 48% of patients and was typical for amyloidosis in all. Common extracardiac symptoms included peripheral neuropathy (44%), carpal tunnel syndrome (40%, bilateral in 30%), gastrointestinal symptoms (36%), autonomic neuropathy (22%), postural hypertension (15%) and tendon rupture (12%); renal dysfunction (eGFR <60 ml/min/1.73 m2) was present in 43% of patients. Treatment for heart failure included diuretics in 90% and b–blockers in 71%, while 45% were receiving drugs blocking the renin-angiotensin axis (ACE inhibitors or ATII blockers) and 50% of patients were on anticoagulants. Regarding amyloidosis-specific treatment, 50% of the patients received tafamidis, 20% doxycycline and 6% antisense oligonucleotide or siRNAs. After a median follow up of 2 years, 36% required hospitalization for management of heart failure, at least once, and the cumulative survival at 1 and 2 years was 96% and 81% respectively. A baseline level of NTproBNP >5000 pg/ml was associated with a 2-year OS of 62% vs 94% for those with lower NTproBNP (p=0.005); a NYHA stage >2 was also associated with a 2-year OS of 54% vs 95% for NYHA stage 2 (p=0.001). The adjusted 2-year OS for those that started tafamidis (from the date of initiation of the drug) vs those that did not receive tafamidis was 85% vs 71% respectively (p=0.289) and remained non-significant after further adjustment for NTproBNP and NYHA stage (p=0.864), probably reflecting the advanced stage of the disease and the advanced age at the time of initiation of the drug.

Conclusions: ATTR CM is a systemic disease; advanced cardiac dysfunction is associated with poor outcomes, despite the use of tafamidis. Early diagnosis and initiation of targeted therapy could probably improve outcomes of patients with ATTR CM

Screening for transthyretin-related amyloidosis in patients with aortic stenosis planned for aortic valve replacement

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Background: Aortic stenosis (AS) is the most prevalent valvular heart disease, leading to a high morbidity and mortality if left untreated. The guideline-directed therapy comprises transcatheter (TAVI) or surgical aortic valve replacement (SAVR). In up to 15% of AS patients, a concomitant cardiac amyloidosis (CA) results in aggravated heart failure and reduced life expectancy. Despite its high prevalence, CA is often overlooked, and targeted pharmacological treatment is not prescribed.

Objective: The SAVER study (Screening for Amyloidosis before Aortic Valve Elective Replacement) aims to establish a simple CA screening tool for AS patients planned for TAVI or SAVR. Here, we present the design and first results of the study.

Material & Methods: The SAVER study is a multicenter, prospective cohort trial started in 2021. AS patients, excluding patients with New York Heart Association (NYHA) class IV, redo-TAVI or -SAVR or ongoing chemotherapy, are enrolled into the study and screened for concomitant transthyretin-related (ATTR) CA in daily clinical practice. The CA screening approach consists of 27 parameters including typical symptoms of amyloidosis, previous diseases, laboratory parameters, an electrocardiogram (ECG) and echocardiographic measurements. Except for the CA-specific patient questionnaire, all examinations are part of routine clinical practice to evaluate AS before treatment. In case of four or more positive indices, the patients are recommended to undergo a bone scan or a magnetic resonance imaging (MRI) and are referred to the Amyloidosis Center Charité Berlin (ACCB), Charité – Universitätsmedizin Berlin, Germany. Further measurements comprise neurological examinations and laboratory tests such as serum and urine immunofixation as well as measurement of serum free light chains to exclude light chain amyloidosis.

Results: The preliminary analysis included 536 AS patients who underwent CA screening. The patients reported the following complaints as part of the CA-specific patient questionnaire: hands that fall asleep (13.6%), an unsteady gait (27.6%), heaviness and numbness of arms or legs (21.8%), gastrointestinal complaints (12.3%) and low blood pressure (8.2%). 14.7% of patients suffered from carpal tunnel syndrome, 9.3% from spinal stenosis, 7.8% from polyneuropathy and 3.2% from biceps tendon rupture. Laboratory measurements, ECGs and echocardiographic examinations will be analyzed to determine the individual risk for CA. Final results are expected in 2023.

Summary & Conclusions: The SAVER approach will provide a simple, rapid, and cost-effective way to screen for ATTR-CA in AS patients in daily clinical practice.

Funding: Pfizer Pharma GmbH, Berlin, Germany

Case series of the treatment journeys of patients who underwent heart transplantation for transthyretin (ATTR) cardiac amyloidosis, with subsequent confirmed orthopedic disease

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BACKGROUND: Heart transplantation for transthyretin amyloidosis (ATTR) ameliorates heart failure symptoms and has survival rates similar to other cardiomyopathies[2]. However, long-term morbidity burden remains high due to extra-cardiac ATTR disease progression[1], including neuropathy due to spinal stenosis, carpal tunnel, and joint pain. Meanwhile, paradigms for surveillance of disease recurrence at cardiac or extra-cardiac sites after heart transplant for ATTR are not well established. The presence of amyloid deposits in ligaments and joints is suspected to be of pathologic significance but is not routinely investigated in ATTR patients who undergo orthopedic surgery. Thus, heart transplant recipients from ATTR cardiomyopathy, with disease-related complications often cannot be prescribed an ATTR stabilizer (Tafamidis) due to the lack of indication for musculoskeletal complications and high drug costs.

OBJECTIVE: Herein, we describe the post-heart transplant course of 3 patients with ATTR, 2 of whom were subsequently treated with Tafamidis.

MATERIAL & METHODS: We reviewed data for 3 heart transplant recipients transplanted for ATTR who had biopsy proven ATTR in skeletal tissues, diagnosed after heart transplant. We examined post-transplant notes for orthopedic symptoms, relevant imaging, and pathology results. All 3 patients had post-transplant heart biopsies that were negative for recurrent cardiac amyloidosis.

RESULTS: All patients were male and in the 7th decade of life at ATTR diagnosis; 2 had wild type (ATTRw), 1 had variant type (ATTRv). Mean age at diagnosis of ATTR was 65 years. Mean age of heart transplant was 67 years. Time from diagnosis of ATTR to heart transplant ranged 1-4 years. Time from heart transplant to orthopedic surgery was another 1-4 years. 2 patients had subsequent ATTR detected in the ligamentum flavum of the spine and 1 in the synovial joint of the hip. Of the 3 cases, 1 received Tafamidis prior to transplant. 2 were diagnosed with ATTR prior to FDA approval of Tafamidis: 1 subsequently started on Tafamadis while 1 was denied insurance coverage, despite multiple appeals. A Quality of life (QOL) assessment by KCCQ-12 was lower in the patient not on Tafamidis.

SUMMARY & CONCLUSION: Tafamidis has not been prospectively studied in ATTR patients after heart transplant. However, recent studies indicate that extracardiac disease progression is common after heart transplant. These data suggest a need to monitor for progression of ATTR at sites other than the transplanted heart, especially in the spine, joints, and transverse carpal ligament[4]. Further studies are needed to understand the pathologic significance of amyloid deposition in these sites and whether initiating or continuing Tafamidis can reduce amyloidosis-related morbidity. Given the safe toxicity profile of TTR stabilizers, consideration can be made to resuming these agents in the post-transplant setting, but such decisions can be affected by denial of drug coverage[3]. Utilization of patient assistance programs and patient advocacy could be helpful in overcoming barriers. Treatment studies focusing on this sub-group of patients are needed to further elicit the utility of TTR stabilizers in preventing extracardiac deposition of amyloid and its effects on QOL. ATTR patients post-heart transplant should

continue to follow with specialized amyloidosis centers to routinely screen for new or worsening systemic symptoms and to potentially participate in future ATTR studies.

TABLE 1:

Age at Dx of ATTR	*ATTR Type*	*Age at Heart Transplant*	*Evidence of Neuro Changes*	*Evidence of GI Changes*	*Age When Ortho Symptoms Noted*	*Positive Orthopedic Biopsy Type*	*Age at Positive Ortho Biopsy*	*Tafamidis After Heart Transplant*	*Age Started on Tafamidis*	*KCCQ Score after Transplant*
63	ATTRv	64	No	No	55	Synovial Joint in Hip	65	Yes	63	92
69	ATTRw	70	Yes	No	71	Ligamentum Flavum	74	No	N/A	53
65	ATTRw	69	Yes	No	61	Ligamentum Flavum	70	Yes	71	N/A

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Factors Associated with Financial Toxicity in Patients with ATTR: Results From Amyloidosis Research Consortium's ATTR Treatment Affordability Patient and Caregiver Survey

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Background: Transthyretin amyloidosis (ATTR) is a rare, progressive and often fatal disease in which the protein transthyretin becomes unstable, misfolds, and deposits in various organs, primarily the heart and the nervous system. ATTR can be categorized into two types, hereditary variant (ATTRv) and wild-type (ATTRwt). Approved therapeutics are able to slow down disease progression and improve survival, hospitalization rates and quality of life; however, the financial cost of these therapeutics can be an obstacle for patients. Financial toxicity is an objective measure of the deleterious effect of financial stress caused by the cost of care on the well-being of patients and their families.

Objective: The objective of this study was to describe the factors associated with financial toxicity and the economic burden of ATTR care and treatment.

Material & Methods: The Amyloidosis Research Consortium (ARC) conducted a US based online survey in patients with ATTR amyloidosis and caregivers from 3Aug2021 – 31Jan2022. Data included demographics, disease characteristics, healthcare resource utilization and costs of treatment and care. Financial toxicity was measured using the Comprehensive Score for financial Toxicity-Functional Assessment of Chronic Illness Therapy (COST-FACIT). Fisher's exact tests and Pearson's chi-squared were used to evaluate differences by financial toxicity groups. A general linear model was performed examining the differences in COST scores and adjusting for ATTR type, annual household income, work status, time since diagnosis, and performance status (ECOG).

Results: 452 patients and caregivers completed the survey. 249 (55%) reported some level of financial toxicity (COST score <26). The mean COST score was 24. Majority of patients had ATTRwt [295 (69%)], were retired [342 (76%)] and reported annual household income <$100,000 [243 (54%)]. Mean (standard deviation (SD)) monthly cost of managing ATTR was $728.69 ($1,711.67) and the mean (SD) monthly cost of ATTR treatment was $645.41 ($2,880.24). To offset some of these costs 97 (21%) patients are currently enrolled in a clinical trial, 192 (45%) receive co-pay assistance, and 195 (43%) patients used savings, borrowed money, or made trade-offs such as not taking a vacation or selling a house. Unadjusted factors that impacted the COST score, causing the score to be lower (indicating more financial toxicity) were having ATTRv, lower household income, being unemployed, having been diagnosed in the past 12 months, choosing a treatment based on cost or insurance coverage, having higher psychological distress, and a reporting a loss of income due to the COVID-19 pandemic (Figure 1). Factors that increased financial toxicity after adjusting for ATTR type, income, employment status, ECOG and time since diagnosis were: choosing a treatment based on cost or insurance coverage; having higher psychological distress; receiving co-pay assistance; paying for treatment with savings, borrowing money, or trade-offs; and reporting a loss of income due to the COVID-19 pandemic (Figure 2). Annual household income had the greatest impact on financial toxicity.

Summary & Conclusion: Financial toxicity is significant in ATTR and comparable to patients with cancer[1,2]. Access to clinical trials and co-pay assistance programs may not be enough to offset the cost of managing and treating ATTR as patients have to make trade-offs for paying for treatment, either using savings and/or borrowing money to pay for treatment and leading to increased financial and/or psychological distress.

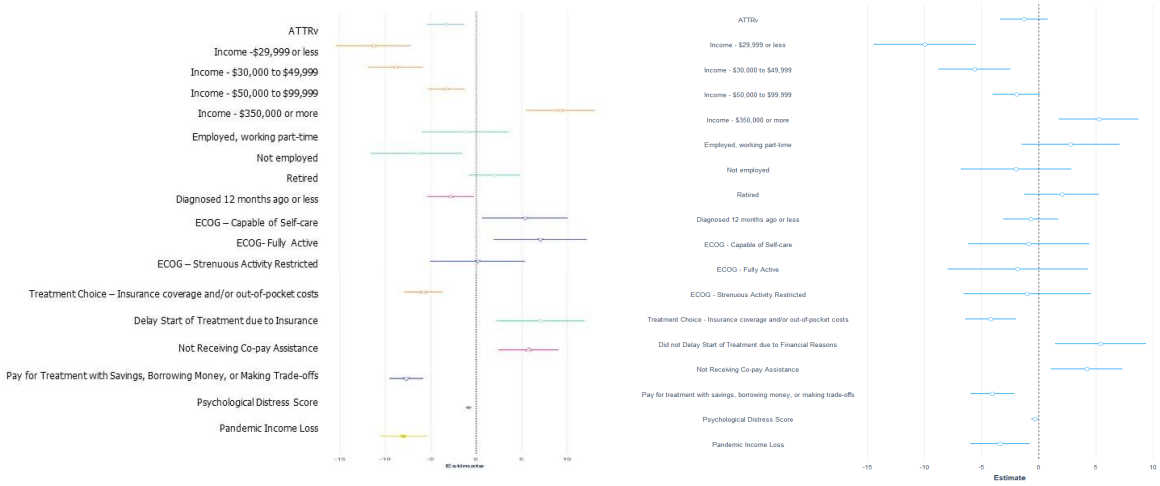

Figure 1: Unadjusted Factors Associated with Financial Toxicity

Figure 2: Adjusted Linear Regression of Factors Associated with COST score

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Characterisation of Austrian Transthyretin Amyloid Cardiomyopathy (ATTR-CM) patients enrolled in a Tafamidis (61mg) early access program

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM) is a rare, progressive and life-threatening disease characterised by the accumulation of amyloid fibrils in the extracellular space of the myocardium.[1] The prognosis of ATTR-CM is generally poor with a median survival of 2.5-3.5 years and 3.6 years in untreated ATTRv (variant transthyretin amyloidosis) and ATTRwt (wild-type transthyretin amyloidosis) patients, respectively.[1,2] Increasing awareness for the clinical presentation of the disease has led to the transformation of ATTR-CM to a relevant condition, that cardiologists and physicians may face in their clinical routine.[3] The availability of real-world evidence (RWE), however, still remains limited due to the rare nature of ATTR-CM.

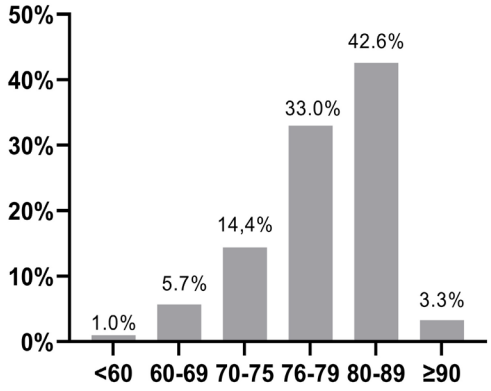
Objective: Aim of this analysis was to describe the baseline characteristics of Austrian ATTR-CM patients enrolled in a local early access program for the prescription of Tafamidis 61mg.

Material & Methods: All patients eligible for enrollment in the Tafamidis early access program were included in this analysis. Eligibility criteria for the early access programm included a confirmed diagnosis of ATTR-CM (via scintigraphy, biopsy or both) and the absence of contraindicatons for the prescription of Tafamidis 61mg (e.g. AL amyloidosis, or NYHA class IV). Physicians were asked to provide baseline characteristics of patients (e.g. age, sex, NYHA class etc.) upon patient enrollment which were extracted from anonymised patient clinical summaries for this analysis.

Results: At the timepoint of data cut-off (03/2020) a total of 209 patients (ATTRwt n=208, ATTRv n=1, 84.7% male) with confirmed ATTR-CM were enrolled in the early access program for Tafamidis 61mg. Mean age at enrollment was 79.1 years (80.8 years in females & 78.7 years in males) (Figure 1). Scintigraphy was used in most patients to diagnose ATTR-CM (n=158, 81.9%) whereas a biopsy was performed in 24 patients (12.4%). In 11 patients (5.7%) a combination of scintigraphy and biopsy was required to confirm the diagnosis of ATTR-CM. According to NYHA class, the majority of patients presented in NYHA II (n=83, 44.9%) at baseline (Figure 2).

Summary & Conclusion: This analysis currently represents one of the most comprehensive Austrian real-world

analyses of baseline characteristics in ATTR-CM patients from a cohort of patients enrolled from centers all over Austria between 12/2018 and 03/2020. Compared to the ATTR-ACT trial cohort, the local cohort analysed consisted of older patients and comprised more females. In terms of NYHA class the Austrian early-access program inlcuded slightly more patients in NYHA II-III compared to ATTR-ACT, whereas less patients in NYHA I were enrolled in the Tafamidis early access program.[4]



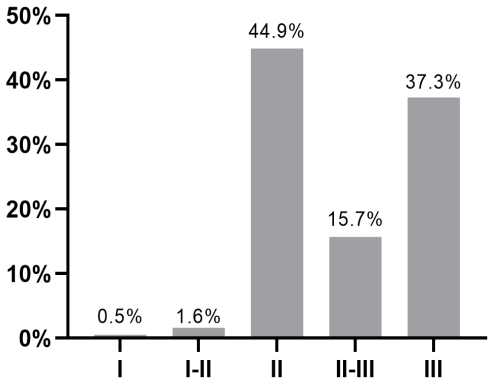


Figure 1.: Age distribution of ATTR-CM patients (n=209) enrolled in the Austrian Tafamidis 61mg early access program

Figure 2.: Distribution of patients enrolled in Austrian Tafamidis 61mg early access program according to NYHA class (n=185)

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Incidence and Risk Factors for Pacemaker Implantation in Light Chain and Transthyretin Cardiac Amyloidosis

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Background: The incidence and risk factors of pacemaker (PM) implantation in patients with cardiac amyloidosis (CA) are largely unexplored. We sought to characterise the trends in the incidence of permanent PM and to identify baseline predictors of future PM implantation in light chain (AL) and transthyretin (ATTR) CA.

Objective: The aim of this study was to characterise the trends in the incidence of PM implantation and to identify baseline parameters able to predict the future need of PM implantation in a large cohort of well-characterised patients with AL and ATTR-CA.

Material & Methods: Consecutive patients with AL and ATTR-CA diagnosed at participating Centres (2017-2020) were included. The diagnosis of AL and ATTR-CA was confirmed by tissue biopsy or through established non-invasive criteria, according to the latest recommendations from the European Society of Cardiology. Clinical data recorded within ±1 month from diagnosis were collected from electronic medical records. The primary study outcome was the need for clinically-indicated PM implantation. Reversible causes of conduction system disease were systematically ruled out, including those drug-related, before implantation in all patients receiving a PM during follow up. For the purpose of estimating the incidence of PM implantation during follow up, patients with PM (n=41) and/or permanent defibrillator in situ (n=13) at CA diagnosis were excluded.

Results: The study population consisted of 405 patients: 29.4% AL, 14.6% variant ATTR and 56% wild-type ATTR; 82.5% were males, median age 76 years. During a median follow-up of 33 months (interquartile range 21-46), 36 (8.9%) patients experienced the primary outcome: 10 AL-CA, 2 variant ATTR-CA and 24 wild-type ATTR-CA (p=0.08 at time-to-event analysis). At multivariable analysis, history of atrial fibrillation (hazard ratio [HR] 3.80, p=0.002), PR interval (HR 1.013, p=0.002) and QRS >120 ms (HR 4.7, p=0.001) on baseline ECG were independently associated with PM implantation. The absence of these 3 factors had a negative predictive value of 92% with an area under the curve of 91.8% at 6 months.

Summary & Conclusion: In a large cohort of well characterized patients with AL and ATTR-CA,

the incidence of PM implantation was high accounting for 8.9% of patients in the 3 years following the diagnosis. History of AF, PR interval and QRS >120 ms on baseline ECG independently predicted the future need of PM implantation in both AL and ATTR-CA, while disease etiology did not. While CA patients with these features might need close monitoring during follow-up for the development of conduction system disease requiring PM implantation, the absence of all risk factors accurately identified patients without need for PM implantation in the first 6 months after diagnosis.

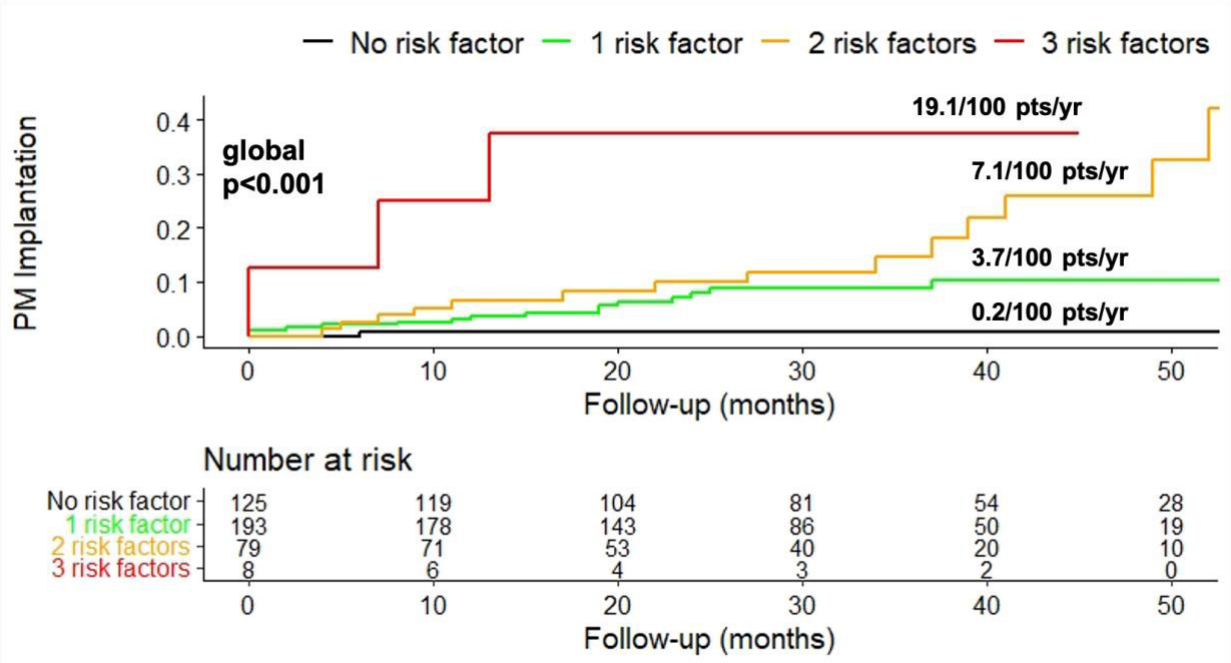

Figure 1.: Incidence rate of PM implantation according to 1) history of AF, 2) PR interval >200 ms, and, 3) QRS >120 ms at baseline. Cumulative incidence is measured as n. of events/ 100 patients/ year. The rate of PM implantation is showed on the y-axis as a percentage. Legend: AF. Atrial Fibrillation; ms. milliseconds; PM. Pacemaker; pts. patients; yr. year.

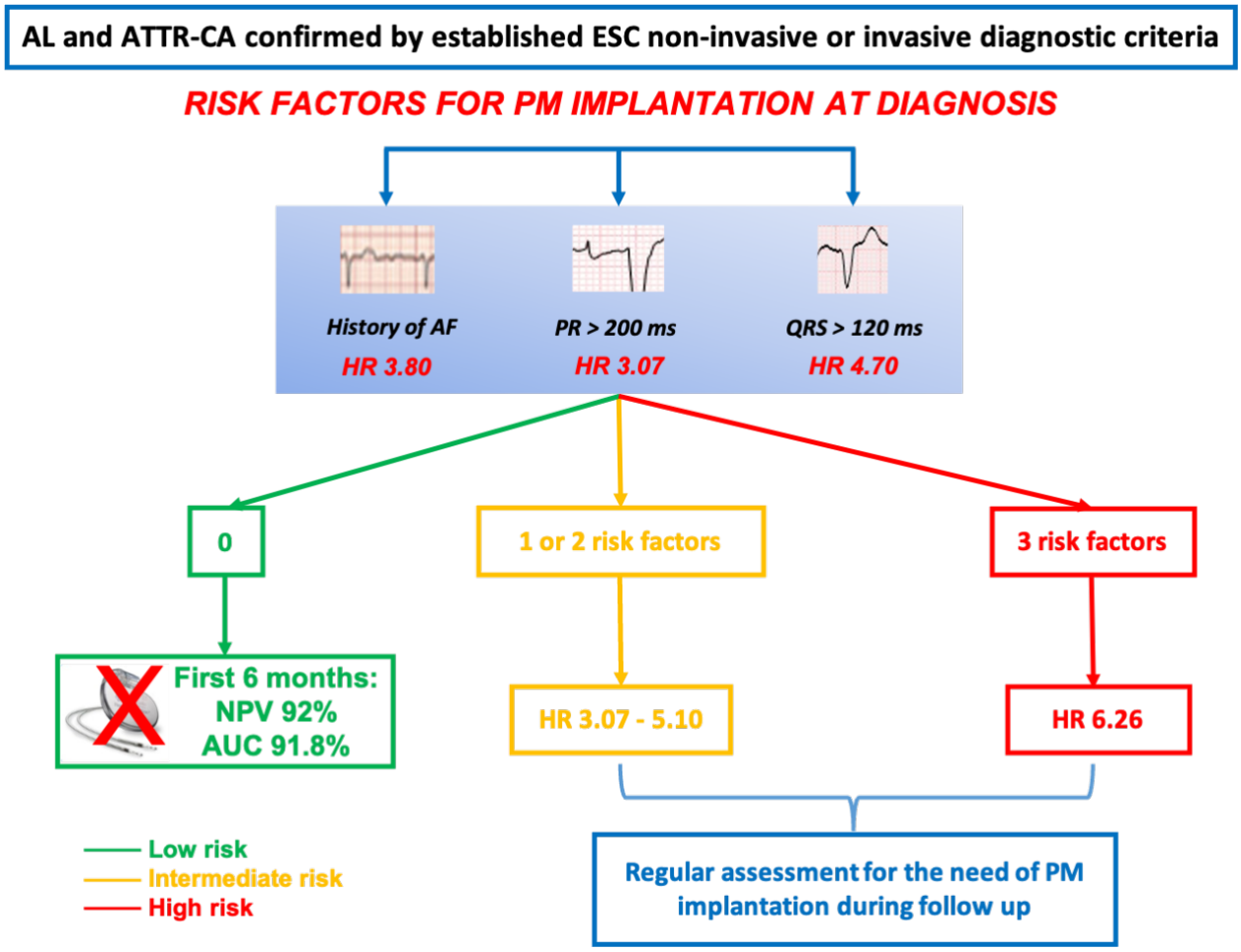

Figure 2.: Proposal of a flow-chart to estimate the risk of PM implantation in patients with AL and ATTR-CA. Legend: AF. Atrial Fibrillation; AL. Light Chain Amyloidosis; ATTR. Transthyretin Amyloidosis; AUC. Area under the Curve, CA. Cardiac Amyloidosis; ESC. European Society of Cardiology; FUP. Follow Up; ms. milliseconds; HR. Hazard Ratio; NPV. Negative Predictive Value; PM. Pacemaker.

The relationship between NT-proBNP and perception of the severity of cardiac symptoms in TTR-CA: the moderating role of anxious and depressive symptoms

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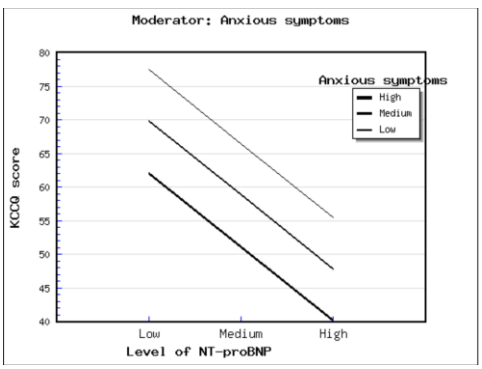
Background: Cardiac amyloidosis (CA) is a significant cause of heart failure (HF) of which a reliable prognostic marker, both of HF and CA, is represented by the level of the N-terminal cleavage of BNP's prohormone (NT-proBNP) (Maleszewski, 2022; Merlini, 2017). In evaluating the severity of cardiac symptoms, the perception that the patient has of own clinical condition is also important, so much that in clinical setting, extensive use is made of self-report questionnaire, such as the Kansas City Cardiomyopathy Questionnaire (KCCQ) (Green et al., 2000). At this regard, however, it is important to underline that the personal assessment of the severity of cardiac symptoms could also be influenced by the patient's psychological state, such as his/her levels of anxiety and depression (Smorti et al., 2016), so that not always the score at the KCCQ can reflect the real personal functioning.

Objective: The aim of this study is to explore the relationship between the level of NT-proBNP and subjective perception of the severity of cardiac symptoms taking in consideration the moderating role of the levels of anxious and depressive symptoms in a clinical group of patients with CA.

Material & Methods: A total of 33 patients (28 males and 5 females) affected by ATTR CA, aged between 62 and 90 (M = 78.74; DS = 6.28) were recruited from the Tuscan Regional Amyloidosis Centre in Florence, Italy. All patients filled out the Italian version of the Hospital Anxiety and Depression Scale (HADS, Costantini et al., 1999) in order to assess the level of anxiety and depression, and the Italian version of the Kansas City Cardiomyopathy Questionnaire (KCCQ; Miani et al., 2003) to measure subjective perception of cardiac symptoms severity. Moreover, the plasma level of NT-proBNP and the glomerular filtration rate (GFR) were also registered within 2 weeks of completing the questionnaires.

Results: The level of GFR did not affect the level of NT-proBNP (ß = -.193, p = .282), HADS_anxiety (ß = -.090, p = .617), and HADS_depression (ß = -.100, p = 580). Moderator analyses shoewd that role of both anxiety (ß = .320, p = .032) and depression (ß = .532, p = .012) on the relationship between NT-proBNP and KCCQ scores. In particular, higher levels of NT-proBNP were more strongly associated with lower levels of KCCQ scores at higher level of anxious and depressive symptoms (Figure 1 and 2). However, the relationship between NT-proBNP and KCCQ scores was non-significant when anxious symptoms were low, and depressive symptoms were high.

Summary & Conclusion: NT-proBNP is a significant predictor of cardiac symptom severity (Müller-Tasch, Krug & Peters-Klimm, 2021): higher level of NT-proBNP predicted a lower self-reported symptom severity. However, predictivity of NT-proBNP on KCCQ scores becomes not-significant for low level of anxiety and for high level of depressive symptomatology. This results could be interpretated considering that low levels of anxious symptoms lead the patient to underestimate their physical sensations, minimizing the importance attributed to their own physical sensations. On the other hand, high levels of depression can lead patients to overestimate the severity of their cardiac symptoms (Skotzko et al., 2000) and to report poorer physical health, with respect to their real clinical condition. These data have relevant clinical implication. The KCCQ is a tool widely used with CA patients as a measure of the severity of HF. However, clinicians need to take into account the assessment of patients' anxiety and depression which could make the use of KCCQ less reliable.



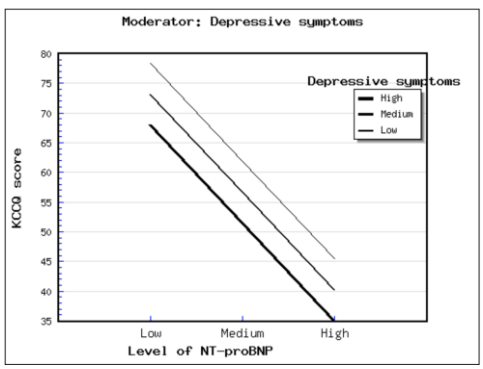


Figure 1.: Interaction between NT-proBNP and anxious symptoms in the prediction of the KCCQ scores.
Note. Significant interaction between NT-proBNP and moderating variables was graphically represented using ModGraph (Joseè, 2013). Moderating variables and independent variables were represented as low (values 1 SD below the mean), medium (values from 1 SD below mean to 1 SD above mean), or high (values 1 SD above the mean).

Figure 2.: Interaction between NT-proBNP and depressive symptoms in the prediction of the KCCQ scores.
Note. Significant interaction between NT-proBNP and moderating variables was graphically represented using ModGraph (Joseè, 2013). Moderating variables and independent variables were represented as low (values 1 SD below the mean), medium (values from 1 SD below mean to 1 SD above mean), or high (values 1 SD above the mean).

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Background: In recent years, awareness for transthyretin amyloidosis has increased among neurologists. Transthyretin amyloidosis has been particularly recognized in the differential diagnosis of polyneuropathy. Previous publications have often assigned typical symptoms to the different etiologies of the disease. Thus, neurological manifestations are usually described for the hereditary variant (ATTRv) but rarely for the acquired wild-type form, underestimating the phenotypic heterogeneity of this multisystemic disease. This leads to a delay in diagnosis. The newly established Amyloidosis Center of Lower Saxony at Hanover Medical School pursues an interdisciplinary approach in diagnosis and treatment of patients with amyloidosis, which includes neurological assessment from the time of first presentation of ATTRv and ATTRwt patients.

Objective: To evaluate neurological involvement in both hereditary and wild-type forms of ATTR at the time of initial presentation at the Department of Neurology of Hanover Medical School.

Material & Methods: Fifty-nine patients presented to our neurologic clinic, of whom eleven (19%) were affected by the hereditary and 48 (81%) by the wild-type variant of transthyretin amyloidosis. The first visit included a detailed anamnesis in addition to a neurological examination. Comprehensive clinical scores like RODS, INCAT, and MRC Sum Score were conducted. Findings were complemented by electrophysiological studies, which were assessed in eleven (100%) ATTRv patients and 35 (73%) ATTRwt patients.

Results & Discussion: As expected, 100% of ATTRv patients had neuropathy symptoms, while 43 (90%) of ATTRwt patients were also diagnosed with polyneuropathy. Clinical scores revealed more severe neurologic disease in ATTRv patients (means: INCAT 4, RODS 32, MRC 68) compared to those with ATTRwt (means: INCAT 2, RODS 39, MRC 79). Within the ATTRv cohort, there were eight reports (73%) of sensory deficits and three reports (27%) of motor weakness. Gait ataxia existed in six (55%) and autonomic dysfunction in five (45%) cases. Meanwhile, clinical examination showed pallhypaesthesia in all patients, paresis in six (55%), and gait ataxia in nine patients (82%). Electrophysiological measurements revealed pathological findings consistent with polyneuropathy in nine (82%) patients. Among the ATTRwt patients deficits in sensitive perception and unsteadiness in gait were mentioned by 18 (38%) and a subjective loss of strength by five (10%). Autonomic complaints were reported by 14 (29%) patients. Pallhypaesthesia manifested in 40 cases (83%), while paresis was determined in eleven (23%) and gait ataxia in 33 (69%) patients. Electrophysiological findings typical of polyneuropathy occurred in 17 (35%) subjects.

Summary & Conclusions: Polyneuropathy occurs in the overwhelming majority of patients with transthyretin amyloidosis, regardless of the etiology of the disorder. The frequency of polyneuropathy diagnoses in patients with ATTRwt is particularly striking. Previous classifications into expected symptom clusters therefore appear to be outdated, underlining the relevance of neurological care for all patients with transthyretin amyloidosis. Furthermore, the discrepancy between the subjective symptom reports and pathological findings during clinical examination highlights that a relevant proportion of patients are unaware of the extent of neurological involvement at the time of initial diagnosis. An interdisciplinary approach to patient care is therefore highly recommended to prevent disease progression at an early stage.

Figures:

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Incidence and factors associated with de novo atrial fibrillation in patients with wild-type transthyretin cardiac amyloidosis

Running title: Incidence of de novo AF in wild-type transthyretin cardiac amyloidosis

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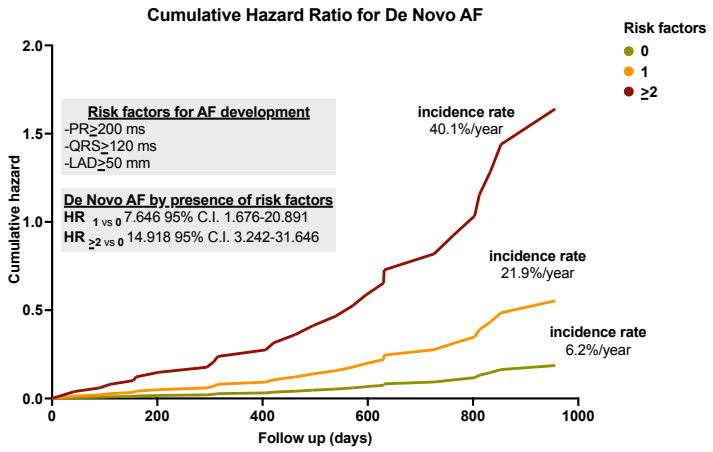
Background: Data on the incidence rate and factors associated with de novo atrial fibrillation (AF) in patients with ATTRwt CA is limited.
We described the incidence and ECG factors associated with de novo AF in patients diagnosed with wild-type transthyretin cardiac amyloidosis (ATTRwt-CA) to drive tailored arrhythmia screening.
Methods: Multicenter, retrospective, observational cohort study performed in six referral centers for CA. All consecutive patients diagnosed with ATTRwt-CA between 2004 and 2020 with >1-year follow up (FU) were enrolled in the study and were divided into three groups according to presence of AF: (1)patients with 'known AF'; (2)patients in 'sinus rhythm' and (3)patients developing 'de novo AF' during FU. Incidence and factors associated with AF in patients with ATTRwt were the primary outcomes.
Results: Overall, 266 patients were followed for a median of 469 days: 148 (56%) with known AF, 84 (31.6%) with sinus rhythm, and 34 (12.8%) with de novo AF. Age and gender were similarly distributed. At multivariable analysis, PR (Hazard Ratio[HR]: 1.008 95% C.I. 1.001-1.016), QRS (HR: 1.022 95% C.I. 1.002-1.043) and left atrial dilatation≥50mm (HR: 3.429 95% C.I. 1.565-7.329) were associated with de novo AF at FU. Patients presenting with at least two risk factors (PR≥200ms, QRS≥120ms or LAD≥50mm) had a higher risk of developing de novo AF compared to patients with no risk factors (HR 14.918 95% C.I. 3.242-31.646).
Conclusions: Incidence of de novo AF in patients with ATTRwt is 20.7%/year. Longer PR and QRS duration and left atrial dilation are associated with arrhythmia onset.

Figure. Cumulative Hazard Ratio for De Novo AF in patients with no risk factors (blue line) and 1 or more risk factors (red line). AF: Atrial Fibrillation



Quality of Life in Patients with Transthyretin Amyloid Cardiomyopathy Treated with Inotersen

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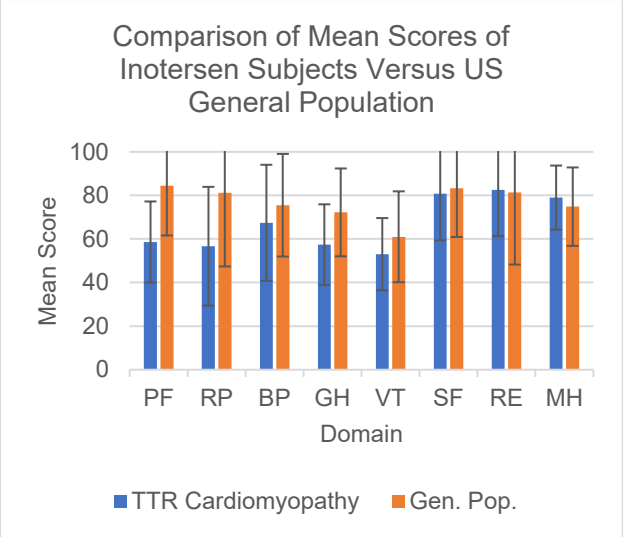
Background: Inotersen, an antisense oligonucleotide, is an effective therapy for familial amyloid polyneuropathy (FAP) but has not been evaluated in amyloid cardiomyopathy due to wild-type or variant TTR. Inotersen is administered as a weekly subcutaneous injection and requires weekly or alternate week blood draws for safety, as it may produce thrombocytopenia and renal impairment. Despite a 22% discontinuation rate in a 15-month trial of inotersen for familial amyloid polyneuropathy (1) drug-treated patients had a stable QoL compared to deterioration in those who received placebo, whether or not concomitant cardiomyopathy was present. Patients with TTR amyloidosis in whom cardiomyopathy is the presenting feature usually have wild-type TTR amyloidosis and tend to be in their 8th decade or above, and the progression of cardiomyopathy may be slower than that of FAP. To date, there is very little data on the effect of TTR silencers on the natural history and QoL among patients with TTR cardiomyopathy. We herein describe the effect of inotersen on QoL treatment. in a group of patients with TTR cardiac amyloidosis receiving inotersen treatment.

Objective: To evaluate the effect of inotersen on QoL in an open-label study of inotersen for TTR cardiomyopathy.

Methods: A 24-month, open-label study to evaluate inotersen was conducted with a sample of 31 patients diagnosed with wild-type (N= 28) or hereditary cardiomyopathy. No patient was taking a concomitant TTR stabilizer (tafamidis or diflunisal). Participants self-administered 300 mg inotersen injections weekly and received alternate week safety blood draws. QoL was evaluated at the Day 1 visit, and subsequent 6-monthly visits using the Short Form 36 Quality of Life Questionnaire (SF36). This questionnaire evaluates 8 domains including physical functioning, mental health, bodily pain, general health, vitality, social functioning, role limitations due to physical health, and role limitations due to emotional health. Each domain is scored between 0-100 (a higher score representing a greater quality of life). Mean baseline (pre-drug) scores were compared to published values in the general population and comparisons were made between baseline and 12-month and baseline and 24-month.

Results: Mean age of participants was 72 ± 17yr, with 30 males. 22 patients remained in the study at 12 months and 14 completed the two years. Worsening heart failure and renal dysfunction were the main reasons for study discontinuation. At baseline, the mean scores of participants in physical health domains were lower than published scores for the normal US population (2), whereas mean scores in mental health domains were similar (Figure 1). None of the 8 individual component domains of the SF36 either improved or deteriorated between baseline and 12 months or 24 months, although there was a very strong trend toward a small improvement in QoL at 2 years among the patients completing the study (Figure 2).

Conclusions: 1. Patients with TTR cardiomyopathy had, as anticipated a lesser QoL in physical domains than published scores for a healthy US population. In contrast, mental health SF36 domains were similar to the normal population, perhaps representing the positive attitudes of patients willing to participate in a clinical trial. 2.Evaluable patients receiving inotersen for TTR cardiomyopathy showed neither an increase nor decrease across the eight domains of QoL, indicating a stability in QoL for those who completed 1 and 2 years of study drug therapy. However, lack of a control group and small study size makes it impossible to determine whether the QoL stability was a drug effect or reflects the natural history of the disease. 3.These data do not account for a presumptive decrease in QoL among patients with side-effects causing study withdrawal, and the high overall withdrawal rate would likely be reflected as an overall decrement in QoL for the group, were these data able to be incorporated.



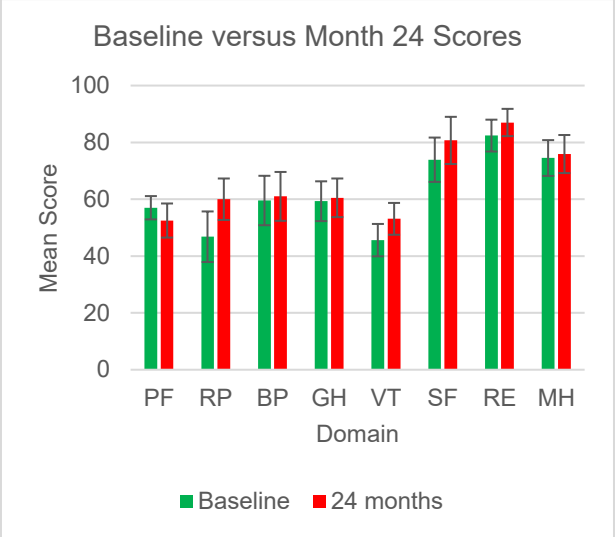


Figure 1 (left): Comparison of the mean scores of TTR cardiomyopathy versus the US general population across the eight domains. The first 4 domains primarily reflect physical wellbeing and are lower than the population norms. The 4 mental health domains are similar to US norms.
Figure 2.(right): Comparison of mean scores at baseline and Month 24 of participants that completed study across the eight domains. Data is from 11/14 subjects who completed 2 years and shows a trend toward improvement in 7/8 domains.

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Support & Funding: The study was sponsored by Akcea Therapeutics. Study design, data collection and analysis were performed independently of the study sponsor.

Prospective evaluation of an applied wt-ATTR-CM machine learning model to a United States (U.S.) health system electronic health record

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Background:
Wild-type transthyretin amyloid cardiomyopathy (wtATTR-CM) is a progressive, life-threatening disease that is an underdiagnosed cause of heart failure (HF). Systematically identifying at-risk patients who can undergo targeted testing provides an opportunity for earlier diagnosis and treatment. To facilliate identification and early diagnosis of ATTR-CM patients, a machine learning model was developed, validated and performance-tested in several large datasets. The model was trained on U.S. medical claims data sourced from IQVIA (including 300 million patients) for the diagnosis of wtATTR-CM, followed by validation in a separate electronic health record-based datasets on a large U.S. dataset. The model was subsequently further validated in the Northwestern Medicine Enterprise Data Warehouse (NMEDW) dataset.[1] Given the retrospective nature of the datasets used to train and validate the machine learning model, there is a need to prospectively apply the model to a large health system to determine its clinical applicability.

Objective: Prospective evaluation of the machine learning model for the identification and diagnosis of patients at-risk for wtATTR-CM.

Material & Methods: A prospective, observational study is underway at Northwestern Medicine, a large, intergrated health system. A random set of participants age ≥ 50 years with a prior diagnosis of heart failure and at least 5 years of data (and at least 50 encounters) in the NMEDW will be identified. Following the Inclusion and Exclusion Criteria (See Figure 1) participants will be designated by a diagnostic threshold of risk of wtATTR-CM. The machine learning model predicted probability will focus on the following 3 tertiles: Low: Predicted probability ≤0.29; Medium: Predicted probability 0.30-0.69; High: Predicted probability ≥0.70. Following completion of study procedures, participants will be classified based upon Table 1. Additional participants will be recruited for each one that is excluded with a goal of 100 participants. For all confirmed cases, the predictive model will score each participant independently and provide a probability of suspected ATTR-CM; the receiver operating characteristic (ROC) curve will be computed based on this probability across all participants in the dataset (wtATTR-CM vs. non-amyloid heart failure controls). Assessment of test characteristics, including sensitivity, specificity, positive predictive value and negative predictive value. Participants found to have ATTR-CM will be referred to clinical care for evaluation of either wtATTR-CM or hATTR-CM following Northwestern's standard of care.

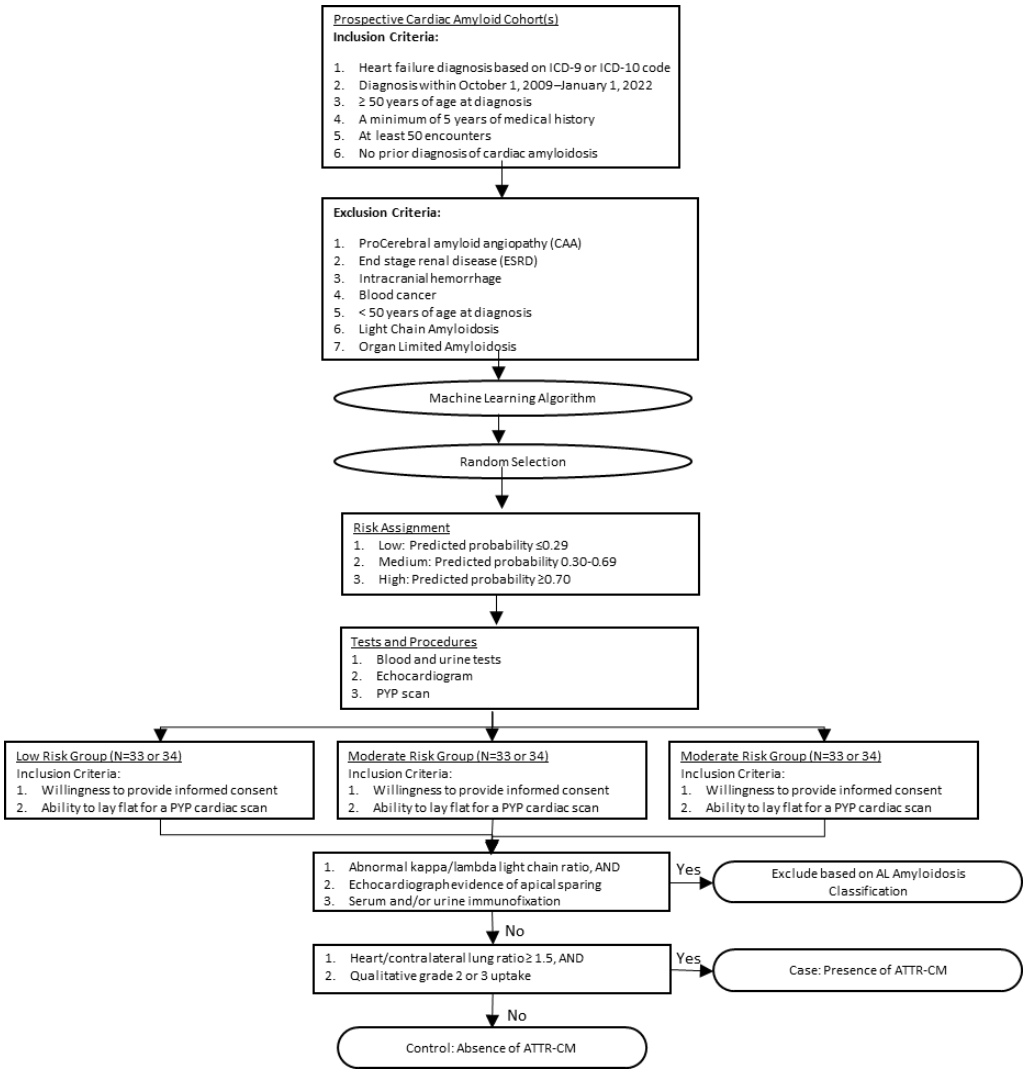
Results: 39,654 participants were queried from the NMEDW for application of the Machine Learning algorithm. Of those meeting the inclusion/exclusion criteria, an invitation to participate is provided. As of April 4, 2022, 30 participants have been enrolled with recruitment completion expected in the fall of 2022.

Summary & Conclusion: A growing need exists to enable a systematic approach to identify ATTR-CM patients earlier in their diagnostic journey. This prospective study informs a practical approach, for systematic adoption and confirmation to a machine learning model in the identification of at-risk heart failure patients for wt-ATTRCM. In addition, it has the potential to inform practical consideration(s) for clinicans based on the predicted probability of at risk patients. A machine learning model approach deployed using electronic health records, in support of an earlier diagnosis of ATTR-CM, may lead to earlier management and improved outcomes for patients with ATTR-CM.

Table 1: Definition of Cases and Controls

Patient Cohort	Definition
AL-Amyloidosis (Excluded)	1. Abnormal kappa/lambda light chain ratio, serum and/or urine immunofixation 2. Echocardiographic evidence of apical sparing
HereditaryATTR-CM (Excluded)	1. PYP scan with qualitative grade 2 or 3 uptake 2. Evidence of *TTR* genetic variant known to cause ATTR-CM
ATTR-CM (Case)	1. PYP scan with qualitative grade 2 or 3 uptake
Heart-Failure (Control)	1. Absence of any of the above

Figure 1.: Prospective Cardiac Amyloid Study Flow



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Support & Funding: Funding provider by Pfizer Inc.

Prognostic Value of Cardiopulmonary Exercise Testing in Patients with Transthyretin Cardiac Amyloidosis

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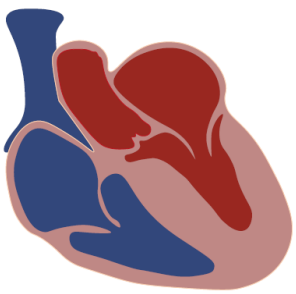
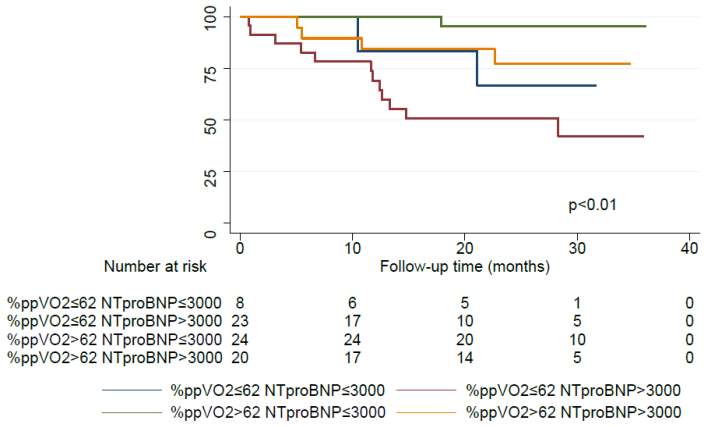
Organisation(s): 1: Tuscan amyloid referral center, Italy; 2: Cardiac Rehabilitation Unit, Department of Experimental and Clinical Medicine, University; 3: Geriatric Medicine and UTIG, Azienda Ospedaliera Careggi, Florence, Italy

Background: transthyretin cardiac amyloidosis (ATTR-CA) is associated with a progressive reduction in functional capacity. The prognostic role of Cardiopulmonary testing (CPET) parameters and in particular of normalized peak VO2 (%ppVO2) remains to be thoroughly evaluated.
Objective: The aim of this study is to evaluate the prognostic value of CPET in a cohort of patients with transthyretin cardiac amyloidosis ATTR-CA.
Material & Methods: In this study 75 patients with ATTR-CA underwent cardiological evaluation and CPET in a National Referral Center for cardiac amyloidosis (Careggi University hospital, Florence).
Results & Discussion: Fifty-seven patients (76%) had wild type ATTR. Median age was 80 (75-83) years, 68 patients (91%) were men. Peak oxygen consumption (14.1±4.1 ml/kg/min) and %ppVO2 (68.4±18.8%) were blunted. Twenty-seven (36%) patients had an abnormal pressure response to exercise. After a median follow-up of 25 (12-31) months the composite outcome of death or heart failure hospitalization was registered in 19 (25.3%) patients. At univariate analysis %ppVO2 was a stronger predictor for the composite outcome than peak VO2. %ppVO2 and NTproBNP remained associated with the composite outcome at multivariate analysis. The optimal predictive threshold for %ppVO2 was 62% (sensitivity: 71%; specificity: 68%; AUC:0.77, CI: 0.65-0.88). Patients with %ppVO2 ≤ 62 %and NTproBNP >3000pg had the worse prognosis with 1- and 2-year survival of 69±9% and 50±10%, respectively.
Summary & Conclusions: CPET is a safe and useful prognostic tool in patients with ATTR-CA. CPET may help to identify patients with advanced disease that may benefit of targeted therapy.

75 patients with ATTRwt underwent cardiopulmonary testing





Tafamidis 61 mg for treatment of ATTR cardiomyopathy in daily clinical practice: an observational study

aus dem Siepen Fabian[1], Hofmann Eva[1], Nagel Christian[1], Locher Valerie[1], Hegenbart Ute[2], Schönlad Stefan[2], Frey Norbert[1], Kristen Arnt V.[1], Hein Selina[1]

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Background:
Since 2020, Tafamidis 61 mg is approved for the treatment of Transthyretin amyloid cardiomyopathy (ATTR-CM) based on the results of the ATTR-ACT study[1]. The European Society of Cardiology (ESC) recommends the use of Tafamidis 61 mg for ATTR-CM patients in NYHA stages I-II, whereas treatment of patients in NYHA stage III remains a case-by-case decision.

Objective:
Since approval of the drug, a therapy with Tafamidis 61 mg was initiiated in 370 patients with ATTR-CM at the amyloidosis center Heidelberg. All patients underwent clinical follow-up visits semi-annual. We sought to investigate the safety profile and the efficacy of the drug in daily routine practice.

Material & Methods:
All ATTR-CM patients under therapy with Tafamidis 61 mg underwent clinical examination, ECG, echocardiography and laboratory testing every 6 months in our outpatient clinic after initiation of therapy. Phone assessment was performed monthly to record general condition, updates on medical history and hospitalizations as well as possible side affects of Tafamidis. Patients with complete data for a minimum follow-up period of 12 months were included in the analysis, overall n= 106 patients with either hereditary (ATTRv, n=28, 26%) or wildtype (ATTRwt, n=78, 74%) ATTR-CM.

Results:
Mean age of the patients was 74 years, predominantly male (98 male, 92 %, 8 female, 8%) in different disease stages (NYHA I 11%, NYHA II 43%, NYHA III 48%). 5 patients died, 2 patients progressed to NYHA stage IV. Only one patient reported nausea and obstipation, no other side affects were reported. No significant change could be observed in hs-Troponin-T, whereas NT-proBNP (3668±4363 vs. 4390±5122 pg/ml, p=0.01) increased and kidney function (eGFR 73±23 vs. 68±26 ml/min, p=0.009) decreased significantly. Echocardiographic parameters (LVEF, IVS, GLS) did not significantly change. Results are shown in table 1.

Table 1:

	Baseline (n=106)	1-year Follow-Up (n=101)	p-value
NYHA stage			
- I	12 (11%)	12 (12%)	
- II	46 (43%)	46 (46%)	
- III	48 (45%)	41 (41%)	
- IV	0 (0%)	2 (2%)	ns
NT-proBNP (pg/mL)	3668±4363	4390±5122	0.01
hs-Troponin-T (ng/L)	45±37	50±29	ns
eGFR (ml/min)	73±23	68±26	0.009
Septum thickness (mm)	17±4	18±4	ns
GLS (%)	-12±3	-11±3	ns

Discussion:
Compared to the study population of the ATTR-ACT study, patients in clinical routine practice were at similar age, but more frequent in NYHA stage III. Despite all limitations of this observational study, we could confirm that Tafamidis was fairly well tolerated by the patients. Biomarkers only slightly increased and echocardiographic findings remained stable in the majority of patients, possibly indicating deceleration of disease progression. However, more longitudinal data is neccesarry to
evaluate long-term effects and therapy response.

Summary & Conclusion:
In a time period of one year, our findings were similar compared to the ATTR-ACT study regarding safety and efficacy. Further osbervation of patients treated with Tafamidis will eludicate long-term effects and long-term safety.

Figure 1:
NT-proBNP at baseline and follow-up

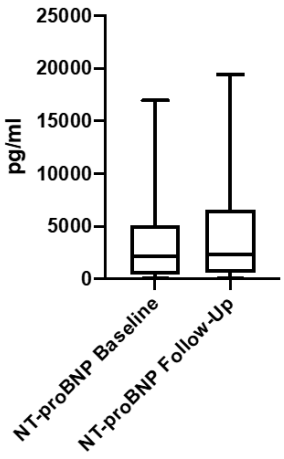
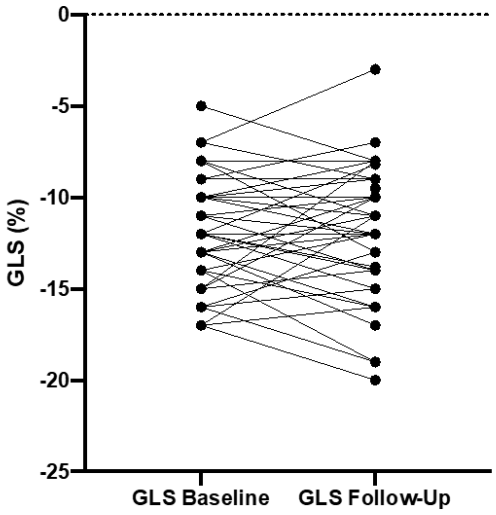


Figure 2:
Global longitudinal strain at baseline and follow-up



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Support & Funding: No funding.

Transthyretin Cardiac Amyloidosis (ATTR-CA) and its rising awareness: Patient characteristics and survival in the Australian context

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[5]*Baker Heart and Diabetes Institute, Australia*

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Background: Despite increasing awareness and treatments for ATTR-CA, true incidence and patient outcomes of the disease in Australia remains unclear.

Objective: We wished to evaluate the epidemiology, characteristics and survival of ATTR-CA at an Australian state amyloidosis service.

Material & Methods: All patients reviewed at the Victorian and Tasmanian Amyloidosis Service with ATTR-CA from July 2014 to July 2021 were included. Patient characteristics and survival data were retrospectively collected.

Results: 245 ATTR-CA patients were identified. Diagnoses have increased more than 440% since 2014. Median age was 80 years old (range 75-84); 228 (93.1%) were male. 51.4% had Gillmore Stage I, 34.3% Stage II and 14.3% Stage III at diagnosis. 11/84 (13.1%) had hereditary disease, 73/84 (86.9%) were wildtype, 34 (14.2%) had an unrelated MGUS.129 (53.5%) had AF at diagnosis. Bone Scintigraphy was the most common investigation (99.2%). 36.1% underwent confirmatory cardiac (13.8%) or bone marrow biopsies (13.1%). 90% commenced specific treatment for ATTR-CA: diflunisal (44.1%), doxycycline (44.0%) green tea extract (48.6%), tafamidis (11.8%) or enrolled onto placebo-controlled trials with acoramidis (6.9%) or patisiran (3.7%). With median follow up of 21 months, estimated overall survival was 71.3, 49.3 and 29.4 months for Gillmore stage 1, 2 and 3, respectively, slightly better than reported in the original Circulation paper.

Summary & Conclusion: ATTR-CA diagnoses in Australia are increasing, with patient characteristic similar to reports elsewhere. Estimated survival in the Australian cohort is better than original reports, possibly reflecting greater uptake of disease-modifying therapies.

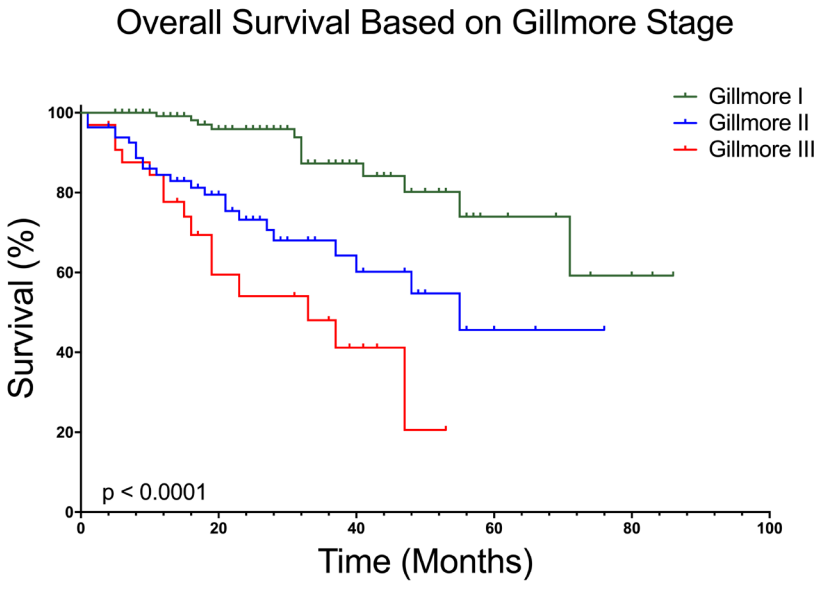

Figure 1.: Kaplan-Meier curve demonstrates estimated overall survival was 71.3, 49.3 and 29.4 months for Gillmore stage 1, 2 and 3, respectively ($p < 0.0001$).

Support & Funding: The authors received no financial support for the research, authorship, and/or publication.

Significant survival benefits with Diflunisal in patients with Transthyretin (TTR) Amyloidosis Cardiomyopathy (ATTR-CM); A retrospective analysis

CHOI, BOYOUN[1,2,3,4,5], LASICA, MASA[1,3,4,6], HUYNH, NATHANIEL[1], SIRDESAI, SHREERANG[1,2], NAGARETHINAM, MEENA[1], TING, STEPHEN[1,3,4], COOKE, JENNIFER[1,3], HARE, JAMES[2,3,4,5], GIBBS, SIMON[1,2,3,4]

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Background: Diflunisal is a non-steroidal anti-inflammatory drug that stabilizes TTR tetramers. However, efficacy and tolerance of diflunisal has been questioned as a long-term therapy for heart failure with ATTR-CM.

Objective: We aim to evaluate its efficacy and toxicity profile of diflunisal in patients with ATTR-CM in Australian population cohort.

Material & Methods: All patients with ATTR-CM who have been treated by the Victorian and Tasmanian Amyloidosis Service between 2015 and 2021 were included in this study. Data were retrospectively collected and analysed.

Results: 108 out of 245 patients were treated with diflunisal in this cohort. Diflunisal group was younger than non-diflunisal group (78 vs 81 years old, p <0.001) and had longer follow up (26 vs 19 months, p = 0.001). Gillmore stages were significantly lower in diflunisal group (p <0.001), although there was no significant difference in eGFR between the groups (p = 0.147). Death rate was significantly lower in diflunisal group (p = 0.015) and estimated survival time was longer in diflunisal group compared to non-diflunisal group (66.6 vs 52.7 months, p = 0.001). Out of 108 patients, 55 patients (45%) discontinued the therapy during follow up. 25 out of 55 patients of these were due to significant adverse events such renal impairment and gastrointestinal adverse events.

Summary & Conclusion: In this retrospective analysis, for those who can tolerate it, diflunisal appears to be a reasonable treatment option with potential benefits in long-term survival in ATTR-CM; as well as being a very affordable alternative to tafamidis. Further prospective trial comparing other TTR specific therapies are required to evaluate the specific long-term efficacy and survival benefit.

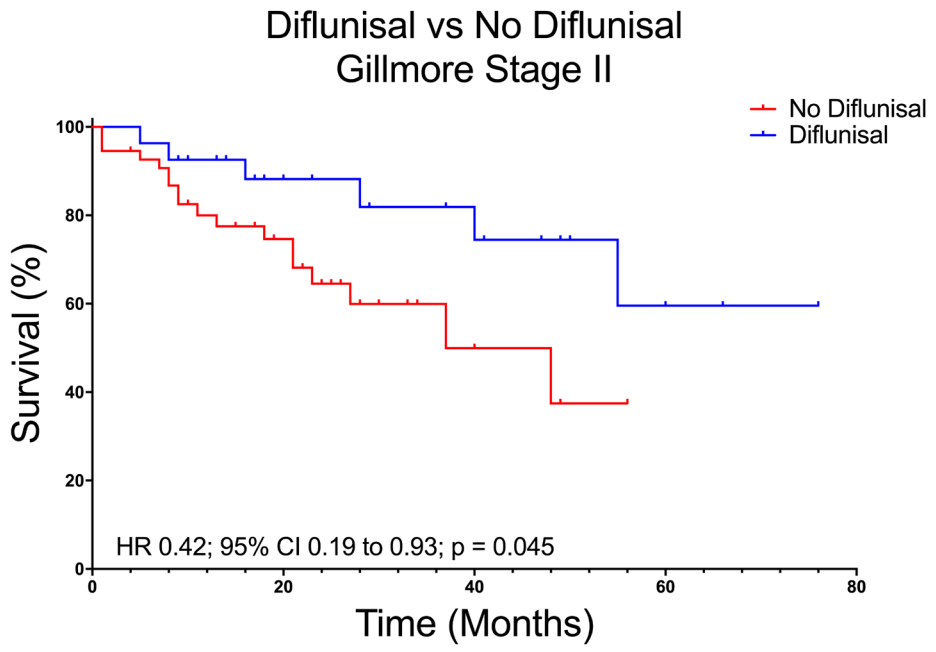

Figure 1.: Kaplan-Meier curve demonstrates that the overall survival of patients with Gillmore Grade 2 who were managed with diflunisal had a significant survival advantage when compared to those who were not treated with diflunisal (HR 0.42; 95% CI 0.19 to 0.93; p = 0.045).

Support & Funding: The authors received no financial support for the research, authorship, and/or publication.

Effect of inotersen on global longitudinal strain in transthyretin cardiac amyloidosis

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Background
Inotersen is a second-generation antisense oligonucleotide targeted to suppress hepatic production of transthyretin messenger RNA, thereby reducing the formation of TTR-derived amyloid fibrils and organ infiltration(1). It is licensed for the treatment of hereditary TTR amyloidosis polyneuropathy in adults(2). Myocardial function measured by speckle tracking echocardiography is known to deteriorate over a 12 -month period in untreated patients but the effect of inotersen on myocardial function using this technique has not been reported in patients with transthyretin amyloid cardiomyopathy (ATTR-CM).

Objective
The purpose of this study was to determine the effect of inotersen on myocardial functional parameters over 12 months of treatment compared to an untreated cohort with ATTR-CM.

Methods
We conducted an open-label prospective study of inotersen in patients with hereditary or wild-type ATTR-CM, New York Heart Association (NYHA) class I-III symptoms and estimated glomerular filtration rate ≥45ml/min/1.73m^2. Inotersen 300mg was administered subcutaneously weekly. No patient received an alternative TTR stabilizing or silencing treatment during study participation. Two-dimensional speckle tracking echocardiography was analysed at baseline and 12 months. Serial left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS) were measured and compared to a retrospective cohort with untreated ATTR-CM.

Results
Thirty-one patients with ATTR-CM were enrolled in the open-label study. Of these, 21 completed 12 months of treatment and had follow-up echocardiography and were compared to a cohort of 22 untreated patients. The untreated cohort were older with a greater proportion having National Amyloidosis Centre stage III disease (Table 1). Over 1 year, absolute global longitudinal strain deteriorated more in the untreated groups by a median of an absolute value of 1.1% (interquartile range [IQR] 0.95) compared with 0.2% (IQR 2.3) in the inotersen group (p=0.02) without a significant change in LVEF (-absolute 2.7±6.4% compared to -0.3±7%; p=0.26).

Summary & Conclusion
In ATTR-CM, inotersen resulted in a lesser deterioration in GLS over a 12-month period compared to an untreated cohort. While this may have been due to slightly worse baseline function in the untreated controls, the deterioration in function in controls is consistent with expected published values and thus the minimal change in the inotersen group suggests a salutary effect of inotersen in preventing deterioration of cardiac function.

Table 1: Baseline patient characteristics stratified by treatment

	No treatment (n=22)	Inotersen (n=21)	P value
Age (years)	78.2 (±6.7)	73.1 (±5.5)	**0.02**
Male sex (n, %)	20 (90.9)	20 (95.2)	0.32
WtATTR (n, %)	21 (95.5)	19 (90.5)	0.58
NAC stage (n, %) **I** **II** **III**	 10 (45.5) 5 (22.7) 4 (18.2%)	 16 (76.2) 5 (23.8) 0	 0.13 0.94 **0.02**
High sensitivity troponin	62.5 (44)	47 (27)	0.13
NTproBNP (ng/L)	2807 (3599)	1416 (2101)	0.08
Prealbumin	22.5 (±4.1)	24.6 (±4.4)	0.12
Creatinine (mg/dL)	1.3 (±0.4)	1.1 (±0.2)	0.08
Systolic BP (mmHg)	120 (29)	113 (20)	0.20
Diastolic BP (mmHg)	70 (12)	68 (9)	0.69
Baseline LVEF (%)	48 (±12.6)	48.5(±13.3)	0.90
Baseline GLS (%)	-9.9 (±2.9)	-11.1 (±3.7)	0.24

BP, blood pressure; GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; NAC, National Amyloid Centre; NTproBNP, N-terminal pro brain natriuretic peptide; wtATTR, wild-type transthyretin amyloid.

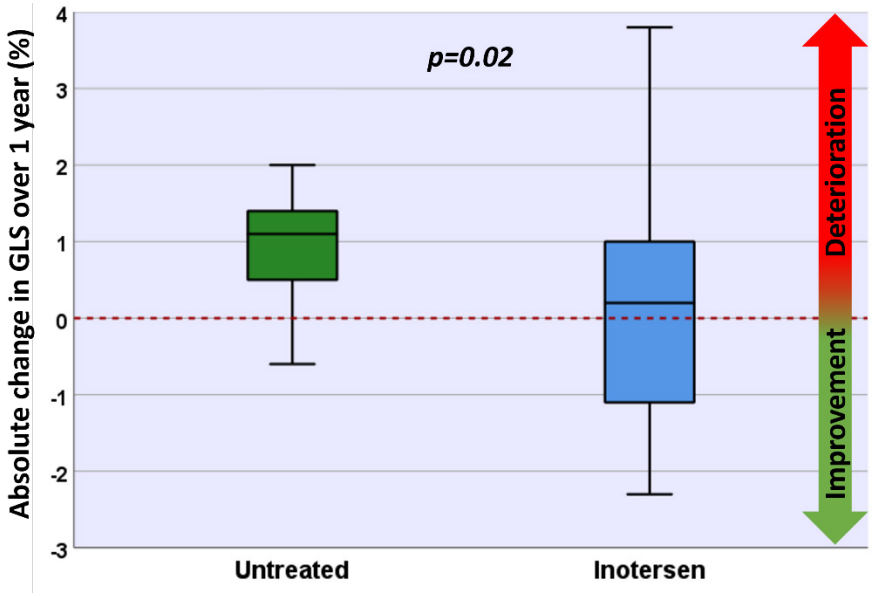

Figure 1: Change in absolute global longitudinal strain at 1 year stratified by treatment.

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Support & Funding

The study was sponsored byAkcea Therapeutics. Study design, data collection and analysis was performed independently of the study sponsor.

Inotersen treatment in transthyretin amyloid cardiomyopathy results in early and sustained serum transthyretin knockdown

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Background
Pharmacological therapies for both wild type and hereditary forms of transthyretin amyloid cardiomyopathy (ATTR-CM) are directed towards stabilization of the TTR tetramer to prevent misfolding or suppression of TTR expression and production by either antisense oligonucleotide or small interfering RNA based therapies. Inotersen is a second-generation antisense oligonucleotide targeted to suppress hepatic production of transthyretin messenger RNA thereby reducing the formation of TTR-derived amyloid fibrils and organ infiltration(1). It is licensed for the treatment of hereditary TTR amyloidosis polyneuropathy in adults(2). There is limited data on its biochemical and clinical effect in either wild-type or hereditary ATTR-CM without polyneuropathy(3).

Objective
The purpose of this study was to prospectively determine the effect and timescale of inotersen on serum transthyretin levels in patients with ATTR-CM.

Methods
We conducted a 24-month open-label prospective study of inotersen in patients with hereditary or wild-type ATTR-CM, New York Heart Association (NYHA) class I-III symptoms and estimated glomerular filtration rate ≥45ml/min/1.73m^2. Inotersen 300mg was administered subcutaneously weekly and serum transthyretin was measured at baseline and monthly during enrolment. No patient received an alternative TTR stabilizing or silencing treatment during study participation.

Results
Thirty-one patients with ATTR-CM (mean age 72.4 ± 6.3 years; 97% male; wild-type ATTR 90%; NYHA II 58%, III 10%) were enrolled; 28 (90%), 22 (71%) and 15 (48%) completed 6, 12 and 24 months of treatment respectively. Mean baseline TTR level was 24 ± 4.7 (figure 1). Inotersen resulted in a mean reduction in circulating TTR of 40.3% ± 21.6 by 1 month and 61.2% ± 19.7 by 2 months. A TTR steady state level was achieved by 4 months with a mean reduction of 69% ± 13.7 below baseline levels. This reduction was sustained throughout the remainder of the 24-month study period.

Summary & Conclusion
In ATTR-CM, treatment with inotersen consistently resulted in an early and sustained knockdown of serum transthyretin, indicative of biochemical efficacy. It is not known what degree of reduction in TTR is required to optimally attenuate progression of cardiac infiltration and further research is warranted to determine the relationship between the degree of TTR knockdown and clinical outcomes.

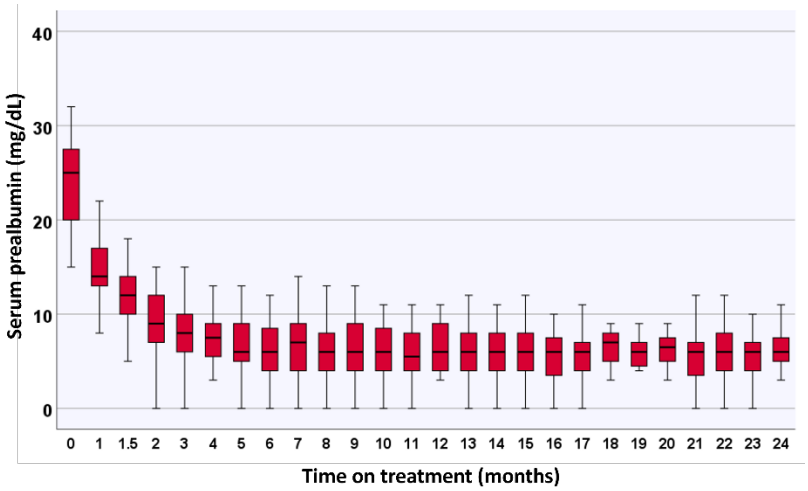

Figure 1: Serum transthyretin levels from baseline during treatment with inotersen.

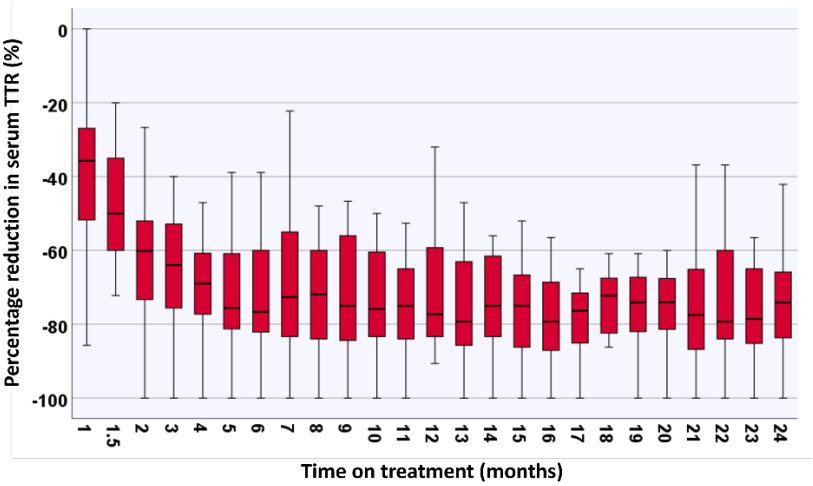

Figure 2: Percentage reduction in serum transthyretin levels from baseline during treatment with inotersen.

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Support & Funding

The study was sponsored by Akcea Therapeutics Study design, data collection and analysis was performed independently of the study sponsor.

Baseline ECG characteristics in ATTR-CM

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Background: ATTR-CM amyloidosis is an increasingly recognised cause of HFpEF in the elderly, with the vast majority of cases being wild-type (wATTR-CM). Alongside heart failure symptoms, patients are at high risk of arrhythmia; including high-grade AV block and atrial and ventricular tachyarrhythmia, due to a proposed combination of ventricular and atrial remodelling, direct infiltration of the myocardium with ATTR amyloid fibrils and subsequent downstream injury and fibrosis. The traditional 'cardiac amyloid' ECG findings of small complexes are relatively unusual in ATTR-CM, and the cohort is poorly defined in terms of recognised baseline ECG characteristics and ECG predictors of severe arrhythmia.

Objective: We set out to better define the baseline ECG characteristics of cardiac-ATTR amyloidosis.

Material & Methods: Patients undergoing screening for a clinical trial at a single study site underwent high-quality triplicate ECG assessment, which was then stored digitally (MUSE). The database was retrospectively examined through the digital system for PR interval, QRS width, QTc interval, AFib/Flutter and R axis. Data was extracted and collated by an automated process, with human oversight. Values were averaged over 3 or more ECGs per volunteer taken on the same day. Definitions used were PR prolongation (>200ms), severe (>300ms), bundle branch block (>120ms) and severe (>150ms) and QTc interval prolongation (>470ms). Axis deviations were as follows: -30 to -110: left axis deviation, -30 to 90: normal axis, 90-180: right axis deviation and 180 to 250: northwest, or 'extreme' axis deviation. Patients were determined as p/AF with at least one irregular ECG captured with no discernible P waves or visible flutter. Additional data on morphology and rhythm was collected and interpreted by a specialist clinician. Patients with right or combined ventricular pacing were excluded.

Results: 296 patients with confirmed wtATTR amyloidosis were screened, with a mean follow-up time of 267 days (0-649) days, covering over 6000 individual ECGs. At baseline, the mean PR interval was 221ms (128-456ms) with 28% of individuals in 1st degree AV block. Approximately 50% of the population had significant left axis deviation, and around 25% had significant right axis deviation. Thirteen percent of the cohort had findings consistent with trifasicular block (BBB and prolonged PR and axis deviation), 1 patient was found to be in complete heart block at diagnosis. 12.8% of all patients had some form of atrial, ventricular or biventricular pacing. A further 42.2% of patients were in AF or atrial flutter at presentation with only 18% of patients in normal sinus rhythm at presentation.
The mean QRS was 122ms, over 50% of the cohort had significant bundle branch block; among those without ventricular pacing, 22.3% had LBBB and 16.2% had RBBB; 23% had a QRS width of >150ms.

Summary & Conclusion: Arrhythmias are prevalent in patients diagnosed with wtATTR-CM. The presence of AF/flutter in 42% at diagnosis suggests that anticoagulation should be considered in all patients. Conduction disease, including AV node and ventricular conduction disease, is common. The role and timing of device implantation in ATTR-CM requires further study.

Support & Funding: None declared.

REAL-WORLD EXPERIENCE WITH TAFAMIS AT CEPARM UNIVERSITY HOSPITAL. FEDERAL UNIVERSITY OF RIO DE JANEIRO. BRAZIL.

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Background:

Hereditary transthyretin amyloidosis (hATTR/ATTRv) is a severe multiorgan disease.Tafamidis is an oral drug that prevents TTR dissociation and deposition in tissues. It is approved worldwide since 2011, and since 2018 is provided to stage I patients in Brazil by the Public Health System

Objective:

To present 10 years real-world (RW) experience with disease progression in patients treated by tafamidis.

Material & Methods:

PND (polyneuropathy disability scale), total NIS (neuropathy impairment score), ECG, echocardiogram intraventricular septum thickness (IVS), NYHA (New York Heart Association) classification for heart failure, body mass index (BMI) and Karnofisky performance status (KPS), were evaluated at day one (D1) of treatment and at a mean time of 36 months after (6-101, SD30), from 2011 to 2021.

Results:

28 V30M ATTR patients were included (13 men) with reevaluation of at least 6 months; 12 were late-onset patients (>50 years); mean time of disease at D1 of 3.4 years (0.5-7, SD1.8); mean age at D1 of 46 years. At D1 5 patients were at PND 0, 17 at I, 5 at II, and 1 at IIIA. At last visit (LV), the corresponding was: 5,13,6 and 4. At D1 27 patients were at Coutinho stage 1, and 1 at 2. At LV: 24 at stage 1, and 4 at stage 2. Mean value of NIS at D1 was 15.8 (0-78.5) and at LV was 31.7 (0-161). 6 patients were considered non-responders with a NIS increase >10 points. At D1 2 patients presented with cardiomyopathy (NYHA of II and III) and at LV both had NYHA of II. ECG at D1 for 24 patients was abnormal in 15, mostly due to conduction abnormalities, and 2 had low voltage. In 14 patients at LV, 11 ECG were abnormal, with similar abnormalities. VS thickness > 12 was present in 4 out of 24 patients at D1, and in 4/ 18 at LV. The mean KPS value at D1 was 84 and at LV was 80. The corresponding values for BMI were: 24.5 at D1 and 24.6 at LV.

Summary & Conclusion:

Based on these limited data, we conclude that tafamidis was well tolerated and effective to treat patients in a real-world practice, although a better selection candidates and option to other treatments is necessary is several cases.

REAL-WORLD EXPERIENCE WITH INOTERSEN AT CEPARM

UNIVERSITY HOSPITAL FEDERAL UNIVERSITY OF RIO DE JANEIRO. BRAZIL.

Waddington-Cruz M; Pedrosa R; Gomes CP; Pinto MV; Pinto LF; Dias M; Santa Rosa R; Amorim, G; Accioli P, Guedes M.

Federal University of Rio de Janeiro, National Amyloidosis Referral Center, CEPARM, Rio de Janeiro, Brazil

Background:

Hereditary transthyretin amyloidosis (hATTR) is a progressive lethal disease.

Inotersen is a subcutaneous antisense oligonucleotide (ASO) that is approved to treat hATTR with polyneuropathy (PN).

Objective:

To evaluate disease progression in patients treated with inotersen in the post-approval trial real world practice.

Material & Methods:

(PND) polyneuropathy disability scale, (NIS) total neuropathy impairment score, ECG, (IVS) intraventricular septum thickness, (CM) cardiomyopathy, (NYHA) New York Heart Association classification for heart failure, (BMI) body mass index, and (KPS) Karnofsky performance status, were evaluated at day one (D1) and at 67 months (55-76, SD 7.6).

Results:

10 ATTRV30M subjects were included (6 men); mean disease duration at D1 of 3 years (1.5-6, SD1.4); mean age at D1 of 45.4 years (31-68, SD 13.9).

At D1 3 patients were at PND I, 5 at II, 2 at IIIA. At last evaluation (LE): 4 patients were at PND I, 3 at II, 1 at IIIA, and 2 at IV. Both at D1 and at LE, 3 patients had CM with 2 at NYHA 1 and 2. ECG at D1 was abnormal for 8 patients, mostly due to conduction abnormalities. At LE, abnormalities persisted in 6 patients; in 2 of those pacemakers were implanted. IVS thickness > 12mm was present in 4 patients at D1 and in 2 at last visit. At D1 mean NIS was 48.2 (12-129.75, SD39.8); KPS was 77 (50-90, SD 12.5); BMI was 22.6 (14-30.5, SD 8.2). At last visit (October 2021), mean NIS was 52.6 (16-141, SD42.4); KPS was 77.7 (50-90, SD12); BMI was 24.8 (18.9-31.4, SD 4.9). NIS progression of >10 was identified in 4/9 patients. There was no case of glomerulonephritis and no platelet decrease grade 4.

Summary & Conclusion:

Based on these limited data, we conclude that inotersen was well tolerated and effective to treat patients.

Neurofilament Light Chain as a Biomarker in Hereditary Transthyretin-Mediated Amyloidosis: 36-Month Data from the Patisiran Global Open-Label Extension

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Background: Hereditary transthyretin-mediated (hATTR) amyloidosis, also known as ATTRv amyloidosis, is a rare, rapidly progressive, and fatal disease. Neurofilament light chain (NfL), a marker of axonal injury, has potential to facilitate early diagnosis of hATTR amyloidosis, and to monitor disease progression and treatment response.

Objective: The objective of this analysis is to evaluate long-term changes in NfL levels in patients treated with patisiran through 36 months of the Global Open-Label Extension (OLE) study (NCT02510261).

Material & Methods: For this post-hoc analysis, NfL plasma levels were measured in healthy controls and in patients with hATTR amyloidosis with polyneuropathy who participated in the Phase 3 APOLLO and Phase 2 OLE parent studies. NfL levels were also measured at 12, 24, and 36 months in patients who rolled into the Global OLE from the two parent studies.

Results & Discussion: Mean NfL levels at APOLLO baseline were 63.2 (placebo, n=47) and 72.0 pg/mL (patisiran, n=111). As previously presented, at 18 months NfL levels significantly increased in the placebo group and significantly decreased following treatment with patisiran. Following 36 months of additional patisiran treatment in the Global OLE, reduction of NfL levels was maintained in the APOLLO-patisiran group (mean 44.8 pg/mL, n=72). Upon initiation of patisiran in the Global OLE, the APOLLO-placebo group experienced a significant reduction in NfL levels through 36 months (mean 40.0 pg/mL, n=15), to a similar level as the APOLLO-patisiran group. NfL levels in patients from the Phase 2 OLE also trended lower following 60 months of patisiran treatment (mean 26.1 pg/mL, n=19).

Summary & Conclusion: NfL may serve as a biomarker of active nerve damage and polyneuropathy in hATTR amyloidosis, making it potentially useful as a biomarker to monitor disease progression and treatment response adjunct to clinical assessments that reflect the overall burden of the polyneuropathy.

Support & Funding: This study was funded by Alnylam Pharmaceuticals. Editorial assistance in the development of the abstract was provided by Adelphi Communications Ltd, UK, funded by Alnylam Pharmaceuticals in accordance with Good Publication Practice (GPP3) guidelines.

Characteristics of patients with ATTR amyloidosis and the Ile107Val mutation: insights from the Transthyretin Amyloidosis Outcomes Survey (THAOS)

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Background: Transthyretin amyloidosis (ATTR amyloidosis) is a clinically heterogeneous disease with spontaneous (wild-type) and hereditary (ATTRv amyloidosis) forms.[1] ATTRv amyloidosis is caused by mutations in the transthyretin (*TTR*) gene.[1] Currently, more than 120 different *TTR* mutations associated with ATTR amyloidosis have been identified,[2] but many are not well characterized.

Objective: The aim of this analysis was to describe the characteristics of patients with the Ile107Val (p.Ile127Val) *TTR* mutation enrolled in the Transthyretin Amyloidosis Outcomes Survey (THAOS).

Material & Methods: THAOS is the largest, ongoing, global, longitudinal, observational study of patients with ATTR amyloidosis, including both hereditary and wild-type disease, and asymptomatic carriers of pathogenic *TTR* mutations (NCT00628745). This analysis described the demographics and clinical characteristics of patients with the Ile107Val mutation at enrollment in THAOS (data cutoff date: January 4, 2022).

Results: There were 45 patients (33/45 males; 73.3%) with the Ile107Val genotype overall, 33 (73.3%) of whom were symptomatic (**Table**). Of the symptomatic patients, 16/33 (48.5%) were enrolled in France, 6/33 (18.2%) in Brazil, and the remaining 11/33 (33.3%) in Germany, Japan, Romania, and the United States. The median (10th, 90th percentile) age at enrollment was similar in France and Brazil, and the median duration of ATTR amyloidosis symptoms was 4.1 (0.5, 16.3) years in France and 3.0 (1.7, 14.9) years in Brazil. Half to two-thirds of symptomatic patients with Ile107Val ATTRv amyloidosis in all countries (18/33; 54.5%) and in France (10/16; 62.5%) had a predominantly neurologic phenotype. One third of patients overall (11/33) had a mixed phenotype, which was the most prevalent phenotype in Brazil (4/6; 66.7%). In the overall population, heart failure (9/33; 27.3%), rhythm disturbance (5/33; 15.2%), and palpitations (4/33; 12.1%) were the 3 most common cardiac symptoms. The 3 most common neurologic symptoms were neuropathic pain/parasthesia (22/33; 66.7%), numbness (19/33; 57.6%), and temperature or pain insensitivity (16/33; 48.5%). In all countries, 14/26 (53.8%) patients had a Karnofsky Performance Status score of 70–90. Of the patients with a predominantly neurologic or mixed phenotype, 6/29 (20.7%) had a modified polyneuropathy disability score of 3b.

Summary & Conclusion: Over half of the symptomatic patients enrolled in THAOS with the Ile107Val mutation had a predominantly neurologic phenotype. A mixed phenotype was observed in almost one-third of patients overall, and in two-thirds of patients in Brazil. Patients were predominantly enrolled at study sites in France and Brazil. The most common presenting cardiac symptom was heart failure, and the most common presenting neurologic symptom was neuropathic pain/parasthesia. These data add to the limited pool of knowledge on the clinical profile of patients with the Ile107Val genotype and emphasize the importance of comprehensive assessment of all patients. THAOS continues to accumulate valuable real-world data on patients with ATTR amyloidosis in general, as well as on specific pathogenic genotypes.

Table. Baseline demographic and clinical characteristics of symptomatic patients with ATTRv amyloidosis and Ile107Val genotype in THAOS

	All countries (N=33)	France (N=16)	Brazil (N=6)
Age at enrollment, median (10th, 90th percentile), years	64.5 (59.1, 74.1)	63.6 (56.7, 73.9)	62.6 (59.1, 78.3)
Sex, n (%)			
Male	29 (87.9)	13 (81.3)	6 (100)
Female	4 (12.1)	3 (18.8)	0
BMI, median (10th, 90th percentile)	26.2 (21.8, 31.7)	26.7 (21.6, 32.6)	27.9 (19.5, 37.8)
mBMI, median (10th, 90th percentile)	972.9 (856.7, 1580.4)	972.9 (862.0, 1760.7)	879.7 (879.7, 879.7)
Duration of ATTRv amyloidosis symptoms, median (10th, 90th percentile), years	4.2 (1.7, 11.7)	4.1 (0.5, 16.3)	3.0 (1.7, 14.9)
Phenotype, n (%)			
Predominantly cardiac	0	0	0
Predominantly neurologic	18 (54.5)	10 (62.5)	2 (33.3)
Mixed	11 (33.3)	4 (25.0)	4 (66.7)
Unknown	4 (12.1)	2 (12.5)	0
Cardiac symptoms, n (%)			
Heart failure	9 (27.3)	3 (18.8)	3 (50.0)
Rhythm disturbance	5 (15.2)	3 (18.8)	2 (33.3)
Palpitations	4 (12.1)	1 (6.3)	3 (50.0)
Dizziness	3 (9.1)	0	3 (50.0)
Neurologic symptoms, n (%)			
Neuropathic pain/parasthesia	22 (66.7)	9 (56.3)	6 (100)
Numbness	19 (57.6)	7 (43.8)	6 (100)
Temperature or pain insensitivity	16 (48.5)	6 (37.5)	5 (83.3)
EQ-5D-5L index score, median (10th, 90th percentile)	0.8 (0.1, 0.9)	0.8 (0.3, 1.0)	0.5 (0.1, 0.6)
Karnofsky Performance Status score, n (%)			
10–30	0	0	0
40–60	9 (34.6)	4 (33.3)	3 (50.0)
70–90	14 (53.8)	5 (41.7)	3 (50.0)
100	3 (11.5)	3 (25.0)	0

BMI was available for 30 patients (France, n=16; Brazil, n=4); mBMI was available for 15 patients (France, n=6; Brazil, n=1); EQ-5D-5L index score was available for 17 patients (France, n=5; Brazil, n=5); Karnofsky Performance Status score was available for 26 patients (France, n=12; Brazil, n=6).
ATTRv amyloidosis, hereditary; ATTR, amyloid transthyretin amyloidosis; BMI, body mass index; mBMI, modified body mass index; THAOS, Transthyretin Amyloidosis Outcomes Survey.

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Analysis of cardiac involvement in hereditary transthyretin amyloidosis after liver transplantation

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Background: Liver transplantation (LT) has been widely performed as the main therapy for hereditary transthyretin (ATTRv) amyloidosis from the 1990s to the early 2010s, and has improved the prognosis of patients suffering from this disease. In long-term survival after LT, progression of cardiac involvement has become a major clinical issue in addition to ocular and leptomeningeal involvement.

Objective: The purpose of this study was to clarify the details of cardiac involvement in the long-term course of ATTRv amyloidosis after LT.

Subjects & Methods: The subjects were 49 patients with ATTRv amyloidosis who underwent LT and had cardiac evaluations. Cardiac involvement after LT were analyzed by electrocardiography, echocardiography, pyrophosphate myocardial scintigraphy, and biomarkers such as BNP levels.

Results: The average observation period from the onset of the disease was 15.9 years (up to 26.9 years). On echocardiography, the average IVSTd, E/e', and EF were 11.4 mm, 12.6, and 64.6%, respectively. Ventricular wall thickening and abnormal accumulation in pyrophosphate myocardial scintigraphy were observed in some cases with late-onset V30M and non-V30M. No significant progression of ventricular wall thickening was observed in cases with early-onset V30M. Twenty-three of 49 cases (46.9%) required pacemaker implantation. The average period from LT to pacemaker implantation was 11.6 years.

Summary & Conclusion: In cases of ATTRv amyloidosis after LT, cardiac conduction disorders continue to progress requiring pacemaker implantation in many cases, even after a long period of time after LT.

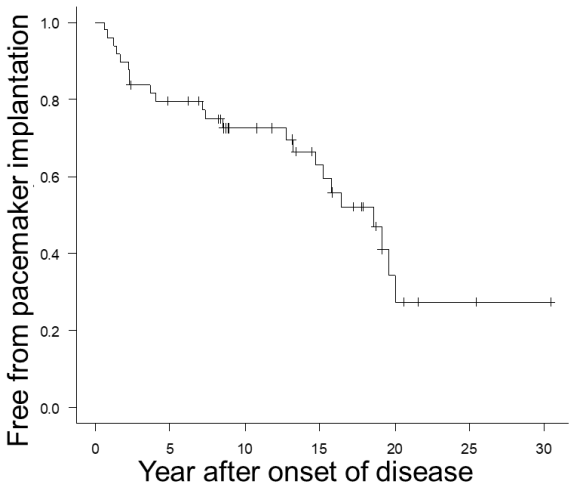

Figure 1.: Kaplan-Meier curve for pacemaker implantation-free in 49 cases ATTRv amyloidosis who underwent LT.

Effect of Patisiran on Polyneuropathy and Cardiomyopathy in Patients with hATTR Amyloidosis with V122I/T60A Variants: A Phase 4 Observational Study

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Background: Hereditary transthyretin-mediated (hATTR) amyloidosis, also known as ATTRv amyloidosis, is a rapidly progressive disease caused by variants in the *TTR* gene. The V122I and T60A variants were historically associated with cardiomyopathy, yet evidence of a mixed phenotype, and even sometimes isolated peripheral neuropathy without evidence of symptomatic cardiomyopathy, is emerging. Patisiran, an RNA interference (RNAi) therapeutic approved for the treatment of amyloidosis with polyneuropathy, targets liver-expressed variant and wild-type *TTR*.

Objective: To assess the effectiveness of patisiran in patients with hATTR amyloidosis with polyneuropathy and a V122I/T60A variant.

Material & Methods: In this multicenter, observational, Phase 4 study of patients in the United States (NCT04201418), the primary endpoint is the proportion of patients with improved/stable polyneuropathy disability (PND) score relative to baseline after 12 months of patisiran treatment. Measures of quality of life (QOL) related to neuropathy (Norfolk Quality of Life-Diabetic Neuropathy [QOL-DN]) and cardiomyopathy (Kansas City Cardiomyopathy Questionnaire [KCCQ]), autonomic symptoms (Composite Autonomic Symptom Score-31 [COMPASS-31]), and extent of cardiac dysfunction (New York Heart Association [NYHA] class) were also assessed. Shift in PND score from baseline to 12 months is reported for patients who completed the full study. Results are based on interim data; full data will be presented at the meeting.

Results & Discussion: As of February 2, 2022, 29 patients had completed 12 months on study: V122I, n=18; T60A, n=11; all with a baseline PND score ≥I. On average, in patients with evaluable baseline data, impaired QOL (mean [SD] Norfolk QOL-DN, 27.0 [35.6], n=5; KCCQ overall summary [OS] score, 62.8 [31.8], n=5), dysautonomia (mean [SD] COMPASS-31, 21.3 [21.6], n=4), and symptomatic cardiac involvement (NYHA class ≥II, n=15) were evident. Fourteen (48.3%) patients had no evidence of symptomatic heart failure. At 6 months, 24 (82.8%) patients had an improved/stabilized PND score vs baseline, increasing to 28 (96.6%) patients at 12 months. In patients with improved PND scores at 12 months (n=8), 5 (62.5%) improved from PND II to PND I, indicating a return to a state of unimpaired ambulation. Patisiran treatment was well tolerated and consistent with the established safety profile.

Summary & Conclusion: Most patients with hATTR amyloidosis with V122I/T60A variants in this Phase 4 study had a mixed phenotype, exhibiting both polyneuropathy and cardiac dysfunction. In addition, a number of patients experienced isolated peripheral neuropathy without symptomatic cardiac involvement. Regardless of genotype and phenotype, patients demonstrated improved/stabilized ambulatory function (as measured by PND score) after 12 months of patisiran treatment. Further, in line with results from other patisiran studies, several patients obtained improvement in their neurologic function in as little as 6 months of treatment.

Support & Funding: This study was funded by Alnylam Pharmaceuticals. Editorial assistance in the development of the abstract was provided by Adelphi Communications Ltd, UK, funded by Alnylam Pharmaceuticals in accordance with Good Publication Practice (GPP3) guidelines.

Transthyretin amyloid polyneuropathy in mainland China: a unicentric study

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Background: Transthyretin amyloid polyneuropathy (ATTR-PN) is a life-threatening autosomal dominant hereditary disease with TTR protein misfolding, tissue deposition, and others caused by TTR gene mutation. The disease is mainly reported in individual cases in mainland China. The clinical phenotypic and genotypic characteristics need to be further summarized.
Objective: We performed genetic and clinical phenotypic analysis of a large group samples of ATTR-PN patients from China..
Material & Methods: A total of 85 patients with ATTR-PN from 60 unrelated families with *TTR* gene diagnosed were included, and the genetic and clinical characteristics of ATTR-PN patients in mainland China were analyzed..

Results: A total of 28 different *TTR* gene mutations were identified in 60 unrelated families, including 15 families with Val30Met (25%) and 7 families with Ala97Ser (11.7%). The mutations not reported in the Chinese mainland population in this study were the Ser77Phe, Ser50Arg, Gly47Val, Glu54Gly, Thr59Lys, Ser23Asn, and Ala19Asp mutations. A total of 14 probands of Val30Met mutations in 15 families were late onset (93.4%) and 11 probands had negative family history at the first visit (73.3%). The mutations of ATTR-PN families in our center were distributed in 19 provinces of mainland China. Among them, the most common mutation (Val30Met) was mainly distributed in the northern region (13 families, 86.7%). However, the second common mutation (Ala97Ser) was mainly distributed in the southern (7 families, 85.7%). The mean age of onset for patients with ATTR-PN was 49.2±12.3 years (range 23 to 77 years). There were 47 (55.3%) late onset cases and 38 (44.7%) early onset cases. Family history was positive in 36 probands (60%). The median course from initial symptoms to the last visit was 4.0 (2.0, 6.0) years. Twelve patients had a long course of disease of 10 to 20 years, mainly with alternating diarrhea constipation, blurred vision and carpal tunnel syndrome as the initial symptoms and stable for a long time. Among 55 cases (64.7%) with distal paresthesia as the first symptom, there were eight cases with onset from upper extremity. The initial symptom of autonomic dysfunction occurred in 20 cases (23.5%), including gastrointestinal symptoms, sexual dysfunction, and orthostatic hypotension. The initial symptom was blurred vision in 12 patients (14.1%). As the disease progressed, all patients developed sensorimotor or sensory neuropathy, 20 patients (23.5%) developed carpal tunnel syndrome, and neurological examinations revealed varying proportions of deep and superficial dysesthesia, muscle weakness, and tendon hyporeflexia in patients with ATTR-PN. Electrophysiological results were mainly manifested as the length-dependent characteristics of sensorimotor involvement, with axonal impairments as the main manifestation. However, 10 patients (14.3%) had mixed neuropathy including axonal and demyelination impairments on electrophysiology, and the demyelination characteristics met the electrophysiological diagnostic criteria of definite CIDP. Patients in the Coutinho stage II or III disease group had a more significant decrease in the detected motor nerve conduction velocity, which was statistically different from that in the Coutinho stage I patients except for the common peroneal nerves ($p<0.05$).

Summary & Conclusion: This study confirmed that Val30Met is the hot mutation in the north of mainland China, while the Ala97Ser mutation is common in the south. The disease is mainly characterized by length-dependent axonal neuropathy, and a few of cases are accompanied by demyelination impairments. .

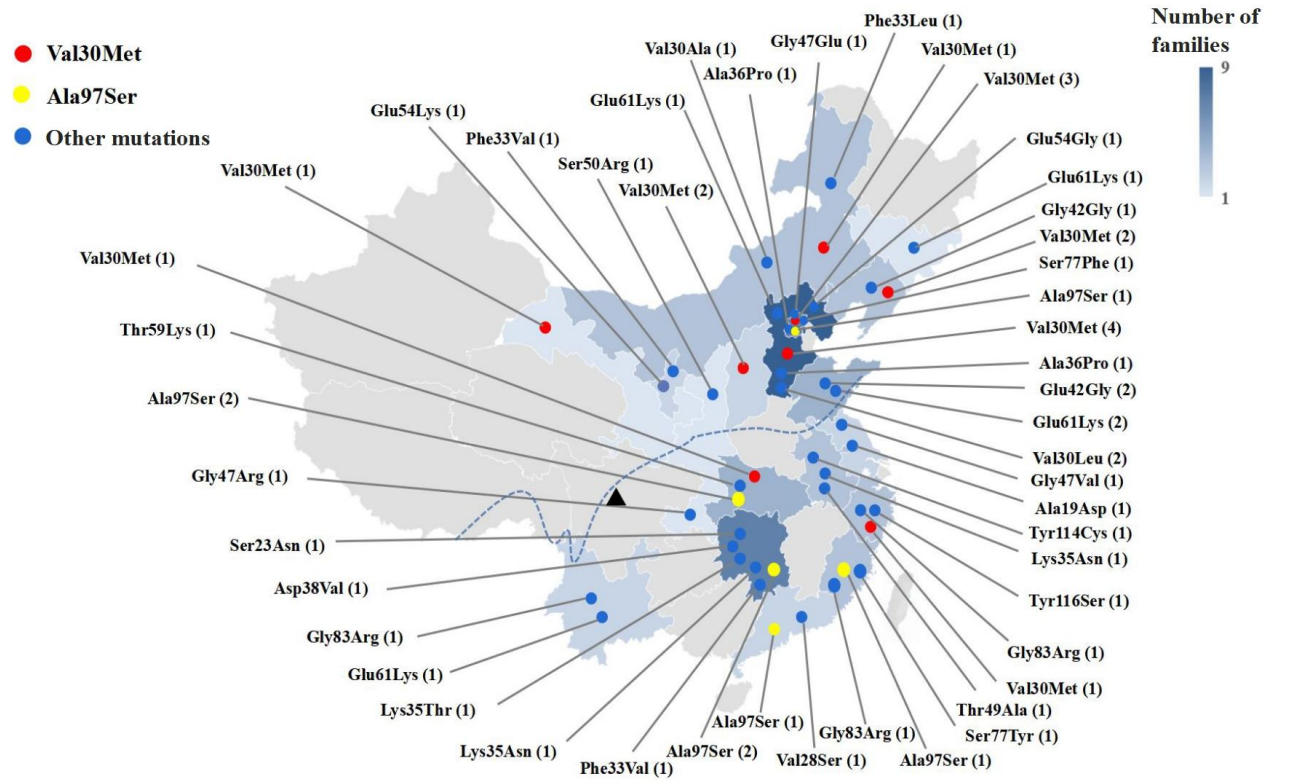

Figure 1.: Geographical distribution of ATTR-PN families in mainland China according to different mutations. ▲: Qinling Mountain_Huaihe River line, bove the line is the north, below is the south

References

Support & Funding: This study was supported by the Beijing Municipal Natural Science Foundation (7194323).

Phenotype and Clinical Outcomes of Hereditary Transthyretin Amyloidosis caused by p.Glu109Lys *TTR* Variant. A new endemic variant in Spain.

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Background: Hereditary transthyretin amyloidosis (ATTRv) is a multi-systemic disease associated with genetic variants in transthyretin (*TTR*). The p.Glu109Lys variant is a rare cause of ATTRv for which the clinical spectrum and prognosis remain unresolved.

Objective: We sought to describe the clinical characteristics and outcomes of p.Glu109Lys ATTRv and assess a potential founder effect in Spain.

Material & Methods: Patients with ATTRv and relatives with the p.Glu109Lys variant from 14 families were recruited at 7 centers. Demographics, complementary tests and clinical course were analyzed. Haplotype analysis was performed in 7 unrelated individuals.

Results: Thirty-eight individuals (13 probands, 15 females, mean age 40.4±13.1 years) were studied. After median follow-up of 5.1 years (IQR, 1.7–9.6), 7 patients died and 7 (19%) required heart transplantation (median age at transplantation 50.5 years; IQR, 45.8–53.2). Onset of cardiac and neurological manifestations occurred at a mean age of 48.4 and 46.8 years, respectively. Disease penetrance was almost complete (96%) by age 60. Ophthalmologic involvement showed incomplete penetrance with a mean age at onset of 52.8 years. Median survival from birth was 61.6 years and no individual survived beyond 65 years. Patients treated with disease-modifying therapies exhibited better prognosis (P<0.001). Haplotype analysis revealed a common origin from an ancestor who lived ~500 years ago in southeast Spain in the province of Jaen (Andalusia) and more precisely in the town of Villacarrillo (10,673 inhabitants).

Summary & Conclusion: p.Glu109Lys ATTRv is a *TTR* variant with a founder effect in Spain. It is associated with near complete penetrance, early onset, and mixed cardiac and neurologic phenotype. Overall, patients have poor prognosis, particularly if not treated with disease-modifying therapies.

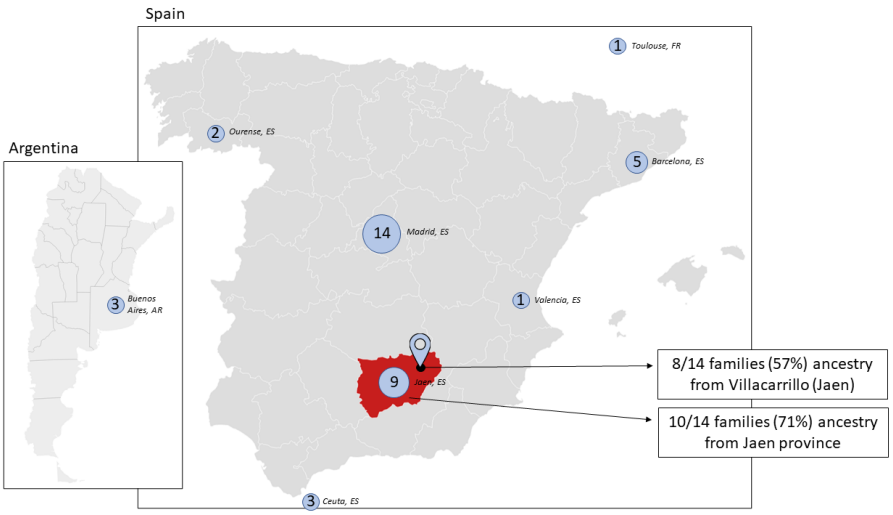

Figure 1: Geographical origin of patients. Numbers displayed represent the province of birth of patients included in the cohort. Jaen province, where most families shared common ancestry, is highlighted in red and the town of Villacarrillo highlighted. AR: Argentina; ES: Spain; FR: France.

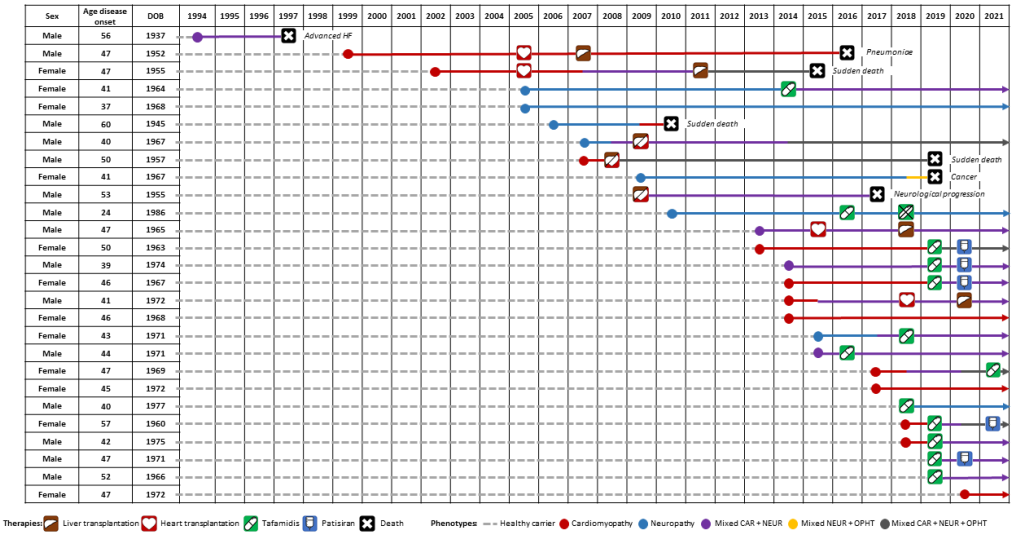

Figure 2: Summary of clinical course of patients from disease onset. DOB: Date of birth; CAR: Cardiomyopathy; NEUR: Neuropathy; OPHT: Opthalmopathy.

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Comparison of disability: intradermal vasomotor nerves, intradermal sudomotor nerves, and intraepidermal sensory nerves in hereditary transthyretin amyloidosis

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Background: Small fiber neuropathy occurs early after onset of hereditary transthyretin (ATTRv) amyloidosis. However, accurate evaluation of the progression of small fiber neuropathy is not easy because notable progression marker for small fiber neuropathies has not been established. The pathophysiology of the small fiber neuropathy in these patients remains to be elucidated.

Objective: The purpose of this study was to clarify the difference in disability at the same time between intradermal vasomotor nerves, intradermal sudomotor nerves, and intraepidermal sensory nerves in ATTRv amyloidosis.

Material & Methods: Thirty-six Japanese ATTRv patients, 5 asymptomatic mutation carriers, and 18 healthy controls were available for this study. We evaluated the feet electrochemical skin conductance (ESC) using SUDOSCAN in all subjects. Moreover, we also measured the intraepidermal nerve fiber density (IENFD) values at the distal leg, sural sensory nerve action potential (SNAP), heat-pain detection threshold (HPDT) at the dorsum of the foot, and the levels of orthostatic hypotension (OH) in all subjects. OH was defined as a systolic BP decrease of 20 mm Hg or more within 4 minutes of tilting.

Results: 1) Significant decrease in the IENFD values was observed not only in patients with ATTRv amyloidosis but also in asymptomatic mutation carriers. 2) The feet ESC levels of ATTRv patients were significantly lower than those of healthy controls. However, the feet ESC levels of asymptomatic mutation carriers were not significantly lower than those of healthy controls. 3) OH was observed in patients with ATTRv amyloidosis but not in asymptomatic mutation carriers. Moreover, no correlation was found between the feet ESC levels and OH levels in ATTRv patients. Although the appearance of OH tended to precede the decline of feet ESC level, it tended to follow the decline of IENFD values.

Summary & Conclusion: Disability of intraepidermal sensory nerve tend to precede not only the disability of intradermal vasomotor nerve and intradermal sudomotor nerve, but also parasympathetic dysfunction leading to myocardial muscarinic receptor upregulation in ATTRv amyloidosis. Thus, skin biopsy to measure the IENFD values may be a notable progression marker for small fiber neuropathies in these patients.

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A descriptive analysis of patients with ATTR amyloidosis and a mixed phenotype from the Transthyretin Amyloidosis Outcomes Survey (THAOS)

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Background: Transthyretin amyloidosis (ATTR amyloidosis) is a rare, life-threatening disease resulting from the deposition of amyloid fibrils in the peripheral nerves, heart, and other tissues and organs.[1] ATTR amyloidosis is caused by pathogenic mutations in the transthyretin (*TTR*) gene (ATTRv amyloidosis) or the aggregation of wild-type TTR protein (ATTRwt amyloidosis).[1] The phenotypic presentation of ATTR amyloidosis can be predominantly neurologic, predominantly cardiac, or a mix of both neurologic and cardiac symptoms.[1]

Objective: This analysis aimed to describe characteristics of patients with ATTR amyloidosis and a mixed phenotype enrolled in the Transthyretin Amyloidosis Outcomes Survey (THAOS).

Material & Methods: THAOS is an ongoing, global, longitudinal, observational survey of patients with ATTR amyloidosis, including both hereditary and wild-type disease, and asymptomatic carriers of pathogenic *TTR* mutations (NCT00628745). This analysis described demographic and clinical characteristics of patients with a mixed phenotype at enrollment in THAOS (data cutoff date: January 4, 2022).

Results: There were 1033 patients (72.3% male; 73.1% White) from 21 countries with a mixed phenotype. Of these, 313 (30.3%) had wild-type disease, 342 (33.1%) had the Val30Met (p.Val50Met) mutation, and 378 (36.6%) had non-Val30Met mutations. The most common non-Val30Met mutations were Val122Ile (p.Val142Ile) (n=84) and Glu89Gln (p.Glu109Gln) (n=68). Median age at enrollment was 69.6 years overall and was higher in patients with wild-type disease than with Val30Met or non-Val30Met mutations (Table). In all patients with a mixed phenotype, 71.7% had sensory neuropathy, 61.4% had autonomic neuropathy, 46.6% had gastrointestinal symptoms, and 42.4% had motor neuropathy. Gastrointestinal symptoms and autonomic, sensory, and motor neuropathy were more common in patients with ATTRv amyloidosis vs ATTRwt amyloidosis, and wild-type patients had lower total scores on the Neuropathy Impairment Score in the Lower Limbs scale. Heart failure was present in 708 (68.5%) patients, most of whom were classified as New York Heart Association (NYHA) functional class II or III at enrollment. Median left ventricular (LV) septal thickness was higher, and median LV ejection fraction lower, in wild-type and non-Val30Met patients vs Val30Met patients. Wild-type patients also had a higher modified body mass index and better quality of life, in terms of EQ-5D-3L derived index and Norfolk Quality of Life – Diabetic Neuropathy scores, compared with patients with Val30Met and non-Val30Met mutations.

Summary & Conclusion: Over 1000 patients with ATTRv amyloidosis or ATTRwt amyloidosis had a mixed phenotype in THAOS. Sensory neuropathy was the most frequently reported neurologic symptom overall and was more common in patients with ATTRv amyloidosis compared with ATTRwt amyloidosis. Gastrointestinal symptoms and autonomic and motor neuropathy were also frequently reported in patients with ATTRv amyloidosis but less so in wild-type patients. Most patients with heart failure were classified as NYHA functional class II or III at enrollment. As the mixed phenotype may be relatively common amidst this rare disease,[2-4] these THAOS data provide important insights into the clinical picture of this population.

Table. Baseline demographic and clinical characteristics of patients with a mixed phenotype in THAOS

	Overall n=1033	ATTRwt amyloidosis n=313	Val30Met ATTRv amyloidosis n=342	Non-Val30Met ATTRv amyloidosis n=378
Male, n (%)	747 (72.3)	287 (91.7)	218 (63.7)	242 (64.0)
Age at enrollment, median (10th, 90th percentile), years	69.6 (46.8, 83.8)	78.9 (69.2, 88.4)	62.3 (37.6, 76.3)	64.4 (49.8, 78.1)
Symptom duration, median (10th, 90th percentile), years	5.8 (1.1, 17.9)	4.4 (0.7, 18.4)	7.1 (2.4, 17.1)	5.2 (1.1, 18.2)
mBMI, median (10th, 90th percentile)	**n=663** 969.3 (711.7, 1255.8)	**n=188** 1034.7 (821.6, 1316.8)	**n=242** 927.3 (649.3, 1198.5)	**n=233** 946.6 (690.9, 1240.3)
Symptoms at enrollment, n (%)				
Motor neuropathy	438 (42.4)	33 (10.5)	227 (66.4)	178 (47.1)
Sensory neuropathy	741 (71.7)	124 (39.6)	324 (94.7)	293 (77.5)
Gastrointestinal	481 (46.6)	27 (8.6)	247 (72.2)	207 (54.8)
Autonomic neuropathy	634 (61.4)	82 (26.2)	295 (86.3)	257 (68.0)
Cardiac disorder	943 (91.3)	223 (71.2)	342 (100.0)	378 (100.0)
Other	521 (50.4)	143 (45.7)	186 (54.4)	192 (50.8)
Derived NIS-LL total score, median (10th, 90th percentile)	**n=425** 16.0 (0.0, 64.0)	**n=72** 4.5 (0.0, 16.0)	**n=177** 36.0 (5.0, 72.0)	**n=176** 14.0 (0.0, 53.8)
Heart failure, n (%)	708 (68.5)	223 (71.2)	149 (43.6)	336 (88.9)
NYHA class[a], n (%)				
I	82 (11.6)	16 (7.2)	37 (24.8)	29 (8.6)
II	351 (49.6)	112 (50.2)	76 (51.0)	163 (48.5)
III	228 (32.2)	79 (35.4)	29 (19.5)	120 (35.7)
IV	26 (3.7)	5 (2.2)	3 (2.0)	18 (5.4)
Missing	21 (3.0)	11 (4.9)	4 (2.7)	6 (1.8)
LV septal thickness, median (10th, 90th percentile), mm	**n=610** 16.8 (12.0, 22.0)	**n=233** 17.0 (13.0, 21.0)	**n=125** 15.0 (9.2, 22.0)	**n=252** 17.0 (13.0, 22.0)
LV ejection fraction, median (10th, 90th percentile), %	**n=578** 55.0 (34.0, 69.0)	**n=235** 53.0 (33.0, 67.0)	**n=94** 61.0 (50.0, 75.0)	**n=249** 53.0 (31.0, 69.0)
EQ-5D-3L derived index, median (10th, 90th percentile)	**n=664** 0.8 (0.3, 0.9)	**n=215** 0.8 (0.5, 1.0)	**n=226** 0.7 (0.3, 0.8)	**n=223** 0.7 (0.3, 0.9)
Norfolk Quality of Life – DN score, median (10th, 90th percentile)	**n=660** 40.0 (5.0, 90.0)	**n=203** 24.0 (2.0, 62.0)	**n=228** 49.0 (10.0, 97.0)	**n=229** 49.0 (6.0, 96.0)

[a]NYHA functional class reported for patients with heart failure only.
DN, diabetic neuropathy; mBMI, modified body mass index; NIS-LL, Neuropathy Impairment Score in the Lower Limbs.

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Hereditary transthyretin amyloidosis in middle-aged and elderly patients with idiopathic polyneuropathy: a nationwide prospective study.

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Background: Hereditary transthyretin amyloidosis (ATTRv) is an adult-onset autosomal dominant disease resulting from TTR gene pathogenic variants. ATTRv often initially presents as a progressive polyneuropathy, and effective ATTRv treatments are available.

Objective: To determine the prevalence of ATTRv in a cohort of patients with age > 50 years with an idiopathic progressive polyneuropathy.

Material & Methods: In this 5 year-long (2017-2021) nationwide prospective study, we systematically analyzed the TTR gene in French patients with age > 50 years with a progressive idiopathic polyneuropathy. The most frequent causes of polyneuropathy, e.g., diabetes mellitus, vitamin deficiency, kidney failure, and alcoholism, were ruled out by extensive clinical and laboratory testing.

Results: 553 patients (70% males) with a mean age of 70 years (60-80) were included in this study. A TTR gene pathogenic variant was found in 16 patients (2.9%), including the Val30Met TTR variation in 11 cases. In comparison with patients with no TTR gene pathogenic variants (n=537), patients with TTR pathogenic variants (n=16) more often presented with orthostatic hypotension (56% vs 21%, p=0.003), significant weight loss (31% vs 11%, p=0.032), rapidly deteriorating peripheral nerve conduction studies (31% vs 8%, p=0.008), and were more often of Portuguese origin (12% vs 2%, p=0.044).

Summary & Conclusion: In this nationwide prospective study, we found ATTRv in 2.9% of patients with age > 50 years with a progressive polyneuropathy, suggesting ATTRv should be systematically investigated in patients with adult-onset idiopathic polyneuropathy. As ATTRv is a treatable disorder, these results are highly important for the early identification of patients in need of disease-modifying treatments.

Support & Funding: This work was supported by a research grant (ID# 53232309) from Pfizer to APHP. However, Pfizer Inc. did not have influence on study design, data processing, or statistical analysis.

Characterization of patients with hereditary Transthyretin Amyloidosis in a Register Study in Germany

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Background:
Systemic transthyretin amyloidosis is caused by deposition of transthyretin (TTR) fibrils especially in the peripheral and autonomic nervous system and the heart[1,2]. It can be either acquired (ATTRwt) or hereditary (ATTRv), while especially in ATTRv, the clinical presentation and age of onset is very variable, even within one genotype. Although the development of effective treatments like TTR stabilizers and gene silencing agents has advanced greatly over the last years, most data on therapy effects and clinical presentation of ATTRv is limited on the few most frequent endemic mutations and typical clinical presentation[3].

Objective:
Within the Amyloidosis Center Charité Berlin (ACCB) we aimed at developing an algorithm for through clinical characterization of patients with ATTRv in order to better understand phenotype and genotype variability especially in rare genotypes of ATTRv to provide better diagnosis, therapy initiation criteria and prognosis.

Material & Methods:
Our algorithm for longitudinal clinical characterization and evaluation of disease progress and therapy effectiveness for patients treated at the ACCB included neurological examination (Neuropathy Impairment Score), questionnaires on live quality (Norfolk QOL, RODS) and autonomic symptoms (COMPASS31 score), and laboratory examinations. Furthermore, histology of diagnostic biopsies, electrophysiology, DPD-scintigraphy, magnetic resonance imaging, and echocardiography were performed depending on individual organ affection. Patients were monitored depending on FAP stage, disease progression, therapy, and induvial needs in 3-12 months intervals.

Results:
Within our register study, we collected patients with various mutations, including p.Val50Met, p.Val142Ile, p.Thr80Ala, p.Leu75Arg, p.Ala129Val, and p.Thr126Asn, as well as variants of unknown significance such as p.Glu27Lys. While patients with p.Val50Met presented with the neuropathy dominant phenotype, patients with p.Val142Ile variants presented with cardiomyopathy. Severe neuropathy was seen in a family with p.Leu75Arg ATTR. Interestingly, in a family with p.Thr80Ala ATTR we detected significant interindividual alterations in phenotype ranging from cardiomyopathy to neuropathy.

Summary & Conclusion:
Patients with ATTRv present with a wide spectrum of mutations within the *TTR* gene, resulting in diverse clinical presentations. While typically, some mutations lead to cardiomyopathy and others to neuropathy dominant phenotypes, we describe patients with differential clinical presentation even within the same mutation. These results reveal a new diversity of ATTRv phenotypes and new challenges in therapy initiation and monitoring. Further research, including pathophysiological background of phenotypical variability and consistent biomarkers for disease onset, progression and therapy effect is needed to provide effective treatment to patients with rare genotypes or atypical phenotypes.

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Hereditary Transthyretin Amyloidosis (ATTRv) in the Middle East: a short report of two confirmed cases

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Background: Hereditary transthyretin amyloidosis (ATTRv) is an autosomal dominant, multisystemic and progressive disease characterized by polyneuropathy, cardiomyopathy and dysautonomia to varying degrees. The number of cases diagnosed with transthyretin Amyloidosis (ATTR) has exponentially increased over the last decade due to increased awareness and improved diagnostic methods. However, there has not been any reports of specific ATTRv mutations from the region of the Middle East. The Abdali National Amyloidosis Center (ANAC) was established 2020 in Amman/Jordan. Run by a multidisciplinary team, it succeeded in diagnosing many patients with immunoglobulin light chain amyloidosis (AL) and ATTR. Here we report 2 cases of ATTRv diagnosed in our center.

Objective: To highlight the TTR mutation and phenotype of 2 Middle Eastern patients with ATTRv

Material & Methods: The medical records of two patients who were recently diagnosed with ATTRv in our center were reviewed. This abstract summarizes their clinical characteristics and phenotypic presentation, and the results of the investigations performed and their outcomes.

A 67-year-old man form north Iraq referred to ANAC on 9/2021 for suspected cardiac amyloidosis. He presented with diarrhea and exertional dyspnea of 5 years duration. ECG showed NSR and low voltage. Echocardiogram showed severe concentric LVH with an EF of 40%, a grade 2 diastolic dysfunction, right ventricular hypertrophy, enlarged right and left atria, thickened IAS, the characteristic apical sparing on global longitudinal strain imaging, and a small posterior PE. NT-proBNP was 22256 pg/L and hs-cTnI was 421.1 ng/L. AL was ruled out. A PYP scan was Perugini grade 3 positive. Gene sequencing was positive for Thr80Ala. Unfortunately, he died before receiving any specific therapy

A 52-year-old man from North Iraq who was referred to ANAC on 10/2021 for suspected cardiac amyloidosis. He complained of cough, anorexia, weight loss and pain in both legs of 1 year duration. Electrocardiogram (ECG) showed a normal sinus rhythm (NSR) and low voltage. An echocardiogram showed concentric left ventricular hypertrophy (LVH) with an ejection fraction (EF) of 45%, thickened mitral and aortic valve leaflets, a small posterior pericardial effusion (PE), and a thickened interatrial septum (IAS). NT-proBNP was 1204 pg/L and hs-cTnI was 83.1 ng/L. AL was ruled out by negative serum and urine immunofixation electrophoresis and normal free light chain assays. A 99mTc-Pyrophosphate (PYP) scan was Perugini grade 3 positive. A nerve conduction study revealed mild bilateral symmetrical, predominantly sensory, axonal polyneuropathy. In addition, there was bilateral mild carpal tunnel syndrome (grade 2/6). Gene sequencing was positive for Thr80Ala mutation. The patient was started on Tafamidis

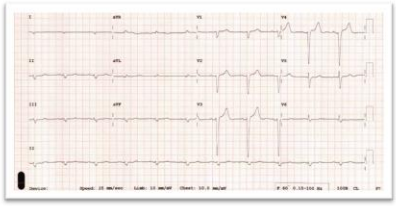
Results & Discussion: Both patients presented with a phenotype of polyneuropathy and cardiac involvement. Both were initially misdiagnosed and empirically treated until they were referred to our center. Once diagnosed, treatment was offered to both, but was only instituted in one due to financial constraints and drug unavailability

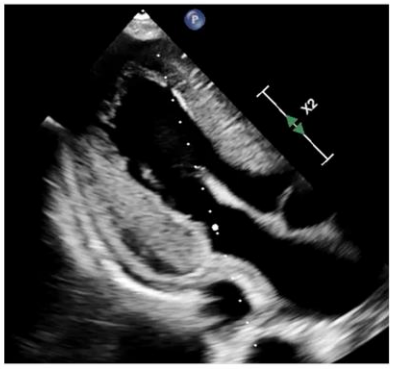
Summary & Conclusions: To our knowledge ATTRv has not been systematically reported from the region of the Middle East. Both cases illustrate the predominance of cardiac and neurological involvement, highlighting the Importance of early suspicion in patients presenting with polyneuropathy or cardiac manifestations. Whether Thr80Ala is the most common type will depend on diagnosing more patients

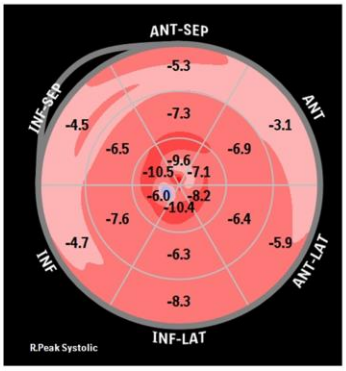
Clinical characteristics

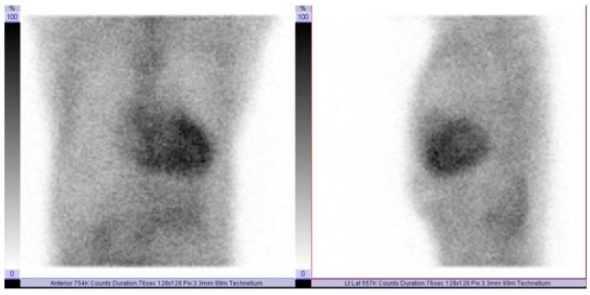
	Patient number 1	Patient number 2
Age (years)	67	52
Gender	Male	Male
Residence	North Iraq	North Iraq
Duration of symptoms (years)	5	1
Initial presentation	Exertional dyspnea and diarrhea	Cough, anorexia, weight loss, leg pain, polyneuropathy, carpal tunnel syndrome
ECG	NSR. Low voltage	NSR. Low voltage
LVH on echocardiogram	Present	Present
LVEF (%)	40	45
GLS	Apical sparing	Not done
NT-proBNP (pg/L)	22256	1204
Hs-cTnI (ng/L)	421.1	83.1
PYP scan result	Grade 3 positive	Grade 3 positive
Gene sequencing	Thr80Ala	Thr80Ala

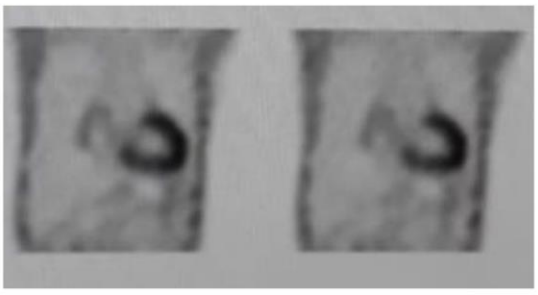
Patient number 1:
ECG, echocardiogram, GLS, planar and SPECT PYP scan











How occupational needs can be obtained? A Semi-Structured Interview with Hereditary Transthyretin Amyloidosis patients.

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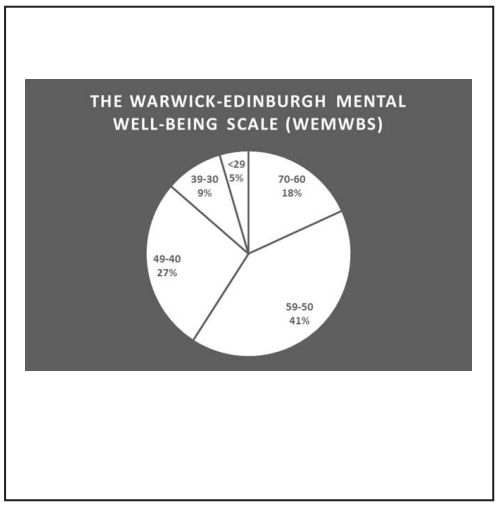
Background: The purpose of this study was to describe a semi-structured interview done in patients with hereditary transthyretin amyloidosis (ATTRv), to better understand patients' occupational situation and needs after diagnosis in order to create a personal and effective occupational therapy intervention.

Objective: To describe a semi-structured interview done in patients with hereditary transthyretin amyloidosis (ATTRv), to better understand patients' baseline situation.

Material & Methods: This is a descriptive, cross-sectional and non-interventional study designed to describe semi-structured interviews done in patients with hereditary transthyretin amyloidosis ATTRv Val50Met. A semi-structured interview created by the research team was used. The first part was a questionnaire consisting of 12 demographic questions, including general data. The second part had two sections, with three main points: physical, psychological and occupational. Physical activity was evaluated using the Spanish version of the Barthel Index (BI) and the Lawton and Brody scale. The Warwick-Edinburgh Mental Well-Being Scale captures a wide conception of well-being. SF-36 evaluated general well-being and Norfolk revealed main symptoms of the diagnosed. Section two consisted of items focusing on the participant's occupational situation.

Results: 44 patients were included in the specific study done in the Hospital Son Llàtzer. The patients were mainly female, 30 (68,18%) and with an average age of 53.06 years. About Occupational aspects, patients were mostly not working because of the ATTRV symptoms or retired 29 (66%), only 15 (34,01%) were employed. Performance of basic activities was found to be influenced by ATTRv disease in 38 (86.36%) were independent, 3 (7%) were slightly dependent and 3 (7%) were moderately dependent. However, all of them were independent due to the early stage of the disease. About Intrumental activities, 50% of the patients interviewed do not have a full punctuation in the Lawton and Brody test, it shows that they found some difficulties to develop their intrumental activities because of symptoms ATTRv disease. Despite the small sample analysed we found a tendency towards correlation between the two scales. As no specific scales for ATTRv have been validated we aimed to include in the design of our study a scale to quantify disability, quality of life and wellbeing. Data regarding the Warwick-Edinburgh Mental Well-Being Scale, thirty-six (82%) of the participants, who answered this scale, refeared that after the diagnosis there was affection in their mental health.Finally, SF-36 and Norfolk have been collected. About Norfolk Scale, all patients 100% blamed symptoms on daily life difficulties, despite the fact that they mostly continue with their daily life activities. Interviewed patients comment that pain and loss sensations in feet and legs 44 (100%), difficulty with fine motor skills, and tiredness were their typical symptoms. Data concerning SF-36 scale punctuations, general well-being, social function, emotional well-being were three most affected items in the scale.

Summary & Conclusion: In conclusion, our data reveal that ATTRv is a severely debilitating disease for patients physically but also psychologically and specifically occupationally. All data obtained in the semi-structured interview could be fundamental information for future Occupational Therapy programmes.



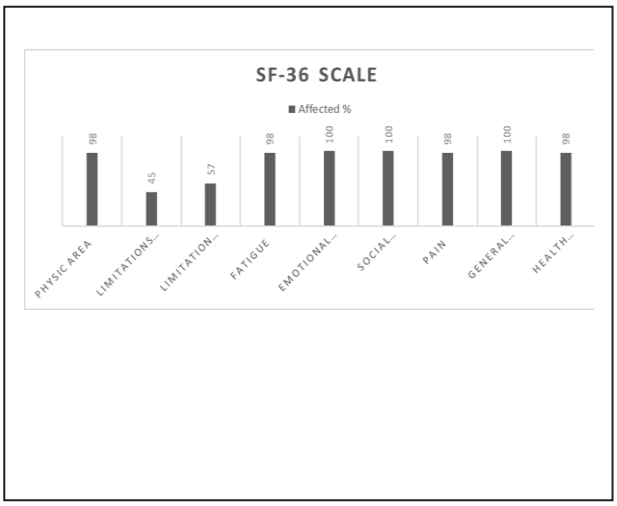


Figure 1.: Patient's responses in Warwick-Edinburgh Mental Well-Being Scale (WEMWBS).

Figure 2.: Results of SF-36 Scale in a group of 44 patients with ATTRv.

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A rare case of late-onset hereditary ATTR Amyloidosis with cardiac and neurologic manifestation

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Background: Hereditary ATTR (ATTRv) amyloidosis is a rare genetic disease caused by mutations of transthyretin (TTR) gene with a wide range of clinical manifestations[1]. There are many reported genetic mutations in the TTR-gene and over 100 have been described as amyloidogenic[2,3].

Objective: p.Thr60Asn, c.179C>A (nomenclature according to the Human Genome Variation Society) is a rare TTR-gene mutation. To date, only few case reports have been published regarding its clinical phaenotype. We present the clinical features of and the diagnostic approach to a 74-year-old patient with late onset p.Thr60Asn ATTRv amyloidosis.

Material & Methods: The patient underwent a comprehensive multidisciplinary assessment focusing on cardiac and neurologic function, structure and imaging. Genetic testing was performed.

Results: A 74-year old woman presented with a 6-month history of exertional dyspnea New York Heart Association-Class II and mild gait instability with occasional vertigo. She underwent bilateral surgery for carpal tunnel syndrome years before. Family history for chronic cardiac and neurologic diseases or amyloidosis was negative. Physical examination revealed no signs of heart failure or congestion. 12-lead electrocardiogramm showed sinus rhythm with normal voltage. Echocardiography revealed biventricular myocardial hypertrophy (interventricular septum 17mm, right ventricular wall 7mm), left ventricular (LV) ejection fraction 64% and higher-grade diastolic dysfunction in absence of significant valvular disease. LV global longitudinal strain was reduced at -14,1%, speckle tracking showed “apical sparing” pattern. The eGFR was 91 ml/min/1,73m², NT-proBNP was 1393 pg/ml. Free kappa/lambda light chain quotient was normal. Immunfixation of serum and urin were unremarkable. Bone scintigraphy showed intensive cardiac tracer uptake with a Perugini Score 3[4]. Neurologic assessment revealed mild gait impairment and distal areflexia at the lower limbs without sensory abnormalities or paresis. Motor and sensory nerve conduction studies were normal. Quantitative sensory testing revealed small fiber dysfunction of the Aδ type. Skin punch biopsies showed distally reduced intraepidermal nerve fiber density without evidence of amyloid deposits. Small-fiber neuropathy was diagnosed according to Devigili et al., 2008[5]. Genetic testing revealed a mutation in the TTR-gene (p.Thr60Asn).
Finally, mixed phenotype ATTRv amyloidosis with cardiac (Stage I according to Gillmore et al.)[6] and early neurologic involvement (FAP Stage I) was diagnosed. A disease-modifiying therapy with Patisiran, a small interfering ribonucleic acid therapeutic, was initiated. Genetic counseling led to initiation of screening for hereditary ATTR amyloidosis in other family members.

Discussion: Although the cinical presentation was suggestive of ATTR wildtype cardiomyopathy, a rare case of p.Thr60Asn ATTRv amyloidosis of mixed phenotype was diagnosed in our multidisciplinary setting using a comprehensive clinical and apparative approach. Predescribed cases with ATTR-v amyloidosis carrying the p.Thr60Asn mutation were all older than 60 years at diagnosis, all had cardiac manifestation, and most of them also neurologic manifestation[7].

Summary & Conclusion: There is a huge variety in onset and clinical manifestation of hereditary amyloidosis [8]. Our case provides additional evidence, that the p.Thr60Asn mutation is associated with late-onset disease with cardiac and neurologic impairment and encourages genetic testing also in eldely patients with TTR amyloidosis.

Figure 1.

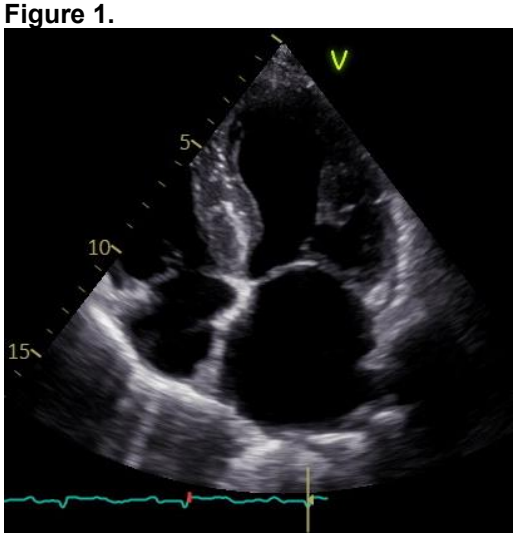

Figure 2.

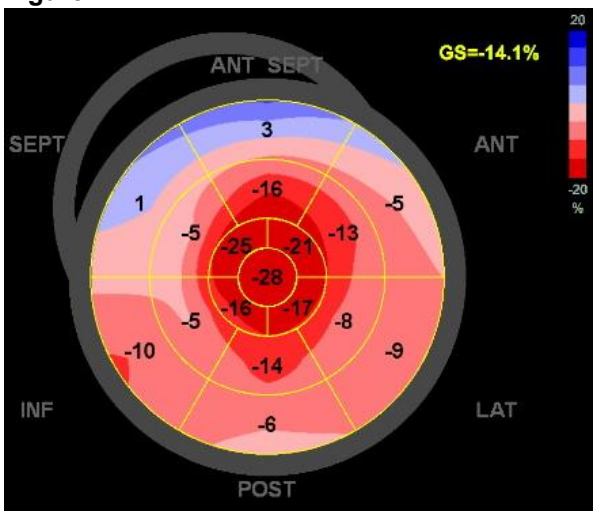

Figure 1.: Transthoracic echocardiography apical 4-chamber view showing remarkable hypertrophy of the interventricular septum and granular sparkling texture of the myocardium.

Figure 2.: Speckle tracking analysis of the left ventricle. Longitudinal shortening is impaired at the basal parts and preserved at the apex, known as “apical sparing” pattern. Global longitudinal strain is reduced at -14,1%.

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Characteristics of patients with ATTR amyloidosis and the Ser77Tyr mutation: insights from the Transthyretin Amyloidosis Outcomes Survey (THAOS)

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[6]Pfizer Inc, New York, NY, USA

Background: Hereditary transthyretin amyloidosis (ATTRv amyloidosis) is a clinically heterogeneous disease resulting from mutations in the transthyretin (*TTR*) gene.[1] Currently, more than 120 different *TTR* mutations associated with transthyretin amyloidosis (ATTR amyloidosis) have been identified,[2] but many are not well-characterized.

Objective: The aim of this analysis was to describe the characteristics of patients with the Ser77Tyr (p.Ser97Tyr) *TTR* mutation enrolled in the Transthyretin Amyloidosis Outcomes Survey (THAOS).

Material & Methods: THAOS is the largest, ongoing, global, longitudinal, observational study of patients with ATTR amyloidosis, including both hereditary and wild-type disease, and asymptomatic carriers of pathogenic *TTR* mutations (NCT00628745). This analysis described the demographic and clinical characteristics of patients with a Ser77Tyr mutation at enrollment in THAOS (data cutoff date: January 4, 2022).

Results: Overall, 75 patients with the Ser77Tyr *TTR* mutation were enrolled in France, Israel, Spain, Italy, and the United States. Of these 60.0% (45/75) were symptomatic at enrollment. Symptomatic patients had a median age (10th, 90th percentile) of 58.9 years (48.4, 69.2) and 68.9% were male (**Table**). Most were enrolled at THAOS study sites in France (53.3% of all symptomatic patients) or Israel (22.2%). At enrollment, symptomatic patients in France had a median age of 57.8 years, a median duration of symptoms of 7.7 years, and 66.7% were male. Symptomatic patients in Israel had a median age of 60.7 years, a median duration of symptoms of 3.6 years, and 80.0% were male. The phenotype of ATTRv amyloidosis in patients with the Ser77Tyr mutation was predominantly neurologic (55.6% overall). A mixed phenotype was observed in 31.1% of symptomatic patients, and a predominantly cardiac phenotype in 2.2%. The most common (in >15% of all patients) neurologic symptoms were neuropathic pain/paresthesia, numbness, temperature/pain insensitivity, and tingling. The most common cardiac symptoms were heart failure and rhythm disturbance. Karnofsky Performance Status score (range 0–100) was 70–100 in 81.8% of symptomatic patients.

Summary & Conclusion: The majority of patients with the Ser77Tyr *TTR* mutation in THAOS presented with a predominantly neurologic phenotype; however, a notable proportion presented with a mixed phenotype. Most symptomatic patients were enrolled at THAOS study sites in France and Israel. Despite some regional differences in ATTRv amyloidosis presentation, the most common cardiac and neurologic symptoms in France and Israel were broadly similar. Accumulating real-world data in THAOS can be used to better understand the differences in ATTRv amyloidosis presentation and natural history of specific mutations.

Table. Demographics and clinical characteristics of symptomatic patients with ATTRv amyloidosis and the Ser77Tyr mutation in THAOS

	Symptomatic patients with the Ser77Tyr *TTR* mutation		
	All countries N=45	**France n=24**	**Israel n=10**
Age at enrollment, median (10th, 90th percentile), years	58.9 (48.4, 69.2)	57.8 (45.0, 69.2)	60.7 (53.6, 66.0)
Male, n (%)	31 (68.9)	16 (66.7)	8 (80.0)
BMI, median (10th, 90th percentile)	**N=43**	**n=24**	**n=10**
	25.5 (20.1, 33.1)	24.7 (19.9, 33.4)	27.9 (20.4, 36.9)
mBMI, median (10th, 90th percentile)	**N=18**	**n=9**	**n=3**
	993.9 (597.9, 1505. 0)	1002.5 (530.0, 1998.3)	994.3 (597.9, 1298.7)
Race/ethnicity, n (%)	**N=21**	**Not collected**	**n=10**
White	20 (95.2)	–	10 (100)
Other	1 (4.8)	–	0
Duration of ATTRv amyloidosis symptoms, median (10th, 90th percentile), years	7.5 (1.3, 14.3)	7.7 (1.5, 12.1)	3.6 (0.4, 21.4)
Phenotype, n (%)			
Predominantly cardiac	1 (2.2)	0	0
Predominantly neurologic	25 (55.6)	15 (62.5)	4 (40.0)
Mixed	14 (31.1)	9 (37.5)	3 (30.0)
Unknown	5 (11.1)	0	3 (30.0)
Cardiac symptoms, n (%)			
Heart failure	15 (33.3)	9 (37.5)	3 (30.0)
Rhythm disturbance	8 (17.8)	7 (29.2)	1 (10.0)
Neurologic symptoms, n (%)			
Neuropathic pain/paresthesia	29 (64.4)	17 (70.8)	7 (70.0)
Numbness	24 (53.3)	12 (50.0)	6 (60.0)
Temperature or pain insensitivity	14 (31.1)	8 (33.3)	3 (30.0)
Tingling	8 (17.8)	1 (4.2)	4 (40.0)
Karnofsky Performance Status score, n (%)	**N=33**	**n=24**	**n=5**
10–30	1 (3.0)	1 (4.2)	0
40–60	5 (15.2)	3 (12.5)	2 (40.0)
70–90	22 (66.7)	16 (66.7)	3 (60.0)
100	5 (15.2)	4 (16.7)	0

ATTRv amyloidosis, hereditary transthyretin amyloidosis; BMI, body mass index; mBMI, modified body mass index; THAOS, Transthyretin Amyloidosis Outcomes Survey; *TTR*, transthyretin.

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1. Ruberg FL, et al. J Am Coll Cardiol 2019;733:2872-91.

2. Rowczenio DM, et al. Hum Mutat 2014;35:E2403-12.

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EFFECTIVENESS OF PATISIRAN FOLLOWING SWITCH FROM TAFAMIDIS FOR THE TREATMENT OF HEREDITARY TRANSTHYRETIN-MEDIATED (hATTR) AMYLOIDOSIS WITH POLYNEUROPATHY

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Background: hATTR amyloidosis, also known as ATTRv amyloidosis, is a rapidly progressive disease caused by variants in the *TTR* gene. Tafamidis, a TTR stabilizer, has shown to slow neuropathy progression in a pivotal study of patients with the V30M variant and has received regulatory approval in select jurisdictions, including the EU, for the treatment of hATTR amyloidosis with stage 1 polyneuropathy. Patisiran, an RNAi therapeutic that targets variant and wild-type TTR production, has shown potential to halt or reverse polyneuropathy and improve quality of life in a wide range of patients, and has received regulatory approval for the treatment of hATTR amyloidosis with stage 1 or 2 polyneuropathy. Real-world data describing the clinical course of patients with hATTR amyloidosis with polyneuropathy switching from tafamidis to patisiran are limited.

Objective: To describe the clinical rationale and effectiveness of treatment switch from tafamidis to patisiran in patients with hATTR amyloidosis with polyneuropathy.

Material & Methods: This observational single-center study at an expert center in France evaluated patients with hATTR amyloidosis previously treated with tafamidis and switched to patisiran. Data were extracted from medical records. Reasons for discontinuation of tafamidis and outcome measures of treatment effectiveness were collected, including polyneuropathy disability (PND) score, assessment of walking difficulties, and neuropathy impairment score (NIS). Safety events leading to unplanned hospitalizations and fatality were also collected.

Results & Discussion: 24 patients were evaluated (male, 67%; V30M, 50%) who received a mean (SD) of 30.1 (17.5) months of tafamidis treatment. Over the tafamidis treatment period, mean (SD) NIS increased by 24.9 (17.1), and approximately one-third of evaluated patients were reported to have a walking status of “stable since last evaluation” (Table 1). In approximately the last year of tafamidis treatment (mean, 11.2 months) and among 16 patients evaluated on PND score, 31% worsened, 69% had no change, and 0% improved. All patients reported neuropathic disease progression as a reason for switch from tafamidis to patisiran, as indicated by worsening in neuropathy symptoms (n=21; 88%; including sensory [n=17; 81%], motor [n=17; 81%], autonomic [n=12; 57%] symptoms), and/or worsening in specific neuropathy measures such as PND score (n=11; 46%) and NIS (n=10; 42%). Concurrent cardiac disease progression was also reported as a reason for switch in 3 (13%) patients. In approximately the first year of patisiran treatment (mean, 11.7 months) and among 19 patients evaluated on PND score, 16% worsened, 74% had no change, and 11% improved. In addition, after switch to patisiran, change in NIS decreased (mean [SD] change from patisiran initiation, 2.4 [12.8]), and two-thirds of evaluated patients were reported to have a walking status of “stable since last evaluation” (Table 1).

Summary & Conclusions: In this study of patients with hATTR amyloidosis who switched from tafamidis to patisiran, stabilization of polyneuropathy impairment was observed in most patients within 12 months of patisiran treatment. However, initiation of patisiran treatment did not help patients regain the level of neurologic function observed prior to initiation of tafamidis. These data highlight the importance of monitoring disease progression and considering timely switch to patisiran in patients who progress. Longer-term follow-up will provide further insights into the potential benefits of patisiran in this patient population.

Table 1: Changes in NIS and level of walking difficulty at each standard-of-care visit

	Initiation of tafamidis	12 months prior to tafamidis discontinuation	6 months prior to tafamidis discontinuation	Discontinuation of tafamidis	Initiation of patisiran	6 months after patisiran initiation	12 months after patisiran initiation
Patients with available data at each milestone, N	24	16	20	24	24	21	19
NIS							
Patients assessed, n (%)	24 (100)	14 (88)	13 (65)	23 (96)	23 (96)	19 (90)	15 (79)
Mean (SD)	36.9 (17.3)	53.4 (16.6)	58.6 (21.1)	61.7 (20.0)	61.7 (20.0)	65.0 (21.5)	59.1 (22.7)
Change in NIS[a]							
Mean (SD)	--	17.0 (18.4)	21.3 (19.7)	24.9 (17.1)	--	5.0 (13.4)	2.4 (12.8)
Change in level of walking difficulties							
Patients assessed, n (%)	17 (71)	16 (100)	19 (95)	24 (100)	24 (100)	18 (86)	18 (95)
Stable since last evaluation, n (%)	5 (29)	5 (31)	4 (21)	9 (38)	8 (33)	15 (83)	12 (67)

[a]Change in NIS at visits associated with tafamidis treatment is calculated as the difference from NIS at tafamidis initiation; change in NIS at visits associated with patisiran treatment is calculated as the difference from NIS at patisiran initiation.

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Characteristics of patients with ATTR amyloidosis and the Ile68Leu mutation: insights from the Transthyretin Amyloidosis Outcomes Survey (THAOS)

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Background: Transthyretin amyloidosis (ATTR amyloidosis) is a clinically heterogeneous disease with spontaneous (wild-type) and hereditary (ATTRv amyloidosis) forms.[1] ATTRv amyloidosis is caused by mutations in the transthyretin (*TTR*) gene.[1] Currently, more than 120 different *TTR* mutations associated with ATTR amyloidosis have been identified,[2] but many are not well-characterized.

Objective: The aim of this analysis was to describe the characteristics of patients with the Ile68Leu (p.Ile88Leu) *TTR* mutation enrolled in the Transthyretin Amyloidosis Outcomes Survey (THAOS).

Material & Methods: THAOS is the largest, ongoing, global, longitudinal, observational study of patients with ATTR amyloidosis, including both hereditary and wild-type disease, and asymptomatic carriers of pathogenic *TTR* mutations (NCT00628745). This analysis described the demographic and clinical characteristics of patients with an Ile68Leu mutation at enrollment in THAOS (data cutoff date: January 4, 2022).

Results: The majority of the 78 patients with the IIe68Leu mutation enrolled in THAOS were in Italy (89.7%; 70/78). Six others were in the United States, 1 in Germany, and 1 in France. Most were symptomatic at enrollment (57.7%; 45/78), with a median (10th, 90th percentile) age of 69.3 (56.5, 80.4) years; 77.8% were male, and 97.7% were White (**Table**). Median duration of ATTR amyloidosis symptoms was 3.0 (0.7, 11.9) years. The majority (42.2%) of symptomatic Ile68Leu patients had a predominantly cardiac phenotype (24.4% neurologic; 26.7% mixed). The most common (in >10%) cardiac symptoms were heart failure (62.2%), rhythm disturbance (28.9%), and palpitations (11.1%), whereas the most common neurologic symptoms were neuropathic pain/paresthesia (20.0%), tingling (15.6%), and numbness (13.3%). Karnofsky Performance Status score (range 0–100) was 70–100 in 90.2% of symptomatic patients.

Summary & Conclusion: The majority of patients with the Ile68Leu *TTR* mutation in THAOS were enrolled in Italy and were symptomatic with a predominantly cardiac phenotype. Substantial proportions of patients also presented with a predominantly neurologic or mixed phenotype. These findings enhance the current understanding of the phenotype of ATTR amyloidosis in patients with the Ile68Leu mutation. Accumulation of real-world data in THAOS may increase knowledge around ATTRv amyloidosis presentation, natural history, and prognosis.

Table. Demographics and clinical characteristics of symptomatic patients with ATTRv amyloidosis and the Ile68Leu mutation in THAOS

	Symptomatic patients with Ile68Leu *TTR* mutation n=45
Age at enrollment, median (10th, 90th percentile), years	69.3 (56.5, 80.4)
Male, n (%)	35 (77.8)
BMI, median (10th, 90th percentile)	25.6 (22.0, 32.4)
mBMI, median (10th, 90th percentile)	1106.4 (690.9, 1539.8) **[n=9]**
Race/ethnicity, n (%)	**n=43**
White	42 (97.7)
African descent	1 (2.3)
Duration of ATTRv amyloidosis symptoms, median (10th, 90th percentile), years	3.0 (0.7, 11.9)
Phenotype, n (%)	
Predominantly cardiac	19 (42.2)
Predominantly neurologic	11 (24.4)
Mixed	12 (26.7)
Unknown	3 (6.7)
Cardiac symptoms, n (%)	
Heart failure	28 (62.2)
Rhythm disturbance	13 (28.9)
Palpitations	5 (11.1)
Neurologic symptoms	
Neuropathic pain/paresthesia	9 (20.0)
Tingling	7 (15.6)
Numbness	6 (13.3)
EQ-5D-5L index score, median (10th, 90th percentile)	0.8 (0.6, 1.0) **[n=39]**
Karnofsky Performance Status score, n (%)	**n=41**
10–30	0
40–60	4 (9.8)
70–90	28 (68.3)
100	9 (21.9)

ATTRv amyloidosis, hereditary transthyretin amyloidosis; BMI, body mass index; mBMI, modified body mass index; THAOS, Transthyretin Amyloidosis Outcomes Survey; *TTR*, transthyretin.

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1. Ruberg FL, et al. J Am Coll Cardiol 2019;733:2872-91.

2. Rowczenio DM, et al. Hum Mutat 2014;35:E2403-12.

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Characteristics of Patients With ATTR Amyloidosis and the Phe64Leu Mutation: Insights From the Transthyretin Amyloidosis Outcomes Survey (THAOS)

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Background: ATTR amyloidosis is a rare, progressive, life-threatening, heterogeneous disease associated with the deposition of transthyretin (TTR) amyloid fibrils in tissues and organs throughout the body, including the peripheral nerves and heart.[1] The hereditary form of ATTR amyloidosis (ATTRv amyloidosis) is caused by pathogenic mutations in the *TTR* gene. Currently, over 120 different TTR mutations associated with ATTR amyloidosis have been identified,[2] but many are not well characterized.

Objective: The aim of this analysis was to describe the characteristics of patients with the Phe64Leu (p.Phe84Leu) *TTR* mutation enrolled in the Transthyretin Amyloidosis Outcomes Survey (THAOS).

Material & Methods: THAOS is the largest, ongoing, global, longitudinal, observational study of patients with ATTR amyloidosis, including both hereditary and wild-type disease, and asymptomatic carriers of pathogenic TTR mutations (NCT00628745). This analysis described the demographics and clinical characteristics of patients with the Phe64Leu mutation at enrollment in THAOS (data cut-off date: January 4, 2022).

Results: In THAOS, 74 patients had the Phe64Leu mutation. Of these patients, 66.2% (49/74) were from Italy, 24.3% (18/74) from the United States, 5.4% (4/74) from Brazil, and 4.1% (3/74) from Argentina. The majority were symptomatic (77.0% [57/74]). Among symptomatic patients with this mutation, the median (10^{th}, 90^{th} percentile) age was 67.5 (53.1, 76.6) years; 71.9% (41/57) were male, and all were White (Table). The median duration of ATTR amyloidosis symptoms was 5.6 (1.2, 15.2) years. Most symptomatic patients with the Phe64Leu mutation (73.7% [42/57]) had a predominantly neurologic phenotype. The most common neurologic symptom was numbness (78.9% [45/57]); the most common cardiac symptoms were heart failure and rhythm disturbance (8.8% [5/57], each).

Summary & Conclusions: Phe64Leu is a rare *TTR* mutation that typically presents with a predominantly neurologic phenotype. Patients with the Phe64Leu mutation were mostly enrolled at THAOS study sites in Italy and the United States. THAOS continues to add valuable real-world data and improve understanding of the genotype-phenotype relationship in people with ATTR amyloidosis and these rare mutations.

Table: Demographics and clinical characteristics of symptomatic patients with ATTRv amyloidosis and the Phe64Leu mutation in THAOS

	Symptomatic patients with Phe64Leu genotype (n = 57)
Age at enrollment, median years (10th, 90th percentile)	67.5 (53.1, 76.6)
Male sex, n (%)	41 (71.9)
Modified BMI, median years (10th, 90th percentile)	n=29 1020.1 (728.0, 1389.1)
Race/ethnicity, n (%)	n=56
White	56 (100)
Duration of ATTR symptoms, median years (10th, 90th percentile)	5.6 (1.2, 15.2)
Phenotype, n (%)	
Predominantly cardiac	0 (0.0)
Predominantly neurologic	42 (73.7)
Mixed	9 (15.8)
Unknown	6 (10.5)
NIS-LL Total Score (derived), median years (10th, 90th percentile)	n = 14 33.6 (2.0, 61.0)
Neurologic symptoms, n (%)	
Numbness	45 (78.9)
Neuropathic pain/paraesthesia	41 (71.9)
Tingling	40 (70.2)
Temperature/pain insensitivity	18 (31.6)
Cardiac symptoms, n (%)	
Heart failure	5 (8.8)
Rhythm disturbance	5 (8.8)
Syncope	4 (7.0)
Palpitations	3 (5.3)
Dizziness	3 (5.3)
EQ-5D-3L index score, median years (10th, 90th percentile)	n=26 0.6 (0.3, 0.8)
Karnofsky performance status, n (%)	n = 44
10-30	0
40-60	13 (29.5)
70-90	25 (56.8)
100	6 (13.6)

Percentages are calculated based on number of non-missing observations for each symptom. (N=57, unless otherwise specified.)
ATTRv amyloidosis, hereditary transthyretin amyloidosis; BMI, body mass index; NIS-LL, Neuropathy Impairment Score-Lower Limbs; THAOS, Transthyretin Amyloidosis Outcomes Survey.

References: 1. Ruberg FL, et al. J Am Coll Cardiol 2019;733:2872-91. 2. Rowczenio DM, et al. Hum Mutat 2014;35:E2403-12.

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Effect of tafamidis on disease progression in patients with non-Val30Met transthyretin amyloid polyneuropathy: a sub-study of the Transthyretin Amyloidosis Outcomes Survey (THAOS)

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Background: Transthyretin amyloid polyneuropathy (ATTR-PN) is a rare, life-threatening disorder characterized by progressive sensorimotor and autonomic polyneuropathy resulting from genetic mutations that destabilize transthyretin (TTR). Over 100 *TTR* single-site variants associated with transthyretin amyloidosis (ATTR amyloidosis) have been identified.[1] Val30Met (p.Val50Met) is the most common variant associated with ATTR-PN, accounting for ~85% of ATTR-PN patients worldwide; the remainder have non-Val30Met mutations.[2,3] The global prevalence of ATTR-PN is estimated at 5,000–10,000 but may be as large as 38,000.[4] Tafamidis, a selective TTR stabilizer, is indicated in >40 countries to delay neurologic progression in adults with ATTR-PN. This Transthyretin Amyloidosis Outcomes Survey (THAOS) sub-study analyzed real-world data in non-Val30Met ATTR-PN patients receiving tafamidis.

Objective: Evaluate disease progression during a pre-tafamidis vs tafamidis treatment period in patients with non-Val30Met ATTR-PN.

Material & Methods: THAOS is an ongoing, global, longitudinal, observational study of patients with ATTR amyloidosis, including both hereditary and wild-type disease, and asymptomatic carriers of pathogenic *TTR* mutations (NCT00628745). This sub-study examined tafamidis use in patients with symptomatic ATTR-PN and a documented non-Val30Met mutation. Patients must have received SOC treatment for ATTR-PN, with data from a pre-tafamidis treatment period of ≥9 months, followed by tafamidis meglumine 20 mg daily. Efficacy analyses compared disease progression in pre-tafamidis SOC vs tafamidis treatment periods. Tafamidis safety was assessed by collecting adverse events (AEs). Follow-up was limited to 24 months for efficacy data but included the full tafamidis treatment period for safety data. Data were collected over a 10 year period (final cutoff date: Aug 1, 2021).

Results: Of 40 patients who met inclusion criteria, 39 (59.0% male; 43.6% White) dosed with tafamidis meglumine 20 mg were included in this analysis; 1 patient received an off-label dose of 61 mg and was excluded. Mean (SD) age was 40.9 (13.6) years at symptom onset and 46.1 (13.9) years at enrollment. Mean (SD) follow-up for SOC and tafamidis treatment periods were 12.1 (6.7) and 17.0 (7.3) months, respectively; mean (SD) tafamidis treatment duration was 3.3 (1.8) years. A slower rate of disease progression was observed in the tafamidis vs pre-tafamidis SOC treatment period on most measures (Table). Differences in the reflex subscore, BMI, mBMI, and Norfolk QoL were statistically significant ($P<0.05$). Total sensory perception score favored the SOC treatment period, although not statistically significant. In the cumulative reporting period, 4 patients died (cardiac failure congestive [n=3]; cardiac failure) and 6 patients had 9 AEs (severe: cardiac failure, cardiac failure congestive [n=2]; moderate: cardiac failure congestive, spinal compression fracture, sinus node dysfunction, transient ischemic attack, anal hemorrhage; mild: diarrhea); all were serious AEs (SAEs) except diarrhea. No AEs/SAEs were tafamidis-related.

Summary & Conclusions: In this real-world analysis of non-Val30Met ATTR-PN patients, efficacy endpoints were directionally favorable for the tafamidis vs SOC treatment period. The nature and incidence of AEs and SAEs reflect the known safety profile of tafamidis or the underlying disease. Results were consistent with the effect of tafamidis in Val30Met ATTR-PN patients and support its use for delaying neurologic progression in patients with non-Val30Met ATTR-PN.

Table. Within-group comparisons of the monthly rate of change in efficacy measures

	SOC pre-tafamidis treatment period		Tafamidis treatment period		Tafamidis – SOC pre-tafamidis treatment period	
Efficacy measure	n	Estimate (95% CI)	n	Estimate (95% CI)	Estimate (95% CI)	*P*-value
MRC motor function						
Total score	28	−0.23 (−0.42, −0.03)	28	−0.06 (−0.22, 0.09)	0.16 (−0.08, 0.41)	0.19
Subscore: ankle + toe	30	−0.09 (−0.16, −0.01)	30	−0.02 (−0.08, 0.03)	0.06 (−0.03, 0.15)	0.16
Sensory perception						
Total score	11	0.11 (−0.13, 0.35)	11	−0.14 (−0.29, 0.01)	−0.25 (−0.53, 0.03)	0.08
Subscore: toe	17	−0.05 (−0.12, 0.03)	17	0.03 (−0.02, 0.08)	0.08 (−0.02, 0.17)	0.10
Reflexes						
Total score	29	−0.09 (−0.15, −0.04)	29	−0.04 (−0.08, 0)	0.06 (−0.01, 0.12)	0.09
Subscore: quadriceps femoris + triceps surae	30	−0.06 (−0.09, −0.03)	30	−0.01 (−0.04, 0.01)	0.04 (0.01, 0.08)	0.02
BMI, kg/m^2	31	−0.07 (−0.11, −0.02)	31	−0.01 (−0.04, 0.03)	0.06 (0.01, 0.11)	0.03
mBMI, g/L	6	−12.35 (−21.39, −3.30)	6	5.74 (−1.12, 12.61)	18.09 (6.49, 29.69)	0.01
Norfolk QoL – DN	11	0.66 (0.19, 1.14)	11	−0.41 (−0.84, 0.01)	−1.07 (−1.71, −0.44)	0.002
Karnofsky PS	29	−0.27 (−0.47, −0.07)	29	−0.13 (−0.29, 0.03)	0.14 (−0.11, 0.39)	0.28

n is the number of patients with non-missing data in a treatment period and non-missing baseline. Differences >0 favor the tafamidis treatment period, except for Norfolk QoL – DN, wherein a difference <0 favors the tafamidis treatment period.

BMI=body mass index; DN=diabetic neuropathy; mBMI=modified BMI; MRC=Medical Research Council; PS=performance status; QoL=quality of life; SOC=standard-of-care

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Cardiological evolution of hereditary transthyretin amyloidosis (AhTTR) in patients with liver transplant

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Background

Liver transplant has been the treatment for hereditary TTR amyloidosis for years. These patients after transplant develop neurological, ophthalmological and cardiological symptoms. In this paper we present the cardiological evolution and treatment establishment of a series of liver transplant patients.

Objetive

To describe cardiological evolution over the years of those patients who received a liver transplant as treatment.

Material & Methods

The variables were collected from the clinical history of these patients. The frequency of events in each group were calculated for subsequent analysis and interpretation.

Results

In our group 25 patients received liver transplant, 6 patients died, 19 patients are currently under follow-up, one of them outside our hospital, therefore we present the data of 18 patients. Patient´s mean age at the time of transplant was 38.6 years CI (28.3-48-9) and the mean age at the time of follow-up was 51.9 years CI (41.9- 61.9).
Regarding cardiac events, 4 patients have implanted pacemakers, of the remaining 12 they present alterations in the electrocardiogram, described in figure 2.
12 of 18 patients presented left ventricle hypertrophy measured by ultrasound, and in all of them the cardiac scintigraphy with 99mTc-diphosphonates was negative. These patients have a LVEF preserved, but the global strain length (GSL) is increased as well as the tissue systolic function parameter S´. In these 18 patients, there are currently 5 of them in treatment with Patisiran (Onpattro).

Summary & Conclusion

At the time of diagnosis all the patients presented a neurological phenotype, without cardiac alterations, for which they received a liver transplant. Years later, a significant percentage of patients have developed new symptoms, the most important being cardiac symptoms, followed by neurological symptoms. This consideration must be taken into account in all patients with liver transplant as a treatment for hereditary transthyretin amyloidosis. Cardiac follow-up should be established to identify alterations mainly due to cardiac hypertrophy, heart failure and cardiomyopathy, causing a mixed phenotype of the disease. Patients who have developed new symptoms have been treated with Patisiran (Onpattro), as it is the only treatment with a clinical trial developed in liver transplanted patients and in addition the only treatment with favorable clinical data.

Figure

	Years (CI)
Age before liver transplant	38,6 (28,3-48,9)
Age follow up	51,9 (41,9-61,9)

Figure 1

	Yes (%)	*No (%)*
Pacemaker	4	14
Pathological EKG	12	6
LVH (Ecography)	12	6
Scintigraphy	0	18
Nt-proBNP elevation	4	14
Troponin T elevation	7	11
Treatment (Patisiran)	5	13
Increase GSL (%)	10	8
Decrease S´ (cm/s)	18	0

Figure 2

Figure 1: Patient´s mean age at the time of liver transplant and follow up age. Figure 2: Description of the evaluations carried out on patients in the follow-up of the disease after liver transplant.

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Hereditary Transthyretin Amyloidosis caused by p.Ser43Asn *TTR* Variant. Insights and possible founder effect in Ecuador.

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Background: The p.Ser43Asn variant is a very uncommon cause of ATTRv. To date only seven cases have been reported in the literature with a clear predominance of cardiomyopathy over neuropathy involvement.

Objective: We sought to describe the clinical characteristics and outcomes of three unrelated ATTRv families with the p.Ser43Asn variant.

Material & Methods: Patients with ATTRv due to p.Ser43Asn variant from 3 unrelated families followed at Hospital Universitario Puerta de Hierro (Madrid, Spain) were studied. Relatives with the p.Ser43Asn *TTR* variant identified through cascade screening were also included irrespective of phenotypic expression.

Results: Eight individuals (3 probands and 5 relatives, 4 males, age range 29-60) were included in the study. The 3 families were not related but all were originally from Southern Ecuador (Loja) and had six additional members not followed at our center with signs/symptoms of ATTRv. Among individuals evaluated at our center, 7 (88%) exhibited signs of ATTRv. All probands showed mixed cardiac and neurologic involvement at first evaluation, whereas among relatives only one showed mixed phenotype, two had isolated neuropathy, one isolated cardiomyopathy and one was a healthy carrier. Median age at disease onset was 49.8 years (Interquartile range [IQR] 44.0-58.8). Five patients received tafamidis and one patient started patisiran as first-line treatment. Cardiomyopathy was defined by advanced symptoms (NYHA II-III in all probands). All patients with neurologic involvement showed isolated sensory symptoms (PND I), median NIS was 3 (IQR 2-11). None progressed to more advanced PND during follow-up. After a median follow-up of 13.2 months (Interquartile range [IQR] 6.2-17.9), 4 patients required admission due to heart failure, 2 developed atrial fibrillation and 2 had complete AV block., One patient was admitted due to cardiogenic shock requiring mechanical circulatory support as bridge to emergent heart transplantation.

Summary & Conclusion: ATTRv caused by the p.Ser43Asn variant is characterized by mixed neurologic and cardiac phenotype although cardiomyopathy seems to determine prognosis. The variant was identified in three unrelated families originally from the same region in Ecuador suggesting a founder effect that might have been unrecognized in their country of origin due to limited access to genetic testing.

	A1	B1	B2	B3	B4	B5	B6	C1
Sex	Male	Female	Female	Female	Male	Male	Female	Male
Age (Years)	50	47	60	58	51	47	29	40
Birth Country	Ecuador	Ecuador	Ecuador	Ecuador	Ecuador	Ecuador	Ecuador	Ecuador
Cardiac involvement	Yes	Yes	No	No	Yes	Yes	No	Yes
NYHA	II	II	-	-	I	I	-	III
AF	No	No	No	No	No	No	No	No
Pacemaker	No	No	No	No	No	No	No	No
Neurologic involvement	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Neurologic assessment	PND I Sudoscan (-) NIS 11	PND I Sudoscan (+) NIS 2	PND I Sudoscan (+) NIS 4	PND I Sudoscan (-) NIS 11	PND I Sudoscan (+) NIS 0	-	-	PND I Sudoscan (-) NIS 2
Other manifestations	CTS	No	CTS, OPHT	No	CTS	No	No	CTS
Treatment	Tafamidis	Tafamidis	Tafamidis	Tafamidis	Tafamidis	None	None	Patisiran
FU time (months)	36.1	17.0	15.6	8.1	18.9	3.0	4.4	10.8
Events during FU	New-onset AF Complete AVB	New-onset AF Cardiogenic shock/MCS/HT	None	None	None	None	None	Complete AVB

Figure 1: Summary of clinical characteristics and events. AF: Atrial fibrillation; AVB: Auriculoventricular block; CTS: Carpal tunnel syndrome; FU: Follow-up; HT: Heart transplantation; MCS: Mechanical circulatory support; OPHT: Ophtalmopathy.

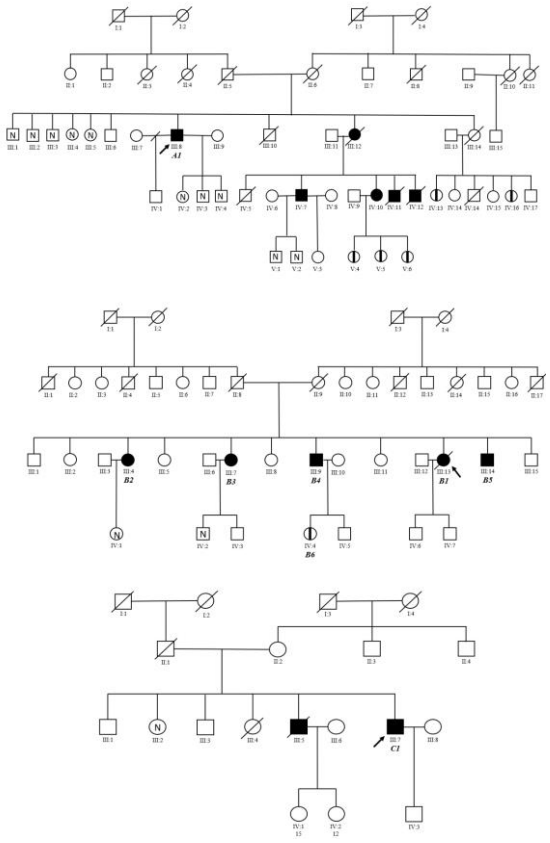

Figure 2: Family trees. Figure displays family trees of three unrelated families included in the study.

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A-V block as presentation of cardiac amyloid caused by conduction tissue infiltration

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Background: Cardiac infiltration by amyloid generates a progressive restrictive cardiomyopathy culminating in diastolic heart failure. Conduction tissue can be affected by amyloidosis particularly in the advanced stage of the disease.

Objective: We sought to describe conduction tissue histologic findings in a previously undiagnosed patient with vTTR amyloidosis presenting with AV block.

Material & Methods: A 68-year-old man with an untreated carpal tunnel syndrome was admitted because of recurrent syncopal episodes. ECG documented sinus rhythm with preserved QRS voltages and diffuse compromise of impulse conduction manifested by right bundle branch block, left anterior hemiblock, 1 and 2 degree A-V block with ventricular rate of 38 beats/min. At 2 D-echo a moderate diffuse thickening of left and right ventricular wall (maximal wall thickness 16 and 10 mm, respectively) was recognized with left ventricular diastolic dysfunction and preserved systolic function (left ventricular ejection fraction 60%). The patient was submitted to invasive cardiac studies including coronary angiography and left ventricular endomyocardial biopsy.

Results Coronary network was normal. Histology of endomyocardial samples showed hypotrophic and degenerated cardiomyocytes surrounded by extensive areas of amorphic material that showed a green birefringence at polarized light after Congo red staining, suggesting cardiac amyloid. In the tissue, samples were included sections of conduction tissue, appearing as small, loosely arranged myocytes positive to potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) immunostaining (figure 1), supplied by a centrally placed arteriole and circumscribed by a fibrous membrane in a fascicle configuration (Monckeberg and Aschoff criteria). Conduction tissue was nearly completely replaced by amyloid deposits positive to immunostaining for TTR. A genetic test revealed a mutation of TTR gene (TTRThr59Lys, p.Thr79Lys) in the affected patient. The patient had a pacemaker implantation and started the treatment with Tafamidis.

Summary & Conclusion: Endomyocardial biopsy documentation of prominent infiltration of conduction tissue by cardiac amyloid causing A-V block and syncope has never been provided before. It's very important to take into account this disease in patients presenting with conduction abnormalities even in relatively young age.

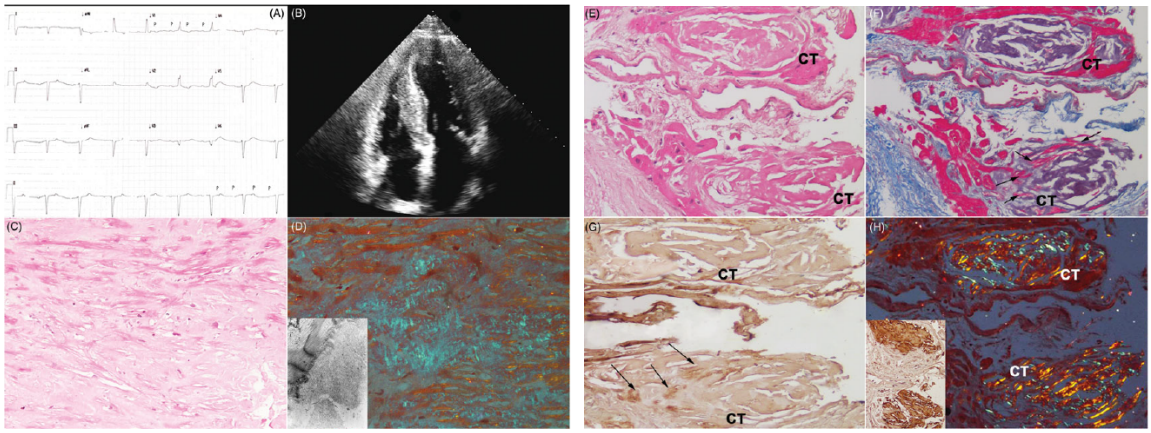

Figure 1. (A) ECG showing a sinus rhythm with preserved QRS voltages, right bundle branch block, left anterior hemiblock, 1 and 2 A-V block. (B) Reveals moderate diffuse thickening of left and right ventricular wall. (C) Sparse hypotrophic cardiomyocytes surrounded by pale pink material (H&E 200). (D) Infiltrating material presents apple-green birifrangence at polarized light after congo red staining suggesting cardiac amyloid. Insert confirms at electron microscopy the material

toconsist of 100 Å wide amyloid fibrils (congo red 200). (E and F) Endomyocardial samples includes sections of conduction tissue (CT) nearly completely replaced by amyloid (H&E and Masson Trichrome 200). (G) Immunostaining for HCN4 showing few spared myocites of conduction tissue (arrows) (200). (H) Congo red staining of CT showing extensive deposition of amyloid (green material). Insert represents positive immunostaining of amyloid material for transthyretin

Comparing patient and clinician perspectives of ATTR amyloidosis: Insights from the development of the Transthyretin Amyloidosis Quality of Life (ATTR-QOL) Questionnaire

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Background: Industry and regulatory guidance for developing patient-reported outcome (PRO) measures emphasize the importance of including both patient and clinician perspectives.[1-5] However, research often shows a disconnect between the concepts these two groups acknowledge and prioritize.[6,7] These competing perspectives may be even more salient for rare diseases with heterogeneous presentations, such as transthyretin amyloidosis (ATTR). Research conducted to develop an ATTR-specific PRO measure—the Transthyretin Amyloidosis Quality of Life (ATTR-QOL) Questionnaire—provided valuable insights into how patients with ATTR and their clinicians perceive the ATTR disease experience.

Objective: To examine similarities and differences in patient and clinician feedback on the ATTR disease experience and the ATTR-QOL.

Material & Methods: To develop the draft ATTR-QOL (US English), the study team first collected input about the disease experience via literature review, concept elicitation interviews (clinicians, patients) and a focus group (patients). After drafting the measure, it was evaluated via 2 modified Delphi panels (MDPs; clinicians, researchers) and cognitive debriefing interviews (patients). Data were analyzed using thematic analysis and/or descriptive statistics.

Results: Study participants included 44 patients (30 hereditary; 14 wild-type) and 20 clinicians (8 cardiology; 6 hematology/oncology; 4 neurology; 2 internal medicine/gastroenterology). Clinicians' backgrounds reflect specialties that frequently treat patients with ATTR.

During concept elicitation interviews, both groups endorsed similar symptoms (e.g., cardiac manifestations, peripheral and/or autonomic neuropathies) and impacts (e.g., physical functioning, mental/emotional wellbeing, work/productivity) for inclusion in a disease-specific PRO measure for ATTR. Patients also stressed impacts on pastimes (e.g., hobbies, recreation) and planning for the future (both short and long term) as key to the ATTR experience, whereas clinicians did not.

When evaluating the tool in MDPs and cognitive debriefing interviews, both patients and clinicians found the draft ATTR-QOL to be useful, comprehensive, and easy to understand; however, feedback on specific items sometimes varied across groups. Several items deemed clear and easy to understand by clinicians (e.g., standing up without assistance, bending down to tie shoelaces) were flagged by patients who needed clarifying examples and more context to respond accurately. Similarly, impact items deemed less relevant by clinicians (e.g., impacts on hobbies, relationships, socializing) resonated strongly with patients. Following the evaluation phase, items were updated to reflect the feedback from both clinicians and patients.

Summary & Conclusion: There was substantial overlap in the concepts endorsed by patients and clinicians; however, perspectives sometimes diverged in notable ways, including patients' greater emphasis on impacts related to recreational activities and social wellbeing. These differences highlight the importance of including both perspectives in PRO development. Feedback from patients and clinicians (as well as other stakeholders) was critical to ensuring the usefulness and broader adoption of the measure for use in ATTR-specific clinical practice, research, and trials. Ultimately, this study resulted in a deeper understanding of where patient and clinician perspectives may differ and a well-rounded instrument that is reflective of both perspectives.

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Peritoneal dialysis is a valid treatment option in hereditary transthyretin amyloidosis

Background: Hereditary transthyretin amyloidosis (ATTRv) has an evolution that may be complicated by end-stage renal disease (ESRD). Available data on the clinical outcomes of patients with amyloidosis undergoing dialysis are limited and reported as inferior to those of other diseases. In our experience, average survival of ATTRv patients on hemodialysis (HD) was 21 months. Probably, the paucity of peritoneal dialysis (PD) descriptions in amyloidosis reflects the fear of an increased risk of peritonitis associated to gastrointestinal disturbances and loss of self-peritoneal dialysis care caused by physical disability. In ATTRv, autonomic neuropathy and cardiomyopathy favours PD due to its capacity to maintain hemodynamic stability by slow fluid and solute removal. Moreover, anticoagulation on HD (on opposite to PD) raises concern of this option in patients with risk for brain haemorrhage related to amyloid angiopathy.

Objective: We conducted the present study to investigate the outcomes of PD in ATTRv patients including liver transplant recipients.

Material & Methods: Single centre retrospective study of patients with ATTRv under PD. Clinical and demographic were collected (patient survival, patient survival on dialysis, dialysis adequacy and dialysis complications). Patients were followed by the same nephrology team that supports ATTR in out/inpatient clinic.

Results: We registered six ATTR V30M patients, all had family history of amyloid nephropathy (demographic and clinical characteristics are summarized in table 1). Mean age at first symptoms was 39 years (range: 32-50) and PD start 50 years (range: 36-60). Mean follow-up period was 30.2 months representing a cumulative follow-up of 241 patient-months. Half of the patients performed dialysis autonomously, this group had less neurological involvement. The inability to obtain a vascular access for HD was the indication for PD in 50% of the cases. All patients were adequately dialyzed and in good fluid control, considering the current standards. There were no reports of peritonitis, catheter-related problems, ultrafiltration failure, and patient or provider fatigue. One case refers to a patient submitted to liver transplantation 24 years prior to dialysis; neither PD technique failure nor impairment of liver graft function were observed. Death was the cause of drop-out in 4 cases, the others suspended PD after major abdominal surgeries not related with technique.

Summary & Conclusion: PD is a valid option in ATTRv patients even in the presence of neuropathy and frailty. PD is effective not only in controlling uremia but also fluid balance which might be favourable in cardio-renal syndromes. Diarrhoea and peripheral neuropathy presumed as contraindications to PD, although prevalent in our cohort, were not responsible for any significant complication which supports the security and suitability of this technique. The withdrawal of anti-amyloid therapies in advanced CKD causes faster decline in health status, increases morbidity and mortality, if not these patients might have better survival and quality of life in PD. Thus, investigations providing additional evidence with larger numbers of patients and longer follow-up are warranted.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Gender	Female	Male	Female	Female	Female	Female
Age ATTRv onset (years)	35	32	34	50	39	44
Age dialysis start (years)	36	42	60	56	48	60
Duration of disease at first dialysis (years)	9	6	4	6	9	6
PD modality	CAPD	APD	CAPD	CAPD	CAPD	CAPD
Self-dialysis	Yes	No	No	Yes	No	Yes
PD follow-up (months)	26	20	36	40	36	23
Indication for dialysis	Fluid overload	Metabolic acidosis	Uremic syndrome	Fluid overload	Uremic syndrome	Uremic syndrome
Indication for peritoneal dialysis	Vascular access failure	Patient choice	Patient choice	Patient choice	Vascular access failure	Vascular access failure

Figure 1.: Demographic and clinical characteristics of patients (PD - Peritoneal dialysis; CAPD - Continuous Ambulatory Peritoneal Dialysis; APD - automated peritoneal dialysis)

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Transthyretin Familial Amyloid Polyneuropathy (TTR-FAP): Electroneuromyographic findings in eighteen newly diagnosed patients

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Background: The classic clinical manifestation of PAF-TTR is progressive length-dependent polyneuropathy, with expressive involvement of small fibers. Although the typical neurophysiological finding of PAF-TTR is axonal sensory-motor polyneuropathy, it is common to observe heterogeneous patterns of presentation in electroneuromyography. This variability may delay diagnosis and impair the initiation of treatment for these patients. In these cases, knowledge about the neurophysiological findings of PAF can be used as an auxiliary tool in the differential diagnosis.

Objective: This study aims to describe the neurophysiological profile of newly diagnosed patients with PAF-TTR.

Material & Methods: The study was performed in two neuromuscular diseases reference centers in Rio de Janeiro, Brazil. We selected 18 patients diagnosed with PAF-TTR confirmed by genetic study and/or nerve biopsy and who underwent electroneuromyography at the time of diagnosis. The neurophysiological data obtained were classified and correlated with clinical and functional findings.

Results: The most common neurophysiological pattern found at the time of diagnosis of these patients was axonal polyneuropathy, present in eleven patients (61.1%). Isolated median neuropathy in the wrist, characterized as Carpal Tunnel Syndrome, was identified in two patients (11%). Four patients (22.2%) showed signs of demyelination in ENMG. All had severe associated axonal involvement. Predominance of axonal sensory-motor polyneuropathy corroborates data found in literature.

Summary & Conclusion: Axonal compromise was predominant in the different mutations evaluated in the study and demyelinization findings are probably secondary to axonal degeneration.

Case series: p.Leu131Met transthyretin amyloidosis in a Danish family: Pure cardiac phenotype?

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Background: Hereditary transthyretin amyloidosis is a rare disease characterised by transthyretin amyloid deposits causing multisystem dysfunction, notably somatic and autonomic neuropathy and cardiomyopathy.[1] A Danish Leu111Met (p.Leu131Met) variant with 100% penetrance has been described and suggested to be with cardiomyopathy only.[2]

Objective: We evaluated the autonomic and somatic small and large nerve-fibre peripheral nervous system to establish if any neuropathy is present in the Danish p.Leu131Met variant.

Material & Methods: Nine p.Leu131Met gene-carriers 29-60 years old participated (6 males). Six had active disease with liver transplantation (LTX, n = 6) and heart transplantation (HTX, n = 2).
The Composite Autonomic Symptom Score (COMPASS-31) and IIEF-5 questionnaires were answered to evaluate autonomic and erectile dysfunction (ED) symptoms.[3-5] Autonomic testing evaluated 1) the parasympathetic nervous system with heart rate variability (HRV) to deep breathing (DB) and Valsalva ratio during Valsalva maneuver (VM) and 2) the sympathetic nervous system with blood pressure (BP) response to VM and Head-Up-Tilt-Table (HUTT) test.[6] A 24-h Ambulatory BP Monitor (ABPM) was used to classify night-time BP dipping status.[7]
The somatic small-fibre peripheral nervous system was clinically evaluated with the Utah Early Neuropathy Score (UENS)[8] and objective measures were provided by a quantitative Sweat (Q-Sweat) test in four locations and a skin biopsy to assess intra-epidermal nerve-fibre density (IENFD).[6,9,10] The large-fibre peripheral nervous system was evaluated with the Neuropathy Impairment Score - Lower Leg (NIS-LL)[11] and motor and sensory nerve conduction study (NCS).[12,13]

Results: All healthy carriers (n = 3) reported autonomic symptoms, but no ED symptoms. Autonomic testing was normal. One healthy carrier scored 2 points in UENS and had a reduced sweat response on the foot, but with normal IENFD and NCS.

All patients with active disease and LTX (n = 4) reported autonomic symptoms. One case scored 26,5 points in COMPASS-31 and had an abnormal BP response to VM, but a normal HUTT, HRV to DB, and 24-h ABPM pattern. She scored 9 points in UENS but had a normal IENFD, Q-Sweat test and NCS. A case with kidney insufficiency and obesity scored 18,6 points in COMPASS-31, reported significant ED symptoms, had an abnormal HRV to DB and a partial dipping pattern on 24-h ABPM. VM and HUTT was not performed. He scored 0 points in UENS and had a normal IENFD and Q-Sweat test, but a decreased amplitude in the sensory median, ulnar and sural nerves on NCS.

Patients with active disease and LTX and HTX (n = 2) reported autonomic symptoms comprised of mainly ED symptoms. Autonomic testing was not performed. One case scored 4 points in UENS and had a normal IENFD and Q-Sweat test. He had a decreased amplitude and velocity of the sensory and motor median nerve. The other case scored 0 points in UENS and had a normal IENFD but a decreased sweat response on the upper thigh.

Summary & Conclusion: Autonomic symptoms were reported in all cases. Decreased HRV to DB revealed cardiovagal dysfunction in one case (LTX). In another case (LTX) an abnormal blood pressure response to VM may be caused by either adrenergic or heart failure. Subclinical small-fibre neuropathy (abnormal IENFD or Q-SWEAT) were present in three cases (healthy carrier, LTX and LTX+HTX). NCS detected median nerve entrapment in another case (LTX+HTX). The case with cardiovagal dysfunction (LTX) had axonal sensory polyneuropathy with no symptoms on clinical examination.

Support & Funding: The study was funded by Lundbeck foundation and Akcea therapeutics.

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20 years of symptomatic and presymptomatic genetic testing for hereditary transthyretin amyloidosis (ATTR) in the Balearic Islands

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Background: The Balearic Islands are composed of four main islands that from the most populated to the least are Mallorca, Ibiza, Menorca, and Formentera. Val30Met (p.Val50Met) is the most common pathogenic variant of the TTR gene worldwide and in the island of Mallorca (Balearic Islands, Spain) where ATTRV50M amyloidosis is considered endemic. ATTRV50M amyloidosis can affect multiple organs and systems, including the nervous and gastrointestinal systems, the heart, kidneys and eyes. It presents in many different forms and with considerable variation in signs and symptoms across individuals and geographic locations. The clinical features of ATTRV50M are mainly neuropathic, with a heterogeneous presentation of peripheral (sensory and motor) and autonomic neuropathy. Gastrointestinal impairment, cardiomyopathy, ocular manifestations and nephropathy are other frequent manifestations of the disease.

Objective: Since 2002, genetic testing has been performed within the unit of molecular and clinical genetics (UDMGC) of Hospital Universitari Son Espases (HUSE) for the public health system of the Balearic Islands (IB-Salut) which covers most of the population of the islands. Testing has identified pathogenic variants in the TTR gene in symptomatic patients and their families (asymptomatic carrier testing) over the last 20 years. Testing was performed by Sanger sequencing of the 4 exons coding for the TTR protein.

Results: We present a retrospective analysis of the last 20 years of genetic testing in the Balearic Islands public health system for mutations in the TTR gene. We have tested 1160 patients (0.1% of the Balearic population of 1.2 milion inhabitants). Of these patients, 58% were male and 42% female and average age of testing was 57 years old. Referral came from the following medical services: Internal medicine 35%, Genetics 26%, Neurology 21%, Cardiology 9%, and Nephrology 4%. Main referral reasons were in this order: affected relatives (presymptomatic testing), polyneuropathy and cardiac involvement. In total, diagnostic yield was 25% and we detected 285 carriers for the V50M variant and 7 carriers for other potentially pathogenic variants (H51N, S77Y, Q89K, T119M and V142I). The prevalence in the Balearic population for these 20 years is 0.0026% (1/3703 individuals). If we break down the carrier data for each island, we find that Mallorca and Menorca show a similar prevalence for the V30M variant of 0.029% and 0.025%, respectively and Ibiza shows a very different scenario with many less carriers and the much lower prevalence of 0.004%. As for the genetic characterization of the predominant mutation, we find that 93% of patients that carry V50M are also carriers of the G26S variant. When we analyze these patients, we observe by exome sequencing that these 2 variants are on the same chromosome (cis conformation). G26S is considered a benign variant although a role as a phenotype modifier in this context cannot be discarded. The presence of G26S and V50M variants on the same chromosome argues in favor of a founder event in the Balearic population.

Summary & Conclusion: A retrospective analysis of 20 years of symptomatic and presymptomatic genetic testing for pathogenic variants in the TTR gene in the Balearic Islands shows a high prevalence of the V50M variant in the islands of Mallorca and Menorca, but not Ibiza. In addition, we present genetic data which argues in favor of a founder event in the origin of the V50M variant in the Balearic Islands.

Cardiac Screening of Amyloid TTR Pathogenic Variant Carriers: Complementary value of Echocardiographic Global Longitudinal Strain Imaging versus Bone scintigraphy

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Abstract

Background: Cardiac involvement is the major determinant of mortality in hereditary transthyretin-derived amyloïdose (ATTRv). Treatment options have recently become available, which make early diagnosis important. Several imaging modalities for screening are proposed including bonescintigraphy, conventional and strain echocardiography but data on their relative value is limited in this population.

Objective: To investigate the value of left ventricular global longitudinal strain (LV-GLS) for screening for cardiac involvement in a population of TTR pathogenic variant carriers.

Material & Methods: Fifty-three TTR pathogenic variant carriers from 2 academic hospitals underwent transthoracic echocardiography and bonescintigraphy as part of routine out-patient care to assess cardiac involvement. LV-GLS was measured by 2 blinded reviewers. Abnormalities in LV-GLS (cut-off -21.5%) and conventional TTE (cTTE) parameters were evaluated in bonescintigraphy positive (≥ grade 1) and negative cases.

Results & Discussion: Carriers with a positive bonescintigraphy (n=20) were 16 years older than those with a negative scan (n=33). When bonescintigraphy was negative, LV-GLS was abnormal in 11 cases (38%). Three out of them had diabetes and/or hypertension as potential additional explanation to ATTRv for the abnormal LV-GLS. In the remaining 11 carriers, cardiac ATTRv was the sole most likely cause of the abnormal LV-GLS. In 85% (17/20) of patients with a positive bonescintigraphy, LV-GLS was equally sensitive in determining the presence of cardiac ATTR as the combination of all cTTE parameters.

Summary & Conclusions: LV-GLS and bonescintigraphy are complementary in screening TTR pathogenic variant carriers for cardiac involvement and should be used in conjunct. LV-GLS can play a significant role in repeated assessment in this population and our results suggest that LV-GLS may detect early disease onset before bonescintigraphy.

Key words: Amyloidosis, echocardiography, global longitudinal strain, speckle tracking, screening, bonescintigraphy

Comparison of Amyloid Detection in the Skin and Tenosynovium of Transthyretin Amyloidosis Patients

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Transthyretin mediated amyloidosis is a progressive, often fatal systemic disease that occurs with genetic (ATTRv) or wildtype (ATTR) transthyretin. Carpal tunnel syndrome (CTS) is often an early manifestation of disease activity. It is important to correctly identify penetrant disease as effective treatment options are now available and are most effective when started early. We assessed the utility of distal leg skin tissue and carpal tunnel synovium to detect amyloid deposition in hATTR carriers and patients with wildtype disease.

15 subjects (11 ATTRv, 4ATTR) mean age 68.6 + 8.9 years diagnosed with median neuropathy at the wrist with or without a diagnosis of hATTR-peripheral neuropathy or cardiomyopathy were assessed. CTS decompression included tenosyonovium sampling (SL) while peripheral nerve evaluations included distal leg skin biopsies, NCV and examination with NIS assessment (MP) and were performed within 1-year of each other. Tissue was processed for intraepidermal nerve fiber density (IENFD), Congo red staining and immunohistochemistry for TTR aggregates.

The mean median amplitude and distal latency on the affected side was 6.6 μV and 6.8 ms respectively. 10 of 15 subjects had PN based upon sural amplitude while 11 of 15 did by IENFD. The sural and IENFD values for those with PN by sural criteria were 1.9 $\pm$ 3.0 μV and 1.9 $\pm$ 3.1 fibers/mm respectively. Amyloid burden was higher in synovium vs skin. Detection of amyloid in skin was improved through immunohistochemistry than Congo red alone. The two techniques led to congruent results in 14 of 15 cases. The only case in which amyloid was present is synovium but not skin was a patient with no evidence of PN by IENFD (11.8 fiber/mm), sural amplitude (25.2 μV) or NIS-LL (4). Three patients had synovium that was negative for amyloid and the severity of their CTS was milder. The presence of amyloid in skin was associated with lower IENFD and sural amplitudes while amyloid burden in skin was positively associated with NIS score.

These results indicate that skin tissue is a sensitive technique to detect amyloid deposition and can be used to assess for both the presence of peripheral neuropathy and disease penetrance. Distal leg amyloid skin biopsy was associated with more advanced peripheral neuropathy and the burden of amyloid was inversely associated with neuropathy severity. Not all CTS in patients with ATTR/v is due to amyloidosis.

A rare TTR mutation determining severe cardiac and neurological amyloidosis

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Background: Systemic amyloidosis is a progressive, severe, and infiltrative disease that results in amyloid deposition in several organs, including the heart. It can be a hereditary form associated with a mutation in the transthyretin gene with deposition of the malformed TTR proteins (ATTRv), due to degenerative processes related to age (wild or wild type (ATTRwt)), or associated with deposition of light chain immunoglobulin secreted by plasma cells (AL).

Objetive: This case alerts to a rare mutatin in TTR that causes severe cardiac and neurological amyloidosis.

Material & Methods: A 35-year-old Brazilian woman presented with progressive peripheral polyneuropathy (Coutinho's staging III) and recently developed progressive dyspnea, easy fatigability, orthopnea, and dry cough, with NYHA functional class III/IV. She had major dysautonomia, diarrhea, significant weight loss, blurred vision, convulsive syncope, and recently developed bradycardia.

Results: Left ventricular (LV) hypertrophy was detected with high levels of N-terminal pro–B-type natriuretic peptide. Laboratory investigations revealed 245 mg/dl of kappa urinary light chains (normal: 170–370 mg/dl) and 124 mg/dl of lambda chains (normal: 90–210 mg/dl). The kappa to lambda ratio was 1.98 (normal: 1.35–2.65). The concentration of serum light chains was 0.734 mg/dl for the kappa isotype (normal: <0.710 mg/dl) and 0.406 mg/dl for the lambda isotype (normal: <0.390 mg/dl). ECG with low-voltage limb leads, pseudo-infarction in the anterior precordial leads (Figure 1A). Transthoracic echocardiogram (TE) showed a mild increase in LV wall thickness (the interventricular septum and posterior wall of the left ventricle were 13 mm thick), infiltration of the interatrial septum and the free wall of the right ventricle, with mildly reduced LV function. The global longitudinal strain (GLS) pattern that is seen in cardiac amyloidosis typically spares the apex of the heart and is characterized by reduced LS in the basal segments with preserved or supranormal LS in the LV tip (Figure 1B). The neurophysiological findings were compatible with an advanced sensory-motor polyneuropathy with a primarily axonal pattern. She had reduced sensitivity and abolished reflexes in the lower limbs. Myocardial scintigraphy with technetium-99m labelled pyrophosphate revealed an increased concentration of the radiotracer in the projection area of the heart against the costal margin, corresponding to Perugini score 3 (Figure 2). Scores >2 and counting ratio between the heart and the contralateral region of >1.5 have a high probability of wild-type or hereditary transthyretin amyloidosis and the genetic testing and was found to have a rare pathogenic mutation in the transthyretin gene, Glu54Lys (p.Glu74Lys).

Summary & Conclusion: The diagnosis was made through clinical and imaging features, such as electrocardiography and TE and myocardial scintigraphy with technetium pyrophosphate with a sensitivity of close to 100%, myocardial scintigraphy with technetium pyrophosphate in grade 3 could confirm an amyloid infiltrate of transthyretin and didn´t require endomyocardial biopsy. In this case, the evolution was dramatic with a disease in an advanced stage and available therapies are limited. The rare mutation, Glu54Lys (p.Glu74Lys), has previously been reported and described in a report from Costa Rica, in a Turkish and a Japanese family, whose members died before age 40 from heart failure

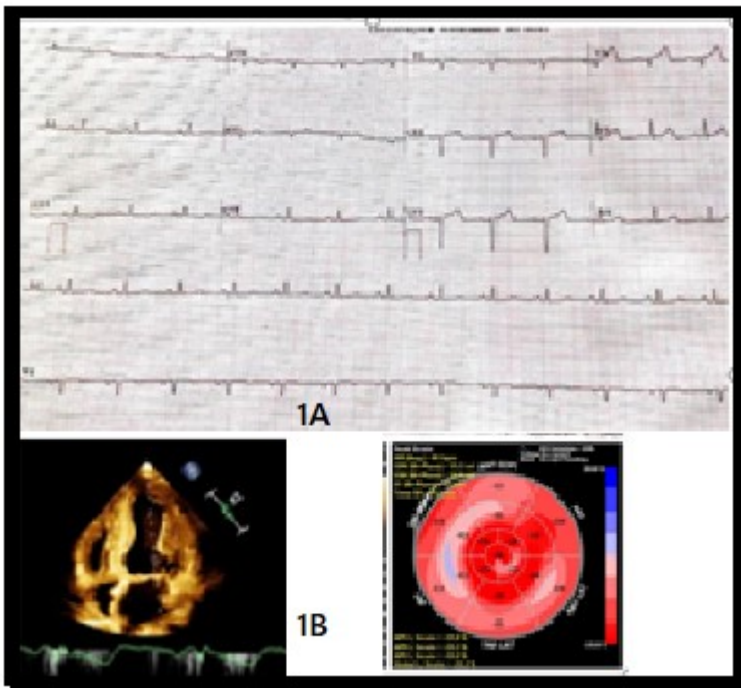

Figure 1.: ECG with low-voltage limb leads, pseudo-infarction in the anterior precordial leads (1A).Four chambers with mild increase in LV wall thickness with mildly reduced LV function. GLS pattern characterized by reduced LS in the basal segments with preserved or supranormal LS in the LV tip (1B).

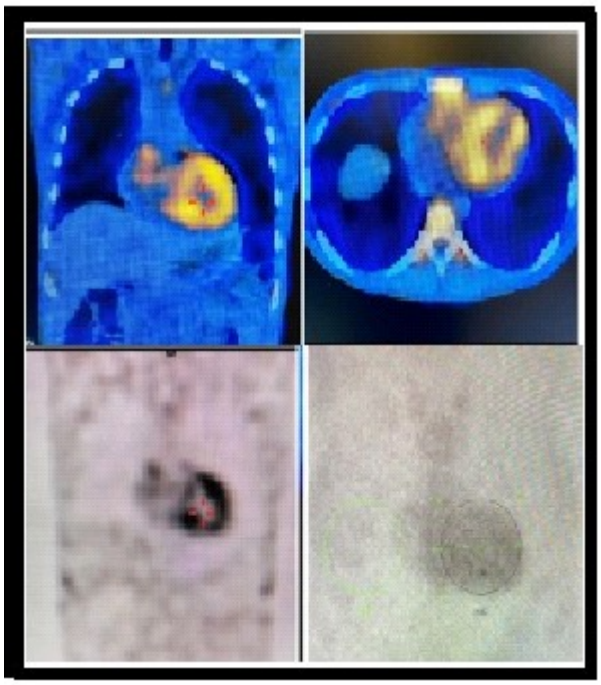

Figure 2.:
Myocardial scintigraphy with technetium pyrophosphate uptake in the heart.

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Lung volume restriction and abnormal cardiopulmonary response to exercise: red warning lights in transthyretin cardiac amyloidosis

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Background: Transthyretin (ATTR) cardiac amyloidosis (CA) is an increasingly recognized condition, symptomatized by restrictive and hypertrophic cardiomyopathies, often resulting in heart failure and death. While dyspnea and exercise intolerance in these patients are often linked to myocardial dysfunction, growing evidence also points towards extra-cardiac causes. Pulmonary involvement seems notably to be a clinically significant manifestation of ATTR-CA. Till now, biological staging systems, such as Gillmore *et al.*'s, applicable to both wild-type (ATTRwt) and hereditary (ATTRv) CA, have been used to stratify disease severity. However, biomarker-based staging might potentially be less discriminating in the earlier phases of cardiac involvement, especially in ATTRv presenting with mixed phenotypes. Reduced peak aerobic capacity (peak VO_2) and lung volume restriction might prove useful in identifying early functional involvement, long before the onset of clinically overt cardiac symptoms. Their combined prognostic value in ATTR-CA has not yet been documented.

Objective: We determined the prognostic value of reduced peak VO_2 and lung volume restriction in ATTR-CA patients. We also analyzed the added predictive value of these 2 functional parameters for adverse outcome, when combined with ATTR biomarker staging.

Material & Methods: In this multicenter real-world evidence study, we retrospectively reviewed records of ATTR-CA patients with pulmonary function (PFT) and cardiopulmonary exercise testing (CPET), seen at 4 expert centers (France, Italy, USA) from 01/08/2005 to 23/12/2021. CPET and PFT were performed using guidelines respectively from the American Thoracic and European Respiratory Societies. Lung volume was considered as normal (Forced Expiratory Volume in 1 second, FEV1 / Forced Vital Capacity, FVC ≥ 0.70 and FVC ≥ 80% predicted values) or restricted (FEV1 / FVC ≥ 0.70 and FVC < 80% predicted values). Patients were prospectively followed until censure (01/04/2022) or study endpoint (MACE: heart failure-related hospitalization or death).

Results: Overall, 82 patients were enrolled: age at testing 70 ± 11 years; 89% men; 57% ATTR-V122I variant; 45% hypertension; 68% NYHA III/IV; 33% non-sinusal rhythm. Median follow-up was 9 months (IQR 4-22), with 31 (38%) MACE. Disease severity, assessed by ATTR biomarker staging, was higher in MACE patients (75% Stages II/III vs 46%; p=0.04). Lung volume restriction concerned 46% of patients (58% vs 39% for "no MACE"; p=0.10), with reduced peak VO_2 (51% vs 66% predicted value) and FVC (74% vs 82% predicted value) in MACE patients. Reduced peak VO_2 and FVC were independent predictors of MACE-free survival, defining 4 risk groups. Peak VO_2 < 50% and FVC < 70% defined the highest risk group (HR 19, 95% CI: 4-83, mean survival: 14 months), as compared to the referent group (peak VO_2 ≥ 50% and FVC ≥ 70%, mean survival 153 months). Combined peak VO_2, FVC and ATTR biomarker staging significantly improved MACE prediction (AUC: 0.83), as compared to ATTR staging alone (AUC=0.68; p=0.03). As such, 89% of patients were correctly reclassified (p<0.01), with 50% of patients assigned a higher risk category and 39% a lower risk category.

Summary & Conclusion: In line with traditional clinical-echocardiographic and cardiac biomarkers, lung volume and aerobic capacity might improve risk stratification in ATTR-CA, particularly in early disease stages. With the advent of novel therapies, their simplicity, non-invasiveness and easy applicability render PFT and CPET potentially highly useful tools for monitoring ATTR-CA.

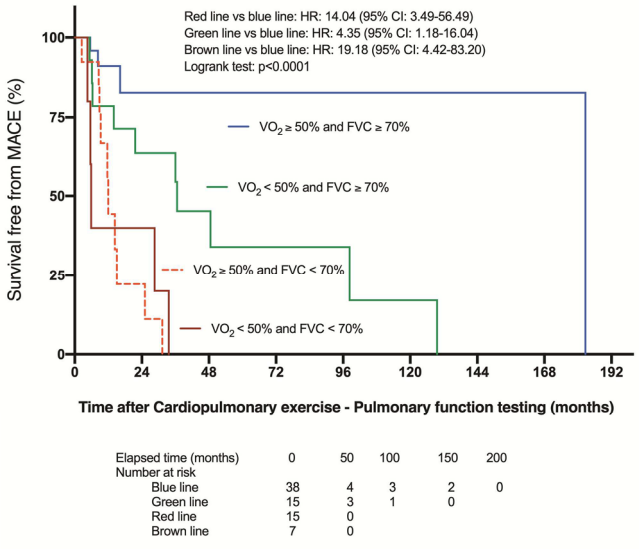

Figure 1: Survival curves for MACE (composite of heart failure-related hospitalization or all-cause death) with the combined use of peak VO_2 and FVC in patients with transthyretin cardiac amyloidosis

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Evaluation and follow-up of the sudomotor function in a cohort of ATTRv patients from a non-endemic area

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Background: Hereditary amyloid transthyretin (ATTRv) amyloidosis is a rare disease with a broad clinical spectrum that varies between endemic and non-endemic areas. Reliable quantification of sudomotor function is essential in the early diagnosis and treatment of this patients. The *Sudoscan* is a non-invasive technique that measures the electrochemical conductance (EC) of the skin (lower EC are associated with more impaired sudomotor function). Its usefulness has been proven in ATTRv patients (1-4). However, there are few studies on the evolution and follow-up of sudomotor function in ATTRv patients.

Objective: To study the measurement and follow-up of the sudomotor function in a cohort of non-endemic ATTRv patients, and to correlate it with clinical parameters.

Material & Methods: Prospective study of patients with ATTRv with at least one *Sudoscan* performed from July 2018 to April 2022. The patients were classified according to the EC in *Sudoscan* **(Fig.1B).** Demographic, clinical, and laboratory data were correlated with mean EC in feet and hands (MEC-FH).

Results: 71 subjects were evaluated; 30 were women (42,3%) and 41 were men (57,7%). At last follow up, 16 were carriers (22,5%) and 55 symptomatic patients (77,5%) (**Fig.1A**). Val50Met mutation was found in 33 subjects (46,5%, 2 with early-onset). Mean age of disease onset was 61 years (SD12.4).
Baseline MEC-FH was significantly higher in carriers vs symptomatic patients, with significant differences depending on the phenotype (p<0,001) (**Fig.1B**). 11 symptomatic patients had a normal baseline sudomotor function (37,9%). A lower baseline MEC-FH was associated to the presence of cardiopathy (p<0,001), neuropathy (p<0,001), significant weight loss (p<0,001), orthostatic hypotension (p=0,005 and p=0,038), abnormal sympathocutaneous response (SCR, p<0,001) and abnormal RR interval variability (RRIV, p=0,047 and p=0,015). MEC-FH was significantly associated to FAP stage (p<0,001), PND stage (p<0,001) and NIS (p<0,001) at any point in the evolution, but not with Compass-31 scale (3).
Patients treated with Tafamidis at last follow-up had a significantly higher baseline MEC-FH than those treated with other drugs (p<0,001) (**Fig.2**). A higher baseline MEC in hands was associated with a good response to Tafamidis (p=0,015) and with less frequency of change of treatment during follow-up (p=0,021). Change in MEC during follow-up was not associated with response to treatment.

Summary & Conclusion: A severe alteration of the sudomotor function in baseline *Sudoscan* was associated to the phenotype, to the presence of neuropathy, cardiac involvement, significant weight loss, orthostatic hypotension, and to an abnormal SCR and RRIV. A normal baseline sudomotor function was associated with a better response to tafamidis and less frequency of change of treatment during follow-up.

FIGURE 1

Fig. 1A. Distribution of phenotypes

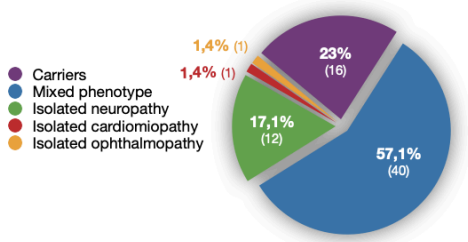

Fig. 1B. Evolution of feet sudomotor function according to phenotype

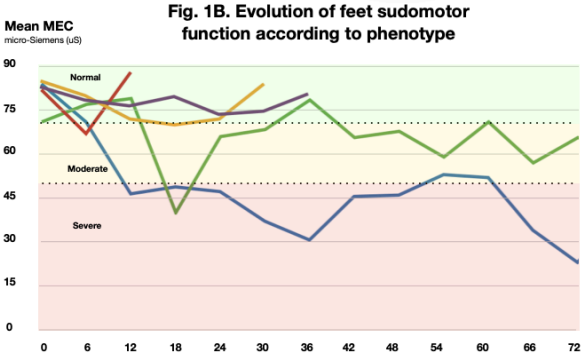

FIGURE 2

Fig. 2A. Treatment at last follow-up

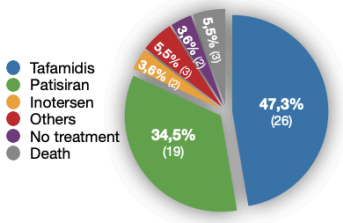

Fig. 2B. Evolution of sudomotor function according to treatment at last follow-up

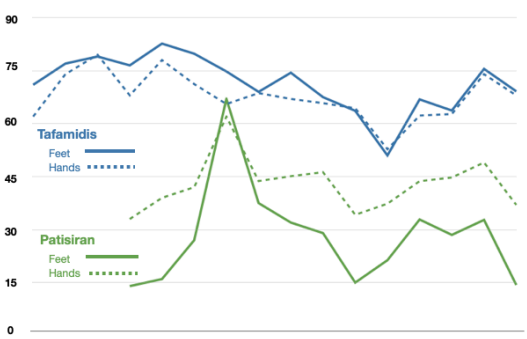

Figure 1.: Sudomotor function and phenotypes.
Figure 2.: Sudomotor function and treatment at last follow-up.

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The Relation between African American Race, Genotype, and Prognosis in Transthyretin Cardiac Amyloidosis

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Background: Previous studies suggest worse outcome in African Americans (AA) with hereditary transthyretin cardiac amyloidosis (hATTR-CA) due to the valine to isoleucine substitution variant (V122I, p.142I) compared to patients with the wild type (WT) form.

Objective: To compare the survival of AA with hATTR due to the V122I variant to both Caucasians and AA with WT in order to help determine whether inferior survival in V122I is **(A)** due to a more aggressive genotype or **(B)** relates to the fact that V122I mainly clusters in AA, who might have a higher risk for all-cause mortality due to racially determined factors.

Methods: Consecutive ATTR-CA patients diagnosed at a single referral center were categorized in 3 categories: (1) Caucasian with WT ATTR-CA (Caucasian WT) (2) AA with V122I ATTR-CA (AA V122I) and (3) AA with wtATTR-CA (AA WT). Assessment of disease characteristics and all-cause mortality was performed using both a univariate and multivariable analysis.

Results: Of 694 ATTR-CA patients, 502 (72%) were Caucasian WT, 139 (20%) were AA V122I, and 53 (8%) were AA WT. Of the total AA cohort, 28% had wtATTR-CA. AA V122I patients presented with higher troponin levels and NYHA class. AA patients (both V122I and WT) exhibited more cardiovascular comorbidities. In an unadjusted analysis, AA patients with ATTR-CA had a higher risk for all-cause mortality (Figure 1). After adjusting for difference in these cardiovascular comorbidities, NYHA class and Troponin, AA WT had similar survival to Caucasian WT, while patients with V122I had worse survival (HR=1.84, 95% CI= 1.20-2.82) in comparison to Caucasian WT (Table 1). The main limitation of the analysis is the smaller number of AA WT patients.

Conclusion: AA V122I patients have a worse survival compared to both AA WT and Caucasian WT patients, even after adjusting for the higher comorbidity burden occurring in AA, suggesting a more aggressive biology of this variant compared to the WT form.

Figure 1

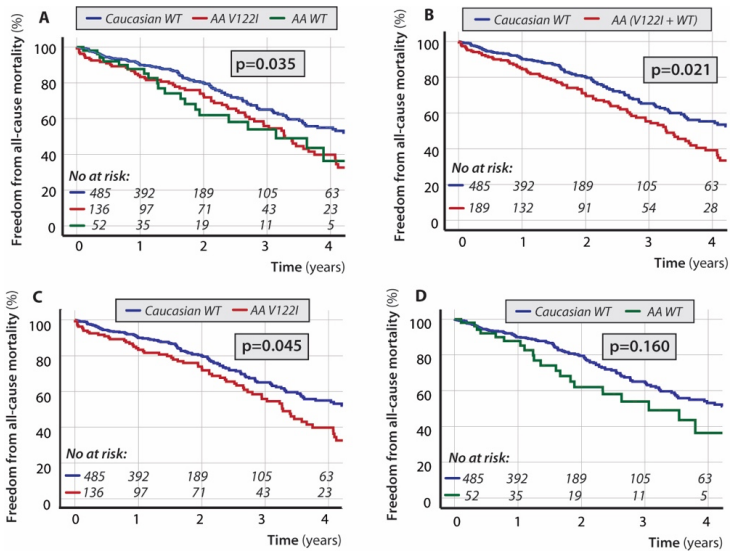

Table 1

Parameter	Hazard ratio	95% CI	P-value
Race genotype categories			
Caucasian WT	reference	reference	reference
African American with V122I	1.84	1.20-2.82	0.005
African American with WT	1.13	0.60-2.11	0.706
Adjusted for following covariates			
Male gender	0.67	0.41-1.11	0.121
Age, years	1.06	1.03-1.08	<0.001
Atrial Fibrillation	1.44	0.97-2.12	0.068
Hypertension	0.90	0.60-1.36	0.623
Diabetes	0.88	0.59-1.30	0.510
Dyslipidemia	1.02	0.67-1.57	0.918
Coronary artery disease	1.64	1.30-2.73	0.009
Stroke	0.85	0.52-1.38	0.500
NYHA-class	1.92	1.44-2.55	<0.001
Troponin	5.61	2.22-14.5	<0.001

Figure 1: Unadjusted Kaplan Meier curves of the different groups

Table 1: Risk of mortality in a Cox-proportional multivariable adjustment hazard model

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Pre-symptomatic genetic testing for hereditary transthyretin amyloidosis: a 20-year single-centre experience

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Background: Effective therapeutic options for hereditary transthyretin amyloidosis demand early diagnosis and timely treatment start for best outcomes. Pre-symptomatic gene testing (PST) followed by tailored clinical monitoring allows early disease recognition in mutation carriers. However, limited experience in PST for ATTRv has been reported to date.

Objective: To report the 20-year experience in pre-symptomatic genetic testing and long-term clinical monitoring of mutation carriers at a single-centre from a non-endemic region for ATTRv.

Material & Methods: PST has been offered at our centre since 2002 in a multidisciplinary genetic counselling setting. The team includes a medical genetist, an internist expert in ATTRv amyloidosis and a psychiatrist available on request. A structured post-test follow-up is offered to all pre-symptomatic mutation carriers. Evaluations are performed every 6 or 12 months according to age, genotype and predicted age of disease onset. Neurological, cardiac, renal and ophthalmologic assessments are consistent with recent recommendations.

Results: 370 at-risk relatives (176 males) from 142 unrelated families have requested genetic counselling and underwent pre-symptomatic testing for ATTRv amyloidosis at our centre since 2002. 85 relatives (23%) were siblings of an index case, whereas 285 belonged to offspring. Median age at PST was 45 years (range 20-86). All subjects who decided to take the test after one or two pre-test counselling sessions returned for the post-test visit in which the result was communicated. No drop-outs occured at this stage of the process. A positive test was reported in 187 (51%) at-risk relatives. Mutation distribution in carriers is as follows: Val30Met 20%, Glu89Gln 16%, Ile68Leu 14%, Phe64Leu 14%, Tyr78Phe 7%, others 29%.170 out of 187 carriers are clinically followed-up at our centre whereas 17 were referred to other centres. After a median time of 6 years (range 0.5-18) from genetic test, 38 carriers developed a symptomatic disease. Median age at disease onset was 56 years (range 27-83). 26% and 10% of symptomatic individuals carried Glu89Gln and Thr49Ala variant, respectively. Disease onset was defined according to the presence of a neurological symptom associated with a positive tissue biopsy or DPD scintigraphy in 15 cases, according to a positive DPD scintigraphy and a CMR/echocardiographic/biomarker abnormality in 11 patients, and according to a definite sign of nerve damage in 12.

Summary & Conclusion: PST for ATTRv appears safe and effective in our experience, with high adherence to periodic clinical monitoring in mutation carriers. Close monitoring of neurological and cardiac signs and symptoms combined with DPD scintigraphy or tissue biopsy allows early recognition of disease onset. A positive DPD scintigraphy is a valuable tool to monitor disease onset particularly in carriers of the Glu89Gln variant.

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Peripheral nerve and cardiac features in hATTR patients presenting with active disease V122I, L58H and late-onset V30M in the US

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hATTR is a rare autosomal-dominant systemic disease with variable penetrance and heterogeneous clinical presentation. Several effective treatments can reduce mortality and disability, though diagnosis remains challenging, especially in the US where disease is non-endemic.We sought to describe the neurologic and cardiac characteristics of common US ATTR variants V122I, L58H and late-onset V30M at presentation. A retrospective case series of patients presenting between January 2008 and January 2020

56 treatment-naïve hATTR subjects with symptoms/signs of peripheral neuropathy or cardiomyopathy and confirmatory genetic testing. Neurologic and cardiac signs, symptoms and results of objective test results were retrospectively collected and compared among patients presenting with Val122Ile, late-onset Val30Met and Leu58His hATTR.

31 V122I, 12 V30M, and 13 L58H patients were included and their age at onset and gender distributions were similar (V122I: 71.5±8.0, V30M: 64.8±2.6, L58H: 62.4±9.8years; 26, 25, 31% female). Only 10% of V122I and 17% of V30M patients were aware of an ATTRv family history, while 69% of L58H were. Peripheral neuropathy was present in all three variants at diagnosis (90, 100, 100%) though neurological impairment scores differed: V122I: 22±16, V30M: 61±31 and L58H: 57±25. Most points (deficits) were attributed to loss of strength. Carpal tunnel syndrome (CTS) and a positive Romberg sign were common across all groups (V122I: 97%, 39%; V30M: 58%, 58%; L58H: 77%, 77%).
ProBNP levels and interventricular septum thickness were highest among V122I subjects (5939±962pg/mL, 1.70±0.29cm) followed by V30M (796±970pg/mL, 1.42±0.38cm) and L58H (404±677pg/mL, 1.23±0.36cm). Atrial fibrillation was present among 39% of V122I cases and only 8% of V30M and L58H cases. Gastrointestinal symptoms were rare (6%) among V122I patients and common in V30M (42%) and L58H (54%) patients.

Important clinical differences exist between ATTRv genotypes. While V122I is perceived to be a cardiac disease, peripheral neuropathy is common and clinically relevant. Most V30M and V122I patients were diagnosed de-novo and therefore require clinical suspicion for diagnosis. A history of CTS and a positive Romberg sign are helpful diagnostic clues.

Prospective MALDI-TOF analysis of blood serum peptidome to predict the onset and progression of hereditary transthyretin amyloidosis.

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Background: Diagnosis in the early stages of hereditary transthyretin amyloidosis (ATTRv) is essential for a timely treatment to halt disease progression. Early recognition remains a challenge, resulting in a delayed diagnosis often due to misdiagnosis [1-2].

Objective: Based on MALDI-TOF proteomic profiling, the goal is to build, assess and validate a predictive model to classify unknown serum samples as belonging to ATTRv asymptomatic carriers, patients or healthy individuals.

Material & Methods: A 12-month study is carried out in the Majorcan endemic focus, including patients, asymptomatic carriers, and healthy volunteer controls. Peptides from serum samples were purified and concentrated using Zip-Tip C18. Proteomic analysis of samples using MALDI-TOF was carried out. The data have been processed and analyzed using linear discriminant analysis (LDA) supervised technique for dimensionality reduction and classification.

Results: Blood samples have been collected from 90 individuals and MALDI-TOF spectra have been obtained. A total of 342 pre-processed spectra passed the intra-quality control, generating a labeled set of serum protein profiles with 75 spectral features (m/z peaks). The three groups have been processed, compared, and the samples have been classified using LDA and a leave-one-out cross validation algorithm. As a result, an accuracy of 74.8% has been achieved. Figure 1 shows the projection of the data into the LD space identifying the three classes. We are currently working on optimizing nonlinear and kernel-based predictive models, along with feature set reduction and selection to achieve better performance in classifying unknown samples.

Summary & Conclusion: Due to the incomplete penetrance of this pathology, as well as its heterogeneity and the irreparable damage caused by amyloid deposition in the absence of treatment, it is urgent to increase diagnostic precision to avoid diagnostic delay and disease progression. Through this proposal, our goal is to increase accuracy to build models of disease progression, as well as to provide a different proteomic approach for sample analysis, which allows the identification of biomarkers.

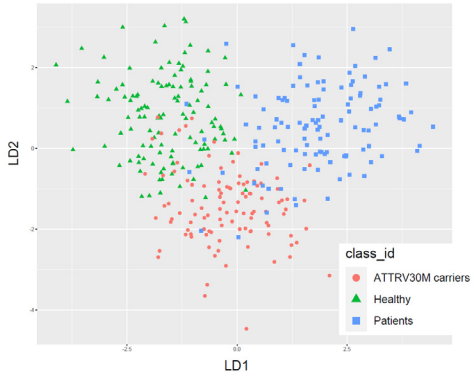

Figure 1.: Class representation after LDA analysis of the sample spectra.

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Support & Funding: This project has been co-financed through Pfizer (IRR ID#64764667) and Sobi.

POSTER PRESENTATIONS TUESDAY, 6TH

Amyloid fibril formation is suppressed by UV irradiation

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Background: Ultraviolet (UV) irradiation from space environment has been increasing due to the destruction of the ozone layer. Although UV damages DNA, peptide and cell structures, it also has the beneficial effects of suppressing excessive immune responses and cell proliferation. However, the effect of UV irradiation on amyloid fibril formation is unknown.

Objective: To examine the effect of UV irradiation on amyloid fibril formation in vitro.

Material & Methods: 1. Human $A\beta_{42}$ samples were irradiated by UV in phosphate-buffered saline (PBS), pH 7.0 at 37°C for 1 or 6 h. 2. After $A\beta_{42}$ samples were incubated in PBS, pH 7.0 for 24 h, the samples were subjected to UV irradiation for 1 or 6 h, respectively. 3. Human wild-type TTR (TTRwt) samples were irradiated by UV in sodium acetate buffer, pH 3.5 at 37°C for 1 h and the samples incubated for 24 h or 5 days, respectively. 4. After TTRwt samples were incubated in acidic buffer for 24 h, the samples were irradiated by UV for 6 h. We used an UVB generator for UV irradiation. We analyzed amyloidogenicity with a thioflavin T (ThT) method and morphological changes by electron microscopy.

Results: ThT fluorescence intensity for human $A\beta_{42}$ samples were decreased by UV irradiation. In the electron microscope analysis for $A\beta_{42}$ sample, the tips of amyloid fibers were thinned by UV irradiation. Although the ThT fluorescence intensity of human TTRwt samples was decreased by UV irradiation, the amount of the aggregate did not differ with or without UV irradiation.

Summary & Conclusion: UV irradiation suppressed $A\beta_{42}$ amyloid fibril formation and TTRwt aggregates. UV irradiation may act to destroy amyloid and amyloid-like substances in various amyloidoses and proteinopathies. Moreover, our experiments suggest that amyloidogenic proteins may form fewer amyloid fibrils in space, which may benefit patients with amyloidosis or proteinopathy who live in space.

Figure 1

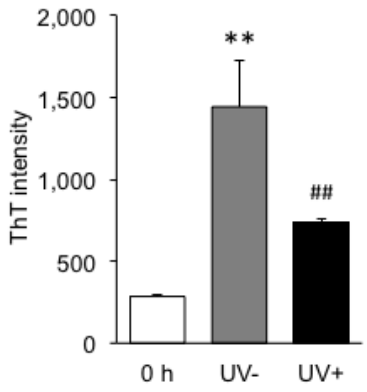

Figure 2

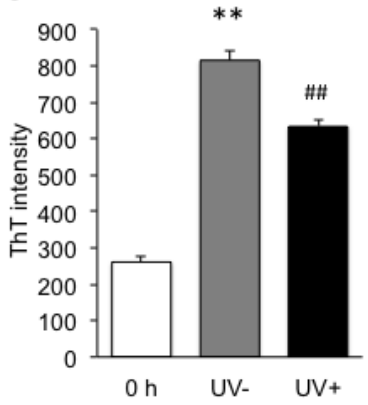

Figure 1: Effect of UV irradiation on $A\beta_{42}$ amyloid fibril formation. 0 h: before examination, UV-: without irradiation, and UV+: with irradiation. $^{**}P < 0.01$: UV- vs. 0 h. $^{\#\#}P < 0.01$ UV+ vs. UV- groups.

Figure 2: Effect of UV irradiation on TTRwt amyloid fibril formation. 0 h: before examination, UV-: without irradiation, and UV+: with irradiation. $^{**}P < 0.01$: UV- vs. 0 h. $^{\#\#}P < 0.01$ UV+ vs. UV- groups.

Support & Funding: This work was supported by Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI grant number 19H03565).

An additive destabilizing effect of two substitutions, T60I/V122I, in heterozygous compound TTRv amyloidosis

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Background: Transthyretin variant-associated (ATTRv) amyloidosis, the most frequent form of hereditary systemic amyloidosis, is usually caused by an amino acid replacement in the protein due to a single point mutation in the *TTR* gene [1]. Compound heterozygous mutations in ATTRv amyloidosis are extremely rare and the contribution from individual TTRv proteins to disease phenotype is not well studied. Recently, we presented a detailed description of the compound heterozygous TTR amyloidosis, T60I/V122I, in an African American female featuring a highly aggressive ATTRv phenotype, and, for the first time, demonstrated the amyloidogenic nature of TTR T60I.

Objective: Current biophysical and biochemical studies explore the structure and stability of recombinant T60I and V122I TTR proteins. Since both T60I and V122I TTR variants are produced simultaneously *in vivo,* they circulate as homo- and hetero-tetramers. To assess the effect of each substitution on the structural integrity of such hetero-tetramers, we studied the binary mixes of T60I and V122I at molar ratios from 0:4 (V122I alone) to 4:0 (T60I alone).

Material & Methods: A synthetic human *TTR* gene cloned into the pQE30 plasmid was used for construction of two TTR variants, T60I and V122I, by site-directed mutagenesis. Recombinant T60I TTR and V122I TTR variants were expressed and purified [2]. CD spectra, melting and kinetic temperature-jump data were recorded by using Jasco J-815 spectropolarimeter. Fluorescence emission spectra were recorded using TECAN microplate reader. Aggregation of the proteins in the presence of SDS micelles (0.5 mM) was monitored by ThT binding / fluorescence.

Results: Far-UV and near-UV CD spectra of T60I and V122I TTR variants were very similar indicating similar secondary and tertiary structure. Similar unfolding rates observed for T60I and V122I in temperature-jump experiments at 85 °C suggest similar kinetic stability. Protein thermodynamic stability was assessed by far-UV CD and intrinsic Trp fluorescence in thermal and chemical unfolding experiments. Compared to V122I, T60I unfolded at lower temperatures indicating its lower thermodynamic stability. Melting curves recorded from the protein mixtures progressively shifted to lower temperatures with increasing of T60I to V122I ratio from 0:4 to 4:0. In chemical denaturation experiments monitored by far-UV CD and Trp fluorescence at 25°C, T60I showed unfolding at lower urea concentrations (C_m=2.4±0.2 M) compared to V122I (C_m=3.4±0.2 M). Similarly to thermal unfolding, urea unfolding data received from the protein mixtures progressively shifted to lower urea concentrations upon increase of T60I to V122I molar ratio from 0:4 to 4:0 (Fig 1). During aggregation in the presence of SDS (Fig 2), T60I protein showed lower increase in ThT emission amplitude compared to V122I, suggesting slower amyloid growth and/or different amyloid structures formed by these two proteins; the mixtures of V122I and T60I showed a weighted average of the data for each individual protein.

Summary & Conclusion: Collectively, these results show that, T60I TTR has lower thermodynamic stability, similar kinetic stability, and distinct amyloid growth kinetics and/or morphology, compared to V122I, and that destabilizing effect of these individual mutations on the TTR stability is additive rather than synergistic. Since the T60I substitution does not alter the protein secondary or tertiary structure its large destabilizing effect likely stems from the altered protein dynamics, which can affect the quaternary structural stability.

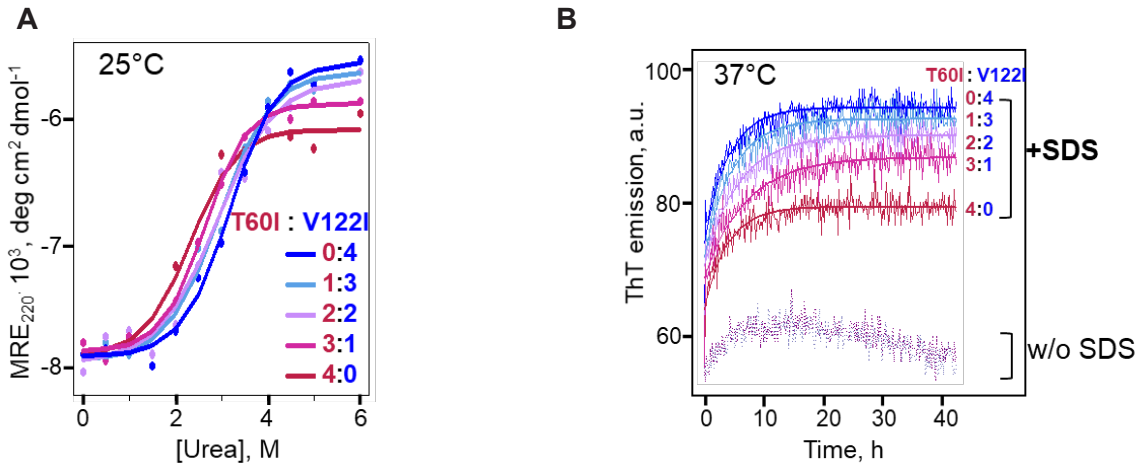

Figure. Effects of T60I and V122I substitutions on chemical stability and aggregation of TTR homo- and hetero-tetramers.
(A) Equilibrium denaturation by urea monitored by CD at 220 nm for secondary structure unfolding after incubation in urea for 96 h. Protein concentrations are 0.2 mg/ml.
(B) ThT binding to amyloid-like structures monitored by fluorescence during protein incubation at 37 °C with shaking, with or without SDS micelles.

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Support & Funding: This study was supported by the Wildflower Foundation and the Gruss Foundation; the Stewart Amyloidosis Endowment Fund, the Young Family Amyloidosis Endowment Fund, and the Amyloidosis Research Fund at Boston University.

Selective recognition of human small transthyretin aggregates by a novel monoclonal antibody

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Background: Biochemical characterization of transthyretin variant TTR Y78F showed that this variant adopts a tetrameric conformation as normal TTR and retains the ability to bind thyroxin (T_4), indicating a functional tetrameric structure. Under acidic pH conditions the Tyr---Phe substitution at position 78 is more prone to form fibrils than non-mutated TTR. It was hypothesized that native TTR Y78F exhibits the characteristics of an intermediate structure in the fibrillogenesis pathway and might represent an early event in TTR amyloidogenesis. Interestingly, this mutation originally designed *in silico* was found associated with peripheral neuropathy and cardiopathy.

Objective: To obtain a monoclonal antibody that identifies specifically amyloidogenic conformational TTR oligomers we immunized TTR knock out mice with recombinant mutant TTR Y78F.

Material & Methods: The best responder animal was used to perform a fusion protocol to obtain hybridomas. The clones obtained were screened for antibody production recognizing recombinant TTR Y78F. One stable hybridoma named CE11, of the IgM isotype, was the result of an additional screening by dot blot and ELISA with several soluble recombinant TTR mutants both amyloidogenic and non-amyloidogenic.

Results: CE11 only recognize the highly amyloidogenic TTR mutants L55P, S52P, A97S, Y78F or acidified TTR WT preparations. At the same time, this clone was negative for TTR V30M, soluble wild type protein or TTR T119M. The reactivity increases with oligomer formation and decreases as mature fibrils grow. The L55P and S52P TTR variants and acidified TTR WT were also characterized by CE11 after size exclusion chromatography (SEC) followed by capture ELISA and native immunoblotting . The mAb recognized two peaks (i) peak 1 present in acidified and in soluble mutant proteins preparations with material above 146 Kdaltons (ii) peak 2 only present in soluble L55P and S52P TTR preparations with material between 66 and 146 Kdaltons . Neither analyses identified tetrameric TTR which is recognized by a polyclonal antibody under the same conditions.

Summary and Conclusion: mAb CE11 is a potential tool to survey therapeutical agents against TTR aggregation.

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Funding

The work was funded by the project Norte-01-0145-FEDER-000008 - Porto Neurosciences and Neurologic Disease Research Initiative at I3S, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (FEDER).

Domain-domain interactions and dimerization of the human λ-III immunoglobulin light chain FOR005 investigated by NMR spectroscopy

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Background: Misfolding of light chain (LC) immunoglobulin and deposition of amyloid fibrils gives rise to systematic amyloidosis. This study aims to characterize variable (VL) and constant (CL) domain-domain interactions in full length LC protein to better understand initial unfolding events.

Objective: We aim to find out whether specific mutations influence domain-domain interactions in light protein. To address this question the structure and misfolding of both patient and germline full length light chains are analysed via solution-state Nuclear Magnetic Resonance (NMR) spectroscopy. Patient mutations are found both in the backbone and the linker region.

Material & Methods: All proteins were expressed using ^{15}N and ^{13}C isotopically enriched media, and purified via anion exchange chromatography. In addition to the patient sequence, protein coding for the germline sequence as well as individual point mutations was prepared.
Results: We obtained high quality solution-state NMR spectra of LC, VL and CL protein. Backbone assignment experiments have been performed for both LC and CL protein. To probe dimerization, concentration dependent HSQC spectra were recorded for LC and CL protein. We established a relationship between the concentration dependence of the NMR chemical shift and the oligomeric states of the respective protein. By comparison of the chemical shifts of VL, CL, and LC protein, we were able to identify the residues that are involved in domain interactions. To find out whether full length LC protein is able to form fibrils, we performed seeding experiments using VL fibril seeds and examined the resulting samples using MAS solid-state NMR. To probe fibril formation kinetics, we carried out Thioflavin T assays as a function of the protein concentration, and in presence and absence of fibril seed.

Summary & Conclusion: Using NMR we were able to get molecular insight into the role of the mutations G136V and C214S for LC aggregation. We found that the LC protein is less likely to form aggregates on its own, but requires VL seeds. We hypothesize that protein unfolding is required for LC fibril formation. Further seeding experiments are currently performed to validate this hypothesis.

Suppoort and Funding: This work was performed in the framework of the Research Unit FOR 2969 (German Research Foundation DFG, subprojects SP01, SP02, SP03, SP04 and SP05). We are grateful to the Center for Integrated Protein Science Munich (CIPS-M) for financial support. We acknowledge support from the Helmholtz–Gemeinschaft.

ALBase: an updated platform to study immunoglobulin light chain sequences

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[*]contributed equally to this work

Background: AL amyloidosis presents a unique research challenge as the immunoglobulin light chain (LC) proteins comprising tissue-deposited amyloid fibrils display extensive sequence variability. For this reason, a large organized database is required to elucidate the links between protein sequence and aggregation. To address these issues, ALBase was created in the Gerry Amyloidosis Research Laboratory at Boston University and made available to the public in 2009. The original database contained 491 amyloidogenic LC sequences, 213 sequences associated with other plasma cell dyscrasias (PCD), and over 3,200 non-PCD associated sequences.

Objective: To create a modern and improved 2nd generation version of ALBase.

Material & Methods: The database and website have been upgraded using the Microsoft .NET framework to conform to current standards and provide a platform for further development. This improvement facilitates frequent updates to ALBase and ensures user access to the latest information. Additional PCD-associated sequences were sourced from GenBank, IMGT®, Uniprot, Protein Database, and the literature; previously deposited redundant sequences have been consolidated or removed. To further extend the number of sequences for analysis, a computational method for identifying clonal sequences from high throughput RNA sequencing datasets has been developed and validated using sequences from the Multiple Myeloma Research Foundation's CoMMpass℠ Study.

Results: A total of 177 PCD-associated sequences have been identified from the literature and added to ALBase. Initial analysis of clonal RNA sequencing data yielded over 600 partial or complete LC sequences associated with multiple myeloma. These clonal LC sequences represent important controls for future research studies as they attain a high concentration in circulation without amyloid deposition. Preliminary analysis of these data provides insights into the over-representation of a subset of genes among amyloidogenic LCs compared to myeloma-associated LCs. Sequence annotation in ALBase also includes information on the biological nature, respective germ-line gene data, identification of the LC gene regions, and mutations with respect to donor germline gene. Various sequence retrieval methods such as clinical category, molecular type, cell type, or accession number are available. Nucleotide or protein sequences can be downloaded in common formats for analysis.

Summary & Conclusion: ALBase contains an extensive compilation of publicly available information on amyloidogenic and other PCD LC sequences culled from multiple sources. This specialized, comprehensive, and up-to-date dataset serves as a powerful tool easily accessible to the research community interested in analyses of nucleotide and protein LC sequences. ALBase can be accessed at https://wwwapp.bumc.bu.edu/BEDAC_ALBase/ or through the IMGT® medical page at http://www.imgt.org/IMGTmedical/Amyloidosis/.

Support & Funding: This project was supported by funding from the Wildflower Foundation and the Boston University Amyloid Research Fund.

Structure-based peptides as novel therapeutic and detection tools in cardiac amyloidosis

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Background: Cardiac ATTR amyloidosis (ATTR-CA) results from the deposition of amyloidogenic transthyretin (ATTR) in the myocardium, leading to hypertrophic cardiomyopathy and heart failure[1]. Despite the presence of pathogenic ATTR deposits in cardiac tissues of approximately 25% of individuals over 80 years of age, the disease is often misdiagnosed or diagnosed too late, resulting in poorer patient prognosis[2,3]. This is likely due to two major issues—a lack of therapeutics that target late-stage amyloid formation, and diagnostic tools that can detect ATTR at early disease stages. Our lab has previously developed small peptides that bind fibrils and hinder their propagation *in vitro* and *in vivo*[4–6]. We have used similar methods to design peptides that serve as detection probes in ATTR-CA tissue. We hypothesize that ATTR-CA fibrils contain unique epitopes that can be targeted by small inhibitors and detection probes.

Objective: Our goal is to harness the structure of ATTR fibrils to design effective late-stage therapeutic agents, as well as detection probes for early diagnosis of ATTR-CA.

Material & Methods: Peptide development was performed by rational design and optimization using the Rosetta-based algorithm ZipperDB. Fluorescent and epitope modifications were added to small peptides to develop detection tools. The effectiveness of peptide inhibitors was assessed by thioflavin-T assays, negative staining electron microscopy, and immunodotblot. The specificity and affinity of probes to ATTR fibrils was assessed through immunodotblot and enzyme-linked immunosorbent assays.

Results: Our results indicate that peptide inhibitors halt aggregation of *ex vivo* fibrils extracted from hearts of ATTR-CA patients (Figure 1). This suggests that they have potential to be used at later stages of disease where aggregation is predominantly driven by amyloid seeding[4] (Figure 1A). Our results show that modified peptide probes are capable of detecting pathogenic ATTR species in human samples of various origin (Figure 2). They detect ATTR aggregates in both crude ATTR-CA heart lysates (Figure 2B,C) as well as *ex vivo* fibrils extracted from hearts (Figure 2D). Their lower detection limit is in picomolar range, and they are specific to ATTR aggregates that are not present in control samples or other systemic amyloid patients.

Summary & Conclusion: ATTR fibrils contain distinct structural components that can be taken advantage of to develop novel late-stage therapeutics or diagnostic tools to improve quality of life for those with ATTR-CA.

Figure 1

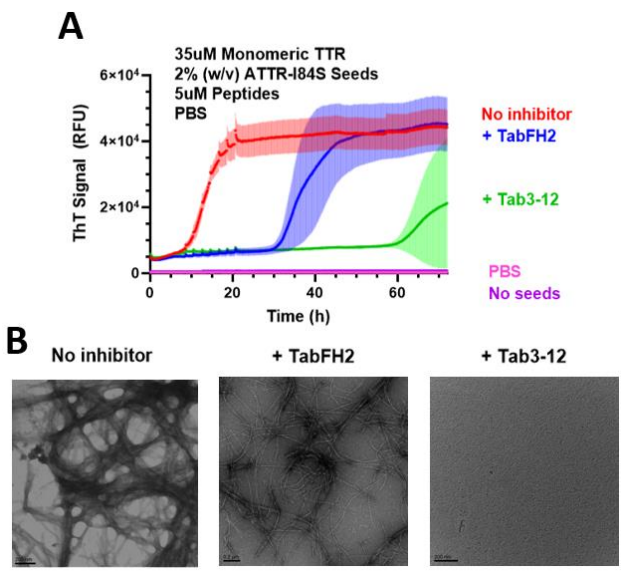

Figure 2

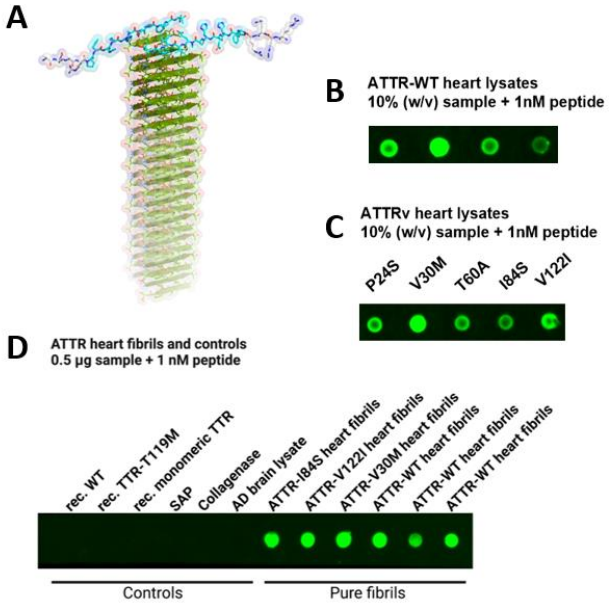

Figure 1. *In vitro* efficiency of structure-based peptide inhibitors. Anti-aggregation peptides display their activity at substoichiometric ratios over longer time courses than previous generations (A). They reduce the amount of total insoluble amyloid over time (B).

Figure 2. Structure-based development of peptide probes. We used the structure of ATTR fibrils to develop probes that target amyloidogenic segments F and H of transthyretin (A). These probes display high affinity to crude heart lysates of both wild type (B) and mutant ATTR-CA (C) patients, as well as fibrils purified from heart tissue (D).

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Support & Funding: This work was supported by the NIH (DP2-HL163810-01, R01AG048120, and RF1AG048120), the People Programme (Marie Curie Actions 298559) of the European Union's Seventh Framework Programme (FP7/2007-2013) the Amyloidosis Foundation (20160759 and 20170827), the American Heart Association (Career Development Award 847236), and UT Southwestern Start-Up funds.

Alternative pathogenic mechanisms and novel pharmacological approaches in gelsolin amyloidosis

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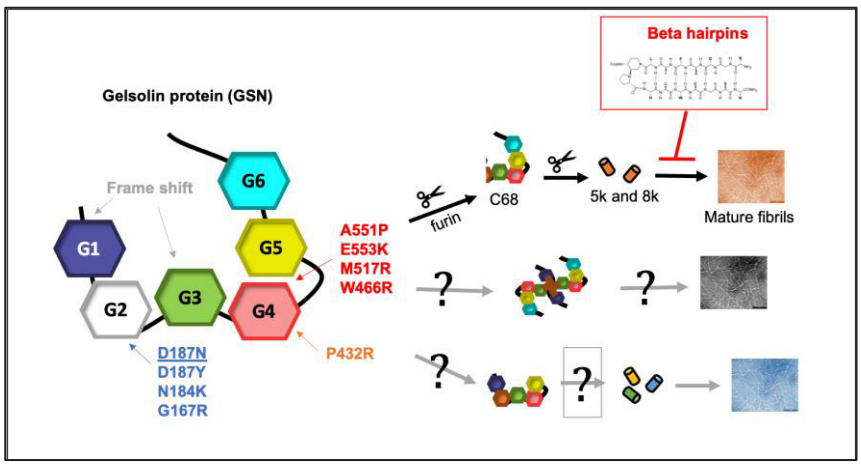
Background: Gelsolin amyloidosis (Agel) is a hereditary misfolding disease caused by pathological aggregation of the multidomain protein gelsolin (GSN)[1]. Several amyloidogenic mutations of GSN have been identified over the years, and most of them localize either in the second domain (G2) or at the interface between domains four and five (G4:G5) (Fig. 1). The well-characterized G2 variants undergo aberrant proteolytic events with the production of two aggregation-prone peptides, named 5k and 8k[2,3]. In contrast, little is known about the molecular events leading to the deposition of the recently described variants. To date, no specific pharmacological therapy for any form of the disease is available. Inhibitors of the aggregation process have been targeted by many studies aiming to slow down pathological aggregation. Among these, the recently identified beta-hairpin mimicking compounds containing a piperidine–pyrrolidine scaffold can modulate the amyloid aggregation process [4,5].

Objective: We aimed at characterizing the three recently discovered G4:G5 interface's variants and developing sequence-specific beta-hairpin inhibitors against the most common pathological variant, namely the D187N/Y substitutions in the G2 domain, for which the amyloidogenic core is known.

Material & Methods: The G4:G5 variants were characterized via thermal and pressure denaturation, fluorometric ThT assays, molecular dynamics, and X-ray crystallography. The *in vivo* proteotoxicity was also evaluated by the *C. elegans*-based assay. Three beta-hairpin peptidomimetics (LBs) were designed starting from the D187N aggregation-prone sequence. These molecules were tested in fluorometric ThT assays for their ability to inhibit the aggregation of the amyloidogenic core of G2 (AMI) and the proteotoxicity of endpoint samples from the ThT assays were assayed in *C. elegans*.

Results: Our data suggest that the G4:G5 variants either promote local destabilization of the interface or a conformational change, leading to the exposure of aggregation hotspots (not yet identified), prone to engage in aberrant protein-protein contacts and aggregation of the full-length protein under physiological conditions[6]. Of the three LBs beta-hairpins tested, two are surprisingly efficient with no measurable aggregation already at a LB:AMI molar ratio equal to 1:1 (Fig. 2A). ThT fluorescence measures both the amount of soluble/insoluble aggregates and their elongation. However, a good anti-aggregation compound should avoid selective inhibition of elongation with further accumulation of the much toxic soluble oligomers. Experiments in *C. elegans* showed that indeed the best *in vitro* performing LB counteracted the proteotoxic effects of aggregated AMI (Fig 2B).

Summary & Conclusion: Our preliminary data on the tested beta-hairpin support the feasibility of the exploited approach, showing that the identification of the sequences of the amyloidogenic trigger, allows the design of molecules able to inhibit the formation of the toxic species. Advanced knowledge of the Agel underlying mechanisms and the identification of other amyloidogenic cores of the protein, will allow us to optimize inhibitors against the G4:G5 variants and more exotic forms of the disease, or, more in general, against other amyloidogenic diseases.



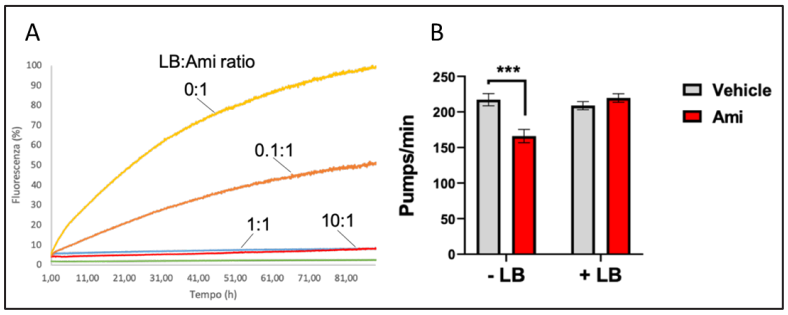


Figure 1.: Agel mutations and mechanisms. Amyloidogenic mutations mapped on the structure of GSN and the putative alternative amyloidogenic pathways triggered by the mutations, the first one being the mechanism triggered by a mutation in G2.

Figure 2.: Antiaggregation activity of a LB beta-hairpin. A) ThT assay of the amyloidogenic core of G2 (AMI) in the presence of increasing concentrations of LB (expressed in LB:AMI molar ratio); B) *C. elegans*-based proteotoxicity assay of AMI incubated in *aggregation-inducing* conditions in the absence (-) and presence (+) of LB (0.1:1, LB:AMI molar ratio).

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Coagulative and fibrinolytic blood proteases efficiently cleave human transthyretin (hTTR) oligomers in vitro to generate the amyloidogenic fragment hTTR(49-127)

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Background: Human transthyretin (hTTR) is a 55 kDa homo-tetrameric protein involved in the pathogenesis of Senile Systemic Amyloidosis. In the vast majority of patients, fibrils of hTTR are composed mainly of C-terminal fragments, the most abundant being fragment 49-127. Hence, the pro-amyloidogenic potential of hTTR is dependent in vivo on hTTR proteolytic cleavage, even though the protease(s) responsible of hTTR proteolysis have not yet been safely identified. Recently, in a hereditary form of hTTR amyloidosis, circulating soluble oligomers of the protein were identified as a diagnostic biomarker and driver of the disease.

Objective: Here, we examined the susceptibility to proteolysis and the conformational features of soluble hTTR oligomers (hTTR-O) produced in vitro. Proteolysis of hTTR-O was tested using different digestive (trypsin), coagulative (α-thrombin, factors VIIa, IXa, Xa, and XIa) and fibrinolytic proteases (plasmin), while their conformational dynamics was investigated by hydrogen-deuterium exchange mass spectrometry (HDX-MS).

Material & Methods: hTTR was purified from human plasma. For oligomer preparation, hTTR was incubated for 7 days at 4°C in 20 mM sodium acetate buffer, pH 4.4 and hTTR-O was purified by size exclusion chromatography (SEC) in PBS buffer, pH 7.4. Reactions with trypsin, plasmin and coagulation factors were conducted at 37°C, 750 rpm at an E/S molar ratio of 1/20 (enzyme/hTTR tetramer). HDX-MS analyses were performed in PBS buffer, pH 7.4, at 20 °C.

Results: Soluble hTTR oligomers showed a wide size distribution, between 160 and 900 kDa, based on SEC gel filtration analysis, higher than native tetrameric hTTR (hTTR-T). Interestingly, hTTR-O was found to be more susceptible to proteolysis by trypsin, plasmin, α-thrombin and factor XIa, compared to hTTR-T. All proteases showed very similar digestion patterns, with the formation of fragment 49-127 (Figure 1A). Global HDX-MS analysis of hTTR-O revealed the presence of a protein pupolation characterized by a higher deuterium incorporation with respect to hTTR-T, indicative of an increased conformational flexibility. Using local HDX-MS analysis, we were able to identify regions of much higher segmental mobility in hTTR-O, compared to hTTR-T, mainly encompassing the protein regions at the dimer-dimer interface in the tetramer structure (Figure 1B). Interestingly, the very first sites of proteolysis in hTTR-O matched with the regions of increased flexibility.

Summary & Conclusion: Proteolysis and HDX-MS experiments demonstrate that soluble oligomers of hTTR are much more flexible (or partially unfolded) than the native tetramer. Importantly, this increase in flexibility allows the hydrolysis of hTTR-O by coagulative and fibrinolytic blood proteases with the formation of the amyloidogenic fragment 49-127. Altogether, these results suggest a novel pathogenic mechanism, in which the formation of soluble hTTR oligomers in vivo can induce a higher susceptibility to proteolysis of hTTR and in turn more efficient fibril deposition.

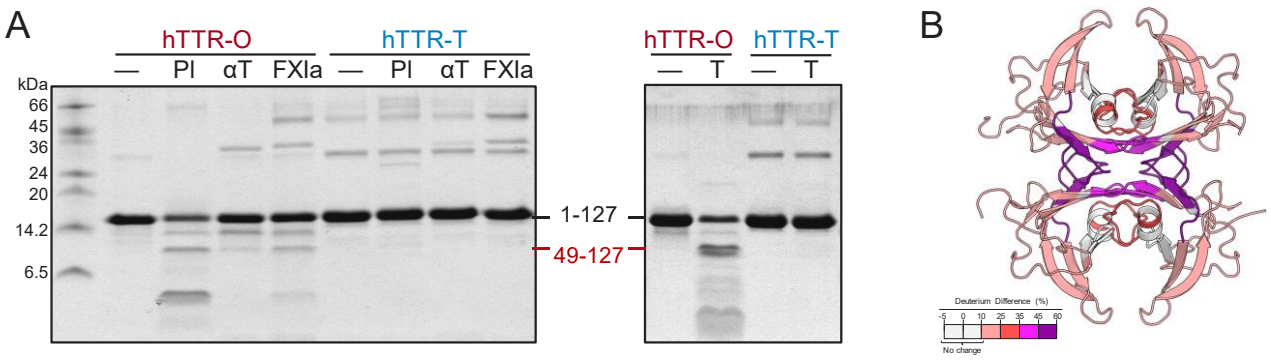

Figure 1: A. SDS-PAGE analysis of the proteolysis reactions of hTTR-O and hTTR-T with plasmin (Pl), α-thrombin (αT), factor XIa (FXIa) and trypsin (T) after 30 min (plasmin and trypsin) and 180 min (α-thrombin and FXIa) of incubation. **B.** Local HDX-MS analysis of hTTR-T and hTTR-O. 3D heatmap of the difference in percent deuterium (%D) uptake between hTTR-O and hTTR-T, after 1-min H/D exchange reaction, projected onto the native tetrameric structure (1rlb.pdb). Regions that are more flexible in hTTR-O are coloured purple.

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Mechanism of Misfolding and Amyloid Aggregation of the λ6 Light Chains.

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Background: A recent study has identified several pro-amyloidogenic hotspots in the variable domain (V_L) of the λ6 light chains(1), a subgroup strongly associated with AL amyloidosis(2). Structural and biophysical studies suggested that the aggregation hotspots located in the complementary determining regions 1 (CDR1) and the β-strands B, D, and E are crucial for amyloidogenesis. Since these hotspots are buried in the V_L core, or have a conformation that hinders aggregation, it was hypothesized that changes disrupting structural motifs that prevent these segments in engaging in edge-to-edge contacts are early events of the aggregation pathway of the λ6 light chains.

Objective: To characterize the conformational changes that occur early during the in vitro aggregation of the λ6 light chains and determine the role of the pro-amyloidogenic hotspots identified in these proteins in the mechanism of amyloid aggregation.

Material & Methods: In vitro fibrilogenesis experiments were performed with the germline-encoded recombinant (r) λ6 V_L proteins 6aJL2(R25) and 6aJL2(G25). The role of the V_L segments with putative protective function, as well as that of previously identified amyloidogenic hotspots in the mechanism of fibrillogenesis, was assessed by site-directed mutagenesis. Conformational changes associated with amyloid aggregation of experimental proteins were evaluated in dot blot assays with five conformation-sensitive rabbit polyclonal antibodies produced agains synthetic peptides of specific regions of the protein 6aJL2(R25) (Figure 1A). Ultrastructural analysis of the aggregates was performed with transmission electron miscroscopy with immunogold labeling (TEM-IGL).

Results: Dot blot and TEM-IGL analysis showed that the conformation-sensitive antibodies recognize both prefibrillar aggregates and amyloid-like fibrils formed by the λ6 rV_L proteins, but not their native state. Dot blot assay revealed that conformational adjustments involving the loop spanning residues 40-60 occur in the early phase (lag phase) of fibrillogenesis of both proteins (Figure 1B-E). As the aggregation proceeds, other regions of the V_L become accessible to antibody recognition. Mutations in key residues of the β-strand A of 6aJL2(R25) make the β-strand B more accessible to antibody recognition, which was associated with a faster kinetics of fibrillogenesis. Similar effect resulted from substituting Arg25 to Gly in 6aJL2(G25) protein (Figure 1C & E). Placement of Asp in several, but not in all positions of the CDR1, inhibited fibrillogenesis of both λ6 proteins.

Summary & Conclusion: Our findings indicate that the early phase of *in vitro* fibrillogenesis of the λ6 proteins 6aJL2(R25) and 6aJL2(G25) is characterized by conformational changes involving the loop 40-60. Site-directed mutagenesis analysis suggests that the highly amyloidogenic hotspot located in the CDR1 plays a central role in the aggregation process.

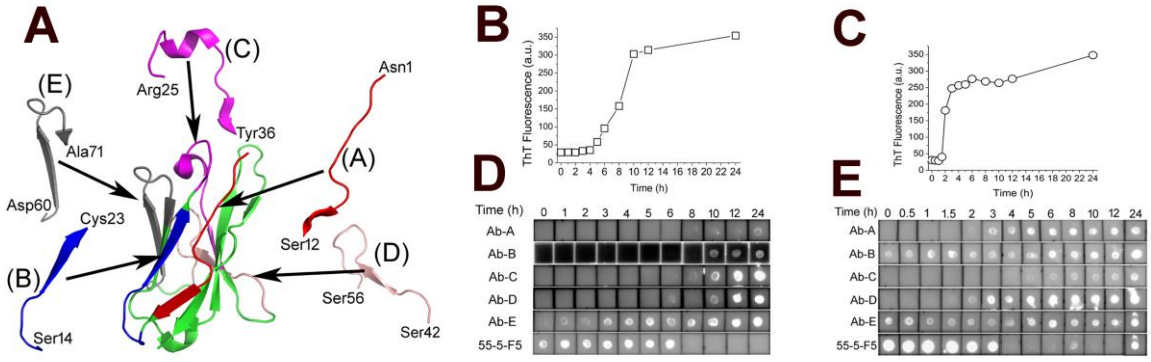

Figure 1. A) Location in 3D structure of the rV$_L$ protein 6aJL2 (PDB 3W0K) of the peptide segments used as immunogen to produce the conformation-sensitive rabbit polyclonal antibodies. Antibodies are named as A up to E. **B)** and **C)** show *in vitro* fibrillogenesis experiments of 6aJL2(R25) and 6aJL2(G25) proteins, followed by thioflavin T (ThT) fluorescence. **D)** and **E)** show dot blot immunoassays performed in aliquots taken during *in vitro* fibrillogenesis of the λ6 proteins as shown in B and C, respecively. The dot blot analyzes were performed at the same time intervale of ThT fluorescence determination, as indicated at the top of the panels.

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Filling the gap for transthyretin amyloidosis: biochemical and structural studies of *in vitro* and *in vivo* assembled amyloid fibrils

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Background: Transthyretin (TTR) amyloidosis is a misfolding disease arising from the intrinsic amyloidogenicity of the wild-type protein and exacerbated by a number of mutations. Its pathological mechanism is still unclear, but recent biochemical and biophysical results suggest that a combination of proteolytic cleavage and biomechanical forces generated by physiological fluid flow and contact with hydrophobic surfaces may be the most plausible cause of disease onset[1,2]. Our current methods for *in vitro* fibril formation produce fibrils that are morphologically and thermodynamically similar to fibrils derived from *ex vivo* material[3].

Objective: To characterize and compare structure of *in vitro* and *ex vivo* S52P TTR fibrils and understand the differences and similarities between the two.

Material & Methods: S52P TTR fibrillogenesis was carried out *in vitro* using the mechano-enzymatic method described in Marcoux et al, 2015, in presence of trypsin or plasmin. Experiments were also conducted in the presence and absence of seeds. Structural studies starting from X-Ray diffraction were performed on *in vitro* S52P, WT fibrils and natural fibrils extracted from the spleen of a patient carrying the S52P TTR mutation following the classical water extraction procedure. Negatively stained transmission electron microscopy (TEM) and an ongoing cryo-TEM analysis (performed on a Titan Krios at ERSF, Grenoble, France) were used to further analyse both *in vitro* and *ex vivo* S52P TTR fibrillar samples.

Results: Using a variety of biochemical and structural techniques, we analysed both *in vitro* and *ex vivo* S52P TTR amyloid fibrils. In particular, electron microscopy analysis of negatively stained samples of *in vitro* fibrils highlighted the presence of polymorphs, that have been recently detected in natural samples from a different mutation[4]. X-Ray fiber diffraction partially oriented amyloid fibrils, both mechano-enzymatic and *ex vivo* showed a common ring at 4.7 Å on the meridional plane. The differences between the two samples on the equatorial plane may be related to the fact that the natural sample is not pure and other species may influence the interspace between the β-sheets.

Summary & Conclusion: A comprehensive study to elucidate the amyloidogenic processes operating under physiological conditions is critical for an understanding of the natural history of the disease, its response to treatment and, crucially, the development of new and more effective therapies. While there is a major drive to develop robust models for *in vitro* TTR amyloidogenesis, it is of crucial importance to relate these to observations from analogous fibrils from *ex vivo* sources given the different pathways that may be involved in their formation. A preliminary cryo-TEM analysis is ongoing to further explore the structure of *ex vivo* S52P TTR fibrils.

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Support & Funding: This work was supported by the Italian Ministry of Research and University Dipartimenti di Eccellenza 2018–2022 grant to the Molecular Medicine Department (University of Pavia)
The cryo-EM experiments were performed on beamline CM01 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The authors thank the ILL Life Sciences Group supported by the Grenoble Partnership for Structural Biology (PSB) and they also used the EM facilities at the Grenoble Instruct-ERIC Center (ISBG ; UMS 3518 CNRS CEA-UGA-EMBL) with support from the French Infrastructure for Integrated Structural Biology (FRISBI ; ANR-10-INSB-05-02)

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Modulation of transthyretin aggregation: effect of preformed fibrils and heparin.

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Background: The metamorphosis of transthyretin (TTR), from the native tetrameric state to a fibrillar non-native state represents a fundamental pathogenic event of systemic amyloidosis by TTR (ATTR). Extensive work has been carried out to recapitulate *in vitro* the TTR metamorphism, from the early methods at non-physiological low pH[1] to the more recent observation on the effect of specific proteases and the identification of the mechano-enzyamtic mechanism of TTR fibrillogenesis[2,3,4]. The two methods share the same conclusion that *in vitro* TTR fibrillogenesis requires tetramer disassembly but the two procedures envisage substantially different pathways. While the prolonged incubation at low pH outlines a two state transition in which the conformers populating the transition state are folded and misfolded monomers, all of which are suitable for aggregation into fibrils, the involvement of a proteolytic event implies a much more complex content of protein conformers (summarized in Figure 1) for which Degradation or Fibrillogenesis become two alternative pathways.

Objective: In the mechano-enzymatic mechanism, the length of the lag phase is most likely directly proportional to the efficiency of the degradation pathway. Once the first nuclei of fibrils are formed and stabilized, a significant shift of the equilibrium between the two processes favouring fibrillogenesis over degradation can be envisaged. The objective of this work was to evaluate the role played by preformed fibrils (seeds) and heparin, used as surrogate of a generic stabilizer of fibrils, in accelerating the shift towards the fibrillogenic pathway.

Material & Methods: Mechano-enzymatic aggregation was carried out using both trypsin and plasmin, two proteases presenting the same specificity but different catalytic efficiency on TTR. We have scrutinized the effect of amyloid seeds and heparin on the fibrillogenesis of two TTR variants responsible for some of the most aggressive forms of cardiac amyloidosis, S52P and L111M TTR, and evaluated their effect on the lag phase and the total yield of amyloid fibrils formed measuring thioflavin T (ThT) emission fluorescence.

Results: The use of the two proteases has revealed peculiar kinectics responses by the two variants that result from the different rates of formation of amyloidogenic fragments, which can favour either the degradative or amyloidogenic pathway. In this context, the addition of amyloid seeds or heparin does not seem to change the rate of formation of amyloidogenic species (k_1; see figure 1) but rather affects the equilibrium between the degradative (k_2 and k_3) and fibrillogenic pathway (k_4 and k_5), shifting it towards the latter.

Summary & Conclusion: Our findings highlight the hypothesis that multiple biologic factors might influence the rate of amyloid growth *in vivo* and that every specific TTR variant might behave differently. These observation are particularly important when it comes to the management of patients affected by different TTR variants, for which a tailored therapeutic strategy may be required to achieve effective results.

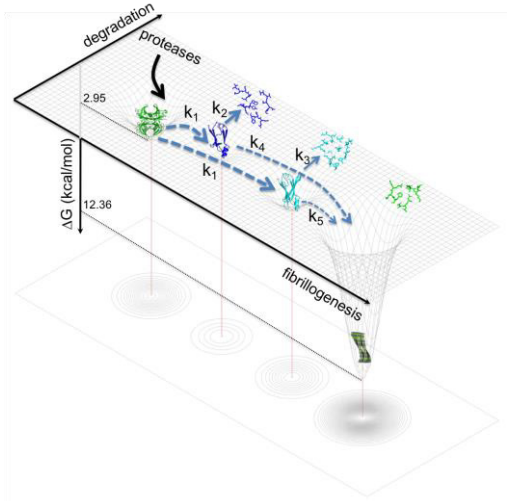

Figure 1.: Mechano-enzymatic mechanism scheme. Energetic funnel of the TTR fibrillogenesis pathway according to

Raimondi et al. (5). Different colours indicate: TTR tetramer (green), truncated monomer (blue), full length monomer (cyan) and fibrils (green-grey). Upper plane: on-pathway intermediates towards fibrillogenesis connected by dashed arrows and kinetic constants k_1, k_4 and k_5. The off-pathway degradation products are characterized by constants k_2 and k_3 and by solid arrows. The ΔG of unfolding for TTR and fibrils are reported.

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Circulating forms of plasma transthyretin in patients with wild-type transthyretin amyloidosis and effects of tafamidis

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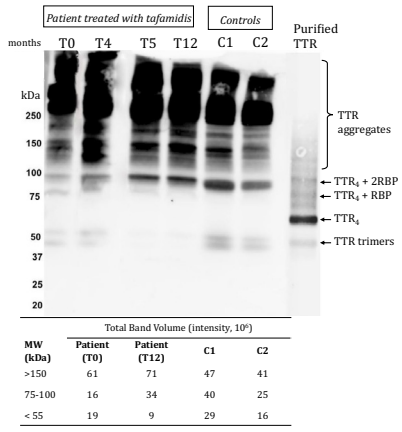
Background: Transthyretin (TTR) is a homotetrameric 55-kDa plasma protein whose function is to transport thyroxin and retinol complexed to retinol-binding protein (holoRBP). TTR misfolding and aggregation can lead to extracellular deposition of amyloid (ATTR). Both wild-type ATTR (ATTRwt) and variant ATTR (ATTRv) amyloidosis are known[1]. *In vitro* studies showed that TTR fibrils assemble through a process that involves tetramer dissociation, misfolding of TTR monomers into an aggregation-prone conformation and the subsequent formation of soluble aggregates, and amyloid fibrils[2-4]. These steps have not been characterized *in vivo*, also because of the lack of methods able to preserve the aggregation state of TTR. For the same reason, the response to TTR tetramer stabilizers such as tafamidis or acoramidis has been evaluated only in terms of total transthyretin levels or tetramer stability.

Objective: We aimed to develop a native electrophoretic method able to characterize the forms of circulating TTR in plasma samples without altering their aggregation state.

Material & Methods: Plasma from ATTRwt patients (n=6) and healthy controls (n=6) were obtained from subjects referred to the Fondazione Toscana Gabriele Monasterio (Pisa, Italy). For all ATTRwt patients, we collected samples before starting tafamidis (T0) and during the first year of treatment. Plasma samples were diluted into native loading buffer (pH 8) and separated on a 4–20% Tris-Gly polyacrylamide gradient gel (pH 8.8) devoid of SDS. Under these conditions TTR migrates mainly according to its size. Separated proteins were blotted onto a nitrocellulose membrane and incubated with anti-TTR (DAKO) or anti-RBP (Siemens Healthineer) antibodies. After incubation of HRP-conjugated secondary antibodies (BioRad), proteins were detected using the Clarity ECL substrate (BioRad). Densitometry analysis was performed using Image Lab software (BioRad).

Results: The patterns of circulating forms of TTR were qualitatively similar between ATTRwt patients at T0 and controls (Fig.1). In both groups, the most represented forms were: TTR dimers or trimers (37-50kDa), TTR tetramers complexed with RBP protein in 1:1 ratio (80kDa,) or 1:2 ratio (100KDa), and high molecular weight (MW) aggregates (>150kDa). Interestingly, neither TTR monomers nor the tetramers were visible. RBP protein was detectable only in association with TTR tetramers, but not with the lower or higher MW fractions (Fig.2). Following tafamidis treatment, all ATTRwt patients displayed a progressive increase of the intensity of the band corresponding to TTR-RBP complexes, in agreement with the drug stabilizing action on TTR tetramers. Interestingly dimers and trimers, detectable at T0, were progressively lost during tafamidis treatment (Fig.1).

Summary & Conclusion: Our preliminary results suggest that in plasma TTR tetramers exist only in association with holoRBP and in equilibrium with low and high MW degradation forms. Likely, when retinol is delivered to tissues, RBP affinity for TTR lowers and thus it might dissociate favoring the disassembling of tetramers into trimers, dimers and monomers. The latter have a high tendency to self-aggregate thus they could generate the high MW TTR fractions, possibly involved in amyloidogenic accumulation. The technique also shows an appreciable effect of tafamidis treatment on the stabilization of circulating TTR tetramers complexed with RBP. The study of circulating TTR fractions can expand our knowledge on mechanisms provoking its destabilization even when not mutated.



Total Band Volume (intensity, 10^6)

MW (kDa)	Patient (T0)	Patient (T12)	C1	C2
>150	61	71	47	41
75-100	16	34	40	25
< 55	19	9	29	16

Figure 1

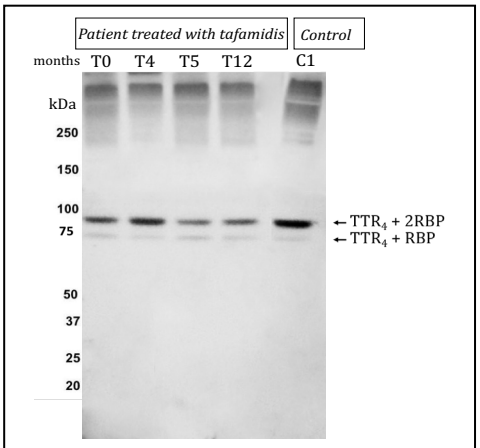


Figure 2

Figure 1.: Western blot analysis for plasma TTR. Electrophoresis was run in a native 4-20% polyacrylamide gel. Lithium-heparin plasma samples (0.5 µl) of two representative healthy subjects (C1, C2) and of one representative patient pre and during tafamidis treatment were analysed. A commercially available purified TTR was used as a marker for the TTR tetramers (TTR4). Densitometric analysis for the patient (0 and 12 months) and controls is reported in the table. TTR: transthyretin; RBP: retinol-binding protein.

Figure 2.: Western blot analysis for plasma RBP performed on the same samples of figure 1. Electrophoresis was run in a native 4-20% polyacrylamide gel. Lithium-heparin plasma samples (0.5 µl) of one representative healthy subject (C1) and of one patient pre and during tafamidis treatment were analysed. TTR: transthyretin; RBP: retinol-binding protein.

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Early Events of Immunoglobulin Light Chain Aggregation: Role of the C-terminus Disulfide Bond

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Background: Studying the early events in immunoglobulin light chain (AL) amyloid formation is of utter importance because some early intermediates formed during the aggregation reaction are highly cytotoxic at low concentrations [2]. These cytotoxic intermediates play a critical role in the initiation of insoluble amyloid fibril assembly [3,4]. Previously, the early events of κI O18/O8 (κI) variable domain (VL) expressed in bacteria were studied at different solution conditions [3,4]. Fibril formation studies have also been conducted on bacteria expressed κI full length (FL) protein [1].

Objective: Study the early events of aggregation of κI FL protein expressed in human cells and determine the possible inhibitory role of the C-terminus disulfide bond in the amyloid formation reaction.

Material & Methods: The protein was purified from Expi293F™ human cells using published methods. The monomeric and dimeric state of the protein was confirmed by SDS-PAGE under non reducing conditions. The *in vitro* aggregation experiment was set up at pH 7.4 at 37ºC with orbital shaking at 300 rpm. High Performance Liquid Chromatography (HPLC) based-sedimentation assay was performed to quantitate the monomer concentration remaining in the solution. The Thioflavin T (ThT) binding assay was executed to determine the extent of fibril formation in the aggregation experiment as a function of time. Circular Dichroism (CD) spectroscopy was performed to observe structural changes during the aggregation reaction. Species diversity was evaluated by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM).

Results & Discussion: κI FL did not present any significant increase in the ThT fluorescence intensity after 800 hours. A significant decrease of monomer concentration was observed in the solution after 600 hours of incubation. At 350 hours, no significant change in the protein's secondary structure was observed. A constant 217nm minimum, corresponding to β-sheet structure, is observed for each time point by CD spectroscopy. The most prominent protein population over the course of the reaction are monomers, observed by DLS. Utilizing TEM, we observe non-fibrillar macromolecular (NFM) intermediates for the κI FL, as previously observed with κI VL [3]. Mass spectrometry analysis of aggregation reaction prtoteins demonstrated that a population of tryptic peptides from κI FL retained the C-terminus disulfide bond. The other population appeared as monomer, suggesting that the disulfide bond was not formed.

Summary & Conclusion: Both monomeric and dimeric species dominate most of the early part of the aggregation reaction. NFMs were observed, rather than amyloid fibrils, as the only aggregates formed. Additionally, fibrillation seems to be inhibited by the presence of a C-terminus disulfide bond in κI FL protein.

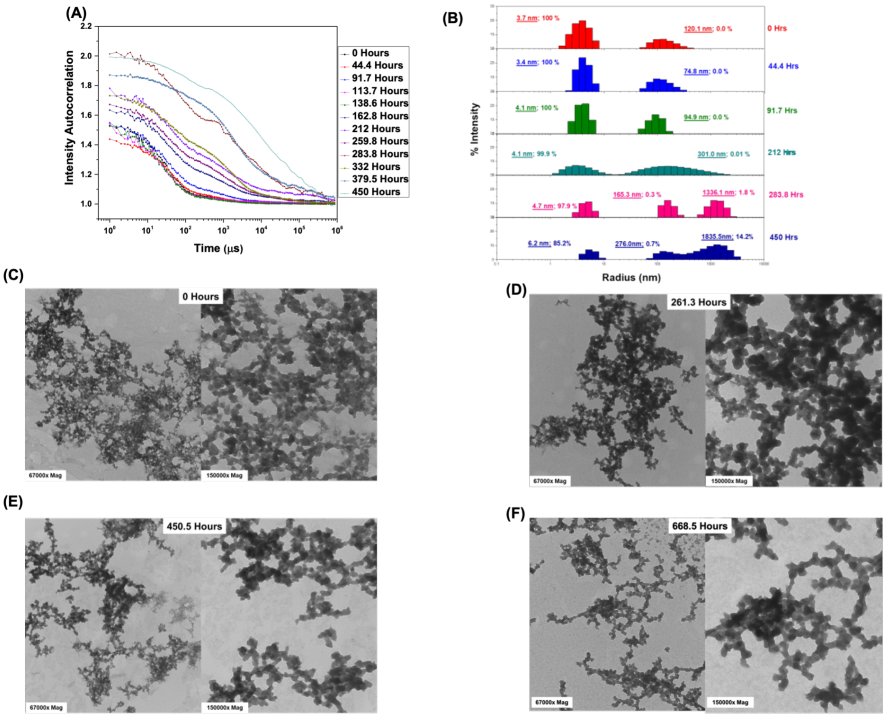

Figure 1.: Results of κI FL Aggregation Experiment. (A) Intensity Autocorrelation analysis obtained by DLS for various time points. (B) Radius analysis obtained by DLS for various time points. (C) TEM at 67000x and 150000x magnification at 0 Hours. (D) TEM at 67000x and 150000x magnification at 261.3 Hours. (E) TEM at 67000x and 150000x magnification at 450.5 Hours. (F) TEM at 67000x and 150000x magnification at 668.5 Hours.

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Recommendations for Addressing the Translational Gap between Experimental and Clinical Research on Amyloid Diseases

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Background: Amyloidoses are generally late-onset diseases in which proteins, after decades of normal service in the body, lose their native function and start aggregating into polymeric forms that are toxic for cells and tissues. Both basic and clinical research are challenged by the heterogeneity, complexity, and time course of amyloidogenesis. Like examples within other specialties, such as oncology, rheumatology and psychiatry, amyloidoses are what philosophers of science call "SCOTCH" (Significant Change Over Time, Complexity, and Heterogeneity) diseases.

Objective: To address translational challenges in amyloid disease research.

Methods: During the international online workshop organized by the LINXS Institute of Advanced Neutron and X-Ray Science in March 2021, it was discussed the existing gap between experimental research and clinical practice and the progress needed to narrow it. The workshop was followed up with a multiple-choice questionnaire with five questions designed to gather participants' opinions on the core issues.

Results and Conclusions: Based on the major issues raised both during the conference and subsequently by the questionnaire, the authors developed a list of recommendations for addressing the translational gap. Key suggestions include improving cross-cultural communication between basic science and clinical research, increasing the influence of scientific societies and journals (vis-à-vis funding agencies and pharmaceutical companies), improving the dissemination of negative results, and strengthening the ethos of science.

Proteolytic stability of amyloid isolated from human cataract eye lens

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Background: Cataract constitute one of the most common eye diseases associated with the blindness of over 18 million people worldwide. Aggregation of lens proteins (i.e., crystallins) is considered a primary cause of the disease. With aging, the lens proteins undergo several post-translational modifications (phosphorylation, glycosylation, truncation, etc.) that lead to their aggregation. It is suggested that the activation of intrinsic proteases could contribute to cataract formation through the formation of various short amyloidogenic peptides that might co-aggregate with native or truncated crystallins and contribute to cataractogenesis[1]. However, the fibrillar morphology of crystallin-derived aggregates is rarely evident in cataract lenses. This led to speculation that amyloid-like protein aggregates might have different structural organization compared to classical amyloids, and a comparative proteolytic susceptibility would be able to provide deeper insight into their molecular structure.

Objective: To probe the differences in the structural organization of classical amyloids and cataract-derived amyloid, it was hypothesized that the crystallin-derived aggregates would display varied susceptibility towards proteolytic digestion. To test this hypothesis, protein aggregates isolated from human cataract eye lenses along with the native soluble crystallin were subjected to proteolysis in the presence of trypsin.

Materials & Methods: Congo red (CR), Thioflavin T (ThT), 8-anilino-1-naphthalenesulfonic acid (ANS), lysozyme, trypsin, phenylmethylsulfonyl fluoride (PMSF), were procured from Sigma Chemical Company, USA. Insoluble protein aggregates were isolated from human cataract eye lenses by using the modified water extraction method [2]. Isolated protein aggregates were then incubated with trypsin solution in the ratio of 10:1 (protein: trypsin), at 37°C for 3 hours on a thermomixer at 800 rpm [1]. Afterward,0.1 mM PMSF was added to stop the enzymatic activity. All samples were characterized by performing a CR absorption shift assay, fluorescence-based ThT, and ANS binding assays to assess the change in amyloid content. SDS-PAGE of the samples has been performed to obtain the banding patterns of proteins.

Results: In the CR absorbance shift assay, trypsin-digested insoluble protein of human cataract eye displayed no shift in the absorbance of maximum wavelength (λ_{max}) in comparison to the un-digested protein aggregate, along with a reduction in ThT binding compared to the un-digested samples (Figure 1). Subsequently, surface hydrophobicity of trypsin-digested protein aggregates has shown a significant decrease from the un-digested one, as analyzed by ANS fluorescence. The observations suggest that upon protease treatment the amyloid contents of protein aggregates are substantially decreased. The SDS-PAGE confirmed the degradation of insoluble protein aggregate by trypsin. Protein aggregates were found to be highly susceptible to trypsin digestion compared to the aggregated lysozyme, taken as control. Heat-induced crystallin aggregates also displayed a similar pattern of proteolytic degradation.

Summary & Conclusion: The study confirms, unlike classical amyloid, protein aggregates in eye lenses are considerably different in their structural organization**.** Proteolytic susceptibility of the crystallin aggregates in

cataract lenses could be further explored for designing protease-based alternative therapeutic strategies for selective clearance of truncated/aggregated lens proteins to improve the lens clarity and restoration of visual acuity.

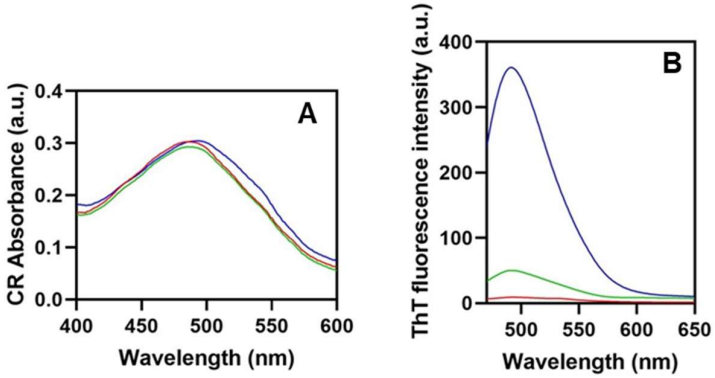

Figure 1. Effect of protease treatment on amyloid-like protein aggregates isolated from human cataract eye lens. (A) CR absorbance shift assay and (B) ThT fluorescence of trypsin digested insoluble protein aggregates isolated from human cataract eye lens. A relative reduction in the λ_{max} of CR absorbance and ThT intensity of the insoluble protein after trypsin treatment has been observed (green line) from the untreated insoluble protein (blue line). The degradation of the amyloid can be clearly identified by analysing this difference. The CR and ThT control are shown by the red line.

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Clusterin in Alzheimer's disease: friend or foe?

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Background: Clusterin, a heterodimeric glycoprotein (*α*- and *β*- chain), is an extracellular molecular chaperone. Clusterin contributes to many physiological and pathological processes. It is involved in Alzheimer's disease (AD), where it keeps the amyloid-β (Aβ) peptide soluble and contributes to its clearance, effectively inhibiting amyloid formation. Even though clusterin co-localizes with Aβ plaques, its role in AD is still not fully understood.

Objective: To investigate the potential effect of clusterin in amyloid formation, we experimentally examined the amyloidogenic potential of its α-chain. Furthermore, we tested whether clusterin's amyloidogenic peptide-analogues can inhibit A*β* fibril formation. Based on our findings, we proposed a putative mechanism in which clusterin prevents amyloid formation.

Material & Methods: AMYLPRED, a consensus algorithm for the prediction of amyloid propensity, was used to locate "aggregation-prone" segments in clusterin's α-chain sequence. In total, 5 regions were predicted. These 5 peptides, a mutant peptide for one of them, which carries a mutation found in Alzheimer's patients, and A*β*42 were synthesized and lyophilized. The clusterin peptide-analogues were studied using transmission electron microscopy (TEM), X-ray fiber diffraction, ATR FT-IR spectroscopy, and Congo Red staining. Each peptide-analogue was co-incubated with equimolar amounts of A*β*42, and the aggregation kinetics were evaluated with Thioflavin T (ThT) fluorescence measurements over time, as well as TEM. The interaction between A*β*42 oligomers and the most potent of the peptide-inhibitors, was examined with molecular dynamics (MD) simulations.

Results: The experimental assays showed that all 5 peptide-analogues exhibit characteristic amyloidogenic properties *in vitro*. Furthermore, ThT kinetics and TEM showed that all peptide-analogues can inhibit or delay A*β*42 fibril formation. In contrast, the mutant peptide-analogue neither exhibits any amyloidogenic properties nor inhibits A*β*42 amyloid formation *in vitro*. Finally, the MD simulations showed that the complex of A*β*42 and clusterin's peptide-analogue dissociates, significantly changing the conformation of the A*β*42 oligomer.

Discussion: Our results indicate that the full-length clusterin could intrinsically exhibit amyloidogenicity, which is contradictory to its function as a molecular chaperone. However, it is possible that the cell prevents toxic amyloid formation under different conditions of stress, by utilizing the intrinsic amyloidogenicity of specific molecular chaperones, which has been shown for other chaperones than clusterin. Amyloidogenesis inhibition for clusterin could be activated under pH-induced stress and achieved through the interaction of its aggregation-prone regions with amyloidogenic proteins, explaining its co-localization with A*β* fibrillar deposits.

Summary & Conclusion: Clusterin has at least 5 aggregation-prone regions in its *α*-chain with a potential role in the inhibition of amyloid-*β* fibril formation. Molecular chaperones with amyloidogenic properties might be implicated in the regulation of amyloid formation, essentially acting as functional amyloids. The ability of molecular chaperones to halt amyloid formation could be vital for tackling AD and other amyloidoses.

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Study of *berry* distinct polymorphism in ATTR amyloidosis fibrils by cryo-EM

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Background: Transthyretin (ATTR) amyloidosis is a systemic disease caused by either point mutations of TTR gene in hereditary ATTR amyloidosis or aging related process in wild-type ATTR amyloidosis (1). Following aggregation of misfolded transthyretin protein into amyloid fibrils, these fibrils deposit extracellularly at multiple organs and tissues which can be fatal. Different ATTR genotypes associate predominantly with certain clinical phenotypes e.g. ATTR-C10R in polyneuropathy; ATTR-WT in cardiomyopathy; and ATTR-P24S, ATTR-T60A and ATTR-I84S in both phenotypes (1).

Objective: We aim to determine the structure of various cardiac ATTR fibrils extracted from patient samples (ATTR-P24S, ATTR-T60A, ATTR_I84S (2 samples), ATTR-V122I and ATTR-WT (2 samples)), and to study any structural differences within these variants.

Material & Methods: Using cryo-EM, we determined the structures of fibrils from ATTR patients with the following variants: ATTR-P24S, ATTR-T60A, ATTR_I84S (2 samples I84S1 and I84S2) and ATTR-WT (2 samples). Ex-vivo preparations of amyloid fibrils were obtained from fresh-frozen human cardiac as described previously (2). For data collection preparation, we used Quantifoil Cu grids R 1.2/1.3, 300 mesh, Vitrobot Mark IV and datasets were collected on a 300 kV Titan Krios microscope (FEI). All datasets were processed using Relion 3.1.0. The reconstructed maps and models were visualized and built using Chimera X, Coot 0.8.9 and Pymol.

Results & Discussion: During 2D classifications, we have identified one common morphology between all ATTR samples which is termed Curvy fibrils (Fig 1A). The Curvy fibrils consist of a twisted single protofilament that have a width that varies from 76-80 Å. The average cross-over length of Curvy fibrils is 684 ± 16 Å. The cross-section of the reconstructed 3D map of ATTR-P24S with a resolution of 3.65 Å, ATTR-T60A (3.3 Å), and ATTR-WT (3.3 Å and 3.7 Å) resemble of a strawberry-shape (Fig 1B). The fibril protein structure of a single layer consists of 2 fragments: the smaller fragment fits the transthyretin sequence from P11 to K35, and the larger fragment fits the sequence from G57 to V121 (Fig 2).
The cross-section of the a ATTR-I84S patient 1, ATTR-I84S1, with a resolution of 3.0 Å, show similarity to the above ATTR fibril maps with significant differences in the fragment at the beginning of the C-terminal sequence (G57 to E63), which shows weaker density map (Fig 1C). Consistently, the fibrils from a second ATTR-I84S patient, ATTR-I84S2, with a resolution of 3.61 Å, do not contain the density corresponding to the fragment G57 to Y69 (Fig 1D). The mutation ATTR-I84S appears to affect the hydrophobic interactions between segment G57 to L58 and A81 to I84,
The structure inconsistency of the same variant I84S could arise from many factors including patient ages, sex, or the relative content of the wild-type form of ATTR in the fibrils. Considering that the overall structures of ATTR fibrils are very similar, the folding event of the segment G57 to Y69 may happen at the very last steps of ATTR fibril formation.

Summary and Conclusion: We have determined the cryo-EM structures of ATTR fibrils from 6 ATTR patients. ATTR fibrils can present more than one morphology.

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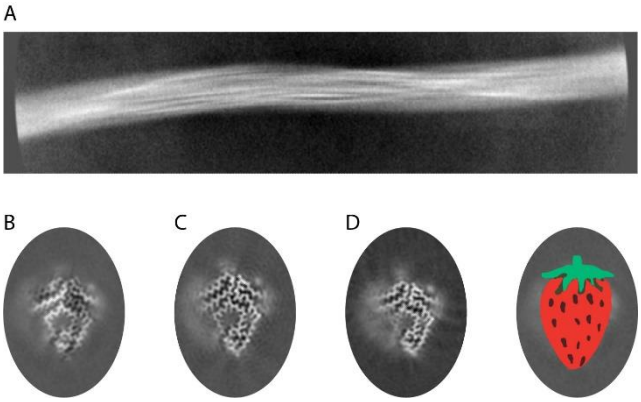

Figure 1: Reconstructions of ATTR fibrils. A, 2D classification of ATTR-P24S, the Curvy class, at box-size 1024 px (1.05 Å/px). B-D, Cross-sections of ATTR-WT (B), ATTR-I84S1 (C), and ATTR-I84S2 after 3D classification. D, Strawberry drawing representing the fibril shape.

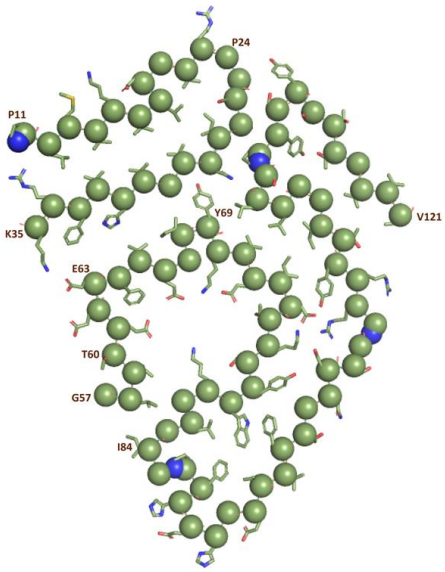

Figure 2: Schematic of single protein layer from ATTR-P24S fibril.

Cardiac proteotoxicity is resulting from a complex interplay of several molecular properties of amyloidogenic Light Chains

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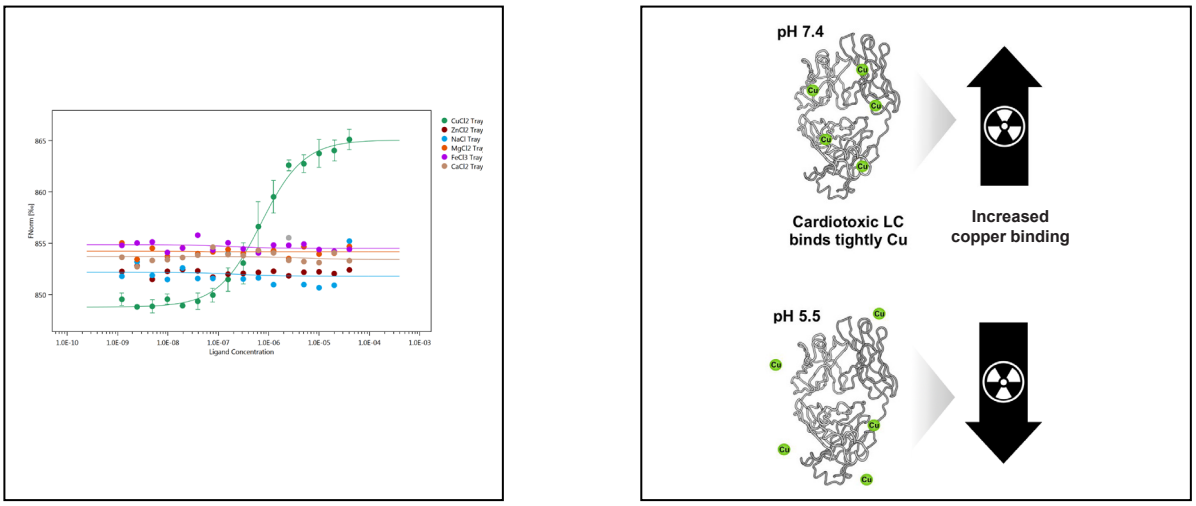
Background: Light chain amyloidosis (AL) is caused by sustained overproduction of amyloidogenic light chains (LCs) by bone marrow B-cells. The resulting high LC concentration results in the formation of amyloid deposits in heart and other target organs. However clinical data indicate that cardiac damage is caused not only by the accumulation of bulky amyloid deposits, but also by soluble LC species [1,2].

Objective: Here we aim to shed light on the molecular bases underlyining the proteotoxicity caused by soluble LCs in AL patients and how binders may tune the biophysical properties of LCs and concomitantly modifiy their toxic effects.

Material & Methods: LCs identified in AL patients with severe cardiatc involvements have been used for this study. Their fold stability has been monitored by circular dichroism and fluorescence spectroscopy, protein dynamics by limited proteolysis and ligand binding by thermophersis and isothermal calorimetry. The *in vivo* toxicity of LCs has been determined by using *C. elegans* as biosensor, able to specifically recognize proteins that cause cardiac invovlvement in AL patients. To this end, the pharyngeal function of worms treated with LCs has been determined.

Results: The LC proteotoxicity observed in AL patients has been recapitulated in a nematode *in vivo* model[3]. Here we show that such toxicity stems from a complex interplay between the biophysical properties of the amyloidogenic LC and the chemical environment, particularly the presence of binders. Specific conservatives mutations on the variable domain (VL) of a highly toxic LC (H6) leads to a H6 variant (mH6) which shows a more cooperative fold, a higher fold kinetic stability and a more rigid structure when compared with H6. When administered to C. elegans, mH6 results significantly less toxic than H6[4]. Counterintuitively, while the specific binding of copper divalent ions to LCs increases proteotoxicity, its binding results in a stabilisation of the VL domain and no effects on protein dynamics are observed[5]. Site-drected mutagenesis on another toxic LC (H7), by introducing two Ala in place of the two His residues present in the CL sequence, results in destabilised CL domain and in an overall slightly less stable protein. Nevertheless, H7 double His-to-Ala mutant displays low toxicity in *C. elegans* compared to H7. Finally, we observed that pH is also tuning LC toxicity. Although the biophysical properties of H7 and H6 at pH 5.5 and 7.4 are comparable, the proteins at pH 5.5 are not toxic when a administred to worms.

Summary & Conclusion:
The above experiments show that LC proteotoxicity can be modulated by changing the LC sequence, by the presence of LC binders, and by the chemical experimental conditions. Some of these data seem pointing at contrasting effects of protein stability and in general of biophysical properties in determining *in vivo* toxicity. These data are likely a glimpse of the complex interplay of biochemical, biophysical and patophysiologic tracts which contribute to the LC soluble proteotoxicity.



Figure

Figure 1.: Thermophoresis experiments showing the specific binding of Cu^{2+} to the amyloidogenic LC H7, while other divalent ions do not bind to H7.

Figure 2.: This figure sketches effects of Cu^{2+} binding on proteotoxicity both at physiologic and slightly acidic pH: when tested in *C. elegans* at pH 7.4, H7 is displaying a singificantly higher toxicity in presence of copper ions. At lower pH, H7 toxicity is abolished both in presence or in absence of Cu^{2+}.

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Assessing Immunoglobulin Light Chain Protein Stability and Stabilization by Pharmacological Chaperones Using Differential Scanning Fluorimetry

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Background: In immunoglobulin light chain amyloidosis (AL), clonal plasma cells over-produce amyloidogenic free light chains (FLC) that subsequently misfold and form toxic soluble aggregates and amyloid deposits. Misfolded FLCs can also be aberrantly proteolyzed before aggregation. Previous studies have found that, compared to non-amyloidogenic FLCs, amyloidogenic lambda FLCs are structurally dynamic and kinetically less stable as dimers. Faster misfolding of amyloidogenic FLC dimers will lead to the formation of higher concentrations of toxic non-native monomers and assemblies thereof. Therefore, a strategy to prevent the formation of toxic FLCs and FLC fragment conformations is to kinetically stabilize the native dimeric fold of the light chain protein, rendering it non-amyloidogenic. This "pharmacological chaperone" or kinetic stabilization approach has previously been used successfully to treat transthyretin amyloid disease.
More needs to be learned about the kinetic and thermodynamic stability of FLCs and how that relates to amyloidogenicity. Knowing that should paint a more complete picture of the forces governing FLC protein misfolding. New methods to assess thermal stability of distinct FLC sequences and FLC–small molecule kinetic stabilizer complexes are therefore needed.

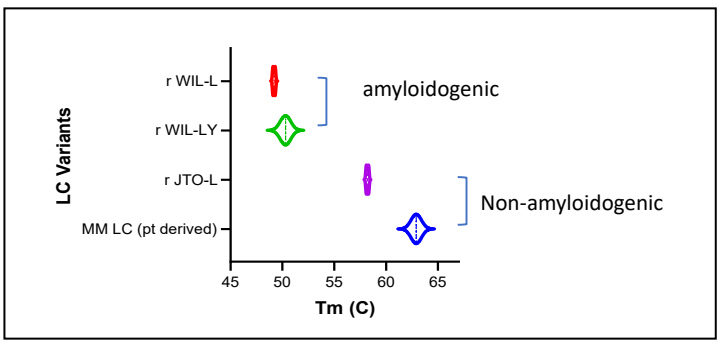
Objective: Develop a Differential Scanning Fluorimetry (DSF) assay to assess the stability of amyloidogenic and non-amyloidogenic FLCs, as well as the stabilizing effects of small molecule kinetic stabilizers.

Material & Methods: Recombinant lambda light chain proteins WIL, JTO and variants were purified to represent amyloidogenic and non-amyloidogenic lambda FLC proteins, respectively. The proteins were produced in E. Coli as inclusion bodies and refolded to homodimeric proteins. FLCs from multiple myeloma (MM) patients were were also purchased from a commercial source. A small molecule fluorophore was identified that monitors the conformational changes of lambda FLCs upon heat-induced protein denaturation. A T_m value is derived from each thermal transistion and used as an indicator for FLC stability. Orthogonal methods such as proteolysis sensitivity and surface plasmon resonance (SPR) are also used to establish correlations.

Results: The DSF assay was optimized and used to assess FLC stability and the stabilization imparted by small molecule lambda FLC dimer kinetic stabilizer binding. Amyloidogenic LC proteins have reduced thermal stability compared to non-amyloidogenic and MM FLCs. LC stabilizers improve protein thermal stability, coinciding with improved resistance to proteolysis.

Summary & Conclusion: We developed a DSF assay to quantify FLC thermodynamic stability and the stabilization effects of small molecule pharmacological chaperones against amyloidogenic lambda FLCs. We demonstrate DSF is an effective, simple and quantitative assay for studying FLC stability and stabilization by pharmacological chaperones.

Figure 1.: DSF assay using recombinant or purified ex vivo FLC proteins, showing Tm for each protein based on triplicate measurements.



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When Amyloid Occupies the Bone Marrow: Are the Responses of CD138-depleted Cells from Marrows with Interstitial Amyloid Archetypal?

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Background: Twenty percent of patients with systemic light-chain amyloidosis (AL) have interstitial amyloid in the marrow space.(*1*) This enables questions concerning how the non-plasma cell (CD138-depleted, 138-) marrow cells in AL marrows may respond to the presence of amyloid and to what degree the responses may be related to precursor processing, hematopoiesis and immunologic pathways.

Objective: Our goal in these studies is to develop a mechanistic understanding of the bone marrow micro-environmental response to the presence of amyloid with the hope of identifying treatment targets.

Materials & Methods: Marrow aspirates were obtained in the same procedure as biopsies, CD138-selection removed plasma cells, and the 138- marrow cells were cryopreserved with DMSO/FBS or stored with methanol fixation. Biopsies were routinely stained with Congo red and assessed for the presence of amyloid. 138- marrow cells were used for Illumina transcriptional profiling or for single-cell RNA sequencing analysis (scRNA-seq). We also performed qPCR and co-immunoprecipitation (IP) and immunoblots (IB) for selected genes or proteins.

Results: Marrow 138- cells from 23 patients were used for these studies; 10 had interstitial and 13 had vascular or no amyloid, with baseline medians of 15% and 13% clonal plasma cells, and no differences in age, gender, light-chain isotypes, or patterns of organ involvement. In Figure 1, a volcano plot (1A) and pathway enrichment analysis (1B) are shown comparing marrows with interstitial vs vascular amyloid. In 1A, among up-regulated genes are *TRIM15*, *TMCC2* and erythroid-related genes. *TRIM15* regulates focal adhesion disassembly critical for cell migration.(*2*) In 1A among down-regulated genes are inhibitors of macrophage activity such as *SIGLEC12*. In 1B, the top 3 pathways are neutrophil degranulation, MHC class II antigen presentation and Rho GTPase signaling, suggesting roles for complement, CD4+ T cells, and cell migration. (scRNA-seq analyses will be presented at ISA 2022.) Both TMCC2 and Rho GTPase signaling may be critical to the pathogenesis of Alzheimer's disease (AD), the former in complex with apolipoprotein E (apoE) and the amyloid protein precursor and the latter playing a role in actin polymerization; pharmacologic modulation of Rho GTPase has been investigated in AD.(*3-5*) In AL,*TMCC2* is expressed in differentiating red blood cells and was significantly up-regulated in 138- cells with interstitial marrow amyloid. However, in Figure 1C, we see IP/IB of TMCC2, apoE and free light chains from 138- marrow cells, demonstrating protein interactions in both groups. Of note, in marrow biopsies with interstitial amyloid, erythroid islands were frequently seen (1D).

Summary & Conclusion: The CD138-depleted cells from marrow aspirates in AL patients display different profiles of gene expression when amyloid is deposited in the interstitium. In the matrix of proteins identified by mass spectrometry of marrow amyloid deposits, both the alpha and beta chains of hemoglobin were seen as were complement co-factors, consistent with erythroid and myeloid presence.(*6*) Our data thus far indicate that cells from marrows with apoE-rich interstitial amyloid may respond with both modulated erythropoiesis and in ways similar to the AD brain, with TMCC2 interacting with apoE and increased Rho GTPase signaling, suggesting an archetypal response. Comparative scRNA-seq may fill the gaps regarding T-cell / macrophage interactions and the trajectory of erythropoiesis, activities possibly influenced by apoE.(*7*)

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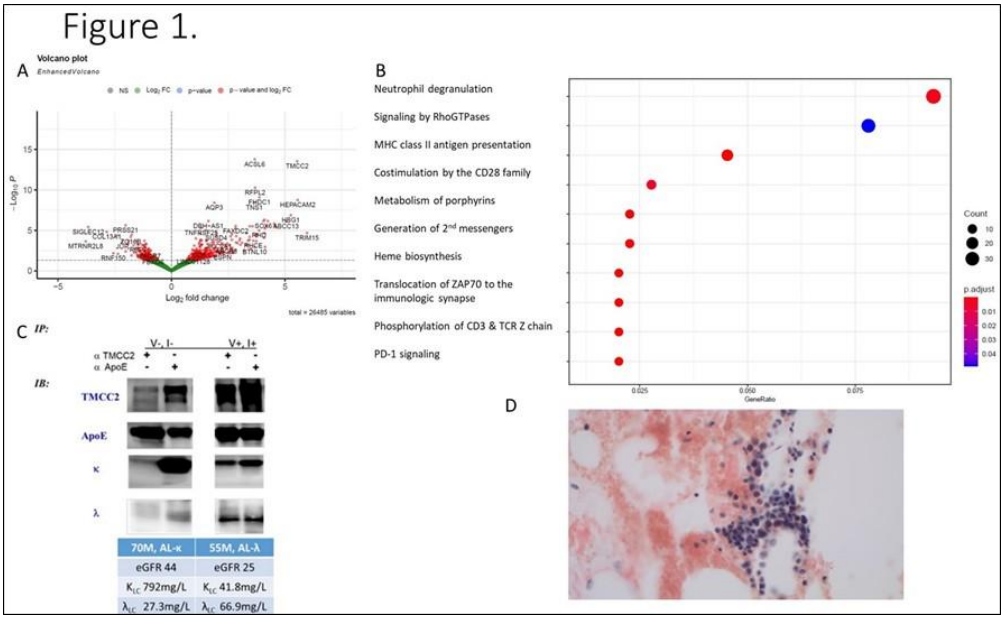

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Exploring the *in vitro* effects of light chain-induced proteotoxicity on primary human cardiovascular system cells

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Background: Direct LC-mediated cardiotoxicity is a crucial pathogenetic aspect in cardiac AL amyloidosis; however, the detailed molecular events leading to cardiac damage are still largely obscure. Cellular models recapitulating the features of LC proteotoxicity are the premise to define the molecular bases of cardiac damage (1-3), and the knowledge of the full repertoire of alterations occurring on major cardiac cell types after LC exposure is a fundamental step in this endeavour. Our previous studies showed that cardiotropic LCs (CT-LCs) impair viability of human cardiomyocytes and cardiac fibroblasts (hCF). In particular, hCF internalize cardiotropic LCs, which localize to endo-lysosomes and mitochondria (1-2).

Objective: In this study, we employ cell biology and biochemical assays to explore and compare the molecular alterations occurring *in vitro* in hCF and aortic endothelial cells exposed to amyloidogenic CT-LCs. The goal is to cast light on the *in vitro* alterations caused by the soluble precursor on cardiovascular system cells, and cast insight on the influence of each cellular species in the definition of cardiac damage in AL amyloidosis.

Material & Methods: Monoclonal amyloidogenic cardiotropic LCs (CT-LCs), and controls (non-amyloidogenic myeloma LCs, MM-LCs), were purified from 24h urine collection of patients, or produced as recombinant proteins in *E. coli* (4). All patients were fully clinically characterized. Commercial primary human cardiac fibroblasts (hCF) and primary human aortic endothelial cells (hAEC) were cultured according to the manufacturer's instructions and exposed to CT-LCs or MM-LCs, testing both young (passage number ≤3) and aged (p≥7) cells. Metabolic activity was evaluated by MTT/ATP quantification, and dose-response curves experiments were performed (LCs concentration range: 1-50 μM). Cell membrane fluidity was investigated using a fluorescent lipophilic probe (pyrenedecanoic acid)-based assay. ROS production and endothelin-1 secretion were assessed with commercial assays.

Results: hCF and hAEC response to LC exposure was divergent. Incubation of hCF with 5μM CT-LCs (3 distinct CLs) significantly reduced metabolic activity at 24h, while incubation with MM-LCs (3 distinct CLs) did not. Cell aging did not worsen the impairment of cell metabolic activity caused by soluble prefibrillar CT-LCs. In contrast, hAEC incubation with LCs in the same experimental conditions showed no significant alterations in cell metabolic activity. Moreover, cell aging had no effect on metabolic activity perturbation, but older cells (p≥7) secreted a significant amount of endothelin-1 only when incubated with CT-LCs. Quantification of pyrenedecanoic acid incorporation by hAEC displayed no significant alterations upon exposure of either type of LCs, while hCF exposed to CT-LCs showed a significantly lower intrinsic signal compared to the non-amyloidogenic controls, suggesting that CT-LCs have a negative impact on membrane fluidity on hCF.

Summary & Conclusion: This work delineates how cardiac and aortic endothelial cells differently respond to CT-LC exposure, underlying the need to explore the whole complexity of cardiovascular system cells to better understand how they influence each other during the development of cardiac damage and to have a more complete picture of the mechanisms of cardiac damage mediated by amyloidogenic light chains.

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Exploring Alzheimer's disease (AD) related human brain proteome with MALDI Imaging Mass Spectrometry in combination with shotgun proteomics

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Background: Neuropathology of AD is characterized by the accumulation and aggregation of Aβ peptides into extracellular senile plaques (SP). The Aβ peptides, composed of forty amino acids, are generated from amyloid precursor proteins (APP) by β- and γ-secretases. Aβ is deposited not only in cerebral parenchyma but also in leptomeningeal and cerebral vessel walls, known as cerebral amyloid angiopathy (CAA). While a variety of Aβ peptides were identified, detailed production and distribution of individual Aβ peptides in pathological tissues of AD and CAA is not fully addressed.

Objective: We develop a novel protocol of MALDI-imaging mass spectrometry (MALDI-IMS) in combination with shotgun proteomics on human autopsy brain tissues to obtain comprehensive proteoform mapping to address a detailed production and distribution of individual Aβ peptides in pathological tissues of AD and CAA.

Material & Methods: Human cortical specimens for IMS were obtained from the Brain bank at Tokyo Metropolitan Institute of Gerontology. Frozen tissue sections were cut on a cryostat at a 10-20 µm thickness onto ITO glass slides. For mass spectrometric measurements, tissue areas were defined using the FlexControl 3.8. and fleXImaging. Spectra were acquired using the rapifleX MALDI Tissuetyper and timsTOF fleX in positive linear mode, whereas ions were detected with spatial resolution of 50-70 µm. Shotgun Proteomics from serial sections of MALDI-IMS were attempted using timsTOF Pro with nanoElute system. Mass spectra as well as annotated proteins and peptides were visualized with flexImaging and SCiLS Lab 2019 Software.

Results: MALDI-IMS with rapifleX MALDI Tissuetyper demonstrated the detailed distributions of both Aβx-40 and Aβx-42 (x = 2, 4, 5, 6, 7, 8, 9, and 11pE) in AD accompanied with moderate CAA brain. Furthermore, MALDI-IMS with timsTOF flex also detected shorter Aβ peptides, including Aβ1-29, Aβ10-40 and N-truncation form of Aβx-42 (x = 3, 3p). Segmentation map obtained with bisecting k-means analysis applied for AD, CAA and non-pathological brains. This clustering method successfully identified plaque-like structures in the parenchyma and vascular structures in subarachnoid space of AD. Moreover, proteomic profile of white matter from AD brains reveals an altered pattern between SP rich vs SP rare areas. As the next step, we have challenged to integrate in depth AD brain proteome with MALDI-IMS and a shotgun proteomics using intact and on tissue digestion technology. Several spots from autopsied brains were selected from white matter, grey matter as well as leptomeningeal vascular structures to be protein and peptide sources through laser capture microdissection. For those shotgun proteomics data, we have compared epitope preference of peptide sequences from identified proteins such as □-synuclein in human brains. The current strategy accelerates the diagnosis and the clarification of the pathogenesis of AD, our current strategy will benefit unravelling molecular mechanisms underlying SP and NFT formation as well as neuronal loss in human brains.

Summary & Conclusion: AD/CAA by visualizing Aβ with undigested native peptide imaging. We have succeeded in visualizing not only the difference in the C-terminal truncation but also the difference in the N-terminal truncation of Aβ. As a next step, we have succeeded in visualizing and identifying proteome of human brains with on tissue digestion method in combination with shotgun proteomics. Current strategy enables us to elucidate AD/CAA pathology in leptomeningeal spaces as well as brain parenchyma through in depth proteomics.

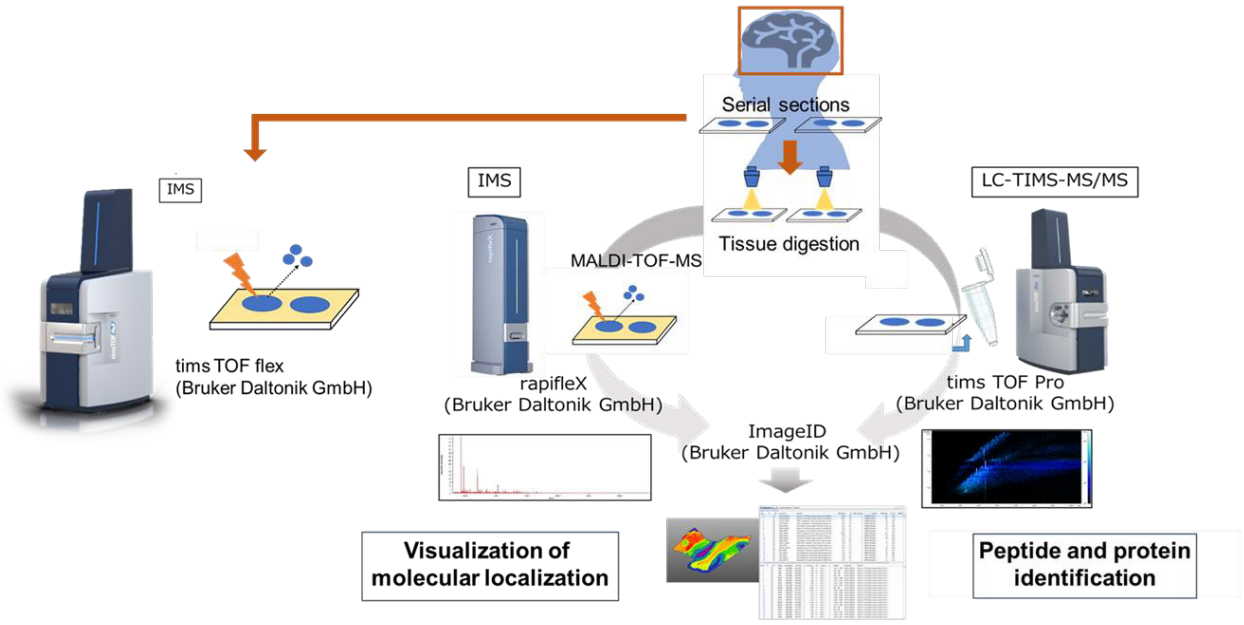

Figure 1. An integrated workflow of MALDI-IMS and shotgun proteomics

Transthyretin (hTTR) Amyloid Fibrils Trigger Plasma Clotting by Activating the Intrinsic Pathway of Blood Coagulation: Implications in Cardiac Senile Systemic Amyloidosis

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Background: hTTR is a 55-kDa homo-tetrameric abundantly present in human plasma (0.18–0.45 mg/ml) and involed in thyroid hormones and retinoic acid transport. Wild-type hTTR can form amyloid fibrils and its aggregation leads to Senile Systemic Amyloidosis (SSA), an acquired amyloid disease mainly affecting elderly people (>75 years) and associated to cardiomyopathy and intracardiac thrombosis (Figure 1).[1-3]. The standard composition of amyloid deposits in SSA patients is a mixture of intact hTTR and C-terminal fragments, with hTTR(49-127) fragment being predominant. The pro-amyloidogenic potential of hTTR is dependent in vivo on proteolytic cleavage, as fragments are more amyloidogenic than intact hTTR.[4,5,7] Recent data suggest that SSA-related cardiomyopathy and intracardiac thrombosis have been often overlooked as a common cause of heart failure in older adults, with an estimated total prevalence of 1–3% in elderly people >75 years of age. Inedded, hTTR amyloid fibrils were found in 25% of post-mortem hearts from patients >80 years of age and in about 15% of older patients with heart failure or aortic stenosis with an estimated total prevalence of 1–3% in elderly people >75 years of age.[1-3]

Objective: Considered that the biochemical pathways leading to intracardiac thrombosis in SSA are unknown, here we explore the possibility that both natural and artificial hTTR fibrils could induce fibrin generation in human plasma and investigate the molecular mechanisms through which the coagulation cascade is activated.

Materials & Methods: Natural hTTR fibrils were isolated from autopsies of deceased patinets with SSA or genetic hTTR-amyloidosis[6]. Wild-type hTTR and hTTR(49-127) were obtained as recombinant species in *E. coli*, while amyloid fibrils were produced by incubating hTTR (0.2 mg/ml, for 72 h, at pH 4.4 and 900 rpm) or hTTR(49-127) (0.4 mg/ml, for 24 h, at pH 7.4 and 900 rpm). Both artificial and natural fbrils were characterized by thioflavin-T binding, dynamic light scattering, and transmision electron microscopy.[5] Fibrils were added (0.5-2 μM monomer concentration) to diluted (1:2) human plasma, and fibrin generation was monitored by turbidimetry, along with SDS-PAGE and Western blot analysis of the fibrin clot. The role of each coagulation factor in plasma fibrin clotting was estabilised using plasma samples depleted with a single coagulative zymogen protease at a time. Confirmnatory experiments were conducted by incubating amyloid fibrlis in PBS with each isolated zymogen and the corresponding chromogenic substrate. Binding of coagulation factors to hTTR fibrils was quantified by ELISA tests.

Results: The data shown in Figure 1 indicate that: i) both natural and artificial hTTR fibrils efficiently and similarly induce, in a concentration-dependent manner, fribin clotting in human plasma; ii) amyloid fibrils of hTTR(49-127) more efficiently trigger fibrin clotting compared to intact hTTR-fibrils. Our results also indicate that amyloid fibrils induce autoactivation of the coagulation factor XII, at the initial steps of the “intrinsic pathway” of blood coagulation cascade. Noteworthy, the amorphous/nonamyoild acid-precipitate of β-casein does not trigger plsama clotting.

Summary & Conclusion: The results of this study provide a novel pathogenetic mechanism, whereby hTTR amylod deposition in the heart chambers may initiate the intrinsic pathway of blood coagulation and lead to intracardiac thrombosis and heart failure in predisposed SSA patients.

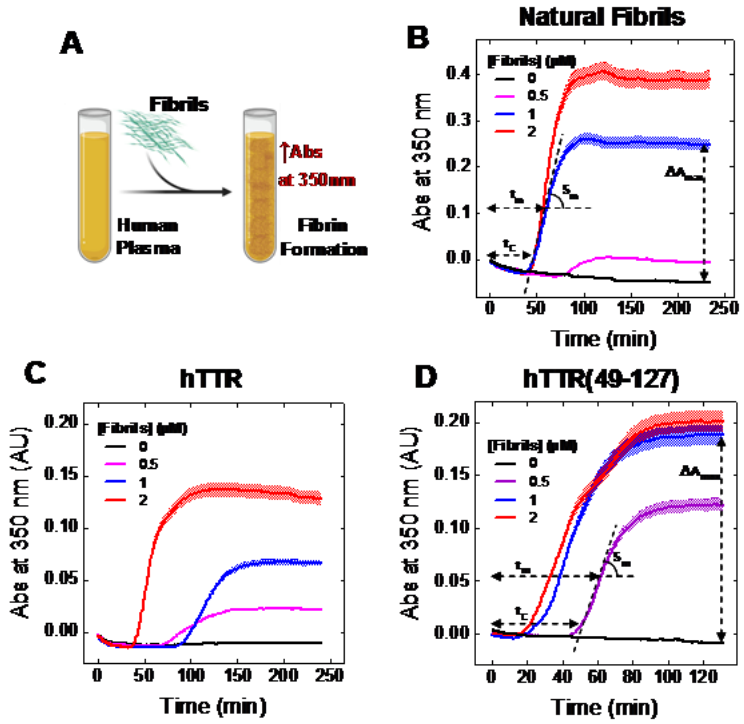

Figure 1. Fibrin generation in human plasma induced by natural and artificial hTTR amyloid fibrils, as indicated.

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V30M TTR animal model displayed a downregulated expression of several chemokines in different immune cell populations

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Background: ATTRV30M disease, formerly known as Familial amyloidotic polyneuropathy is an autosomal dominant neurodegenerative disorder associated with deposition of mutant transthyretin (TTR) aggregates and amyloid fibrils, particularly in the peripheral nervous system (PNS), ultimately leading to axonal fiber degeneration and cell death[1]. The mutation responsible for the disease consists in a substitution of a methionine for a valine at position 30 (TTR V30M) that predisposes TTR to form aggregates and fibrils[2]. Nerve biopsies from ATTRV30M patients display increased cytokine production, but intriguingly no immune inflammatory cellular infiltrate is observed around TTR aggregates, and this lack of response contributes to disease aggravation[3]. Moreover, in nerve biopsies from ATTRV30M patients, Schwann cells (SCs) abnormalities have been reported, including multimembraneous bodies, vacuoles, and fibrous materials in the cytoplasm[4]. Additionally, ATTRv patients display quantitative and qualitative abnormalities in tissue resident macrophages, and these abnormalities may accelerate TTR amyloid deposition in some organs[5]. Regarding to animal models, V30M mice display a downregulated innate immune response in response to nerve injury when compared to WT mice[6].

Objective: In this study, we decided to further investigate the inflammatory immune response in different cell populations, namely SCs and bone marrow derived macrophages (BMDM) on the animal model of the disease and compare with the WT TTR mice.

Material & Methods: RNA was extracted from sciatic nerve of the mouse model of the disease and the respective control group with 6 months of age and the levels of several chemokines were assessed by RT-PCR. To investigate the role of SCs in the disease, mouse SCs were incubated with human WT or V30M TTR for 24 hours and after RNA extraction the expression levels of several chemokines were assessed. Moreover, to address the macrophage response to TLR4 stimulation, we incubated BMDM of either genetic background with LPS and measured the expression of several chemokines 6 hours later by RT-PCR.

Results: First, we show a significant impairment in the production of several chemokines in the PNS of a pre-clinical model of TTR V30M amyloidosis. In addition, we observe that *in vitro* mouse SCs are activated by incubation with WT TTR contrariwise to incubation with V30M TTR and respond with the production of several chemokines and cytokines, such as CCL20, CXCL3, CXCL2 and CCL8. Furthermore, we demonstrate a downregulated expression of several chemokines in BMDM generated from a transgenic mouse model for human V30M TTR upon stimulation with agonists for TLR4 receptor.

Summary and Conclusion: In conclusion, the presence of V30M TTR impacts the expression of several chemokines in different immune cell populations, pointing towards a close interface between the absence of chemokine response with the development of the disease. Elucidating how these mediators interact in the context of ATTRv may be crucial to explain the immunological impairment observed in peripheral nerves of ATTRv patients.

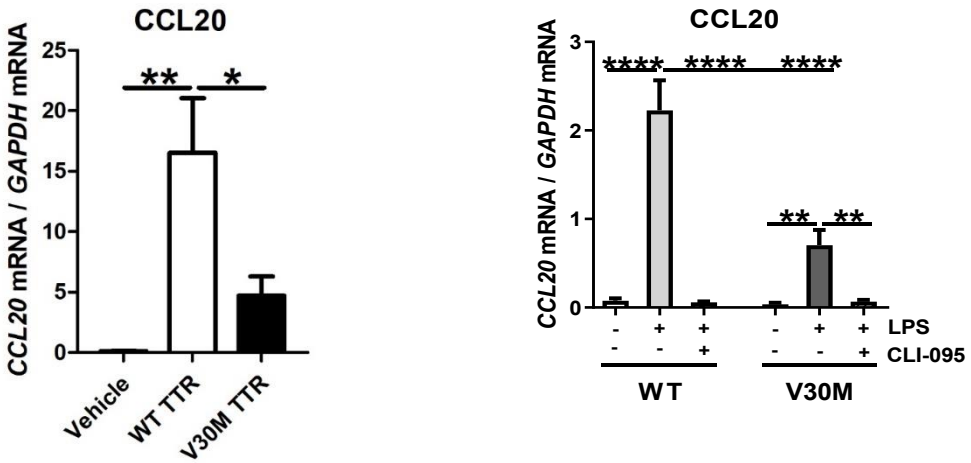

Figure 1.: The expression of chemokines was downregulated in Schwann cells incubated with V30M TTR. Reduced levels of CCL20 was determined by RT-PCR in SCs incubated with V30M TTR when compared to WT TTR

Figure 2.: BMDM derived from WT and V30M TTR animals responds differently to stimulation with TLR4 agonist. BMDM from V30M TTR mice displayed a downregulated expression of CCL20.

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Funding

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In vitro treatment of light-chain amyloidosis plasma cells to characterize response to venetoclax

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Background: Studies have demonstrated that light-chain amyloidosis (AL) plasma cells (PC) inherently differ from multiple myeloma (MM) PC, warranting dedicated research on AL PC biology and response to therapy[1]. Venetoclax is a small-molecule inhibitor of pro-survival BCL-2 protein that has shown clinical potential in both MM and AL. In MM, expression of BCL-2 and BCL-xL were shown to be predictors of response to venetoclax.[2]

Objective: The aim of our research was to study AL amyloidosis PC survival in a hydrogel-based 3D-culture model and to characterize response to venetoclax, including the relation with t(11;14) and protein expression of BCL-2 and BCL-xL.

Material & Methods: Bone marrow samples from patients with AL were gathered at the University Medical Center Utrecht, the Netherlands, and the Policlinico San Matteo Pavia, Italy. All included patients provided written informed consent. Bone marrow mononuclear cells were cultured for 7 days in a Puramatrix hydrogel-based model, supplemented with pro-survival cytokines IL-6 and APRIL at 100 ng/ml[3]. AL cell lines were cultured in IMDM with addition of IGF-1 and IL-6[4]. To study the response to BCL-2 inhibition, we added venetoclax for 24 hours at 100 nM. To correlate venetoclax sensitivity with expression of pro-survival proteins, intracellular protein expression of BCL-2 and BCL-xL was measured using flow cytometry.

Results: 22 patient-derived AL bone marrow samples were included. After 7 days *in vitro*, we observed an increase in surviving PC in Puramatrix hydrogel compared to conventional 2D culture ($p = 0.039$) (Figure 1A). Additionally, there was a significant increase in surviving PC at day 7 in the presence of both IL-6 and APRIL compared to culture without pro-survival cytokines or APRIL or IL-6 alone ($p = 0.004$) (Figure 1B-C). Next, we characterized response to venetoclax. In the AL cell lines, ALMC-2 expressed a higher level of BCL-2 protein and simultaneously had a higher sensitivity for venetoclax than ALMC-1 ($p = <0.001$) (Figure 2A). Protein expression of BCL-xL was similar for the two cell lines. In line with previous reports on MM, we found a difference in BCL-2 and BCL-xL protein expression in relation to t(11;14) status in patient-derived AL PC, albeit with high heterogeneity in the t(11;14) positive group (Figure 2B). However, unlike in MM, neither t(11;14) nor BCL-2 or BCL-xL protein expression did not predict *in vitro* venetoclax sensitivity.

Conclusion: In summary, we established an *in vitro* method to study response to treatment in patient-derived AL PC. We demonstrate a benefit to AL PC survival for both the Puramatrix hydrogel and the combination of pro-survival cytokines IL-6 and APRIL. We have characterized response to venetoclax *in vitro,* and related it tocytogenetic status and protein expression of BCL-2 and BCL-xL. In contrast with MM, sensitivity to venetoclax in AL seems to be mediated by factors other than BCL-2 and BCL-xL expression alone. These results possibly reflect the distinct differentiation status of AL PC from MM PC. Our results support further validation of the *in vitro* use of primary AL PC to work towards personalized medicine for AL amyloidosis patients.

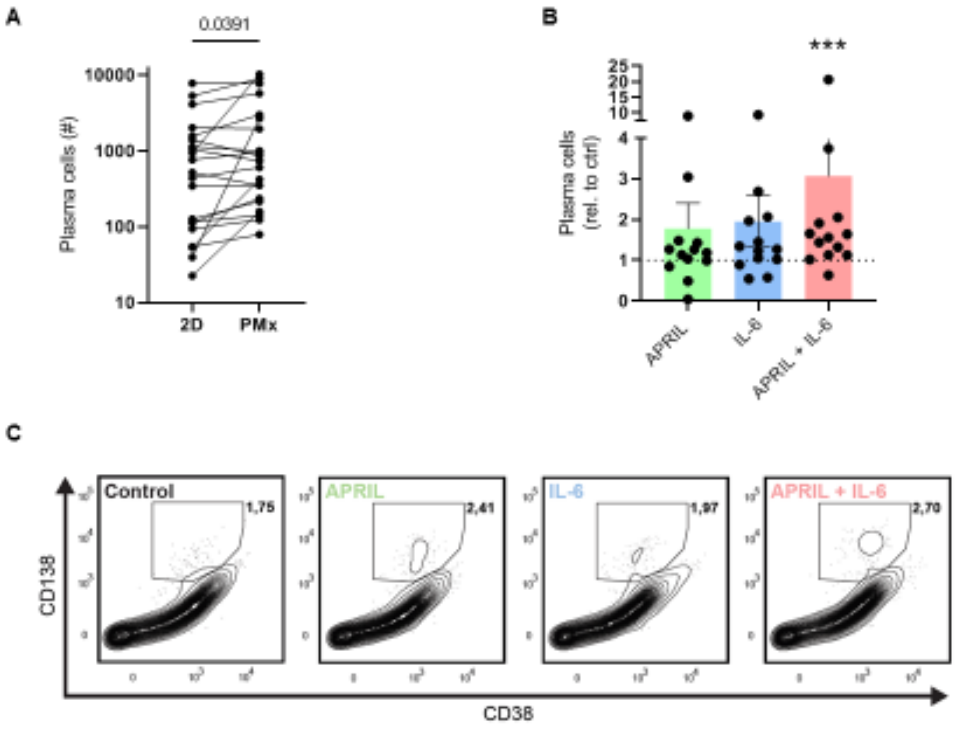

Figure 1. A) Benefit of Puramatrix (Pmx) on number of surviving plasma cells at day 7, compared with 2D culture. **B)** Effect of pro-survival cytokines IL-6, APRIL and the combination on number of surviving plasma cells at day 7, compared to untreated control (ctrl). **C)** Representative flow cytometry plots of influence of pro-survival cytokines on plasma cell population.

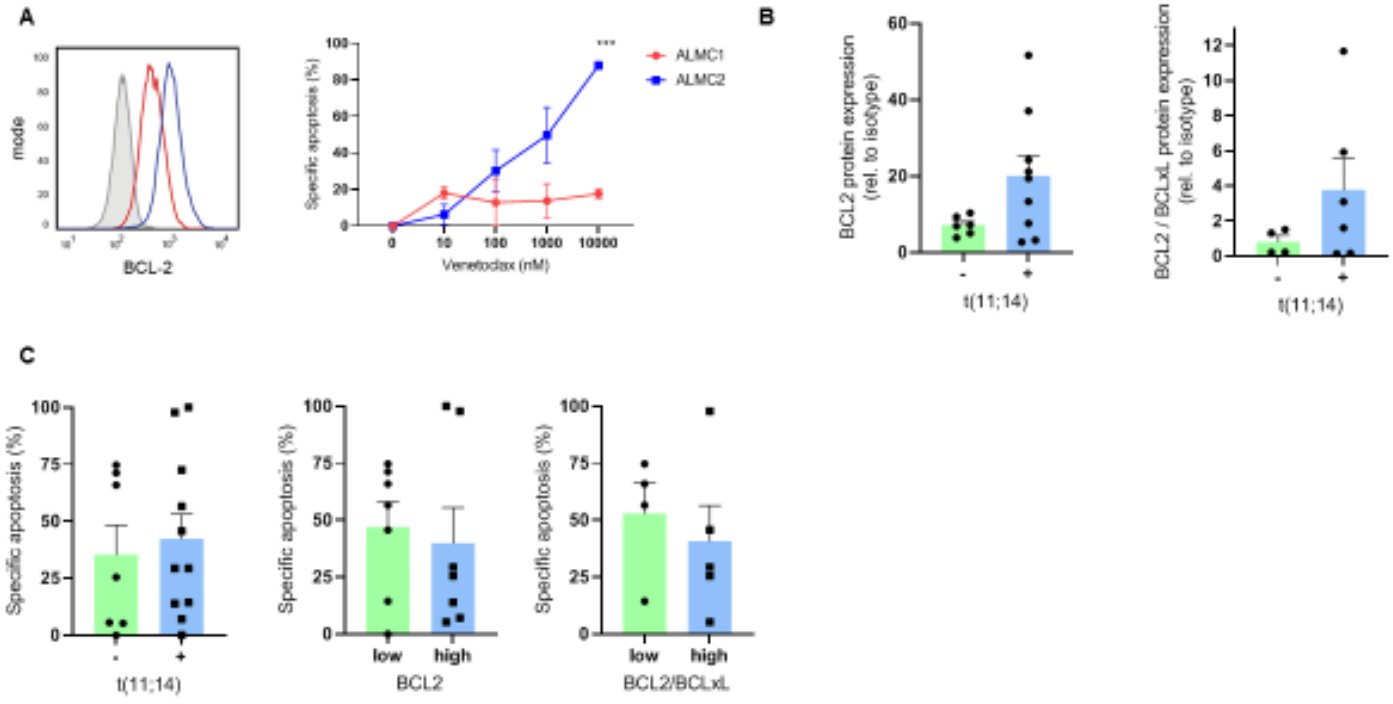

Figure 2. A) Sensitivity to venetoclax of AL cell lines ALMC-1 and ALMC-2 in relation to protein expression of BCL-2. B) Protein expression of BCL-2 and expression ratio of BCL-2/BCL-xL in patient-derived AL PC in relation to t(11;14). C) Sensitivity to venetoclax in patient-derived AL PC in relation to t(11;14), BCL-2 protein expression and ratio of BCL-2/BCL-xL expression.

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Frequent Occurrence of Fibrinogen Amyloidosis in Japanese Squirrels (*Sciurus lis*)

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Background: Fibrinogen amyloidosis is a hereditary systemic amyloidosis in humans that is caused by the deposition of amyloid derived from fibrinogen alpha chain[1]. In animals, there are five case reports of fibrinogen-suspected amyloidosis (two martens and three dogs) [2,3], but no definitive identification has been made and no epidemic outbreak also has been reported.

Objective: In the present study, systemic amyloidosis with severe glomerular amyloid deposition were frequently observed in Japanese squirrels (*Sciurus lis*), and pathological analysis of these cases was performed to identify the pathogenesis.

Material & Methods: Twenty-eight Japanese squirrels that had been kept in five zoos in Japan and died were used in the research. As far as we know, consanguinity between individuals was not confirmed. Formalin-fixed paraffin-embedded (FFPE) tissue sections of the systemic organs were prepared and stained with hematoxylin and eosin and Congo red. Immunohistochemistry was performed for fibrinogen alpha chain, serum amyloid A, immunoglobulin lambda chain, immunoglobulin kappa chain, and transthyretin. To clarify the protein composition in amyloid deposits, glomeruli with amyloid deposits were dissected from FFPE, digested by trypsin and LC-MS/MS was performed. The MS/MS data was analyzed using Mascot server with the protein database of Eastern gray squirrel (*Sciurus carolinensis*).

Results: In 21 of the 28 cases examined, there was mild to severe amyloid deposition in the glomeruli and nodular or polypoidal amyloid deposits in the systemic vascular walls. Electron microscopy revealed clusters of unbranched fine fibers approximately 10 nm in diameter in the glomeruli. Severe amyloid deposition was also observed in the papillary muscles of the heart, aortic valve, peritoneum, and pyloric lamina propria, although these varied from individual to individual. In the kidney, there was amyloid deposition in the glomeruli, but no deposition in the tubular interstitium. Immunohistochemistry showed that amyloid deposits were positive for the fibrinogen alpha chain and negative for serum amyloid A, immunoglobulin lambda/kappa chain, and transthyretin. LC-MS/MS results revealed the fibrinogen alpha chain as a primary candidate protein for glomerular amyloid.

Summary & Conclusion: Based on the results of immunohistochemistry and mass spectrometry, the systemic amyloidosis observed in this study was diagnosed as a fibrinogen amyloidosis. The pattern of renal amyloid deposition in Japanese squirrels, such as severe deposition in glomeruli and absent in the medulla, closely resembled the pattern of human fibrinogen amyloidosis[1]. This suggests the involvement of similar pathogenic factors in the formation of glomerular pathology in Japanese squirrels, as in humans. This study suggests that the Japanese squirrel may frequently develop fibrinogen amyloidosis and be a potential model for the pathological research of human fibrinogen amyloidosis. We are currently conducting genetic analysis of the Japanese squirrels.

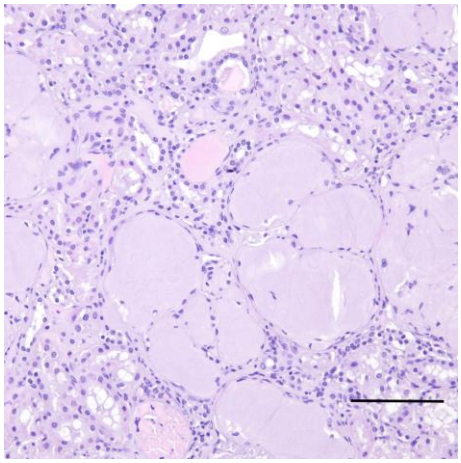

Figure: Glomeruli of a squirrel affected with severe fibrinogen amyloidosis (hematoxylin and eosin). The native structure is replaced by eosinophilic substance (amyloid). Bar = 100 µm

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Exercise suppresses mouse systemic AApoAII amyloidosis through enhancement of the p38 MAPK signaling pathway

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Background: Exercise interventions are beneficial for reducing the risk of age-related diseases. Recent animal studies demonstrate that exercise could modify amyloidosis pathophysiology through direct effects on precursor protein metabolism, as well as indirect effects on physiological adaptive response.

Objective: Exercise-stimulated skeletal muscle release endocrine factors, so-called myokines, in order to crosstalk with other organs for metabolic adaptation. Therefore, we aimed to evaluate the beneficial effect of exercise in systemic organs and to understand the differences in protective response between organs using systemic mouse AApoAII amyloidosis, which is an inducible model by inoculation of tissue-extracted AApoAII fibrils[1].

Material & Methods: We previously reported that R1.P1-*Apoa2*^{c} is an inducible model of AApoAII amyloidosis by intravenous injection of tissue-extracted AApoAII fibrils[1]. Ten-week-old female mice were subjected to the AApoAII induction and speed-controlled treadmill exercise for 16 weeks with the following protocols: (1) High-intensity interval training (IT); The 30 min training sessions involved five sets of 3 min low-intensity running at 30% speed of V_{max} followed by 3 min of high-intensity running at 70% speed of V_{max}. (2) Moderate-intensity continuous training (CT) The 30 min running session at 50% speed of the pre-checked max speed (V_{max}).

Results: As the results of treadmill exercise for 16 weeks, mice showed upregulation of gene expression levels of myokine *IL6*, mitochondrial regulator *Ppargc1a*, glucose transporter *Glut4* and fatty acid oxidation biomarker *Pdk4* in skeletal muscle. We observed decreased levels of hepatic and splenic amyloid burden without changes in circulating serum ApoA-II levels in both CT and IT groups. To investigate the molecular mechanisms of exercise-mediated prevention of amyloidosis, we analyzed the changes in hepatic transcriptome by RNA-seq. Based on KEGG pathway analysis, exercise activated the p38 mitogen-activated protein kinase (p38 MAPK) signaling, resulting in upregulation of tumor suppressor p53 and small heat shock protein beta-1 (HSPB1) in the liver. Furthermore, amyloid deposition additively enhanced the same signaling pathway, and exercise-induced accumulation of phosphorylated HSPB1 to the extracellular AApoAII deposits.

Summary & Conclusion: We previously reported that extracellular amyloid deposits induced tissue-specific adaptive responses including activation of endoplasmic reticulum (ER) stress[2]. Small heat shock proteins have shown to be involved in the proteostasis of both intra- and extracellular environments by chaperoning the misfolded proteins during ER stress[3]. Our findings that the activation of p38 MAPK signaling followed by upregulation of HSPB1 could be a protective adaptation pathway against amyloid deposition. Importantly, exercise also activated the p38 MAPK signaling independent of ER stress-related pathway, as well as induced HSPB1 phosphorylation and accumulation to the extracellular amyloid deposits. These findings suggest that exercise may assist stress-related protective adaptation pathways against amyloidosis.

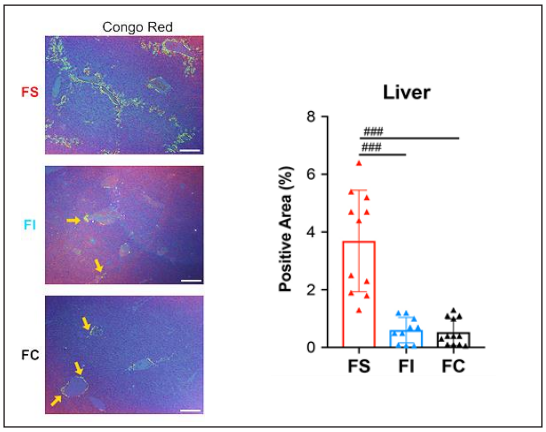

Figure 1

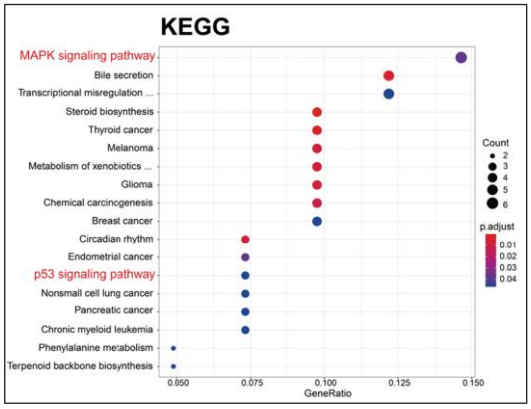

Figure 2

Figure 1.: Representative light microscope images of hepatic AApoAII deposition stained by congo red. Amyloid deposits (indicated by orange arrows) were identified under a polarized light microscope (left panels) and quantified using ImageJ software (Right panels). FS: without exercise, FI: with IT exercise, FC: with CT exercise, Scale bars: 100 μm.

Figure 2.: KEGG pathway analysis of exercise-mediated differentially expressed genes.

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Characterization of heterozygous ATTR Y114C amyloidosis-specific iPS cells

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Background: Hereditary transthyretin (TTR) amyloidosis (ATTR amyloidosis) is autosomal dominant and caused by mutation of *TTR* gene. Of different point mutations, ATTR Y114C (replacement of tyrosine with cysteine at position 114) amyloidosis is a rare and lethal disease, mainly shows rapid progressive dementia that presents with cerebral amyloid hemorrhage (CAA) by its amyloid deposition in cerebral blood vessels, unlike ATTR V30M amyloidosis with mainly peripheral neuropathy. However, since the pathogenesis of ATTR Y114C amyloidosis has yet to be elucidated, no specific treatments for ATTR Y114C amyloidosis is available. Disease-specific induced pluripotent stem (iPS) cells exhibiting potential to differentiate into various disease-responsible cells with maintaining genetic contexts, are very useful tools for elucidating the pathogenesis of rare diseases. We previously established the heterozygous ATTR V30M amyloidosis-specific iPS (V30M iPS) cells, and demonstrated that V30M iPS could be very useful for elucidating the pathogenesis of ATTR V30M amyloidosis.

Objective: We established and characterized heterozygous ATTR Y114C amyloidosis-specific iPS (Y114C iPS) cells.

Material & Methods: Y114C iPS cells were differentiated into disease-responsible hepatocytes-like cells (HLCs) by using 2D feeder free method. Y114C iPS cells were differentiated into definitive endoderm cells with Activin A, and then, differentiated into HLCs from Y114C iPS cells (Y114C HLCs) with Oncostatin M & heptocyte growth factor. Mass spectrometry (MS) analysis was used to evaluate the expression pattern of both wild-type and Y114C TTR in Y114C HLCs.

Results: We successfully differentiated heterozygous Y114C iPS cells into HLCs. The endoderm marker SOX17 expression was observed on day 4, while the pluripotent marker OCT3/4 was markedly decreased. On day 27 after differentiation, the expression of hepatocyte maker albumin was detected, and TTR expression was significantly increased in Y114C HLCs. MS analysis indicated that both wild-type & Y114C TTR protein was indeed expressed in heterozygous Y114C HLCs, suggesting that heterozygous Y114C HLCs expressed the wild-type & Y114C TTR protein at different level. Because it is documented that wlild-type & Y114C TTR protein was existed different levels in the serum of heterozygous ATTR Y114C amyloidosis patients, our heterozygous Y114C iPS cells may be a usueful pathological model.

Summary & Conclusion: We demonstrated that our heterozygous ATTR Y114C amyloidosis-specific iPS cells differentiated into disease-responsible hepatocyte indeed expressed both wild-type & Y114C TTR differently. Future investigasion will focus on the more detailed molecular pathological dynamics of the wild-type & Y114C TTR expression to elucidate the pathogenesis of heterozygous ATTR Y114C amyloidosis.

BiP deletion leads to decreased cell viability and antibody production in multiple myeloma cell lines

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Background: Despite drug development in multiple myeloma and AL amyloidosis, these diseases remain incurable and are accompanied by significant morbidity to both the patient and the health system. This is partially attributed to a minimal understanding of plasma cell biology. The majority of a plasma cell's resources go to the production of antibodies.[1] In order to ensure antibodies are folded properly, the endoplasmic reticulum (ER) has several chaperones that assist in this process.[2] If unfolded protein accumulates, a sensing pathway called the unfolded protein response (UPR) is activated through dissociation of the protein BiP from three transmembrane protein sensors (IRE1, ATF6 and PERK).[3] BiP dissociation leads to activation of the sensors which enact a number of adaptive changes to manage the increased unfolded protein load (Figure 1A).[4] BiP is the central regulator of this process.

Objective: The objective of this study was to understand the role of BiP expression and function in relation to cell survival and antibody production in dysplastic plasma cells that underlie the pathology of AL amyloidosis. Secondary objectives were to determine the effects of BiP deletion on activation of the UPR and the resultant change in the cell's ability to adapt to further stressors.

Materials & Methods: The myeloma cell lines KMS11, RPMI8226 and MM1.S were cultured in RPMI 1640 media (mediatech) with 10% fetal bovine serum. Cell lines were transduced with lentivirus containing FUCas9Cherry (addgene: 70182) selected for mcherry expression by flow sorting and then transduced a second time with lentivirus containing the doxycycline inducible and GFP tagged plasmid FgH1tUTG (addgene: 70183) with a small guide RNA (sgRNA) against the HSPA5 gene. Cells were sorted for double GFP and mCherry positivity. Doxycycline treatment expressed HSPA5 targeted sgRNA and induced deletion. Non-targeted sgRNA were used as controls. Subtilase AB toxin (SubAB), a BiP inhibitor, was purified from plasmids obtained from Dr. Herbert Schmidt. Wild type and BiP deleted myeloma cell lines were treated with subAB, thapsigargin (THG) or bortezomib (BTZ) respectively and assessed for viability and surface light chain by flow cytometry on a BD Symphony A3 lite.

Results: Wild type multiple myeloma cell lines treated with subAB for 72 hours in vitro showed decreased viability compared to saline treated controls (Figure 1B). BiP deleted myeloma cell lines had decreased ability to adapt to ER stressors. Treatment with BTZ, a proteasome inhibitor, and THG, a SERCA Ca^{2+} pump inhibitor, leads to increased ER stress through accumulation of unfolded proteins tagged for degradation and ER calcium depletion, respectively. BiP deleted myeloma cells significantly decreased viability compared to non-targeted sgRNA controls when treated with BTZ (Figure 1C) and THG (Figure 1D) in two of three cell lines. Moreover, BiP deletion led to a decrease in basal surface light chain expression compared to non-targeted controls in 2 of 3 cell lines (Figure 2).

Summary & Conclusion: BiP is an important mediator of the UPR, a prominent survival and adaptive pathway in plasma cells. BiP deletion led to decreased ability of myeloma cells to handle ER stressors, including the clinically used drug bortezomib. It also led to decreased expression of light chains, the main effector of tissue damage in AL amyloidosis. Though preliminary, these results suggest BiP and possibly other UPR elements could be targets for drug development in AL amyloidosis in both cytotoxicity and curtailing secretion of toxic antibodies.

Figure 1

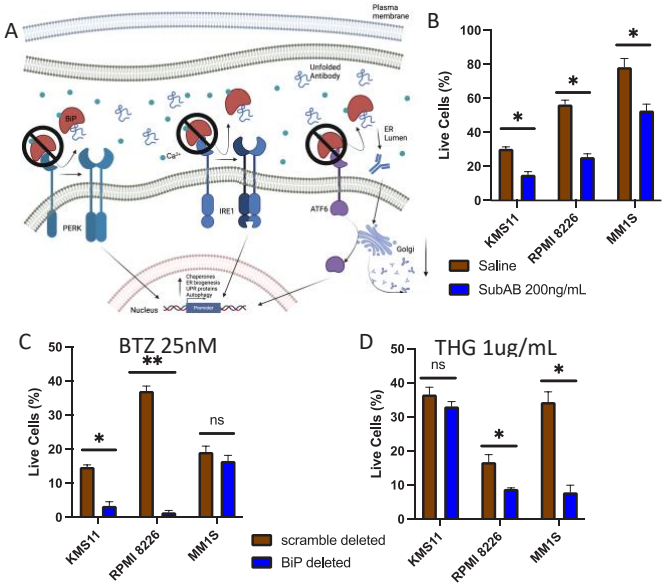

Figure 2

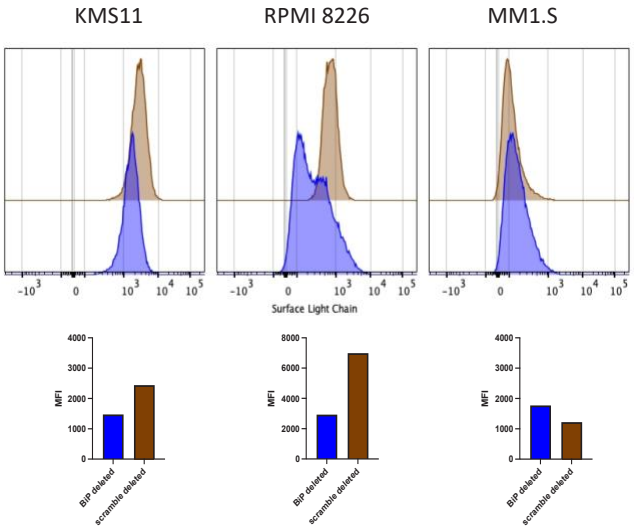

Figure 1.: BiP inhibition and deletion sensitizes myeloma cell lines to death during ER stress
A) KMS11, RPMI8226 and MM1.S myeloma cells lines treated with BiP targeted toxin SubAB for 72 hours in vitro. B) BiP deletion should lead to maximal activation of the UPR and its absence should decrease the protein folding capacity of the cell and thereby decrease antibody production and sensitize to cell death. C) Proteasome inhibition with bortezomib (BTZ) 25nM for 72 hours in vitro compared to controls with non-targeting sgRNAs (scramble). D) ER stress induction through calcium depletion with thapsigargin (THG) at 1ug/mL for 72 hours in vitro compared to controls with non-targeting sgRNAs. *p<0.05, **p<0.005

Figure 2.: BiP deletion leads to decreased light chain expression
Intensity of surface stained light chain on BiP deleted KMS11 (kappa light chain), RPMI 8226 (lambda light chain) and MM1.S (lambda light chain) (Blue) vs. non-coding sgRNA containing controls (brown). Mean fluorescence Intensity (MFI) for each cell line group is noted below the histograms with the same color layout at intensity.

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Age-related amyloid deposition in C57BL/6 mice: Pathological findings and characterization of the renal damage

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Background: The C57BL/6 mouse strain is the most widely used for studying aging mechanisms and age-related diseases. However, little attention has been paid to age-related amyloid deposition, which can damage some organs in mice. It was recently reported that spontaneous amyloidosis commonly occurs in the aged C57BL/6 strain[1], but the pathophysiological and biochemical characterizations of amyloidosis in C57BL/6 mice have not been clarified. We have identified the amyloid protein associated with mouse senile systemic amyloidosis found in the senescence-accelerated mouse strain (SAMP1) as Apolipoprotein A-II (ApoA-II), which is the second most abundant protein constituent of high-density lipoprotein[2]. We have revealed that AApoAII amyloidosis occurs with aging in C57BL/6 and SAMP1 mouse strains.

Objective: Although biomedical researchers have been using C57BL/6 mice as a standard model for basic aging research, the onset of spontaneous amyloidosis and the effects of amyloid deposition on various organ functions in aged mice may cause inaccurate analysis of experimental data. The present study investigated the age-related prevalence of amyloidosis in C57BL/6 mice, the type of amyloid protein, and the effects of amyloid deposition on organ functions.

Material & Methods: The C57BL/6 mice were purchased from the Jackson Laboratory Japan, Inc. At 12-86 weeks old, we examined the degree of amyloid deposition in multiple organs and identified the amyloid protein by immunohistochemistry, immunoblotting, and mass spectrometry. In addition, we induced amyloidosis by injecting amyloid fibrils extracted from aged mice into the tail vein of young mice and evaluated amyloid-induced impairment of organ function at 40 weeks old to distinguish between the effects of aging and amyloid deposition.

Results: 1. Amyloid deposits were first observed in the small intestine and tongue between 36-40 weeks old and gradually expanded to multiple organs, except for brain tissue, with increasing age. Mice over 70 weeks old exhibited moderate-to-severe amyloid deposition in multiple organs, including the heart, kidney, intestine, tongue, stomach, lungs, and skin. 2. The results obtained from immunohistochemistry, immunoblotting, and mass spectrometry suggest that the amyloid protein of the spontaneous amyloidosis occurring in aged C57BL/6 mice was ApoA-II and excluded the possibility of other protein deposits such as Serum amyloid A (SAA), Apolipoprotein A-I (ApoA-I), Transthyretin (TTR), and Apolipoprotein A-IV (ApoA-IV). 3. Amyloid deposition caused impairment of renal function in mice exhibiting significant proteinuria, and electron microscopical and Immunofluorescent analysis results suggest that amyloid deposition damaged the intra-glomerular mesangial cells and podocytes.

Summary & Conclusion: We found AApoAII mouse senile amyloidosis in SAMP1 mice with type C amyloidogenic ApoA-II. C57BL/6 mice have type A ApoA-II, and we detected AApoAII amyloidosis in the tissues of all C57BL/6 mice aged over 40 weeks. Our results strongly suggest that deposition of ApoA-II as amyloid fibrils is the leading cause of spontaneous amyloidosis in C57BL/6 mice. This research suggests that aged C57BL/6 mice have a high prevalence of renal dysfunction associated with severe amyloid deposition in the glomerulus of amyloidosis-induced mice (40 weeks old) comparable to renal disfunction in old mice (80 weeks old). We will investigate the deleterious effects of amyloid deposition in other organs in subsequent work,

Figure1

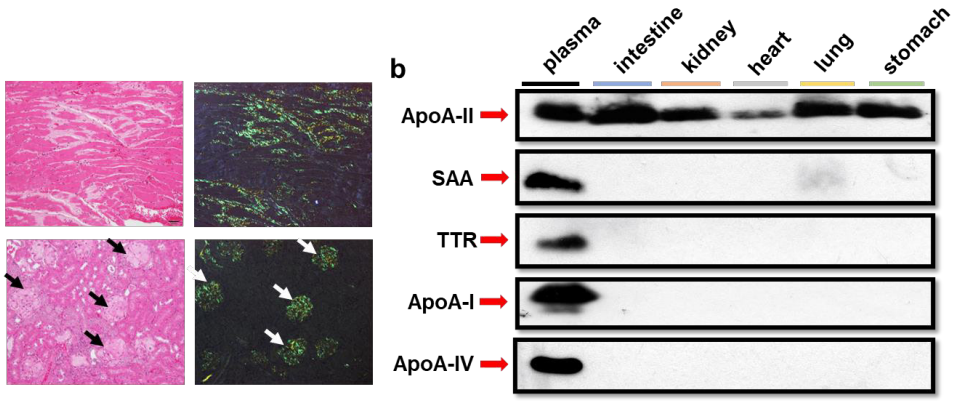

Figure2

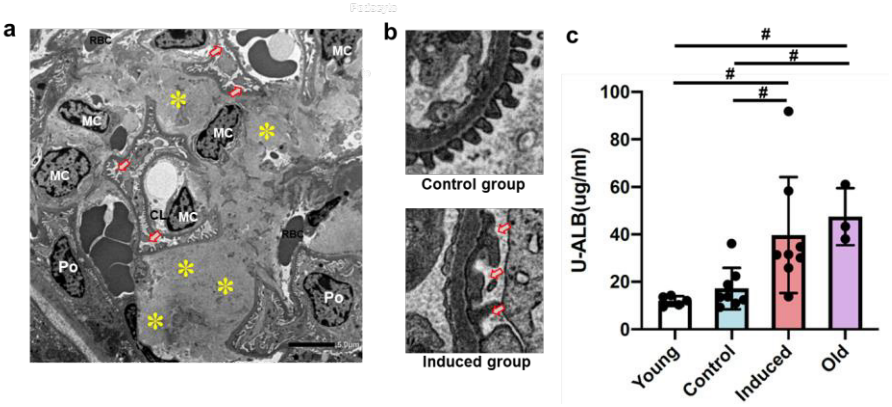

Figure 1. Spontaneous AApoAII amyloidosis in aged C57BL/6 mice. a. Representative amyloid deposition in the heart (top) and kidney (bottom) of 80-week-old C57BL/6 mice. Arrow, glomerulus. **b.** ApoA-II was detected in Amyloid fibrils extracted from various organs. Positive control, plasma.

Figure 2. Amyloid deposition damages the glomerular structure and causes albuminuria. a. Representative TEM image of 40-week-old mice with induced amyloidosis. Arrow, foot process; asterisk, amyloid fibrils; MC, mesangial cell; Po, podocyte. b. Glomerular foot process in the control group and amyloidosis-induced group. c. Albumin level in the urine. Young, 12 weeks old; Control, 40 weeks old with no amyloidosis; Induced, 40 weeks old with amyloidosis; Old, 80 weeks old with spontaneous amyloidosis. #, $P<0.05$.

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HEMOSTASIS DYSFUNCTION INDUCES SENILE APOA2 AMYLOIDOSIS IN A MOUSE MODEL

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Background: Senile amyloidosis has been observed in breeding mice, and described as an APOA2 amyloidosis. This amyloidosis type is characterized by the formation of amyloid fibrils from a misfolding of the plasma protein Apolipoprotein 2 and is associated with the type C APOA2 (APOA2c). Our team observed severe senile amyloidosis in a transgenic mouse with a knockout of Serpine-1, the gene encoding for Plasminogen Activator Inhibitor-1 (PAI-1). Mice developped amyloidosis, starting at 12-18 months of age, affecting kidney, liver, spleen and to a lower extent, heart. PAI-1 has an important role in hemostasis through the regulation of plasmin activity. The objective of this study is to characterize this new senile amyloidosis model, to try to understand the relationship between hemostasis dysfunction and amyloid deposits and to evaluate the value of this model for therapeutic investigations.

Materials & Methods: 18-month-old mice with severe amyloidosis were analysed. Amyloid deposits were identified by Congo red staining , electron microscopy and laser microdissection coupled with mass spectrometry (LMD-MS). Amyloid fibrils were isolated from mice spleen using standard method (1). Atomic Force Microscopy (AFM) was perfomed on the purified fibrils to confirm the presence of amyloid fibrils and mass spectrometry was carried out to identify the major proteins in fibrils. Western blot on purifed fibrils, immunohistochemistry and immunofluorescence on frozen sections were performed using specific antiserum against mouse APOA2. Serum APOA2 levels were quantified using immunoenzymatic assay. The APOA2 variant was determined by Sanger sequencing. Renal amyloidosis in vivo was monitored with urine collections. We evaluated the accelerated development of amyloidosis by seeding PAI-$1^{-/-}$ mice with amyloid fibrils from previous PAI-$1^{-/-}$ mice with severe amyloidosis.

Results: LMD-MS indicates that APOA2 is the major component of deposits. We confirmed the colocalization of APOA2 with congo-red positive amyloid deposits in spleen, heart, liver and kidney. Electron microscopy on organs and AFMidentify typical amyloid fibrils.Western Blots performed on two differents water-suspension fraction of fibrils purification showed monomeric form of APOA2. Serum APOA2 concentrations is normal in young mice but seem to decrease between 12-18 months, the age of amyloidosis developement. MS analysis of purified fibrils showed the presence of APOA2 together with other amyloid protein signature such as APOE and vitronectin. Sequencing analysis revealed that PAI-$1^{-/-}$ mice express the non-amyloid APOA2a allele. Seeding with ApoA2 fibrils accelerated amyloid formation. Finally, albuminuria appeared to be correlated with the extent of amyloid deposits in the kidneys and can be to use to follow pathology development.

Summary & Conclusion: We herein described a new mouse model of systemic amyloidosis directly link to the APOA2a protein variant which was never described as naturally amyloidogenic (2) This model of APOA2 amyloidosis mimics multiple organ damages, is easily inducible and can be monitored for kidney function. Hence, it could represent a exquisite amyloidosis model for pre-clinical trials. Further studies are needed to understand the link between the absence of PAI-1 and APOA2 amyloidosis in this model.

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Endocytic inhibitory drugs protect *C. elegans* from the toxicity of amyloidogenic light chains

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Background: The knowledge of the mechanisms underlying the cardiac damage in immunoglobulin light chain amyloidosis (AL) is essential for developing novel therapies and improving patients' outcomes. We used the nematode *Caenorhabditis elegans*, whose pharynx is evolutionarily related to the vertebrate heart (1). Its muscle cells have autonomous contractile activity, reminiscent of cardiac myocytes, and the electrical coupling between muscle cells, calcium-based action potentials, and high mitochondrial density resemble those present in the mammalian heart. Only light chains (LCs) isolated from patients suffering from amyloid cardiomyopathy caused in *C. elegans* a persistent pharyngeal dysfunction accompanied by the increase of radical oxygen species, ultrastructural alteration in the pharynx, and mitochondrial damage similar to that observed in human hearts of cardiac AL patients (2,3). Whether cellular internalization of LCs, relevant for cellular dysfunction and amyloid deposition in cardiac fibroblasts (4), may contribute to the pharyngeal damage remains to be investigated.

Objective: Our goal was to investigate whether the internalization of soluble cardiotoxic LCs by pharyngeal cells contributes to their proteotoxicity *in vivo*.

Material & Methods: Bristol N2 worms were treated with 100 µg/ml of recombinant cardiotoxic LC or 1 mM hydrogen peroxide in 10 mM PBS, pH 7.4. Control worms were incubated with non-amyloidogenic LC or 10 mM PBS, pH 7.4. The pharyngeal pumping rate, measured by counting the number of times the terminal bulb of the pharynx contracted over a 1-min interval, was scored 20 h later (2,3). Worms were also pretreated for 30 min with the inhibitors of endocytosis (10 µM cytochalasin B, 100 µM genistein, or 6 µM chlorpromazine) before the exposure to LC or hydrogen peroxide.

Results: The effect of three drugs able to inhibit different endocytic pathways was investigated. Genistein, which interferes with caveolae-mediated endocytosis, and chlorpromazine able to inhibit the clathrin-dependent endocytosis, were employed. Based on the knowledge that worm's muscle cells have phagocytic activity modulated by actin rearrangement that can be inhibited by cytochalasin B (5), this inhibitor was also used. All these compounds significantly counteracted the pharyngeal dysfunction caused by the administration of the cardiotoxic LC (Fig. 1). Since genistein and chlorpromazine have exhibited strong antioxidant activity in various experimental models (6,7), we evaluated whether their protective effect was related to their anti-oxygen radical generation activity. As shown in Figure 2, these two compounds but not cytochalasin B counteracted the toxic effect of hydrogen peroxide.

Summary & Conclusion: These results indicate that endocytic drugs can protect from the toxicity caused by cardiotoxic LC *in vivo*, in *C. elegans*. This effect may be ascribed to a reduced pharyngeal cell endocytic internalization of LC by cytochalasin B and, for genistein and chlorpromazine, to their ability to act also as antioxidant drugs.

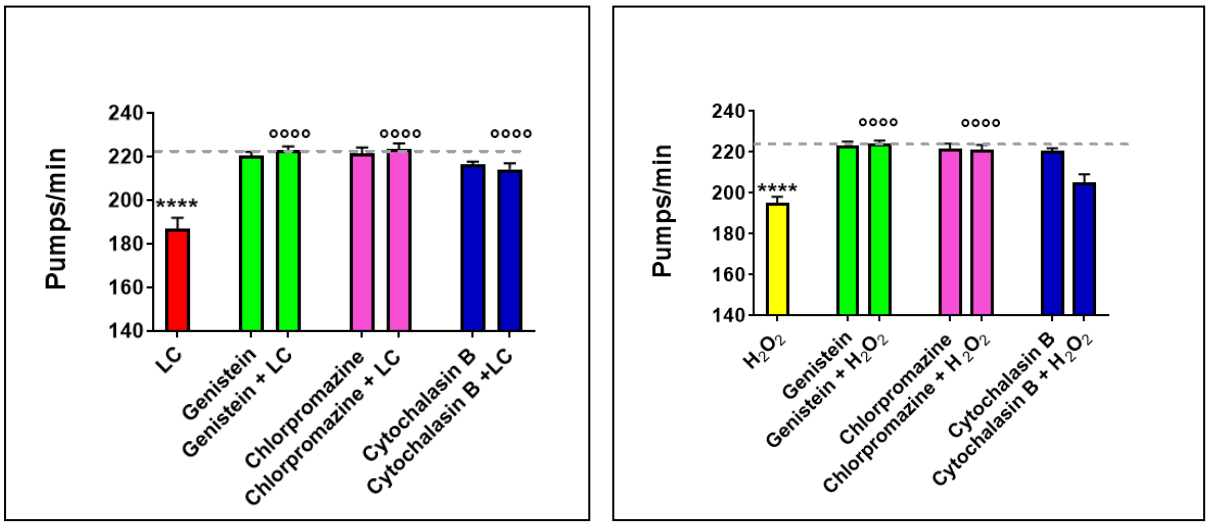

Figure 1.: Effect of endocytic inhibitory drugs on the pharyngeal dysfunction caused by cardiotoxic LC. ****p<0.0001 *vs* Vehicle (dotted line) and °°°°p<0.0001 *vs* LC, one-way ANOVA and Bonferroni's *post hoc* test.

Figure 2.: Effect of endocytic inhibitory drugs on the pharyngeal dysfunction caused by hydrogen peroxide. ****p<0.0001 *vs* Vehicle (dotted line) and °°°°p<0.0001 *vs* H_2O_2, one-way ANOVA and Bonferroni's *post hoc* test.

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Is *C. elegans* a good model of β_2-microglobulin related amyloidosis?

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Background: β_2-microglobulin (β_2m), is one of the most extensively studied amyloidogenic protein[1]. The wild-type (WT) protein is responsible for dialysis related amyloidosis (DRA) which occurs in long-term dialysed patients as a result of the persistently high plasma concentration of circulating β2m in a time frame of 15/20 years. On the other hand, the natural pathogenic variant of β_2m (D76N) causes amyloid deposits despite a normal plasma concentration and in a time frame of 50 years or more, according to the natural history of the disease (1).
To date, no mice models reproducing the clinical manifestations of β_2m-related diseases have been successfully obtained. Given the crucial importance of animal models to understand the disease mechanisms, we have extensively investigated the possibility to reproduce crucial aspects of the disease *in vivo* using *Caenorhabditis elegans* (*C. elegans*) (2).

Objective: To establish transgenic *C. elegans* strains as suitable models of DRA and hereditary D76N β_2m related amyloidosis and characterize their pathological phenotypes, proteomic and metabolic alteration associated to β_2m expression.

Material & Methods: The nematodes expressing the β_2m isoforms were generated as previosuly described by Faravelli and coauthors (3). Characterization of the pathological phenotypes was carried out by using the INVertebrate Automated Phenotyping Platform (INVAPP) and the Paragon algorithm (3). The metabolomic profiles were defined by an NMR-based untargeted approach. Metabolite assignment was based on reference data from the literature and from HMDB, and MMCD web databases.
Protein extracts from different worm cultures were processed and analyzed by untargeted label-free quantitative proteomics. The samples were digested and analyzed by LC-MS/MS with a data-dependent acquisition (DDA) method using an Orbitrap mass spectrometer. The LC-MS/MS data were analyzed using appropriate softwares (MaxQuant and Perseus) for protein identification and quantification.

Results: Two transgenic nematode strains were established and compared. We generated and characterized one line expressing WT β_2m at high concentration to mimic what happens in subjects under dialysis, and another line expressing D76N β_2m at low levels, as occurring in human carriers of the pathogenic mutation.
Using an automated system for worms' phenotyping we established that β_2m concentration dictates the pathologic features of these strains. Whereas at low concentration the WT is less toxic than D76N β_2m variant (2), the situation is reversed at high concentration of WT versus low concentration of the D76N β_2m variant.
Preliminary data obtained by NMR indicate that the expression of different β_2m isoforms in nematodes is strongly associated with metabolomic alterations. Moreover, a comprehensive proteomic analysis carried out on our models suggests that the proteome profile is deeply affected by the presence of β_2m.

Summary & Conclusion: Our data highlight the fundamental role played by the concentration of β_2m in determining its toxicity *in vivo*. Furthermore, these data fit with the features of the disease in patients, in which high concentration of WT β_2m causes the amyloid deposition earlier than the amyloidogenic variant at physiological (low) concentration.

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TITLE: Efficient transient expression of exogenous immunoglobulin light chain (AL) full length proteins from cultured human cells

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Background: Studies targeting the characterization of AL full length (FL) proteins require abundant quantities of purified protein. Prokaryotic expression, typically from *Escherichia coli*, has long been the gold standard for *in vitro* production of AL proteins due to the speed, scalability and relative low cost of such systems. However, unstable proteins like amyloidogenic light chains tend to form inclusion bodies in bacterial expression systems, complicating their extraction and purification. Structural and cell-based studies can be better served by transient expression of proteins under physiological conditions in mammalian cell systems, where native post-translational modifications, including glycosylation, result in appropriately folded functional proteins.

Objective: To express functional AL FL proteins utilizing an adapted 293F-derived human cell line, cultured under physiological conditions, and harvested as clarified cell culture supernatant, in an efficient and cost-effective manner.

Materials & Methods: Gene fragments for κIO18/O8 FL (κI germline protein) and AL-09 FL (patient-derived amyloidogenic variant) were PCR amplified from existing pET12a recombinant plasmid constructs utilizing gene-specific custom primers designed to incorporated N-terminal *AgeI* and C-terminal *KnpI* restriction digest sites into the sequences of interest to facilitate subcloning into specific mammalian expression vectors. The resulting PCR products were ligated into pHL-FcHis and pHL-6XHis vectors and screened by Sanger sequencing. The recombinant vectors were transfected into the Expi293F™ human cell line with linear polyethylenimine (PEI) transfection reagent and incubated in serum-free Expi293™ Expression Medium suspension culture at physiological conditions (37°C, 8% CO_2 humidified, 125 rpm) for 6 days. The culture media were cleared of cells and debris by successive application of low and high-speed centrifugation, followed by 0.2 μm filtration. Quantitative analyses of protein expression were done using SDS-PAGE, Coomassie stain and western immunoblotting utilizing primary antibodies to human kappa free light chain.

Results: κIO18/O8 FL and AL-09 FL light chain proteins expressed by mammalian cell cultures are produced in sufficient quantities to facilitate subsequent biophysical analyses, though yields can ultimately be variant-dependent. Milligram quantities of κIO18/O8 FL germline were regularly produced from relatively small cell culture volumes (150 mL). Alternatively, AL-09 FL yields were markedly lower (1mg/150 mL media) than germline protein (6.4 mg/150 mL media). However, this is the same behavior observed in bacteria. Expressed AL-09 FL is still detectable via western immunoblot analysis, and sufficient protein is achievable by increasing culture volume. The pHL expression vectors produce soluble His-tagged proteins, which allow for simple purification over HisPur™ cobalt resin, eliminating chemical processing and laborious size exclusion chromatography.

Summary & Conclusion: Transient expression of AL light chains from mammalian cell culture shows to be a reliable and consistent mode of *in vitro* protein production. The use of appropriate expression vectors and the Expi293F™ cell line simplify post-expression harvest and purification processes. Subcloning is further expedited by use of cost-effective custom gene fragments, eliminating need for amplification of AL FL plasmid constructs via PCR. Ultimately, this expression system will prove to be a powerful tool in AL amyloidosis research.

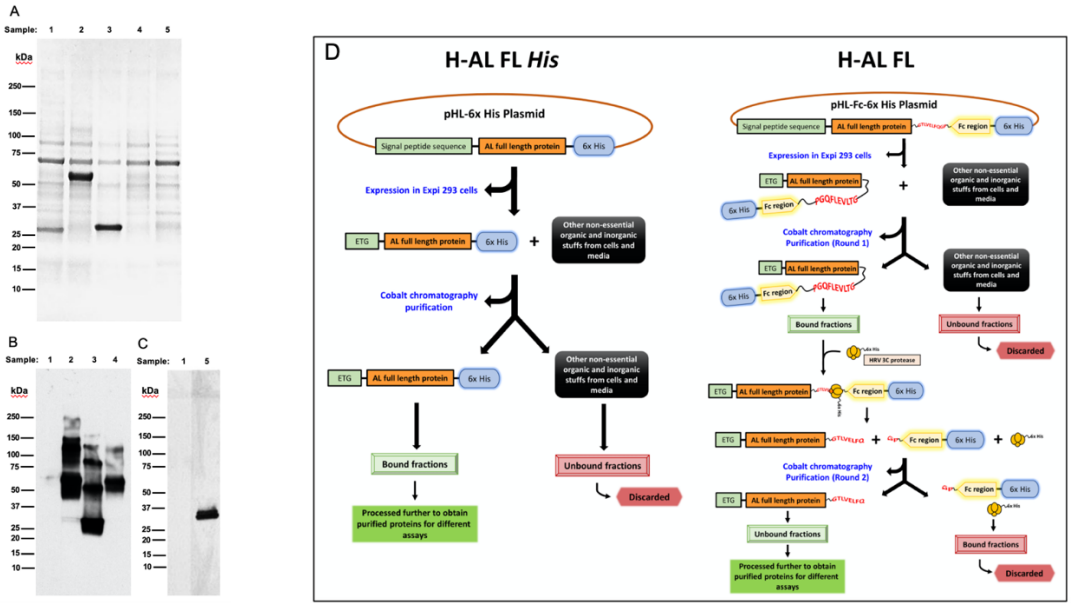

Figure 1. SDS-PAGE, western immunoblot analysis and purification schematic of κIO18/O8 FL and AL-09 FL transiently expressed over 6 days in Expi293F™ human cell line culture. (A) Coomassie Brilliant Blue staining results. Sample 1, sfGFP control. Sample 2, κIO18/O8FL.pHL-FcHis. Sample 3, κIO18/O8FL.pHL-6XHis. Sample 4, AL-09FL.pHL-FcHis. Sample 5, AL-09FL.pHL-6XHis. (B,C) Western blot results using anti-human κ free light chain primary antibody, and rabbit-anti-sheep IgG-HRP secondary antibody, with the same corresponding sample identifiers. (D) Purification schematic for proteins expressed from pHL-FcHis and pHL-6XHis vectors.

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Strategies to induce amyloid light chain deposition in a transgenic mouse model

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Background: Immunoglobulin light chain amyloidosis (AL) is the most frequent type of systemic amyloidosis, affecting mainly the heart and kidney. This disease is due to free monoclonal light chains (LC) produced in excess by a plasma cell clone. However, the mechanism of fibril formation in this disease is still incompletely understood, as well as the amyloidogenic precursor form(s). Recent *ex vivo* and *in vitro* studies highlighted a major involvement of the variable domain (VL) of the light chain in fibrils composition and formation **(1, 2)**. But the precise role of the VL *in vivo* it is still under debate **(3)**. Despite years of efforts to generate an animal model that could allow to better understand this disease, no model has yet succeeded in reliably reproducing the pathophysiology of this disease.

Objective: In the present study, we aimed at reproducing AL amyloidosis in a transgenic mouse model. Such model could help studying the pathophysiology of these diseases at their earlier stages. It could also be a valuable support for assessing the involvement of each one of the species related to AL amyloidosis, namely the full length LC, its VL or the mature fibrils, and their direct toxicity in organs.

Material & Methods: We developed an original transgenic approach using an insertion of a human pathogenic LC gene in the endogenous mouse kappa locus, such as the LC is produced by the naturally Ig producing B and plasma cells. Then, to avoid the association of human LCs with endogenous murine HCs, we backcrossed this strain with the DH-LMP2A mice, characterized by a high number of plasma cells devoid of endogenous HC. This strategy leads to a production of the human free LC similar or higher than in patients and proved efficient to reproduce in mice several monoclonal gammopathies of clinical significance (MGCS)**(4)**.

Results: Despite strong LC production, mice did not naturally develop AL amyloidosis nor seemed to exhibit any organ toxicity due to the circulating amyloidogenic free LC. In vitro, the full length LC was resistant to amyloid formation at physiological conditions but the variable domain (IGLV6) showed high propensity to form fibrils. A single injection of amyloid fibrils and/or seeds, obtained from the variable domain (VL) of the human LC gene, led to amyloid deposits starting at 48 hours post-injection, especially in the heart, spleen, liver and, to a lesser extent, in the kidney. Interestingly, the injection of the soluble form of this VL was also able to induce AL deposits in the same organs, starting at 4 months, demonstrating the high amyloidogenicity of the VL for the first time *in vivo*. We confirmed that the deposits contain the full-length human LCs, which elongate VL fibrils *in vivo*.

Summary & Conclusion: This is, to our knowledge, the first transgenic mouse model of AL amyloidosis closely reproducing human lesions, especially in heart. Further studies are needed to better understand the early biochemical events leading to AL amyloidosis *in vivo*, but this model already shows that a partial degradation of the LC is likely required to initiate amyloid fibrils and that once seeded, the full length LC can elongate these fibrils. This mouse model opens new perspectives to better understand the toxicity of amyloid LC, their involvements in different biological processes and organ dysfunction and of course, to test new therapeutic approaches.

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Prognostic Implication of Longitudinal Changes of Left Ventricular Global Strain after Chemotherapy in Cardiac Light Chain Amyloidosis

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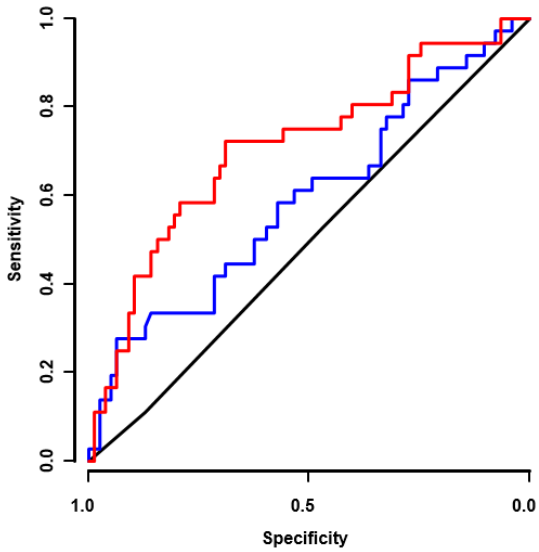
Background: Cardiac involvement is the main prognostic determinant in AL amyloidosis.
Objective: We sought to determine the prognostic significance of longitudinal change of left ventricular (LV) global longitudinal strain (GLS) in cardiac light chain (AL) amyloidosis patients undergoing chemotherapy.
Material & Methods: We retrospectively investigated 117 cardiac AL amyloidosis who underwent chemotherapy from 2005 to 2019. All patients underwent comprehensive 2D conventional transthoracic echocardiography at baseline and after completion of first line chemotherapy (median time interval: 7.0 months). Speckle tracking analysis of images were performed offline. Absolute value of LV GLS was expressed as [LV GLS] and change of [LV GLS] after chemotherapy was expressed as Δ [LV GLS] (Post-chemotherapy [LV GLS] – Pre-chemotherapy [LV GLS]). Cardiac response was defined as a reduction of NT-proBNP >30% of baseline level at 12 months after initiation of the first line of chemotherapy. Clinical outcomes including hematologic responses, cardiac response and all-cause mortality were analyzed.

Results: The median follow up was 25.0 months (10.0-47.0 months). Complete hematologic responses (CR) were achieved in 34 (29.1%) patients. Baseline clinical and echocardiographic parameters were similar in patients with and without CR. Δ [LV GLS] significantly differed between CR and non-CR group (Δ [LV GLS] 0.4 ± 2.8% in CR group vs. -0.6 ± 2.5% in non-CR group, p value 0.046). Δ [LV GLS] showed satisfactory predictive performance for all-cause mortality (cut-off value=0.8, AUC 0.643, 95% CI [0.537 - 0.748], p-value=xx). Adding Δ [LV GLS] to 2012 Mayo stage + pre-chemotherapy [LV GLS] showed incremental prognostic value (c-index: 0.637 vs. 0.708; Relative Integrated Discrimination Index 0.07, p value= 0.003; Net Reclassification Improvement 0.54, p value <0.001). Δ [LV GLS] showed significant correlation with cardiac response (AUC 0.820, 95% CI [0.737-0.904]).
Summary & Conclusion: In cardiac amyloidosis patients who underwent chemotherapy, longitudinal change of [LV GLS] after chemotherapy showed significant association with overall survival as well as cardiac response.

Addictive prognostic value of Δ [LV GLS] and Pre [LV GLS] in 2012 Mayo stage



	Models	C-index	95% CI	P value	Relative IDI	P value	NRI	P value
Model 1	Mayo Stage (I+II, III, IV)	0.554	0.471-0.636					
Model 2	Mayo Stage + Pre [LV GLS]	0.637	0.545-0.728	0.123	0.02	0.201	0.06	0.754
Model 3	Mayo Stage + Pre [LV GLS] + Δ [LV GLS]	0.708	0.625-0.790	0.059	0.07	0.003	0.54	<0.001

Figure 1.: Addictive prognostic value of Δ [LV GLS] and Pre [LV GLS] in 2012 Mayo stage

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Amyloid Valvular Heart Disease: A Look Beyond the Ventricular Walls

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Background: Cardiac amyloidosis (CA) refers to deposition of fibrillar proteinaceous material within myocardial interstitium (1, 2). Traditionally, transthoracic echocardiography (TTE) reveals ventricular wall thickening, systolic and diastolic dysfunction, and abnormal global longitudinal strain (GLS). Valvular involvement of amyloidosis has been identified in increasing frequency recently (3-5). Rarely, CA might present with atypical features of normal/borderline increased wall thickness with predominantly thickened valves, which could make diagnosis challenging.

Objective: Review atypical imaging of “valvular” CA, recognize associated valvular regurgitation/stenosis, and understand the high index of suspicion needed for accurate diagnosis

Methods: We retrospectively identified 3 cases of histologically proven CA of differing amyloid types with predominantly valvular abnormalities on TTE imaging from our institutional cardiac amyloid database using an electronic medical record.

Results: Case 1 is a 70-year-old man who presented to our institution for evaluation of non-ischemic cardiomyopathy and severe mitral valve regurgitation (MR). TTE showed normal ventricular wall thickness with left ventricular (LV) enlargement, severely thickened mitral valve, and ejection fraction (EF) of 34% (Figure 1A). Although the suspicion of CA was low based on these images, given a family history of amyloidosis, further workup revealed a positive 99m technetium pyrophosphate bone scintigraphy (99m Tc-PYP) scan. An endomyocardial biopsy confirmed wildtype transthyretin amyloidosis (ATTRwt-CA). Given his significant heart failure symptoms secondary to severe MR, he underwent mitral valve replacement with histopathologic examination of the mitral valve disclosing dense deposition of amyloid (Figure 1B). Case 2 is a 61-year-old man with a history of bilateral carpal tunnel release and macroglossia who underwent a TTE as part of a preoperative evaluation. It revealed normal LVEF, borderline increased wall thickness, significantly thickened mitral and tricuspid valve leaflets with moderate regurgitation, and abnormal GLS of -11% (Figure 2A). Although the relatively normal wall thickness was thought to be unusual for CA, further testing revealed monoclonal gammopathy. Subsequent fat pad and bone marrow biopsy revealed amyloidosis that was typed via tandem mass spectometry as light chain (AL) amyloidosis. He was subsequently started on daratumumab plus cyclophosphamide, bortezomib, and dexamethasone (DARA-CyBorD) and underwent autologous stem cell transplantation. Finally, case 3 is a 60-year-old woman who presented with jaundice and elevated liver enzymes and was found to have extensive nodular hepatic lesions on imaging. TTE showed mild ventricular wall thickening with LVEF 54%, severe “globular” thickening of the tricuspid valve, and associated tricuspid stenosis (Figure 2B). A liver biopsy confirmed APOA1-type amyloidosis. Her TTE findings were thought to represent endocardial valvular CA. She ultimately underwent combined liver-kidney transplant.

Summary and Conclusions: These three cases highlight a phenomenon of atypical TTE findings of three separate subtypes of CA with predominant valvular abnormalities. The relatively normal ventricular wall thickness is explained by predominant endocardial amyloid deposition causing valve thickening, sometimes with minimal or mild interstitial myocardial involvement (6). Recognition of the variability of pathologic distribution of amyloid is important to understand the spectrum of cardiac imaging phenotypes.

Figures:

Figure 1: A. Apical 4 chamber view demonstrating LV enlargement and thickened mitral valve leaflets. B. Scattered Congo red deposits were identified throughout the mitral valve with green birefringence evident when viewed between cross polarized light, confirming amyloid.

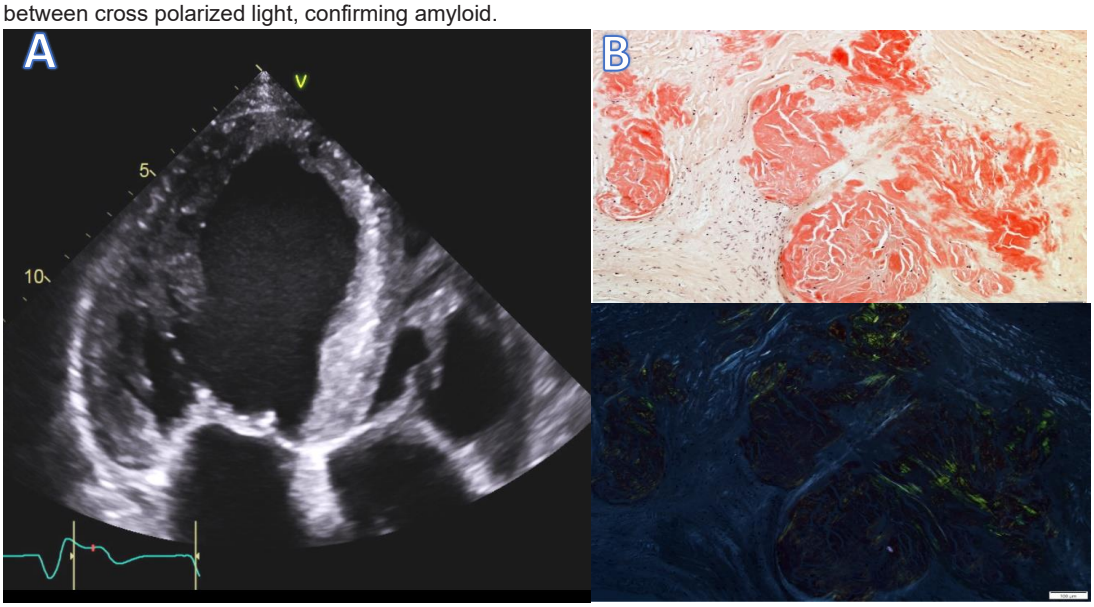
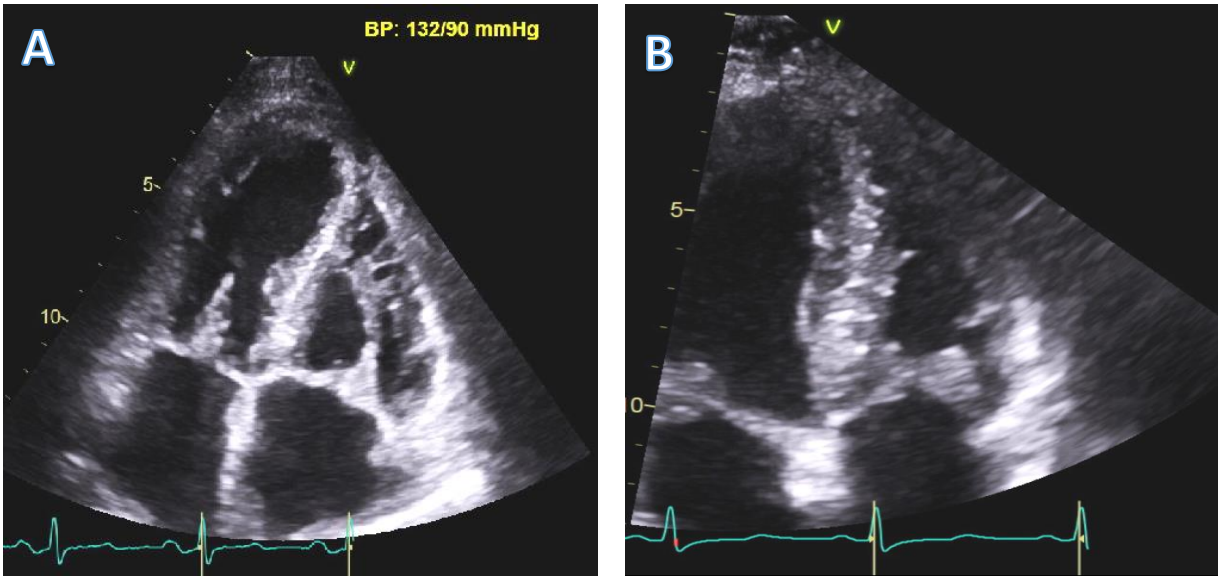


Figure 2. A. Apical 4 chamber view showing borderline enlarged ventricular wall thickness and significantly thickened mitral and tricuspid valves. B. RV dedicated view showing severe and "globular" thickening of the tricuspid valve, concerning for endocardial amyloid.



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Scintigraphy Scan with planar and SPECT imaging of the chest using Technetium 99m Pyrophosphate for the assessment of AL Amyloidosis: Comparison with an ATTR cohort of patients

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Background: AL amyloidosis (AL) is a low tumor burden plasma cell disorder characterized by deposition of insoluble fibrils composed of immunoglobulin light chains.[1] Nuclear Scintigraphy is a non-invasive tool with diagnostic potential in the assessment of cardiac amyloidosis (CA). Various tracers have been used in the past for scintigraphic assessment of CA. Recently, a large cohort of patients with AL cardiac amyloidosis reported a positive 99mTc-DPD scintigraphy planar uptake in ~40% of cases, including grade 2-3 in 10% of all patients.[2]

Objective: The primary objective of this study was to assess the role of Pyrophosphate (PYP) scan with planar and SPECT imaging of the chest in patients with recently/newly diagnosed AL amyloidosis patients treated with Bortezomib-containing regimens (BCR) as first line therapy and to compare with a cohort of ATTR amyloidosis patients seen at the Amyloidosis Program of Calgary.

Material & Methods: Patients with newly/recently diagnosed AL amyloidosis treated upfront at our Institution with BCR were identified. From these, patients undergoing PYP Scintigraphy for the assessment of cardiac amyloidosis were evaluated. In addition, patients with ATTR amyloidosis undergoing PYP scintigraphy were used for comparison. A p-value of <0.05 was considered significant.

Results: 94 consecutive patients with AL amyloidosis treated with upfront BCR and 152 with ATTR seen at the Amyloidosis Program of Calgary from 01/12 to 03/22 were identified. From these, 41 AL and 126 ATTR amyloidosis patients underwent PYP imaging. Clinical characteristics are seen in **Table 1.** Median age at diagnosis was 68 and 83 years the AL and ATTR cases, respectively (p<0.001). According to the Mayo Clinic staging criteria (2012)[3]: 9 patients were classified as stage I (21.4%), 8 Stage II (19%), 10 Stage III (23.8%), and 12 Stage IV (12%). In the ATTR group, stage I was seen in 49 (38.9%), II in 43 (34.1%) and III in 26 (20.6%).[4] PYP planar imaging at 1-hour was reported as score 0 in 12 (29.2%), 1 in 22 (53.6%), 2 in 6 (14.6%) and 3 in 1 patient (2.4%) with AL amyloidosis. In contrast, planar grade 2-3 uptake represented 94.4% of cases with ATTR amyloidosis (**Table 1**). Median NTproBNP for PYP score 2-3 patients was 2366 ng/L in AL amyloidosis and 3032 ng/L for those with ATTR (p0.001). Most AL amyloidosis patients with planar grade 2-3 were classified as Mayo Clinic stage III and IV in 71.4% (5 out of 7). A trend towards better overall survival was noted for patients with PYP score 0-1 compared to patients with score 2-3 (NR vs 47.2 months, p=0.09) (**Fig. 1**).

Summary & Conclusion: PYP scintigraphy is a useful tool to assess cardiac involvement by amyloidosis. In patients with ATTR, visual planar grade 2-3 uptake is more common. We describe here a group of AL amyloidosis with cardiac uptake, with grade I uptake being the most common grade. These data emphasize on the importance of ruling out AL amyloidosis in all patients with suspected CA. The role of PYP in the assessment of cardiac function and outcomes in patients with AL warrants further investigation.

Table 1. Clinical Characteristics of patients with AL and ATTR amyloidosis undergoing PYP at the Amyloidosis Program of Calgary

Characteristic	N=41 (AL Amyloidosis)	N=126 ATTR Amyloidosis	P value
Age (median)	68	83	<0.001
Gender			
Male	24 (58.5%)	111 (88.1%)	
Female	17 (41.5%)	15 (11.9%)	
Creatinine (µmol/L)	92	127	0.15
Albumin (g/L)	30	35	0.005
Stage I	9 (21.4%)	49 (38.9%)	NA
Stage II	8 (19%)	43 (34.1%)	
Stage III	10 (23.8%)	26 (20.6%)	
Stage IV	12 (12%)		
Unknown	3 (7.1%)	8 (6.3%)	
LDH (IU/L)	201	223	0.2
Troponin T (ng/L)	29	52	0.2
NTproBNP (ng/L)	1446	2853	0.4
Organ involvement			
Cardiac	28 (68.2%)	121 (96%)	<0.001
Nerve	7 (17%)	43 (34.1%)	0.038
Renal	27 (65%)	0	<0.001
Visual Planar Grade			<0.001
0	12 (29.2%)	1 (0.07%)	
1	22 (53.6%)	6 (4.7%)	
2	6 (14.6%)	31 (24.6%)	
3	1 (2.4%)	88 (69.8%)	
Score 2-3	7 (17%)	119 (94.4%)	<0.001

A kit method for direct radiolabeling the amyloid reactive peptide p5+14 with technetium-99m (^{99m}Tc) for the detection of cardiac amyloidosis by SPECT/CT imaging

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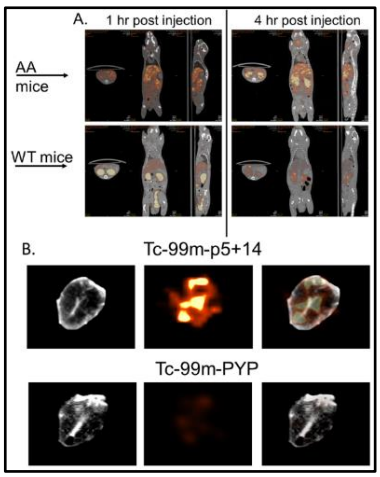
Background: Amyloid deposition is now understood to be a common cause of restrictive cardiomyopathy. Therefore, there is a need for a facile imaging agent to screen for cardiac amyloid in this patient population. In a first-in-human imaging study (NCT03678259), the radioiodinated (I-124) amyloid-binding peptide, AT-01 (formerly p5+14), was shown to be an effective radiotracer for the detection of multiple organs in many types of amyloid by PET/CT imaging. Peptide p5+14 has previously been evaluated as a Tc-99m-labeled reagent in preclinical studies (1). A facile synthesis of ^{99m}Tc-p5+14 for SPECT/CT or planar gamma scintigraphic imaging would provide cardiologists with an easily accessible and sensitive imaging agent, that binds directly to amyloid deposits, for the detection of cardiac amyloidosis of any type.

Objective: Commercial radiopharmacies generally do not employ complex chemical syntheses to generate clinical radiotracers, but rather rely on kit methods for production, especially for Tc-99m-labeled probes. A radiolabeling kit has been developed for the facile synthesis of ^{99m}Tc-p5+14.

Materials & Methods: Peptide labeling kits were prepared using adaptations of the reagents and concentrations previously published (1). The kits contain 20 µg $SnCl_2$ and 100 µg peptide in 0.03 N NaOH. Product was produced by addition of 1-2 mCi of pertechnetate. The ^{99m}Tc-p5+14 product was purified and evaluated for radiochemical purity, radiochemical yield, and bioactivity. The storage conditions and stability of the kit formulation were evaluated over a 3-month period. Amyloid reactivity of ^{99m}Tc-p5+14 (100 - 200 µCi) was evaluated *in vivo*, using a murine model of systemic AA amyloidosis, by SPECT/CT imaging and tissue biodistribution measurements. In certain studies. ^{99m}Tc-PyP was used as a comparator. Heart and liver tissue sections containing AL and ATTR amyloid deposits were used in overlay autoradiography studies to demonstrate binding of the probe to human amyloid.

Results: The ^{99m}Tc-p5+14 tracer was produced with >75% yield, >85% radiopurity and >90% bioactivity in a fibril pulldown assay. The labeling kits were stable for more than 3 months when stored at -80°C, with no significant decrease in production efficiency. When injected into mice, ^{99m}Tc-p5+14 detected hepatosplenic AA amyloid, the major sites of amyloid deposition in this model, as well as the scant deposits in the hearts of AA mice using *ex vivo* SPECT/CT imaging of isolated heart (Fig. 1). The distribution of the radiotracer in peripheral amyloid deposits in the AA mouse model were consistent with the findings using the I-124 radiolabeled tracer. Micro-autoradiography studies demonstrated that ^{99m}Tc-p5+14 effectively and specifically bound human amyloid deposits in the liver with AL and in both AL- and ATTR-containing cardiac tissue sections (Fig. 2).

Summary & Conclusion: This kit method for production of ^{99m}Tc-p5+14 would provide a facile method for generating an easily accessible, highly effective, amyloid-binding radiotracer for the detection and diagnosis of cardiac amyloidosis using gamma scintigraphy or SPECT/CT imaging.



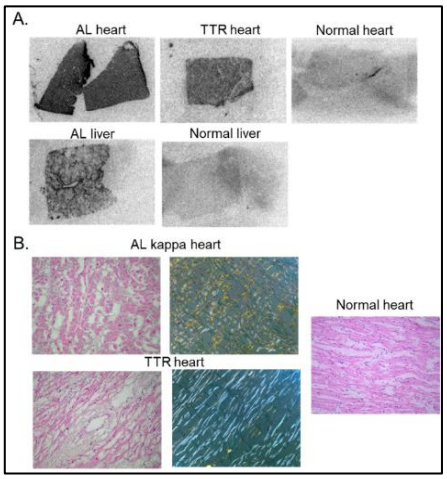


Figure 1: A Whole-body SPECT/CT of AA (upper) and WT (lower) mice injected with 100 µCi, 20 µg of ^{99m}Tc-p5+14 tracer either 1 h (left) or 4 h (right) post-injection. **B** *Ex vivo* imaging of hearts excised (1 h post injection) from AA mice injected with ~ 200 µCi of either ^{99m}Tc-p5+14 (top) or ^{99m}Tc-PYP (bottom) and perfused with contrast agent. CT only (left), SPECT only (middle) and merged SPECT/CT (right).

Figure 2: A Images from a phosphor imaging screen exposed to tissue sections of AL, ATTR and normal heart that had been incubated with ^{99m}Tc-p5+14 tracer. **B** Micro-autoradiographs of the same sections developed after 1 day exposure and lightly counterstained with H&E (left panels) or Congo Red for amyloid (right panels).

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Quantitative changes in organ-specific amyloid load in a patient with AL amyloidosis, measured by ^{124}I-AT-01 PET/CT imaging, correlate with serum biomarkers

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Background: The amyloid imaging agent, ^{124}I-AT-01 (aka ^{124}I-p5+14), is being developed for the detection and diagnosis of systemic amyloid deposits, of any type, by PET/CT imaging (1,2). This reagent could provide a facile, first-line method for the diagnosis of amyloidosis as well as permit quantitative longitudinal imaging for monitoring organ-specific progression or regression of disease. In the first-in-human imaging study at the University of Tennessee Medical Center (NCT03678259), one patient with multi-organ AL amyloidosis was imaged twice, 23 months apart, using ^{124}I-AT-01.

Objective: Here we report a case study of a patient with AL amyloidosis who had responded positively to plasma cell immunotherapy who underwent repeat PET/CT imaging. The goal was to quantitatively assess changes in amyloid load (based on ^{124}I-AT-01 retention), in the heart, liver, spleen and kidneys assessed from PET/CT images by using both manual (single slice) and automated (fully 3D) methods. These data were evaluated in the context of serum biomarkers of organ function and free light chain levels.

Material & Methods: A 70-year-old male presented with abnormal liver function tests, notably increased alkaline phosphatase. Biopsy of the liver and celiac nodes both showed presence of AL amyloid. The patient received ~2 mCi ^{124}I-AT-01 and PET/CT imaging in May 2019 with repeat imaging in April 2021. The patient was on daratumumab-based therapy during this time. PET/CT images were acquired at 5 h post injection. The entire liver, spleen, kidneys, heart, and aorta were automatically segmented on the CT image using a pretrained 3D U-net convolutional neural network (TRAQinform IQ technology, AIQ Solutions). The contours were used to quantify the mean standardized uptake value (SUV) in each organ normalized by the mean aorta lumen radioactivity (3D $SUVR_{mean}$). Independently, a fully manual 2D ROI analysis was performed on a single representative slice of each organ, resulting in 2D $SUVR_{mean}$ outputs. Serum free light chain (FLC) and biomarkers of hepatic (alkaline phosphatase; (ALKP)) and renal function were collected as standard of care. NT-proBNP was collected coincident with imaging.

Results: The PET images indicated amyloid uptake of the radiotracer in the heart, spleen, liver, kidneys, pancreas and bone marrow, with physiological distribution of radioactivity in the parotid and salivary glands, stomach lumen and urinary bladder. In the second scan, hepatosplenic radioactivity had visually decreased relative to heart and kidney. Quantitative analysis of PET/CT images by manual 2D and automated 3D methods correlated significantly (r_P < 0.98; p < 0.02). Changes in hepatic, splenic, renal and cardiac radiotracer uptake were -22.6%, -53.2%. +13.1%, +18.2% for the manual method, and -25.5%, -56.3%, +12.9%, +19.64% for the automated method. Concurrent with the ~24% decrease in hepatic amyloid, serum ALKP decreased 40% (~200 IU/mL to ~120 IU/mL), both occuring in the context of a hematologic response and 40% decrease in serum FLC.

Summary & Conclusion: Organ-specific changes in amyloid load can be visualized and quantified using ^{124}I-AT-01. In this patient, changes in the hepatic amyloid load were consistent with improved organ function based on serum ALKP measurements. However, reduction in hepatosplenic amyloid may have occured in the context of stable or slightly increasing amyloid deposition in the heart and kidneys. Organ-specific amyloid regression and progression, and biomarker-based response to therapy following anti-plasma cell therapies, remains a complex phenomenon in patients with systemic AL amyloidosis. PET/CT imaging using ^{124}I-AT-01 may play a valuable role, not only in the diagnosis of amyloidosis, for monitoring changes in organ-specific amyloid load.

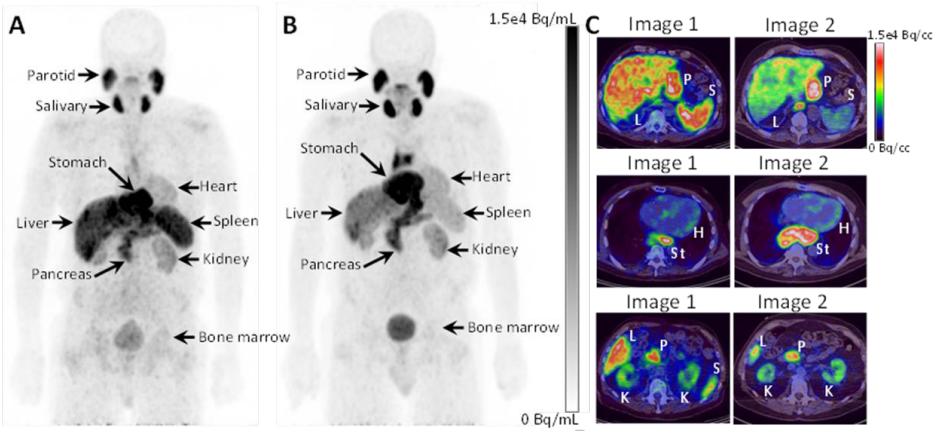

Figure 1.: Repeat PET/CT imaging of a patient with systemic amyloidosis using ^{124}I-AT-01. Maximum intensity projection (MIP) images (A) of the patient and comparison with those acquired 23 months thereafter (B). Axial PET/CT images of first infusion (Image 1) and repeat infusion (Image 2) showing a visual decrease in hepatosplenic amyloid, but not cardiac or renal deposits (C). All images are thresholded to the same maximal value.

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The role of speckle tracking echocardiography in the diagnostic assessment of cardiac amyloidosis and Fabry disease

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Background: Differential diagnosis of infiltrative cardiomyopathies, such as cardiac amyloidosis (CA) and Fabry disease (FD), is often challenging using standard echocardiographic imaging. In recent years, new echocardiographic tools including 2D speckle tracking imaging (2D STE) were introduced. Following these developments, the longitudinal left ventricular (LV) strain using 2D STE, i. e. apical sparing in CA and an impaired posterolateral longitudinal function in FD, have become established as new diagnostic indices.

Objective: The present subanalysis of our study registry aimed to evaluate layer specific radial LV strain and longitudinal right ventricular (RV) strain as potential diagnostic markers to distinguish CA from FD.

Material & Methods: We retrospectively measured layer specific radial LV strain as well as global and free wall RV strain with 2D STE in patients with CA or FD. Patients with insufficient image quality were excluded. Echocardiography was performed using a Vivid E9 or E95, GE Vingmed, Horton, Norway (M5S 1.5–4.5MHz transducer). Statistical analysis comprised Mann-Whitney-U test or *t*-test to compare both groups and receiver operating characteristic (ROC) curve analysis to evaluate diagnostic accuracy of echocardiographic parameters.

Results: Sixty-eight patients were retrospectively analysed, including 43% with diagnosed CA and 57% with genetically confirmed FD. Echocardiographic measurements observed significantly reduced radial LV strain in the epicardial, mid and endocardial layer as well as lower values of radial LV strain gradient (difference between endocardial and epicardial layers) in CA compared to FD patients. Moreover, CA patients showed impaired global and free wall RV strain in comparison to FD. In detail, free wall RV strain was reduced in all three segments but reached significance only in the mid and basal area. Of the described echocardiographic parameters, endocardial radial LV strain and global RV strain showed the highest diagnostic accuracy (area under the curve [AUC] 0.81, 95% confidence interval [CI] 0.70-0.92, and AUC 0.80, 95% CI 0.69-0.92, respectively) to differentiate CA from FD.

Summary & Conclusions: CA patients presented reduced radial LV and longitudinal RV function as assessed by 2D STE in comparison to FD patients. Therefore, 2D STE should be integrated into differential diagnostic assessment of infiltrative cardiomyopathies.

Funding: The study was financially supported by Alnylam Pharmaceuticals (Cambridge, MA, USA).

Assessment of Incidental Cardiac Uptake in Bone Scintigraphy across Spain. ECCINGO Study

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Background: Myocardial uptake on bone scintigraphy with diphosphonates has become a useful tool for the early detection of transthyretin cardiac amyloidosis (ATTR-CM), the prevalence of which remains unknown.[1]

Objective: The objective of this study was to assess the prevalence of myocardial uptake in bone scintigraphy in a representative sample of patients without previous clinical suspicion of cardiac amyloidosis (CA).

Material & Methods: Observational, retrospective, multicenter study in 21 hospitals across Spain. All scans performed for non-cardiac reasons during the months of September, October and November 2019 were reviewed. Scans with positive cardiac uptake (Perugini scores 1-3) were evaluated by a central laboratory. The medical histories of the patients with positive uptake scintigraphies were reviewed.

Results: 9864 scans were reviewed. Cardiac uptake was observed in 71 patients 0.72%) over 18 years of age (85.90% male) (. In these positive patients the most used radiotracers were 99mTc-HMDP (39.40%) and 99mTc-HDP (33.80%). Main diagnosis for requesting scintigraphy were prostate cancer (70.40%) and breast cancer (11.30%). Scan requests came from urology (49.30%) and oncology (31.00%). Prevalence of cardiac uptake increased with age: ≤ 65 y.o. (0.02%), > 65 - ≥ 75 y.o. (0.27%), > 75 - ≤ 80 y.o. (0.92%) and > 80 y.o. (3.77%). According to the central laboratory assessment the distribution in the Perugini grading scale was, grade 1 (23.90%), grade 2 (29.60%) and grade 3 (46.50%). Heart/Contralateral ratio (H/CL) was available for 24 patients with a mean of 2.1 ± 1.1 (95% CI 1.7 – 2.6). A third of the patients (33.80%) also presented extracardiac uptake, being the most common: bone metastasis (7.04%) abdominal wall (5.60%) and lower limbs muscular mass (4.20%). A previous heart failure (HF) diagnosis had been found in the 16.90% of the patients with positive uptake, being most of them 57.10% in NYHA class II. Ten patients with cardiac uptake were subsequently diagnosed with ATTR amyloidosis (ATTR-Y) with a mean delay of 10.8 months (95% CI: 6.0-16.8). All ATTR-Y patients were > 70 y.o. and 90.00% were male. All ATTR-Y patients had left ventricular hypertrophy (LVH) compared to 25.49% of patients in whom a diagnosis of ATTR (ATTR-N) was not confirmed (p=0.00003). Patients in the ATTR-Y group presented some episode of orthostatic hypotension and involuntary weight loss in the clinical history (30.00% in both) compared to 3.77% (p=0.025) and 5.88% (p=0.05) of the ATTR-N group respectively.

Summary & Conclusion: This national study found a prevalence of 0.72% of incidental cardiac uptake in bone scintigraphies performed for non-cardiac reasons in adult patients. The results of this study bring light on the profile of the elderly patients with incidental cardiac uptake and reconfirm the connection between age and cardiac findings in bone scans. The follow-up and referral of patients with incidental cardiac uptake can facilitate the early diagnosis of CA with the consequent impact on the management and prognosis of patients.

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Valve disease in cardiac amyloidosis: an echocardiographic score

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Background: Cardiac amyloidosis (CA) may affect all cardiac structures, including the valves.
Objective: We performed the first systematic assessment of the echocardiographic features of valvular CA, we synthesized these features in a score and evaluated its diagnostic and prognostic value.
Material & Methods: From 423 patients undergoing a diagnostic workup for CA we selected 2 samples of 20 patients with amyloid transthyretin (ATTR-) or light-chain (AL-) CA, and age- and sex-matched controls. We chose 31 echocardiographic items related to the mitral, aortic and tricuspid valves, giving a value of 1 to each abnormal item.
Results: Patients with ATTR-CA displayed more often a shortened/hidden and restricted posterior mitral valve leaflet (PMVL), thickened mitral chordae tendineae and aortic stenosis than those with AL-CA, and less frequent PMVL calcification than matched controls. Score values were 15.8 (13.6-17.4) in ATTR-CA, 11.0 (9.3-14.9) in AL-CA, 12.8 (11.1-14.4) in ATTR-CA controls, and 11.0 (9.1-13.0) in AL-CA controls (p=0.004 for ATTR- vs. AL-CA, 0.009 for ATTR-CA vs. their controls, and 0.461 for AL-CA vs. controls; **Figure 1**). Area under the curve values to diagnose ATTR-CA were 0.782 in patients with ATTR-CA or matched controls, and 0.773 in patients with LV hypertrophy (**Figure 2**).
Summary & Conclusion: Patients with ATTR-CA have a prominent impairment of mitral valve structure and function, and higher score values. The valve score is quite effective in identifying patients with ATTR-CA among patients with CA or unexplained hypertrophy.

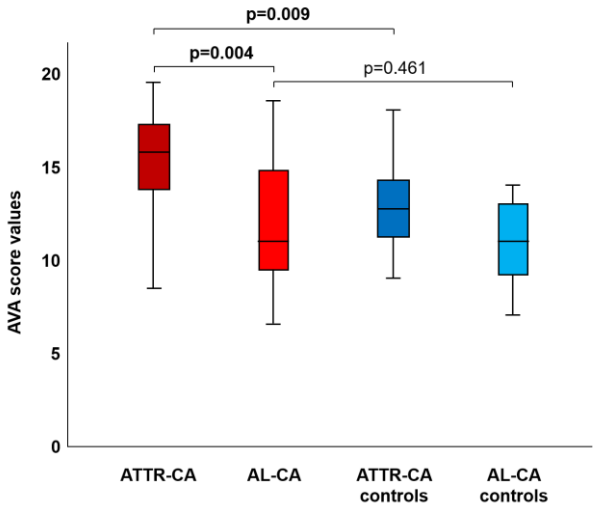

Figure 1. Amyloid valve score values in patients with amyloid transthyretin (ATTR) or light-chain (AL) cardiac amyloidosis (CA) and matched controls.

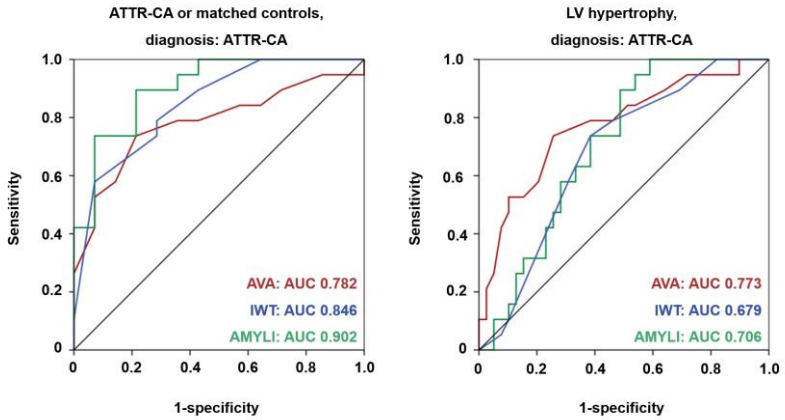

Figure 2. The valve, Increased Wall Thickness (IWT), and AMYLI scores for the diagnosis of amyloid transthyretin cardiac amyloidosis (ATTR-CA).
Area under the curve (AUC) values are reported. The scores were evaluated in patients with ATTR-CA or matched controls (*left*) or in those with left ventricular (LV) hypertrophy (*right*). p values for all comparisons were non-significant (ATTR-CA or controls: valve score vs. IWT, p=0.524; valve score vs. AMYLI, p=0.205; IWT vs. AMYLI, p=0.313; LV hypertrophy: valve score vs. IWT, p=0.243; valve score vs. AMYLI, p=0.402; IWT vs. AMYLI, p=0.704).

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Coronary flow reserve by PET 13N-Ammonia in patients with Hereditary Transthyretin Amyloidosis with and without cardiac involvement.

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Background: Transthyretin Cardiac amyloidosis (ATTR) presents with diffuse deposition of amyloid fibrils in the heart. Biomarkers of cardiac involvement such as troponins are often elevated even in early disease stages, reflecting direct cardiomyocyte damage and coronary microvascular dysfunction by amyloid deposits.

Objective: Evaluate global and segmental coronary flow reserve (CFR) by PET 13N-Ammonia in patients with hereditary Transtirretina Amyloidosis with and without cardiac involvement.

Material & Methods: Thirty-eight ATTR mutation carrier patients (18 with cardiac amyloidosis and 20 without cardiac amyloidosis underwent CFR study by PET 13N-Ammonia. Cardiac involvement was defined by means of echocardiography, with an end-diastolic interventricular septal wall thickness ≥ 12 mm or positive endomyocardial biopsy or grade II or III nuclear scintigraphy using bone avid radiotracers. The Mann-Whitney test was used to compare the values of coronary flow reserve between the groups. The effect size was calculated using the Wilcox method and 95% confidence intervals, obtained by bootstrapping. A multivariate linear regression model was applied to identify the main determinants of global coronary flow reserve.

Results: Compared with patients without cardiac involvement, patients with cardiac involvement were older (69 ± 8 vs. 41 ± 11 years old; p<0,001), presents worse NYHA functional classification, higher left ventricular mass index (198 ± 51 g/m2 x 75 ± 19 g/m2; p<0,001), thicker interventricular septum (17,9 ± 3,4 mm vs. 8,7 ± mm; p<0,001]. Coronary flow reserve values were significantly lower in the cardiac amyloidosis group globally (1,8 ± 0,4 vs. 2,9 ± 0,7; p<0,001) and in all analyzed segments (p<0.01). The apical segments were also affected when comparing group with and without cardiac involvement (2,0±1 vs. 3,0±0,7; P=0,002), suggesting absence of microvascular apical preservation. In multivariable linear regression analyses, age and BNP were the main factors associated to coronary reserve flow. The reduction in global CRF decreases by 0,25, 0,08 and 0,17 points, on average, for each decade of life, for each 100 mg/dl of serum BNP and for 0,1 heart to contralateral lung ratio uptake in 3-hour caption on TcPYP scintigraphy respectively.

Summary & Conclusion: Patients with cardiac amyloidosis present lower coronary flow reserve both globally and segmental compared to mutation carrier without cardiac amyloidosis. The evaluation of Coronary flow reserve by PET 13N-Ammonia may be a worthwhile tool in cardiac involvement in TTR patients.

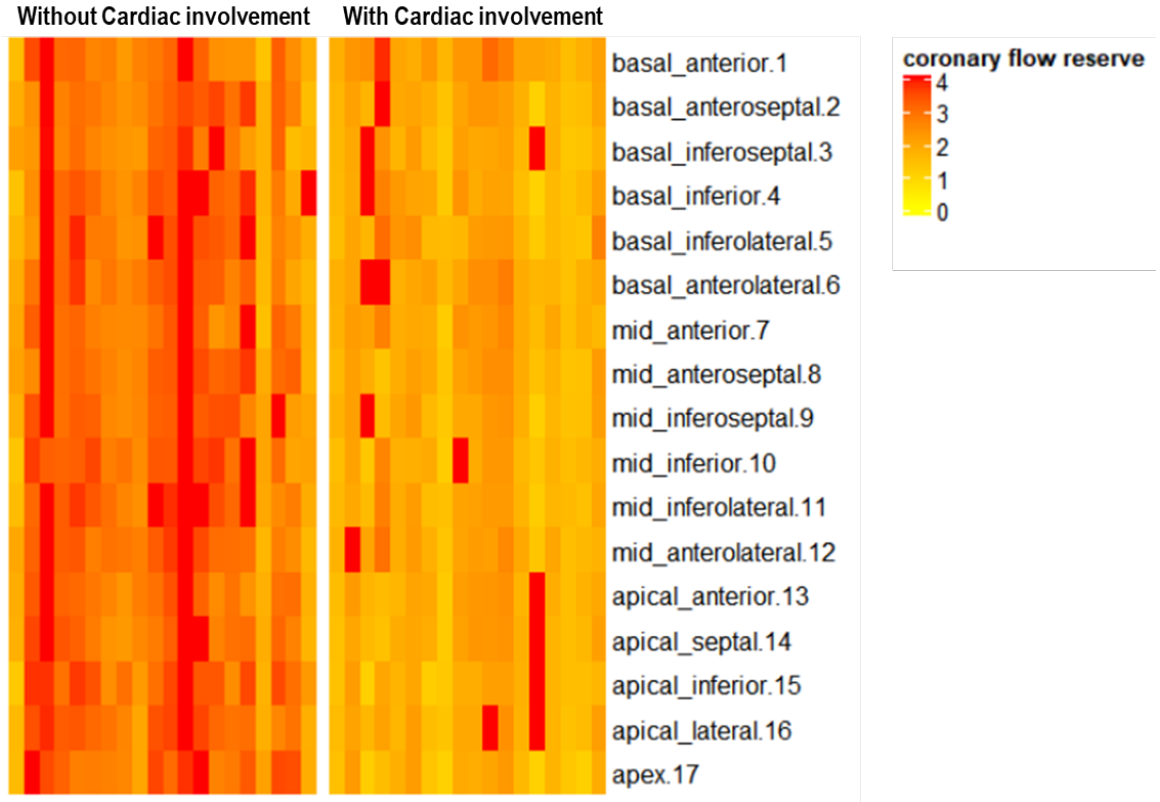

Figure 1.: Heatmap Coronary Reserve Flow, seen by segments. Represents an overview of each patient's coronary flow reserve values. Each row of the heat map illustrates an anatomical segment of the heart, and each column represents the coronary flow reserve values in each of the patients. Darker colors illustrate higher values of coronary flow reserve. Coronary flow reserve values were significantly lower in the case group ($p<0.01$) in all segments studied.

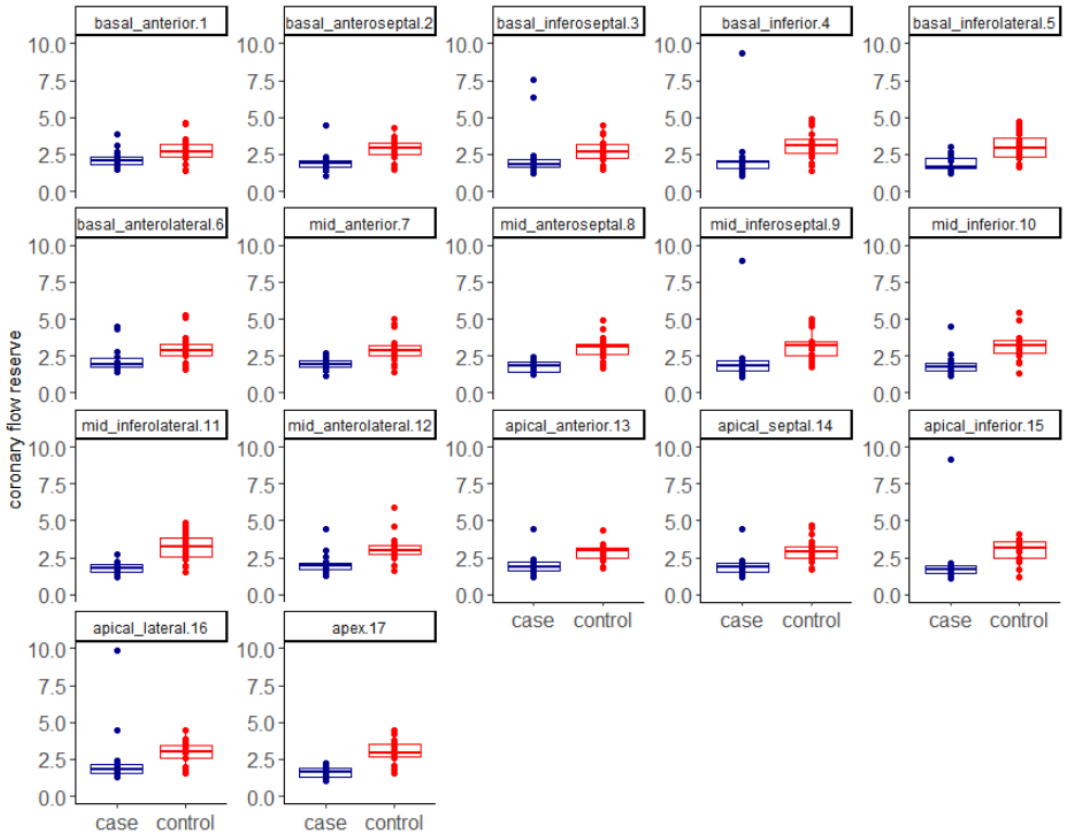

Figure 1. Coronary flow reserve values according to study group and cardiac segment.

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Differentiation of ATTR amyloidosis based on abdominothoracic organ-specific uptake of ^{124}I-AT-01 (^{124}I-p5+14) assessed by PET/CT imaging

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Background: Systemic amyloidosis is a multi-organ disorder with variable presentation due to the heterogeneity of organ involvement in patients with amyloidosis. The major forms of the disease result from the deposition of transthyretin (ATTR) or monoclonal immunoglobulin light chains (AL). Cardiac amyloidosis is a common clinical feature of both. At present, there are no approved methods for imaging systemic amyloid in all abdominothoracic organs. This capability would be clinically relevant given the diverse multi-organ distribution of the pathology in patients. In addition to diagnosis, visualization of whole body amyloid load, and monitoring response to therapy, there would be clinical value if the imaging agent could, with acceptable specificity, differentiate ATTR (the most prevalent form of systemic amyloidosis and for which multiple therapeutic interventions are available) from other forms of the disease. Agents such as ^{99m}Tc-PyP and ^{18}F-florbetaben have demonstrated the ability to differentiate ATTR from AL amyloidosis based on selective uptake and differential washout kinetics, both related to the relative affinity of the reagent for the deposits (1, 2). Herein, we present an alternative method based on the relative organ uptake of ^{124}I-AT-01 (^{124}I-p5+14) seen by quantitative PET/CT imaging (an analysis performed on data collected in a Phase 1/2 study of the radiotracer in patients with amyloidosis, NCT03678259).

Objective: The goal of this post-hoc analysis was to investigate potential methods for differentiating patients with ATTR amyloidosis from patients with other forms of the disease by comparing quantitative uptake of ^{124}I-AT-01 in the heart, liver, spleen and kidney in PET/CT images. A cohort of patients with positive visual cardiac uptake of the radiotracer were included in the analysis (n=16 non-ATTR and n=17 ATTR).

Material & Methods: The ^{124}I-AT-01 study enrolled a total of n=57 subjects (>18 years of age). Fourty-eight subjects (n=48) had systemic amyloidosis, two (n=2) subjects had localized AL amyloidosis, two (n=2) subjects were asymptomatic ATTRv carriers, and a cohort of five healthy volunteers (n=5) were imaged. Thirty-three patients (n=33) had uptake of ^{124}I-AT-01 in the heart based on visual examination of the images by a nuclear medicine physician. The radioactivity in the heart, liver, spleen, kidney and blood pool (lumen of thoracic aorta) was quantifed (Bq/cc) by manual region of interest analysis and a standard uptake value ratio (SUVR) for each organ determined using the blood pool as the reference tissue. The SUVR and heart SUVR-to-organ ratios from patients with ATTR and non-ATTR amyloidosis were asessed for statistical assumptions and then compared using two-tailed t-tests or Mann-Whitney U tests (α=0.05). Receiver operator characteristic (ROC) analyses were performed using the Wilson/Brown method (3).

Results: There was significantly less amyloid (^{124}I-AT-01 retention) in the liver, spleen and kidney of patients with ATTR as compared to the non-ATTR cohort (p<0.005). Similarly, the heart-to-organ (heart:liver, heart:spleen, heart:kidney, heart:(liver+spleen), heart:(liver+kidney)) ratios were significantly lower in the non-ATTR patients (p<0.02) due to the larger denominator in these analyses. These data indicate that an ROC analysis could identify a cut-off value for discriminating ATTR amyloidosis. The area under the curve (AUC) values for all analyses were >0.7 and highly significant. Optimal cut-off values for discriminating ATTR patients were obtained using the spleen SUVR (AUC=0.81, p=0.002; 88% sensitivity and 76% specificity) and heart SUVR:spleen SUVR ratio (AUC=0.83, p=0.001; 82% sensitivity and 63% specificity).

Summary & Conclusion: PET/CT imaging of patients with amyloidosis using ^{124}I-AT-01 allows for quantitative detection of pathology in major abdominothoracic organs. Analysis of radiotracer uptake in the heart, liver, spleen and kidney can be used to differentiate ATTR from non-ATTR forms of amyloidosis with high sensitivity and may provide additional insight on disease type following whole body PET/CT imaging with ^{124}I-AT-01.

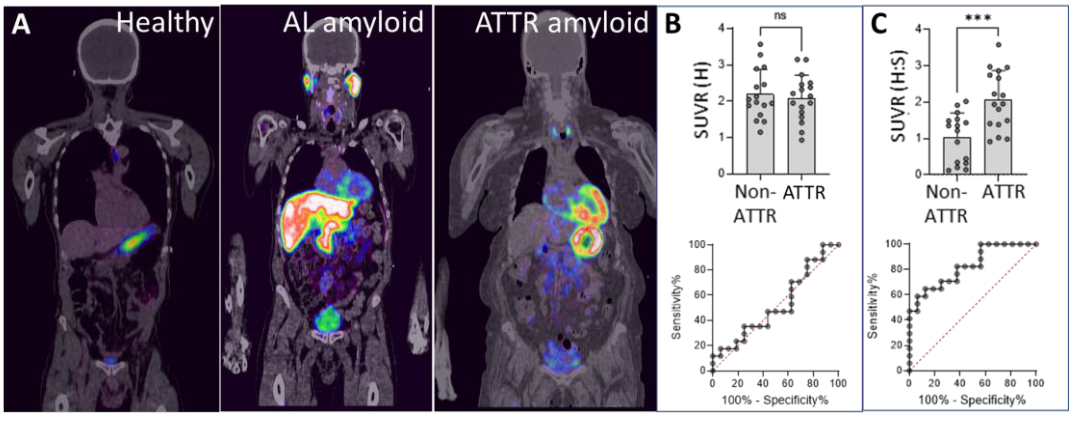

Figure 1.: Method for differentiating patients with ATTR amyloidosis from those with other (non-ATTR) forms of amyloidosis. A) Representative images of ^{124}I-AT-01 PET/CT images at 5 h post injection in a healthy subject, and patients with AL or ATTR showing the characteristic intense multi-organ uptake of radiotracer in the AL patient and activity restricted to the heart in the patient with ATTR amyloidosis. B) Patients included in the analysis were those with positive cardiac uptake (*n*=16 Non-ATTR and *n*=17 ATTR) and no significant difference in amyloid load, based on radiotracer uptake in the LV wall and IVS SUVR. C) Heart SUVR-to-spleen SUVR ratio is significantly different in patients with non-ATTR and ATTR amyloidoses. ROC analyses of this ratio shows a significant difference that can be used to support identification of patients with ATTR with >80% sensitivity (AUC = 0.83, *p*=0.001).

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Support & Funding: Support for this work comes from the Amyloidosis and Cancer Theranostics Program gift fund. We appreciate the support of the Cancer Institute and Department of Nuclear Medicine at the University of Tennessee Medical Center, notably Bryan Whittle, Robin Geldrich, and Barbara Marine. Attralus owns intellectual property associated with ^{124}I-AT-01.

Detection of extracardiac amyloid in patients with ATTR amyloidosis by PET/CT imaging using the amyloidophilic radiotracer ^{124}I-AT-01 (^{124}I-p5+14)

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Background: Transthyretin-associated (ATTR) amyloidosis is the most common form of systemic amyloidosis worldwide. Patients with a diagnosis of ATTR amyloidosis are generally categorized as either dominated by symptoms of peripheral neuropathy or cardiomyopathy, which defines the clinical management of the patient and the course of treatment available. Despite the classification into these two major pathologies, the anatomic distribution of ATTR amyloid deposits in the patient population can be extensive and heterogenous. This is supported by autopsy studies where numerous extracardiac and extraneuronal amyloid deposits have been identified (1). However, due to the paucity of amyloid specific whole body imaging technologies, these sites of deposition and their clinical importance have not been fully appreciated. We have reviewed the ^{124}I-AT-01 PET/CT images from ATTR amyloidosis patients (*n*=20) who were enrolled in the Phase 1/2 imaging study (NCT03678259) for extracardiac uptake of radiotracer, which is indicative of the presence of amyloid.

Objective: The goal of this analysis was to assess, by visual evaluation of ^{124}I-AT-01 PET/CT images, the uptake of radtiotracer in extracardiac anatomic sites, which might indicate the presence amyloid.

Material & Methods: The trial enrolled a total of *n*=57 subjects (>18 years of age) including patients with a diagnosis of ATTR amyloidosis (*n*=20). Both ATTRv (*n*=15) and ATTRwt (*n*=5) patients were imaged. All patients had a confirmed diagnosis of amyloidosis (based on biopsy findings, genotyping, or imaging studies). Subjects were administerd a single IV dose of ^{124}I-AT-01 (<2 mCi I-124 and <2 mg AT-01). PET/CT images were acquired ~5 h post injection using a low dose CT (120 kVp, 50 effective mAs) from crown to thighs. The images were reviewed by a radiologist who was blinded to the clinical status of the subject, and uptake of the radiotracer in organs and tissues was recorded. To assess the physioloigcal distribution of radioactivity, a chort of healthy subjects (*n*=5) was recruited and similarly evaluated.

Results: Uptake of ^{124}I-AT-01 was observed in at least one anatomic site in 19 ATTR patients (sensitivity = 0.95; 95% CI: 0.77, 1.00; *n*=20). Of the eleven (11/20) ATTR patients diagnosed with ATTR cardiomyopathy, cardiac uptake of ^{124}I-AT-01 was seen in all (sensitivity = 1.00; 95% CI: 0.74, 1.00; *n*=11). Extracardiac uptake of ^{124}I-AT-01 in ATTR amyloidosis patients was observed principally in the: joints, including shoulders, spinal discs and facets (11/20); thoracic and lumbar spine (6/20); spleen (7/20); kidney (9/20); liver (5/20); and muscle (4/20) (Fig. 1). Additionally, uptake in other anatomic sites including the lung, pancreas, adrenal glands, and pituitary gland were also observed in the PET/CT images. For the entire ATTR cohort, a total of 17 non-cardiac organs or tissues were deemed positive by ^{124}I-AT-01 imaging.

Summary & Conclusion: ATTR amyloidosis is clinically considered to principally involve the heart and/or peripheral nerve. However, musculoskeletal abnormalties, including carpal tunnel syndrome, bicep rupture and lumbar spinal stenosis, which may portend ATTR amyloidosis, can precede the appearance of ATTR cardiomyopathy and neuropathy by many years. Thus, ATTR amyloid is known to deposit in multiple anatomic sites; however, the extent of the deposition and the clinical importance of extracardiac amyloid has yet to be appreciated. Using PET/CT imaging of ^{124}I-AT-01, we have shown heterogeneous and often extensive ATTR amyloid deposits in numerous extracardiac sites, commonly the joints and spine. Extensive systemic deposition of ATTR has been recognized in autopsy studies and may now be evaluated non-invasively by PET/CT imaging. Despite the small cohort of patients in this study, these preliminary observations warrant further investigation to determine their importance to clinical management and the quality of life of patients with ATTR amyloidosis.

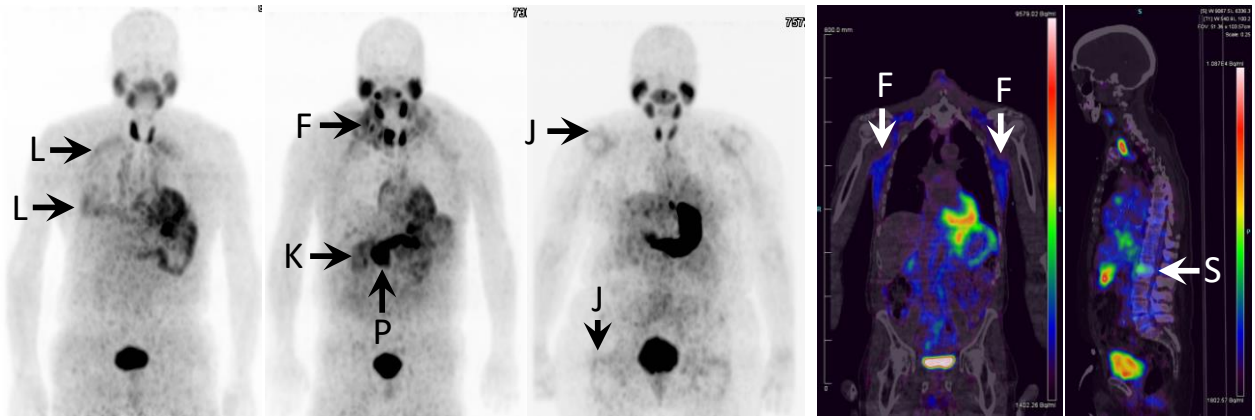

Figure 1.: Retention of ^{124}I-AT-01 in extracardiac anatomic sites of patients with ATTR amyloidosis by PET/CT imaging. Maximum intensity projection (black and white) images showing retention of radiotracer in the lung (L), fat (F), kidney (K), pancreas (P) and joints (J). Coronal (left) and sagittal (right) PET/CT images of patients with retention of ^{124}I-AT-01 in fat tissue and the spine/vertebral disc (S).

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Support & Funding: Support for this work comes from the Amyloidosis and Cancer Theranostics Program gift fund. We appreciate the support of the Cancer Institute and Department of Nuclear Medicine at the University of Tennessee Medical Center. We acknowledge the support of Bryan Whittle, Robin Geldrich, and Barbara Marine for imaging and nursing assistance. Attralus Inc. owns intellectual property associated with ^{124}I-AT-01.

Myocardial stiffness evaluation using atrial kick in healthy controls and patients with cardiac amyloidosis: a pilot study

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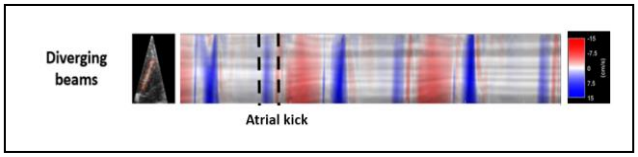
Background: Increase in myocardial stiffness is an early manifestation of myocardial amyloid infiltration [1, 2]. Therefore, measures of myocardial stiffness may provide incremental value beyond traditional echocardiographic measures for assisting in the earlier diagnosis of cardiac amyloidosis. But, simple tools to measure myocardial stiffness are currently limited. Left ventricular (LV) filling following atrial kick in late diastole generates LV myocardial stretch that propagates with a wave speed that is directly related to myocardial stiffness [1]. We implemented an ultrasound-based cardiac stiffness measurement, intrinsic elastography, which is based on naturally occurring cardiac waves generated by atrial kick during LV filling: the higher the wave speed, the higher the myocardial stiffness.

Objective: The goal of this study was to evaluate the feasibility of LV myocardial stiffness measurement using intrinsic cardiac elastography in a group of healthy volunteers and patients with transthyretin (ATTR) cardiac amyloidosis. It is our hypothesis that LV myocardial propagation velocity will be higher in ATTR cardiac amyloidosis patients compared to healthy controls.

Material & Methods: We prospectively enrolled 9 patients with ATTR cardiac amyloidosis, diagnosed by grade 2 or grade 3 myocardial uptake on 99m technetium pyrophosphate scintigraphy. Patients with atrial fibrillation, cardiac pacing, or fusion of the transmitral E and A waves were excluded (N=4). We also recruited 17 healthy volunteers with no history of heart failure. We implemented a diverging beam mode with a frame rate of 327 frames/sec using Philips X5-1 probe and EPIQ scanner [3]. The field of view was reduced to encompass only the interventricular septum (Figure 1). All measurements were collected over at least two cardiac cycles and were repeated three times per volunteer during one scanning session.

Results & Discussion: Over all participants and heartbeats, an average (± standard deviation) wave speed of 3.12 (± 2.04) m/s was measured in amyloidosis patients; while it was 1.42 (± 0.24) m/s in healthy volunteers. Fig. 2 shows the wave speed variability of 17 volunteers and 5 amyloidosis patients across all acquisitions and heartbeats. The coefficient of variation across all measurements was 16.87% and 52.56%, respectively in healthy volunteers and patients with amyloidosis. Such values are very close to the velocities measured in amyloidosis patients and healthy controls reported by Pislaru et al. (3.2 ± 1.0 m/s for cardiac amyloidosis, and 1.6 ± 0.2 m/s for healthy controls) [1].

Summary & Conclusion: These preliminary results confirm the feasibility of intrinsic elastography for measurement of myocardial stiffness in healthy volunteers and patients with ATTR cardiac amyloidosis. The results from this study represent the first step toward the development of an ultrasound-based method for quantifying differences in myocardial stiffness properties between healthy and myopathic ventricles. Future clinical studies will explore the ability of this method to quantify myocardial stiffness in pathologies such as left ventricular hypertrophy, hypertrophy cardiomyopathy, and myocardial infarction.



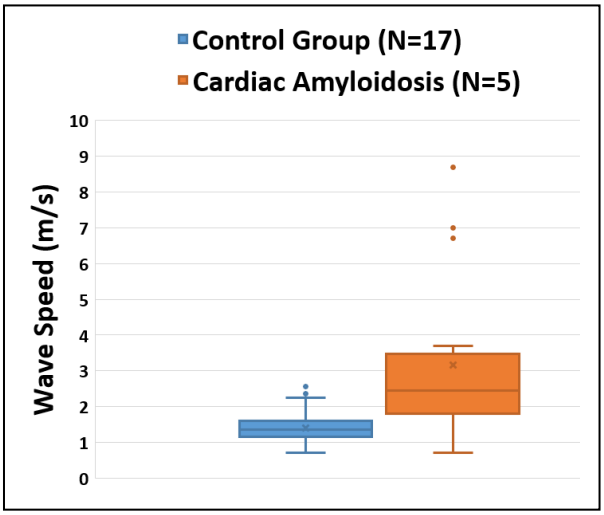


Figure 1. (left): Exemplary tissue velocity M-Mode spanning the interventricular septum obtained from the diverging beam. The atrial kick part of the tissue M-mode used for post-processing is shown in dashed lines.

Figure 2. (right): Myocardial wave speed (median and interquartile range) in 17 healthy volunteers and 5 amyloidosis patients using diverging and focused beams.

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[99mTc]Tc-DPD Scintigraphy Associating Semi-Quantitative Methods for the Diagnosis of Cardiac Amyloidosis: Experience in an Endemic Area

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Background: Balearic Islands are the fifth endemic focus worldwide for hereditary transthyretin amyloidosis(ATTRv), being Val50Met the most predominant mutation. In the imaging field of amyloid, it is well stablished a cut-off value in cardiac scintigraphy with 99mTc-pyrophosphate(PYP) performed after 1-hour post-injection for differentiating between ATTR and AL(≥ 1.5 suggestive of ATTR), but with 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid(DPD) performed 3-hour post-injection is not yet established.

Objective: Assess the usefulness of semi-quantitative methods in the diagnosis of cardiac amyloidosis(CA) in [99mTc]Tc-DPD scintigraphy, establishing a cut-off value and compare it to the Perugini-visual-scale(PVS).

Material & Methods: Retrospective study including patients who underwent [99mTc]Tc-DPD scintigraphy for suspected CA in our center. Thoracic planar images were acquired 3-hour post□injection performing anterior, left-anterior-oblique, left-lateral projections and single photon emission Computed Tomography/Computed Tomography(SPECT/CT) in some cases. Myocardial uptake was graded from 0-3 using PVS, considering 2-3 positive scans. Heart-to-contralateral(H/CL) ratio was calculated using the mean counts within ROI placed over the heart and mirrored over the contralateral chest. H/CL corrected(H/CLc) ratio also was performed subtracting the background activity. Relationship between PVS and semi-quantitative ratios was analyzed. Data obtained were compared with each CA subtype (ATTRv,ATTRwt,AL) by means of the available clinical data, other imaging techniques (Echocardiography/cardiac-MRI), analytical(NT-proBNP/Immunofixation), genetic(Val50Met-mutation) and histopathological findings.

Results: 374 planar studies and 13 SPECT/CT were performed from April/2014-December/2021 in 349 patients (mean age: 71 years [22-95]; 61% male). Using PVS, 292/374(78%) patients had a negative scan and 82/374(22%) had a positive scan. 11/13(85%) SPECT/CT performed in positive scans, showed a higher myocardial uptake in interventricular septum(9/11) and lateral wall of left ventricle(2/11). 47/374(13%) showed extracardiac uptake: only abdominal(20), only pulmonary(9), only liver(4), abdominal + pulmonary(13) and pulmonary + liver(1). 42/47(89%) showed grade 3. Hepatic uptake was attributable to non-ATTR causes in 4 scans. Final diagnosis in the 81 patients with positive scans(1patient with 2 scans) was: ATTRv(43), ATTRwt(36) and AL(2). Mean values of the H/CL and H/CLc ratios related to PVS grading were respectively: 1.0±0.10SD and 1.0±0.11SD for grade 0(n=284); 1.2±0.19SD and 1.3±0.17SD for grade 1(n=8); 1.8±0.21SD and 2.2±0.30SD for grade 2(n=14); 3.2±0.95SD and 4.2±0.94SD for grade 3(n=68). H/CL and H/CLc values showed a significative ascending trend along with the PVS grading (p=0.001). ROC analysis were performed between the semi-quantitative-ratios and a binary visual scale, cutoff values of 1.45(AUC=0.99) and 1.5(AUC=1.0) were established for HCL and HCLc, respectively; yielding sensitivity and specificity values of 100% and 99.7% for both ratios. Values of H/CL and H/CLc were not statistically different among the different subtypes of amyloid deposition(ATTRv, ATTRwt and AL).

Summary & Conclusion: In our experience, H/CL and H/CLc ratios showed a significant concordance with the visual assessment through PVS in the diagnosis of CA. Semi-quantitative-methods are not able to discriminate between the different subtypes of CA. Extracardiac abdominal and pulmonary uptake seems to be exclusively associated with advanced stages of disease

	Perugini visual Scale			
	0 (n=284)	**1 (n=8)**	**2 (n=14)**	**3 (n=68)**
H/CL (mean+/-SD)	1.0+/-0.10	1.2+/-0.19	1.8+/-0.21	3.2+/-0.95
H/CLc (mean+/-SD)	1.0+/-0.11	1.3+/-0.17	2.2+/-0.30	4.2+/-0.94

Figure 1.: The mean values of the H/CL and H/CLc ratios related to PVS grading were respectively in this table.

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Title: Myocardial Contraction Fraction (MCF): A Simple Measure of Myocardial Shortening That is Associated with Longitudinal and Circumferential Strain in Transthyretin Cardiac Amyloidosis.

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Background: The measurement of left ventricular ejection fraction (LVEF) does not accurately reflect systolic myocardial performance in small and thickened hearts, such as in patients with Transthyretin cardiac amyloidosis (TTR-CA). Less fiber shortening is required to produce a similar LVEF in a thick-walled compared with a thin-walled ventricle. Myocardial strain was developed as a means to objectively quantify regional myocardial function and represents the fractional change in length of a myocardial segment. Conceptually, myocardial contraction fraction (MCF) is a measure of the degree to which the overall myocardium contracts during systole (assessed by stroke volume) when considered in relation to its capacity to contract (assessed by myocardial volume).

Hypothesis: We evaluated the correlation between MCF and the various measures of strain, including global longitudinal strain (GLS), circumferential strain (SC), and radial strain (SR) with the hypothesis that MCF is associated a parameter that represents various strains.

Methods: Echocardiograms on 70 patient, 56 patients with TTR-CA (46 wild type patients, 10 variant patients), along with 14 patients without TTR-CA (3 patients without LVH, and 11 patients with LVH) were systematically evaluated. LV volumes and ejection fractions were assessed using the biplane Simpson's equation from apical 4- and 2-chamber views. All strain measurements were performed off-line using automated software (EchoPAC Advanced Analysis Technologies; GE Medical Systems,

Milwaukee, Wisconsin). For each segment, global longitudinal strain (GLS), peak global circumferential strain (peak G SC), end-systolic circumferential strain (SC(ES)), peak global radial strain (peak G SR), end systolic radial strain (SR(ES)) were recorded and averaged. Additionally end-systolic rotation (Rot(ES)) was recorded. Segmental longitudinal strain (GLS) was determined for epi-myocardium, mid-myocardium, and endo-myocardium. Correlations among various strains and MCF were determined by Pearson's correlation coefficient.. A p value <0.05 was considered statistically significant.

Results: Participants with ATTR-CA were older, more often males with thicker LV walls than controls and thus higher LV mass. As expected, ATTR-CA has lower longitudinal, circumferential and radial strain than controls. As shown in the table below, there was a strong correlation between GLS and MCF (both AFI r=0.77 and Q-analysis r= 0.77) among all subjects studied. There was moderate correlation between peak G SC and MCF (r=0.6) and SC(ES) and MCF (r=0.4) as well. There was a weak correlation between MCF and Peak G SR (r=0.39), which was also statistically significant. Segmental LGS from the epi-myocardium, mid-myocardium, and endo-myocardium, was differentially correlate with MCF, with epi-myocardium having the lowest GLS and endo-myocardium having the highest GLS ($p < 0.001$).

Correlation of MCF with Various LV Strains

Parameter	MCF and GLS (AFI)	MCF and GLS (Q-analysis)	MCF and peak G SC	MCF and SC(ES)	MCF and Peak G SR	MCF and SR (ES)	MCF and Rot(ES)
Coefficient r	-0.77	-0.77	-0.67	-0.47	0.39	0.14	-0.15
N	70	70	70	70	70	70	70
T statistic	-9.95	-9.95	-7.44	-4.39	3.49	1.17	-1.25
DF	68	68	68	68	68	68	68
P value	<0.001	<0.001	<0.001	<0.001	0.001	0.25	0.11

Conclusion: MCF is significantly associated with all segmental strains though the strength of the association is greatest for GLS, followed by circumferential strain (SC) and then radial strain. These data demonstrate the MCF is a volumetric measure of myocardial shortening analogous to strain.

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Histological validation of cardiac 99mTc-DPD uptake in patients with cardiac transthyretin amyloidosis

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Background: Cardiac transthyretin (ATTR) amyloidosis is a progressive and fatal disease caused by the extracellular deposition of misfolded ATTR protein in the myocardium. In an era where new therapies are rapidly emerging, development of non-invasive imaging modalities to quantify amyloid burden over time is of utmost importance. Although endomyocardial biopsy (EMB) remains the gold standard in amyloid detection and typing, 99mTechnetium-3,3-diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) scintigraphy is a widely available and accurate tool for non-invasive diagnosis of cardiac ATTR amyloidosis. However, it remains to be determined whether the degree of cardiac 99mTc-DPD uptake correlates with the histological amyloid infiltration at EMB – thus, justifying 99mTc-DPD scintigraphy as a disease monitoring tool.

Objective: This single-centre observational study aimed to compare the extent of histologic amyloid burden on EMB with the quantification of cardiac 99mTc-DPD uptake on planar images and SPECT/CT acquisitions in cardiac ATTR amyloidosis.

Material & Methods: 26 patients with cardiac ATTR amyloidosis were enrolled. Patients were included in case of (1) EMB-proven ATTR amyloidosis and (2) availability of 99mTc-DPD scintigraphy (reference activity: 550 MBq). Visual interpretation using the Perugini score, quantitative analysis of cardiac 99mTc-DPD uptake by planar whole-body imaging and SPECT/CT using regions of interest (ROI) were performed, and heart to whole-body ratio (H/WB) was measured. Histological amyloid load was quantified as percentage of the analysed myocardial tissue using Sulfated Alcyan Blue staining and the Fiji-ImageJ programme. Pearson's and Spearman's correlation coefficients were used for correlation analysis and assessment of agreement.

Results: ATTR patients had a median age of 77 [73-79] years and were predominantly male (85 %). An abnormal Perugini score (i.e. 2 or 3) was present in 25 patients (96 %), whereas 1 patient was assigned Perugini score 1 (4 %). Increased cardiac tracer uptake was documented in all patients, both on 99mTc-DPD planar scintigraphy [ROImean 129 ± 37] and SPECT/CT [ROImean 369 ± 142]. Histologic amyloid burden on EMB was 32 ± 19 % on average. It significantly correlated with Perugini score ($p=0.003$ $r=0.56$), as well as with cardiac 99mTc-DPD uptake (planar: $p=0.006$ $r=0.54$, SPECT/CT: $p=0.018$ $r=0.48$) and H/WB ($p=0.046$ $r=0.41$).

Summary & Conclusion: We have demonstrated a good correlation between histological amyloid infiltration at EMB and cardiac 99mTc-DPD uptake on scintigraphic planar images and SPECT/CT scans, illustrating the potential of 99mTc-DPD scintigraphy to yield reliable quantitative information on cardiac amyloid burden. Further investigations with a larger number of patients are required to confirm our findings, and to ultimately implement thresholds in cardiac 99mTc-DPD uptake measurements for being used for guiding disease and therapy management.

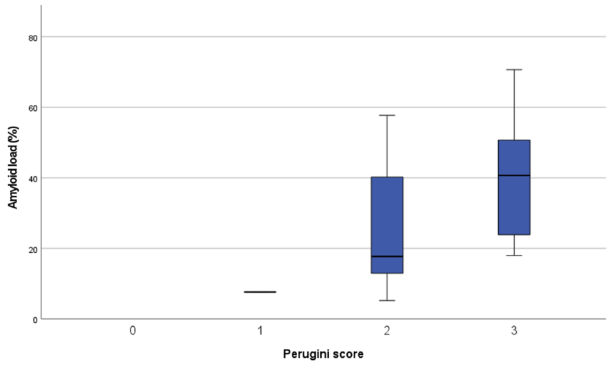

Figure 1.: Correlation between Perugini score and histological amyloid load in EMB

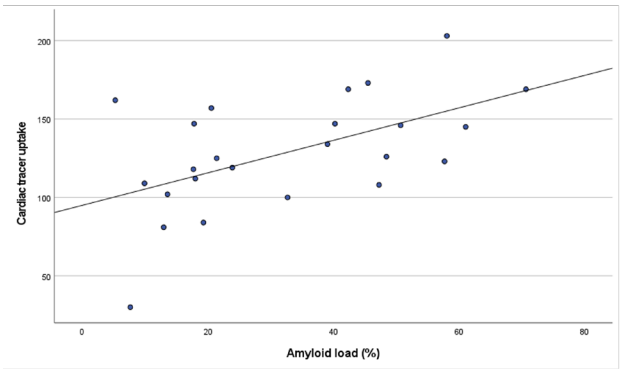

Figure 2.: Correlation between histological amyloid load in EMB and the degree of cardiac tracer uptake on scintigraphic planar scans

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Support & Funding: Pfizer

Is a change of the current echocardiographic red flag for left ventricular wall thickness useful in cardiac amyloidosis screening?

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Background: Cardiac amyloidosis (CA) is a progressive and fatal infiltrative cardiomyopathy caused by the extracellular deposition of insoluble misfolded proteins in the myocardium. As new disease-modifying therapies are increasingly emerging, prompt and specific diagnosis is fundamental to avoid treatment delay. Diagnosis of CA is challenging owing to non-specific clinical and echocardiographic findings. Nevertheless, transthoracic echocardiography remains the most often used first-line imaging tool for amyloidosis screening. A left ventricular wall thickness ≥15 mm of unknown etiology is considered a red flag for underlying CA. However, it is very likely that with this cut-off many patients, especially in the early stages of the disease, will not be detected. Reducing the diagnostic wall thickness threshold will certainly increase the sensitivity of the amyloidosis screening. It is to be feared, however, that this reduction of the cut-off will lead to a large number of patients being unnecessarily subjected to further testing, and substantially increase costs.

Objective: This study aimed to test the hypothesis that reducing the cut-off of the LV wall thickness to ≥13 mm in echocardiographic screening increases the sensitivity for early diagnosis of CA substantially without an unproportionally increase in effort and costs.

Material & Methods: We retrospectively analysed the echocardiographic reports from August 2021 to December 2021 at our department. Each individual LV wall thickness was documented to determine the frequency of patients with LV wall thicknesses ≥12 mm, ≥13 mm, ≥14 mm and ≥15 mm of unknown etiology. This was to give an idea of how many patients were potentially eligible for further amyloidosis screening solely due to changes in the echocardiographic cut-off value.

Results: 1.007 echocardiographic reports were analysed in a retrospective manner. Of these, 900 included a valid LV wall thickness value, while 107 (mainly exclusions of pericardial effusion) did not. From LV wall thickness ≥15 mm (5.4%), to ≥14 mm (10.0%), to ≥13 mm (18.6%) to ≥12 mm (32.0%) an exponential increase in patient number was recorded. With regard to CA diagnosis, it can be assumed that systematic screening of patients with LV wall thickness ≥12 mm will certainly result in increasing sensitivity but in turn decreasing specificity. On the basis of the patient number within each LV wall thickness category we conclude that amyloidosis screening of patients with an LV wall thickness ≥13 mm is feasible and reasonable.

Summary & Conclusion: We herein conclude that a change of the current echocardiographic red flag for LV wall thickness from ≥15 mm to ≥13 mm is useful in cardiac amyloidosis screening. In case of LV wall thickness <13 mm the use of further red flags is of utmost importance in order to prevent unnecessary amyloidosis screening and disproportionate increase in costs.

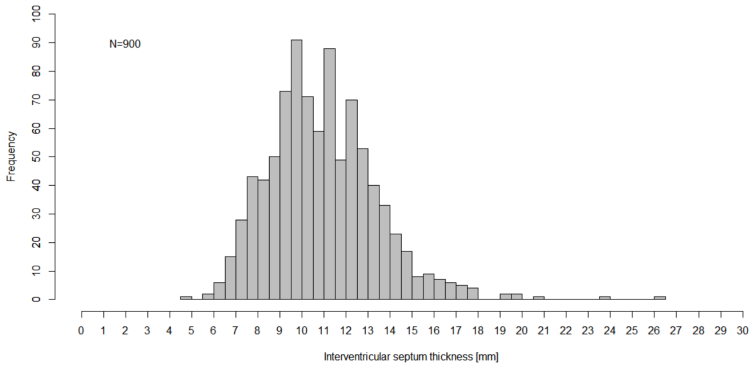

Figure 1.: Distribution of LV wall thickness among 900 patients referred to our department for routine echocardiography

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Support & Funding: Sobi

Prognosis of light chain amyloidosis with biopsy-proven cardiac involvement

Matthias Aurich, Julian Bucur, Johannes Vey, Sebastian Greiner, Fabian aus dem Siepen, Ute Hegenbart, Stefan Schönland, Norbert Frey, Hugo A. Katus, Derliz Mereles

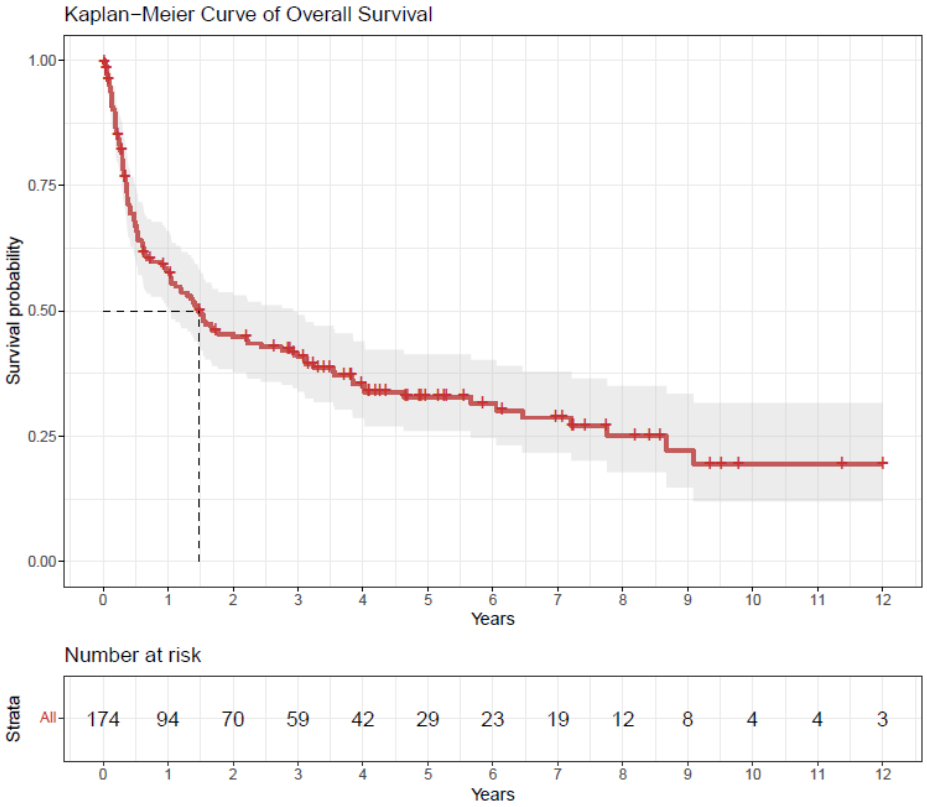
Background: Cardiac involvement (CI) is one of the main determinants of mortality in light chain (AL) amyloidosis. So far, the number of studies investigating survival of patients with biopsy-proven cardiac AL amyloidosis is limited. The aim of this study is to analyze different clinical, laboratory, electro- and modern echocardiographic parameters for their prognostic value in the assessment of patients with AL amyloidosis and definite CI.

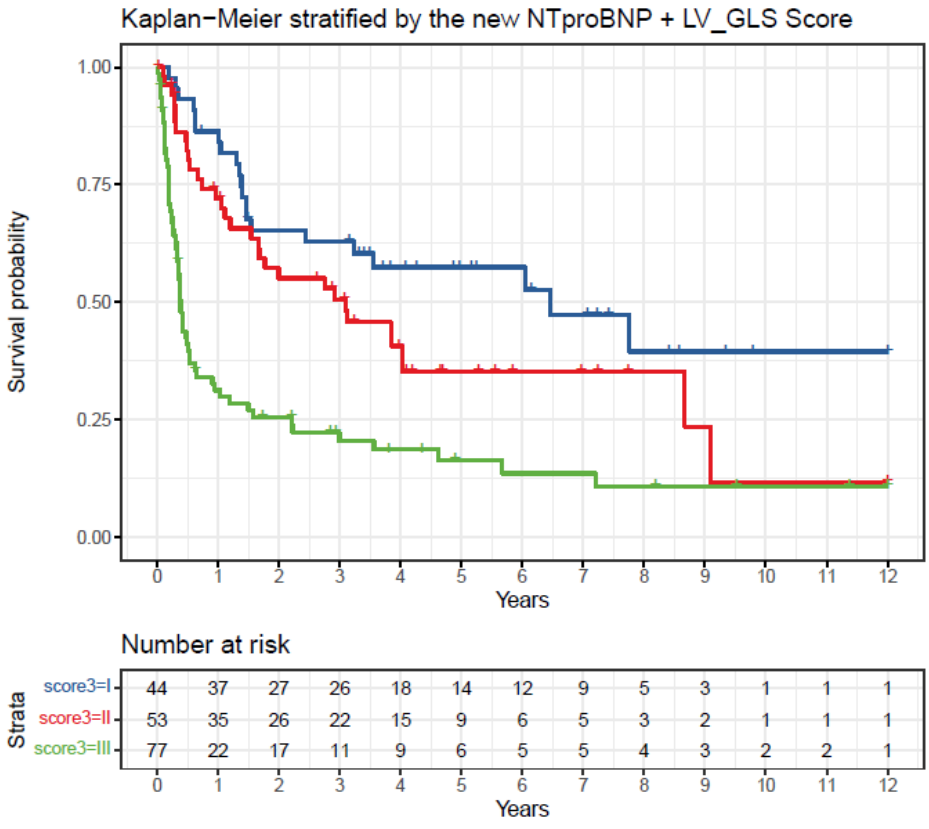
Methods: All patients with AL amyloidosis who had their first visit at the amyloidosis center at the university hospital Heidelberg between 2006 and 2017 (n = 1.628) were screened for having additional CI proven by endomyocardial biopsy (EMB). Patients with former treatment specific for AL amyloidosis, with significant cardio-vascular or renal co-morbidity were excluded from further investigation. Patients who met all inclusion criteria were divided into survivors and non-survivors and mortality markers were detected by uni- und multivariate analysis. Cut-off values for each parameter were calculated and survival over time analyzed by Log-Rank Test.

Results: One-hundred seventy-four patients with AL Amyloidosis and CI confirmed by EMB could be identified. Median follow-up time for the complete cohort was 5.2 years. At the end of the investigation period 115 patients have died. Among all parameters male gender, blood pressure, New York Heart Association functional class (NYHA-FC), low voltage pattern, left ventricular (LV) end-systolic volume (ESV), ejection fraction, longitudinal ventricular function, pericardial effusion, N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitive Troponin T, difference in free light chains (dFLC) and creatinine were associated with poor outcome but only NYHA-FC > II (HR: 1.649; 95 % CI: 1.088 - 2.500; p = 0.019), LV global longitudinal strain (GLS) (HR: 1.120; CI: 1.032 - 1.215; p = 0.007), LV-ESV (HR: 1.020; CI: 1.008 - 1.032; p = 0.001), pulmonary artery pressure (HR: 0.977; CI: 0.956 - 0.997; p = 0.027), NT-proBNP (HR: 1.568; CI: 1.166 - 2.109; p = 0.003), dFLC (HR: 1.302; CI: 1.050 - 1.615; p = 0.017) and creatinine (HR: 2.092; CI: 1.014 - 4.315; p = 0.046) were independently predictive in multivariate analysis. By combination of the cardiac biomarker NT-proBNP with a cut-off value of 5.762 ng/ml and the functional cardiac imaging parameter GLS with a cut-off value of -8.9 % a good risk stratification regarding survival could be achieved.

Conclusion: Cardiac biomarkers and modern imaging techniques are important predictors of survival in patients with AL amyloidosis and CI. The combination of NT-proBNP and GLS for risk-stratification is able to separate patients according to prognosis and has therefore the potential to optimize current staging systems.

Keywords. Cardiac light chain amyloidosis, Survival, Echocardiography, Strain Imaging


Kaplan−Meier Curve of Overall Survival
Survival probability
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12
Years
Number at risk
Strata
All
174 94 70 59 42 29 23 19 12 8 4 4 3
0 1 2 3 4 5 6 7 8 9 10 11 12
Years


Kaplan−Meier stratified by the new NTproBNP + LV_GLS Score
Survival probability
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12
Years
Number at risk
Strata
score3=I 44 37 27 26 18 14 12 9 5 3 1 1 1
score3=II 53 35 26 22 15 9 6 5 3 2 1 1 1
score3=III 77 22 17 11 9 6 5 5 4 3 2 2 1
0 1 2 3 4 5 6 7 8 9 10 11 12
Years

Multi-imaging characterisation of cardiac phenotype in different types of amyloidosis

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Background: Bone scintigraphy is extremely valuable when assessing patients with suspected cardiac amyloidosis (CA), but the clinical significance and associated phenotype of different degrees of cardiac uptake across different types is yet to be defined.

Objective: We sought to define the phenotypes of patients with varying degrees of cardiac uptake on bone scintigraphy, across multiple types of systemic amyloidosis using extensive characterization comprising of biomarkers, echocardiographic and cardiac magnetic resonance (CMR) imaging.

Material and methods: A total of 296 patients (117 immunoglobulin light-chain [AL] amyloidosis,165 transthyretin [ATTR] amyloidosis, 7 apolipoprotein-A1-amyloidosis [AApoAI],and 7 apolipoprotein-A4-amyloidosis [AApoA4]) underwent deep characterisation of their cardiac phenotype. All CMR scans were scored using the following grading system: no features of CA (normal left ventricular [LV] mass, no late gadolinium enhancement [LGE] and normal extracellular volume [ECV]), early cardiac amyloid infiltration (normal LV mass, raised ECV and/or subendocardial LGE), and characteristic of CA (increased LV mass, diffuse subendocardial or transmural LGE, altered gadolinium kinetics and raised ECV).

Results: AL-amyloidosis patients with grade 0 myocardial radiotracer uptake spanned the spectrum of CMR findings from no evidence of CA to characteristic features of CA, while AL-amyloidosis patients with grade 1-3 always produced characteristic CMR features. In ATTR-amyloidosis the CA burden strongly correlated with myocardial tracer uptake (R=0.88, 95%CI[0.84-0.91], P<0.001), except in patients with the Ser77Tyr variant. All ATTR-amyloidosis patients with grade 0 uptake had no features of CA. Most ATTR-amyloidosis patients with grade 1 uptake had no features of CA on CMR (56.8%) or early cardiac amyloid infiltration (31.8%); while a minority had characteristic features of CA (11.4%) (all of which had the Ser77Tyr variant). All ATTR-amyloidosis patients with grade 2 or 3 cardiac uptake had characteristic features of CA. AApoAI-amyloidosis presented with grade 0-1, and unique features of disproportionate right sided involvement such as disproportionate right ventricular (RV) uptake on ^{99m}Tc-DPD, RV free wall thickening, and tricuspid valve thickening and dysfunction. Within our cohort AApoAIV-amyloidosis always presented with grade 0, and characteristic CA on CMR. AL-amyloidosis grade 1 patients had characteristic CMR features of CA (n=48,100%), while only ATTR-amyloidosis grade 1 patients with the Ser77Tyr variant had characteristic CA on CMR (n=5,11.4%). Following exclusion of Ser77Tyr and AApoAI, a CMR showing characteristic CA or an extracellular volume >0.40 had a sensitivity and specificity of 100% for AL-amyloidosis.

Summary and conclusions: Deep characterisation of the cardiac phenotype in different types of amyloidosis, across a range of ^{99m}Tc-DPD cardiac uptake grades has identified clear differences between each amyloidosis type. The distinctive characteristics in each cohort has allowed the development of a diagnostic pathway to help define the diagnostic differentials and the clinical phenotype in each individual patient, following comprehensive assessment with ^{99m}Tc-DPD scintigraphy and CMR.

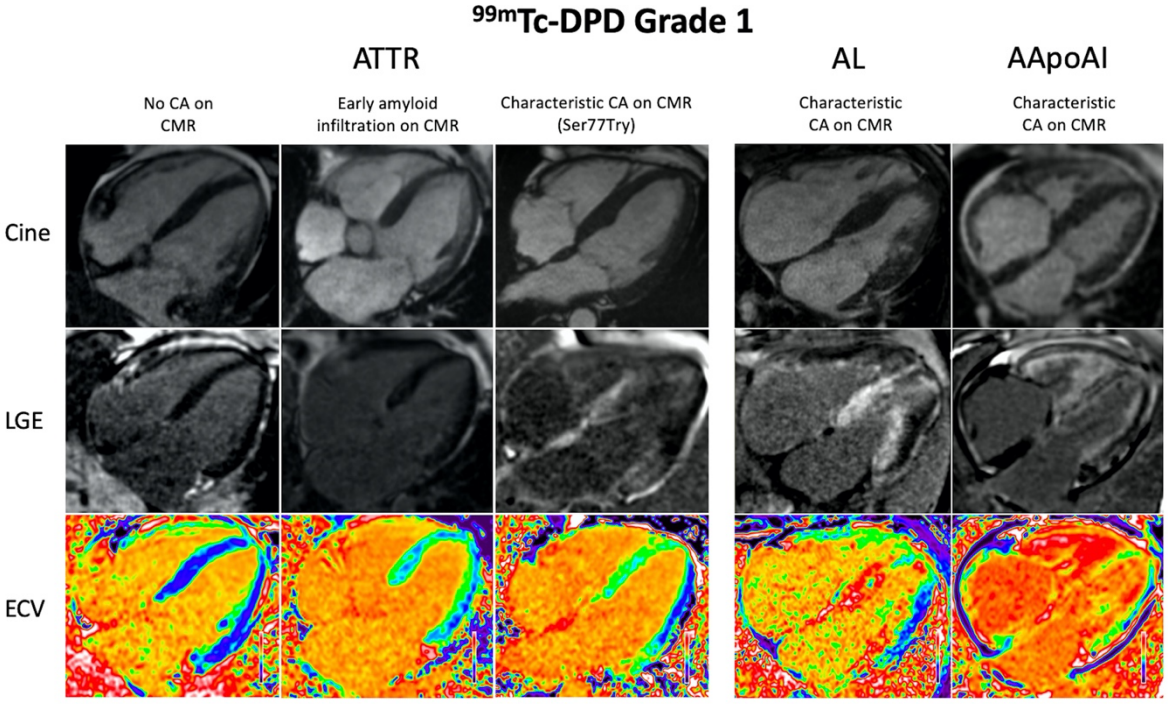

Figure 1. Cardiac magnetic resonance imaging in grade 1 ^{99m}Tc-DPD cardiac uptake

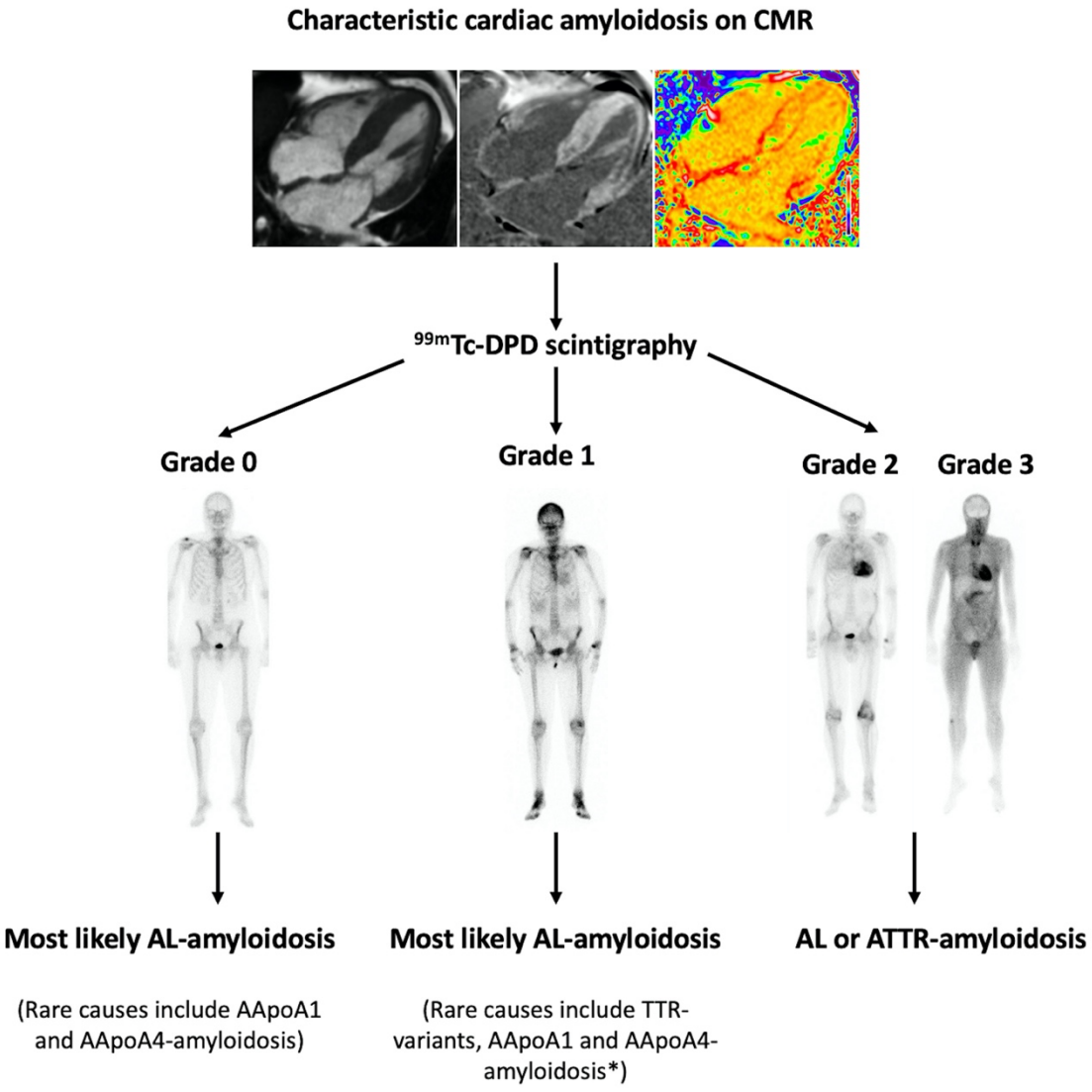

Figure 2. Diagnostic pathway for patients with characteristic features of cardiac amyloidosis on CMR. *Although there were no cases in our study cohort, there have been sparse reports of mild myocardial tracer uptake in AApoA4-amyloidosis.

Prognostic Implications of Clinical Phenotype and Severity of Cardiac Involvement in Patients Presenting with Immunoglobulin Light Chain Amyloidosis

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Background: Patients with systemic immunoglobulin light chain (AL) amyloidosis may present with a wide array of signs and symptoms due to the multi-systemic organ involvement. The presence of cardiac involvement is the key determinant of survival. Cardiac magnetic resonance (CMR) has the unique ability to measure the continuum of cardiac amyloidosis (CA) infiltration providing a deep characterisation from early CA involvement to severe degree of CA burden.

Objective: The aim of this study was to characterise the clinical profiles and the severity of organ involvement in patients presenting with AL amyloidosis and to investigate implications for long-term outcome.

Material & Methods: Patients newly diagnosed with AL amyloidosis at the National Amyloidosis Centre underwent comprehensive clinical, laboratory and instrumental work up, including CMR imaging with evaluation of left ventricular (LV) mass, late gadolinium enhancement (LGE) and extracellular volume (ECV). Patients' clinical phenotype was classified according to the symptoms at presentation: heart failure (cardiac phenotype), renal impairment (renal phenotype) and other symptoms ("other" phenotype). The presence and severity of CA was investigated by "CMR grade" as: 0= no features of CA (normal LV mass, no LGE and normal ECV); 1=early cardiac amyloid infiltration (normal LV mass, raised ECV no LGE); 2= characteristic of CA on tissue characterisation with normal mass (diffuse subendocardial or transmural LGE, altered gadolinium kinetics and raised ECV); 3= characteristic of CA on tissue characterization with elevated mass (diffuse subendocardial or transmural LGE, altered gadolinium kinetics and raised ECV). The main outcome was all-cause mortality.

Results: The study population included 453 AL patients presenting with cardiac (24.1%, n=109), renal (29.4%, n=133), cardiac and renal (31.8%, n=144) and other (14.8% n=67) phenotypes. During a median follow up of 32 (IQR 6-53) months, cardiac phenotype either in isolation or in combination with renal phenotype was associated with a higher rate of all-cause mortality compared to the others ($p<0.001$) (Figure 1). On CMR imaging, 36.1% (n=70/194) of patients without cardiac phenotype at presentation had characteristic features of CA (CMR grade 2 and 3) vs 12.2% (n=30/245) of patients with cardiac phenotype had no features of CA (CMR grade 0) ($p<0.001$). With Kaplan Meier analysis, the risk of all-cause death increased in patients with cardiac phenotype alongside with CMR score (both $p<0.001$) (Figure 2). At multivariable analysis, age at diagnosis (hazard ratio [HR] 1.03, $p<0.001$), clinical phenotype at presentation (HR 1.19, $p=0.048$) and ECV (per each 5% increase) measured by CMR (HR 11.35, $p<0.001$) emerged as independent prognostic parameters.

Summary & Conclusion: Patients with newly diagnosed AL amyloidosis present most frequently

with renal and cardiac phenotypes. CMR detects CA in >35% of patients with non-cardiac phenotype. ECV is an independent predictor of all-cause mortality across the full clinical spectrum of AL amyloidosis.

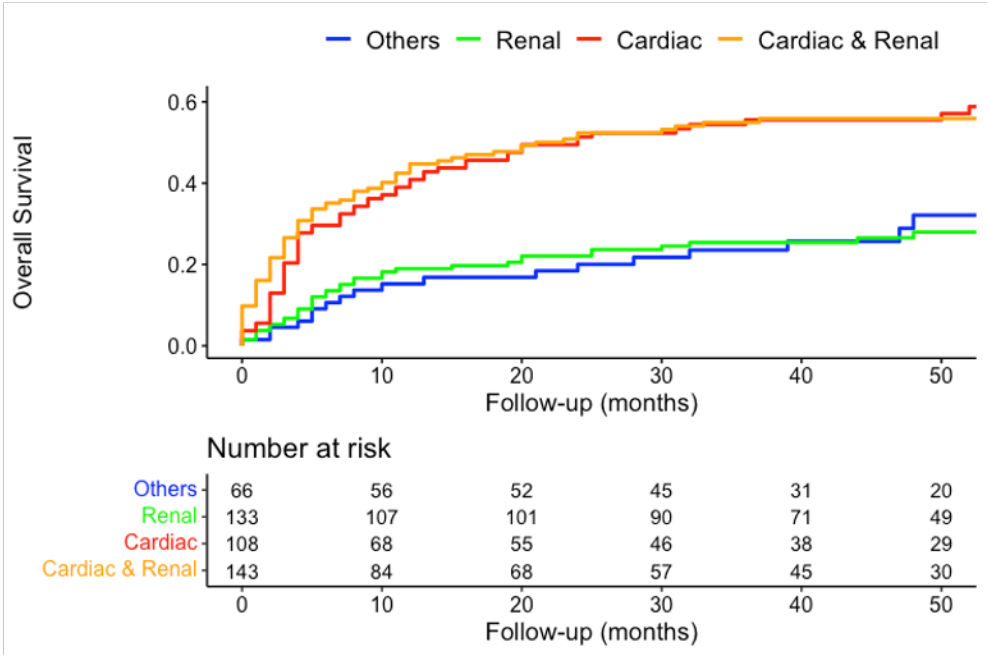

Figure 1.: Overall survival in newly diagnosed light chain amyloidosis according to the clinical phenotype at presentation.

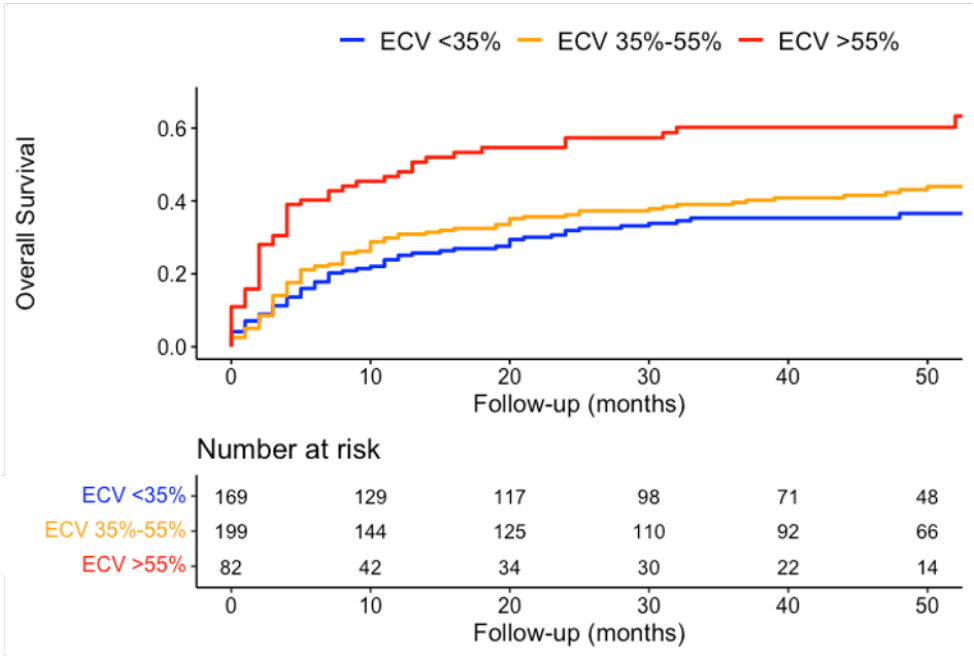

Figure 2.: Overall survival in newly diagnosed light chain amyloidosis according to the extent of cardiac involvement measured by ECV at cardiac magnetic resonance. Legend: ECV, extracellular volume.

Support & Funding: M Fontana is supported by a British Heart Foundation Intermediate Clinical Research Fellowship (FS/18/21/33447).

Diagnostic performance characteristics of quantitative and semi-quantitative parameters of Tc^{99m} pyrophosphate imaging for diagnosis of transthyretin (ATTR) cardiac amyloidosis: The SCAN-MP Study

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Background: Transthyretin cardiac amyloidosis (ATTR-CA) can be diagnosed non-invasively, without a requirement for cardiac biopsy, by combining imaging with Tc99m pyrophosphate (PYP) scintigraphy and concurrent assessment for monoclonal proteins[1]. While now accepted as standard of care[2], validation studies of this methodology were conducted in referral populations with a high prevalence of ATTR-CA[1,3]. The optimal heart-to-contralateral chest (H/CL) ratio threshold for non-invasive diagnosis of ATTR-CA using PYP imaging in a population with low pretest probability is not known.

Objective: The SCAN-MP (Screening for Cardiac Amyloidosis with Nuclear Imaging in Minority Populations) study is a prospective cohort study designed to define the prevalence of ATTR-CA in older adult (age >60 years) Black or Caribbean Hispanic patients with heart failure and an increased wall thickness but without clinically diagnosed ATTR-CA. We report findings from the first 229 patients in SCAN-MP who underwent PYP imaging at 3 hours after tracer injection.

Material & Methods: Using myocardial PYP retention by SPECT as the reference standard, we evaluated the diagnostic performance of different semi-quantitative and quantitative (H/CL ratio) planar parameters obtained from 3-hr PYP imaging in SCAN-MP. Planar PYP imaging at 3 hours was followed by SPECT if the semi-quantitative cardiac uptake (Perugini grade) was 2 or greater on the planar images to clarify myocardial versus blood pool uptake. Differences in test performance thresholds were compared by ROC analysis with Youden index to determine the optimal discrimination threshold and by logistic regression. The sensitivity, specificity, positive predictive value, negative predictive values, false positive and false negative rates of both semi-quantitative and quantitative parameters used to diagnose ATTR-CA were determined.

Results: The prevalence of ATTR-CA in this subset of patients enrolled in SCAN-MP was 6.1%. Demographic, clinical and imaging characteristics are listed in **Table 1**. Patients with ATTR-CA were older, had lower left ventricular ejection fraction, lower left ventricular stroke volume and thicker left ventricular walls. Semi-quantitative grade 0 uptake was observed in 77% of scans. All grade 3 scans were adjudicated as ATTR-CA (false positive rate was 0%). There were 11 scans interpreted as grade 2, but ATTR-CA was diagnosed in only 4 (36%). Test characteristics for discrimination of ATTR-CA employing different H/CL thresholds from 1.3 – 1.5 or semi-quantitative grades 2 to 3 can be found in **Table 2**. An H/CL threshold of 1.4 resulted in a false positive rate of 0.47% with specificity of 99%, but at a cost of missing 1 case (7% false negative rate). By comparison, the H/CL threshold of 1.3 or a semi-quantitative score of 2 or 3 did not miss any cases of ATTR-CA but each criterion was associated with a false positive rate of 4.2% and 3.3% respectively. ROC analysis demonstrated that H/CL thresholds of 1.3, 1.4 and 1.5 were associated with Youden indices of 0.96, 0.93 and 0.87 respectively (**Figure 1**).

Summary & Conclusion: We demonstrate the diagnostic performance of PYP imaging in a low-prevalence but at-risk older, minority patient cohort with heart failure. Our data confirm that planar grade 2 scan results should be interpreted with caution and we demonstrate that the current guideline-suggested diagnostic H/CL threshold of 1.3 was limited by its false positive rate. Further expansion of our cohort will determine the optimal combination of imaging metrics to diagnose ATTR-CA.

Table 1. Demographic, clinical and imaging characteristics of the study population.

Variable	Negative for ATTR-CA (n=215, 93.9%)	Positive for ATTR-CA (n=14, 6.1%)	p-value
Age	72.5±8.8	82.5±9.1	<0.01
Male sex	103 (47.9%)	9 (64.3%)	0.23
Black race	180 (95.7%)	14 (100%)	1.0
Weight (Kg)	90.3±21	81.1±16.1	0.11
Body mass index	33±7	29.6±5.2	0.08
NYHA Class			0.20
Class I	54 (25.2%)	1 (7.1%)	
Class II	110 (51.4%)	7 (50%)	
Class III	46 (21.5%)	6 (42.9%)	
Class IV	4 (1.9%)	0 (0%)	
eGFR	64.1±25	52.1±21	0.08
LVEF (%)	61.7±9.7	53.2±13.4	<0.01
LV stroke volume (ml)	72.7±21.9	59.1±21.5	0.03
Septal wall thickness (mm)	13±2	16±3	<0.01
Posterior wall thickness (mm)	13±2	15±3	<0.01
E/e'	15.1±6.4	23.4±9.3	<0.01
Global longitudinal strain (%)	-14.4±3.9	-11.5±3	0.02
Underwent SPECT			
H/CL ratio	1.1±0.09	1.6±0.17	<0.01
Semi-quantitative grade			<0.01
Grade 0	167 (77.7%)	0 (0%)	
Grade 1	41 (19.1%)	0 (0%)	
Grade 2	7 (3.3%)	4 (28.6%)	
Grade 3	0 (0%)	10 (71.4%)	

NYHA indicates New York Heart Association; eGFR, estimated Glomerular Filtration Rate; LVEF, Left Ventricular Ejection Fraction; SPECT, Single Photon Emission Computed Tomography; H/CL ratio, Heart to Contralateral Chest ratio

Continuous variables are displayed as mean +/- standard deviation

Table 2. Diagnostic characteristics of various H/CL ratios and semi-quantitative grades

	H/CL ≥ 1.3	H/CL ≥ 1.4	H/CL ≥ 1.5	Grade 3	Grade 2 or 3
Sensitivity	100%	93%	79%	73%	100%
Specificity	96%	99%	100%	100%	97%
False Positive Rate	4.2%	0.47%	0%	0%	3.26%
False Negative Rate	0%	7%	21%	27%	0%
PPV	60%	93%	100%	100%	67%
NPV	100%	99%	99%	98%	100%

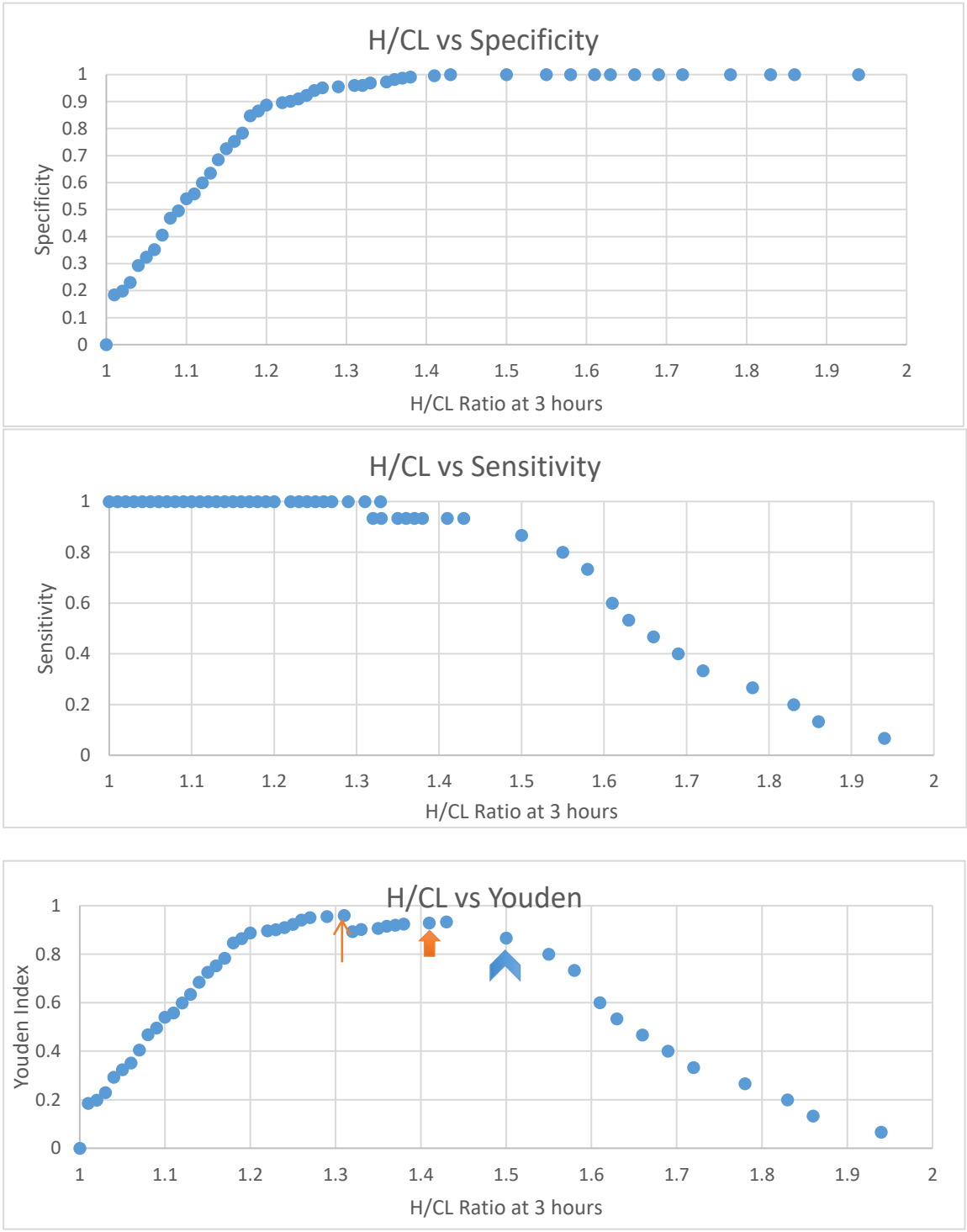

Figure 1. Specificity, Sensitivity and Youden indices associated with different heart to contralateral chest (H/CL) ratio thresholds. X-axis demonstrates H/CL ratios ranging from 1 to 2. Y-axis demonstrates specificity, sensitivity and Youden indices in panels 1, 2 and 3 respectively. The 3 arrows in panel 3 highlight Youden indices of 0.96, 0.93 and 0.87 associated with H/CL ratios of 1.31, 1.41 and 1.5 respectively.

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DPD scintigraphy – a biomarker of microcalcifications rather than amyloid

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Background: Diagnosis of transthyretin (ATTR) amyloidosis is often difficult, particularly in the wild-type (wt) form where deposits may be very limited in peripheral tissues. Scintigraphy with technetium-labelled bone markers has become an increasingly used method to identify patients with cardiac involvement of transthyretin (ATTR) amyloidosis. By unknown mechanisms, hearts with such deposits bind the ligands more strongly and rapidly than the skeleton (1). It has been speculated that ATTR fibrils themselves cause the affinity but a clear explanation has not been given.

Objective: The aim of the study was to investigate ATTRwt hearts extensively for calcifications.

Material & Methods: Blocks from the heart of three patients with extensive ATTRwt amyloidosis and one with systemic AL amyloidosis, remaining after a thorough study of the conductive system (2) were used. Adjacent sections from all parts of the hearts were stained with Congo red, immunolabelled for ATTR or stained according to von Kóssa (3). Selected sections were used for autoradiography with [^{99m}Tc]-DPD. A selected area of one ATTR heart block was re-imbedded in epon and processed for transmission electron microscopy (EM), including immune EM. Finally, some markers for autophagocytosis were used.

Results: Irregularly distributed clouds of myriads of microcalcifications were identified, particularly in the atrio-ventricular area of each ATTR heart (Fig 1 A, B) and to lesser degree in the AL heart. Interestingly, only much fewer microcalcifications were seen more peripherally in the ventricles or in the atria. The clouds were seen as often in connective tissue as in amyloid areas and amyloid deposits were frequently free of any calcifications. The microcalcifications were roundish and tiny, usually below 1-2 µm in diameter. They clearly bound [^{99m}Tc]-DPD (Fig 1C). Markers for autophagocytosis as well as iron were positive in some of the particles Their submicroscopic appearance seemed remarkably structured, often surrounded by a membrane-like coat, followed by different layers. Clear regions, probably indicating material lost during preparation, were prevalent (Fig 1D).

Summary & Conclusions: Bone scintigraphy with [^{99m}Tc]-DPD does not label the amyloid deposits but seem to reflect a disease-related process yet to be explored. Consequently, this kind of scintigraphy is a surrogate marker for amyloid and further studies are needed to determine to which degree the amount of microcalcifications really reflect the amount of amyloid.

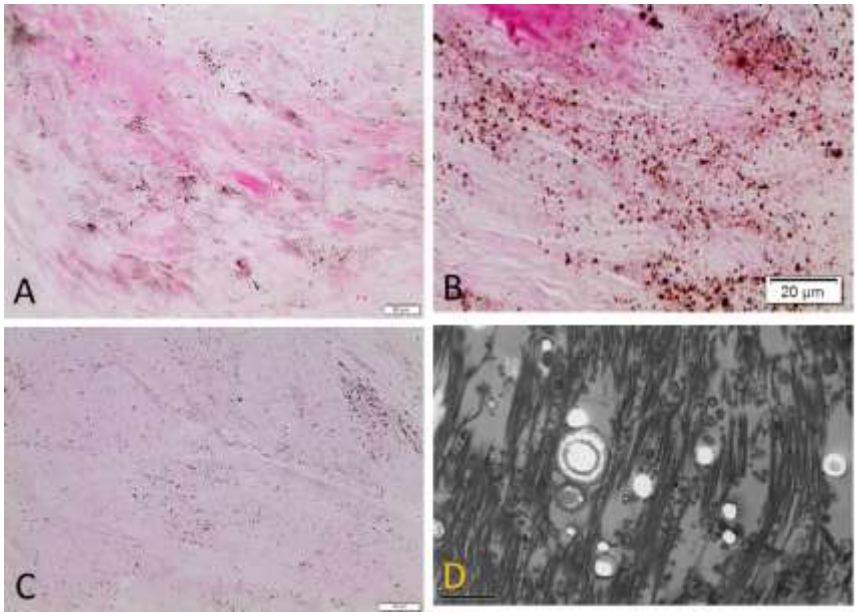

Figure 1. A. Von Kóssa staining showing clouds of very small calcifications in the atrioventricular area of a heart with ATTRwt amyloid. B. The same phenomenon at higher magnification. C. Autoradiography with [^{99m}Tc]-DPD

showing labelling of the small calcifications. E. Transmission electron microscopic picture from an area with calcifications. Bar in A and C, 50 µm, in B, 20 µm and in D, 1 µm.

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Prognostic Role of Echocardiographic Right Ventricular Parameters in Patients with Wild-Type ATTR Cardiac Amyloidosis

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Background:
Right ventricular (RV) involvement in AL amyloidosis has been reported to portend a poor prognosis. We previously demonstrated that RV functional and structural abnormalities are common in wild-type transthyretin (ATTRwt) cardiac amyloidosis. However, there is limited data on the prognostic role of RV involvement in ATTRwt cardiac amyloidosis.

Objective:
To determine the prognostic role of right ventricular parameters obtained by transthoracic echocardiography in predicting mortality in ATTRwt cardiac amyloidosis.

Material & Methods:
A total of 116 patients with confirmed ATTRwt cardiac amyloidosis between January 1, 2018 to December 31, 2018from Mayo Clinic, Rochesterwere retrospectively identified and transthoracic echocardiograms obtained within 90 days of diagnosis were reviewed. Conventional echocardiographic measurements and right heart measurements, including dimension, thickness, area, fractional area change and free wall longitudinal strain were analyzed. Patients who were deceased at the end of follow-up period (February 1, 2022) were recorded as the outcome event. Survival analysis was performed using Cox regression univariate analysis.

Results:
There were 29 death events observed at the end of the follow-up period. Median values of NT-proBNP, left ventricular ejection fraction and RV parameters in the event and non-event groups are summarized in Table 1. The median value of RV end-diastolic area was 23.90 (21.00, 28.00) cm^2 in the non-event group and 28.00 (24.20, 32.20) cm^2 in the event group. There was a non-statistically significant trend for a reduced RV strain in the event group. Survival analysis (Table 2) was notable for a statistically significant association with increased RV end-diastolic area [HR 1.12 (CI 1.03-1.20); p=0.005] and RV end-systolic area [HR 1.11 (CI 1.01-1.21); p=0.031]. RV linear dimensions, wall thickness and strain were not associated with an increased incidence of death. The study results could be limited due to the small sample and event size.

Summary & Conclusion:
Right ventricular structural and functional abnormalities are common in ATTRwt cardiac amyloidosis. Increased RV end-diastolic area and RV end-systolic area were associated with an increased incidence of death. Our findings highlight the need for larger studies or studies with multimodality imaging to determine the prognostic ability of RV data in ATTRwt amyloidosis.

Table 1:

Variable	Non-Event Group (N=72), median (Q1, Q3)	Event Group (N=29), median (Q1, Q3)
NT-proBNP, pg/ml	1831.00 (876.25, 3436.25)	3484.50 (1824.00, 7132.00)
LV Ejection Fraction	54.00 (43.50, 58.00)	52.50 (42.25, 61.75)
RV Dimensions, mm		
Base	44.50 (37.75, 48.00)	44.00 (39.50, 52.00)
Mid	30.00 (26.00, 37.00)	36.50 (31.25, 42.50)
Length	79.00 (72.00, 85.00)	81.00 (74.00, 87.75)
RV Free Wall Thickness, mm	8.00 (7.00, 9.00)	8.00 (7.00, 10.00)
RV End-Diastolic Area, cm^2	23.90 (21.00, 28.00)	28.00 (24.40, 32.20)
RV End-Systolic Area, cm^2	16.00 (12.25, 18.00)	18.00 (15.20, 20.00)
RV Fractional Area Change, %	35.50 (31.00, 41.50)	34.00 (27.80, 40.50)
RV Strain, %	-17.00 (-21.00, -14.00)	-13.000 (-17.50, -10.75)
Right Atrial Area, cm^2	24.50 (21.75, 28.50)	24.80 (21.50, 33.00)
RV S', m/s	0.10 (0.08, 0.12)	0.09 (0.07, 0.11)
TAPSE, mm	15.50 (13.00, 18.25)	15.00 (14.00, 17.00)
RV Systolic Function		
Normal	22 (32.8%)	6 (23.1%)
Mild	24 (35.8%)	5 (19.2%)
Moderate	21 (31.3%)	15 (57.7%)
Estimated RV Systolic Pressure, mmHg	38.50 (32.00, 47.250)	40.00 (34.75, 52.25)
TR Gradient, mmHg	29.00 (25.00, 34.25)	27.00 (25.00, 36.25)
TR Severity		
Mild	39 (58.2%)	13 (50.0%)
Moderate	24 (35.8%)	10 (38.5%)
Severe	4 (6.0%)	3 (11.5%)

Table 2:

Variable	Hazard Ratio (Confidence Interval)	P-value
NT-proBNP	1.00 (1.00-1.00)	0.008
LV Ejection Fraction	1.00 (0.97-1.04)	0.967
RV Dimensions		
Base	1.04 (0.98-1.10)	0.179
Mid	1.03 (1.00-1.06)	0.033
Length	1.03 (0.99-1.07)	0.195
RV Free Wall Thickness	1.04 (0.80-1.35)	0.770
RV End-Diastolic Area	1.12 (1.03-1.20)	0.005
RV End-Systolic Area	1.11 (1.01-1.21)	0.031
RV Fractional Area Change	0.98 (0.94-1.02)	0.308
RV Strain	1.07 (0.96-1.19)	0.199
Right Atrial Area	1.06 (0.99-1.12)	0.091
TAPSE	0.99 (0.88-1.11)	0.853
RV Systolic Function		
Mild Dysfunction	0.81 (0.25-2.66)	0.731
Moderate Dysfunction	2.24 (0.87-5.79)	0.094
Estimated RV Systolic Pressure	1.02 (0.98-1.06)	0.305
TR Gradient	1.00 (0.95-1.05)	0.922
TR Severity		
Moderate	1.21 (0.53-2.76)	0.653
Severe	2.34 (0.66-8.23)	0.185

Table 1: Comparison of variables between event (death) and non-event groups using Kruskal-Wallis Rank Sum Test and Pearson's Chi-Squared Test. LV: Left Ventricle, NT-proBNP: N-terminal Pro B-Type Natriuretic Peptide, RV: Right Ventricle, TAPSE: Tricuspid Annular Planar Systolic Excursion, TR: Tricuspid Regurgitation.

Table 2: Cox Regression Models for Survival (Confidence Interval level=0.95). LV: Left Ventricle, NT-proBNP: N-terminal Pro B-Type Natriuretic Peptide, RV: Right Ventricle, TAPSE: Tricuspid Annular Planar Systolic Excursion, TR: Tricuspid Regurgitation.

References: Bodez D et al, Prognostic value of right ventricular systolic function in cardiac amyloidosis. *Amyloid*. 2016 Sep;23(3):158-167. PMID: 27348696.

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False positive bone-scintigraphy in elderly hypertrophic cardiomyopathy

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Background: Differential diagnosis of cardiomyopathies with hypertrophic phenotype is crucial because some secondary forms of cardiac hypertrophy can be susceptible of specific treatment aimed to revert and/or stabilize the increase in wall thickness. A positive nuclear scintigraphy with hydroxy bisphosphonate bone tracer (99mTc-HPD) is believed to have high sensitivity (>99%) and specificity (91%) for the diagnosis of transthyretin (TTR) amyloid cardiomyopathy[1].

Objective: We report the case of an old patient with increased thickness of ventricular walls and a positive bone scintigraphy, who was unexpectedly found not affected by cardiac TTR amyloidosis at left ventricular endomyocardial biopsy.

Material & Methods: An 85-year-old man with systemic arterial hypertension was admitted because of pre-syncope associated to evidence at Holter monitoring of both bradiarrhythmias (1st degree atrioventricular block with PR interval of 300 ms and several episodes of 2nd degree Mobitz type 1 atrioventricular block with electrical pause greater than 3 s) and tachyarrhythmias. He suffered from a bilateral carpal tunnel syndrome that underwent surgery 2 years earlier. Routine laboratory tests were normal except for elevated cardiac troponin HS (0.295 mcg/L, nv < 0.014 mcg/L) and N-terminal pro-B-type natriuretic peptide (384.3 pg/mL, nv = 0–254 pg/mL). Because of clinical suspicion of senile amyloidosis, nuclear scintigraphy with hydroxy bisphosphonate bone tracer (99mTc-HPD) was performed, that revealed a grade 3 myocardial uptake. Serum-urine immunofixation and immunoglobulin free light-chain was negative for monoclonal gammopathy. Neurological examination was unremarkable. Genetic test did not show TTR mutation. Thus, a diagnosis of wild-type TTR cardiac amyloidosis was suggested. The patient, in view of Tafamidis treatment, after informed consent, underwent a LV endomyocardial biopsy.

Results: Six specimens were drawn from the LV (3–5 mm2 each), cut and processed for histology, immunohistochemistry, and transmission electron microscopy. Unexpectedly, histology failed to confirm amyloid deposition but revealed the presence of severely hypertrophied disarrayed cardiomyocytes fragmented in short runs by interstitial and replacement fibrosis, denoting a sarcomeric hypertrophic cardiomyopathy. Congo red staining at polarized light scored negative for areas of apple-green florescence. Immunohistochemistry for transthyretin, kappa/lambda light chain and SAA was negative. Transmission electron microscopy failed to demonstrate the presence of rigid unbranched amyloid fibrils. Thus, a final diagnosis of hypertrophic cardiomyopathy was reached.

Summary & Conclusion: A positive cardiac signal of bone scintigraphy can occur in other pathologic settings. Future studies are needed to investigate the incidence of false-positive TTR cardiac amyloidosis to bone scintigraphy compared with endomyocardial biopsy.

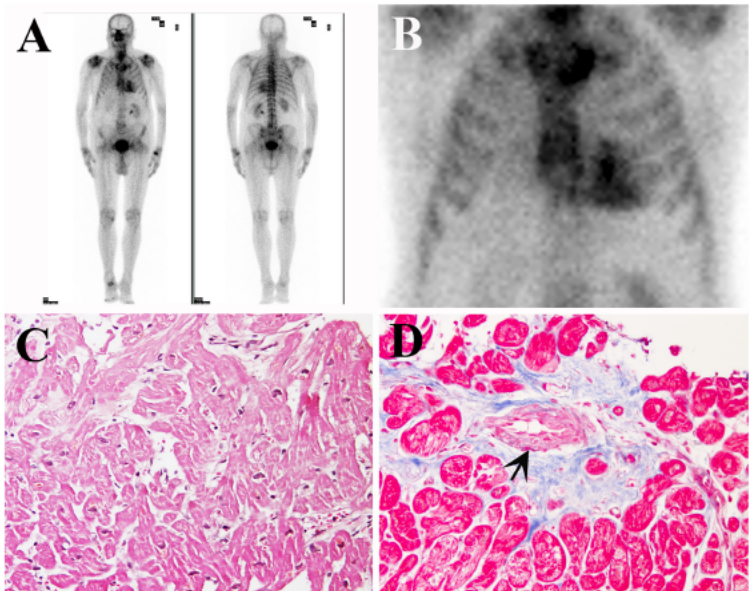

Figure 1.: Bone scintigraphy and LV Endomyocardial biopsy findings. (A, B) Whole body (A) and planar (B) images of the chest 90 minutes after 360 MBq of 99mTc diphosphonates i.v. administration, showing an evident accumulation of the radiopharmaceutical in the cardiac region (Perugini score 3). (C–D) LV endomyocardial biopsy revealing the presence of severely hypertrophied and disarrayed cardiomyocytes (C) interrupted in short runs by interstitial and replacement fibrosis, with severe lumen narrowing of a small artery due to hypertrophy and hyperplasia of smooth muscle cells (D).

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Dual-echo turbo-spin-echo and 12-echo multi-spin-echo sequences are equivalent techniques for obtaining T2-Relaxometry data in hereditary transthyretin amyloidosis

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Background: T2-Relaxometry, a quantitative magnetic resonance neurography (MRN) technique, has been successfully used to characterize and quantify different diffuse neuropathies in recent years. Typically, multi spin echo (MSE) sequences are applied for obtaining T2-relaxometry data as they provide defined echo times (TEs). However, due to their time-consuming acquisition, they are frequently replaced by turbo spin echo (TSE) sequences that in turn bear the risk of systematic errors when analyzing small structures such as peripheral nerve fascicles or lesions within nerve fascicles.

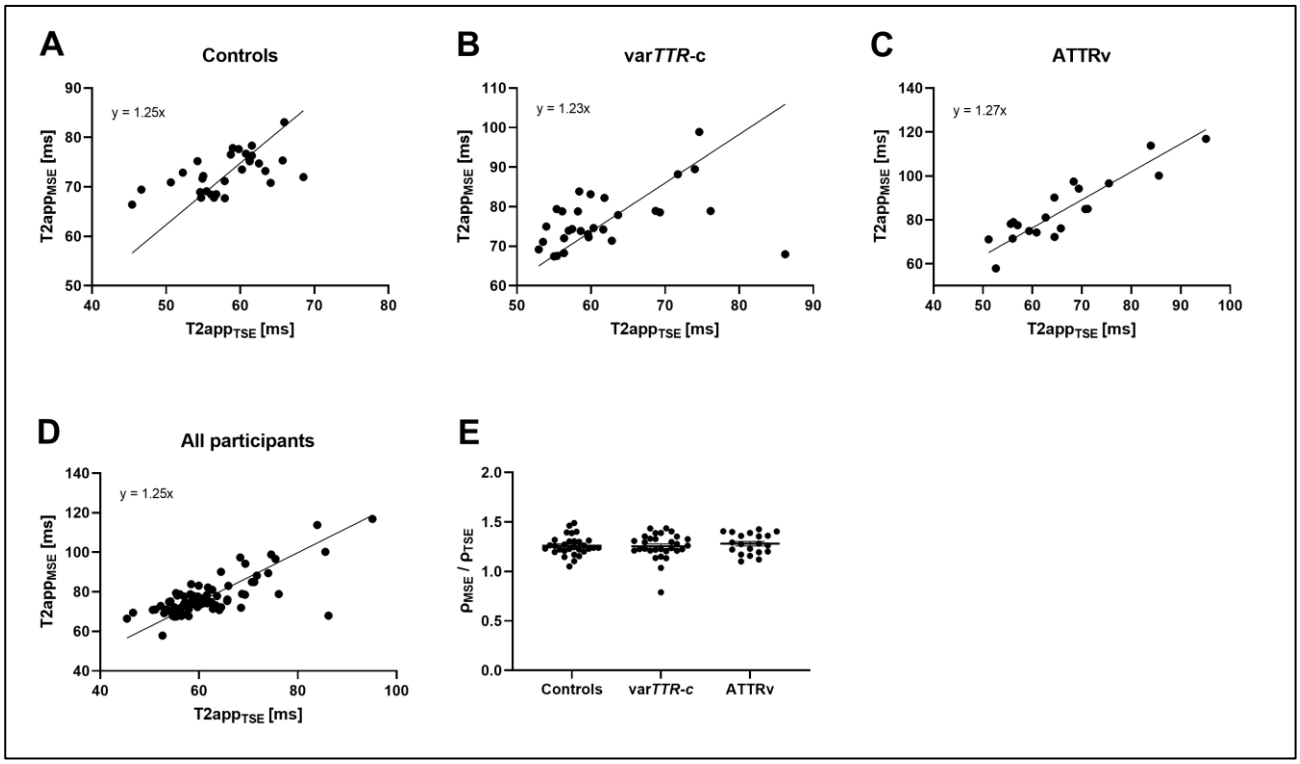
Objective: To test whether T2-relaxometry data derived from either dual-echo TSE or 12-echo MSE sequences are equivalent for quantifying peripheral nerve lesions in hereditary transthyretin (ATTRv) amyloidosis. ATTRv amyloidosis is an ideal surrogate disease for this approach, as it allows the inclusion of both asymptomatic carriers of the variant *transthyretin* gene (var*TTR*) and symptomatic ATTRv amyloidosis patients.

Material & Methods: 20 clinically symptomatic ATTRv amyloidosis patients (4 females, 16 males; mean age, 61.8 years; range, 33-76 years), 30 asymptomatic var*TTR*-carriers (18 females, 12 males; mean age, 43.1 years; range, 21-62 years), and 30 healthy volunteers (13 females, 17 males, mean age 41.3 years, range 22-73) were prospectively included and underwent 3 Tesla magnetic resonance neurography. T2-relaxometry was performed at the right mid to lower thigh by acquiring an axial 2-dimensional dual-echo TSE sequence with spectral fat saturation (TE1/TE2 = 12/73 ms, repetition time 5210 ms, acquisition time 7:30min), and an axial 2-dimensional MSE sequence with spectral fat saturation and with 12 different TE (TE1 = 10 ms to TE12 = 120 ms, ΔTE = 10 ms, repetition time 3000 ms, acquisition time 11:23 min). Sciatic nerve regions of interest were manually drawn on 10 central slices per participant and sequence, and the apparent T2-relaxation time ($T2_{app}$) and proton spin density (ρ) were calculated individually from TSE and MSE relaxometry data.

Results: Linear regression showed that T2app values obtained from the dual-echo TSE ($T2_{app}$TSE), and those calculated from the 12-echo MSE ($T2_{app}$MSE) were mathematically connected by a factor of 1.3 throughout all three groups (controls, var*TTR*-carriers, symptomatic ATTRv amyloidosis), whereas a factor of 0.5 was identified between respective ρ values. $T2_{app}$ calculated from both TSE and MSE, distinguished between symptomatic ATTRv and controls ($T2_{app}$TSE p=0.0028; $T2_{app}$MSE p<0.0001), whereas differences between var*TTR*-carriers and ATTRv amyloidosis were only observed for $T2_{app}$MSE (p=0.0082). Sciatic nerve ρ differentiated well between healthy controls vs. var*TTR*-carriers (ρTSE p=0.0027; ρMSE p=0.0398) and vs. symptomatic ATTRv amyloidosis (ρTSE and ρMSE p<0.0001, respectively), but also between var*TTR*-carriers vs. ATTRv amyloidosis (ρTSE; p=0.0001; ρMSE, p<0.0001).

Summary & Conclusion: Dual-echo TSE and 12-echo MSE sequences provide equally robust and reliable T2-relaxometry data when calculating $T2_{app}$ and ρ in symptomatic and asymptomatic ATTRv amyloidosis. Therefore, the dual-echo TSE might be proposed as a preferred sequence in future MRN protocols as it requires shorter acquisition times, therewith reducing motion artifacts and increasing cost- and time-efficiency, while also achieving higher image resolution. Both relaxometry techniques allow for the quantification of nerve injury in ATTRv amyloidosis, and can thus contribute to a better understanding of the pathomechanism of amyloid related neuropathies.[1]

Figure 1: Calculated T2appTSE and T2appMSE values of the sciatic nerve were plotted against each other individually for each group (A: controls, B: asymptomatic var*TTR*-carriers, C: symptomatic ATTRv amyloidosis) and cumulative over all groups (D). Note the linear relationship that was identified in each group. A linear regression was respectively pictured by a straight line and the corresponding equation y=ax (y:T2appMSE, x:T2appTSE, a:slope of the straight line). Calculated slopes were 1.25 for healthy controls, 1.23 for asymptomatic varTTR-carriers, and 1.27 for symptomatic ATTRv amyloidosis. Graph E shows the distribution of individual factors resulting from ρMSE / ρTSE for each subject, respectively plotted for each group.



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CARDIAC IMAGING FOR ASSESSING INVOLVEMENT IN AL AMYLOIDOSIS PATIENTS: EXPERIENCE IN A SINGLE TERTIARY HOSPITAL

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Background: Cardiac involvement is the main prognostic factor for patients with immunoglobulin light chain (AL) amyloidosis. In 2012, the Mayo Clinic proposed a prognostic classification system based on the levels of cardiac biomarkers (NT-proBNP and c-TNT) and the differential of serum free light chains (dFLC). This system identifies a group of poor prognosis that corresponds to those patients with advanced cardiac involvement (stage IV) with a median survival of only 6 months, however this prognostic system does not include any cardiac imaging parameter that might add a potential prognostic value. In this regard, imaging-based novel cardiac biomarkers are needed to provide a more precise assessment of the degree of cardiac involvement.

Objective: To describe the degree of cardiac involvement in newly-diagnosed AL amyloidosis patients in a tertiary care hospital based on cardiac imaging (echocardiography and cardiac magnetic resonance (CMR)) and to investigate a pattern that could provide a more accurate risk-assessment.

Material & Methods: The database of the Hematology Department at the University Hospital of Salamanca, Spain, that includes patients from 1999 to 2022, was retrospectively analyzed, and AL amyloidosis patients who had a baseline echocardiogram were selected. We identified 41 patients, 21 of them also had baseline CMR. In the first part of the study cardiac imaging parameters were collected for a descriptive analysis of cardiac involvement. In the second part of the study, hematological parameters, treatments and outcomes were also collected.

Results: A total of 41 patients with a median age of 66 years [54-76] were studied. Twenty-two were men (53.7%) and 19 were women (46.3%). 37 patients were classified according to the 2012 Mayo Clinic risk stratification scale: Stage I, 2 patients (5.4%); Stage II, 10 (27.07%); in Stage III, 8 patients (21.6%) and in Stage IV, 17 patients (45.94%). Among the echocardiographic parameters, left ventricle (LV) relative thickness was above >0.6 in 24 patients (61.5%) out of a total of 39 who have this parameter, E/e' was >11 in 17 patients (58.6%) of 29 and tricuspid annular plane systolic excursion (TAPSE) <1.9 in 20 patients out of 33 (60.6%) and Vol AI was >=34 in 17 patients out of 30 (56.67%). In CMR, T2 mapping >54 ms was presented in 15 of the 18 patients (83.3%) who have this measure. Regarding extracellular volume (ECV), the upper normal limit (2 DS) was 26%, with 100% of our patients being above this value. For patients with suspected cardiac amyloidosis, the cut-off point was 0.4 (40%) and 12 of 16 patients in whom the ECV was calculated were above this value (75%). The area of the right and left atria was >= 15 in all our patients, meaning that there was atrial dilation in both. We observed 14 patients who presented gadolinium enhancement at diagnosis (66.7%).
A selection of patients who were within Stages III and IV (high-risk groups) was made. According to the echocardiogram parameters, it was observed that 18 (75%) of the high-risk patients had a septum of >12 mm. Fourteen patients (73.7%) had an E/e' >11. As for the TAPSE, in 13 patients (59.1%) it measured <1.9. LV relative thickness was >0.6 in 16 patients (66.7%). The AI Vol was >=34 ml/m2 in 11 patients (52.4%). Regarding CMR, 10 patients had an ECV >0.4 (90.9%) and a T2 >=54 ms, 11 patients (84.5%).

Summary & Conclusion: a descriptive analysis of cardiac imaging parameters in AL amyloidosis patients was obtained. As expected, ECV assessed by CMR was above normal limit in all AL cardiac amyloidosis patients. In advanced cardiac involvement an specific imaging pattern was identified. Analysis of correlation between serum cardiac biomarkers, sFLC, response and multimodal imaging parameters is ongoing and will be presented at the meeting.

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Phenotyping of hypertrophic cardiomyopathies using echocardiography: amyloid, Anderson-Fabry and hypertensive heart disease

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Background: Differentiating between phenotypes of hypertrophic cardiac disease is essential for management and prognostication. Endomyocardial biopsy is gold standard, but is invasive and associated with complications. Echocardiography is economical, safe and widely used. It can be useful as a screening test jSzKto proceed to further testing, such as Cardiac MRI or bone scintigraphy scan. Simple echocardiographic markers may be able to distinguish between different types of infiltrative cardiomyopathy.

Objective: We sought to identify echocardiographic markers that distinguish Cardiac Amyloid (CA), Anderson-Fabry disease (AFD) and hypertensive (HT).

Material & Methods: Patients were recruited from Westmead Hospital, Sydney and Princess Alexandra Hospital, Brisbane. All patients had mean wall thickness ≥11mm. Left ventricular (LV) structure and function parameters, diastolic markers, global (GLS) and segmental longitudinal strain were analysed. Other parameters including relative apical sparing (RAS), LV ejection fraction to strain ratio (EFSR), mass to strain ratio (MSR) and AMYLI score (relative wall thickness*E/e') were evaluated.

Results: We evaluated 209 patients, comprising of 120 CA (58 ATTR, 62 AL), 31 AFD and 58 HT patients (mean age 64.1±13.7yrs (75% male)). LV measurements were significantly different across the three groups (figure 1 - p<0.05). EFSR and MSR differentiated between infiltrative (CA + AFD) vs non-infiltrative (HT) cardiomyopathy (ROC-AUC of 0.80 and 0.91 respectively). RAS and AMYLI score best differentiated between CA vs AFD and HT (ROC-AUC of 0.81 and 0.78 respectively). Linear discriminant analysis was performed with significant variables (LV mass indexed, average e', LV GLS, and basal strain); the resultant model predicted 78.6% of all cases (figure 2). Additionally, MSR was the only parameter that differentiated ATTR and AL CA (mean 13±6.5 vs10.2±5.0 respectively, p=0.006).

Summary & Conclusion: Echocardiographic parameters can be used to differentiate between hypertrophic cardiac phenotypes and have potential utility as a screening tool to guide further investigations. Further validation of these markers are required to confirm these findings.

	Amyloid		Fabry		Hypertension	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	66.6	11.8	46.1	11.0	68.6	10.8
LVEF biplane (%)	53.1	8.2	61.2	8.6	60.8	3.8
MWT (mm)	15.0	2.9	13.7	2.5	12.3	1.0
LVMI (g/m2)	134.7	40.3	139.8	37.1	92.5	17.4
RWT	0.67	0.19	0.55	0.15	0.58	0.09
E/A (m/s)	1.54	1.11	1.35	0.47	0.85	0.24
Average e' (cm/s)	5.0	1.7	7.8	2.0	6.5	1.8
E/e' (cm/s)	17.7	8.8	11.0	3.8	11.3	3.8
LAVI (ml/m2)	51.2	21.9	37.5	13.8	32.4	7.5
GLS (%)	-13.1	3.8	-16.2	3.9	-18.9	2.2
Basal Strain (%)	-8.9	4.1	-14.1	3.5	-16.6	2.3
Mid Strain (%)	-12.3	3.8	-15.6	3.8	-18.6	2.6
Apical Strain (%)	-18.0	5.1	-19.3	5.2	-22.4	4.0
EFSR	4.3	1.1	4.0	1.3	3.3	0.4
MSR	11.6	5.9	9.8	5.9	5.0	1.1
RAS	0.92	0.31	0.66	0.16	0.64	0.12
AMYLI	12.3	7.7	6.1	2.7	6.7	3.0

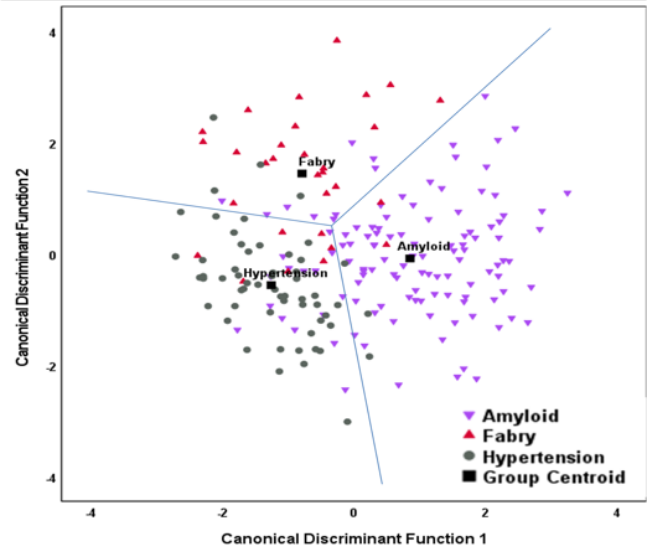

Figure 1. (left): Differences in echocardiographic parameters between amyloid, Anderson-fabry and hypertensive heart disease (p<0.05 for each marker across all groups). *AMYLI score: RWTxE/e', E/A: Mitral inflow E/A velocity ratio, e': Mitral valve tissue doppler e' velocity, EFSR: LVEF to strain ratio, GLS: Global longitudinal strain, LAVI: left atrial volume indexed, LVEF: left ventricular ejection fraction, LVMI: Left ventricular mass indexed, MSR: LV mass to strain ratio, MWT: Mean wall thickness, RAS: relative apical sparing, RWT (relative wall thickness).*

Figure 2. (right): Linear discriminant analysis model derived using significant relevant echocardiographic variables significant variables (LVMI, average e', LV GLS, and basal strain). Linear discriminant functions are plotted against the 3 groups. The model was good at discriminating the 3 cases, correctly predicting 78% amyloid, 70% Anderson-fabry, and 85% hypertensive heart disease.

References

nil

Support & Funding: Nothing to disclose.

Multimodality cardiac imaging for differential diagnosis of infiltrative cardiomyopathy

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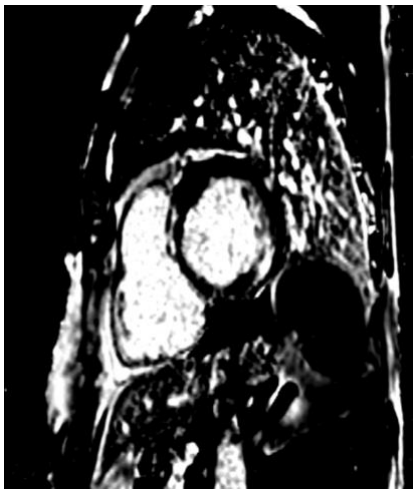
[4] *Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*

Background: Hereditary transthyretin (ATTRv) amyloidosis is a rare disease but the prevalence is higher in Val30Met (p.Val50Met) endemic regions such as parts of Portugal, Sweden, and Japan.[1] The prevalence of cardiac sarcoidosis, an inflammatory infiltrative cardiomyopathy of unknown etiology, is also rare with an annual incidence of approximately 1/100,000 in Sweden.[2] Clinically differentiating early stage cardiac amyloidosis from sarcoidosis may be difficult since both cardiomyopathies may have a similar clinical presentation including heart failure, atrial arrhythmias, and high-degree atrioventricular block. Here, we present a case where both diseases were present and where cardiac magnetic resonance imaging (CMR) was an important part of the diagnostic work-up.

Case Description: A 71-year old male patient with a history of hypertension was seeking medical attention in 2019 because of paroxysmal atrial arrhythmia, shortness of breath, and lower extremity edema. He had previously been hospitalized with pleural and pericardial effusion and cardioverted once. There was a known family history of Val30Met-associated familial amyloid polyneuropathy (FAP). His father was diagnosed with FAP at the age of 73 and died at the age of 77 due to progressive polyneuropathy. Two cousins had previously been diagnosed with FAP and undergone liver transplantation. The patient experienced two years of progressive balance disorder and numbness, as well as pain in feet and hands. Neurophysiological examination showed sensorimotor axonal polyneuropathy and carpal tunnel syndrome on the right side. ECG showed atypical atrial flutter and a left anterior fascicular block. NT-proBNP was markedly elevated (2530 ng/L, ref<194 ng/L) and hs-troponin T was slightly elevated (17 ng/L, ref<15 ng/L). Echocardiography showed enlarged atria, mildly reduced systolic function with left ventricular ejection fraction 50% and a mild septal hypertrophy (13mm). Global longitudinal strain (GLS) was -16% but no apical sparing sign was present. Abdominal fat pad biopsies was negative for amyloid. Genetic testing confirmed the presence of the Val30Met mutation known in the family and ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scan was positive with Perugini grade 3 uptake. Hence a diagnosis of vATTR mixed phenotype with polyneuropathy and early cardiomyopathy was made and the patient started treatment with TTR-stabilizer.
CMR showed no clear signs of cardiac amyloidosis but a late gadolinium enhancement (LGE) pattern in the inferolateral wall and also enlarged mediastinal and hilar lymph nodes that raised suspicion of sarcoidosis. A computer tomography scan was consistent with stage 1 pulmonary sarcoidosis. Further examination with ^{18}fluorodeoxy glucose positron emission tomography−computed tomography (^{18}FDG-PET-CT) showed pathologic uptake in the myocardial wall consistent with the findings on CMR. Endobronchial biopsy of a subcarinal lymph node with pathologic uptake revealed presence of non-necrotizing granuloma. The patient was subsequently diagnosed with cardiac
sarcoidosis and is now treated with corticosteroids

Discussion: This is a rare case of patient with concomitant cardiac amyloidosis and sarcoidosis that after diagnosis received etiological treatment for both diseases. The presence of sarcoidosis in this case could easily have been missed and highlights the importance of a thorough diagnostic work-up including multimodality cardiac imaging and the use of CMR for differential diagnosis.



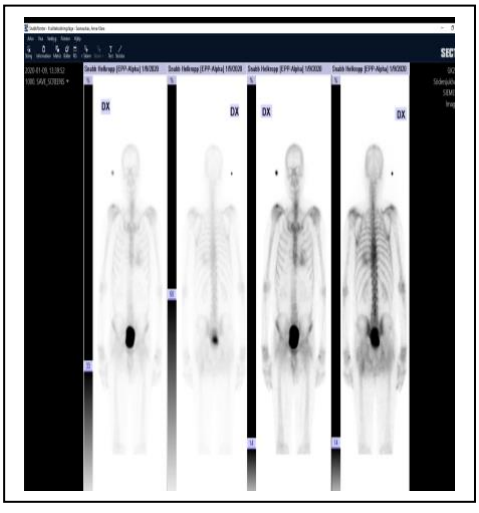


Figure 1. CMR showing no clear signs of cardiac amyloidosis but an LGE-pattern in the inferolateral wall and also enlarged mediastinal and hilar lymph nodes (not clearly visualized here) that raised suspicion of sarcoidosis.

Figure 2. DPD scan showing grade 3 cardiac uptake.

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Support & Funding: None of the authors have relationships relevant to the contents of this paper to disclose.

Quantification of left ventricular amyloid using ^{124}I-p5+14 (AT-01) and ^{18}F-florbetapir positron emission tomography in AL and ATTR amyloidosis

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Background: In light-chain (AL) and transthyretin (ATTR) amyloidosis, methods to quantify left ventricular (LV) amyloid burden are still limited. ^{18}F-florbetapir can image cardiac amyloidosis in positron emission tomography (PET), but with higher uptake in AL than ATTR, despite typically higher amyloid content in ATTR. The peptide p5+14 (AT-01) is a novel amyloid target and was shown to image multiple types of amyloid in mice (as ^{99m}Tc-p5+14) and in humans (as ^{124}I-p5+14).

Objective: This pilot study aims to quantify and compare LV amyloid activity using ^{124}I-p5+14 and ^{18}F-florbetapir PET in participants with AL and ATTR cardiomyopathy (AL-CMP, ATTR-CMP), and in non-amyloid controls.

Material & Methods: Participants with AL-CMP or ATTR-CMP were included based on standard diagnostic criteria with proof of cardiac involvement by imaging and/or biopsy. All AL-CMP and ATTR-CMP participants underwent PET/CT with ^{124}I-p5+14 and with ^{18}F-florbetapir, while controls underwent PET with ^{124}I-p5+14 only. LV cardiac amyloid activity (standardized uptake value, SUV) was calculated for the entire LV volume, defined by voxels with uptake > 2 times that of the blood pool (mean left atrial uptake). LV cardiac amyloid activity volume was estimated as mean LV amyloid activity times LV myocardial volume with activity. LV amyloid SUV values were compared across groups using the Wilcoxon rank-sum test and across tracers using the Wilcoxon signed-rank test (paired).

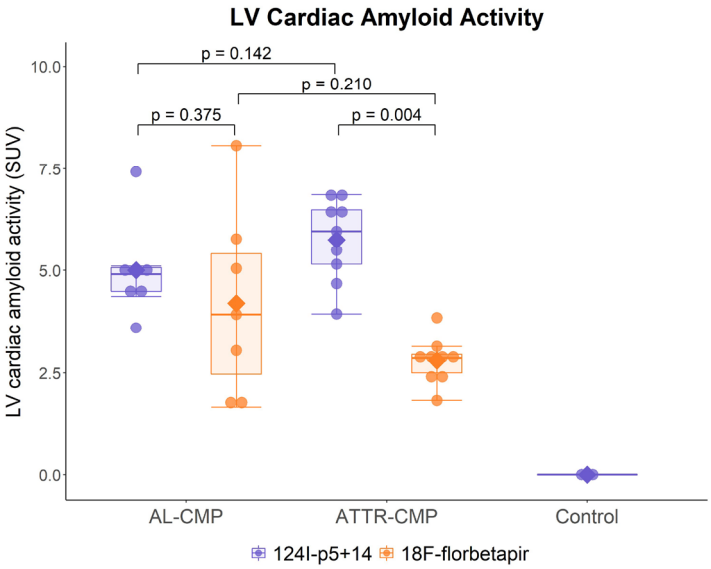
Results: We included 18 participants: 7 AL-CMP (39%), 9 ATTR-CMP (50%), and 2 controls (11%). Median age was 72 years (IQR 66–77) and 16 participants were men (89%). The median ^{124}I-p5+14 and ^{18}F-florbetapir doses were 35.7 MBq (IQR 32.8–39.7) and 273.5 MBq (255.7–315.6), respectively.

^{124}I-p5+14 LV cardiac amyloid activity (SUV) was somewhat higher in ATTR-CMP than in AL-CMP [median 5.96 g/mL (IQR 5.14–6.49) vs. 4.90 g/mL (4.48–5.06); p=0.14, Figure 1]. ^{124}I-p5+14 LV cardiac amyloid activity volume was similar in AL-CMP vs. ATTR-CMP [median 972.5 SUV*ml (IQR 783.4–1085.8) vs. 958.2 SUV*ml (625.0–1252.2), p >0.99]. In controls, no voxels were above 2 times the blood pool, resulting in 0 SUV and 0 SUV*mL.

^{18}F-florbetapir LV cardiac amyloid activity (SUV) was similar in ATTR-CMP and AL-CMP [median 2.85 g/mL (IQR 2.50–2.95) vs. 3.93 g/mL (2.47–5.40); p=0.21, Figure 1], with more spread in AL-CMP. The median ^{18}F-florbetapir LV cardiac amyloid activity volume was similar in ATTR and AL-CMP [median 1143.8 SUV*ml (IQR 870.4–1364.5) vs. 953.3 SUV*ml (749.9–1333.3); p=0.61).

When comparing ^{124}I-p5+14 vs. ^{18}F-florbetapir, LV cardiac amyloid activity was similar among AL-CMP participants (p=0.38), with more spread using ^{18}F-florbetapir. But among ATTR-CMP participants, LV cardiac amyloid activity was significantly higher with ^{124}I-p5+14 (p=0.004). The median ^{124}I-p5+14 and ^{18}F-florbetapir cardiac amyloid activity volumes were similar in ATTR and AL-CMP.

Summary & Conclusion: ^{124}I-p5+14 LV cardiac amyloid activity was similar in ATTR and AL cardiomyopathy, and was higher in amyloidosis participants than in controls, who showed no myocardial uptake above 2 times the blood pool. Compared to ^{18}F-florbetapir, ^{124}I-p5+14 showed significantly higher LV cardiac amyloid activity in ATTR cardiomyopathy, but similar activity volume. Thus, this novel tracer, ^{124}I-p5+14, is potentially valuable for quantification of cardiac amyloid in AL and ATTR cardiomyopathy, and may appropriately provide higher uptake than ^{18}F-florbetapir in ATTR cardiomyopathy. The significance of these findings warrants further study.



Support & Funding: We are extremely grateful to the BWH-Attralus study participants without whom this study would not be feasible. We are grateful for research funding from Attralus, the National Institutes of Health: K24 HL157648 (PI Dorbala S), and International Society of Amyloidosis and Pfizer (Clerc O).

Changes in Left Ventricular Myocardial Composition Following Targeted Plasma Cell Therapy in Light Chain Amyloidosis: A Cardiac Magnetic Resonance Study

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[1] *Cardiac Amyloidosis Program, Brigham and Women's Hospital, Boston, USA*
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[3] *Stanford University, Stanford, USA*

Background: In systemic light chain (AL) amyloidosis, the severity of cardiomyopathy (CMP) is an essential prognostic factor. Plasma cell therapies have substantially improved survival. However, no discernible cardiac changes appear on echocardiography within the first year. Cardiac magnetic resonance (CMR) provides quantitative data on myocardial composition, but whether CMR can detect such changes early after AL therapy is not well known.

Objective: This study aimed to longitudinally analyze myocardial composition on CMR in patients with systemic AL amyloidosis, with and without CMP, at baseline, at 6 months, and at 12 months after targeted plasma cell therapy.

Material & Methods: Participants with newly diagnosed, biopsy-proven systemic AL amyloidosis were enrolled prospectively into groups of AL-CMP or AL-non-CMP, based on cardiac biomarkers and echocardiography. The AL-CMP cohort underwent 3 T CMR at baseline, 6 months, and 12 months after initiation of AL therapy, while AL-non-CMP cohort did so at baseline and 6 months. LV myocardial composition was assessed using CMR cine imaging, native T1 and T2 mapping. Extracellular volume (ECV) was estimated from native and post contrast T1 mapping. Data were compared using Wilcoxon rank-sum test (unpaired) and Wilcoxon signed-rank test (paired). P-values were adjusted for multiple testing using the Holm procedure.

Results: This study included 80 participants: 60 AL-CMP and 20 AL-non-CMP. Median age was 62 years and 57% were males. Among participants, 41 AL-CMP (68%) and 18 AL-non-CMP (90%) underwent ≥1 follow-up visit. In AL-CMP, 13 participants died within 12 months (22%). Using personalized treatment regimens with cyclophosphamide, bortezomib, dexamethasone, and/or daratumumab, 33 AL-CMP (92% of 36) and 13 AL-non-CMP (76% of 18) participants reached very good partial response (VGPR) or complete response (CR) at 6 months. On follow-up, LV mass, LV ejection fraction, and T2 time did not significantly change in either of the groups. However, significant changes were observed in native T1 time, and ECV.

Native T1 time: In AL-CMP (Figure 1), native T1 time increased gradually from baseline to 6 months and to 12 months (baseline median 1200 ms [IQR 1149–1375] vs. 6 months 1293 ms [1223–1400]; p=0.004; and 12 months 1362 ms [1225–1423]; p=0.016 vs. baseline). In AL-non-CMP, T1 increased significantly from baseline to 6 months (median 1191 ms [IQR 1096–1232] vs. 1219 ms [1150–1261], p–0.002).

ECV: In AL-CMP (Figure 2), baseline median ECV was 53.1% (IQR 47.2–58.1) which initially showed an increasing trend at 6 months to 55.7% (49.6–58.4; p=0.18 vs. baseline) and then decreased at 12 months to 53.2% (47.9–55.4; p=0.008 vs. 6 months). In AL-non-CMP, baseline median ECV was 29.8% (IQR 27.5–38.2), which increased at 6 months to 34.5% (29.3–41.1; p=0.009).

Summary & Conclusions: In newly diagnosed systemic AL amyloidosis, gross LV structure and function were abnormal at baseline and remained unchanged at 12 months despite successful therapy in the vast majority. ECV (reflecting interstitial volume) was abnormal at baseline, expanded at 6 months, but decreased at 12 months to baseline values. Yet native T1 times (reflecting myocardial composition) increased gradually from baseline to 6 and 12 months. Together, these findings suggest that, in patients with and without CMP, myocardial composition continuously changes after therapy despite the absence of substantial modifications in interstitial volume, which may indicate remodeling of the interstitium and cardiomyocytes.

Fig1. LV Native T1 Time Over Time

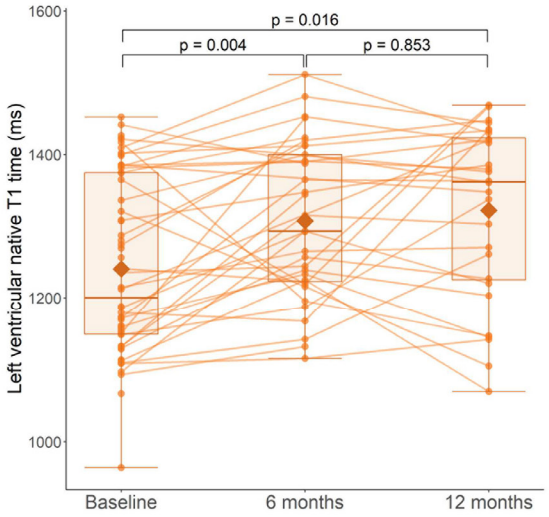
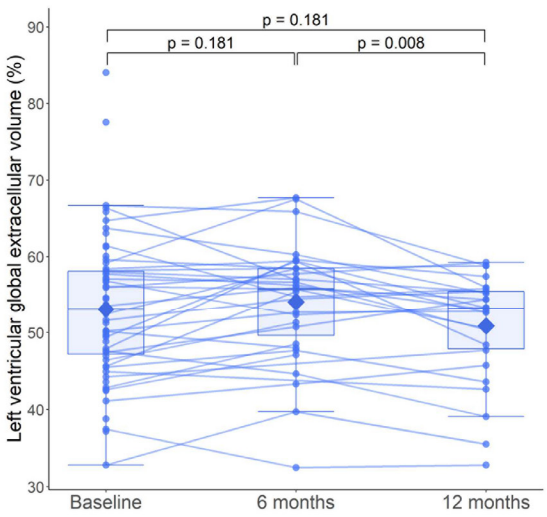


Fig 2. LV Extracellular Volume Over Time



Support & Funding:
We are extremely grateful to the MICA study participants without whom this study would not be feasible. We are grateful for research funding from the National Institutes of Health: R01HL130563 (PI: Dorbala S and Falk RH); R01HL150342 (PI: Dorbala S), K24 HL157648 (PI Dorbala S), the American Heart Association: 16CSA28880004 (PI: Liao R and Dorbala S; 19SRG34950011 Liao R and Dorbala S), and International Society of Amyloidosis and Pfizer (Clerc O).

Evaluation of Echocardiographic Parameters for Prognostication in Patiens with Systemic Light Chain Amyloidosis

Ahmet Mursel Ulusan[1], Ali Yildiz[1], Bernard Kim[2], Noa Biran[3], Pooja Phull[3], Linda Schmidt[3], David Vesole[3], David S Siegel[3], Harsh Parmar[3]

Organisation(s): 1: Department of Internal Medicine, Hackensack University Medical Center, Hackensack, NJ, United States of America; 2: Heart failure and PAH Program, Hackensack University Medical Center, Hackensack,NJ, USA; 3: John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, NJ, USA

Background: Systemic immunoglobulin light chain amyloidosis (AL) is a multisystem disorder characterized by extracellular deposition of amyloid fibrils that commonly affects the cardiac, renal and the GI systems. Current staging is based chiefly on cardiac biomarker levels including NT-pro-BNP, BNP, Troponin T or Troponin I (per the Mayo Clinic and BU staging). We evaluated the utility of echocardiographic parameters including Left Ventricular Mass Index (LVMI) and Left Ventricular Ejection Fraction (LVEF) in predicting survival in patients with AL amyloidosis

Objective

To determine the prognostic utility of LVMI and LVEF in patients with AL amyloidosis

Material & Methods

We retrospectively reviewed 57 patients, who were treated at Hackensack University Medical Center, diagnosed with AL amyloidosis between 2010-2020. We evaluated the impact of LVMI and LVEF in terms of predicting mortality. The median LVMI for our group of patients was found to be 112g/m^2, while median LVEF was found to be 57.5%. We therefore stratified patients according to a left ventricular mass index >=112g/m^2 compared with those with values lower than 112g/m^2. We also assessed patients with an ejection fraction of those less than 57.5% compared with those >=57.5%. Survival analysis was performed using the Kaplan-Meier method. We hypothesized that patients with an increased LVMI or decreased LVEF would have a higher risk of death.

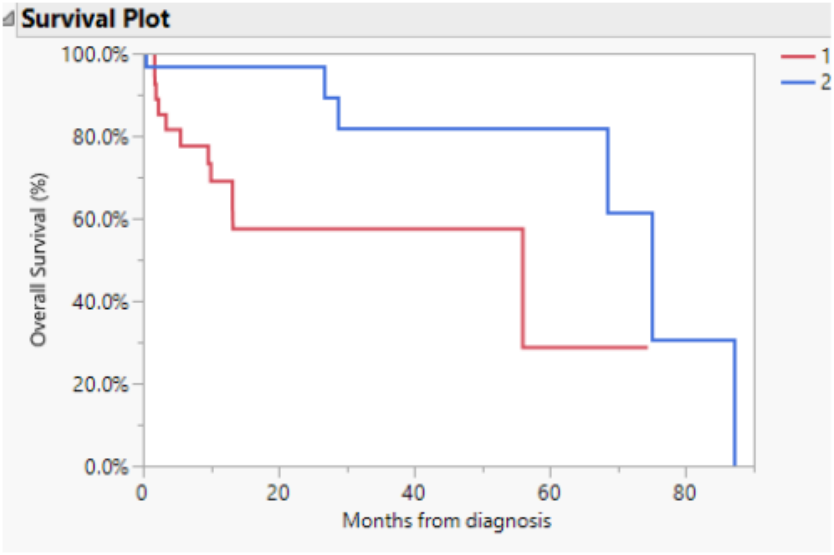
Results & Discussion

30 patients were found to have an LVMI>=112g/m^2 (cohort 1), while 27 patients had an LVMI<112g/m^2 (cohort 2). 39 patients had an LVEF of >=57.5% (cohort 3) while 18 patients had an LVEF<57.5% (cohort 4). The median OS for cohort 1 was significantly lower than that of cohort 2 (55.9 months vs 74.9 months, p=0.0058). Similarly, the median OS for cohort 4 was inferior when compared with cohort 3. (13.3 months vs 87.1 months, p<0.0001)

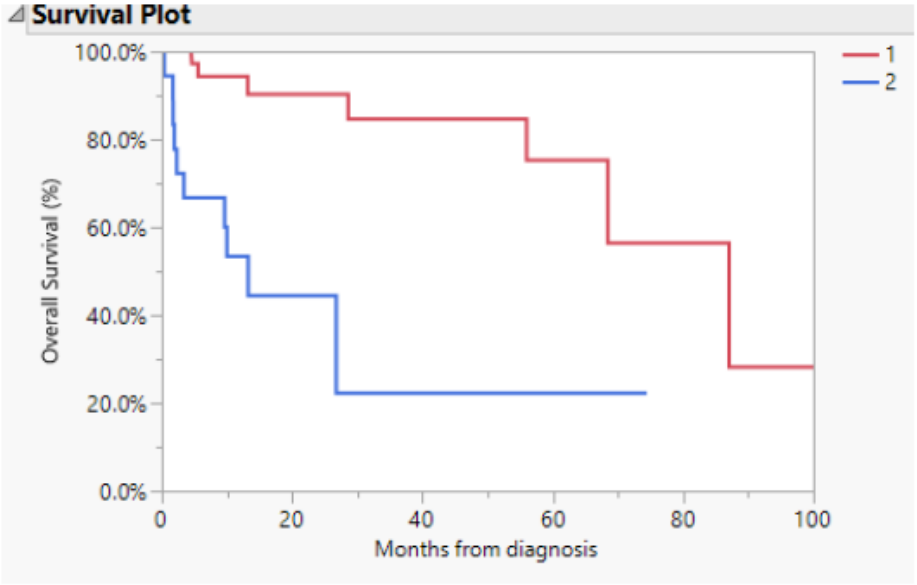
Summary & Conclusions

In our small group of patients with AL amyloidosis, those who had an LVMI of over 112g per meter squared or those with an EF of less than 57.5%, OS was found to be significantly shorter. These findings are indicative of an overall poor cardiac function associated with an increased mortality and highlights the importance of a multidisciplinary approach involving cardiologists and medical oncologists

Figure 1.



Patients with an LVMI>=112 (Red curve) had an inferior median OS (55.9 months) compared with patients with an LVMI<112 (Blue curve) (74.9 months)



Patients with an EF<57.5% (Blue curve) had an inferior median OS (13.3 months) compared with patients with an EF>=57.5% (Red curve) (87.1 months)

Central Nervous System damage in hereditary Transthyretin Amyloidosis: A multimodal MRI study

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Background: Hereditary transthyretin amyloidosis (hATTR) is an autosomal dominant disease caused by mutations in the transthyretin gene (*TTR)*. More than 140 mutations in the TTR gene have been identified, but significant variability exists in penetrance and clinical presentation according to the genotype [1]. The main clinical manifestation is due to the accumulation of misfolded protein aggregates in the peripheral nerve leading to a sensorimotor polyneuropathy. Other classically compromised systems are the cardiac, gastrointestinal, renal, and ophthalmologic systems [2]. The involvement of the central nervous system (CNS) is poorly studied so far. There are only few case descriptions, mostly based in late stage patients who presented major vascular CNS complications[3]. No comprehensive neuroimaging evaluation was reported using MRI in an earlier cohort of patients.

Objective: To determine the pattern of gray and white matter CNS involvement in hATTR using multimodal quantitative MRI.

Material & Methods: We evaluated twenty-one patients with genetically confirmed hATTR and symptoms consistent with the diagnosis, and twenty-one age and sex matched controls. Patients and controls underwent high-resolution MRI on a 3 Tesla Achieva-Intera PHILLIPS Scanner. The Freesurfer software v.7.2 was used to measure cortical thickness and deep gray matter volumes in this study. The FSL software v6.0 was used to create maps of fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD). Patients and controls were compared using the tract based spatial statistics (TBSS) algorithm. In TBSS, voxelwise analyses of the diffusion parameters is automatically done as a part of the algorithm. All analyses were corrected for multiple comparisons correction. P-values<0.05 were deemed significant.

Results: Mean age of patients and disease duration were 52.4±16 and 7.9±7 years, respectively. TBSS demonstrated FA reduction predominantly in the cingulate and superior frontal gyrus white matter. MD was increased in cingulate and superior frontal gyrus, in the cerebellum and occipital white matter. AD and RD were increased in the same white matter regions. Abnormalities had a predominance towards the left hemisphere. FreeSurfer demonstrated a reduction of cortical thickness in patients relative to controls predominantly in the left medial frontal (p-value 0.0027), middle occipital (p-value 0.006), left superior temporal (p-value 0.0035) and right cingulate cortices (p-value 0.009).

Summary & Conclusion: The results described above suggest that there is structural CNS damage in the disease targeting both gray and white matter. MRI is able to capture these structural changes early in the disease course. The clinical correlates of these CNS changes are probably cognitive deficits - especially memory and visuospatial complaints, which have been increasingly recognized in hATTR amyloidosis.

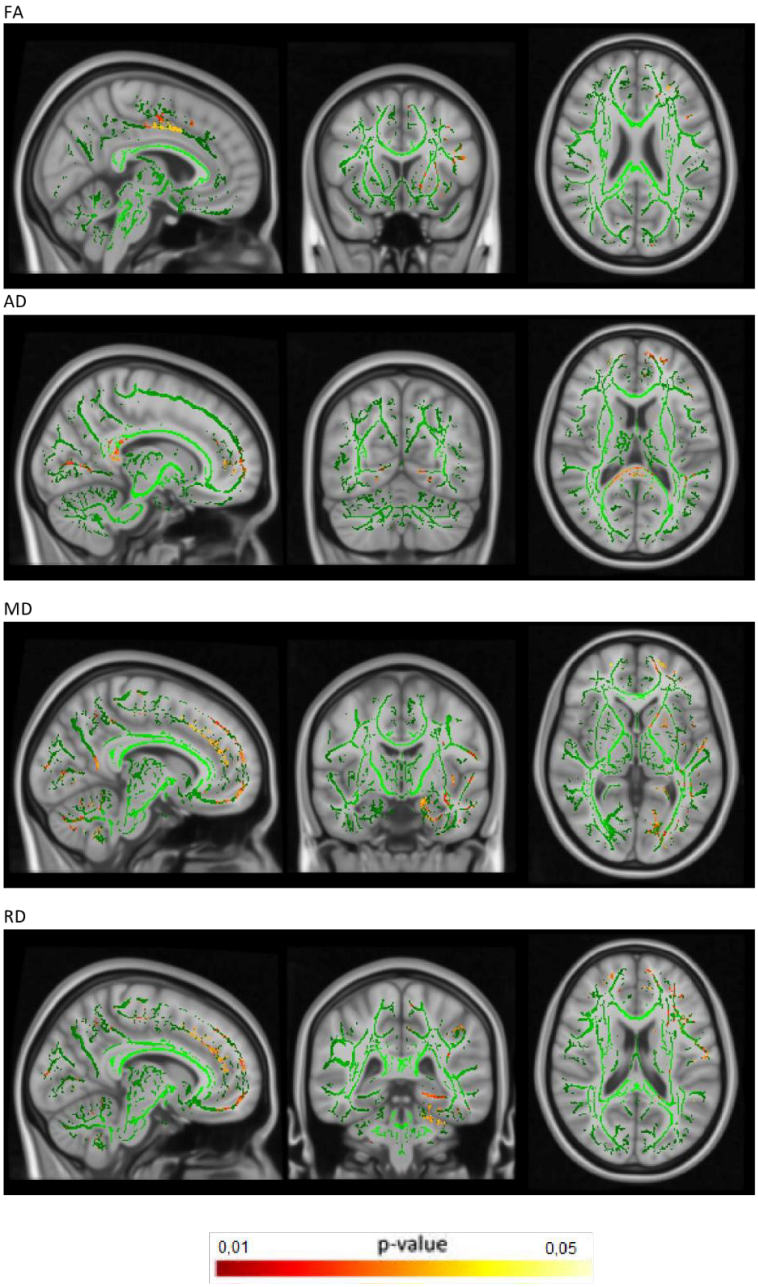

Figure 1: TBSS analyses showing CNS structural damage in hATTR

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Neurology. 2021 Dec 14;97(24):1111-1119.

Support & Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and PFIZER.

LATE BREAKING POSTER PRESENTATIONS TUESDAY, 6TH

Amyloid Multidisciplinary Hybrid Clinic: A novel model of care in the age of telehealth

Gorrie, Natasha [1,2,3],Fatkin, Diane[1,2,3], Smyth, Renee[1], Carroll, Antonia[1,4], McCaughan, Georgia [1,3,5], Bart, Nicole [1,2,3]

1 St Vincent's Hospital Sydney
2 Victor Chang Cardiac Research Institute
3 University of New South Wales
4 University of Sydney
5 Garvan Institute

Background:

Amyloidosis is a complex multi-system disease which is underdiagnosed and undertreated due to the varied clinical presentation and lack of clinician awareness (1). Access to Amyloid services offering diagnostic biopsy and specialist care is limited and only available in quaternary centres in capital cities. Approximately 30% of Australian's live in rural or regional locations, with a greater percentage of Indigenous persons. The health care needs are greater in regional areas with higher rates of morbidity and mortality but significantly less clinicians, in particular specialists, per capita (2). In Australia, patients may need to travel up to 10 hours to reach their nearest centre for care, which poses a significant barrier to optimal care for rare diseases, such as amyloidosis. Despite this there are no regional services for Amyloidosis, or any multidisciplinary care outreach for any disease, in Australia. A recent meta-analysis showed telehealth services in heart failure was more effective at reducing hospitalisation, all-cause mortality and cardiac mortality compared with traditional clinic models (3). Despite this telehealth services in Australia have previously been limited due to access and funding issues (4). The COVID pandemic has increased the infrastructure and funding of telehealth care and provided an opportunity to improve our models of care (5).

Objective:
We sought to develop a new model of Amyloidosis care during the COVID pandemic utilising telehealth technology, with a goal to address barriers to care in Australia and improve quality of care and collaboration between specialists and local health care providers.

Methods:

We developed a novel, multidisciplinary approach to standardising care of all Amyloidosis patients. All referrals to St Vincent's Hospital for suspected amyloidosis were invited to be seen in the Amyloid multidisciplinary team (MDT) clinic. Patients were offered a one-time collaborative visit encompassing investigations, cardiac imaging, neurophysiology studies and clinical care. Clinical care included review by a Cardiologist, Neurologist, Haematologist and genetic counsellor all with specialised amyloidosis and transplant training, to address diagnosis, management and screening for complications. All reviews and follow up were offered via telehealth where feasible.

Results & Discussion:

Between September 2021 and June 2022, 30 patient encounters occurred. A total of 57% were performed via telehealth with 37% of patients from regional or rural areas. It is estimated more than 120 hours of travel time was saved by patients utilising telehealth. The clinic resulted in 20 new amyloidosis diagnosis and 2 early cardiac transplant referrals. Modification of therapy was made in all new diagnoses.

Summary & Conclusions:
Hybrid multidisciplinary care of amyloidosis patients is feasible and results in better collaboration between specialists and more equitable access to care.

Figures:

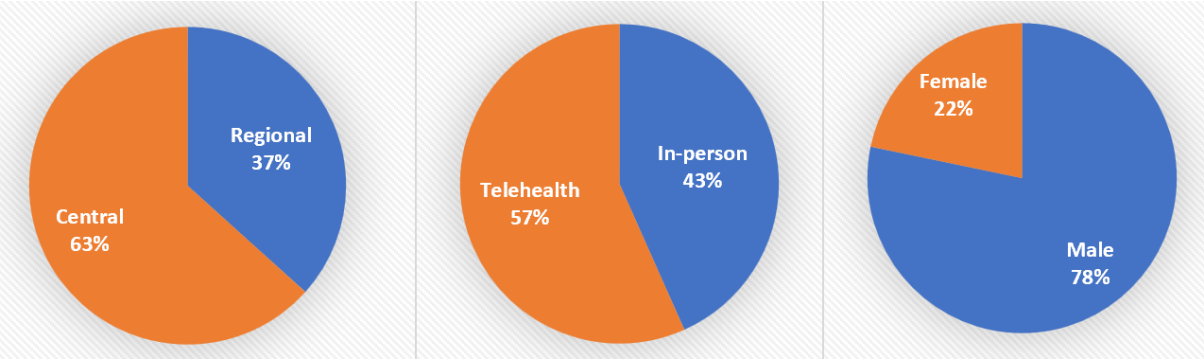

Figure 1: Demographics of the Amyloid MDT Hybrid clinic.

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Burden of transthyretin amyloid cardiomyopathy in patients and caregivers: interim analysis of a large, ongoing, non-interventional study

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Background: Despite being a fatal disease with significant symptomology, the burden of transthyretin amyloid cardiomyopathy (ATTR-CM) is poorly characterized.[1]

Objective: We report interim findings from an ongoing, multinational, cross-sectional, non-interventional study characterizing the burden of ATTR-CM on patients and caregivers. The final study will include around 200 patient and caregiver pairs, providing measures of mental and physical health, health care resource use, work impact, and economic burden.

Material & Methods: Patients with ATTR-CM untreated with disease-modifying medication and their non-paid primary caregivers are enrolled at major amyloidosis referral centers. Patients with a heart/liver transplantation, a left ventricular assistance device, light-chain amyloidosis, or a predominantly neuropathic phenotype are excluded. Caregivers with diseases that significantly impact their quality of life are excluded. Data are obtained through survey responses and review of each patient's medical record. This interim analysis describes demographics and clinical characteristics including self-reported caregiver data from the 22-item Zarit Burden Interview (ZBI, range 0–88; a well-validated measure of burden) and the Hospital Anxiety and Depression Scale (range 0–21; subscores ≥8 used to indicate probable anxiety or depression).

Results & Discussion: In the interim analysis, 105 patient and caregiver pairs were included from 14 sites in 8 countries (in Europe, Canada, Russia, and Australia). Patients had a median age of 81 years (Q1: 75.0, Q3: 85.0), 83% were male, 79% were married/in a domestic partnership, and few (3%) were employed. Median time since diagnosis was 0.7 years (Q1: 0.3, Q3: 1.5), with the median age at first symptoms and diagnosis being 78 and 80 years, respectively. The majority of patients had wild-type ATTR-CM (92% of 96 with genetic testing). Symptoms were multisystemic but often cardiac-related (83% heart failure, 70% shortness of breath, 48% atrial fibrillation, 40% fatigue, 31% leg/ankle swelling, 31% weakness, 22% gastrointestinal/urinary problems, 22% insomnia, 21% leg pain). Of the 102 patients with data, a New York Heart Association classification of II (58%) or III (24%), and a left ventricular ejection fraction of 55%–70% (53%) or 40%–54% (39%) were most common.

Caregivers had a median age of 65 years, 86% were female, and most were spouses (54%) or direct descendants (43%) of the patient (Table). 38% were employed. The median duration of caregiving was 2 years. Caregivers spent a median of 5 hours a week providing care and 10% recalled days in the prior 3 months where they had been unable to complete household chores due to caregiving responsibilities (median 9 days).The median ZBI score was 16 (Q1: 5.5, Q3: 26.0; n=104), similar to that previously reported for patients with heart failure.[2,3] When split by category, 39% of caregivers reported at least a mild to moderate burden of care (score >20; Figure). Probable anxiety or depression was reported by 41% and 31% of caregivers, respectively.

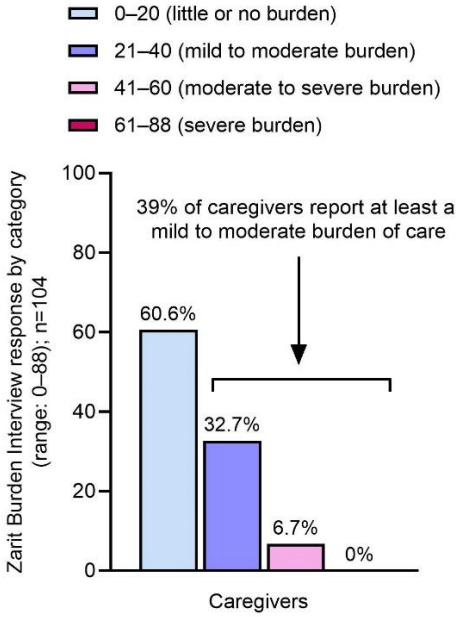
Summary & Conclusion: This is the first large study to comprehensively characterize the burden of ATTR-CM in patients and caregivers. In this interim analysis, demographics and clinical characteristics of patients were typical of those with early-stage ATTR-CM. Caregivers were generally female spouses or direct descendants of the patient. Though they had been providing care for a relatively short period of time, 39% reported at least a mild to moderate burden, and anxiety and depression were common.

Figures

Table. Demographics and characteristics of caregivers

	Caregivers n=105
Age, median (Q1, Q3), years	65.0 (56.0, 74.0)
Female (%)	85.7
Relationship to patient (%)	
Spouse	54.3
Direct descendant	42.9
Other	2.9
Resides with the patient (%)	61.9
Employed (%)	37.5
Employment status change due to caregiving responsibilities (%)	2.9
Time spent providing care/week, median (Q1, Q3), hours	5.0 (0.0, 35.0) **[n=91]**
Duration providing care, median (Q1, Q3), years	2.0 (0.3, 4.0) **[n=89]**
Proportion reporting days in past 3 months unable to complete household chores due to caregiving responsibilities (%)	9.7 **[n=103]**
If yes, how many, median (Q1, Q3), days	9.0 (3.0, 25.5) **[n=8]**

Figure. Zarit Burden Interview findings



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Support & Funding: This study was sponsored by Pfizer.

Epidemiology of cardiac amyloidosis in Germany: a retrospective analysis from 2009 to 2018

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Background and Objective: Improved imaging modalities contributed to increasing awareness of cardiac amyloidosis. Contemporary data on frequency trends in Germany are lacking.

Material and Methods: In a retrospective study using health claims data of a German statutory health insurance patients with diagnostic codes of amyloidosis and concomitant heart failure between 2009 and 2018 were identified.

Results: Prevalence increased from 15.5 to 47.6 per 100,000 person-years, and incidence increased from 4.8 to 11.6 per 100,000 person-years, with a continuous steepening in the slope of incidence trend. In patients with amyloidosis and heart failure age and male gender significantly increased whereas prevalence of myeloma and nephrotic syndrome significantly decreased over time. Median (IQR) survival time after first diagnosis was 2.5 years (0.5 to 6 years), with a 9% (95% CI 2-15%, p=0.008) reduced risk of death in the second compared to the first five years of observation. In the first year after diagnosis mean total health care costs were 21,955 € (median 9,873 €, IQR 3,922 to 24,714€) per person.

Conclusion: The rise in cardiac amyloidosis has continuously accelerated in the last decade. Considering the adverse outcome and high health care burden further effort should be put on early detection of the disease to implement available treatment.

Key words: *cardiac amyloidosis, light-chain amyloidosis, transthyretin amyloidosis, amyloid cardiomyopathy*

Funding disclosures. None to declare

Ixazomib maintenance following initial therapy in patients with high-risk immunoglobulin light chain (AL) amyloidosis.

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Background: AL amyloidosis is a clonal plasma cell disorder characterized by the production of abnormal monoclonal light chains (LC) that misfold and deposit in tissues disrupting organ structure and function. Burden of plasma cell disease at diagnosis impacts event-free (EFS) and overall survival (OS).[1,2] Patients (pts) with AL and > 10% clonal bone marrow plasma cells (BMPCs) have inferior outcomes which suggests that BMPCs that can expand their niche beyond 10% have a distinct relationship to the bone marrow microenvironment and more aggressive biology. Treatment of AL is directed at LC producing BMPCs and depth of hematologic response is a critical determinant of outcome.[3] Eradication of minimal residual disease (MRD) is likely clinically relevant since continuous production of amyloidogenic LCs even below the threshold of conventional serologic detection may impede critical organ recovery.[4] This multicenter phase II trial explored the use of Ixazomib as maintenance therapy in pts with high-risk AL.

Objectives: To determine the EFS (hematologic progression (PD) or organ progression, next therapy or death) of pts with high-risk AL defined by > 10% BMPCs at diagnosis receiving Ixazomib maintenance. To assess depth of hematologic response, frequency of organ response, OS and explore use of mass spectrometry (MS) to detect MRD.

Material & Methods: Eligible pts had AL, >10% BMPCs at diagnosis, received 1 line of induction therapy, were within 12 months (mos) of starting therapy and achieved at least partial hematologic response (PR). Ixazomib 4mg on days 1, 8, 15 of a 28 day cycle for up to 24 cycles was given. Hematologic and organ responses were assessed every cycle x 4 cycles, then every other cycle. Pts were enrolled from 8/2018 and 6/2021; however, enrollment ceased from 3/2020 to 6/2021 due to the pandemic.

Results: 13 pts, 54% male, 77% white, median age 65 years (range, 56-67) with renal (n=7), cardiac (n=4), gsastrointestinal (n=3), soft tissue (n=1) and neuropathic (n=1) involvement enrolled. Median BMPCs at diagnosis was 20% (range 12-40%). As part of initial therapy, 46% received autologous stem cell transplant, 31% prior Ixazomib and 38% daratumumab. Hematologic response at enrollment was PR (n=1), VGPR (n=9) and CR (n=3). 1 patient was MRD negative by flow cytometry at study entry. Pts received a median of 20 cycles (range 3-24) of ixazomib; 2 remain on treatment (both cycle 13). 5 pts discontinued therapy, 4 for PD and 1 for toxicity. Estimated EFS was 60% (38%, 95%) at 24 mos after study entry (**Figure 1**) and OS is 100%. Heme response deepened in 3 pts (1 PR to VGPR and 2 VGPRs to CRs); 2 converted from MRD positive to negative by flow cytometry (sensitivity 10^{-5}). Despite this, peripheral blood MS detected clonotypic LCs in all pts with samples tested (example, **Figure 2**). Cumulative incidence of organ response increased over time from 36% at 12 mos to 52% at 18 and 24 mos following study entry.

Summary & Conclusion: Ixazomib maintenance was well tolerated in pts with high-risk AL and deepened heme responses, increased organ responses and resulted in favorable EFS. Clonotypic LCs were trackable in peripheral blood using MS even in pts who achieved the deepest responses and may prevent organ improvement in some pts. The study was hampered by challenges imposed by the pandemic. Daratumumab's approval has also changed the treatment paradigm. However, long term follow up of pts on the study will characterize clonal LC dynamics with sensitive methods and inform future studies evaluating maintenance in AL.

Figure 1

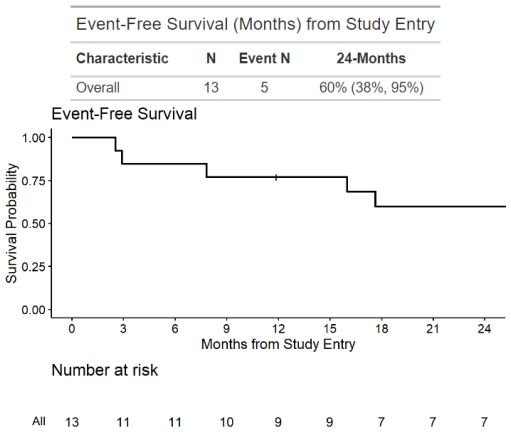

Figure 2

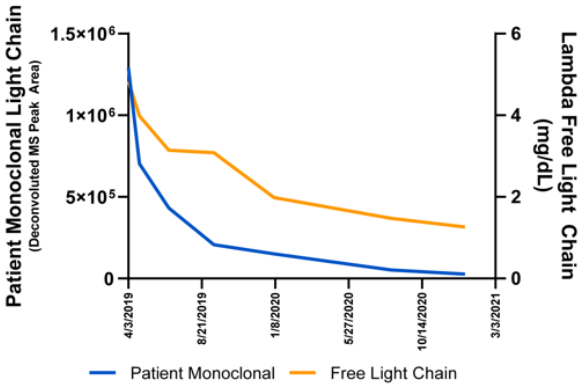

Figure 1. Event free survival defined by hematologic or organ progression, initiation of next therapy or death.

Figure 2. The patient's lambda monoclonal immunoglobulin is detected in peripheral blood by liquid chromatography-mass spectrometry (QTOF) at all time points available. Reconstructed mass spectrum peak area for the patient specific monoclonal light chain is plotted and compared to the lambda free light chain assay levels.

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Mid-term analysis of the Clinical Amyloidosis Registry in Germany

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Background: Currently there are no data available on the epidemiology of systemic amyloidosis in Germany. Previous studies in other regions estimated an incidence of AL amyloidosis of 8 to 14 cases per million population per year but the frequency and distribution of other types of amyloidosis is unknown.

Objectives: Our aim was to collect epidemiological data on amyloidosis diseases in Germany to determine the distribution of amyloidosis types. Also data on diagnosis, prognosis, overall survival (OS) and quality of life (QoL) were collected.

Methods: The analyzed registry population of 963 patients consists of all reported cases of newly diagnosed amyloidosis patients with a Congo Red positive tissue sample or unequivocal findings in bone scintigraphy (ATTR amyloidosis) between 4.1.2018 - 21.11.2019. Data cutoff of the analysis was June 30, 2021. QoL data of patients were collected every 6 months (EQ-5D-5L and SF36-v2) and every 12 months (EORTC QLC-C30).

Results: The 963 patients were primarly included through the amyloidosis outpatient clinic (n=581, 60.3%) and mainly male (n=706; 73%). The median age at diagnosis was 72 years. The subgroup distribution was as follows: 45.5% AL, 42.8% ATTR, 6.8% local, 3.1% AA, 1.2% others and 0.4% type unknown. Whilst biopsy (88%) more frequently led to diagnosis than bone scintigraphy (12%), heart (76%) and kidney (34%) were the most common involved organs. Data on survival were available in 897 cases and analyzed using the Kaplan-Meier estimate. With a median follow-up time of 27.0 months since diagnosis, 245 patients died (153 AL and 60 ATTRwt, 32 other subgroups), amyloidosis being the most frequent cause of death (n=191, 78,0%). In contrast to ATTRwt, patients with AL amyloidosis had a higher mortality risk (1y OS: 94.3% AL, 79.0% ATTRwt) and a shorter estimated median survival (AL: 41.4 months, ATTRwt: NR). In multivariate analysis increasing age (HR 1.04; $p<0.001$) for AL amyloidosis and advanced cardiac involvement (CI) were associated with worse OS. Compared to patients with stage I/II, patients with heart stage III had a much higher hazard of death (AL: HR 2.33, $p=0.001$; ATTRwt: HR 5.69; $p<0.001$) (Dispenzieri et al., 2004; Gillmore et al., 2018). QoL was analyzed using a generalized estimating equations model (GEE) regarding time, age and sex and CI for AL amyloidosis. According to the EORTC QLQ-C30 for patients with AL amyloidosis, no CI implied a higher global score (+7.41 pts; $p=0.002$). Global score improved in the first 12 months after inclusion in the registry (+12.34 pts; $p<0.001$). While fatigue decreased after 12 months by 6.22 pts ($p<0.001$), a missing CI indicated less fatigue (-8.88 pts; $p=0.004$). For ATTR patients, the global score decreased (-0.75 pts; $p<0.001$), whilst the fatigue score increased (+1.24 pts; $p<0.001$) per year of age, respectively.

Summary: The analysis of 963 patients observed a high number of ATTRwt patients in the German population, and a worse OS for AL amyloidosis compared to ATTRwt after 27 months of follow-up time. For AL amyloidosis patients, QoL improved after 12 months, whilst CI was associated with more fatigue. For ATTR patients a higher age was related to a lower QoL and a higher level of fatigue.

Conclusion: This mid-term analysis of the registry allows a more extensive insight of clinical data of patients with systemic amyloidosis, it encompasses a prolonged surveillance of OS and includes for the first time data on QoL for AL and ATTRwt patients. Future evaluations of the registry will allow to estimate the incidence of amyloidosis in Germany.

Keywords: German registry, survival, QoL
Category: Diagnosis and prognosis of AL amyloidosis

On bead de-glycosylation coupled with MALDI-TOF mass spectrometry provides a simple method for confirming light chain glycosylation and provides a sensitive method for residual disease detection

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Background: Intact light chain matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) assays are emerging as a high throughput alternative to standard electrophoretic techniques and have recently been approved by the International Myeloma Working Group for use in lieu of immunofixation. In addition to providing high sensitivity, these assays are able to identify patients with spectral patterns suggestive of light chain N-linked glycosylation based on the presence of broad “hedgehog” shaped peaks with a higher mass than non-glycosylated light chain. Comparable MALDI-TOF MS assays have been demonstrated to be able to detect residual disease in some patients with systemic AL amyloidosis in complete haematological response (CR) (1). However, de-glycosylation testing may offer a way of enhancing assay sensivity further as it will consolidate the broad peak representing multiple glycoforms into a single de-glycosylated monoclonal FLC peak.

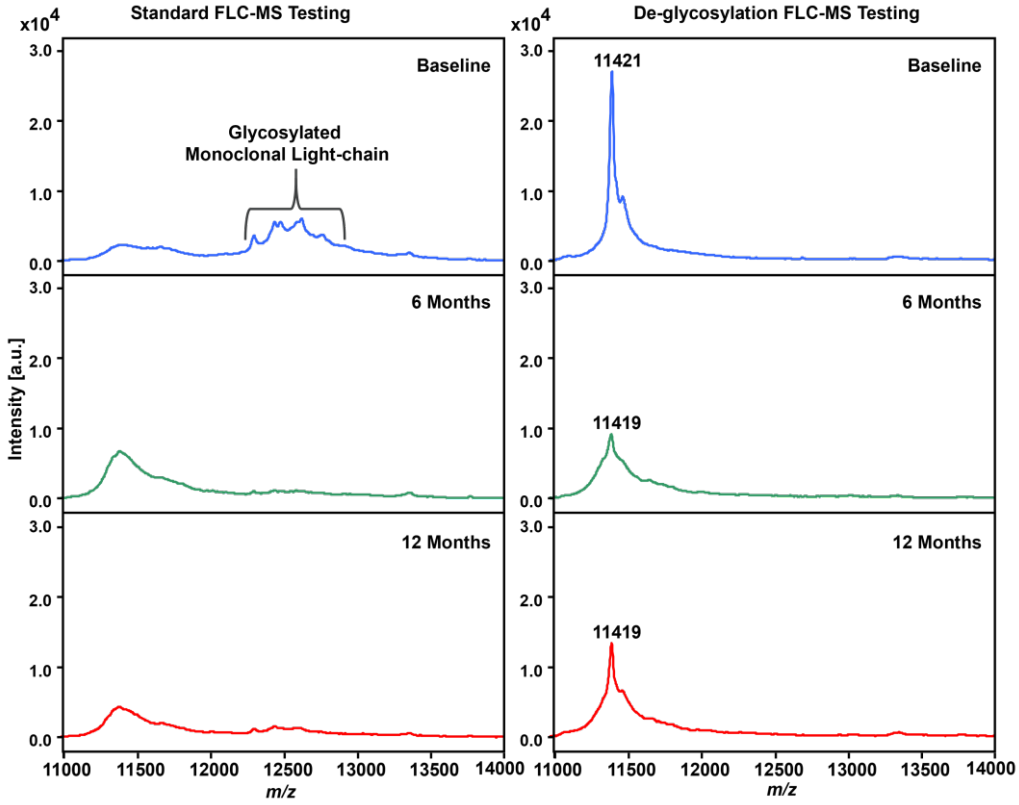
Objective: To investigate whether on-bead de-glycosylation coupled with free light chain mass spectrometry (FLC-MS) testing could improve the sensitivity of FLC-MS analysis for residual disease detection in patients with light chain N-linked glycosylation; and to compare its sensitivity to serum immunofixation (sIFE), serum free light chain (sFLC) and Bence Jones protein (BJP) assessment.

Material & Methods: Samples from five patients with systemic AL amyloidosis undergoing treatment at the UK National Amylodosis Centre who were identified as having spectra suggestive of FLC N-linked glycosylation and became FLC-MS negative on follow-up samples at 6 and/or 12 months post treatment initiation were included in this study. Samples from all time points underwent immune precipitation and on-bead deglyosylation followed by MALDI-TOF MS analysis. Briefly, serum was incubated with magnetic microparticles covalently coated with antisera specific for kappa and lambda FLC and washed to remove non-FLC proteins. The immobilised FLC were resuspended in water, incubated overnight with PNGase F, washed once with de-ionised water to remove the PNGase F, and eluted. The eluates were spotted onto target plates and analysed by MALDI-TOF MS. MALDI-TOF MS results were compared to sIFE, sFLC and BJP results.

Results: PNGase F treatment confirmed the presence of FLC N-linked glycosylation in 5/5 baseline samples. 4 samples were available for testing at 6 months post treatment initiation. Residual disease was detectable by standard techniques (BJP positive and abnormal kappa FLC) in 1/4 (25%) samples. By contrast, FLC-MS followed by PNGase F treatment identified residual monoclonal FLC in 3 out of 4 (75%) samples at this time; including the sample positive by standard methods. At 12 months post treatment initiation, residual monoclonal protein was detectable in 1/5 (20%) samples using standard techniques. FLC-MS combined with PNGase F treatment identified residual monoclonal FLC in this sample, and in 2 further samples that were negative by standard electrophoretic techniques and sFLC assessment, thus providing positive results for 3/5 (60%) patients at this time. Figure 1 shows a case example of residual monoclonal FLC expression becoming apparent by FLC-MS upon de-glycosylation of the monoclonal FLC.

Summary & Conclusion:. FLC-MS testing coupled with on-bead deglycosylation provided greater sensitivity for the detection of residual disease compared to electrophoretic techniques and sFLC assessment.

Figure 1: Monoclonal lambda FLC were detectable at baseline using standard FLC-MS and on-bead de-glycosylation (mass-to-charge ratio (*m/z*) 11421 for the doubly charged FLC) in a patient with lambda FLC AL amyloidosis (sIFE positive, BJP positive, serum free lambda 311.4 mg/L, sFLC ratio 0.03). At 6 months and 12 months post treatment initiation the patient had no detectable residual disease using standard techniques, however residual monoclonal lambda FLC with the same *m/z* as the monoclonal FLC identified at baseline were detected using FLC-MS coupled with on-bead de-glycosylation.



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Support & Funding:

The Binding Site Ltd provided funding for the reagents for the mass spectrometry testing.

Nori M et al
ISA RW PAC Submission

Real World Patient, Advocate, and Caregiver Perspectives on Amyloidosis: Awareness, Knowledge Gaps, and Psychosocial Impact

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Background: All types of amyloidosis are rare, progressive, potentially fatal disorders that are difficult to diagnose and treat. Correct diagnosis of amyloidosis is often delayed due to the nonspecific nature of symptoms and misdiagnoses leading to delays in treatment initiation.[1,2] This delay results in considerable medical and financial burden on patients and their caregivers.[3-5] While standardized quality of life instruments used in randomized controlled trials yield helpful data for healthcare professionals providing care for patients with amyloidosis, they may not fully capture the patient perspective on living with their disease. Further, the COVID-19 pandemic placed new and sometimes unanticipated burdens on patients, caregivers, and medical personnel. We report here the results on patient, advocate, and caregiver (PAC) perspectives during the initial stages of the COVID-19 pandemic from an online community of people involved with amyloidosis.

Objectives: To augment our understanding of amyloidosis by sharing the PAC experience.

Material & Methods: Over the last 2 years, 17 single-question polls and 7 short surveys were posted in an online community for people involved with amyloidosis (oneAMYLOIDOSISvoice.com). In addition, >1000 social wall posts by members of the amyloidosis community were evaluated to capture real-world insights into the impact amyloidosis has on patient lives. The data are reported as summary statistics.
Results: For the 17 polls, there were a total of 1370 responses of which 1135 (83%) were from patients, 131 (9%) from caregivers, and the remaining 104 (8%) from others. The 7 surveys comprised a total of 64 questions answered by 73 patients, 3 caregivers, and 1 other. The social wall consisted of free-form comments and posts and identified as “Post”, “Reply”, and “Reply to Reply”. There were 1099 messages of which 233 (21%) were original posts, 501 (46%) were replies to the posts, and 365 (33%) were replies to replies.
The main results of the polls and surveys are summarized in the **table**. In addition, members posted their gratitude for companies that provided assistance in obtaining treatments through the COVID-19 period, eg, “*I had been going to a hospital, but when they wanted to switch to home infusions, [commercial program] stepped in and helped facilitate that. I had three home infusions at no out-of-pocket cost.*” Others posted on the cost of treatment, eg, “*[The hematologist] said he would look for some help in paying $225,000 a year for the cost. I applied to the insurance company for some relief and after numerous calls to the doctor to send the confirmation; they gave me the ok. The price will delete all my savings in a few years.”*
Summary & Conclusion: The data reported here reflects the real-world experiences and concerns of patients with amyloidosis and their caregivers. Such data provide insights into what is important to the PAC community. Single-question polls and short surveys in a disease specific on-line community offer a quick and easy method to gain insight into life with amyloidosis that has heretofore been unpublished. While the results can only be reported descriptively, they nonetheless are highly informative for those treating patients with amyloidosis. This is the first step in obtaining comprehensive PAC input into living with amyloidosis.

Table. Summary of Responses on Polls

Topic	Yes (%)	No (%)
Personal Impact of Amyloidosis		
Amyloidosis has interfered with personal life (n = 276)	69.2	30.8
Ability to take care of oneself (n = 25)		
Can take care of myself independently	76.0	
Can take care of myself with minimal assistance	20	
Unable to take care of myself	4	
Feelings about managing one's disease (n = 221)		
Has made me feel stronger	62.9	
I put on a brave face	19.9	
No change in my feelings	12.2	
I am unsure of my feelings	5.0	
Patient/Caregiver Response to Diagnosis		
Important for me to inform others (n = 165)	86.7	13.3
>4 hours/week spent researching disease (n = 19)	36.8	63.2
Type of information sought most (n = 46)		
Treatments available	76.1	
About the disease	65.2	
Availability of community support	58.7	
Clinical trials	56.5	
Genetic counselling (n = 23)		
Received it	52.2	
Considering it	21.7	
Not interested	26.1	

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Renal histopathological scoring of amyloid deposits is crucial to assess disease progression in light-chain (AL) amyloidosis: a multicentre retrospective study

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Background: Renal involvement is common in light-chain (AL) amyloidosis (60%-80% cases). To date, renal biopsy is not recommended as a routine examination in patients with AL amyloidosis and renal involvement. However, renal histology can have an important prognostic role.

Objective: Amyloid load might be linked to both systemic disease severity and temporal exposure to amyloid deposition in different organs.
We proposed an amyloid load scoring system in order to predict renal and overall survival in patients with AL amyloidosis.

Material & Methods: We retrospectively collected AL cases who underwent to renal biopsy from 5 Italian Institutions. The primary composite outcome includes time to death and time to end-stage kidney disease development.
We applied an Amyloid load score characterized by a semiquantitative evaluation for amyloid deposition in glomeruli, interstitium, and vessels. Each lesion was scored from 1 to 3. The sum of damage (0–9) associated with amyloid deposition was calculated, indicating total numeric codes of renal pathologic damage.

Results: Between 2008 to 2021, we recruited 162 patients. Their median age at diagnosis was 66.5 (±10) years, serum creatinine was 1.95 (±1.92) mg/dl and proteinuria was 5.4 (±4.6) grams per 24hr.
After a median follow-up of 4.1 (± 3.6) years and a 6-month landmark analysis, the primary composite outcome was achieved by 46/122 (38%) patients.
Seventy-eight patients (60%) experienced a hematologic response, classified as a complete response or very good partial response, while 49 (37.4%) patients obtained a renal response (Figure 1). Among them, 39 (80%) patients had an amyloid score ≤4 and only 4 had an amyloid score> 5.
Higher values of Amyloid load score, as an expression of amyloid load linked to both disease severity and temporal exposure, were significantly associated with an increased risk of achieving the composite primary outcome (log rank 39.92, p<0.0001) (Figure 2).
Interestingly, 6/162 (3.7%) patients had a negative Congo red stain in renal biopsy (AL amyloidosis diagnosis through an abdominal fat pad aspiration plus monoclonal restriction at immunofluorescence staining or amyloid fibrils detected by electron microscopy). Moreover, 49/156 (31.4%) patients did not show a monotypic (kappa or lambda) immunofluorescence staining.
Despite the widely accepted clinical definition of renal amyloidosis is “more than 0.5 g/24hr of non-Bence Jones proteinuria in presence of a positive fat pad aspiration”, 8/162 (4.9%) patients with a biopsy-proven renal AL amyloidosis showed less than 0.5 g/24hr of non-Bence Jones proteinuria.
AL amyloidosis showed to overlap in 14/153 (9.2%) patients with other Monoclonal gammopathy of renal significance (MGRS), such as LCDD (4 cases), C3 nephropathy (4 cases), light-chain proximal tubulopathy (2 cases), light chain-related tubulointerstitial nephritis (2 cases), cast nephropathy (1 case), type 1 crioglobulinemia (1 case).

Summary & Conclusions: Renal histopathological scoring of amyloid deposits is crucial to assess disease progression in patients with AL amyloidosis, and in particular a score ≥5 identifies patients at greater risk of evolution of renal damage and mortality.

Figure 1 Hematologic and renal response in our cohort

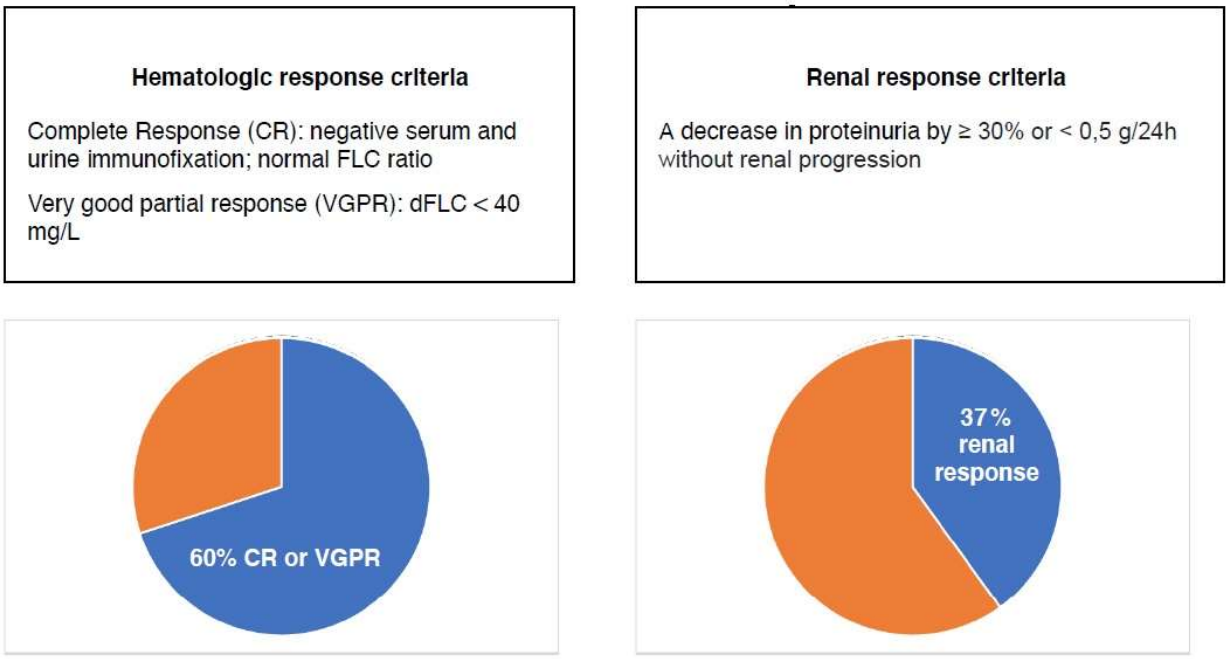
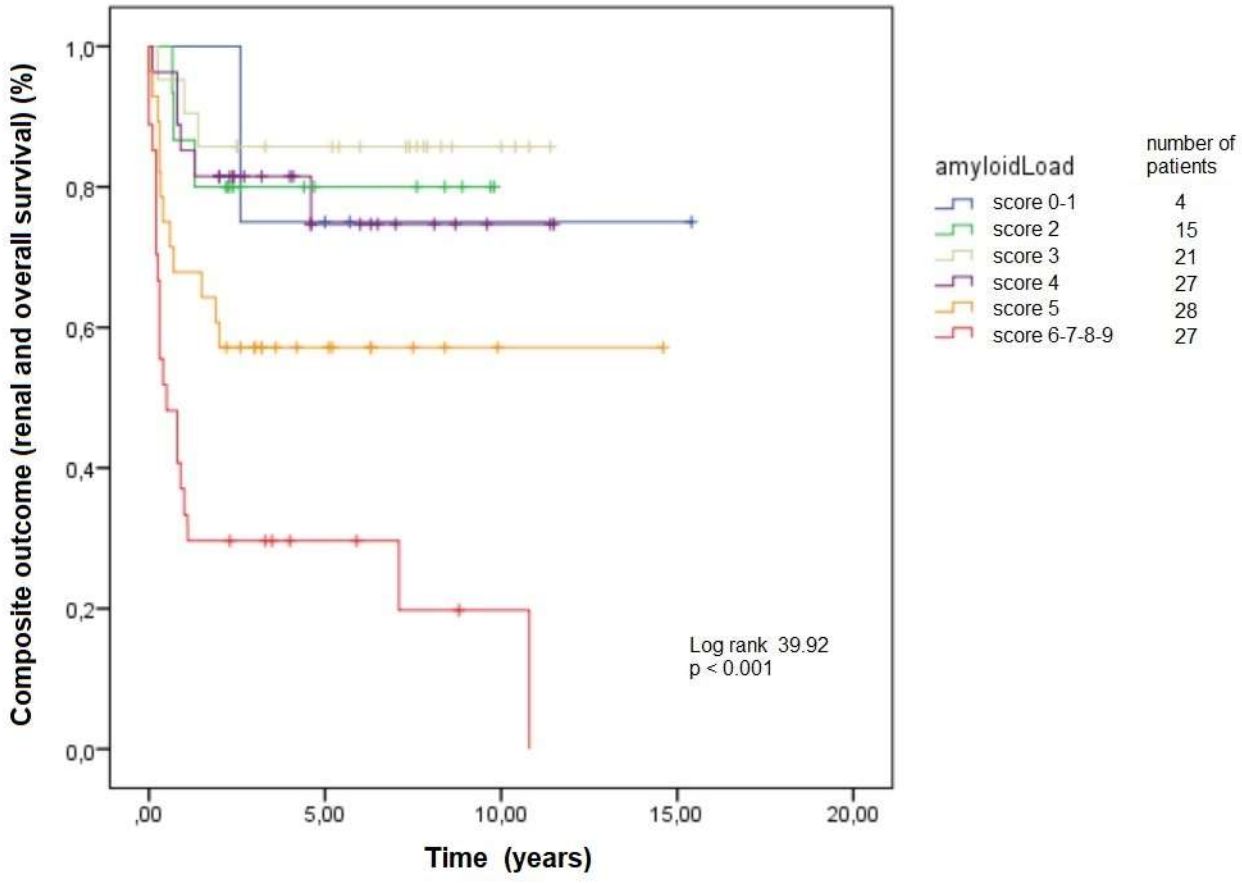


Figure 2 Renal and overall survival in relation to renal amyloid score



Response rates to second line treatment with Daratumumab Bortezomib (Velcade) Dexamethasone (DVD) in relapsed/refractory light chain (AL) amyloidosis after initial bortezomib based regime

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Background: Bortezomib based regimes are the most common first line treatments in patients with systemic AL amyloidosis (1); addition of Daratumumab (Dara-VCD) is licensed in this setting. The impact of 2nd line daratumumab-bortezomib-dexamethasone (DVD) in patients who have relapsed after or are refractory to front line bortezomib has not been reported.

Objective: The primary objective was to assess the impact of second line DVD in patients who received Bortezomib therapy upfront analysing outcomes based on depth and duration of haematological response to front line therapy.

Methods: All patients from a prospective observational study of newly diagnosed AL amyloidosis (ALCHEMY) who received a frontline Bortezomib based therapy and second line DVD were analysed. The best haematological response achieved following DVD was compared to the response to front line therapy and stratified accroding to whether second line therapy was initiated within 12 months or beyond 12 months of the start of front line therapyand were designated '<12 months' vs '>12 months'.

Results: 116 patients were included, 75 (64.7%) male and median age at diagnosis 64 years. 40 (34.5%) patients were Mayo stage 3a/3b, 72 (62.1%) had cardiac involvement and 63 (54.3%) patients had 2 or more organs involved. 85 (73.3%) patients had λ AL-type and 30 (25.9%) were κ AL-type. Median involved free light chains (iFLC) at diagnosis was 217mg/l (range 6.8 – 10,109mg/l) with median dFLC 197mg/l (range -24.6 – 10,132mg/l).

Median number of cycles of front line therapy received was 6 and median time to next line of treatment was 17 months (range 1 – 86) with 44 (37.9%) requiring second line treatment within 12 months from diagnosis. 41 patients (35.3%) achieved a complete haematological response response (CR), 43 (37.1%) very good partial response (VGPR) and 32 (27.6%) achieved a partial response (PR) or no response (NR).

All patients received the second line DVD. At 6-month assessment 74 (63.8%) patients achieved a VGPR/CR and at 12 months 60 (51.8%) a VGPR/CR. Overall 81 (69.8%) patients achieved a VGPR/CR at any time point and the best haematological response was a CR in 42 (36.2%) , VGPR in 36 (31.1%) and PR/NR in 35 (30.2%). There was no significant difference in proportion of patients achieving a CR/VGPR to DVD irrespective of whether they had late (>12 month) vs. early (<12 month) relapse (Table 1). However, of patients achieving PR/NR to front line treatment, only 21% and 26% respectively achieved a CR and VGPR to DVD compared with 51% achieving a CR at second line in those who had previously achieved CR to 1st line.

17 patients required third line therapy with median time to next treatment of 13 months (range 0 – 34 months) and 22 (19%) patients died. Proportion of patients surviving 24 months after DVD was 96%/73%/52% in those achieving CR/VGPR/PR to second line DVD

Conclusion: Patients with proteasome inhibitor sensitive disease (i.e. CR/VGPR to 1st line treatment) show excellent response to 2nd line treatment with DVD, irrespective of the duration of response to 1st line treatment. However, those achieving less than VGPR to initial bortezomib based regime have poor responses to DVD. DVD remains a treatment option for relapsed proteasome inhibitor sensitive patients with AL amyloidosis.

Table 1 – Hematological response to second line therapy based on response to first line therapy subdivided on the requirement for second line therapy within 12 months.

Second line therapy >12 months from first line					
		Best second line response			
		CR	VGPR	PR/No response	Total
Best first line response	CR (%)	18 (51%)	9 (26%)	8 (22%)	35
	VGPR (%)	13 (46.4%)	10 (35.7%)	5 (17.9%)	28
	PR/NR (%)	1 (11.1%)	2 (22.2%)	6 (66.7%)	9
	Total	32	21	19	72
Second line therapy <12 months from first line					
		Best second line response			
		CR	VGPR	PR/No response	Total
Best first line response	CR (%)	4 (66%)	1 (16%)	1 (16%)	6
	VGPR (%)	1 (6%)	11 (73.3%)	3 (20%)	15
	PR/NR (%)	5 (21.7%)	6 (26.1%)	12 (52.2%)	23
	Total	10	18	16	44

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Retrospective Cohort Study of treatment with BCL-2 inhibitor Venetoclax in relapsed or refractory AL amyloidosis

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BACKGROUND: In systemic AL amyloidosis, treatment strategy is the suppression of the plasma cell clone within the bone marrow. Numerous treatment regimens have been established based on therapy protocols for multiple myeloma. Venetoclax is an oral Bcl-2 inhibitor known for its application in the treatment of chronic lymphatic leukaemia and acute myeloid leukaemia. The drug has been shown to also be effective in t(11;14)-positive multiple myeloma (1). This genetic aberration is present in 50 % of patients with AL-amyloidosis (2). Recently, Venetoclax has been used in refractory or relapsed AL-amyloidosis revealing promising results (3).

OBJECTIVE: In this study, we aim to evaluate the benefit of treatment with Venetoclax in patients with t(11;14)-positive AL-amyloidosis at the University Hospital of Heidelberg.

MATERIAL & METHODS: 28 patients who presented with relapsed or refractory AL amyloidosis from September 2020 to July 2022 were retrospectively analysed. Each patient underwent treatment with Venetoclax after one or more prior treatment lines. Hematologic response was assessed after 3, 6 and 12 months of treatment.

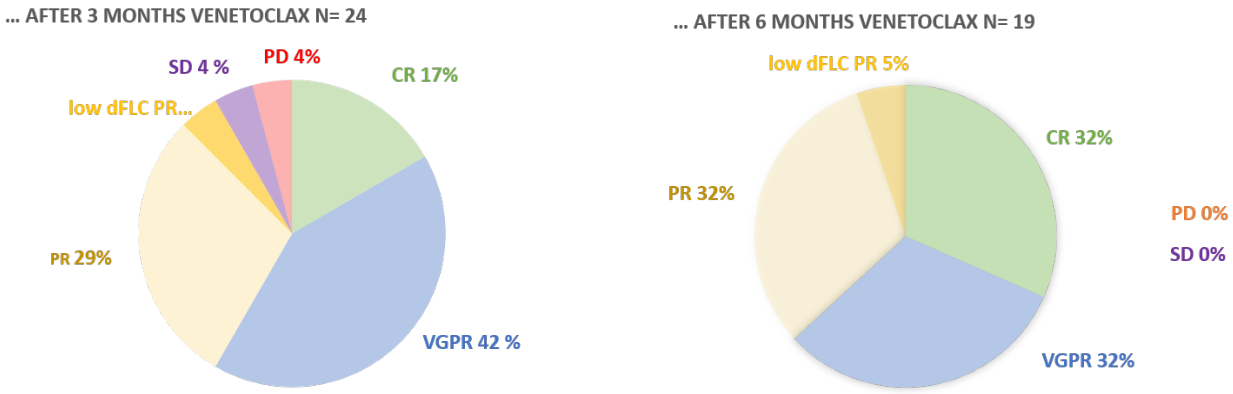
RESULTS: Median age was 68, 14 were male. 21 had AL λ-type (6 κ -type). Median dFLC at baseline was 117 mg/L (0–1178 mg/L). Cardiac involvement was most common (93%, baseline NT-proBNP 4168 pg/ml; range 459-27511 pg/ml), followed by renal (68%, baseline eGFR 26ml/min; range 19-96ml/min, baseline proteinuria 8,3g/d; range 0,8-26 g/d) and liver involvement (21%; baseline AP 586 U/l; range 88-1084 U/ml); 82% had ≥2 organs involved. 93% had t(11;14) by FISH. 2 patients were included without proven t(11;14) – in one cases iFISH has not been accomplished, in the other case the underlying disease was a lymphoplasmacytic lymphoma. 79% of patients received ≥2 prior lines of therapy. Prior therapy lines included Daratumumab (93%), Bortezomib (79%), Lenalidomid (54%), Pomalidomide (32%), Melphalan (32%), Cyclophosphamide (25%) and autologous stem cell transplantation (11%). Venetoclax treatment was feasible and well tolerated in most patients. Hematologic response rates after 3 months showed CR in 16%, VGPR 42%, PR in 29%, low dFLC PR in 4%. SD and PD was seen in 4%, respectively. Remission was improved in 61 % to PR or better. 4 patients were excluded from this analysis as no three-month data have been obtained yet. Overall, cardiac response was seen in 4 patients, renal and liver responses in 2 patients, respectively. One patient died under treatment with Venetoclax within 3 months due to cardiac arrest, another patient with VGPR died while the rest has ongoing responses.

SUMMARY AND CONCLUSION:

The data of our analysis support the promising results of Venetoclax in t(11;14)-positive relapsed or refractory AL amyloidosis patients (even in advanced stages of the disease). Two questions remain. Should we move Venetoclax to earlier lines? And can we even improve the efficacy in some patients by adding other anti-clonal therapies?

FIGURES:

HEMATOLOGIC RESPONSE



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Tafamidis medication adherence in patients with transthyretin cardiac amyloidosis (ATTR-CM) in a Japanese medical claims database

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM) is an underdiagnosed, potentially fatal condition characterized by deposits of amyloid protein fibrils in the walls of the heart. The International Society of Amyloidosis (ISA) has recently recommended tafamidis for treatment of patients with transthyretin amyloid with mixed phenotype, neuropathy and ATTR-CM.[1] Tafamidis, a benzoxazole derivative that lacks nonsteroidal anti-inflammatory drug activity, has been demonstrated to improve all-cause mortality and cardiovascular related hospitalizations in clinical trials.[2] Pre-defined treatment adherence (≥ 80% of scheduled doses) was high for patients receiving either tafamidis or placebo (97.2% and 97.0%, respectively).[2] Medication adherence as measured in clinical trials may not reflect real-world adherence in usual care settings, thus, understanding tafamidis adherence in a real-world Japanese population is important for assessing its full impact on the ATTR-CM population since its approval in March 2019 in Japan.

Objective: The objective of this study was to describe baseline characteristics and adherence among patients with ATTR-CM treated with tafamidis (VYNDAQEL®) in Japan using the Japanese Medical Data Vision (MDV) database.

Material & Methods: This study was a non-interventional, retrospective cohort study of adult (≥18 years old) patients in the Japanese MDV claims database diagnosed with ATTR-CM[3] and with at least two tafamidis prescriptions of dose strength 4x20 mg/day between March 1, 2019 and August 31, 2021. The date of the first prescription was defined as the index date, with follow-up time defined as the time between the first and last prescription plus the days' supply from the last refill. Baseline characteristics were assessed during a 12-month pre-index period. Adherence was measured using two metrics: (1) the modified medication possession ratio (mMPR), calculated by taking the sum of days supplied for all fills within the follow-up period, divided by the number of days of follow-up, and reported as a percentage, with patients classified as adherent with an mMPR of ≥80%, and (2) the proportion of days covered (PDC), calculated by taking the total number of days' supply dispensed during the follow-up period divided by number of days of follow-up, adjusting for any days' supply overlap.[4]

Results: A total of 210 patients were identified, the mean age of the cohort was 78 years, and the majority (89%) were male. The most common baseline cardiovascular comorbidities were heart failure (85%), ischemic heart disease (66%), hypertensive diseases (49%) and diabetes (35%). 75% of patients received heart failure medications in the 12 months prior to index, with the most common being beta-blockers (49%), diuretics (48%), angiotensin receptor blockers (ARBs) (30%), angiotensin-converting enzyme inhibitors (ACEIs) (22%), and sodium/glucose cotransporter-2 inhibitors (SGLT2i) (8.1%).

Over 426.5 days of average follow-up, mean mMPR was 96% with a median of 100% (interquartile range [IQR]: 97%, 101%). 93% of patients were adherent (defined as an mMPR ≥80%). In the same follow-up period, mean PDC was 93.6% with a median of 99% (IQR: 93%, 100%).

Summary & Conclusions: This study found high adherence rates to tafamidis in this real-world Japanese patient population. Adherence rates in this study were similar to those reported by the tafamidis clinical trial and a previously published US commercial claims adherence analysis.[5] Further studies should be conducted to assess the impact of real-world adherence on real-world outcomes.

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The impact of renal histopathology on the renal outcome for newly diagnosed patients with AL amyloidosis

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- **Introduction/Background**

Light chain amyloidosis (AL) is the most common form of renal amyloidosis. Renal involvement greatly impacts the morbidity and predicting the renal outcome is crucial in the management. Few studies were done to assess the impact of histopathological scoring of amyloid deposits on the renal outcome.

- **Objectives**

The goal of the study was to evaluate whether degree of global glomerulosclerosis and interstitial fibrosis for patients who have AL amyloid with renal involvement were associated with change in kidney function (slope of eGFR) over time while adjusting for age and heart involvement.

- **Methods**

We studied 37 patients newly diagnosed renal AL amyloidosis. All had renal biopsy at the time of diagnosis. We calculated the eGFR using the CKD-EPI and global sclerosis was calculated as a proportion (0-1, i.e. 25/60=0.42). Fibrosis was combined into groups as follows: 1) none, minimal, mild, 2) moderate and severe.

- **Results**

In a model adjusting for age, heart involvement and fibrosis, we found no significant interaction between time and global sclerosis (P=0.84), suggesting that global sclerosis is not significantly associated with change in renal function (slope of eGFR). It should be noted that the patients with higher global sclerosis started with much lower eGFR at baseline (Table 1).

Similarly in a model adjusting for age, heart involvement, and global sclerosis, we found no significant interaction between time and moderate to severe fibrosis vs. none/minimal/mild (P=0.95), suggesting that fibrosis is not significantly associated with change in renal function (slope of eGFR). It should be noted that the patients with moderate/severe fibrosis started with much lower eGFR at baseline (Table 2).

- **Conclusions**

The degree of global sclerosis and interstitial fibrosis are not significantly associated with the change in eGFR in patients with renal AL amyloid. Those results correlate with Hoelbeek et al. who found that composite scarring injury score (The sum of the percentage of global sclerosis and percentage of tubulointerstitial fibrosis) correlated to baseline eGFR at diagnosis but did not correlate with progression to end stage kidney disease for AL patients.

Table 1. Observed eGFR at baseline, over time and change at 6 months by tertile of global sclerosis

eGFR	N missing	Overall (N=37)	Global sclerosis 0-0.12 (N=12)	Global sclerosis 0.13-0.32 (N=13)	Global sclerosis 0.33-0.86 (N=12)	p-value
Baseline	0	50.9±28.1	64.0±21.0	58.6±32.5	29.5±15.5	0.003[a]
3 months	1	50.1±30.8	61.4±29.5	53.2±31.7	34.1±26.6	0.093[a]
6 months	0	48.0±31.5	62.5±31.1	56.2±31.5	24.8±17.4	0.004[a]
Change from 0 to 6 months	0	-2.9±14.6	-1.5±21.0	-2.4±12.0	-4.7±9.6	0.87[a]

Table 2.Observed eGFR at baseline, over time and change at 6 months by fibrosis group

eGFR	N missing	Overall (N=36)	None mild minimal (N=26)	Moderate to Severe (N=10)	p-value
Baseline	0	51.6±28.2	60.4±27.1	28.6±15.7	0.001[a]
3 months	1	50.7±31.1	58.0±29.7	32.5±28.0	0.026[a]
6 months	0	48.6±31.7	57.6±30.6	25.4±22.2	0.005[a]
Change from 0 to 6 months	0	-2.9±14.8	-2.8±15.3	-3.2±14.1	0.94[a]

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Nori et al.
ISA PED Submission

The patient voice: development and results of a pilot patient experience data (PED) survey

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Background: AL amyloidosis is a complex rare disease that is difficult to diagnose and treat.[1] Collectively, rare diseases are very poorly represented in medical literature.[2] Furthermore, our research shows that the patient and caregiver perspectives are practically absent, regardless of the rare disease.[2] It is essential that patient and caregiver voices be heard and incorporated into clinical practice to hasten diagnosis, initiate treatment early, and improve their quality of life in ways that are meaningful to them.
Objective: To develop a validated survey that captures patient experience data for patients with amyloidosis.
Methods: We fielded a 62-question pilot survey to understand patients' diagnostic journey and experiences with amyloidosis from January 6-12, 2022 on www.oneAMYLOIDOSISvoice.com, an online community of patients, advocates, caregivers, researchers, physicians, and other stakeholders. The survey was limited to self-identified patients with amyloidosis. Responses from those who completed <50% of the survey were censored. The survey was designed with16 sets of questions to determine internal consistency between responses. The data are reported as descriptive statistics.
Results: Of 45 responders, 39 met the prescribed respondent criteria. The results are summarized in the table. Twenty-six (66.7%) responders were in the 61 to 80-year-old group. There were 27/2418 (1.1%) responses with internal inconsistencies. Most (69.2%) patients were unhappy to receive their diagnosis and two-thirds underwent at least 5 tests to receive a correct diagnosis.
Conclusions: The pilot survey provided valuable insights into the impact of amyloidosis on patient psychosocial experiences and their diagnostic journey. It also revealed areas for improving clarity of questions for survey validation. A revised survey based on learnings from the pilot has been developed and fielded.

Table: Summary of Survey Responses

Parameter	Number (%) of responders
Amyloidosis type	
Immunoglobulin light chain (AL)	18 (46.2)
Wild-type transthyretin (ATTRwt)	12 (30.8)
Hereditary transthyretin (ATTRv)	6 (15.4)
Other	3 (7.7)
Patients with comorbidities	18 (46.2)
Response to diagnosis	
Relieved to receive diagnosis	7 (18.0)
Unhappy about diagnosis	27 (69.2)
Family/personal relationship	
Informed family	39 (100)
Worried that disease was passed on to children	7 (18.0)
Family was supportive	30 (76.9)

Improved family relationships	12 (30.8)
Worsened family relationships	2 (5.1)
Relationship with friends	
Informed friends	37 (94.9)
Friends were supportive	31 (79.5)
Improved friendships	9 (23.0)
Worsened friendships	1(2.6)
Work relationships	
Informed colleagues	25 (64.1)
Diagnosis interfered with work life	20 (51.3)
Diagnosis interfered with personal relationships	14 (35.9)
Diagnosis had negative impact on self esteem	15 (38.5)
Enjoyment of life	
Enjoying life as much as before diagnosis	18 (46.2)
Not enjoying life as much as before diagnosis	19 (48.7)
Diagnosis	
Underwent multiple (≥2) diagnostic tests	32 (82.1)
Underwent ≥5 tests to obtain correct diagnosis	26 (66.7)
Misdiagnosed	12 (30.8)
Life expectancy	
Expect life to be shortened	24 (61.5)
Do not expect life to be shortened	12 (30.8)
Treatment	
Receiving treatment for amyloidosis	31 (79.5)
Satisfied with treatment received	31 (79.5)
Seeing ≥3 specialists for treatment	18 (47.4)

References:
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The role of local complement expression in renal amyloidogenic light chain amyloidosis

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Background: Amyloidogenic light chain (AL) amyloidosis is a systemic protein misfolding and deposition disease, which most commonly affects kidney, heart or both. Recently, a potential role of the complement activation in the pathology of amyloidosis has been proposed, and both C9 and to a lesser extent C3 has been detected within amyloid deposits in the kidney [1].

Objective: With some complement factors occurring within AL amyloid, the aim of this study was to narrow down the potential complement activation pathways and to investigate the role of kidney cells as a potential source of the complement integrated into amyloid.

Material & Methods: To confirm the presence of complement factors C1q, C3c, C5b9 and C3d, immunohistochemistry was performed on kidney biopsies from 31 lambda and 10 kappa AL patients. Staining for complement factors and amyloid was evaluated and scored compartment-specifically in glomeruli, tubules and vessel walls. To further determine whether this complement response could be facilitated by kidney cells stimulated with AL fibrils, human mesangial cells and proximal tubular cells were co-incubated with AL protein fibrils. These fibrils were either mature fibrils isolated from the explanted heart of an AL patient (FibPat-C) or generated under laboratory conditions using the recombinant variable domain derived from the same patient (Fib Pat) or the corresponding germline sequence (Fib wt). After 24h of co-incubation, RNA was isolated and gene expression of 12 complement related genes was analysed using NanoString multiplex RNA analysis technology (C1s, C3, C5, CCN2, CD44, CD46, CD55, CD59, CFB, CFD, CFH, SERPING1).

Results: In renal biopsies from both lambda and kappa AL patients, the membrane attack complex C5b9 was detected in blood vessels and some glomeruli, and correlated with amyloid deposition. However, the most prominent staining in all compartments was observed for C3d, while only few cases had deposits positive for C3c and C1q. *In vitro*, the expression of most complement genes was unaffected by co-incubation with fibrils regardless of their genesis. Yet, in human mesangial cells some activating and inhibitory complement factors appeared to be downregulated after stimulation with FibPat-C or FibPat compared to cells treated with PBS.

Summary & Conclusion: In summary, both C3d and C5b9, correlated spatially with amyloid in glomeruli and vessel walls, but only C3d was also found tubulo-interstitially. In contrast, complement factors of neither the classical nor the alternative pathway of complement activation were enriched. I*n vitro* stimulation of mesangial cells with AL fibrils failed to induce expression of complement factors, suggesting that they are either expressed locally by other cells or enter the kidney from the bloodstream. However, further studies are needed to investigate the influence of the potential local inhibitory complement response to amyloid fibrils on renal AL pathology.

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Support and Funding: This work was supported by a DFG grant in the context of the research unit FOR 2969 (AM_93/13-1). We would also like to thank all our colleagues of the FOR 2969, who did not directly participate in this project, especially the team of the Institute of Protein Biochemistry Ulm, who kindly provided us with some of the protein utilised in this study.

Transthyretin tetramer destabilization, marked by lower plasma transthyretin, is causally associated with increased risk of all-cause and cardiovascular mortality in the general population

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Background: Transthyretin tetramer destabilization is the rate-limiting step in transthyretin cardiac amyloidosis (ATTR-CA), an underrecognized contributor to mortality in older adults. Evidence suggests that increasing transthyretin tetramer destabilization is marked by lower plasma transthyretin concentrations.

Objective: We tested the hypothesis that increasing transthyretin tetramer destabilization, marked by lower plasma transthyretin, was causally associated with incident all-cause and cardiovascular mortality, and whether plasma transthyretin at or below the 5th- percentile was associated with the same endpoints in the general population.

Material & Methods: We genotyped 102,204 individuals from two studies of the Danish general population, the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS), and measured plasma transthyretin concentrations in a subset of 20,694 individuals. We first tested whether genetic variants in the transthyretin gene (*TTR)* which were associated with stepwise lower transthyretin tetramer stability and lower plasma transthyretin, were associated with higher risk of all-cause and cardiovascular mortality and whether this association was causal using Mendelian randomization. Second, we tested observationally whether extreme low plasma transthyretin concentration at or below the 5th-percentile versus 6-95th-percentile in the CGPS, was associated with higher risk of all-cause and cardiovascular mortality and validated the results in the CCHS.

Results: Compared to p.T139M (n=485 heterozygotes), a well-known transthyretin stabilizing variant, *TTR* genotypes which associated with increasing tetramer destabilization, were also associated with stepwise lower plasma transthyretin of an average -20% for wild-type (n=20,132) and -30% for heterozygotes for "Other mutations" (p.V142I, p.H110N, p.D119N; n=77). The corresponding hazard ratios (HRs) for all-cause and cardiovascular mortality, using p.T139M as the reference, were 1.37 (1.06-1.77) and 1.63 (0.92-2.89) for wildtype and 1.66 (0.95-2.88) and 2.23 (0.78-6.34) for "Other mutations" (Figure 1). In instrumental variable analyses, a one standard deviation genetically determined lower plasma transthyretin, was associated with incidence ratio ratios of 2.34 (1.21-4.52) for all-cause mortality and 10.8 (2.61-44.9) for cardiovascular mortality, suggesting a causal relationship between transthyretin tetramer destabilization and risk of all-cause and cardiovascular mortality (Figure 2). In observational analyses in individuals in the CGPS with plasma transthyretin concentrations ≤5th percentile versus 6-95th percentile (reference), multifactorial adjusted HRs were 1.39 (1.17-1.64) and 1.66 (1.17-2.38) for all-cause and cardiovascular mortality, respectively, with similar findings in the CCHS.

Summary & Conclusion: Transthyretin tetramer destabilization, marked by lower plasma transthyretin, is causally associated with increased risk of all-cause and cardiovascular mortality in the general population.

Figure 1.

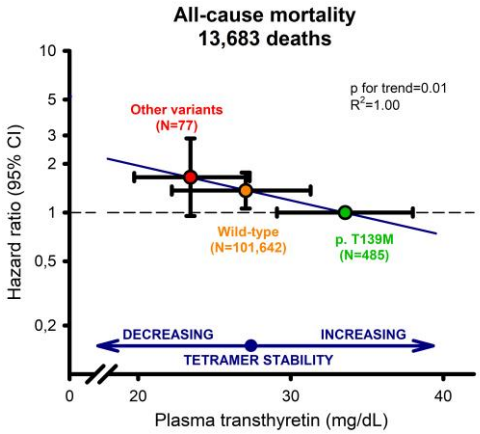

Figure 2.

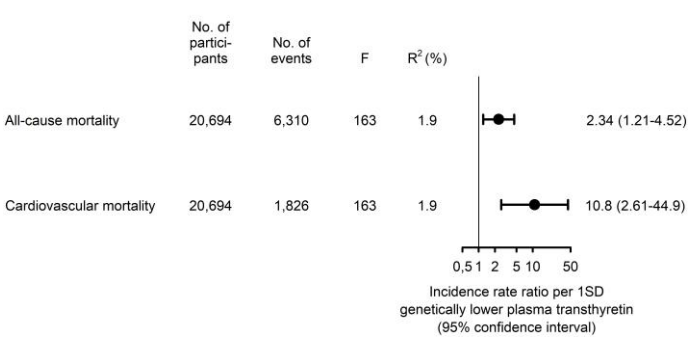

Figure 1.: Risk of all-cause mortality as a function of genetically determined plasma transthyretin concentration using *TTR* p.T139M as the reference group in the Copenhagen City Heart Study and the Copenhagen General Population Study combined. “Other variants” include *TTR* p.H110N, p.D119N and p.V142I.

Figure 2.: Risk of all-cause and cardiovascular mortality for a 1 standard deviation genetically lower transthyretin concentration in the Copenhagen City Heart Study and the Copenhagen General Population Study combined. Risk ratios were derived from instrumental variable analyses and were adjusted for age, sex, and population. R^2 is the proportion of plasma transthyretin explained by the genetic instrument. The F-statistic is a measure of the strength of the genetic instrument. An F-statistic above 10 is indicative of an instrument of sufficient strength.

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Two-year follow-up of the first case of systemic light chain amyloidosis treated with anti-B cell maturation antigen -CAR T cells

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Background: Chimeric antigen receptor T cell (CART) therapy is a groundbreaking approach in the treatment of relapsed/refractory multiple myeloma (MM). Occasionally, a patient with MM will develop a light-chain (AL) amyloidosis with organ dysfunction, which is currently an exclusion criterion for CART clinical trials**.**

Objective: In 2021, we reported the first case to our knowledge of a patient with MM who evolved to an AL amyloidosis treated with B cell maturation antigen (BCMA) CART therapy[1]. Here we present the recently achieved two-year follow-up after infusion of the BCMA CART ARI0002h.

Material & Methods: Our institution developed an academic second generation humanized 41BB-based CART targeting BCMA, called ARI0002h, which is currently being tested on the CARTBCMA-HCB-01 clinical trial for patients with relapsed/refractory MM. The patient underwent treatment with ARI0002h as a compassionate use after approval by the local Research Ethics Committee and the Spanish Agency of Medicines and Medical Devices. An informed consent form was obtained. Patient medical records were collected for analysis.

Results: A 61-year-old woman was diagnosed with an IgA-lambda symptomatic MM in 2014. She received several lines of treatment including an autologous stem cell transplantation, proteasome inhibitors, immunomodulatory drugs and the CD38-targeted antibody daratumumab. Despite the previous therapies, the patient relapsed with a serum M-protein of 20.7 g/L, lambda serum free light-chain (FLC) of 231 mg/L and bone marrow infiltration by 23% plasma cells (normal FISH), without evidence of extramedullary disease by PET-CT or other CRAB signs. However, she developed edema and significant non-selective albuminuria (24-hour proteinuria of 2626 mg with urinary M-protein of 307 mg). Therefore, subcutaneous fat aspirate and renal biopsy were performed showing amyloid deposits of lambda type. Diagnosis of systemic AL amyloidosis with renal involvement (revised Mayo Stage II) was stablished. Cardiac involvement was ruled out. BCMA expression in bone marrow plasma cells before CART infusion was 23%. The patient received 3 x10^6 ARI-0002h cells/kg in a fractionated manner (3 doses) after lymphodepletion with fludarabine (90 mg/m^2) and cyclophosphamide (900 mg/m^2), developing a grade I cytokine release syndrome, treatment-related grade 4 neutropenia and grade 2 thrombocytopenia, with no evidence of neurotoxicity. The patient achieved a hematologic partial response at 1 month and a complete response (CR) at 3 months after CART infusion, with negative minimal residual disease (MRD) in the bone marrow by next generation flow cytometry maintained at 24 months. A renal response[2] was also obtained at 6 months and remained stable at 24 months (Figure 1). ARI0002h peak of expansion and persistence in peripheral blood (PB) by both flow cytometry and quantitative RT-PCR techniques are also depicted in Figure 1; ARI0002h disappearence in PB by RT-PCR around month 2 from infusion has not precipitated a hematologic relapse after 2 years of follow-up.

Summary & Conclusion: A patient with an AL amyloidosis evolved from a MM and treated with a BCMA-directed CART remains in hematologic CR and maintains the renal response 2 years after CART infusion. Considering the importance of obtaining fast and deep hematologic responses in the treatment of AL amyloidosis, CART could be a

promising therapeutic strategy for this disease.

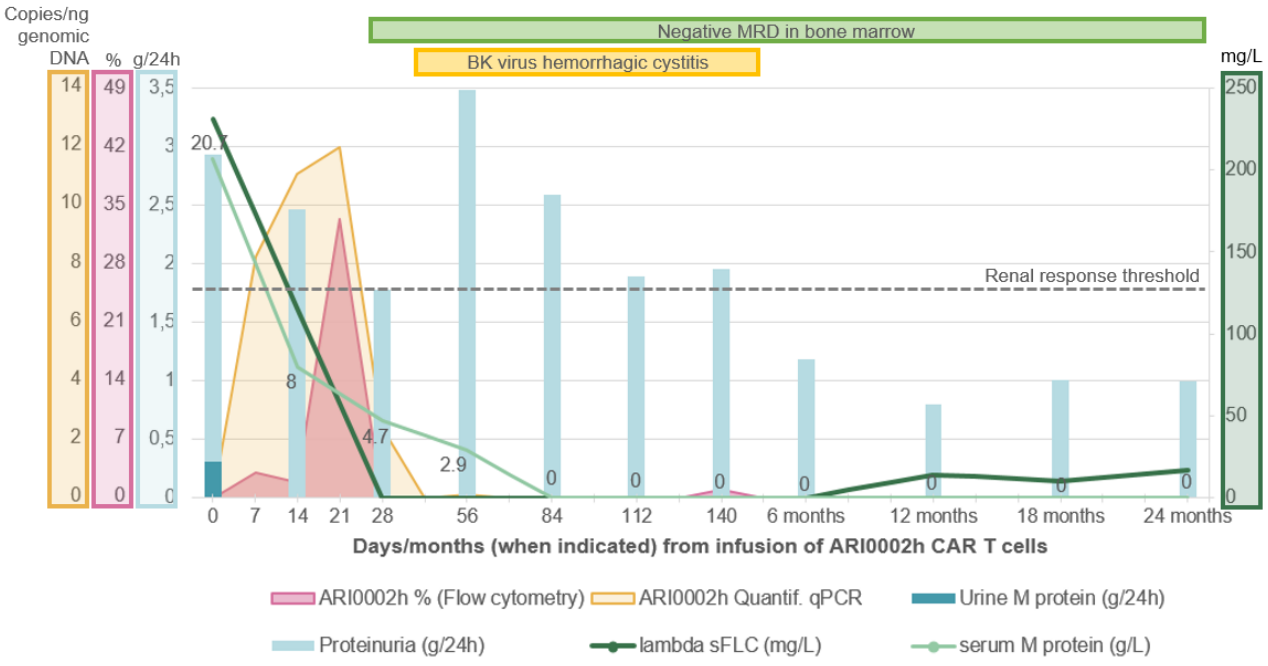

Figure 1.: Evolution of clinical and laboratory findings after infusion of ARI0002h in the course of the 24 months of follow-up. Numbers inside the graph refer to the serum M protein values at each timepoint (light green line).

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POSTER PRESENTATIONS WEDNESDAY, 7TH

Quantitative Sensory Testing: a good tool to differentiate between an asymptomatic carrier from an early symptomatic ATTRv amyloidosis patient?

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Background: Neurophysiological tools to evaluate early neurodegeneration in ATTRv amyloidosis have been widely studied showing a low sensitivity despite its high specificity. Quantitative sensory testing (QST) has been one of the tools used in clinical trials for follow-up and disease progression assessment.

Objectives: To detect the utility of QST in differentiating between an asymptomatic carrier from an early symptomatic ATTRv amyloidosis patient.

Material and Methods: A total of 208 ATTRV30M amyloidosis carriers were assessed with QST with vibratory (VDT) and cooling (CDT) detection thresholds, an intermediate heat–pain response (HP 5.0), heat–pain detection threshold (HP 0.5) and stimulus– response slope (HP 5.0–0.5). Subjects were divided in asymptomatic TTRV30M carriers and patients, all in stage 1 of disease, grouped according to Polyneuropathy Disability Modified score (mPNDs 0-2). Nonparametric statistics (Kruskal-Wallis H test) were used for group comparisons. Post-hoc pairwise comparisons were performed using Dunn's procedure, with a Bonferroni correction for multiple comparisons.

Results: A total of 208 ATTRV30M amyloidosis carriers (124 females) were included. Of these, 112 subjects (53,8%) were considered asymptomatic TTRV30M carriers and 96 (46,2%) symptomatic TTRV30M carriers (patients). All patients were in stage 1 of disease: 22 (22,9%) with organ involvement other than signs of sensory loss (mPNDs 0); 57 (59,4%) with mPNDs 1 and 17 (17,7%) with mPNDs 2 .

A male predominance (p = 0.025) and an older age (p= 0.013) is observed in the PNDs 2 group. Total Neuropathy Impairment Score (NIS) correlates with disease severity measured by mPNDs (p<0,001).

Statically significant differences were seen in CDT, HP 5.0 and HP 0.5 between asymptomatic carriers and patients with any score of mPNDs (p < =.05) consistent with small fiber involvement in very early stages of disease.

Asymptomatic carriers and patients with mPND 0 could be distinguished from patients with mPNDs 1 and 2 (p= 0.000) by VDT, substantiating the involvement of large fibers in more advance stages of polyneuropathy.

Summary & Conclusions: QST, in particular CDT and HP 0.5 modalities presents itself as a good tool to predict the transition from asymptomatic to early symptomatic stage in ATTRv amyloidosis, granting an early diagnosis and a suitable treatment to prevent or delay disease progression.

The Journey to Diagnosis of ATTR Amyloidosis: Burden of Early Disease

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Background:

Transthyretin-mediated (ATTR) amyloidosis is an underdiagnosed, progressive, debilitating, and fatal disease caused by misfolded TTR protein, and is classified as hereditary (hATTR amyloidosis), caused by pathogenic *TTR* gene variants, or wild-type (wtATTR amyloidosis), with no variant present. Multisystem involvement, including sensorimotor, autonomic, and cardiovascular (CV) manifestations, has been seen in both disease types.

Objective:

To use a patient survey to gain insights on patients' medical histories on their path to ATTR amyloidosis diagnosis.

Material & Methods:

In 2020, an on-line survey was conducted in collaboration with Amyloidosis Support Groups (ASG) in the US in ≥18 yrs old patients with a diagnosis of ATTR amyloidosis. Patients reported signs/symptoms, diagnoses, and procedures pre-ATTR amyloidosis diagnosis.

Results & Discussion:

Responses were obtained from patients with hATTR amyloidosis (n=20; male, 45%; mean age at diagnosis, 65.6 yrs) and wtATTR amyloidosis (n=27; male, 89%; mean age at diagnosis, 72.5 yrs). A substantial symptom burden was observed pre-diagnosis, with >7 disease-typical symptoms reported in 55% hATTR and 19% wtATTR amyloidosis patients. Neuropathy- and CV-related symptoms with a severe/very severe impact on daily life were reported in both hATTR and wtATTR amyloidosis. In hATTR amyloidosis, neuropathy symptoms were most common (tingling in feet [60%], numbness in hands [55%]/feet [55%]), while in wtATTR amyloidosis CV symptoms were most common (fatigue, shortness of breath [both 63%]), with neuropathy symptoms reported at a lower prevalence (4%-56% depending on the type of neuropathy). Symptoms were reported >10 years pre-diagnosis in both disease types; however, the mix of symptoms differed. In general, both neuropathy and CV symptoms were present in ≥10% of hATTR amyloidosis patients >10 years pre-diagnosis, and persisted up to diagnosis. In contrast, the only symptom potentially related to neuropathy in ≥10% of wtATTR amyloidosis patients (male only) at >10 years pre-diagnosis was erectile dysfunction, and CV and other neuropathy symptoms became more frequent <10 years pre-diagnosis. In both disease types, some of the earliest neuropathy and CV symptoms had a moderate/severe impact on daily life, and the number of different symptoms that reached ≥10% prevalence increased approaching diagnosis. Patients with both disease types reported orthopedic- diagnoses >10 years before their diagnosis; however wtATTR amyloidosis patients reported the most orthopedic procedures (56% had carpal tunnel syndrome [CTS] release surgery). Specific CV-related diagnoses were present in ≥10% of wtATTR amyloidosis patients 0–10 years from diagnosis, yet only reached this prevalence later in hATTR amyloidosis patients. The most common orthopedic, CV, and neuropathy diagnoses in both disease types were bilateral CTS, thickened LV wall, and irritable bowel syndrome (hATTR)/sleep apnea (wtATTR), respectively.

Summary & Conclusion:

Patients with ATTR amyloidosis experience a myriad of burdensome symptoms and other diagnoses on their journey to ATTR amyloidosis diagnosis. Symptoms start early and accumulate over time. Neuropathy- and CV-related symptoms are seen in both disease types in the 10 years pre-diagnosis, highlighting the multisystem nature of the disease and the importance of considering patients' full medical histories in identifying the disease at an early stage.

Figure 1. Signs or symptoms with a prevalence of ≥ 10% from 0 to 10+ years before diagnosis of ATTR amyloidosis.
In the survey, patients could only report one time point at which they first experienced a particular manifestation. For each time category (0 to 3; 4 to 10 and >10 years), each of the manifestations shown here were reported by at least 10% patients. Bolded manifestations are considered cardiac-related, non-bolded manifestations are neuropathy-related.

	> 10 years	4–10 years	0–3 years before ATTR diagnosis
hATTR Amyloidosis	**Heart palpitations/heart fluttering**	Shortness of breath	Shortness of breath
	Fatigue	**Fatigue**	**Fatigue**
	Dizziness/lightheadedness	Tingling in hands	**Swelling in your lower legs/feet**
	Numbness in hands	Tingling in feet	**Fainting**
	Numbness in feet	Unusual sensation/discomfort in feet	**Dizziness/light headedness**
	Tingling in hands	Unable to regulate body temperature	Numbness in hands
	Tingling in feet	Alternating constipation and diarrhea	Numbness in feet
	Unable to regulate body temperature		Tingling in hands
			Tingling in feet
			Unusual sensation /discomfort in hands
			Unusual sensation /discomfort in feet
			Electric shocks
			Loss of ability to sense temperature
			Muscle weakness
			Alternating constipation and diarrhea
			Nausea
			If male: erectile dysfunction
			Voice changes
			Loss of bowel control
			Difficulty walking
			Difficulty swallowing/choking
wtATTR Amyloidosis	Erectile dysfunction	**Heart palpitations/heart fluttering**	**Heart palpitations/heart fluttering**
		Shortness of breath	**Shortness of breath**
		Fatigue	**Fatigue**
		Muscle weakness	Swelling in your lower legs/feet
		Erectile dysfunction	Dizziness/light headedness
		Sexual dysfunction	Numbness in your hands
			Numbness in your feet
			Tingling like pins and needles in your hands
			Tingling like pins and needles in your feet
			Unusual sensation or discomfort in your hands
			Unusual sensation or discomfort in your feet
			Muscle weakness
			Alternating constipation and diarrhea
			Difficulty walking

Key: Cardiovascular-related; Neuropathy-related

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Manifestations of chronic heart failure in patients with AL-amyloidosis and ATTR-amyloidosis. Prospective observation data.

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Background.

Amyloidosis is a systemic disease, characterized by extracellular deposition of a specific soluble precursor protein that aggregates in the form of insoluble fibrils in various organs and tissues, leading to a violation of their structure and functions. The urgency of the problem is associated with the variety of clinical manifestations and the difficulty of diagnosis in everyday medical practice, leading to late diagnosis and untimely appointment of targeted therapy.

Objective.

To evaluate the clinical manifestations of chronic heart failure in patients with AL - amyloidosis and ATTR amyloidosis.

Material & Methods.

A prospective study with an annual comprehensive clinical and instrumental examination included 19 patients with a confirmed diagnosis of AL (15 patients) or ATTR (4 patients) amyloidosis. The average age was 61.2 ±8.72 and 71.25±6.63 years, respectively, among them men – 11 (58%), women - 8 (42%). Among patients with AL amyloidosis of men – 7 (47%) and women - 8 (53%), among patients with ATTR amyloidosis of men – 4 (100%), women - 0. Median follow-up 1 [0.5; 5] year.

Results & Discussion.

The time from the onset of the first clinical manifestations in the groups was 3.5±2 and 7±3.5 years, respectively.

In a survey of complaints related to the assessment of the degree of chronic heart failure, patients with AL – amyloidosis noted shortness of breath in 14 cases (74%), with ATTR amyloidosis in 3 (75%), p= 0.289, edema of the lower extremities – 13 (87%) and 2 (50%), p= 0.110, dizziness – 5 (33%) and 2 (50%), p= 0.540, clinical manifestations of orthostatic hypotension – 4 (27%) and 0, p= 0.246.

The degree of CHF (NYHA) among patients with AL - amyloidosis and ATTR - amyloidosis was grade 1 in 4 (27%) and 0 patients, respectively, p=0.246, grade 2 in 3 (20%) and 2 (50%) patients, p= 0.227, grade 3 in 6 (40%) and 2 (50%) patients, p= 0.719.

According to echocardiography, the ejection fraction in patients with AL -amyloidosis was 45±12%, with ATTR amyloidosis - 51±6%, the thickness of the interventricular septum – 1.7± 0.2 cm and 1.8± 0.7 cm, stroke volume – 46.6±15 ml/m2 and 45±6.6 ml/m2, the systolic pressure in the pulmonary artery – 41.1±12.8 mmHg and 28.6±16.8 mmHg, hydropericardium – 6 (40%) and 1 (25%), p= 0.581, respectively.

Ascites was observed in 4 (27%) patients with AL - amyloidosis and 0 patients with ATTR amyloidosis, p= 0.246.

In patients with AL – amyloidosis, monotherapy with loop diuretics was prescribed in 10 (67%) patients, therapy with two or more diuretics - in 5 (33%). Among patients with ATTR-amyloidosis, monotherapy was prescribed in 3 (75%) patients, therapy with two diuretics in 1 (25%).

13 patients with AL-amyloidosis underwent specific therapy, two patients did not receive specific therapy due to terminal CHF.

Therapy with tafamidis at a dose of 20 mg per day is carried out in 3 patients with ATTR -amyloidosis.

During the follow–up period, 5 deaths were registered among patients with AL – amyloidosis: 1 – as a result of pulmonary embolism, 1 – as a result of pneumonia, 3 - as a result of terminal CHF. There were no fatal cases among patients with TTR amyloidosis.

Summary & Conclusions. In patients with AL – amyloidosis, manifestations of heart failure debut at an earlier stage of the disease, are more severe in nature compared to patients with ATTR - amyloidosis.

Artificial Intelligence Enhanced Electrocardiogram: A Simple Tool to Monitor for Clinical Improvement in Cardiac Amyloidosis?

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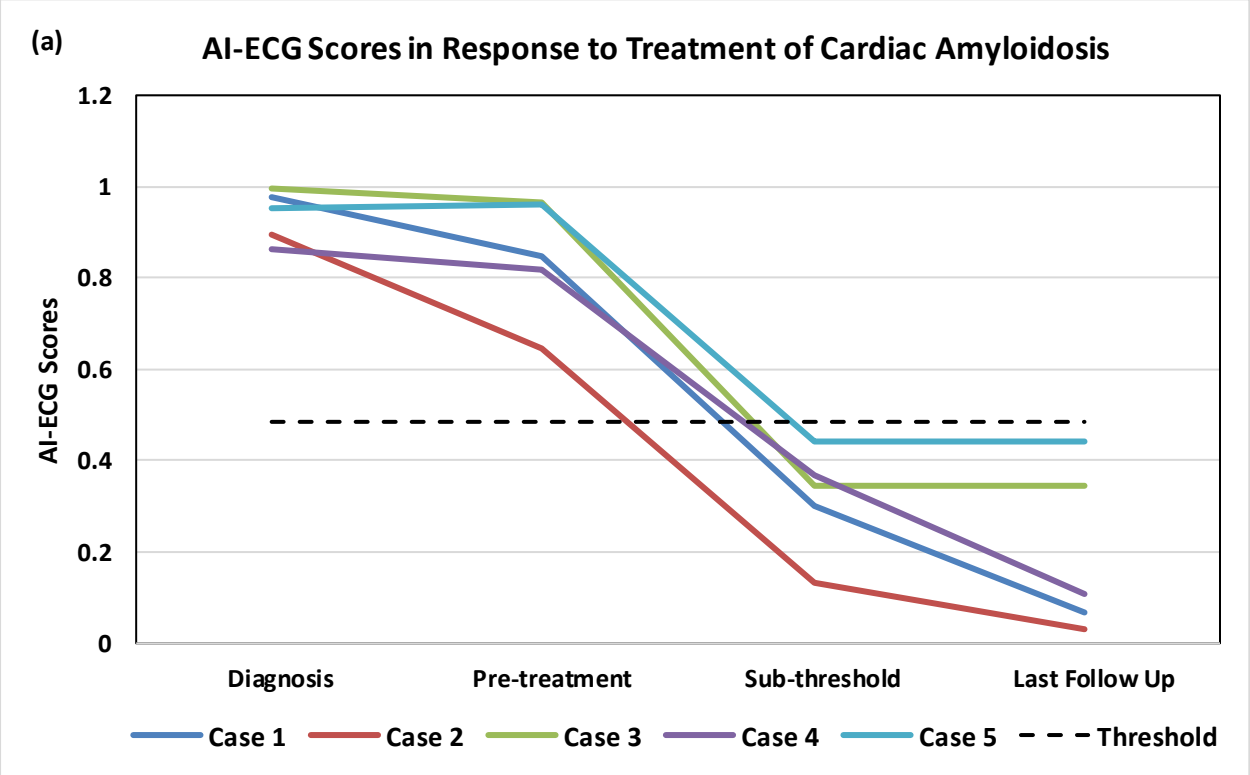
Background: Artificial intelligence enhanced electrocardiogram (AI-ECG) is an effective tool for detection of all forms of cardiac amyloidosis, which may promote early diagnosis (1, 2). AI-ECG score has also been shown to hold value in prognostication by predicting survival in patients with cardiac amyloidosis (3). However, the role of AI-ECG in monitoring impact of therapeutic interventions is not well defined.

Objective: To identify the role of AI-ECG in monitoring response to therapy patients with immunoglobulin light chain (AL) and transthyretin (ATTR) cardiac amyloidosis.

Material & Methods: We identified 5 cases of cardiac amyloidosis (4 AL and 1 ATTR) from our institutional cardiac amyloidosis database who had a high AI-ECG probability of cardiac amyloidosis at the time of diagnosis with subsequent decrease in the AI-ECG score below the optimal probability threshold of 0.485 (previously reported by Grogan et al [2]) after therapeutic interventions. These cases were reviewed via the institutional electronic health record system.

Results & Discussion: Of 5 patients, 2 were females. Mean age was 64 years (range: 60-72 years). All patients were evaluated and treated with response to treatment as detailed in the Table below. Mean AI-ECG score at the time of diagnosis, pre-treatment, first sub-threshold AI-ECG score and last follow up were 0.937 (range: 0.863-0.996), 0.656 (range: 0.646-0.965), 0.229 (range: 0.134-0.442) and 0.199 (range: 0.0309-0.442), respectively, as shown in Figure, panel (a). Time for AI-ECG scores to reach below threshold ranged from 33 days to 46 months. Corresponding laboratory and echocardiographic data are listed in the Table below.

Summary & Conclusions: This series of cases demonstrates that AI-ECG may be a useful tool to monitor for clinical improvement after treatment of cardiac amyloidosis. Future studies can focus on validation in a larger cohort to define the role of AI-ECG in monitoring for clinical improvement as well as surveillance during remission in cardiac amyloidosis.



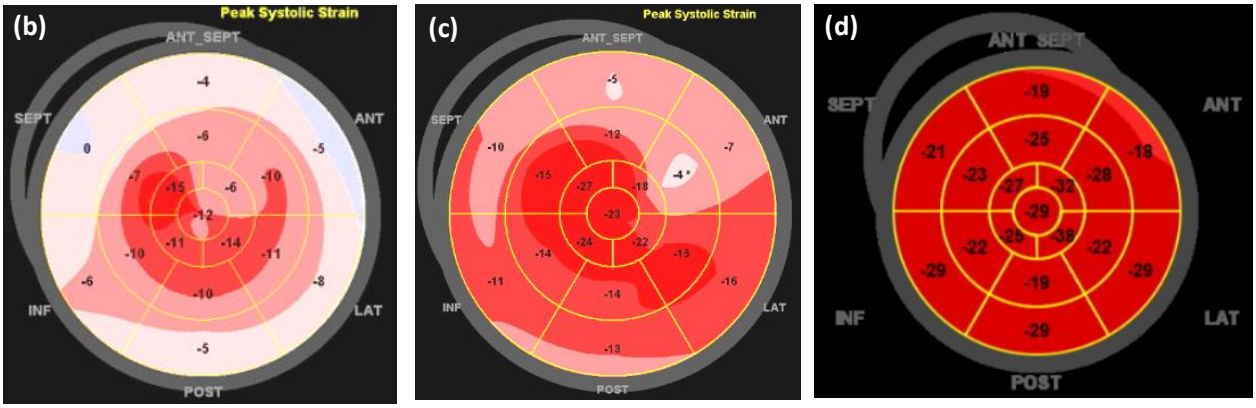


Figure: (a) AI-ECG scores of all 5 cases at different points during clinical course with scores decreasing below the optimal probability threshold of 0.485 in all patients after therapeutic interventions.
(b) Polar maps from case 5 at the time of diagnosis showing abnormal GLS (-7%) with apical sparing pattern commonly seen in cardiac amyloidosis.
(c) Improvement in GLS (-15%) and apical sparing pattern on follow up after treatment with inotersen.
(d) Sample polar map showing normal global longitudinal strain or GLS (-25%) with a normal pattern.

Table: Basic Characteristics and Results

	Case 1	Case 2	Case 3	Case 4	Case 5
Amyloid type	AL	AL	AL	AL	hATTR (T60A)
Age at Diagnosis (years)	63	61	62	60	72
Gender	Male	Female	Female	Male	Male
Treatment	ASCT → RCT: Ixazomib v SOC	Dara-CyBorD → ASCT	CyBorD → ASCT	ASCT	Inotersen
Response to therapy	Complete HR	Complete HR	Very good partial HR	Very good partial HR	Good clinical response
Troponin T, 4th generation (ng/mL)^ or 5th generation (ng/L)*					
Diagnosis	<0.01^	177*	27*	<0.01^	<0.01^
Peak Value	0.02^	177*	39*	0.02^	43^
Last Follow Up	16*	47*	20*	63*	43^^
NT-proBNP (pg/mL)					
Diagnosis	1350	847	1904	215	1512
Peak Value	2113	17316	1904	1137	2155
Last Follow Up	385	481	540	66	234^^
Posterior Left Ventricular Wall Thickness (mm)					
Diagnosis	14	9	16	12	15
Last Follow Up	12	11	-	10	-
Global Longitudinal Strain (%)					
Diagnosis	-13	-17	-10	-17	-7
Last Follow Up	-14	-15	-	-20	-
Artificial Intelligence enhanced ECG (AI-ECG) Scores					
Diagnosis	0.977	0.895	0.996	0.863	0.954
Pre-treatment	0.849	0.646	0.965	0.819	0.961
Sub-Threshold**	0.301	0.134	0.345	0.367	0.442
Last Follow Up	0.0676	0.0309	0.345	0.108	0.442
Time to Sub-Threshold	46 months	33 days	10 months	17 months	41 months

AL: Light chain Amyloidosis. ASCT: Autologous Stem Cell Transplant. Dara-CyBorD: Daratumumab + Cyclophosphamide + Bortezomib + Dexamethasone. hATTR: hereditary Transthyretin Amyloidosis. HR: Hematologic Response. NT-proBNP: N-terminal pro B-type Natriuretic Peptide. SOC: Standard of Care. T60A: Threonine to Alanine substitution at position 60
^^Values taken from the time of last follow up and do not correlate with AI-ECG scores at the time of last follow up
**Sub-threshold: defined as a decrease in AI-ECG score below the optimal probability threshold of 0.485

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Diagnostic and therapeutic center for amyloidosis at Kumamoto University

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Background: The Amyloidosis Center at Kumamoto University was established in 2012. We support diagnosis of systemic and localized amyloidosis and provide consultation service for physicians about current treatments for amyloidosis. Our amyloidosis center team includes neurologists, cardiologists, hematologists, nephrologists, radiologists, ophthalmologists, orthopedists, transplant surgeons, and pathologists. Treatable amyloidosis patients are taken care of our center team members.

Objective: To analyze cases with suspected amyloidosis in the Amyloidosis Center at Kumamoto University.

Material & Methods: We performed histopathological, genetical and proteomical analyses in our center. For amyloid-typing, at first, we perform immunohistochemistry (IHC) using amyloid-specific antibodies panel in our center. If identifying amyloid precursor protein by IHC was difficult, we perform laser microdissection-liquid chromatography tandem–mass spectrometry (LMD-LC–MS/MS) analysis.

Results: In 2021, 909 cases were analyzed. Amyloidosis typing by IHC was performed on 680 cases, mass spectrometry on 36 samples, and TTR genetic testing on 193 samples. In 680 cases, 76% was amyloid positive but 24% was negative. The amyloid proteins were identified in 96% via IHC using our antibody panel. ATTRwt was 60%, AL was 33%. ATTRv was 3%, AA was 2%. Number of cases with ATTRwt amyloidosis was increasing year by year in our center. In 4%, amyloid-typing was difficult by IHC. We performed LMD-LC-MS/MS analysis for 36 cases and amyloid proteins were identified in 28 cases (88%). Proteomic amyloid typing was difficult in cases with a small quantity of amyloid deposits or a small tissue sample.

Summary & Conclusion: We supported diagnosis of amyloidosis by means of histopathological, genetical and proteomical analyses in our center. Number of cases with ATTRwt amyloidosis was increasing year by year.

Figure 1

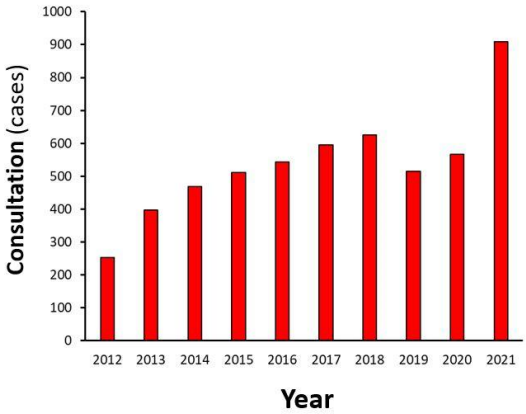

Figure 2

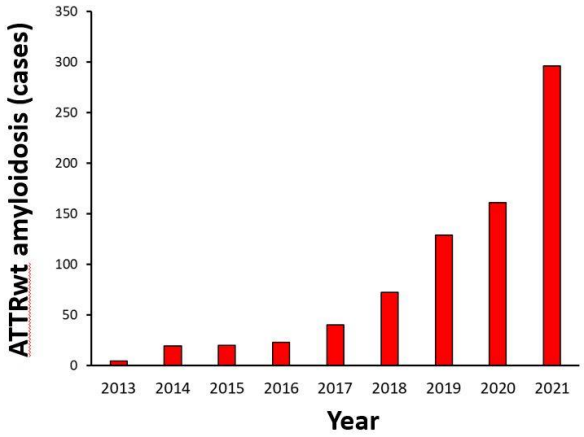

Figure 1.: The number of consultation on diagnosis for amyloidosis in the amyloidosis center at Kumamoto University.
Figure 2.: The number of cases with ATTRwt amyloidosis diagnozed in the amyloidosis center at Kumamoto University.

Support & Funding: This study was supported by Global Bridges at Mayo Clinic and Pfizer Global Medical Grants.

EXPERIENCES AND DECISION-MAKING IN CONFIRMED AND POTENTIAL CARRIERS OF ATTR-RELATED GENETIC VARIANTS

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Background: Transthyretin (TTR) amyloidosis (ATTR) is a progressive, debilitating form of amyloidosis caused by the aggregation and deposition of misfolded TTR protein in organs and tissues, resulting in damage and dysfunction. TTR becomes unstable due to inherited variants or aging. There is a current lack of research regarding the experience and considerations of asymptomatic individuals with family members who are diagnosed with hereditary ATTR (ATTRv) and why they may choose to pursue or not pursue genetic testing to discover whether they, too, are carriers of variants. Individuals with at least one family member experiencing symptomatic ATTRv and who have received a positive genetic test have been defined as "confirmed carriers" and those who have not done genetic testing have been defined as "potential carriers."

Objective: The objective of this study was to identify differences in attitudes, beliefs, and behaviors related to genetic testing, disease awareness, and interest in preventative medicine between confirmed and potential carriers.

Material & Methods: To understand the experiences of confirmed and potential carriers, a series of five virtual focus groups with geographically dispersed individuals (confirmed carriers=12; potential carriers=8) across the U.S., U.K., and E.U. were conducted by third party moderators.

Results: 67% of confirmed carriers and no potential carriers reported being followed by an ATTR specialist or receiving regular tests to screen for ATTR symptoms. Key differences in the perspectives of confirmed versus potential carriers were uncovered and are summarized in fig 1. Confirmed carriers reported that resolving uncertainty and potential prevention were catalysts in the decision to pursue testing. Potential carriers indicated that specific life events, such as the development of symptoms, family planning, or aging, were potential to pursue testing. Individuals within the U.S. reported concerns regarding the impact of genetic test results on insurance eligibility. This concern was not reported by individuals in the U.K. and E.U.

Summary & Conclusion: These findings indicate that asymptomatic individuals with at least one family member diagnosed with hereditary ATTR may choose to pursue or not pursue genetic testing and seek preventative care for a variety of reasons, some of which are determined by personality, family experience, attitudes about preventative care, and regional regulations related to insurance privacy.

POTENTIAL CARRIERS	CONFIRMED CARRIERS
Typically **uninformed** about ATTR & treatment options	Typically **informed** about ATTR & treatment options
Consider themselves **healthy**	Consider themselves **at risk**
View ATTR as **manageable & treatable**	View ATTR as **serious & debilitating**
Not motivated to proactively monitor symptoms	**Motivated** to proactively monitor symptoms
Hesitant to take experimental preventative treatments	**Interested** in taking experimental preventative treatments

Figure 1.: Key differences in attitudes about ATTR, relative risk, and preventative care between confirmed and potential carriers.

Support & Funding: Support and funding was provided by BridgeBio Pharma.

Potential sources of error in the identification and referral of amyloidosis to a tertiary center

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Background: The diagnostic approach to amyloidosis is changing, particularly with the widespread acceptance of ^{99m}technetium-based pyrophosphate (^{99m}Tc-PYP) scintigraphy as non biopsy criteria for detecting transthyretin (ATTR) cardiac amyloidosis. Greater awareness and ease of workup may lead to more testing for amyloidosis in the community, which has the potential to produce a higher false positivity rate. Thus, there is a need to understand the limitations of amyloid-related diagnostic testing.

Objective: To describe the common sources of error in the identification and referral of amyloidosis.

Material & Methods: We conducted a retrospective reviewof all referrals to the Boston University Amyloidosis Center between 2010 and 2021, in order to identify false positive referrals—defined as patients with suspicion of amyloidosis who were determined to have absence of amyloid pathology after an extensive workup at our center. Information about the factors leading to referral were collected from medical records. Data accrual from consented patients was approved by the Institutional Review Board.

Results: Of 2424 referrals of suspected amyloidosis, 162 (7%) demonstrated an absence of amyloid pathology based on extensive assessment at our center. This percentage increased over time, from 4% in 2010 to 15% in 2021. False positive referrals were younger in age (median 62 vs. 65 years, p=0.002) and more likely female (48% vs. 38%, p=0.015) or a racial minority (23% vs. 17%, p=0.040), compared to true positives. Thirty-three/sixty-two (20%) individuals were asymptomatic and amyloidosis was suspected solely based on incidental laboratory, imaging or histological findings.

The most common sources of inaccuracy in disease identification (Table 1) were erroneous staining in the community of tissue specimen with Congo red (n=66, 41%), followed by suspicious findings on cardiac imaging that turned out not to be amyloid in nature (n=42, 26%). In recent years, misinterpretation of ^{99m}Tc-PYP scans emerged as a major source of false positive referrals (n=20, Figure 1), most often due to blood pooling artifact (n=8) and high myocardial calcium content (n=4). There were 8 (5%) patients who received treatment for amyloidosis prior to referral, which was discontinued after assessment at our center.

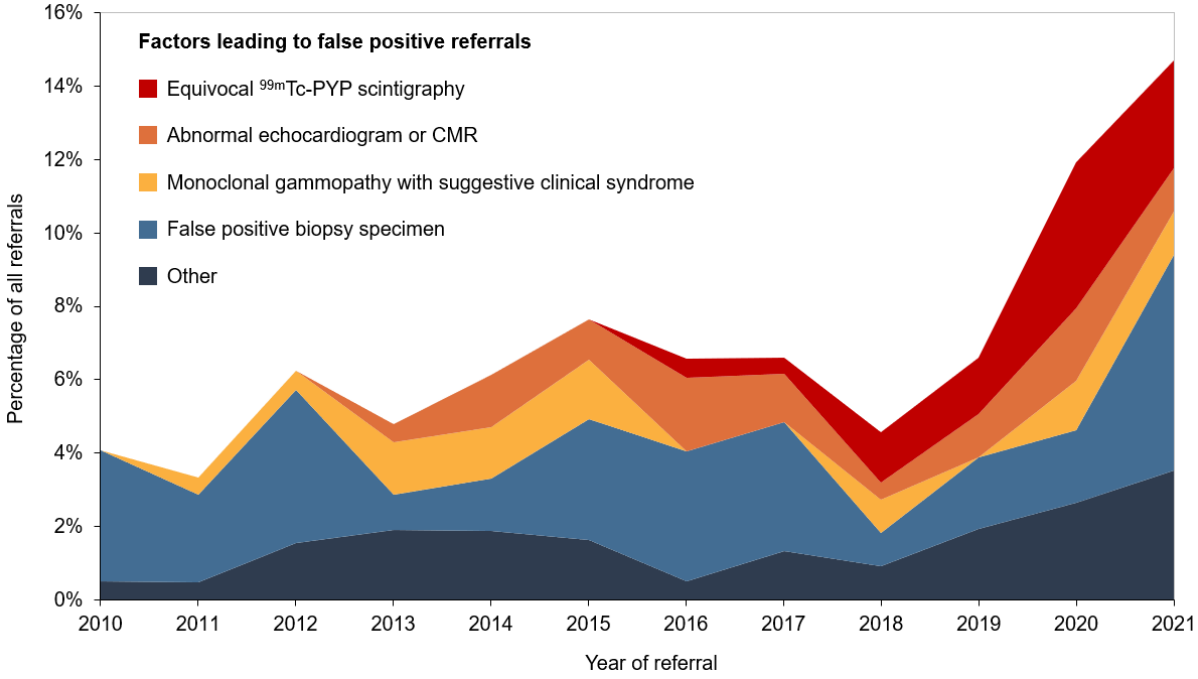
Summary & Conclusion: Having an awareness about these common sources of diagnostic error in the workup of amyloidosis can improve patient care. Referring patients with suspected amyloidosis to centers of excellence serves an important role in the diagnostic process, by helping to ensure accurate diagnosis and prevent mistreatment.

TABLE 1: Factors leading to false positive referrals to the Amyloidosis Center (N=162).

	n (%)
False positive Congo red staining of tissue specimen	66 (41%)
Suspicious findings on cardiac imaging*	42 (26%)
^{99m}Tc-PYP scintigraphy	20
Echocardiogram	14
CMR	8
Monoclonal gammopathy with suggestive clinical syndrome	17 (11%)
Genopositivity for a transthyretin variant without active amyloid pathology	15 (9%)
Suggestive clinical syndrome without monoclonal gammopathy	12 (7%)
Family history of amyloidosis	6 (4%)
Other	4 (2%)

Abbreviations: ^{99m}Tc-PYP, 99mtechnetium pyrophosphate; CMR, cardiac magnetic resonance imaging.

FIGURE 1: The emergence of cardiac imaging as a major source of false positive referrals of amyloidosis. Abbreviations: ^{99m}Tc-PYP, 99mtechnetium pyrophosphate; CMR, cardiac magnetic resonance imaging.



Support & Funding: The Amyloidosis Center database is supported by the Amyloid Research Fund of Boston University School of Medicine.

Higher Length of Stay and Readmission Burden in Heart Failure Patients with Cardiac Amyloidosis Than Those without

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Background:
Recognition of cardiac amyloidosis (CA) and initiation of novel targeted therapies are associated with improved survival and reductions in heart failure (HF) readmission. Despite the rising prevalence, it is unknown whether these outcomes remain discrepant between CA and non-CA HF patients in this contemporary era.

Objective:
Comparing trends of readmission and LOS between CA and non-CA HF patients.

Material & Methods:
We collected hospitalization data for all HF patients undergoing evaluation of CA between January 2018 and December 2020. CA was confirmed by a combination of PYP imaging, cardiac magnetic resonance imaging, and assessment of paraproteinemia. Data on HF readmission and length of stay (LOS) were collected by review of electronic medical record.

Results & Discussion:
A total of 108 patients underwent evaluation for CA during the study period, of which 26 (24%) had CA (transthyretin=23; light-chain=3). Patients with CA were older than patients without (mean age 77 vs 69 years, respectively, p=0.003). Gender and race distribution were similar between the two groups.

Patients with CA had similar HF readmission rates compared to those with non-CA HF (1.0 vs 0.9 admission/patient/year, p=0.736), however time to readmission was significantly shorter in CA patients (0.6 vs 0.2 months, p<0.001). Cumulative LOS was significantly longer in patients with CA (10.0 vs 3.6 days/patient/year, p<0.001). Patients with CA had longer cumulative LOS prior to (4.5 vs 2.3 days/patient/year, p=0.002) and after (3.1 vs 1.2 days/patient/year, p=0.002) diagnosis, compared to non-CA HF patients. Patients without CA had a significant decline in cumulative LOS after the exclusion of CA, this effect was not significant in patients with CA [Figure].

Summary & Conclusions:
Despite confirmatory diagnosis, patients with CA have a shorter time to HF readmission and

greater LOS. As expected, mortality remained higher for HF with CA. Novel clinical approaches need to be instituted for CA patients to reduce HF readmission.

Figures:

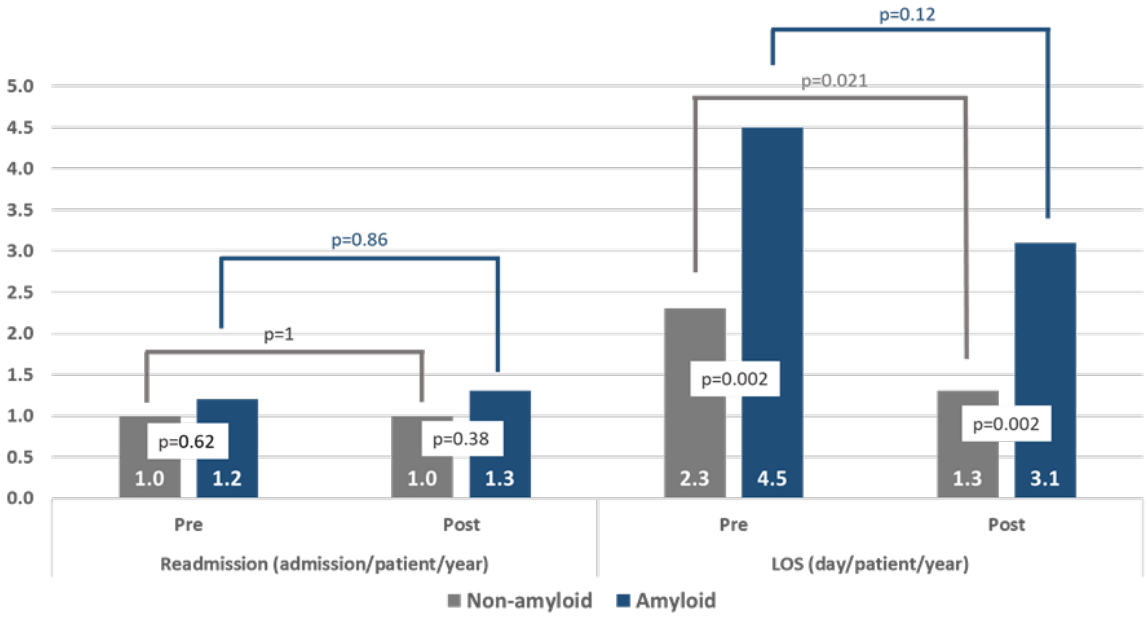

Figure: differences in LOS and readmission between CA and non-CA HF patients and within each group before and after diagnosis.

References:
None

Support & Funding:
None

MANAGEMENT AND PATIENT ESTIMATION OF AMYLOID LIGHT-CHAIN (AL) AMYLOIDOSIS IN PORTUGAL: RESULTS FROM A PHYSICIANS' SURVEY

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Background: Light-chain (AL) amyloidosis is a clonal, non-proliferative plasma cell disorder in which fragments of immunoglobulin light or heavy chain are deposited in tissues in the form of amyloid fibrils.[1] According to an incidence-to-prevalence model from 2019, the estimated incidence in Portugal is 12.50 cases per million individuals, with an equal 5-year prevalence of 35.41 cases per million individuals.[2]

Objective: Assess the AL Amyloidosis patient journey, understand treatment strategies in Portugal as well as unmet needs. Estimate AL Amyloidosis patient numbers, including potential underdiagnosis, according to epidemiology, public hospital Diagnosis Related Group (DRG) database and key opinion leaders' insights.

Material & Methods: Market research study supported by qualitative interviews with 4 hematologists key opinion leaders (KOL's). Total AL Amyloidosis patients were estimated through epidemiologic data available for Portugal and supported by Hospital production quantified using the National Health Service (NHS) hospital Diagnosis Related Group (DRG) database.

Results: Most patients begin their medical process through General Practice. After referral, patients are ultimately transferred to Hematology for final diagnosis and follow-up. HCPs report a time to diagnosis of up to one year with multiple referrals needed and limited treatment options. Patients eligible for transplant may reach a median overall survival of 4-5 years, while non-eligible patients tend to live 1 to 2 years after diagnosis. Only 10% of patients are eligible for Autologous Stem Cell Transplant (ASCT), with the preferred choice of treatment being Cyclophosphamide, bortezomib, dexamethasone (VCD). On the other hand, most patients (~90%) are not eligible for ASCT, where VCD triple combination or combinations of proteasome inhibitors are reported Standard of Care (SoC) for frontline treatment. Since effective therapies for this disease are lacking, such results may lead to a widespread use of Daratumumab, as suggested in the recently published Mayo Clinic treatment guidelines.[3] A forecast model was developed, considering a medium value for AL Amyloidosis prevalence in MM patients of 12.5%, and assuming a MM prevalence of 2 262 patients in Portugal.[4] Between 2017 and 2018, only 68 patients were treated with an official diagnosis of AL Amyloidosis in Portuguese hospitals, which is lower than the reported prevalence in the literature. Considering NHS hospital DRG database and assuming the 12.5% prevalence of AL amyloidosis in MM patients, there is an estimate of 351 AL amyloidosis patients in Portugal, from which only 19.4% are properly diagnosed, while 80.6% are potentially miscoded or undiagnosed.

Summary & Conclusion: AL amyloidosis pathway is not well established, and hematologists report a long time to diagnosis and treatment. Physicians highlight an urgent need for well-tolerated therapies that can tackle organ involvement, with less frequent adverse effects and less severe toxicity. The lack of awareness in early detection, as well as patients developing AL Amyloidosis after a MM diagnosis that are not classified with the second pathology, are the main reasons claimed by physicians that lead to a possible underdiagnosis in Portuguese hospitals.[5] The undeniable gap between the estimated prevalence of AL amyloidosis patients in Portugal and treated patients with an AL amyloidosis diagnosis coding in Portuguese public hospitals, reinforces the pressing need to adopt health policies that may prevent misdiagnosis/delayed diagnosis.

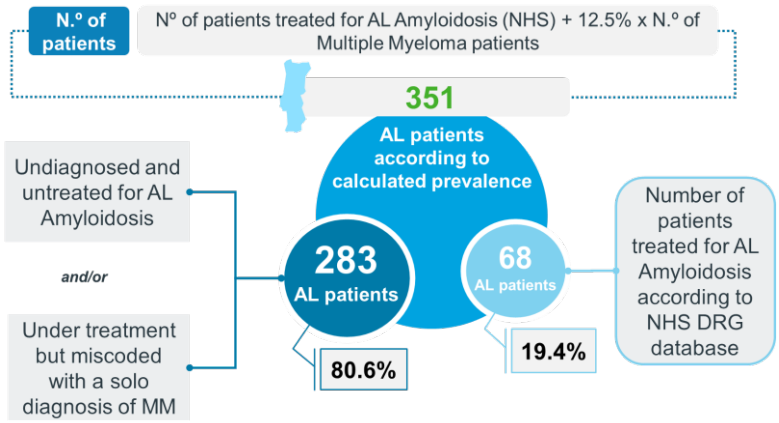

Figure 1.: Estimated AL Amyloidosis prevalence in Portugal, including diagnosed patients and undiagnosed/misdiagnosed patients

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Prevalence of pauci-symptomatic amyloid transthyretin cardiac amyloidosis in the general population

Alberto Aimo[1,2], Iacopo Fabiani[2], Vincenzo Castiglione[1], Chiara Arzilli[2], Andrea Barison[2], Valentina Spini[2], Giuseppe Vergaro[1,2], Francesco Gentile[2], Elisa Poggianti[2], Claudia Taddei[2], Daniela Puccianti[2], Annalisa Picerni[2], Martina Niccolai[2], Giorgia Panichella[1], Elena Iacopini[3], Gaspero Pitti[3], Stefano Moscardini[3], Claudio Passino[1,2], Michele Emdin[1,2]

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Background: Amyloid transthyretin cardiomyopathy (ATTR-CM) has become treatable. Wild-type ATTR-CM is an age-related disorder. Establishing the exact prevalence of ATTR-CM in elderly subjects from the general population may be useful for healthcare providers and policy makers.
Objective: To define the prevalence of ATTR-CM in elderly subjects from the general population.
Material & Methods: We are conducting a population screening in an area of Tuscany (Italy) where there is no cluster of variant ATTR. From March to December 2021 we screened 500 subjects aged ≥65 years followed by 3 general practitioners (GPs) in Terricciola (about 40 km from Pisa). From March 2022, we are evaluating individuals followed by GPs based in the city of Pisa. All subjects undergo first-line exams including clinical history and physical examination, electrocardiogram, transthoracic echocardiogram, and blood sampling. The following elements are searched: 1) any clinical red flag of amyloidosis (history of carpal tunnel syndrome, lumbar spine stenosis, etc.), 2) interventricular septal thickness ≥12 mm or other echocardiographic red flags, 3) hs-troponin T higher than the reference value (r.v.; 14 ng/L). Patients with any of these elements are referred to second-line exams (serum free light chain measurements, serum and urine electrophoresis, and ^{99m}Tc-hydroxymethylene diphosphonate scintigraphy). Further exams are decided on an individual basis to reach a definite diagnosis of CA and its subtype.
Results: As of April 12, 2022, 761 subjects ≥65 years have been evaluated for possible participation (697 in Terricciola and 64 in Pisa). Among them, 198 (26%) could not be contacted, were reluctant to enter the study, or were bedridden. The other 563 subjects (74%) have undergone first-line exams. Thirty-eight percent of individuals (n=214) have been referred to second-line exams (search for a monoclonal protein and diphosphonate scintigraphy), which have been performed in 118 of them (55%). Two subjects have been found to have no monoclonal protein and an intense cardiac bone tracer uptake (Perugini score 2). They were both women, aged 83 and 78 years. The first patient had been operated to both hands for carpal tunnel syndrome, and the second one had a history of atrial flutter and pericardial effusion. The echocardiogram showed an interventricular septal thickness of 12 and 14 mm, respectively, with normal left ventricular (LV) ejection fraction (and 60%, respectively), and LV mass index of 90 and 94 g/m^2, respectively; both patients had grade 1 diastolic dysfunction and none had moderate or severe valve disease. hs-troponin T (26 and 90 ng/L, respectively; r.v. <14 ng/L) and N-terminal pro-B-type natriuretic peptide (249 and 2041 ng/L, respectively; r.v. <125 ng/L) were elevated in both patients, while renal function was within normal limits. Both patients complained of mild dyspnea on effort (New York Heart Association class II), and the second patient was on oral furosemide 25 mg daily (0.37 mg/kg). The search for *TTR* gene mutation was negative in both cases.
Summary & Conclusion: Based on these findings, the prevalence of pauci-symptomatic wild-type ATTR-CM in the elderly population can be calculated as 2/467=0.4%, which is well above the threshold for a rare disease (5 in 10,000). Nonetheless, it is important to notice that this prevalence was calculated on a small cohort and following the identification of individuals with early-stage disease.

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Redefining the epidemiology of cardiac amyloidosis. A systematic review and meta-analysis of screening studies

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Background: An algorithm for non-invasive diagnosis of amyloid transthyretin cardiac amyloidosis (ATTR-CA) and novel disease-modifying therapies have prompted an active search of CA.
Objective: To define the prevalence of CA in different disease settings based on the available literature.
Material & Methods: We performed a systematic search for screening studies on CA, focusing on the prevalence, sex and age distribution in different clinical settings. The review was registered on PROSPERO (CRD42022306259).
Results: The prevalence of CA in different settings was as follows: bone scintigraphy for non-cardiac reasons (n=5 studies), 1% (95% confidence interval [CI] 0-1%); heart failure (HF) with preserved ejection fraction (n=6), 12% (95% CI 6-20%); HF with reduced or mildly reduced ejection fraction (n=2), 10% (95% CI 6-15%); conduction disorders warranting pacemaker implantation (n=1), 2% (95% 0-4%); surgery for carpal tunnel syndrome (CTS; n=3), 7% (95% CI 5-10%); hypertrophic cardiomyopathy phenotype (n=2), 7% (95% CI 5-9%); severe aortic stenosis (n=7), 8% (95% CI 5-13%); autopsy series of "unselected" elderly individuals (n=4), 21% (95% CI 7-39%). The average age of CA patients in the different settings ranged from 74 to 90 years, and the percentage of men from 50% to 100%. Many patients had ATTR-CA, but the average percentage of patients with amyloid light-chain (AL) CA was up to 18% (**Figure 1**).
Summary & Conclusion: Searching for CA in specific settings allows to identify a relatively high number of cases, who may be eligible for treatment if the diagnosis is unequivocal. ATTR-CA accounts for many cases of CA across the different settings, but AL-CA is not infrequent. Median age at diagnosis falls in the eighth or ninth decades, and many patients diagnosed with CA are women.

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Support & Funding: none.

Finding a balance between specialist and local care: amyloidosis patient perspectives on a single-centre approach

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Background: The model for amyloidosis care in the UK is a single specialist service, the National Amyloidosis Centre (NAC). Since the NAC was established in 1999, the service has grown. In 2012 the centre saw 3473 patients; this increased to 5926 in 2020. The NAC makes every effort to promote equal access to the service, but regional discrepancies in diagnosis and referral exist(1)potentially leading to inequity of access to care and patient outcomes. The COVID19 pandemic and travel restrictions have also highlighted the vital need for local expertise.

Objective: The aim of this study was to seek the opinion of amyloidosis patients about the concept of local amyloidosis hubs across the UK, and a move away from a single specialist centre
Material & Methods: UK Amyloidosis support groups were contacted and feedback obtained via an online survey. Patients were asked about delays in their diagnosis or treatment, and where these delays occurred. There was the opportunity for qualitative feedback.

Results & Discussion: A total of 22 patients responded. All patients were satisfied with the service they received at NAC, but relayed difficulty in accessing the centre. One elderly patient travelled by car, train, plane and taxi to reach the centre. She voiced that a centre closer to home would significantly benefit her rural area. Over two thirds (68.2%) of patients experienced delays, including being referred from their general practitioner to local specialist (32.1%) and also in seeing an amyloidosis expert in London (28.6%) (Figure 1).Qualitative feedback focused on the impact of delays:

- *"We can have the best drugs in the world but if diagnosis is too late then what's the point. It's no good getting a referral to a specialist centre if delays have been caused by not enough awareness or specialist knowledge."*
- *"The way forward is more centres of excellence to increase publicity around the condition, new centres will raise interest and awareness about amyloidosis which is key."*
- *"It would be good to see services expanding as more and more patients are being diagnosed."*

Summary & Conclusions: Amyloidosis care in UK has had a centralised model of care, with expertise focused in a single centre. The strengths of this model are high quality diagnostic assessment and research, but necessitates lengthy travel to the South East of England. This study explored patient opinions about the current service. All patients were complementary about the care provided by NAC, but reported difficulties with access, lack of local expertise all leading to diagnostic delays. This reflects previous research where patients reported having to travel for treatment as inconvenient and a practical hardship(2) due to the social and physical demands of travel(3).The COVID19 pandemic has also stressed the importance of building resilience into a service. A single specialist centre risks limitations in resources, information, systems and processes. Although this study is small, the patient feedback obtained supports the creation of new specialist treatment centres, in geographically appropriate locations, so patients can receive care closer to home. Service development to a hub-and-spoke model would expand the reach of the NAC, allowing the centre to accommodate a larger number of patients, from a greater geographical area. This would improve accessibility, increase awareness, and improve the resilience of the service by allowing shared resources, including staffing. We hope this study prompts discussion around existing models of amyloidosis care in the UK and globally.

Figures

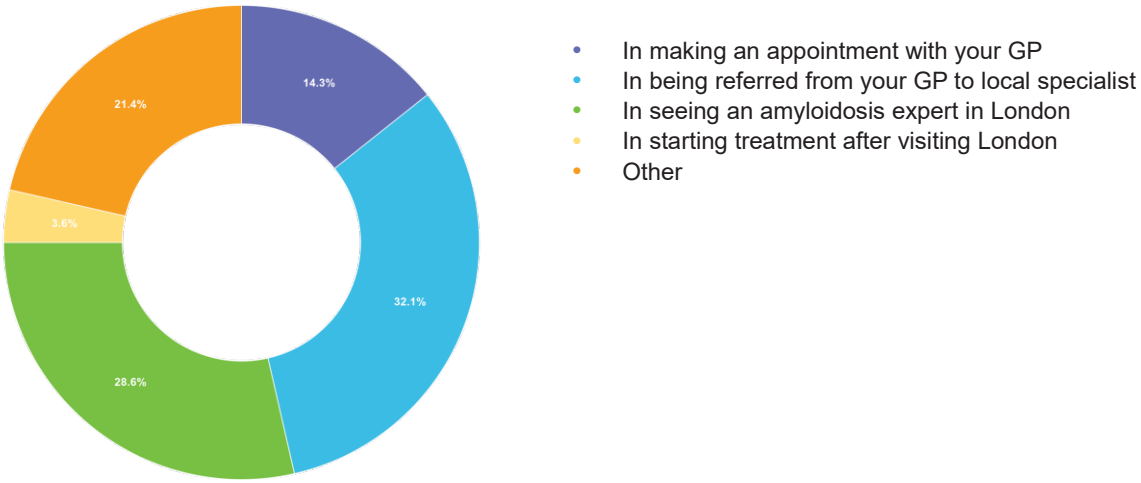

Figure 1. Patient feedback from an online questionnaire asking: 'if there was a delay in your patient journey, what step did the main delay occur?'

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Multidisciplinary approach for the early detection of amyloid in patients who undergo carpal tunnel syndrome or lumbar stenosis surgery. Preliminary results of an ongoing study.

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Background: Symptomatic carpal tunnel syndrome (CTS) and lumbar stenosis (LS) seem to precede the cardiac manifestations of cardiac amyloidosis (CA) due to transthyretin amyloidosis (ATTR), so they could represent early markers of CA. CTS is more commonly present in CA due to wild-type ATTR (ATTRwt), although it can also be present in its hereditary variant (ATTRv) and in primary amyloidosis caused by immunoglobulin light chain deposition (AL).

Objective: Evaluate the prevalence of amyloidosis in patients undergoing CTS or LS surgery, in an endemic area of the TTR mutation Val50Met (ATTRv).

Material & Methods: To date, 179 operated patients have been included (42 SL, 137 CTS) in whom an intraoperative biopsy was obtained (ligamentum flavum in SL; synovial tissue or flexor retinaculum in CTS) for histopathological analysis using Congo Red staining for amyloid detection and immunohistochemistry (IHC) for subtyping in amyloid A (AA), kappa, lambda and ATTR. Blood and urine test to rule out a monoclonal component and a cardiac scintigraphy (CS) with 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) to detect myocardial uptake were performed. CS was reported using the visual Perugini scale (0 = negative; 1 to 3 = increasingly positive). A cardiac SPECT/CT was performed in cases with a positive planar imaging, according to the ASNC/EANM Cardiac Amyloidosis Practice Points. Depending on the results of the three tests performed, patients with the diagnostic of amyloidosis or suspected amyloidosis ("positive amyloid patients") will be referred to Internal Medicine, Cardiology and Haematology departments for characterization, follow-up and assess the possibility of an early systemic amyloidosis, CA, or AL, respectively, by clinical and analytical evaluation, electrocardiography, other imaging techniques (echocardiography and cardiac MRI) and genetic test.

Results: A total of 150 biopsies were obtained (39 SL, 111 CTS), 9 of them were amyloid positive (6 SL, 3 CTS). All IHC were negative for AA, kappa, and lambda. IHC for ATTR is still pending. 26 operated patients without biopsy (3 SL, 23 CTS). Three biopsies are pending for histopathological result. 124 laboratory tests and CS have been performed: 2/124 CS were positive (grade 2-3) and underwent CTS surgery, one of them without biopsy and the other one with positive biopsy for amyloid. 122/124 CS were negative (grade 0). In 6/124 laboratory tests, a monoclonal component was detected. 67/79 completed cases with all three tests have completed the study. To date, positivity for amyloid has been obtained in 10/179 cases (5.6%): 7 positive biopsies (one of them with positive blood test for monoclonal gammopathy of undetermined significance (MGUS), one with positive CS and 5 with negative blood/urine test and CS), 2 positive biopsies with normal CS and the remaining blood/urine test still pending, and 1 CS positive without biopsy and normal blood/urine test. In the follow-up of positive cases, all of them have shown a negative diagnostic work-up for systemic amyloidosis, cardiomyopathy, or AL. In two patients with amyloid positive biopsy by SL: one had a low risk GMSI, and another had a non-Val50Met variant of undetermined significance (VUS) in genetic test.

Summary & Conclusion: The estimated prevalence of amyloidosis in our series of surgically treated patients with CTS or SL is 5.6% to date, with no signs or symptoms suggestive of localized or systemic amyloidosis in the follow-up. These are preliminary data in an ongoing study.

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Artificial Intelligence-Enhanced Models To Predict Light Chain Amyloidosis From Patients With Monoclonal Gammopathy Of Undetermined Significance And Smoldering Multiple Myeloma

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Background: Early diagnosis of AL amyloidosis remains a diagnostic challenge, mainly due to the low prevalence of the disease and heterogeneity of presentation. Artificial intelligence (AI) is a tool which can assist in improving disease recognition.

Objective: To establish an AI-based model to accurately discriminate patients with AL amyloidosis from patients with monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM), who are at risk of developing AL amyloidosis.

Material & Methods: We screened all consecutive patients from the dysproteinemia database at Mayo Clinic Rochester with the diagnoses of MGUS, SMM and AL amyloidosis who were diagnosed between January 1, 2000, and December 31, 2018 and evaluated within 90 days of their diagnosis. Patients who had more than one diagnosis were excluded. The choice of variables to be included in the models were those in whom data was available in >50% of the patients (with one exception being serum free light chain assay which was available in 44% of patients but deemed important for modeling). In a subset model, we incorporated the AI-enhanced electrocardiogram (AI-ECG) score for cardiac amyloidosis (available in routine clinical practice at Mayo Clinic). This variable is AI-based captures from standard 12-lead ECG to predict the probability of cardiac amyloidosis.[1] We randomly divided the final study cohort in a 1:1 ratio to training and validation set. Random forest algorithm was used for modeling to predict the probability of AL amyloidosis diagnosis versus non-AL amyloidosis diagnosis (i.e., MGUS/SMM).

Results: 20, 579 patients were screened, of which 4,087 patients (19.9%) were included in this study (MGUS n=2716, AL amyloidosis n=1217, SMM n=154). The first model (ALERT 20) included 20 variables, of which 7 were clinical and 13 were common blood count and blood chemistry testing (Figure). The validation set yielded an AUC-ROC of 0.946 for accurate classification of AL amyloidosis against MGUS/SMM. The second model (ALERT 20-ECG model) included all variables used in the ALERT 20 mode plus the AI-ECG score. By adding the AI-ECG score, the AUC-ROC in the validation set for accurate disease classification increased modestly to 0.958. As disease prevalence decreases the positive predictive value (PPV) and increases the negative predictive value (NPV) of a model, we tested the models' performance in various prevalence scenarios (Figure), including the estimated prevalence of AL amyloidosis among MGUS/SMM patients (estimated at 1/100-1/1000 person years). Although the PPVs of ALERT 20 and ALERT 20-ECG are low (<1-27.6%), the NPV is over 99%.

Summary & Conclusion: Two AI-based algorithms to aid clinicians in recognizing patients with AL amyloidosis and distinguishing them from patients with MGUS/SMM are presented. These models, when tested in populations at risk of AL amyloidosis (where the prevalence of AL amyloidosis is sufficiently elevated), have a plausible PPV to be used as screening tool for this uncommon, yet life-threatening, disease.

Reference

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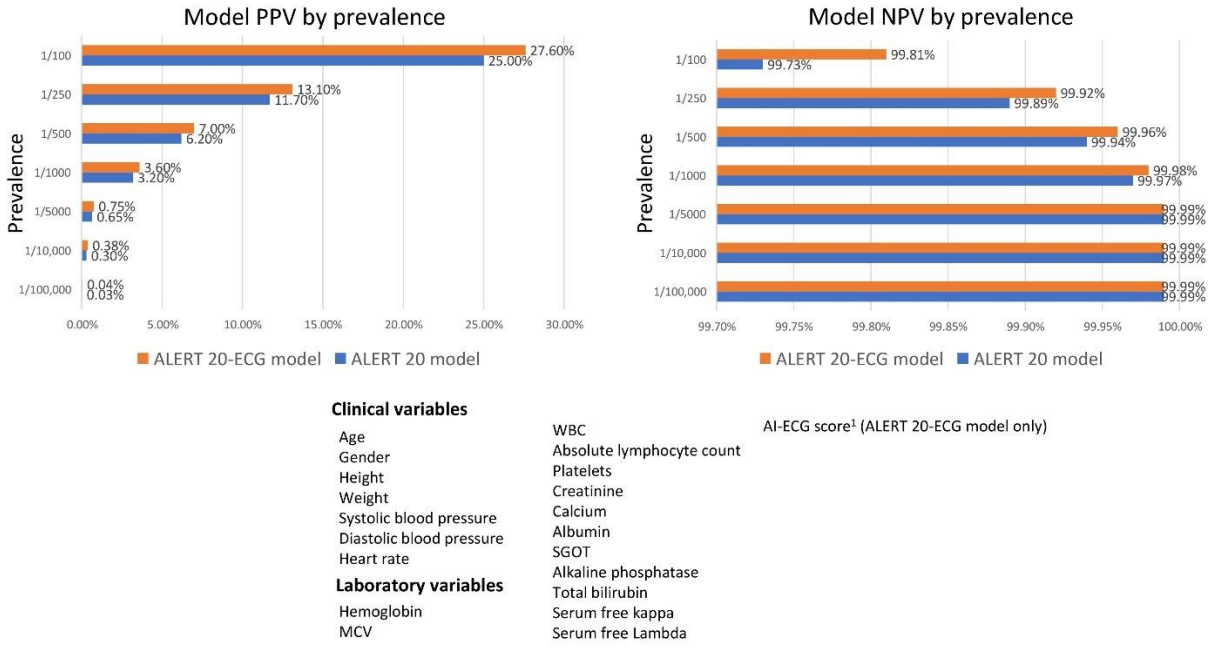

Clinical variables

Age
Gender
Height
Weight
Systolic blood pressure
Diastolic blood pressure
Heart rate

Laboratory variables

Hemoglobin
MCV
WBC
Absolute lymphocyte count
Platelets
Creatinine
Calcium
Albumin
SGOT
Alkaline phosphatase
Total bilirubin
Serum free kappa
Serum free Lambda

AI-ECG score[1] (ALERT 20-ECG model only)

Figure: NPV and PPV of ALERT 20 and ALERT 20-ECG models across various AL prevalences scenarios. The models' variable usage is listed at the bottom.

Support & Funding: none

Lumbar spinal stenosis syndrome as surrogate for transthyretin cardiac amyloidosis

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Background: Wild type transthyretin cardiac amyloidosis (ATTR-CA) is an underrecognized condition[1]. Lumbar spinal stenosis (LSS) often predates ATTR-CA by 5-10 years[1] and ATTR deposits were found in many ligament specimens after LSS surgery[2-3] but the association between LSS diagnosis and/or surgery with development of ATTR-CM over time is yet to be clarified.

Objective: We hypothesized that if LSS is a prodromal condition for ATTR-CA, patients (pts) with LSS should have higher rates of cardiovascular (CV) events and a worse overall survival (OS). If proven to be true, routine histological evaluation of ligamentum flavum at surgery as a means to earlier diagnosis of this life-threatening disease would be indicated.

Material & Methods: We performed a retrospective cohort study of pts aged 50 to 90 years with a history of LSS from 1995 to 2015, identified through the Rochester Epidemiology Project. History of LSS diagnosis and/or surgery was based on ICD codes. Three age- and sex-matched controls (CNTRL) were randomly selected from the same population. We evaluated several endpoints: survival, congestive heart failure (CHF), atrial fibrillation/flutter (AF) and pacemaker/defibrillator (PM/ICD) placement. The composite cardiac endpoint included any of these cardiac enpoints.

Results: A total of 6371 LSS cases and 16924 CNTRL were included. Median age of the LSS cases was 68 years, 45% were male. Of the cases, 17% also had a surgical procedure code indicating LSS surgery (operative cohort). Incidence of comorbidities was significantly higher in LSS cases vs matched CNTRL. LSS diagnosis significantly increased the risk of CHF vs. CNTRL (**Figure 1**, HR=1.78, 95% CI: 1.74 - 1.82; p<0.0001), although the operative cohort did not demonstrate the same risk (HR=0.89, 95% CI: 0.85 - 0.93; p<0.0001). Findings were similar regarding incident AF and PM/ICD placement. Cumulative incidence and risk of a composite outcome including CV events possibly related to ATTR-CA (CHF, AF or PM/ICD placement) was significantly higher in those with LSS diagnosis (**Figure 2**, HR=1.62, 95% CI: 1.62 - 1.66; p<0.0001). A total of 7013 deaths were reported with a median follow-up of 8.3 years (95% CI: 8.2 - 8.5). OS was significantly worse in LSS cases vs CNTRL (HR=1.10, 95% CI: 1.05 - 1.15; p<0.0001).

Summary & Conclusion: LSS diagnosis was associated with increased risk of CV events, possible manifestations of ATTR-CA, and worse OS. This risk was not demonstrated in the operative cohort, likely due to selection bias. We believe these data support routine histological evaluation of ligamentum flavum specimens to foster early diagnosis of ATTR-CA.

Figure 1

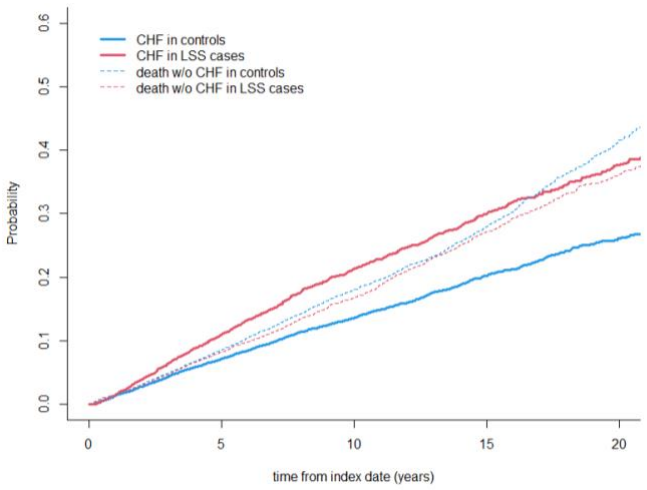

Figure 2

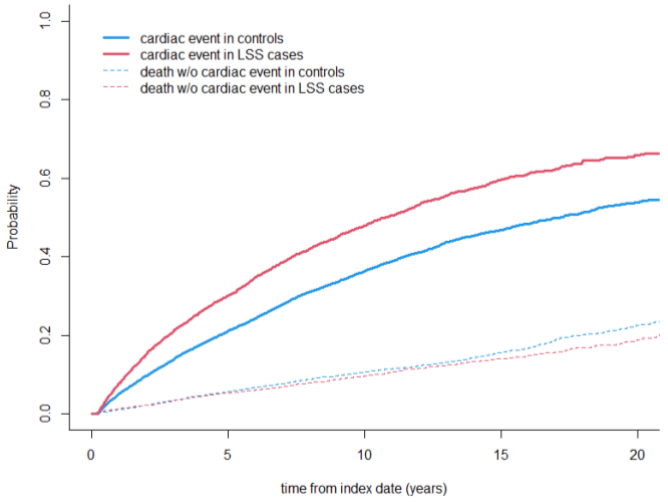

Figure 1.: Cumulative incidence of CHF including death as a competing risk, among patients with LSS and matched controls in those without a prior history of CHF.

Figure 2.: Cumulative incidence of the composite outcome of CV events (including CHF, AF or PM/ICD placement), including death as a competing risk, among patients with LSS and matched controls in those without a prior history of the cardiac events of interest.

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Expert recommendations for improving the implementation of nuclear scintigraphy to support accurate diagnosis of cardiac amyloidosis in a non-specialist setting

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Background: Nuclear scintigraphy (NS) is a powerful tool to support the diagnosis of cardiac amyloidosis. Compared with traditional invasive biopsy, the relative cost, ease of implementation, and non-invasive nature of NS has resulted in its broad uptake. While societal guidance documents support an accurate application of NS, experience gaps remain in the non-expert setting, impacting the quality of image and diagnostic accuracy.

Objective: To improve the acquisition and interpretation of NS for the diagnosis of cardiac amyloidosis.

Material & Methods: An international expert panel convened to identify gaps in guidance for non-experts.

Results: Experts identified several challenges experienced by non-experts implementing NS, including the absence of training and first-hand, practical experience in imaging protocols, the lack of widespread access to instruments and software to support optimal imaging approaches, the low levels of access to complementary procedures such as time allocated for SPECT and TTR sequencing, and a significant proportion of NS being performed without the required evaluation for the presence of a clonal dyscrasia; the latter being of paramount importance given the rapidly progressing nature of light-chain cardiac amyloidosis (AL-CM). In addition, a paucity in confidence among non-experts was noted across several areas, including patient eligibility, quantification methods for evaluating signal intensity, specific protocols for the use of different radiotracers, and clarity on scenarios that require additional confirmatory evaluation. Together these challenges impact the ability of some non-experts to confidently use NS to support a diagnosis of cardiac amyloidosis.
Several recommendations were made to improve the application of NS for non-experts: 1. Education on the inability of NS in isolation to diagnose transthyretin amyloid cardiomyopathy (ATTR-CM), with particular emphasis on the essential role of serum and urine biochemical tests to exclude AL-CM; 2. Consideration of cardiac magnetic resonance imaging prior to invasive cardiac biopsy in patients showing grade 1 cardiac uptake of radiotracers; 3. The need to adapt approaches based on the radiotracer used; 4. The essential requirement for scanning 3 hours post injection (versus 1 hour) in non-expert settings and in the absence of SPECT imaging; sites with expertise to read SPECT scans 1 hour post injection should extend to 3 hours whenever blood pooling is evident; 5. To avoid false-positive diagnosis, SPECT is strongly recommended (even when scanning 3 hours post injection) in addition to planar imaging, to avoid blood pooling being perceived as myocardial signal; SPECT and planar imaging should be combined, where possible using SPECT/CT.

Summary & Conclusion: Identification of knowledge gaps by multidisciplinary partners and sharing of expert knowledge will facilitate an accurate and standardised approach, enhancing the diagnostic rate and accuracy of NS for cardiac amyloidosis.

Support & Funding: This project was supported by Pfizer.

Amyloidosis and its multifaces: A case report

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[3]Hospital Pró-Cardíaco, Rj – Brasil

[4]Grupo Fleury - Brasil

Background: Amyloidosis is a systemic disease caused by the deposition of insoluble proteins in several organs, including the heart, especially by light chain (AL) or transthyretin (TTR), with a range of possible treatments, which are modifiers and reduce cardiovascular events, death and hospitalization.

Objetive: This case alerts to the multifacets of the disease and the care in the amyloid differentiation according to the European Position and published in 2021 and Positioning of the Brazilian Society of Cardiology (SBC) published in October 2021, v.117 by Arq. Brazil Cardiol.

Material & Methods: A case report of 75-year-old man undergoing an initial investigation of fatigue, edema and paresthesia of the lower limbs, denies chest pain, dyspnea or syncope. Sporadic report of change in bowel habit and postprandial fullness. Recent weight loss, not associated with changes in eating habits. He denies comorbidities, alcoholism or smoking.

Results: Renal function preserved, no anemia, normal transaminases. Serium Free Light (FL) Kappa (k) 7.72 mg/L and lambda (L) 1.4mg/L, K/L ratio 5.68, and serium FL K/L ratio 8,1, Nt-proBNP 5222 pg/dL. Cardiorespiratory physical examination without significant changes, symmetrical lower limb edema 2+/4. Echocardiogram with SIV 25mm, PP 22mm, LVEF 55%, grade 3 diastolic dysfunction, preserved biventricular function, with no change in contractility. Electroneuromyography with axonal pattern sensorimotor polyneuropathy, bilateral carpal tunnel syndrome. He underwent bone marrow biopsy and histopathology with hypocellular marrow with a slight amount of polyclonal plasma cells, Congo red negative. Abdominal fat biopsy performed with positive Congo red under polarized light and mass spectrometry detected TTR. Genetic testing configures mutation at the Val142Ile position in the TTR gene. Myocardial scintigraphy with grade 3 technetium pyrophosphate.

Summary & Conclusion: According to the Brazilian and European Positioning, the increase in free K and L light chains and in their K/L ratio initially directs the patient to the hematological route, which requires biopsy for confirmation of AL amyloid deposit. In this case, TTR deposit was detected in abdominal fat, and the laboratory result was interpreted as monoclonal gammopathy of uncertain significance, significantly more prevalent in man and blacks than in whites, a common premalignant plasma cell disorder in over 50 years. The diagnosis was a TTR hereditary amyloidosis and in this way it can be directed to the correct treatment.

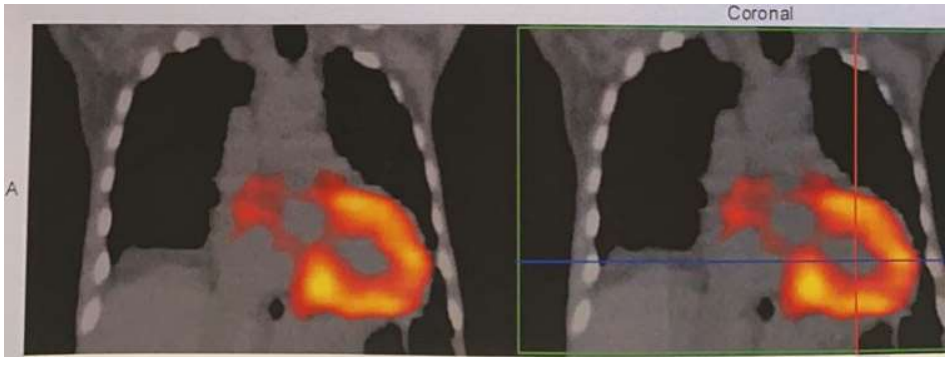
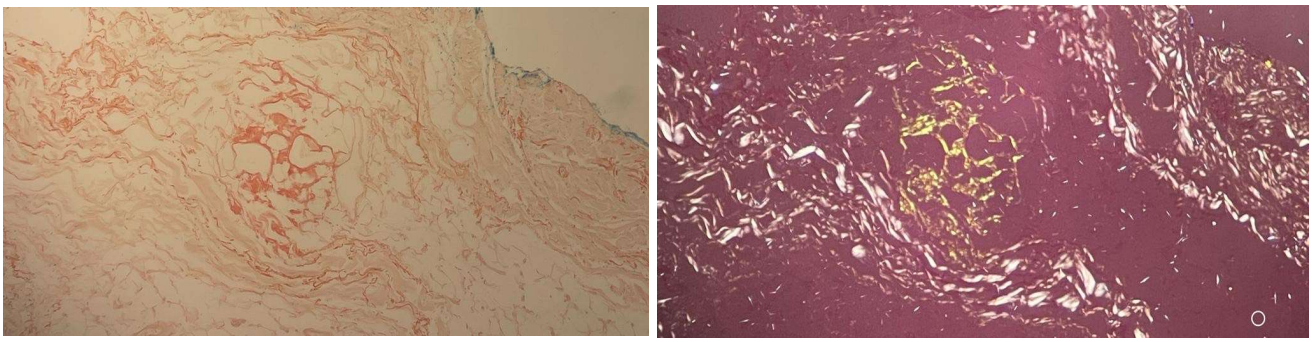


Figure 1.:
Myocardial scintigraphy with technetium pyrophosphate uptake in the heart.



A B

Figure 2.: A. Congo Red of fat biopsy. B. birefringence “apple green” in Congo red color.

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Real-life evaluation of an algorithm for the diagnosis of cardiac amyloidosis

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Background: The French referral centre Henri Mondor Amyloidosis Network, France, used algorithm of Gillmore et al. (1), to diagnose cardiac amyloidosis (CA) with bone scintigraphy (BS), gammopathy testing (GT), biopsies (extracardiac and cardiac), and genetic testing to diagnosis CA**.**

Aims: To evaluate the real-life use of a modified Gillmore algorithm for the diagnosis of cardiac amyloidosis (CA) at the French National Reference Centre for Cardiac Amyloidosis

(Henri Mondor Hospital, Créteil, France). In a "one-stop shop" approach, bone scintigraphy (BS), a monoclonal gammopathy test (GT), a salivary gland biopsy (SGB), and genetic testing were performed at the same time.

Method and Results: This retrospective cohort study was conducted from June 2008 to May 2019. All patients having undergone BS and GT after referral to the Henri Mondor reference centre for suspected amyloidosis were included in the study. A total of 1222 patients were included: 349 had no cardiac uptake on BS and negative GT (BS-/GT-), 276 were BS-/GT-positive (GT+), 420 patients were BS+/GT-, and 177 were BS+/GT+. Our "one-stop shop" amyloidosis check-up enabled us to diagnose 892 (73%) patients; only 330 (27%) patients required additional examinations, such as mass spectrometry and/or a cardiac biopsy. This subset notably included 112 patients with amyloid light chain (AL) amyloidosis. Over 64% of the patients with transthyretin (TTR) amyloidosis (ATTR-CA) or another type of amyloidosis were diagnosed during the "one-stop shop" visit. BS had a sensitivity of 99% and a specificity of 96% for the diagnosis of ATTR-CA. GT had a sensitivity of 100% and a specificity of 76% and SGB had a sensitivity of 54% and a specificity or 100% for the diagnosis of AL amyloidosis. 205 (17%) of the 910 TTR genetic tests detected TTR mutations - most of which were found in patients aged between 55 and 84.

Conclusion: The results of our real-life cohort study confirmed the ability of a "one-stop shop" approach with a modified Gillmore algorithm to diagnose CA and thus emphasized the importance of simultaneous testing for earlier diagnosis. The SGB has diagnostic value because it is easy, quick and less invasive than a cardiac biopsy. We also found that the diagnostic yield for a TTR mutation test was low in patients aged 85 or more.

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Funding: The French National Reference Centre for Cardiac Amyloidosis (Henri Mondor Hospital, Créteil, France) has received an institutional grant from Pfizer.

Optimal Patient Selection for Referral to Tc-99m-PYP Scanning for Transthyretin Cardiac Amyloidosis

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Background: Diagnosis of transthyretin cardiac amyloidosis (ATTR-CM) can be facilitated using echocardiography by identification of typical features, including several deformation-based ratios[1-3] and novel multi-parametric scores[4,5]. These measurements have been suggested to discriminate ATTR-CM from other causes of increased left ventricular wall thickness among patients referred for ATTR-CM evaluation, but limited data inform their relative predictive accuracy.

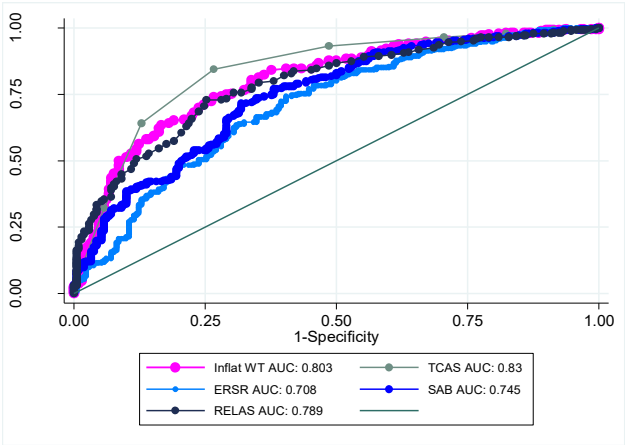
Objective: We sought to identify predictive echo parameters and compare these strain-based ratios and multi-parametric scores in patients with suspected ATTR-CM referred for 99mTc-pyrophosphate (PYP) scintigraphy.

Methods: Echocardiograms from 598 patients referred for ATTR-CM evaluation by 99mTc-PYP SPECT from 3 major amyloidosis referral centers were analyzed for standard measures of structure and function including longitudinal strain (LS). A binary logistic regression model was used to identify the echo parameters that best associated with definitive ATTR-CM adjudicated by PYP scan results. The relative apical sparing ratio (RELAS)[1], septal apical to base (SAB) LS ratio[2], and ejection fraction to strain ratio (EFSR)[3], as well as the increased wall thickness (IWT) score[4] and the Mayo clinic derived ATTR score (TCAS)[5] were calculated. Comparison of the differential diagnostic capacity of the fore-mentioned indices was performed by means of multiple receiver operating characteristic curves comparison based on the methodology by Delong et al.

Results: Over half of the subjects (54.2%) referred for 99mTc-PYP scan were subsequently diagnosed with ATTR-CM [78% were male, median age of 76 years (interquartile range 70, 81)]. A combination of age, inferolateral wall thickness, and mid-wall basal LS had the highest cross-validated AUC of 0.90. Using published cut-offs for the various ratios and scores and a cut of ≥ 14 mm for inferolateral wall thickness performed as well as TCAS (AUC: 0.80 vs 0.83, p=0.17), and was superior to IWT score, EFSR and RELAS; a cut-off of ≥-8% for average basal LS had a similar AUC to TCAS (0.84 vs. 0.83, p=0.87), and outperformed the other indices (Figure 1).

Conclusion: As more patients are screened for ATTR-CM, echocardiographic features are essential to aid detection of disease and improve patient selection for 99mTc-PYP. Several deformation-based ratios and multiparametric scores have been proposed for these purposes, yet in this population with suspected ATTR-CM increased inferolateral wall thickness or a reduced basal average longitudinal strain were robust predictors of disease and outperformed many of these more complex metrics.

A



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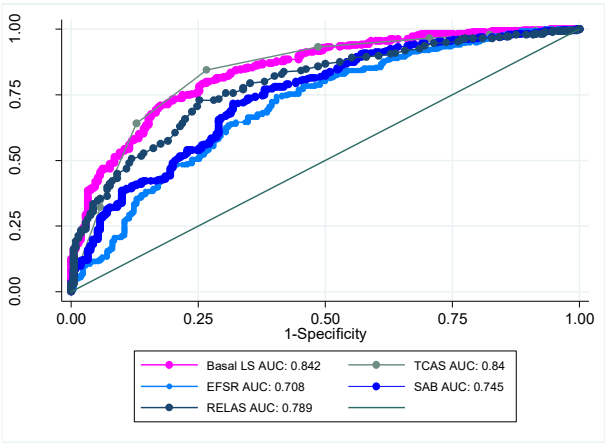

Figure 1: Comparison of receiver operating characteristic curves of A) inferolateral wall thickness and B) average basal longitudinal strain to deformation ratios (RELAS, SAB, EFSR) and the TCAS multiparametric score to diagnose cardiac transthyretin amyloidosis.

AUC, area under the curve; EFSR, ejection fraction to strain ratio; PWd, posterior wall diameter/inferolateral wall thickness; LS, longitudinal strain; RELAS, relative apical sparing ratio; SAB, septal apical to base LS ratio; TCAS, Mayo clinic derived ATTR score.

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Cardiac amyloidosis screening in a cohort of patients with spinal stenosis: a case series

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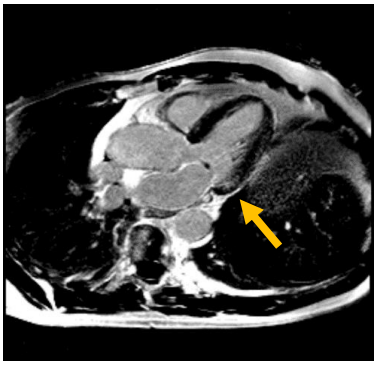
Background: Systemic amyloidosis comprehends a heterogeneous group of pathological processes that eventually lead to formation and deposit of unsoluble proteins in various organs, including the heart, alterating the structure and function of the diseased organ. The forms derived from transthyretin are due to inherited or acquired mutations in the TTR-gene. Evidence in literature supports the hypothesis that systemic amyloidosis and cardiac involvement may be preceded by amyloid deposits in structures such as ligaments by several years[1]. Proof of amyloid deposits have been described in the ligamentum flavum of patients who underwent surgery to treat spinal stenosis.

Objectives: We aimed to screen for signs of infiltrative processes involving the myocardium 28 patients referred to our Centre for spinal stenosis and evidence of amyloid in biopsies of ligamentum flavum after lumbar laminectomy for symptomatic lumbar stenosis (except for one awaiting for surgery). Our goal was to assess the cardiovascular status of these patients by means of a comprehensive evaluation with medical history, biomarkers, TT-echocardiography, cardiac magnetic resonance and genetic testing.

Material and methods: We analyzed 28 subjects in a single point in time collecting NT-pro-BNP and ST-2 levels. We evaluated heart function with TT-echocardiography with a standard-of-care protocol. We evaluated dimensions, function and the structure of the myocardium with magnetic resonance. All patients were also tested for genetic mutation responsible of TTR-amyloidosis.

Results: We analyzed 28 subjects (females 39.9%). Immunochemistry characterization of amyloid was available for 23 subjects and it showed TTR-amiloid deposits in all of them (23/23). 2/28 patients had a confirmed history of carpal tunnel syndrome. 1/28 patients had a cervical stenosis[2] and no lumbar stenosis. 7/28 patients (24%) had a previous history of coronary artery disease. The mean NT-pro-BNP level was 313.76 ng/L (SD 394.36) and the mean ST-2 level was 9.50 pg/L (SD 9.50). GLS had a mean value of -17.20 (SD 3.88). Ejection fraction, indexed end-diastolic diameter of the left ventricle, LV mass and ECV at MRI were normal in the majority of subjects (mean EF 64%, SD 6.9; mean LVEDVi 67.23 ml/mq, SD 11.91; mean LV mass 63.71 gr/mq, SD 11.91; mean ECV 22.38, SD 2.67). LGE signs were found in 5/29 subjects (17%). The genetic testing for TTR mutation was negative in all of the patients. Bone scintigraphy was available for 2/28 patients and positive with a Perugini-score of 2 in the only patient that concomitantly showed signs of LGE at cardiac MRI. No significant statistical correlation was found between GLS and NTproBNP or ST-2.

Summary and conclusions: We found signs of cardiac amyloidosis in only one patient. However, we cannot exclude that the others will eventually develop cardiac amyloidosis[3]. Two of our patients also presented a positive history of carpal tunnel syndrome and one of them was also the subject with a diagnosis of cardiac amiloidosis. We can speculate that these patients represent a high risk population for cardiac amyloidosis development. The amyloid deposits could precede the cardiac involvement and represent a premature sign of cardiac amyloidosis development, potentially leading to early diagnosis and treatment. Prospective randomized and multi-centre trials are warranted to evaluate the optimal timing of follow-up with cardiac MRI of these patients. Limitations are the little number of patients and the presence of CAD that poses a bias to cardiac evaluation.



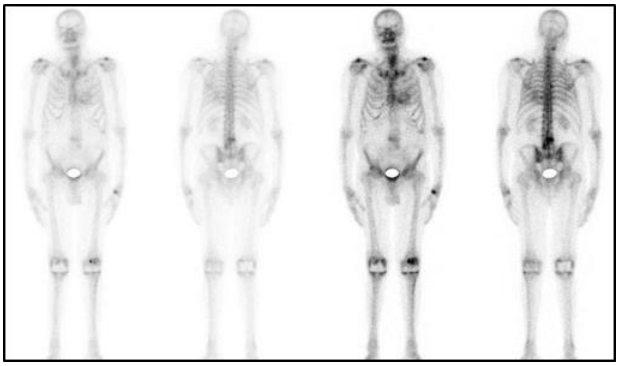


Figure 1: MRI showing the presence of late-gadolinium enhancement compatible with fibrosis in the middle-mural basal inferior wall of the left ventricle.

Figure 2: bone scintigraphy of one patient of our series; Perugini score 2.

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Changes in the Journey to Diagnosis of Cardiac Amyloidosis in the Past 10 Years: Results from the Amyloidosis Research Consortium's 2017 Cardiac Survey and 2022 Amyloidosis Community Survey

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Background: Cardiac amyloidosis (CA) is a severe, progressive, and often fatal disease. Since 2019, a number of new therapies have become available. There has been a growth of interest in the amyloidosis diseases across the scientific community and industry, with an increasing number of initiatives aimed at raising disease awareness and improving speed and accuracy of diagnosis in recent years.

Objective: To gain an understanding of the current experience of patients and caregivers in their diagnositic journey of cardiac amyloidosis, and how that experience has changed over the past decade.

Material & Methods: The Amyloidosis Research Consortium (ARC) conducted an online survey in 2017 focused on diagnosis, barriers to healthcare, and disease state for patients with cardiac amyloidosis and their caregivers. ARC launched a subsequent online survey for patients and their caregivers of all types of amyloidois in April 2022. Patients or their caregivers with cardiac amyloidosis [either light chain amyloidosis (AL, hereditary variant (ATTRv) or wild-type transthyretin amyloidosis (ATTRwt)] and a time since diagnosis of 5 or less years in the 2017 survey and 3 or less years in the 2022 survey to avoid overlap were included. Data collected included demographics, disease characteristics, and pathway to diagnosis. Results were stratified by survey and differences between the two surveys were examined.

Results: A total of 702 patients and caregivers were included in this analysis, 330 from the 2017 survey and 372 from the 2022 survey. Survey engagement by patients and caregivers with ATTR increased from 99 (30% of respondents) to 259 (70% of respondents), with the largest increases noted in the wild-type ATTR (ATTRwt) group (Table). Caregivers were more involved and represented 12% of respondents in 2022 vs 4% in 2017. Diagnosis by a cardiologist (vs. other specialities) was similar across the surveys, with the greatest number in the ATTRwt cohort, and patients with ATTRwt were much more likely to be diagnosed by the first provider in 2022 vs 2017 (27% vs 19%). Overall, the rate of misdiagnosis reported by patients decreased from 144 (44%) from the 2017 survey to 114 (31%) in the 2022 survey. For ATTR, the number of patients who had an organ biopsy as part of their diagnostic work-up decreased between surveys from 86% in 2017 to 34% in 2022, while the rates of organ biospsies for patients with AL remained similar between the two surveys (59% in 2017 vs 66% in 2022). The rates of diagnoses made by imaging (without biopsy) went up for ATTRwt from 38 (63%) in the earlier survey to 174 (88%) in the recent survey but remained relatively similar for AL and ATTRv.

Summary & Conclusion: Since the approval of multiple therapies for systemic amyloidosis, efforts to promote amyloidosis disease awareness have increased. In the roughly five-year span between surveys, the number of participants has increased, as has the proportion of ATTR patients. Disease awareness efforts appear success with more patients diagnosed by the first doctor they see for amyloid-related symptoms and more non-invasive diagnoses made in the ATTRwt cohort. While patients continue to see multiple doctors and endure misdiagnosis on their journey, these results are encouraging and suggest that educational efforts to increase disease awareness are having an impact and should be continued. Additional efforts are needed to reduce time to diagnosis.

Characteristics	AL		ATTRwt		ATTRv	
	2017 Survey N = 231	2022 Survey N = 113	2017 Survey N = 60	2022 Survey N = 197	2017 Survey N = 39	2022 Survey N = 62
Region						
Asia-Pacific	17 (7.4)	9 (8.0)	3 (1.5)	11 (4.3)	4 (10)	1 (1.6)
Europe	77 (33)	10 (8.8)	6 (3.0)	22 (8.6)	10 (26)	9 (15)
North America	137 (59)	94 (83)	188 (95)	224 (87)	25 (64)	52 (84)
Gender						
Male	122 (54)	51 (45)	54 (95)	152 (78)	22 (59)	31 (50)
Age						
71 or older	39 (17)	40 (35)	44 (73)	151 (77)	9 (23)	15 (24)
Race						
White	193 (90)	107 (95)	42 (93)	190 (96)	27 (79)	53 (85)
Number of organs affected by amyloidosis						
1 (Heart)	13 (5.6)	32 (28)	6 (10)	124 (63)	1 (2.6)	16 (26)
2 or more	218 (94)	81 (72)	54 (90)	73 (37)	38 (97)	46 (74)
Number of doctors seen before amyloidosis diagnosis						
1	27 (12)	15 (13)	11 (19)	52 (27)	5 (14)	10 (16)
2 or more	204 (88)	98 (87)	49 (81)	145 (73)	29 (86)	52 (84)
Received a misdiagnosis before amyloidosis diagnosis	103 (45)	45 (40)	23 (38)	41 (21)	18 (46)	28 (45)
Diagnostic Testing Performed						
Organ Biopsy	128 (59)	75 (66)	54 (93)	61 (31)	31 (86)	26 (42)
Imaging	165 (76)	89 (79)	38 (66)	174 (88)	32 (89)	49 (79)

Table 1.: Characteristics of Patients and Caregivers with Cardiac Amyloidosis in 2017 and 2022 Surveys by Amyloidosis Subtype

Support & Funding: None

Prognostic Value of an Artificial Intelligence Enhanced ECG Model in Cardiac Amyloidosis

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Background: We have previously demonstrated that an artificial intelligence (AI) enhanced model using a standard 12-lead electrocardiogram (ECG) accurately predicts cardiac amyloidosis (CA) in both immunoglobulin light chain (AL) and transthyretin amyloid (wild [ATTRwt] and hereditary [ATTRv]) types (1). We hypothesized that the AI-ECG score would be prognostic for survival in that same population of CA.

Objective: To determine if AI-ECG score would be prognostic for survival in CA patients.

Materials & Methods: 2,539 patients with CA (1,839 AL, 530 ATTRwt, 170 ATTRv) were included. Each patient was assigned an AI-ECG score reflecting the probability of CA predicted by the AI algorithm as a continuous variable. CA stage was calculated using the European modification of the Mayo 2004 criteria for AL and 2016 Mayo score for ATTR. Risk of death was modeled using Cox proportional hazards and Kaplan-Meier was used to estimate survival.

Results & Discussion: CA patients had median age of 67 years (IQR 59, 74), a left ventricular ejection fraction of 59% (IQR 49%, 65%) and 71.6% were male. Median survival in months was 23.1 (95% CI 19.3, 28.2) in AL, 50.0 (CI 43.1, 52.3) in ATTRwt, 59.9 (CI 47.2, 75.4) in ATTRv. On multivariate analysis, AI-ECG score was a significant predictor for mortality (Hazard Ratio per unit change 2.76; 95%CI 2.27, 3.39). On subset analyses, AI-ECG score was independent of AL and ATTR biomarker stage in prediction of survival (Table 1).

Summary & Conclusion: An AI-ECG score predicts survival in AL and ATTR CA patient, independent of biomarker staging systems. In future, these variables may prognosticate mortality risk and intervention in CA patients.

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Drs Grogan, Lopez-Jimenez, Dispenzieri, Attia, AbouEzzeddine, Kapa, Friedman, and Murphree and Mayo Clinic have licensed the algorithm described in this work to Anumana and may benefit from its commercialization.

Table 1: Multivariate analysis of mortality in cardiac amyloidosis subtype including patient age, AI-ECG score and biomarker staging systems.

		Multivariate Analysis	
	Number of patients	**HR (95%CI)**	**P**
AL group	1839		
Age		1.03 (1.03, 1.04)	**<0.0001**
AI ECG score		2.11 (1.66, 2.70)	**<0.0001**
Modified 2004 AL Stage[1]			
I	74	Ref	
II	497	1.65 (1.10, 2.48)	0.015
IIIa	460	2.58 (1.71, 3,87)	**<0.0001**
IIIb	257	5.31 (3.51, 8.05)	**<0.0001**
No data	551	3.38 (2.69, 3.86)	**<0.0001**
ATTR group	700		
Age		1.05 (1.04, 1.06)	**<0.0001**
AI ECG score		2.79 (2.02, 3.86)	**<0.0001**
Mayo ATTR Stage[2]			
I	224	Ref	
II	100	1.86 (1.34, 2.59)	0.0002
III	89	3.51 (2.52, 4.88)	**<0.0001**
No data	287	1.83 (1.41, 2.37)	**<0.0001**

Abbreviations: HR – hazard ratio; CI – confidence interval; CA – cardiac amyloidosis; ECG – electrocardiogram; AL – light chain amyloidosis; ATTR – transthyretin amyloidosis; Ref – reference group.

[1] Modified Mayo 2004 AL Stage: Thresholds for troponin T and NT-proBNP are 0.035 ng/mL and 332 pg/mL. Stage I, II and III represent neither high, one high, and both high respectively. Stage III is further split by ultra-high NT-proBNP (> 8500 pg/mL).

[2] Mayo 2016 ATTR Stage: Thresholds for troponin T and NT-proBNP are 0.05 ng/mL and 3000 pg/mL. Stage I, II and III represent neither high, one high, and both high respectively.

Retrospective analysis of a patient cohort with suspicion of systemic amyloidosis, finally not confirmed

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Background: Due to non-specific symptoms amyloidosis is often misdiagnosed and there are many diseases presenting with similar symptoms which leads to a wide spectrum of possible differential diagnoses (1, 2). Differential Diagnosis (DDX) generators are computer programs aimed to aid physicians in the diagnosis of difficult cases by enabling the user to type in findings of patients and suggesting possible differential diagnoses. One of the DDX generators is Isabel (Isabel Healthcare Ltd. 2022, Bunch Lanen, Haslemere, UK). The user types in findings and Isabel ranks the differential diagnoses according to how well they match with the entered data.

Objective: Retrospective analysis of symptoms, findings and final diagnoses in a cohort of patients with suspected amyloidosis leading to referral to the amyloidosis center Heidelberg, but where amyloidosis could not be confirmed in the course of the diagnostic work-up. Furthermore, the DDX generator Isabel was evaluated concerning its usefulness in the diagnostic process of amyloidosis.

Material & Methods: Out of 2829 patients referred to the amyloidosis center between January 2014 and December 2020, 351 without amyloidosis were identified. For the evaluation, medical reports of the amyloidosis center as well as other clinics, have been used. To gather information about the further course of their illness, patients either received a questionnaire or came in for a second investigation. In order to test the usefulness of the DDX-Generator Isabel, 90 patients without amyloidosis were selected from this cohort. The data of these non-amyloidosis patients were compared to another cohort of 30 random patients with confirmed amyloidosis.

Results: At the first visit we could give 18 out of the 351 patients a final diagnosis. Out of the remaining 333 patients, we received follow-up information of 185 patients leading to a new final diagnosis in another 25 patients. For further detail, see Figure 1. A summary of the most common reasons for referral and final diagnoses is given in Figure 2. An interesting finding was that 41 patients were referred with a biopsy showing amyloid, which could not be confirmed through a second investigation. Concerning the evaluation of the DDX-generator Isabel, 90 patients without amyloidosis were included: 30 presented primarily with a polyneuropathy (8 had a monoclonal gammopathy), 30 with a cardiomyopathy (4 had a monoclonal gammopathy) and 30 with unspecific symptoms (8 had a monoclonal gammopathy). In this cohort, the DDX generator Isabel listed amyloidosis as a possible differential diagnosis in the top ten differential diagnoses in 25 of 90 cases (27,8%). These 90 patients were compared to 30 patients with amyloidosis. Out of these 30 patients, 18 had an AL amyloidosis (all with a monoclonal gammopathy), 7 an ATTRwt amyloidosis, 4 an ATTRv-Amyloidosis and 1 an AA amyloidosis. Amyloidosis was significantly more often present in the top ten differential diagnoses in this group (28 out of 30 cases, 93,4%, p<0,00001).

Summary & Conclusion: As anticipated, patients in the non-amyloidosis cohort presented with a wide spectrum of differential diagnoses. In many patients the cause of the disease remained unknown as we only received a final diagnosis in 43 patients (18,6% of evaluable patients), while in 11,7% the amyloid deposits could not be confirmed. The DDX-Generator Isabel can aid physicians to consider amyloidosis as a possible diagnosis. Nevertheless, there is room for improvement, as the results vary depending on how the entered symptoms are phrased.

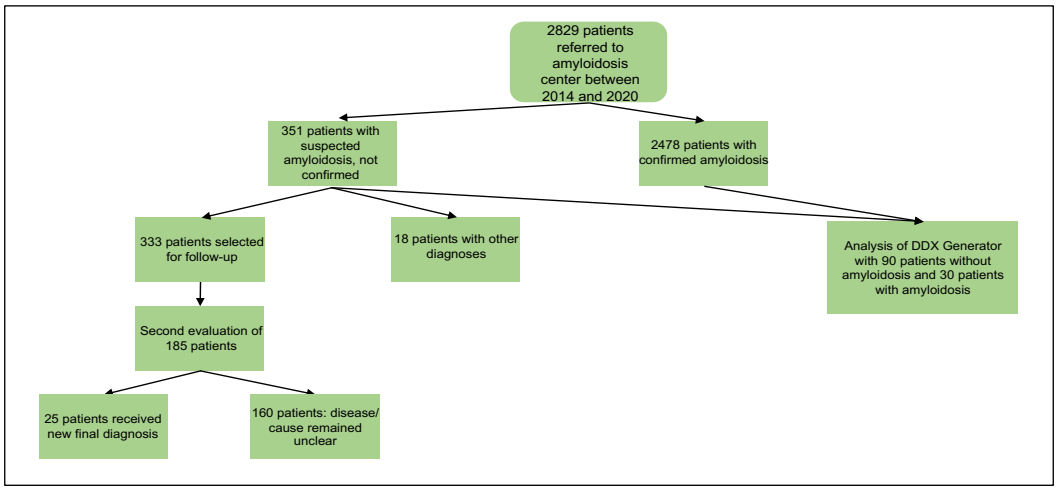

Figure 1: Flow-chart depicting work process. Mean follow-up time was 34,5 months ± 24,2 months.

Reason for referral	N	%	Final diagnoses	N	%
Referred patients in total*	**351**			**351**	
Plasma cell dyscrasia - Plasma cell dyscrasia only - with neurological symptoms - with cardiological symptoms - with renal symptoms - with other symptoms	**129** 7 74 17 14 17	**36,8**	Plasma cell dyscrasia related diseases: • IgM-associated Polyneuropathy • Waldenstrom macroglobulinemia • POEMS-Syndrome • AL-Amyloidosis** • MGRS	**17** 7 7 1 1 1	**4,8**
			Multiple other diseases not related to plasma cell dyscrasia (Polyneuropathy, Cardiomyopathy, …) Plasma cell dyscrasia ruled out	**105** **7**	**30** **2**
Neurological symptoms (without plasma cell dyscrasia)	**79**	**22,5**	Polyneuropathies: • Polyneuropathy of unknown cause • Polyneuropathy of known cause Other neurological diseases Other non-neurological diseases	**70** 62 8 **5** **4**	**19,9** **1.4** **1,1**
Cardiovascular diseases (without plasma cell dyscrasia)	**77**	**22**	Cardiomyopathies: • Hypertrophic cardiomyopathy • Other cardiomyopathies Other cardiological diseases Systemic inflammatory diseases Other diseases	**63** 27 36 **8** **3** **3**	**17,9** **2,3** **0,9** **0,9**
Renal findings (without plasma cell dyscrasia)	**10**	**2,8**	Chronic kidney disease of unknown origin Other kidney diseases	**5** **5**	**1,4** **1,4**
Other symptoms or findings (without plasma cell dyscrasia)	**56**	**16**	Multiple other diseases	**56**	**16**

Figure 2: Overview of most frequent reasons for referral and final diagnoses made in the amyloidosis center Heidelberg and in the follow-up. *351 patients referred in total: mean age at time of referral: 59,6 ± 12,8 years (29 years – 85 years), 201 males, 150 females, 13 patients died during follow-up **Diagnosis AL-Amyloidosis was made in the follow-up after 30 months

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Title: **Validation of Amylo-AFFECT, a self-reported questionnaire to assess health-related quality of life and to determine the prognosis in cardiac amyloidosis**

Short title: Quality of life questionnaire dedicated to cardiac amyloidosis

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Aims : Self-reported questionnaires are useful to estimate the health-related quality of life (HR-QoL), impact of interventions and prognosis. To our knowledge, no HR-QoL questionnaire has been created in cardiac amyloidosis (CA). The aim of this study was to develop and validate a specific tool to assess HR-QoL and its prognosis value in CA.

Material and Method: A self-reported HR-QoL questionnaire "Amylo-AFFECT" composed by 34 items identified for CA discomfort related domains, was designed based on the literature and reports from patients. Construct and validate theoretical model, internal consistency, and convergent validity were assessed, in particular correlations between our questionnaire and the HR-QoL Minnesota Living Heart Failure (MLHF) questionnaire. Prognostic values were calculated.

Results and Discussion: Amylo-AFFECT was completed by 515 patients, among them, 425 (82.5 %) had CA. Wild type and hereditary amyloidosis (ATTRwt and ATTRv), immunoglobulin light-chain amyloidosis (AL) were diagnosed in 47.8%, 14.7% and 18.8% of cases, respectively. The best HR-QoL evaluation was obtained with 5 dimensions: "Heart failure", "Vascular dysautonomia", "Neuropathy", "Gastrointestinal and urinary dysautonomia" and "Skin or mucosal involvement". Significant positive correlations were found between the global scores of Amylo-AFFECT and MLHF ($r_s = 0.72$, $p < 0.05$). Patients with AL had a higher score in the 1st (7.3 vs 5.5) and 5th (4.4 vs 1.7) dimensions, and in the 3rd dimension (8.8 vs 5.6) for ATTRv when compared to other diagnosis (all p-value < 0.01). Patients with the worst prognostic value have a greater risk of death or heart transplant after 1-year of follow-up (log-rank < 0.01).

Amylo-AFFECT demonstrates good psychometric properties and is useful to quantify HR-QoL and estimate the prognosis in CA. It may help to improve the overall management of patients with CA.

Keywords:

Cardiac Amyloidosis, Quality of Life, Prognostic, Transthyretin, Self-reported Questionnaire

The landscape of amyloidosis in Switzerland: Report of the Amyloidosis Registry

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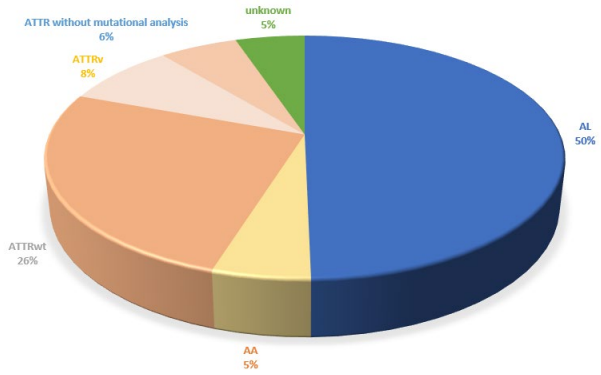
Background and Objective: Systemic amyloidoses are rare protein folding diseases with heterogeneous and often nonspecific clinical presentation. To better understand systemic amyloidosis and to apply state of the art diagnostics and treatment, the interdisciplinary Amyloidosis Network was founded in 2013 at the University Hospital Zurich, Switzerland. In this respect, a registry was implemented to study the landscape of amyloidosis within the network. Patient data were collected retrospectively from 2005 - 2014 and prospectively from 2015 onwards. Here we present the first analysis from this amyloidosis registry.

Material & Methods: Patients 18 years or older, diagnosed with all subtypes of systemic amyloidosis were eligible for inclusion, if they were treated in one of the four referring centers (Zurich, Chur, St. Gallen, Bellinzona). Baseline data were captured at the time of diagnosis. Follow-up data were assessed half-yearly for the first two years, followed by an annual record.

Results: Between 01.01.2005 and 29.02.2020, 247 patients were screened, and 155 patients with confirmed systemic amyloidosis were included in the present analysis.
The most common amyloidosis type was light-chain (AL) (49.7%; n=77), followed by transthyretin amyloidosis (ATTR) (40.6%, n=63) and amyloid A (AA) amyloidosis (5.2%, n=8). The majority of patients (63.2%, n=98) presented with multi organ involvement. Nevertheless, single organ involvement was seen in all types of amyloidoses, most commonly in AA amyloidosis (75%, n=6).
Survival analysis was done for AL and ATTR patients. Observation time of the surviving patients was 2.16 years (95% CI 0 – 23); 3.31 years (95% CI 0 – 20) in AL patients and 1.53 years (95% CI 0 – 23) in ATTR patients, respectively. One, 3 and 5 year survival rates were 87%, 72.5% and 71% in AL amyloidosis and 89.8%, 83.7% and 75.5% in ATTR amyloidosis patients respectively.

Conclusion: Our amyloidosis registry data reflects data from major international centers. In particular, the prospectively collected data will facilitate multicentre outcome research and should lead to a standardization of diagnosis and treatment in these patients suffering from rare diseases. Our joint effort aims at positively influencing quality of life and outcome of this fragile patient population in need of innovative treatment and interdisciplinary care.



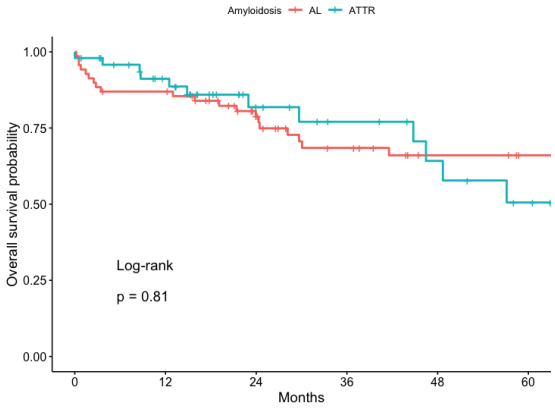


Figure 1: Pie-chart showing the percentage of patients included in the Amyloidosis Registry for each type of amyloidosis. N=155
Figure 2: Overall survival in AL and ATTR patients

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Low QRS voltages in cardiac amyloidosis: echocardiographic correlates and prognostic value

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Background: Low QRS voltages (LQRSV) are common in electrocardiograms (ECGs) of patients with light chain (AL) or transthyretin (ATTR) cardiac amyloidosis (CA)[1,2,3].

Objective: To assess the prevalence of LQRSV in two different etiologic types of cardiac amyloidosis. To identify clinical and echocardiographic determinants of LQRSV. To investigate the prognostic significance of LQRSV in AL- e ATTR-CA subtypes.

Material & Methods: This is a multicenter, retrospective, observational study performed in six Italian referral centers for CA including consecutive patients with AL- and ATTR-CA patients. LQRSV were defined as QRS amplitude ≤0.5 mm (0.5 mV) in all peripheral leads. The study outcome was cardiovascular (CV) death.

Results: A total of 411 (n=120 AL, n=291 ATTR) patients were included. LQRSV were detected in 169 CA patients (41%), 66 (55%) with AL-CA and 103 (35%) patients with ATTR-CA (p<0.001). In AL-CA, LQRSV were more frequently observed in patients with younger age (p=0.046), NYHA class>2 (p<0.001), younger age (p=0.046), greater left ventricular (LV) thickness and more severe LV diastolic and right ventricular (RV) systolic dysfunction. In patients with ATTR-CA, LQRSV were more common in those with AF (p=0.013), pericardial effusion (p=0.005) and RV dysfunction (p=0.033). At a follow-up visit (after averagely 22 months), no significant differences in QRS score and voltage-to-mass ratio were detected in both groups, although a trend towards increasing QRS voltages (29 vs 31 mV, p=0.494) and voltage-to-mass ratio (0.25 vs 0.27, p=0.095) was evident in AL patients (Figure 1). During a median follow up of 36 months, 61 (15%) patients died for CV causes, 33 (28%) with AL-CA and 28 (10%) with ATTR-CA. LQRSV were independent predictors of CV mortality both in AL-CA (hazard ratio [HR]: 3.143; 95% confidence interval [CI]: 1.414-6.982; p= 0.005) and in ATTR-CA (HR: 2.605; 95% CI: 1.230-5.516; p= 0.012) (Figure 2).

Summary & Conclusion: LQRSV are a common, but not ubiquitous ECG pattern in patients with CA, more prevalent in AL than ATTR subtype. In both conditions, LQRSV reflect more frequently an advanced disease stage and independently predict worse CV survival.

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Support & Funding: None.

Figure 1: Differences in QRS score and voltage-to-mass ratio between baseline and follow-up visit in AL-CA (Panel A) and ATTR-CA (Panel B) patients.

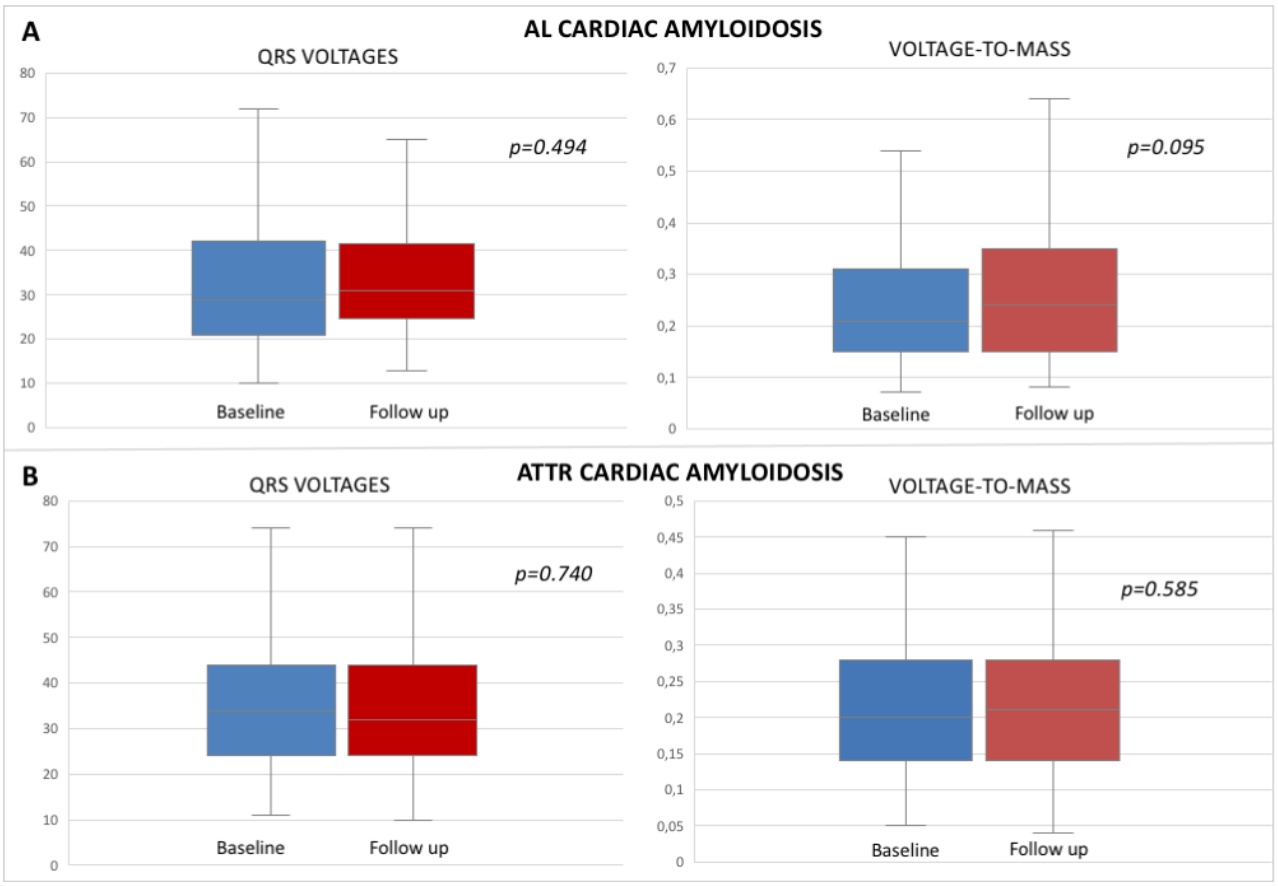
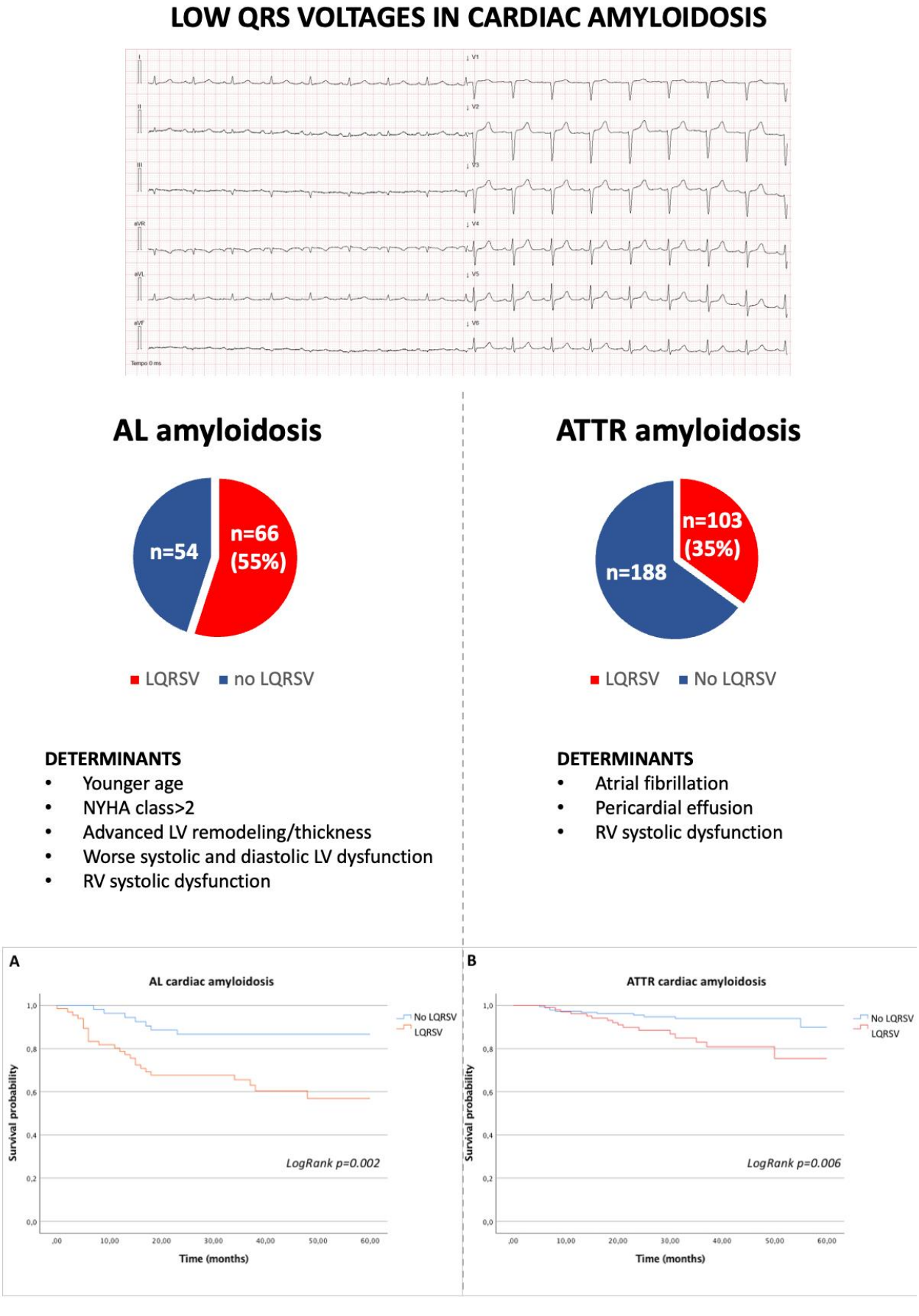


Figure 2: Low QRS voltages prevalence, determinants and prognostic significance in AL- and ATTR-CA.



Clinical impact of musculoskeletal pathology in patients with transthyretin-associated amyloidosis (ATTR): retrospective analysis of the case series from our center

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Background: Early diagnosis of transthyretin-associated amyloidosis (ATTR)-cardiomyopathy (CM) is crucial to improve the outcome of these patients. Several authors have proposed new extra-cardiac manifestations: Carpal Tunnel Syndrome (CTS), Trigger Finger (TF), Lumbar Canal Stenosis (LCS), Cuff Rotators (CR), Knee osteoarthritis (KO) and Hip osteoarthritis (HO) that may be significantly relevant in the early diagnosis of the disease. Our hypothesis is that musculoskeletal manifestations precede the diagnosis of ATTR and may serve as predictors to prevent cardiac manifestations. In addition, we think that those extra-cardiac manifestations related to ATTR may present a more aggressive course of the disease and may be a more powerful and specific predictor of the disease.

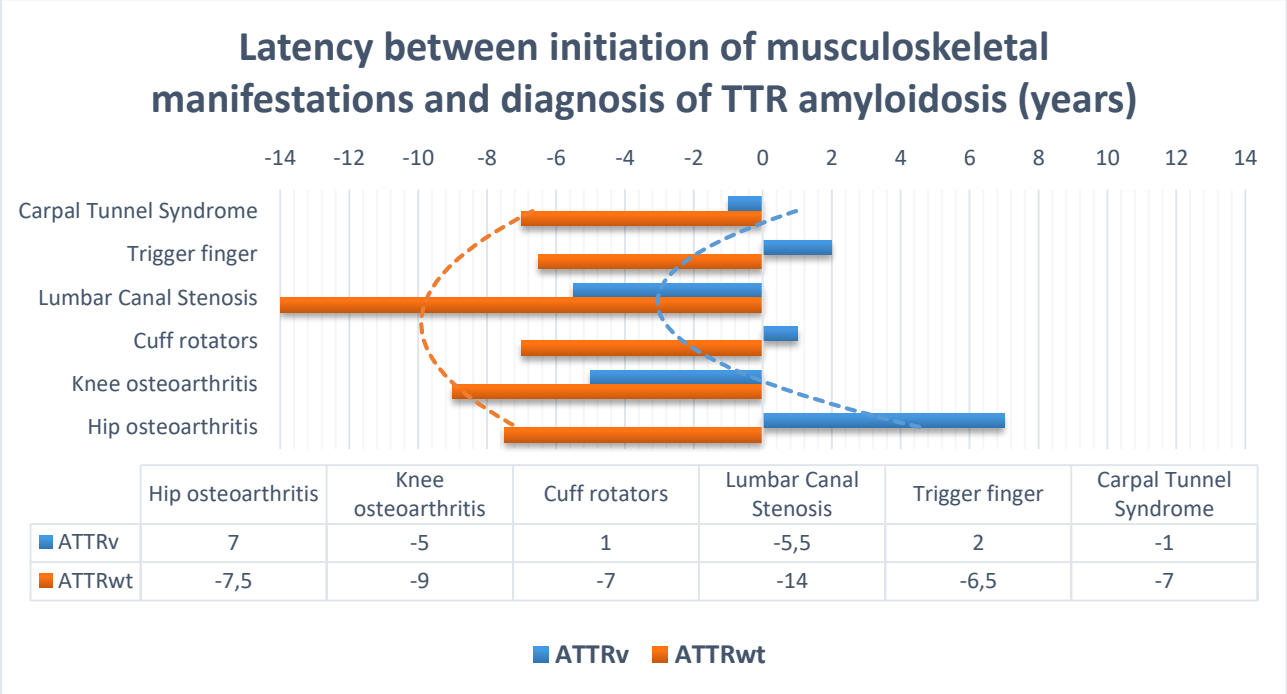
Objective: The objective of this study is to evaluate the incidence of cardiovascular events (CV) events (death, Heart Failure (HF) decompensation or admission for CV causes) and the natural course of the disease in patients affected by ATTR in its variant and wild-type forms according to the presence or absence of concomitant musculoskeletal manifestations.

Material & Methods: A retrospective study of a sample of 128 patients with ATTRwt, ATTRv or genetic carriers treated in a comprehensive HF management program and included in the THAOS registry with follow up until September of 2021. A stratification of the sample was carried out, obtaining 99 patients with confirmed diagnosis of ATTRwt or ATTRv. Statistical analysis was carried out using the IBM SPSS Statistics 23 program.

Results: Patients with ATTRwt had a statistically significantly higher prevalence of musculoskeletal manifestations (CTS p0.001; TF p0.002; LCS p0.002; CR p0.037; KO p0.001; HO <0.001). In addition, a greater number of adverse cardiovascular manifestations appeared in patients with ATTRwt (AF/Flutter p0.001; HF <0.001; Heart Failure (HF) decompensation p<0.001).
Latency between musculoskeletal manifestations and first diagnosis of TTR amyloidosis was significantly longer in ATTRwt compared to ATTRv except in Knee and hip osteoarthritis: CTS 7,00 years (0,00-14,00) p0,042, TF 6,50 years (2,00-11,00) p0,027, LCS 14,00 years (8,00-17,00) p0,001, CR 7,00 years (3,50-11,50) p0,035, KO 9,00 years (5,00-13,00) p0,130, HO 7,50 years (2,00-14,50) p0,131.
Finally, we performed Kaplan Meier curves to assess the difference in survival until the non-fatal heart failure event according to the presence of musculoskeletal manifestations, in which patients with ATTRwt presented lower survival with Log Rank (Mantel-Cox) p<0.001.

Summary & Conclusion: As seen in the literature, musculoskeletal manifestations may precede the diagnosis of ATTR and may serve as predictors to prevent cardiac manifestations. In addition, we think that those extra-cardiac manifestations related to ATTR may present a more aggressive course of the disease and may be a

more powerful and specific predictor of the disease.



	Hip osteoarthritis	Knee osteoarthritis	Cuff rotators	Lumbar Canal Stenosis	Trigger finger	Carpal Tunnel Syndrome
ATTRv	7	-5	1	-5,5	2	-1
ATTRwt	-7,5	-9	-7	-14	-6,5	-7

Figure 1.: Triguero et, al. Latency between initiation of musculoskeletal manifestations and diagnosis of TTR amyloidosis, no (years). Significant p-value in all the musculoskeletal manifestations except in Knee and hip osteoarthritis. Year 0 corresponds to the diagnosis of ATTR. Therefore, musculoskeletal manifestations may appear earlier or later. Being in the case of ATTRwt predecessors to the diagnosis of ATTR in most cases significantly compared to ATTRv.

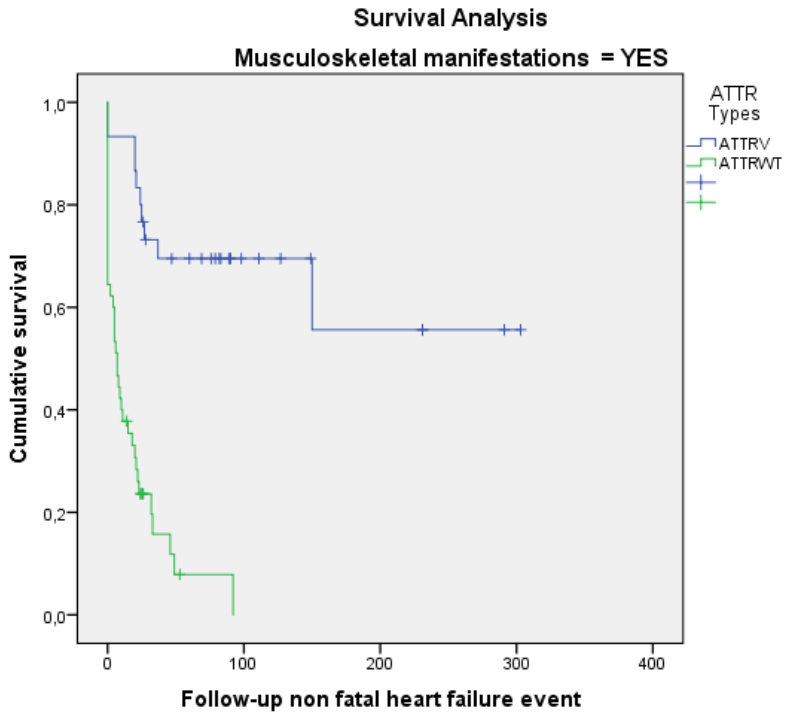


Figure 2.: Kaplan Meier curves to assess the difference in survival until the non-fatal heart failure event according to the presence of musculoskeletal manifestations, in which patients with ATTRwt presented lower survival with Log Rank (Mantel-Cox) p<0.001.

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EARLY DETECTION OF HEREDITARY AMYLOIDOSIS

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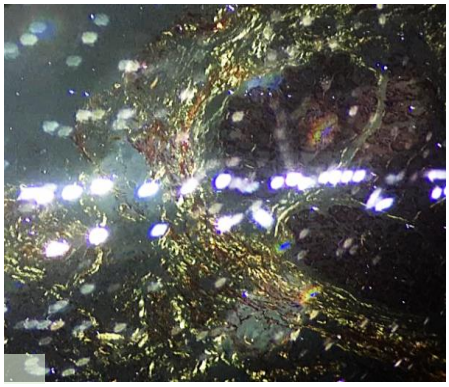
Background: Hereditary amyloidosis transthyretin is a rare, autosomal dominant systemic disease caused by extracellular deposition of insoluble amyloid fibrils formed by the mutated transthyretin protein. The disease has a heterogeneous clinical presentation and symptoms are mainly neuropathic, including autonomic, sensory and motor impairment and also may be associated with cardiac, gastrointestinal, renal and ocular involvement. Diagnosis in the early stages of ATTRh is essential to allow adequate treatment and to prevent disease progression.

Objective: To report two cases of hereditary amyloidosis in asymptomatic carriers through active search from index cases

Material & Methods: Asymptomatic individuals carrying the hereditary amyloidosis gene are screened and submitted to annual assessments that include a neurological examination, a questionnaire on symptoms of dysautonomia, complementary tests that include electroneuromyography and screening for dysautonomia, such as Quantative Sensory Test(QST), heart rate variability HRV) and reflex sympathetic response (RSR). We identified abnormal findings in two young women carriers of the hereditary amyloidosis transthyretin.

Results: A 32-year-old female carrier of Phe84Leu gene took her first evaluation in 2018, and presented symptoms of dysautonomia (blurred vision, dry eyes, increased sweating in her hands, orthostatic intolerance and constipation). The electroneuromyography showed mild carpal tunnel syndrome bilateral. The reflex-sympathetic response was abolished in the four limbs. She was then submitted to a salivary gland biopsy which revealed deposit of amyloid material. (Figures 1 and 2). Her father was the index case and at the age of 64 he started a condition of polyneuropathy and dysautonomia, receiving a late diagnosis and treatment was not possible dying from disease complications. The second case was a 22-year-old female carrier of Val50met gene. She had reflex sympathetic response abolished in the 4 segments and the quantative sensory test (QST), revealed warm detection and warm pain thresholds were higher and the cold detection and cold pain thresholds were smaller when compared to a sex and age matched healthy control. She also underwent salivary gland biopsy, which also confirmed the deposition of amyloid material in the tissue. Her aunt was the index case and presented polyneuropathy and dysautonomia at the age of 38, and treatment was not possible because it was in an advanced stage.

Summary & Conclusion: Hereditary transthyretin amyloidosis is a devastating disease. The clinical presentation and the age of onset varies. The disease is often rapidly progressive, so an early diagnosis is important for a an effectively treatment. It is necessary to establish the predicted age of onset of symptomatic disease (PADO) to start a regular follow-up of those carriers of TTR mutation.The PADO depends on the particular mutation, the typical age of onset for that mutation and the age of onset in family members. Once the PADO has been determined, the monitoring should begin 10 years before this date. Follow-up should initially be annual and should increase in frequency as carriers approach their PADO, particularly for genotypes associated with rapid progression. In our case, both patients were diagnosed before the time suggested for the start of monitoring and if we were to start the follow-up years later, they would already be symptomatic and possibly out of therapeutic possibility. Therefore, the question remains of when it would really be ideal to start the follow-up.



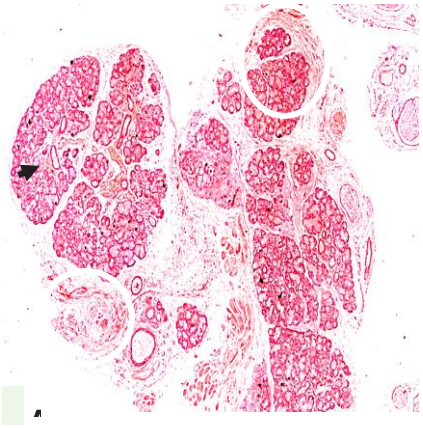


Figure 1.: Congo Red, 100x. Amyloid deposits under polarized light microscopy show an apple-green color.

Figure 2.: Congo Red, 40x. Histological sections show minor salivary gland with deposit of amorphous eosinophilic material located perivascularly and around glandular ducts and acini.

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ROLE OF COMBINING AI-ECG TO CLINICAL RISK SCORES FOR THE PREDICTION OF TRANSTHYRETIN AMYLOID CARDIOMYOPATHY IN HEART FAILURE WITH PRESERVED EJECTION FRACTION

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM) can mimic heart failure with preserved ejection fraction (HFpEF). ^{99m}Tc-pyrophoshate cardiac scintigraphy (PYP) provides a highly-specific, non-invasive diagnositic technique for ATTR-CM. We recently developed and validated a ATTR-CM score comprised of 3 clinical (age, male sex, and hypertension diagnosis) and 3 echocardiographic (ejection fraction, posterior wall thickness and relative wall thickness) variables that predicts increased risk for ATTR-CM in HFpEF cohorts with variable ATTR-CM prevalence (1). An ECG-based artificial intelligence tool (AI-ECG) has also been shown to be effective in screening general populations for amyloid cardiomyopathy (2).

Objective: To evaluate the impact of combining AI-ECG to the clinical ATTR-CM score for the prediction of increased risk of ATTR-CM in HFpEF.

Material & Methods: A cohort of patients with validated HFpEF (EF ≥ 40%) was constructed from patients undergoing PYP at Mayo Clinic (n=484, 38% with ATTR-CM). The ATTR-CM score is a multivariable logistic regression model converted to a simple point score, comprised of clinical and echocardiographic variables (Figure 1) whereby a score ≥ 6 indicates increased risk for ATTR-CM to warrant PYP screening. The AI-ECG tool is a deep neural network trained to predict cardiac amyloidosis from a single 12-lead ECG. Both the ATTR-CM score and the AI-ECG tool were applied to the HFpEF cohort. The predictive characteristics of the ATTR-CM score and AI-ECG for the diagnosis of ATTR-CM were assessed individually and in combination.

Results: See table 1. Both the ATTR-CM score and AI-ECG tool had strong predictive characteristics when used individually. Combining the AI-ECG tool and the ATTR-CM score provided minimal improvement in the predictive characteristics for ATTR-CM. The inclusion of an interaction term did not improve the performance of the combined model.

Summary & Conclusion: Both the clinical ATTR-CM risk and the AI-ECG tool effectively predict ATTR-CM with an excellent negative predictive value to rule out ATTR-CM and a positive predictive value sufficient to warrant specific ATTR-CM testing (PYP). Combining the AI-ECG to the clinical ATTR-CM risk minimially improved upon the predictive characteristics of either approach for identifying HFpEF patients at increased risk of ATTR-CM.

Clinical Variable	Value	Points
Age (years)	60-69	**2**
	or 70-79	**3**
	or ≥ 80	**4**
Sex	Male	**2**
Ejection Fraction	< 60%	**1**
Posterior Wall Thickness	≥ 12 mm	**1**
Relative Wall Thickness	> 0.57	**2**
Hypertension History	Present	**-1**

Figure 1: ATTR-CM score for prediction of transthyretin cardiac amyloidosis (ATTR-CA) in patients with heart failure with preserved ejection fraction (HFpEF). A score of ≥ 6 is considered high risk for ATTR-CM.

	AUC†	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
ATTR-CM Score	0.89 (0.86, 0.92)	92 (88, 96)	64 (58, 70)	62 (56, 67)	93 (89, 96)
AI-ECG Tool	0.77 (0.73, 0.81)	77 (70, 83)	66 (61, 72)	58 (52, 64)	82 (77, 87)
Combined	0.90 (0.88, 0.93)	92 (87, 95)	68 (62, 73)	64 (58, 69)	93 (89, 96)
Combined, with interaction term	0.90 (0.88, 0.93)	92 (87, 95)	69 (64, 74)	65 (59, 71)	93 (89, 96)

Table 1: Predictive characteristics of the ATTR-CM score and AI-ECG tool individually and in combination (without and with an interaction term) in the referral cohort of HFpEF patients with high (38%) prevalence of ATTR-CA. Results presented with 95% confidence interval. †AUC calculated using score and AI-ECG as continuous variables. Combined models used cutoff value fixed to match the sensitivity of ATTR-CM method.

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Prevalence and Implications of Classic ECG Findings in a Contemporary ATTR Cohort

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Background: The prevalence of the classic ECG findings of low voltage and a pseudo-infarct pattern in contemporary patients with ATTR cardiac amyloidosis, and their association with mortality are unknown.

Objective: To determine the prevalence of a pseudo-infarct pattern and/or low voltage ECG in ATTR-CA and their association with mortality.

Material & Methods: Patients who underwent a Tc-99m PYP scan for suspected CA were included. Diagnostic evaluation included serum studies, and an endomyocardial biopsy (EMB) when needed. ATTR-CA was diagnosed by a positive PYP scan AND negative serum studies for a paraprotein, or by EMB. ECG tracings closest to the date of PYP imaging were interpreted specifically for this analysis. What was mean or median follow up?

Results: Of 271 patients analyzed, 75 (28%) were diagnosed with ATTR-CA. A pseudo-infarct pattern was seen in 42 (16%) patients. Of these, 18 (42%) had a diagnosis of ATTR-CA. A pseudo-infarct pattern was not associated with an increased odds of a diagnosis of ATTR-CA (OR 0.44, 95% CI 0.22 – 0.87) and was not associated with mortality (OR 0.61, 95% CI 0.21 – 1.81). A low voltage pattern was seen in 22 (8%) patients of whom 12 had a diagnosis of ATTR-CA diagnosis. A low voltage EKG was not associated with an increased odds of a diagnosis of ATTR-CA (OR 0.28, CI 0.12 – 0.69), and was not associated with mortality [OR 2.13, 95% CI 0.61 – 7.46]. The presence of both pseudo-infarct and low voltage pattern was seen in only 6 (2%) patients, of whom 3 had a diagnosis of ATTR-CA. There was no significant association between these ECG findings and a diagnosis (OR 0.37, 95% CI 0.07 – 1.89). Two patients with ATTR-CA and both pseudo-infarct and low voltage pattern died [OR 0.70, 95% CI 0.31 – 1.64].

Summary & Conclusions: With increasing awareness and earlier diagnosis, clinical phenotypes may differ from classic descriptions. Our data suggest that classic ECG findings of a pseudo-infarct pattern and low voltage are not associated with the diagnosis or outcome of ATTR-CA. Awareness of contemporary phenotypes are essential for early recognition of disease.

Figures: None

References: None

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Prevalence of Daytime and Nighttime Central Apneas in Patients with Cardiac Amyloidosis

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Background: Cardiac amyloidosis (CA) is an infiltrative cardiomyopathy characterized by the myocardial deposition of amyloid fibrils consisting of misfolded proteins, most commonly deriving from either transthyretin (ATTR-CA) or immunoglobulin light chains (AL-CA). Cardiac pseudo-hypertrophy and diastolic dysfunction are the hallmarks of CA, which can lead to the development of overt heart failure (HF)[1]. Central apneas are a common comorbidity in HF patients, secondary to hemodynamic impairment and chemoreflex hypersensitivity. Central apneas are associated with neuro-hormonal activation, functional impairment, and worse outcome, particularly when observed both at daytime and at nighttime[2]. A single polysomnographic study has reported a high prevalence of sleep apneas in patients with CA so far,[3] while the prevalence of central apneas over the 24-hour period remains unexplored in this clinical scenario.

Objective: To evaluate the prevalence and the clinical correlates of daytime and nighittime breathing disorders in patients with CA.

Material & Methods: Patients referred to the "Fondazione Toascana Gabriele Monasterio" (Pisa, Italy) with a definitive diagnosis of CA were recruited. Patients underwent a comprehensive clinical, biohumoral, and echocardiographic evaluation, and a 24-hour ambulatorial cardiorespiratory monitoring, as previously validated[1]. An apnea/hypopnea index (AHI) ≥5 events/hour identified patients with unstable breathing, classified as having either central or obstructive apneas according to the prevalent phenotype at daytime, nighttime, and over the 24-hour period. The time with oxygen saturation of <90% (T-90) was used to assess desaturation burden.

Results: Out of 40 patients enrolled (aged 75±10 years, 70% men, mean left ventricular ejection fraction 47±12%, NYHA class I-II/III-IV 45/55%, BMI 27±4 kg/m2, NT-proBNP 4603 [2050-9406] ng/L), 24 (60%) and 16 (40%) had a diagnosis of ATTR-CA or AL-CA, respectively. Over the 24-hour period, 35 (88%) patients had an AHI ≥5 events/hour, of whom 24 (60%) had an AHI ≥15 events/hour. The median AHI over the 24-hour period, at daytime, and at nighttime were 18 [9-31], 13 [5-25], and 27 [14-36] events/hour, respectively. Central apneas were more common than obstructive apneas both at daytime (73% vs. 8%) and at nighttime (55% vs. 35%). Although such trends were similar between ATTR-CA and AL-CA (p>0.05), AL-CA patients had more frequently an AHI ≥15 events/hour (75% vs. 50%, p=0.03) and a higher desaturation burden (T-90, 15 [13-28] vs. 10 [4-16] minutes, p=0.02). No significant association was observed between the apnea burden and clinical, echocardiographic, and biohumoral parameters.

Summary & Conclusion: Breathing disorders and, most notably, central apneas are highly prevalent in patients with CA both at daytime and nighttime, and are more severe in patients with AL-CA than in those with ATTR-CA. Future studies should assess the specific pathophysiological determinants and the prognostic impact of central apneas in CA patients.

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The distribution of amyloidosis diseases in Germany: National Clinical Amyloidosis Registry

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Background: Currently there are no data on the epidemiology of systemic amyloidosis in Germany. The registry was initiated in 2018 and collected data auf 1200 patients until March 2020 (part I). We included only patients who gave written consent to analyse their clinical data.

Objectives: Our aim was to collect more epidemiological data on amyloidosis diseases in Germany by including patients with amyloidosis reported by German referral pathologist in part II. These data will be used to determine incidence and distribution of amyloidosis types.

Methods: Part II consists of 1000 reported cases of newly diagnosed amyloidosis patients between 01.04.20 - 22.03.21. Data cutoff of the analysis was 28.02.22. Patients are included in the registry by two ways: arm A: pseudononymized reports of amyloidosis biopsies by two referral pathologists. Arm B: personal presentation at the amyloidosis outpatient clinic or phone contact of the attending physician to the Amyloidosis Center. Inclusion criteria were either a Congo Red positive tissue sample or unequivocal findings in bone scintigraphy (ATTR amyloidosis); here we also collect patients characteristica, prognostic factors and survival.

Results: 986 patients could be evaluated. 14 patients had to be excluded as they did not fulfill inclusion criteria. 411 patients (341 heart and 79 kidney biopsies) were included in arm A and 575 patients in arm B. 127 patients were registered in both arms (identified by biopsy number). Patient characetristica and distribution of subtypes in arm A and B are shown in the table.

Parameter	Arm A (n=411)	Arm B (n=575)
Age (median/range)	78 / 32-92	73 / 27-90
Sex m/f	315 / 96	419 / 156
AL	126 (30,6%)	240 (41,7%)
ATTRwt	1	217 (37,7%)
ATTRv	0	25 (4,3%)
ATTR not classified	263 (64%)	45 (7,8%)
AA	12 (2,9%)	6 (1%)
AL local	na	37 (6,4%)
Other	1	3
Not classiefied	0	2

Table 1: Characteristic of registered patients in arm A and B

In arm B the most common involved organs were heart (72%) and kidney (58%) in AL and the heart in ATTR (97%). Median time from first symptoms to diagnosis was 292 days for AL and 699 days for ATTR patients. Data on survival (local amyloidosis excluded) were available in 536 cases (median follow-up of 15.4 months). 82 patients died (20 ATTRwt and 55 AL, 7 other). The most frequent cause of death was amyloidosis itself (n=74 pts., 90%). 6% of patients died due to therapeutic complications. Compared to ATTRwt patients with AL amyloidosis had a higher risk to die, one/two-year survival were 97/80% and 85/73%, respectively.

Summary: First results of the expanded registry show that AL and ATTR amyloidosis are the most common forms of amyloidosis in Germany. Within one year 859 newly diagnosed patients could be identified. Due to the still limited number of patients incidence cannot yet be estimated. The time between first symptoms and diagnosis was longer in ATTR patients which reflects the different biologic behaviour of both diseases. The delayed diagnosis especially in AL patients leads still to a high early death rate. Two limitations of the study are that the sickest patients are often not included in arm B due to organizing reasons and we have not covered all referral pathologists for arm A.
Outlook: The registry will include patients for 3 years and a follow-up time of 2 years. The goal is to estimate incidence of AL and ATTR amyloidosis in Germany.

The registry part II was financially supported by Janssen.

Amyloidosis diagnoses and shifting distribution of ATTR and AL from 2019 to 2021: a German single center experience.

Timon Hansen, Hämatologisch-Onkologische Praxis Altona, Hamburg

Background:

Currently there are no data about the distribution of different amyloidosis subtypes in newly diagnosed patients in Germany in the recent 2 years available.

Objective:

The aim of this analysis was to collect recent data about the distribution of different amyloidosis subtypes in patients with newly diagnosed amyloidosis and about a potential change since the approval of specific treatments for systemic wild type transthyretin (ATTRwt) and light chain (AL) amyloidosis in 2020 and 2021. Two-year evaluation of the German Clinical Amyloidosis Registry has been published at ASH annual meeting 2021 including data of patients diagnosed between January 2018 and November 2019, but more recent data regarding the two following years is pending.

Meterial & Methods:

Data about age, sex, amyloidosis subtype and overall survival (OS) were prospectively documented from all newly diagnosed patients who presented at our Outpatient Amyloidosis Center in Hamburg between 01.01.2019 and 31.012.2021. In patients with ATTR, also data about the way of diagnosis (biopsy vs. no biopsy) were collected. Data cutoff of the analysis was April 2022. Inclusion criteria were Congo Red positive tissue sample with amyloidsubtyping by immunohistochemistry or, in case of transthyretin amyloidosis, pathognomonic findings in bone scintigraphy (Perugini Grade 2 or 3) in combination with a second cardiac imaging suggesting cardiac amyloidosis (echocardiography or cardiac MRI).

Results & Discussion:

During the 3 years time period, 151 patients presented with the above mentioned inclusion criteria. 36 were female and 115 male. Most frequently detected amyloidosis subtypes were ATTR in 54% (95% wild type, 5% variant) and AL in 32% of patients. Median age was 76,1 years in patients with ATTR (70,5 years in 8 patients with soft tissue involvement only) and 60,4 years in patients with AL. The remainder cases were localized AL (9%), unknown subtype (2%), AA (1%), Aß (1%) and seminal vesicle amyloidosis (1%). Notably the distribution of subtypes changed over time from 2019 to 2021 as follows: In 2019 it was 45% ATTR and 37% AL, in 2020 49% ATTR und 35% AL an in 2021 68% ATTR and 25% AL (Figures 1 + 2). The way ATTR amyloidosis was diagnosed was 57% by biopsy (70% cardiac, 30% extracardiac) and 43% by cardiac imaging regarding the whole time period. Herein a shift from biopsy to non-invasive imaging diagnosis was detected over the 3 years: In 2019 only 23% were made without histological verification while in 2020 and 2021 the proportion of non-invasive diagnoses was 48% and 53% respectively. Survival data will be presented at the ISA meeting.

Summary & Conclusions:

Systemic ATTR and AL amyloidoses are the most frequent subtypes presenting in our outpatient Amyloidosis Center. A shift from only slight overbalance of ATTR to AL to a more than doubled proportion of patients with ATTR amyloidosis (mainly wild type) was seen from 2019 to 2021. Reasons could be an increasing awareness and the implementation of diagnostic algorithms for cardiac amyloidosis pushed by the approval of the TTR stabilizer Tafamidis for the treatment of ATTR Cardiomyopathy in 2020. Additionally as a confounding factor we realize fewer referrals of patients

with AL Amyloidosis since the approval and wide availability of Daratumumab-based first line treatment in 2021. Diagnosis of ATTR Amyloidosis is meanwhile predominantly done non-invasive.

Figures:

Figure 1

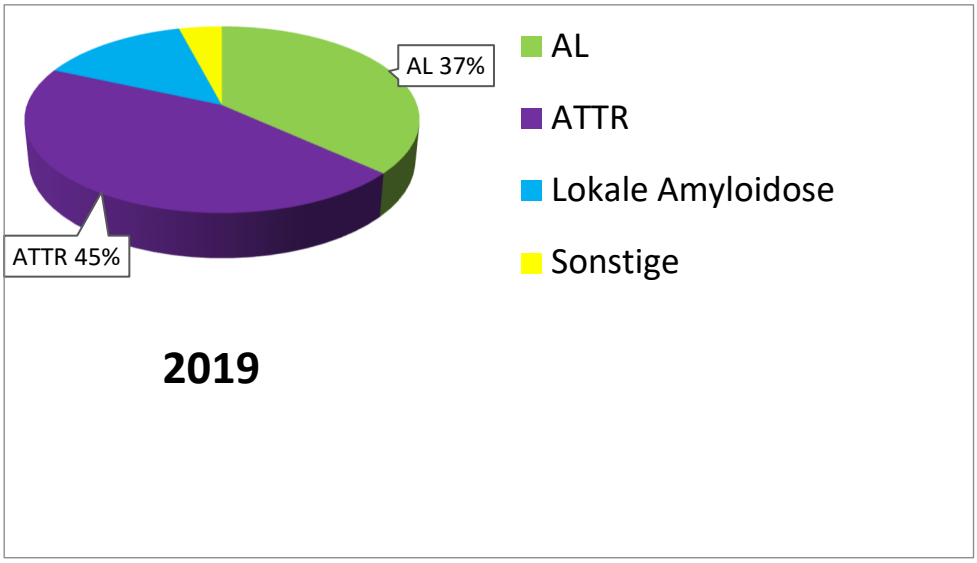
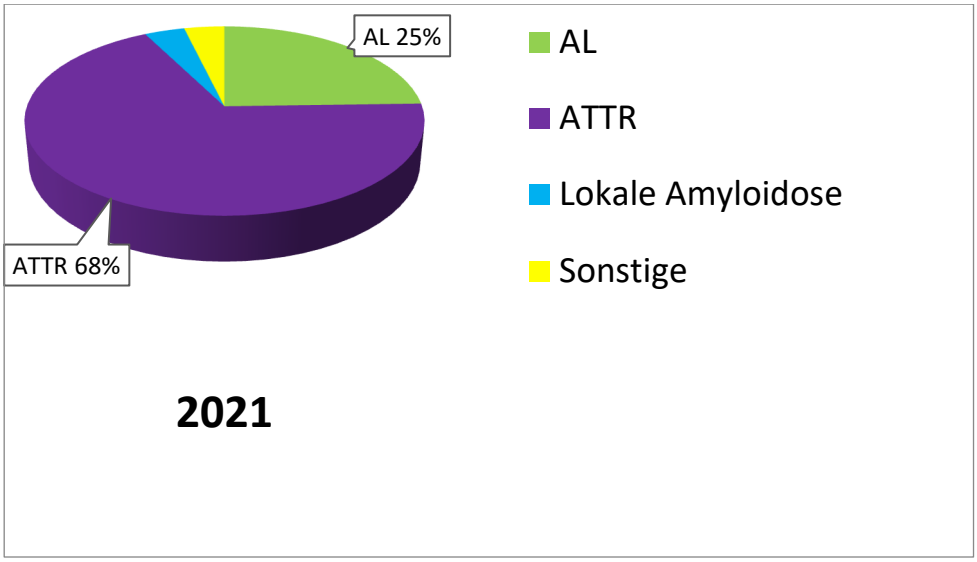


Figure 2



Initial Experience of a Private Amyloidosis Center

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Background: Amyloidosis is a systemic disease caused by the deposition of insoluble proteins in several organs, including the heart, especially by light chain (AL) or transthyretin (TTR), with a range of possible treatments, which are modifiers and reduce cardiovascular events, death and hospitalization. According to the 2021 - European Position of amyloidosis (EP) and Positioning of the Brazilian Society of Cardiology (PASBC)-GEMIC-DEIC recommends the development of Reference Centers as a care model seeking to integrate a specialized multidisciplinary approach and in a collaborative environment for the development of clinical research.

Objetive: To show the initial experience of diagnosis and follow-up of patients with systemic amyloidosis in a brazilian amyloidosis private center created in August 2021.

Material & Methods: According to the EPA and PASBC: From the establishment of clinical suspicion by generalists, neurologists, cardiologists or hematologists, based on clinical history, physical examination with suggestive findings – the investigation of monoclonal immunoglobulin light chains should be carried out, for an effective screening for the presence of AL amyloidosis, protein electrophoresis and dosage with ratio of kappa and lambda free light chains (LC), and the detection of an abnormal relationship between k/L chains (greater than 1 ,65, with attention to chronic kidney disease with possible increased ratio) and this detection of monoclonal LC makes it necessary to refer the the Hematological Route in the Flowchart, and the performance of tissue biopsy - to confirm the deposit of amyloid protein and the elaboration of the therapeutic strategy. If this is normal, the cardiological route directs the myocardial scintigraphy with uptake of technetium pyrophosphate in the heart, the contralateral heart ratio and SPECT, being grade 2 or 3 Perugini confirms the TTR diagnosis in a non-invasive way, without the need for endomyocardial biopsy. Genetic testing directs mutation or non-mutated - wild type. Patients who will be followed up by research protocols with multispecialty and multidisciplinary care and directed to appropriate treatment according to availability and indication.

Results: There are 40 patients currently being followed up until this moment, 22 with hereditary transthyretin amyloidosis (ATTRv), 3 ATTRv associated with gammopathy of uncertain significance (MGUS), 3 with light chain (AL), 6 wild type, 6 still with incomplete diagnosis. There are 16 patients with symptomatic ATTRv -Val50met and 9 patients - Val142Ie, with cardiac and neuropatic involvement. Most of the hereditary and AL forms with electroneuromyography with axonal pattern sensorimotor polyneuropathy, bilateral carpal tunnel syndrome. Four patients underwent bone marrow biopsy and Abdominal fat biopsy performed with positive Congo red under polarized light and mass spectrometry detected AL, one of this patients detected TTR with MGUS. The ATTRv and ATTRw patients underwent myocardial scintigraphy with grade 3 technetium pyrophosphate.

Summary & Conclusion: The amyloidosis center aims to develop clinical research and develop protocols for diagnosis, better monitoring and prognosis, the possibility of treatment in addition to the clinical study to this underdiagnosed disease.

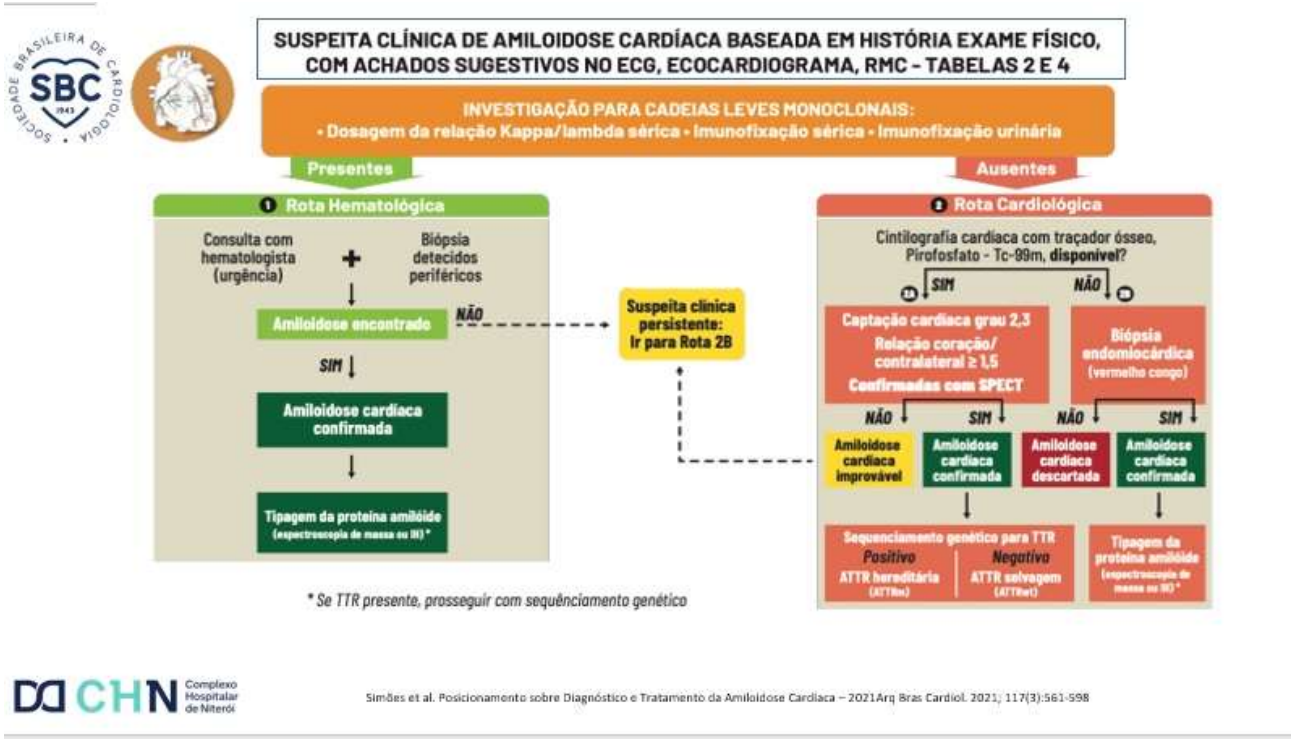

Figure 1. Brazilian Flowchart

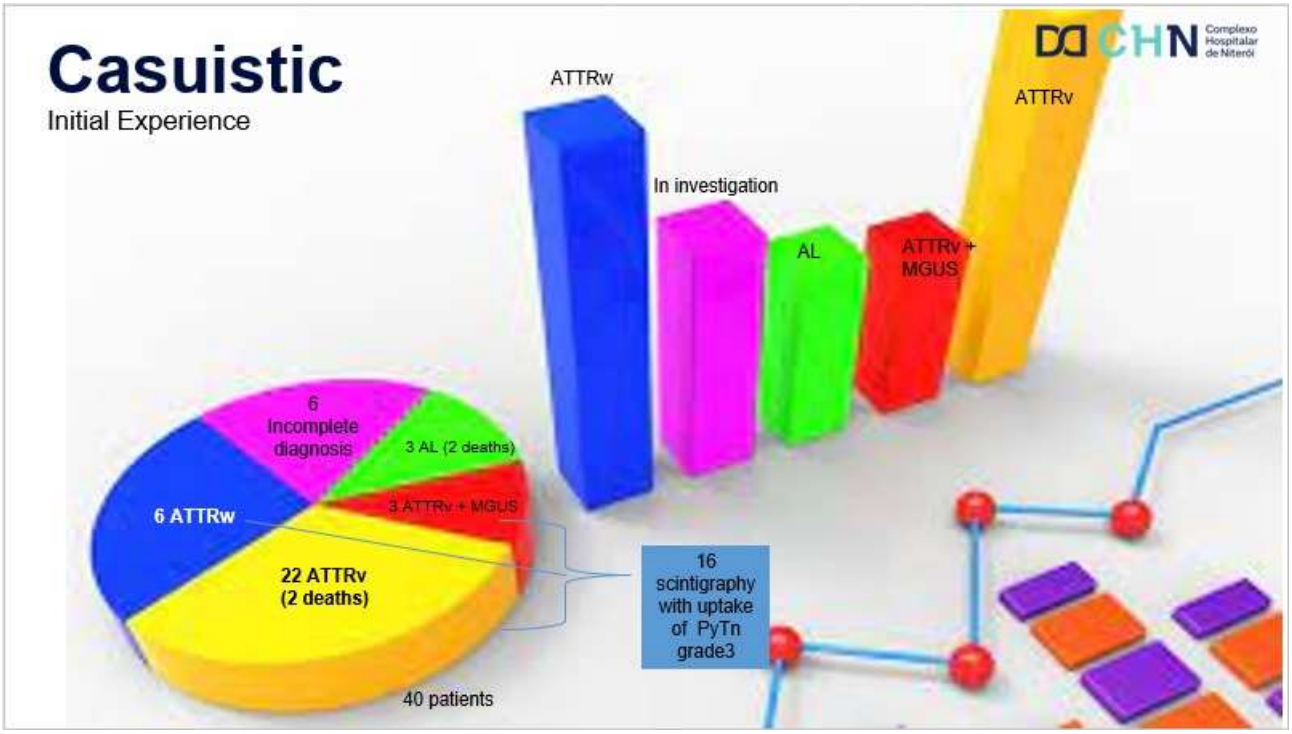

Figure 2. Initial experience of a brazilian private center of amyloidosis

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From symptoms and signs to diagnosis – Development of a simple screening tool for hereditary transthyretin amyloidosis (AmyloScan©)

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Background: Hereditary transthyretin (ATTRv) amyloidosis is a rare, progressive, systemic disease. In addition to cardiac dysfunction, it is mainly characterized by a length-dependent polyneuropathy with a broad spectrum of sensorimotor and autonomic symptoms. Due to the heterogenous clinical presentation and rarity of the disease misdiagnosis is common and leads to a delayed start of treatment. Since the median survival for patients is reported to be about 7 years from disease onset [1], an easy-to-use screening tool for early identification of ATTRv amyloidosis is needed.

Objective: The aim of this study is to identify characteristic symptoms and signs of ATTRv amyloidosis with polyneuropathy and, based on this, to develop a simple screening tool for early diagnosis and differentiation from other neuropathies (AmyloScan©).

Material & Methods: So far, 10 patients with ATTRv amyloidosis with polyneuropathy, 14 patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and 15 patients with diabetic polyneuropathy (dPNP) were investigated. Patients were phenotypically characterized by DFNS quantitative sensory testing (QST) and bedside-QST, heart-rate-variability, tilt table test, and sudomotor function test. Sensory testing was performed on the dorsum of one hand, one foot and a border zone area, i.e., the most proximal area that was still clinically affected (most frequently at the lower limb). Several validated questionnaires were used to further assess the pain quality (NPSI, PainPREDICT, PainDETECT), autonomic symptoms (CADT), and quality of life (SF-36).

Results: Regardless of the underlying disease, all patients reported at least one sensorimotor symptom at the lower limb, and most also reported one at the upper limb. However, in contrast to CIDP and dPNP patients, the majority of ATTRv amyloidosis patients reported symptoms that indicated additional organ manifestations besides polyneuropathy, most frequently cardiac symptoms, i.e. dyspnoea and palpitations. A carpal tunnel syndrome was more frequent in ATTRv amyloidosis compared to CIDP (70% vs 14.3%, p=0.005) and dPNP (26.7%, p=0.032). Autonomic symptoms were more common in ATTRv amyloidosis than CIDP, i.e., 63% vs 8% reported a combination of at least two autonomic symptoms (p=0.001), most frequently orthostatic dizziness, gastrointestinal complaints and erectile dysfunction. Sensory signs at the hand characteristic for ATTRv amyloidosis were: an impaired cold detection threshold compared to CIDP (p=0.015), a higher vibration detection threshold compared to dPNP (p=0.006) and pressure pain hyperalgesia compared to both CIDP (p=0.022) and dPNP (p=0.019). The latter was also present at the border zone area (CIDP: p=0.006, dPNP: p<0.001).

Summary & Conclusion: Although ATTRv amyloidosis patients showed some similar symptoms and signs to CIDP and dPNP patients, several characteristics could be identified to distinguish the diseases from each other. Multiple organ manifestations, a carpal tunnel syndrome in the patients' history and autonomic symptoms combined with impaired thermal detection and pressure pain hyperalgesia seem to be characteristic features of ATTRv amyloidosis that might be used as part of a new screening tool for early diagnosis of ATTRv amyloidosis with polyneuropathy.

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Determining amyloid subtype: a retrospective comparative study between a clinical, laboratory, imaging, and pathological model and mass spectrometry

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Background: Systemic amyloidosis results from the deposition of protein fibrils in tissues, leading to multiorgan dysfunction and potentially death. Diagnostic journey is complex with frequent delays in diagnosis. The correct diagnosis of amyloid subtype allows the institution of proper treatment. Until mass spectrometry(MS) became the gold standard for defining the precursor protein, the amyloid subtype was inferred according to clinical manifestations, laboratory tests, imaging methods, and pathological techniques. As MS is not widely available, defining the precursor protein is still a limiting factor in the diagnostic process, leading to delays in the initiation of treatment.

Objective: To assess the accuracy of the a “clinical, laboratory, imaging and pathologic model(CLIPM)” in defining amyloid subtype compared to MS.

Material & Methods: We included all patients with biopsy-proven diagnosis of systemic amyloidosis from 2009 and 2020 in a tertiary, public, university center (Hospital das Clínicas, University of Sao Paulo). Amyloidosis subtype previously defined by the CLIPM was assessed by retrospectively reviewing medical records. A confirmed subtype was considered if the protein was identified by immunohistochemistry(IHC) and/or indirect immunofluorescence, or a genetic mutation. Probable subtype was defined as follows: AL if evidence of a monoclonal protein; ATTR if cardiac uptake grade 2-3 on PYP scintigraphy, classical neurological symptoms with positive familial history, or liver transplant from a donor with ATTRv amyloidosis (domino transplant); and AA in the presence of an underlying systemic chronic inflammatory condition. If >2 features were present suggestive of different subtypes without any confirmatory criteria, the amyloid subtype was considered inconclusive. Laser microdissection followed by liquid cromatography coupled to MS was performed in available biopsies. CLIPM was compared to MS results. Consent form was obtained from alive patients and the project was approved by Ethics Commitee.

Results: One hundred and fourty-three patients were identified with a biopsy-proven diagnosis of systemic amyloidosis. Table 1 shows patients´ characteristics and their diagnostic journey. MS was performed in 38 biopsies until this preliminary result of the study. Among them, biopsy sites were kidney 37%, heart 17%, fat pad, gastrointestinal and nerve 11% each, lung 6%, bone marrow and liver 3% each. The following subtypes were identified by MS: AL 62%, 71% being lambda, ATTR 24%, AA 8%, AFib 3% and AH 3%. In 30 cases, a correct diagnosis was made by the CLIPM (accuracy of 79%). In 2 cases classified as AL by the CLIPM (positive IHC for light chain), MS evidenced ATTR. The AH case was diagnosed as AL by the CLIPM, and in 4 cases MS was not able to identify the precursor protein (sample quality issues).

Summary & Conclusions: The correct diagnosis of the amyloidosis subtype is mandatory to properly treat patients. Although the combined interpretation of clinical presentation, laboratory, imaging and immunopathological methods may suggest the precursor protein, they are not always sufficient to establish a reliable diagnosis. Nonspecific reactions leading to false-positive results in immune-mediated techniques such as IHC may occur, as shown in 2 cases in our study. Misdiagnosis can lead to inappropriate treatments. MS remains the gold standard method for typing amyloid and should be available at referral centers.

Characteristic	Amyloidosis Subtype				
	AL	ATTR	AA	AFib	Inconclusive
	n = 97 (%)	n = 19 (%)	n = 12 (%)	n = 6 (%)	n = 9 (%)
Gender					
Male	49 (50.5)	15 (78.9)	4 (33.3)	4 (66.7)	5 (55.5)
Female	48 (49.5)	4 (21.1)	8 (66.7)	2 (33.3)	4 (44.4)
Age at diagnosis (years)					
Mean (SD)	60.3 (±11.3)	59.3 (±15.2)	46.2 (±16.4)	57.6 (±5.8)	61.8 (±15.3)
Educational level					
Elementary	58 (67.4)	9 (47.4)	6 (54.5)	3 (50.0)	6 (75.0)
Secondary	20 (23.2)	8 (42.1)	3 (27.3)	2 (33.3)	2 (25.0)
University	8 (9.3)	2 (10.5)	2 (18.2)	1 (16.7)	0 (-)
ECOG					
≥ 2	55 (59.1)	16 (94.1)	3 (30.0)	1 (25.0)	8 (100)
< 2	38 (40.9)	1 (5.9)	7 (70.0)	3 (75.0)	0 (-)
Initial clinical manifestation					
Renal disorders	62 (63.9)	2 (10.5)	5 (50.0)	6 (100)	2 (22.2)
Heart disease	37 (38.1)	12 (63.2)	2 (20.0)	0 (-)	7 (77.7)
Neuropathy	19 (19.6)	12 (63.2)	2 (20.0)	1 (16.7)	1 (11.1)
Gastrointestinal symp	23 (23.7)	4 (21.1)	5 (50.0)	0 (-)	2 (22.2)
Cachexia	40 (41.2)	6 (31.6)	3 (30.0)	0 (-)	2 (22.2)
Number of specialties consulted until diagnosis					
1 to 2	43 (44.3)	13 (68.4)	3 (25.0)	4 (66.7)	6 (66.7)
≥ 3	54 (55.7)	6 (31.6)	9 (75.0)	2 (33.3)	3 (33.3)
Types of specialties consulted until diagnosis					
General practitioner	63 (64.9)	9 (47.4)	5 (41.6)	1 (16.7)	4 (44.4)
Nephrologist	50 (51.5)	2 (10.5)	5 (41.6)	6 (100)	1 (11.1)
Cardiologist	33 (34.0)	11 (57.9)	2 (16.6)	1 (16.7)	8 (88.8)
Neurologist	3 (3.1)	10 (52.6)	4 (33.3)	0 (-)	2 (22.2)
Gastroenterologist	13 (13.4)	3 (15.8)	3 (25.0)	0 (-)	2 (22.2)
Time from symptoms onset to diagnosis (months)					
Median (range)	9.0 (0.5-90.3)	30.6 (1.9-108.0)	40 (3.8-74.7)	10.4 (0.9-36.9	2.4 (2.8-114.0)

Missing values: educational level (9.0%); ECOG (9.0%); time from symptoms onset to diagnosis (4.0%)
AL = Light Chain Amyloidosis, ATTR = Transthyretin Amyloidosis, AA = Serum Amyloid A Amyloidosis, AFib = Fibrinogen Amyloidosis

Table 1: Characteristics of patients according to the clinical, laboratory, imaging, and pathological model.

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Cardiac amyloidosis in Latin America: Opportunities to increase disease awareness among clinicians. Findings from the AMILO-LATAM research group.

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Background: Cardiac amyloidosis (CA) is an under-diagnosed disease presenting as restrictive cardiomyopathy associated with high morbidity and mortality.[1,2] Wild-type transthyretin amyloid cardiomyopathy (ATTR-CM) is mainly seen in elderly patients, increasing as life expectancy grows.[3] Nowadays prevalence of the disease has been demonstrated to be higher than previously thought and novel imaging techniques (nuclear medicine, cardiac MRI, and ECHO) allow accurate noninvasive diagnosis without the need for endomyocardial biopsy. Novel-specific therapies have been recently developed so early identification of affected individuals is essential. We have recently created an international working group on CA (AMILO-LATAM) composed of cardiac imaging specialists from Latin American (LA) countries, aiming to identify potential needs in technical resources, training, and education in our region.

Objective: To investigate the knowledge of LA clinicians regarding ATTR-CM including utilization of 99mTc-PYP scans.

Material & Methods: An anonymous online survey was distributed among clinicians of different LA countries as a cross-sectional, observational study. We asked ten questions about general awareness of CA and different clinical or imaging findings (red flags) commonly associated with the disease. Spanish and Portuguese questionnaires were promoted by digital means for two months (June-July 2021). The information was automatically entered into a spreadsheet for tabulation and descriptive statistical analysis (Microsoft Excel 2016).

Results: We received 406 responses from professionals from 17 of the 20 LA countries. The respondents were cardiologists (65%), followed by general practitioners/family doctors (17%), others (12%), and internists (6%). 47% considered to have an intermediate degree of knowledge about CA, 33% regarded as adequate, and 20% said to have little knowledge. A large majority (93%) believe that reaching a diagnosis in patients with suspected CA is important because it can change the therapeutic strategy. The majority of participants (74%) believe that the prevalence of CA in older adults is low, although higher than perceived; only 15% believe it is high. Nevertheless, 53% identify being elderly and having HFpEF as a red flag, while 73% consider that older patients with LVH and a discordant ECG should be tested for CA. In patients with a suspicious ECHO, the main next study to be indicated is cardiac MRI (43%), only 38% considered nuclear medicine as the next one. 65% of responders had not ordered a single 99mTc-PYP scan during the year before the survey.

Summary & Conclusion: A significant lack of awareness about CA was identified. The red flags are unclear, especially in the elderly population, and PYP scans are not recognized as the study of choice. Very few patients were referred for evaluation of ATTR-CM with nuclear techniques in most countries. As expected, having requested a nuclear scan was directly related to the degree of knowledge on the subject. The results demonstrate the need for educational programs and other measures to increase clinical awareness and early detection of CA so that disease management can be improved through timely diagnosis and treatment.

Figures

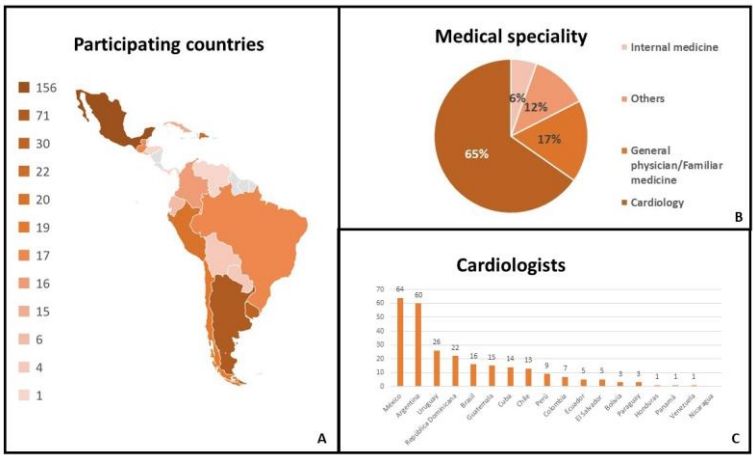

Figure 1. A)Participating countries. B)Medical specialty of the respondents. C)Countries of the participant cardiologists

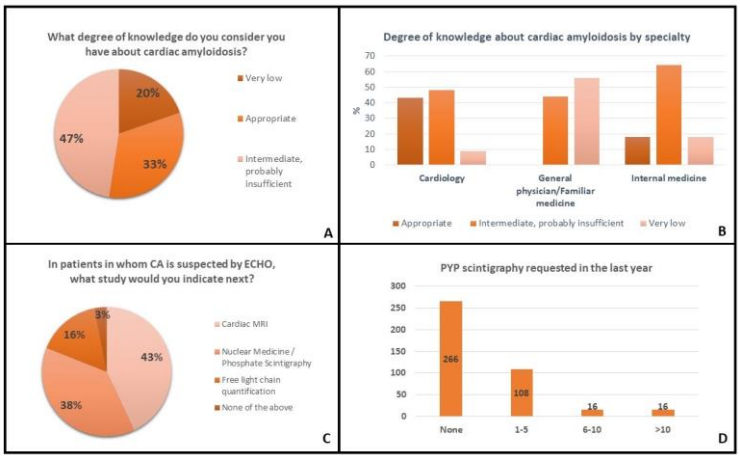

Figure 2. A and B)Degree of knowledge about CA globally and by specialty. C)Election study to continue with the study approach with ECHO suspected of CA. D) 99mTc- PYP scintigraphy requested in the last year by clinicians participants.

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SPREAD-ATTR: Quality assessment of teaching in transthyretin amyloidosis.

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Background: The lack of knowledge about amyloidosis and the delay in diagnosis are some of the main public health problems related to the disease. Unfortunately, the patient currently only receives the diagnosis after going through several specialists, almost always in tertiary or quaternary health services. Basic health care lacks information about the disease, its diagnosis and treatment possibilities. It is also observed that students who are completing the medical course are generally unaware of the disease and its impact. Thus, we have a vast field where much knowledge can still be sown, in order to fill the gaps of misinformation, optimizing patient access to diagnosis and treatment as early as possible.

Objective: Expand knowledge of transthyretin amyloidosis, including primary care medicine professors, medical interns, and other healthcare professionals, based on currently known best practices, to increase clinical suspicion of the disease and shorten the patient's journey to diagnosis and treatment, with all the support and support needed by the network.

Material & Methods: The project will be divided into 4 phases, where 1000 students and professors from 6 faculties of medicine from the same city in our country will be trained separately. Fot all phases, training will be given in the form of synchronous and asynchronous physical and online classes, with teaching material available to students for later reference. All training students will take a pre-test for general knowledge about the disease, and a post-test after 6 months, to analyze the degree of knowledge acquired and retained. The focus is to present the warning signs that generate the suspicion of the disease, developing and presenting a clinical evaluation checklist, which will also serve as a form for referring patients to an amyloidosis specialist.

Results: The project is still in the implementation phase. At the end, the degree of retention of knowledge of each student will be analyzed. Different data will also be analyzed, with emphasis on the total number of people trained, patients screened and effectively diagnosed with the disease and how long the journey to diagnosis of the disease took. Project metrics: (1) knowledge retained after 6 months of training; (2) number of students trained; (3) number of teachers trained; (4) number of trained health professionals; (5) number of participating Basic Health Units; (6) number of patients screened; (7) number of newly diagnosed cases and (8) time between symptom onset and diagnosis.

Summary & Conclusion: We believe that adequate training for students who are completing their graduation and for their primary care teachers will allow earlier diagnosis of the disease, decrease in the patient's journey to diagnosis, better management of complications presented by patients, in centers of excellence in neurology. , cardiology, hematology and nephrology, it will strengthen the referral network in the Unified Health System for patients and their families with the disease and will lead to the creation of a culture in the country about amyloidosis.

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Glomerular and tubular renal function in patients with Hereditary Transthyretin Amyloidosis (ATTR)

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Background: Transthyretin mutation hereditary amyloidosis (TTR) is a systemic, progressive and disabling disease with an autosomal dominant character and variable penetrance. It is the most common form of hereditary (familial) amyloidosis. The clinical presentation is predominantly neurological and cardiac, but amyloid deposits have been demonstrated in the tubulointerstitial and glomerular compartments of the kidneys. Therefore, changes in glomerular or tubular renal function may be early manifestations of renal impairment in this population.

Objective: To evaluate the glomerular and tubular renal function in patients with ATTR.

Material & Methods: Cross-sectional study including outpatients with ATTR, older than 18 years old, with GFR>45ml/min/1.73m^2 estimated by the CKD-EPI equation. We collected blood samples for electrolyte and blood venous gas analysis, and urine sample after 12 hours of water restriction for analysis by dipstick, urinary osmolality (UOSM) by freezing point, urinary pH (UpH) by potentiometry, urinary ammonium (UNH_4^+) by spectrophotometry and titratable acidity (UTA) by NaOH. We performed urinary measurement of gamma-glutamyl transferase (UGGT) as a biomarker of tubular lesion by 5-Amino-2-Nitrobenzoate (14-79U/gcreat). Proteinuria, albuminuria, citrate and electrolyte measurements were performed in 24-hour urine. Results expressed as media±SD or median (interquartile range). Comparison between groups was performed using non-parametric tests (Mann-Whitney).

Results: We evaluated 49 patients (46 carriers of the Val50Met mutation), 44.8±13.5 years of age, 6.5±7.5 years of diagnosis of the disease, 63% female, 84% Caucasian, BMI 25.1±4.9kg/m^2 and GFR 85.2±22.4ml/min/1.73m^2. 57% of patients were symptomatic. Serum bicarbonate 25.1mmol/L (23.9-26.5); Proteinuria 107mg/24h (73-190), Albuminuria 3.8mg/24h (3.8-8.6) with prevalence of 25% (pathological proteinuria) and 9% (microalbuminuria), respectively. Urine specific gravity 1,015 (1,011-1,020), UOSM 653mOsm/Kg/H_2O (448-851) with 49% of decreased urinary concentration capacity. UpH: 0h 5.06 (5.07-5.80); UNH_4^+: 30.1µEq/min/1.73m^2 (20.8-41.3); UTA 21.2µEq/min/1.73m^2 (3.5-40.4); urinary citrate: 308mg/24h (218-528) with 22% of renal tubular acidosis; UGGT 49.3U/gcreat (9.5-100.0) with 35% of patients presenting elevated biomarker. When comparing the asymptomatic versus symptomatic groups, there was a significant difference in UOSM ($p<0.001$), urine specific gravity ($p=0.001$) and urinary citrate ($p=0.008$).

Summary & Conclusion: Renal glomerular function was relatively preserved with low levels of tubular proteinuria. The prevalence of renal tubular function disorders was high, both in asymptomatic and symptomatic patients. We suggest that early diagnosis of subclinical renal tubular dysfunctions is important to minimize the risk of progression to chronic kidney disease in this population.

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Targeted sequencing of functionally selected genes in patients with wild-type transthyretin amyloidosis

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Background: Wild-type transthyretin (ATTRwt) amyloidosis is caused by the misfolding and subsequent deposition of the transthyretin protein (TTR) in the absence of mutations in the TTR gene. Several studies in patients with the variant form of ATTR amyloidosis (ATTRv) suggest that the presence of SNPs in certain genes other than the *TTR* may influence the development of the disease (1–4), affecting the age of onset. Regarding ATTRwt amyloidosis, it is currently unknown the existence of other genetic factors involved in its aetiopathogenesis.

Objective: This work aims to study the presence of sequence variants in genes selected for their potential involvement in ATTR amyloidosis, in patients with the wild-type form of the disease.

Material & Methods: In this study, we performed targeted sequencing of a panel of genes in a cohort of 27 patients diagnosed with ATTRwt following the criteria of Gillmore et al (5). The panel included 84 protein-coding genes that, according to their function, could impact on the TTR protein and thus play a role in the pathogenesis of the disease: genes encoding proteases potentially involved in TTR cleavage, genes encoding TTR-interacting proteins, extracellular chaperones and extracellular matrix related proteins, as well as genes described in literature as altered in different types of amyloidotic diseases.

Results & Discussion: According to the criteria established by the ACMG-AMP, a total of 15 gene variants of uncertain significance as well as one likely pathogenic variant were identified in 14 different genes. Among the patients included in the study, 5 patients carried only one variant, 4 patients had 2 variants, and intriguingly, one patient was carrier of 4 different variants, including that classified as likely pathogenic. This latter variant, rs144607263 (c.1083C>T: p.Ala361Ala), could lead to a loss-of-function of its gene encoded protein, ECM1, an extracellular matrix protein whose changes in the expression level have been associated with non-amyloidotic cardiac conditions (6,7). To date, it had been no previously described in patients with ATTRwt amyloidosis.

Summary & Conclusions: This study provides novel data about the identification of variants in diverse protein-coding genes that could somehow play a role in the etiopathogenesis of ATTRwt amyloidosis. Although most of the selected genes have been previously associated with the development of ATTRv amyloidosis, this is the first time that variants in such genes are identified in patients with ATTRwt amyloidosis. Importantly, we found a variant classified as likely pathogenic. Given the small sample size of our study cohort, no causality can be inferred between the presence of the variants and the development of the ATTRwt amyloidosis. Consequently, further studies regarding larger sample sizes should be conducted.

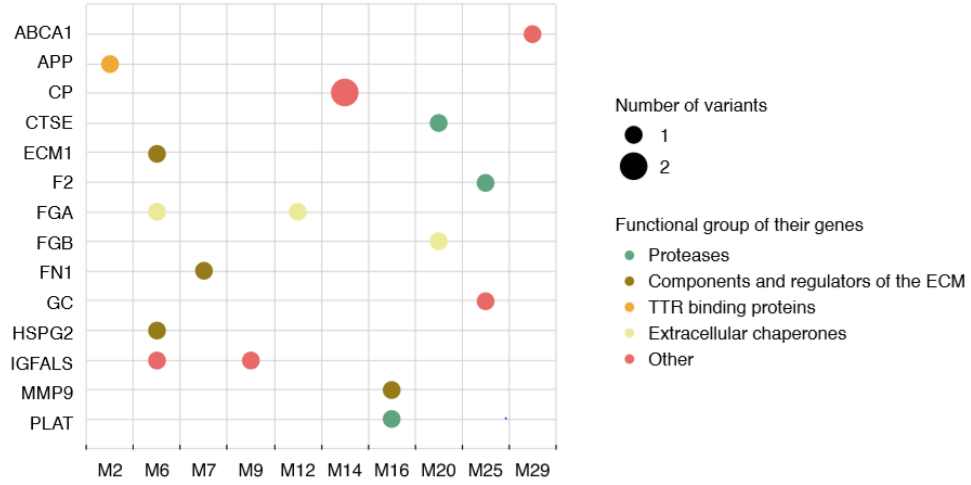

Figure 1: Matrix bubble plot displaying the genes where sequence variants were identified and their corresponding ATTRwt amyloidosis carrier patients (M). Genes are classified by color according to their functional group. ABCA1: ATP binding cassette subfamily A member 1, APP: Amyloid beta precursor protein, CP: Ceruloplasmin, CTSE: Cathepsin E, ECM1: Extracellular matrix protein 1, F2: Coagulation factor II - thrombin, FGA: Fibrinogen Alpha Chain, FGB: Fibrinogen

Beta Chain, FN1: Fibronectin 1, GC: GC vitamin D binding protein, HSPG2: Heparan sulfate proteoglycan 2, IGFALS: Insulin like growth factor binding protein acid labile subunit, MMP9: Matrix metallopeptidase 9, PLAT: Plasminogen activator- tissue type, ECM: Extracellular matrix.

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Detection of amyloidogenic FLC in serum as a non-invasive tool to facilitate the diagnosis of AL amyloidosis

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Background: Accurate diagnosis of amyloidosis requires detection and typing of amyloid deposits in a tissue biopsy. Widely available immunohistochemical techniques underperform in identifying the amyloid type, with unacceptable high false positive and false negative rates. Proteomic typing approach based on laser micro-dissection of amyloid fibrils coupled with mass spectral analysis is now considered as the gold standard for the molecular amyloid typing. However, these technologies are expensive and still not widely implemented in most medical centers. Moreover, in some cases, biopsy is risky or declined, while the commonly used non-invasive tests are diagnostically insufficient. The results of our previous studies based on serum free light chain (FLC) Western blot analysis showed that increased level of monoclonal FLC dimers and high ratio values of involved/uninvolved dimeric FLC may serve as molecular markers of AL amyloidosis [1- 4].

Objective: The primary goal of this study is the development and validation of a reliable serum-based laboratory test to support the diagnosis of AL amyloidosis. In this study we assessed whether the light chain type (κ or λ) and amino acid sequences *of monoclonal FLC dimers in patients sera* matched those *of light chain deposits in tissue biopsies.*

Material & Methods: Serum samples were collected from 10 patients in whom diagnosis was confirmed by biopsy examination and typing was performed using laser micro-dissection coupled with liquid chromatography – tandem mass spectrometry (LC-MS/MS). Serum FLC were analysed using sodium dodecyl sulfate (SDS)-electrophoresis based Western blotting in order to reveal abnormal FLC monomer-dimer patterns typical of AL amyloidosis [1- 4]. The gel containing FLC dimer band was excised and subjected to mass spectrometry. Briefly, proteins-in-gel were reduced, alkylated and digested by trypsin. The resulting tryptic peptides was resolved by capillary reverse-phase chromatography followed by tandem MS analysis by the Q exactive plus mass spectrometer. The data were analyzed by proteome discoverer 2.4 vs the human proteome and the unreviewed sequences of the light chains from the uniport database, with 1% FDR. The obtained sequences of the identified serum FLC dimers were compared to the sequences of light chain deposits in the tissue biopsies.

Results: FLC patterns of 9 out of 10 patients with biopsy-proven AL amyloidosis (pts. 1-6, 8-10) demonstrated FLC monomer-dimer patterns typical of AL amyloidosis (Fig.1) according to the criteria previously suggested [3,4]. The type of monoclonal dimers (κ or λ) matched the amyloid type detected in the MS-based biopsy typing. Serum samples from 6 patients with the most abundant FLC dimers (pts. 1,3-5,7,9) were subjected to mass spectral analysis. Analysis of the dimeric FLC from these patients revealed that the variable region sequences of the most intense light chains matched the results of biopsy examination of the same patients (Table 1). Mass spectral analysis of the remaining 4 samples is underway.

Discussion: Monoclonal FLC are direct precursors of amyloid deposits in AL amyloidosis. The importance of dimeric FLC levels was highlighted in previous studies as a non-invasive marker of AL amyloidosis. In the present study, we further enhanced the clinical utility of this technique by demonstrating amino acid sequence homology of the circulating FLC dimers with that of light chain sequences in the amyloid deposits in tissue biopsy obtained from the same patients.

Summary & Conclusion: Serum FLC analysis based on Western blotting allows detection of circulating amyloidogenic FLC with homologous light chain sequencing as observed in tissue biopsy from the same patients using **LC-MS/MS**. This test, if further validated across various light chain amyloidogenic sequences, can be used as a non-invasive test to support the diagnosis of AL amyloidosis, when tissue biopsy is negative but index of suspicion remains elevated.

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Support & Funding: The study is supported by Pfizer Global Medical Grants; program 65925007.

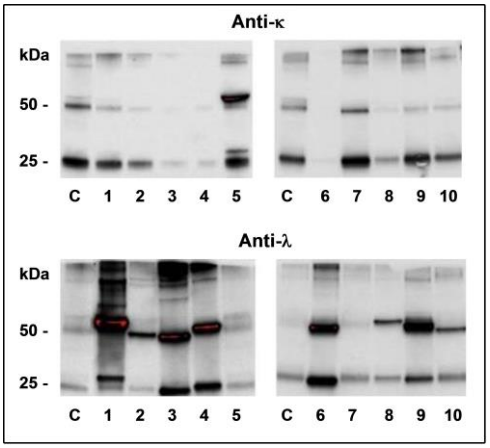

Figure 1. Western blot analysis of FLC in sera of AL patients (pts.1-10). Abnormal FLC monomer-dimer patterns are demonstrated compared to control sample (C) representing a mixture of serum samples from healthy individuals. AL patients showed highly increased levels of monoclonal dimers (λ:,1,3,4,6,9; κ: 5), abnormally high dimerization of monoclonal dimers (dimer/monomer ratio) (λ:,1,9; κ: 5); abnormally high ratio of involved to non-involved FLC dimers (1-6, 8-10); abnornal migration of monoclonal FLC (λ: 1; κ: 5) .

Table 1. Homology of light chain amyloid deposits and circulating monoclonal FLC in six AL patients with most abundant circulating FLC dimers

IGLV family						
Patient No.	**1**	**3**	**4**	**5**	**6**	**9**
Biopsy	**LV2-14**	**LV3-21**	**LV3-21**	**KV4-1**	**None**	**LV-47, LV1-44**
Serum FLC dimers	**LV2-14**	**LV3-19, LV1-44, LV3-21**	**LV3-21**	**KV4-1**	**LV2-18**	**LV1-44**

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Immunoglobulin high-throughput sequencing in Monoclonal Gammopathy of Clinical Significance (MGCS): experience of the French Amyloidosis center

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Background: Our laboratory have a historical immunoglobulin (Ig) sequencing activity in monoclonal Ig-related diseases. In the past, we used SANGER sequencing to determine monoclonal Ig sequences from bone-marrow samples. Now, we have developed a new highly sensitive high-throughput sequencing method called RACE-Repseq[1]. This method was adapted from Turchaninova[2] to detect small B or plasma cell clones in patients. It allows us to obtain not only the full-lenght sequence of the mononoclonal Igs but also the dominant B cell repertoire.

Objective: Identification of the monoclonal Ig and its underlying clone in MGCS[3] (Monoclonal Gammopathy of Clinical Significance) is essential for the diagnosis, the management and assessing disease response of these patients. In some cases, the hematologic evaluation may be limited using laboratory routine techniques (flow cytometry, electrophoresis, immunofixation and free light chain assay), complicating the diagnosis and assessment of response to chemotherapy. We investigated if our Ig-sequencing method can help clinicians to manage these patients.

Material & Methods: Bone-marrow samples from patients with various types of MGCS-related disease were analysed. To determine the monoclonal Ig sequence, total RNAs were extracted from these bone-marrow samples, retrotranscribed to cDNA and amplified using 5' RACE-PCR (adding a unique molecular indentifier to correct amplifications errors and amplification biais). Then, specific primers of the heavy and/or light chain constant domains were used for the amplification of Ig repertoire. An asymetric paired-end sequencing strategy is used to obtain full-length Ig sequences on an Illumina MiSeq sequencer. Finally, analysis was done using Ig Blast, Imgt/V-quest, Vidjil and homemade softwares.

Results: We managed to identify the monoclonal Ig in almost all the analyzed samples. For example, in a series of biopsy-proven AL amyloidosis with low free light chain levels at diagnosis, we found the implicated clones in 100% cases althought laboratory routine were not contributive in most cases for monitoring the disease[1]. We also showed that the full-lenght sequence of the mononoclonal Igs obtained by this approach could be useful to help diagnosis in another rare disorder, the POEMS syndrome[4].

Summary & Conclusion: Our results indicate that RACE-RepSeq is a sensitive method to detect small plasma cell clones in patient's bone-marrow likely due to the high amount of Ig transcripts produced by antibody secreting cells. This tool appears promising for the management of patients with AL amyloidosis but also any other MGCS diseases to confirm the diagnosis and to adapt the therapy. Further studies are ongoing to evaluate the strenght of this method in blood samples, to assess minimal residual disease or to test its complementarity with mass spectrometry-based diagnosis.

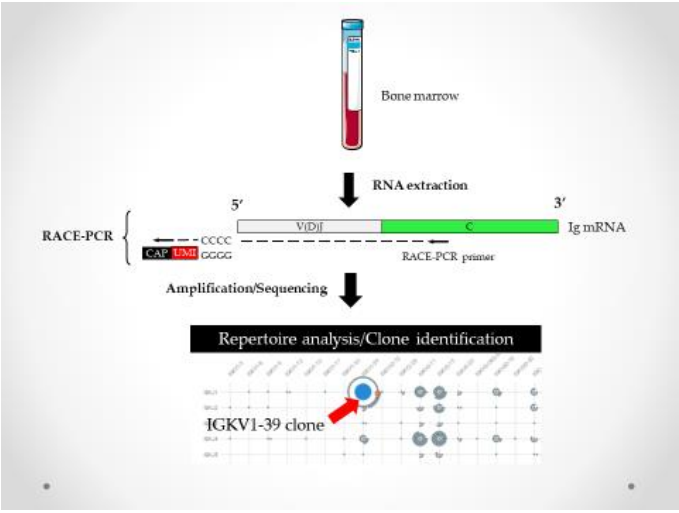

Figure 1: Schematic representation of immunoglobulin high-throughput sequencing method for identification of plasma cell clone in patient's bone-marrow or B-cell repertoire analysis. (UMI=unique molecular identifier).

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Neurofilament light chain, an early biomarker for polyneuropathy in hereditary transthyretin-related (ATTRv) amyloidosis

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Background: Serum neurofilament light chain (sNfL) is a sensitive marker for polyneuropathy (PNP) in hereditary transthyretin-related (ATTRv) amyloidosis patients and correlates with the severity of polyneuropathy (1-4). We hypothesized that sNfL may diagnose neuronal damage in patients with ATTRv amyloidosis before the onset of symptoms and before PNP can be detected by electromyography (EMG) examination.

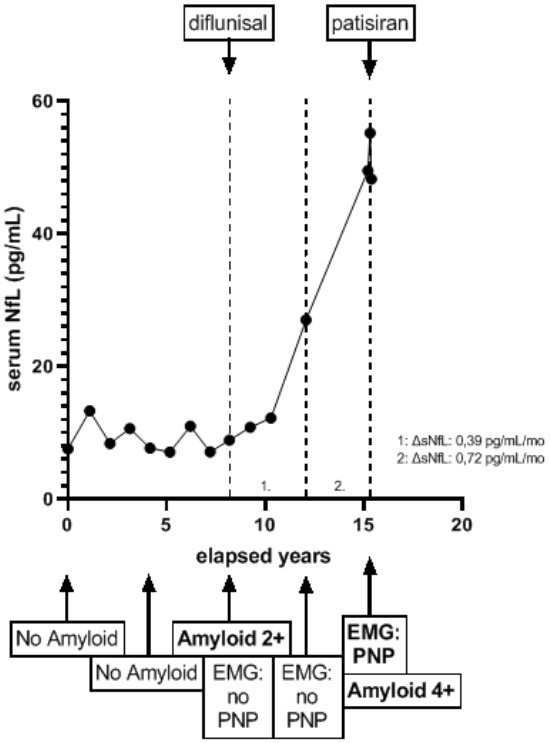
Objective: To establish the course of sNfL in three different groups: 1. persistently asymptomatic variant carriers (with and without detected amyloid), 2. ATTRv amyloidosis patients with PNP on treatment, 3. variant carriers who develop PNP.

Material & Methods: sNfL levels were assessed longitudinally in asymptomatic variant carriers (with and without detectable amyloid), ATTRv amyloidosis patients with PNP on treatment (either a transthyretin (TTR) stabilizer or a TTR-silencer), and variant carriers who developed PNP. PNP was established by EMG examination. The single-molecule array (SIMOA) assay was used to assess sNfL levels.

Results & Discussion: sNfL levels significantly increased over 1 year in 20 persistently asymptomatic carriers ($p < 0.001$), with the strongest increase in variant carriers (N=8) with detectable amyloid in the subcutaneous abdominal fat tissue. In 21 symptomatic ATTRv amyloidosis patients with PNP on treatment with a TTR-stabilizer, sNfL levels remained stable over 1 year. In 24 patients treated with a TTR-silencer, sNfL levels significantly decreased after 1 year of treatment ($p = 0.01$). In 8 out of 9 variant carriers who developed PNP a rise in the sNfL level could be observed before the onset of symptoms and establishment of PNP by EMG examination (figure 1).

Summary & Conclusions: sNfL is a marker for early neuronal damage since a rise in sNfL level occurs before abnormalities can be detected by EMG examination. Our data support the use of sNfL in monitoring disease progression, screening asymptomatic variant carriers and monitoring of treatment effect.

Figure 1: Variant carrier who developed polyneuropathy.



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The Clusterin/von Willebrand Factor Ratio Is Significantly Lower in Marrow Plasma from AL λ-type Than from λ-isotype Monoclonal Gammopathy Patients

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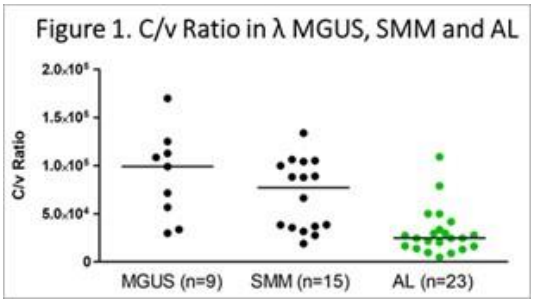
Background: Previous studies have shown that clusterin (apolipoprotein J) may play a variety of roles in protein folding disorders; in AL amyloidosis, clusterin is found in amyloid deposits and serum levels of clusterin are reduced in patients with cardiac involvement.(*1-4*) A previous study has also shown that von Willebrand factor (vWF) levels are significantly elevated in AL patients, possibly due to endothelial cell dysfunction.(*5*) Patients with monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM) with free light chain abnormalities may be at risk of AL and those with AL-related *IGVL* genes encoding their clonal light-chain producing plasma cells may be at further risk.(*6-8*) Seeking additional parameters for likelihood of AL in SMM patients, we asked whether the ratio of clusterin to vWF (C/v) in marrow plasma differed between AL, MGUS and SMM patients.

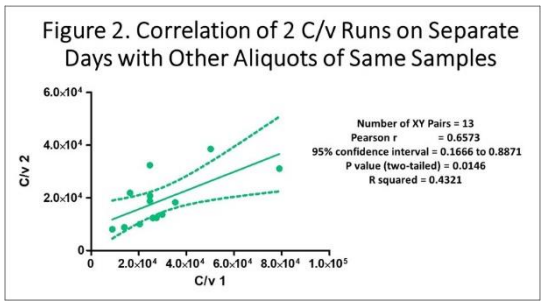
Objective: Our primary objective is to develop methods that enable early diagnosis of AL or risk of AL during the prodromal decade prior to symptomatic presentation.(*8*) The presence of a monoclonal gammopathy is an established risk factor for AL but additional parameters are required in order to develop a testable likelihood algorithm.

Materials & Methods: λ-isotype MGUS or SMM patients from multiple sites in the USA with a difference between involved and uninvolved FLC > 23mg/L and a κ-to-λ ratio below normal and no evidence of amyloid on prior studies consented to participate in a clinical study screening for AL (NCT04615572) and had bone marrow aspirates sent overnite to Tufts. In addition, newly diagnosed AL patients seen at Tufts consented to have marrow aspirates obtained for research on an IRB-approved study. All aspirates were heparinized and had plasma separated initially and aliquoted for storage at -20° C. All patients consented to having their clinical findings put into a REDCap database. Clusterin and vWF ELISA were performed on thawed plasma following manufacturers' instructions. GraphPad PRISM V5 was used for statistical analyses.

Results: Nine MGUS and 16 SMM patients, all λ-isotype, enrolled on NCT04615572, met FLC criteria and had marrow shipped to Tufts; 1 SMM patient was found to have AL. Twenty-two AL λ-type patients had marrow aspirates obtained in the same time period. There were no differences in age or gender, but the percentage of marrow plasma cells in SMM and AL patients were significantly greater than in MGUS patients. iFLC λ medians were 167, 107 and 80mg/L in AL, SMM and MGUS patients. Marrow plasma from AL patients had significantly lower C/v ratios (Figure 1). There was no correlation between FLC λ and the C/v ratio (r = -0.20, P > 0.05). Runs on different days with the same AL samples were significantly correlated (r = 0.66, P = 0.01, Figure 2).

Summary & Conclusion: These preliminary data suggest that the C/v ratio should be explored as a parameter of interest in the construction of a likelihood algorithm for AL in SMM patients. The rationale for the ratio is that with AL progression during the prodromal period prior to significant organ damage clusterin may decrease while vWF may increase. Major issues of methodology and antibody and sample validation remain to be addressed, and whether peripheral blood provides a more useful source of plasma is unclear particularly given the potential for platelet activation. And whether ratios below a threshold may signal risk of AL, and whether changes in the ratio over time may have significance, remain questions for exploration.





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A spectrum of clinical phenotypes associated with p.(Arg54Gly) TTR variant.

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Background: Renal amyloidosis has been reported in Portuguese individuals with hereditary ATTR (hATTR) amyloidosis associated with p.(Val50Met) (1); however, it is rarely described as a presenting symptom of hATTR amyloidosis.
Objective: We report a patient who presented with nephrotic range proteinuria and was subsequently diagnosed with hATTR amyloidosis caused by the p.(Arg54Gly) TTR variant and describe the clinical phenotype associated with this variant in 3 other patients.
Material & Methods: A 37-year-old male from Albania presented with nephrotic syndrome. He underwent renal biopsy at his local hospital, which showed evidence of amyloid deposition and was referred to the National Amyloidosis Centre for further investigations and to elucidate the type of amyloidosis.
Results: At the time of referral he had nephrotic range proteinuria but preserved renal function. He had no symptoms of neurological involvement or bowel disturbance; no breathlessness, chest pains or palpitations and no dizziness or syncope. He had no family history of amyloidosis or renal disease. There was had no evidence of a plasma cell dyscrasia (no serum or urine monoclonal protein on immunofixation, normal serum free light chains) or history of inflammatory disease. There was no evidence of cardiac amyloidosis by echocardiography or cardiac biomarkers. ^{123}I-SAP scintigraphy showed visceral amyloid deposits in the kidneys and spleen.

Congo red staining of his renal biopsy revealed amyloid deposits in the glomeruli and vessels (Fig 1A). There was specific staining of the amyloid deposits with anti-TTR antibody (Fig 1B) and no staining with antibodies against other known amyloid fibril proteins. Laser Micro Dissection (LMD) liquid chromatography and tandem Mass Spectrometry (MS) confirmed the immunohistochemistry showing transthyretin was the amyloid fibril protein. DNA examination using our targeted hereditary amyloidosis NGS gene panel and Sanger sequencing revealed the patient was heterozygous for c.160 A>G resulting in p.(Arg54Gly) TTR variant.

6 months after diagnosis the patient showed symptoms of early amyloid-related peripheral neuropathy, PND stage 1, but no clinical evidence of autonomic neuropathy. His ^{99M}Tc-DPD scan showed cardiac uptake as well as significant soft tissue uptake (Perugini grade 3) and a CMR was consistent with early cardiac amyloidosis. He was referred for an ophthalmologic examination. He started treatment with patisiran, which was well tolerated.
Summary & Conclusion: hATTR amyloidosis represents a diagnostic challenge due to the heterogeneous clinical presentation. We report an Albanian patient with p.(Arg54Gly)-associated hATTR amyloidosis, who presented with nephrotic syndrome. Interestingly, we have diagnosed two unrelated patients, also from Albania, with this mutation who did not have renal disease, but presented with bilateral floaters in their mid-50s and were diagnosed with ocular amyloidosis on a conjunctival biopsy. They subsequently developed symptoms of peripheral and autonomic neuropathy, which is entirely in keeping with the phenotype of p.(Arg54Gly)-associated hATTR amyloidosis reported previously by Koutsis et al., also in an Albanian patient (2). To date 4 Albanian patients with p.(Arg54Gly) were diagnosed with hATTR; p.(Arg54Gly) is extremely rare and not reported on population databases, thus, there might be a hot-spot region in Albania were the incidence of hATTR amyloidosis associated with this particular TTR variant is high. This case highlights the need to perform comprehensive clinical and laboratory investigations to elucidate the amyloid type in all cases of systemic amyloidosis, particularly given the availability of promising novel therapeutics to treat various amyloidosis syndromes.

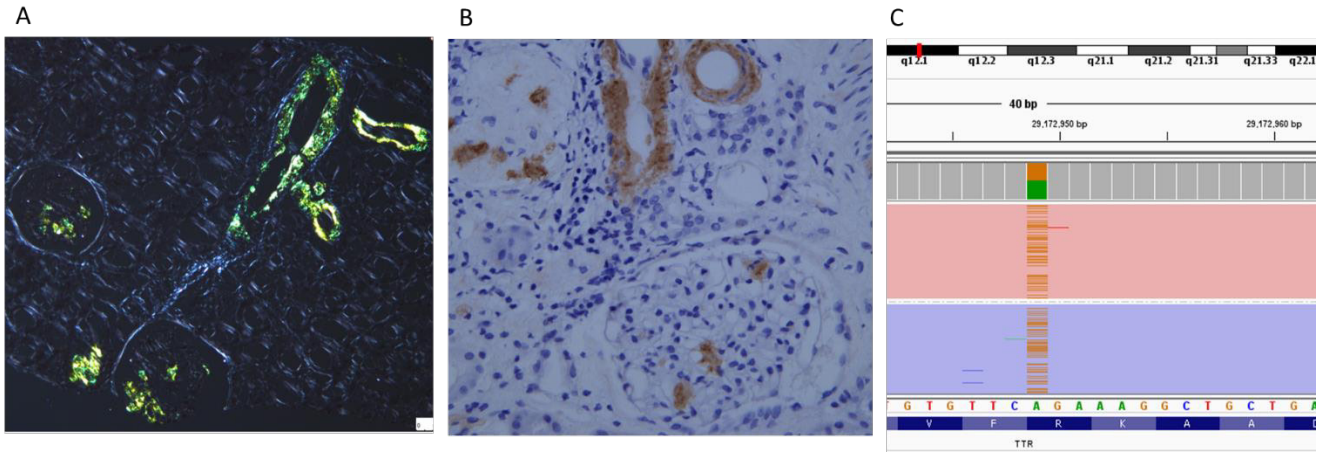

Figure 1. (A) Congo red and (B) anti-TTR serum staining of the amyloid deposits in the renal biopsy (C) NGS result showing patient is heterozygous for c.160 A>G nucleotide substitution in the TTR gene.

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Neurofilament light chain as early biomarker for hereditary transthyretin amyloidosis – the Swedish experience

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Background: Hereditary transthyretin amyloidosis (ATTRv) is a multi-systemic disease with extensive damage on the peripheral nervous. Neurofilaments have a crucial rule in the stability of the neurons and when a damage occurs in neurons the neurofilaments will be released into circulation and can be detected. Neurofilament light chain (Nfl) has been considered to be a sensitive biomarker for peripheral nerve damage.

Objective: The aim of this study was to assess the levels of Nfl in the Swedish ATTRV30M patients and evaluate if Nfl levels differs between patients with fibril type A compared to fibril type B.

Material & Methods: Forty patients with ATTRV30M amyloidosis (20 with amyloid fibril type A and 20 with fibril type B), 18 asymptomatic gene carriers and 20 healthy controls without any signs of peripheral neuropathy were included in the study. Nfl in serum was measured using sandwich ELISA.

Results: Nfl levels were significantly increased in patients compared with controls, $p< 0.001$. No statistical difference in Nfl levels could be detected between asymptomatic gene carriers and controls. Patients with fibril type B showed elevated levels of Nfl compared to controls, $p<0.001$ as did patients with fibril type A, $p<0.03$. A statistical significance regarding Nfl levels could be detected between patients with fibril type B and gene carriers, $p=0.07$, while no statistical difference could be detected between patients with fibril type A compared to gene carriers. No difference could be detected in levels of Nfl between fibril type A compared to fibril type B.

Summary & Conclusion: ATTRv amyloidosis is characterized by axonal damage and Nfl is therefore a good candidate as a disease biomarker. Recently, studies have shown elevated plasma concentrations of Nfl. Our study confirms that Nfl seems to be a reliable biomarker for this disease.

Support & Funding: This work was supported by County of Västerbotten, patient organisation FAMY/AMYL and Wallenberg Centre for Moleculllar Medicine.

Neurofilament light chain measurement in hereditary transthyretin-related amyloidosis patients with myocardial sympathetic neuronal damage: substitution for ^{123}I-meta-iodobenzylguanidine scintigraphy?

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Background: The use of iodine-123 labelled metaiodobenzylguanidine (^{123}I-MIBG), a chemical modified analogue of norepinephrine, plays an important role in the evaluation of sympathetic innervation in cardiac amyloidosis. MIBG normally accumulates in vesicles in sympathetic nerve endings close to myocardial cells. Reduced uptake and increased loss of MIBG probably reflects damage of the cardiac sympathetic nerves. Serum neurofilament light chain (sNfL) is a biomarker for neuronal damage and levels of sNfL reflect polyneuropathy severity in hereditary transthyretin-related (ATTRv) amyloidosis. Unexpectedly, sNfL levels also correlated with troponin T, a biomarker for cardiac damage. This observation has not yet been explained. It can be hypothesized that cardiac neuronal damage explains the correlation between sNfL and troponin T.

Objective: To establish the possible relation between sNfL and myocardial sympathetic neuronal damage in patients with ATTRv amyloidosis.

Material & Methods: Levels of sNfL were measured in ATTRv patients who had undergone an ^{123}I-MIBG scintigraphy, autonomic function testing and nerve conduction studies (N=36). The single-molecule array (SIMOA) assay was used to assess sNfL levels. Myocardial sympathetic neuronal damage was detected using ^{123}I-MIBG scintigraphy: either late heart-to-mediastinum ratio (HMR) <2.0 or wash-out >20% was considered as abnormal ^{123}I-MIBG scintigraphy parameters.

Results & Discussion: sNfL levels were significantly increased in patients with an abnormal ^{123}I-MIBG scintigraphy compared to patients with a normal ^{123}I-MIBG scintigraphy ($p = 0.0006$). Late HMR negatively correlated with sNfL ($r= -0.60$ $p= <0.0001$) and wash-out rate correlated positively with sNfL ($r= 0.65$, $p= <0.0001$). Univariate analysis indicates a relation between sNfL and polyneuropathy, autonomic neuropathy, troponin T, late HMR, wash-out rate and abnormal ^{123}I-MIBG-scintigraphy. In multivariate regression analysis polyneuropathy and troponin T were independent predictors of sNfL levels ($F(4,29)= 21.180$, $p= <0.000$, $R^2= 0.745$).

Summary & Conclusion: Myocardial sympathetic neuronal damage based on ^{123}I-MIBG scintigraphy correlates with sNfL, but it is not an independent predictor of sNfL. Increased sNfL levels in ATTRv patients with an abnormal ^{123}I-MIBG scintigraphy are probably caused by the presence of polyneuropathy in these patients.

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Figure 1: sNfL in patients with a normal/negative ^{123}I-MIBG scintigraphy (MIBG -) and an abnormal/positive ^{123}I-MIBG scintigraphy (MIBG +).

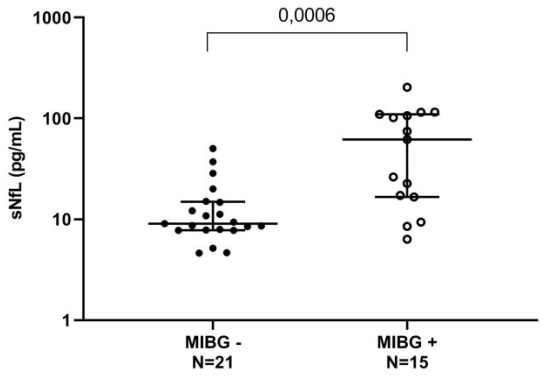

Table 1: Univariate and multivariate linear regression analysis for log10 sNfL in carriers of a TTR mutation and ATTRv patients.

	Univariate analysis		Multivariate analyses (R^2 =0,745)	
	B (% CI)	***p-value***	***B (% CI)***	***p-value***
Age (years)	0,007 (-0,005 - 0,019)	0,230		
Polyneuropathy (yes/no)	0,599 (0,346 - 0,851)	0,000	0,252 (0,026 - 0,478)	0,030
Autonomic neuropathy (yes/no)	0,529 (0,219 - 0,839)	0,001	0,207 (-0,004 - 0,417)	0,054
Small fiber neuropathy (yes/no)	0,425 (0,018 - 0,833)	0,041		
Troponin T	0,019 (0,014 - 0,025)	0,000	0,014 (0,008 - 0,020)	0,000
Late HMR	-0,370 (-0,530 - -0,210)	0,000		
Wash-out rate	0,021 (0,013 - 0,030)	0,000		
Abnormal baseline MIBG scintigraphy (yes/no)	0,565 (0,303 - 0,827)	0,000	0,031 (-0,218 - 0,280)	0,800

B refers to influence on log10 (sNfL).

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Prevalence of variant genotype in patients with suspected cardiac ATTR amyloidosis

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Background: Emerging therapeutic options and availability of non-invasive diagnostic tools are increasing disease awareness and recognition of cardiac ATTR amyloidosis worldwide. However, the relative prevalence of hereditary ATTR amyloidosis may be underestimated, particularly in aged males in whom the disease may be assumed to be wild-type and *TTR* genetic test not performed. *TTR* gene variants associated with a predominant cardiac phenotype are known to result in worse prognosis. Identification of a pathogenic variant may not only guide treatment strategy but also allow for genetic counselling and cascade testing in at risk relatives.

Objective: To identify the prevalence and type of genetic mutations in patients referred to our Centre for suspected cardiac ATTR amyloidosis.

Material & Methods: We evaluated consecutive patients referred to our Centre from January 2017 to December 2021 for suspected cardiac ATTR amyloidosis based on signs of heart involvement on echocardiogram or magnetic resonance, a Perugini score 2 or 3 bone scintigraphy or a tissue biopsy proving TTR amyloid deposition, no family history, no signs of neurological or other organ involvement. All patients underwent clinical evaluation and laboratory analysis including serum and urine immunofixation, serum free light chains, and *TTR* genetic testing. As AAPOA-I amyloidosis is reported in Northern Italy we also routinely perform *APOA1* genetic test in patients with suspected cardiac amyloidosis. Diagnosis was ultimately established on a tissue biopsy in patients in which a monoclonal component (CM) was identified.

Results: 481 patients were included, mean age at presentation was 76 years (min-max 50-93), 40 (8%) were women. In all patients with CM (29%), tissue biopsy confirmed TTR amyloid deposition by immunoelectron microscopy or proteomics. 439 patients (91%) did not carry *TTR* or *APOA1* variants, while 42 (9%) had a heterozygous pathogenic mutation. 41 patients have a pathogenic *TTR* mutation the most frequent being Ile68Leu (n=28; 67%), followed by Tyr78Phe, Val122Ile and Val94Leu (n=3/2/2 respectively). One novel mutation, namely Pro43Thr, was identified. In one patient *APOA1* Leu75Pro variant was identified and diagnosis of AApoA-I amyloidosis confirmed by typing on endomyocardial biopsy. Patients' characteristics at presentation are reported In Table 1. No significant differences were found between ATTRwt and ATTRv patients apart from gender distribution. Although age did not differ among the two groups, the prevalence of a variant genotype raises when considering only patients younger than 70 years (14%). 20% of women carry a pathogenic variant, irrespective of age. Interestingly, among women with ATTRwt (n=32), almost one third (31%) was diagnosed with hip dysplasia in childhood, requiring multiple orthopaedic surgeries and prothesis implantation.

Summary & Conclusion: In our cohort, one out of ten patients referred for suspected cardiac ATTR amyloidosis was ultimately diagnosed with ATTRv. Our results emphasize the importance of performing genetic testing irrespective of age at presentation. Diagnosis of ATTRv is relevant for monitoring disease progression, for defining treatment strategy and for offering genetic counselling and presymptomatic test in at risk relatives. Finally, the high prevalence of hip dysplasia among women with ATTRwt deserves further investigation to define whether it may represent a novel, gender-related red flag for this disease.

Patient characteristics	All cohort (n=481)	ATTRwt (n=439)	ATTRv (n=41)
Age at diagnosis, years	76 (±7,7)	76,4 (±7,5)	74,1 (±8)
Male, n	441 (92%)	407 (93%)	34 (83%)
NYHA class (I/II/III)	21%, 63%, 16%	20%, 63%, 17%	23%, 67%, 10%
Gillmore Stage (I/II/III)	40%, 46%, 14%	39%, 48%, 13%	52%, 32%, 16%
IVS (mm)	17,6 (±2,6)	17,6 (±2,7)	17,3 (±2,5)
EF (%)	50 (±10)	51 (±10)	52 (±8)

Table 1.: Demographic, clinical and laboratory characteristics for all cohort, ATTRwt (wild-type transthyretin amyloidosis) and ATTRv (hereditary transthyretin amyloidosis). All data are expressed as mean (± standard deviation) and prevalence as %. NYHA, New York Heart Association, classification of hearth failure; IVS, interventricular septum; EF, ejection fraction.
P-value is >0,05 for all parameters except “Male” for which p-value 0,023.

Engraftment Syndrome After ASCT is Associated elevated IL-10 and IP-10 (CXCL10) levels

Background

Autologous stem cell transplant (ASCT) is an effective therapy for systemic light chain amyloidosis (AL) and other plasma cell dyscrasias. AL patients undergoing ASCT have a higher risk of developing engraftment syndrome (ES) which is associated with increased peri-transplant morbidity. Acute kidney injury (AKI) is common in ES of AL patients and incidence of AKI has been reported to be up to 26%. ES is a poorly understood phenomenon; several cytokines such as IL-1beta, IL-1RA, IL-6, IL-12, IL-4, and IL-13 have been implicated in its pathogenesis.

Objective

We prospectively analyzed inflammatory marker levels in patients with AL and other plasma cell dyscrasia undergoing ASCT with or without ES

Material & Methods

Subjects with plasma cell dyscrasias undergoing ASCT were prospectively enrolled in the study. Serial blood samples were taken at baseline pre-ASCT, time of engraftment, and following discharge. ES was defined according to the criteria by Maiolino. Samples from the time of engraftment were obtained prior to initiation of corticosteroids if needed for ES. Plasma cytokine levels were measured using BioRad Bio-Plex Pro 48-Plex Human Cytokine Screening Luminex Kit. Cytokine levels were analyzed by the Mann-Whitney test adjusted for multiplicity using the Holm-Šídák method. This study was approved by the institutional review board at Columbia University Irving Medical Center.

Results & Discussion

25 subjects were enrolled. Baseline data and demographics are shown in table 1. Subjects included 9 (36%) with AL, 15 (60%) with multiple myeloma and 1 (4%) with POEMS. Nine (36%) subjects developed ES. ES developed in 4/9 (44.4%) amyloid and 4/15 (26.7%) MM patients. When analyzed by diagnosis, no significant difference was observed in cytokine levels. When analyzed by the incidence of ES, levels of IFN-a2, LIF, GM-CSF, IL-1b, M-CSF, and SCGF-b were significantly elevated in subjects with ES at baseline but this significance was lost when adjusted for multiplicity. Levels of MIG, IFNg, Eotaxin, IP-10, IL-10, and M-CSF were significantly elevated in subjects at time of engraftment but only IP-10 (Fig 1 a) and IL-10 (Fig 1-b) remained significant when adjusted for multiplicity. IP-10 and IL-10 may reflect increased macrophage activity in subjects with ES, with increased activity of M1 and M2 polarized macrophages. IP-10 and recruitment of CXCR3+ T cells has been shown to be involved in colitis and GVHD. Finally, IL-10 may have a paradoxical pro-inflammatory effect in scenarios of IFNa-dependent immune dysregulation e.g. SLE. IL-10 has been reported to increase expression of IFNg inducible genes such as IP-10 in IFNa primed macrophages.

Summary & Conclusions

The observed inflammatory changes are compatible with a type I/II interferon-signature and therefore suggest that blocking the JAK/STAT pathway may be a promising strategy to prevent development of ES in high risk patients.

Support and Funding:

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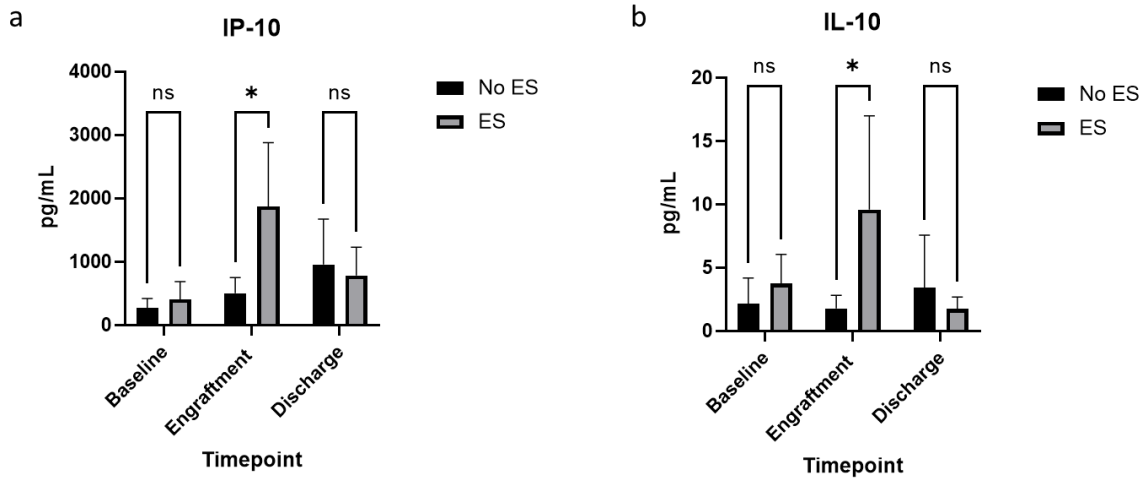
Figures

Figure 1. Levels of IP-10 (1a) and IL-10 (1b) in pg/mL by timepoint.



Tables:

Table 1. Baseline demographics and characteristics of the study population.

Characteristic	ES N=9	No ES N=16
Age, median (range), yr	62 (30-75)	62 (42-72)
Gender, male %	6 (66.6%)	10 (62.5%)
Diagnosis n(%)		
AL	4 (44.4%)	5 (32.3%)
MM	4 (44.4%)	11 (68.8%)
POEMS	1 (11.1%)	--

Genetic modifiers in hereditary and acquired TTR amyloidosis: a genome-wide association study

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Background

Hereditary transthyretin (ATTRv) amyloidosis is a rare systemic and life-threatening condition caused by mutations in the transthyretin (TTR) gene. To date, considerable variability has been observed in age of onset (AOO), penetrance, progression rate and response to treatment, both across families and countries. This variability in ATTRv amyloidosis cannot be entirely explained by the specific mutation in TTR gene, as well as factors favoring wild-type TTR deposition in acquired age-related amyloidosis (wtATTR), are still unknown. Therefore, a role for genetic modifiers has been hypothesized for these two conditions

Objective

The aim of this multicentre study is to identify genetic modifiers in ATTRv and wtATTR amyloidosis, employing an unbiased genome-wide approach

Material & Methods and Results

In patients with ATTRv amyloidosis we will perform a genome-wide association study (GWAS) to identify loci harboring genetic variations that alter 1) age of neurological onset; 2) clinical phenotype and progression; 3) response to anti-amyloidogenic treatments. Patients affected by wtATTR amyloidosis will also be tested in a complementary case-control GWAS. The TTR locus as well as significant loci from the GWAS will be further explored by long-read sequencing

Summary & Conclusions

The proposed research will identify novel genetic risk factors, informing disease prognosis and guiding monitoring and treatment. Also, it will provide seminal information about the mechanisms involved in amyloid deposition, potentially leading to the identification of novel drug targets. The study is actively enrolling, and we would be pleased for additional Centres to join (if interested: andrea.cortese@unipv.it; l.obici@smatteo.pv.it)

Skin Biopsy Has A High Diagnostic Yield In Patients With Systemic Amyloidosis And Neuropathy

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Introduction. Since small fiber neuropathy is an early and common feature of several amyloidosis subtypes, we aimed to assess the detection yield of skin biopsy for amyloid in patients with confirmed amyloidosis and symptoms of peripheral neuropathy.

Methods. The study investigated three groups of patients: 1) transthyretin and light chain amyloidosis (ATTRv, ATTRwt and AL), 2) non neuropathic controls (NNC) and 3) other neuropathic disease controls (ONC). All subjects underwent neurological examination, electroneuromyography and 3 mm skin biopsy at the ankle and thigh. Sections were immuno-stained with anti-PGP9.5 and Congo red. Intra-epidermal nerve fiber density (IENFD), amyloid index and burden and large fiber involvement were analyzed.

Results. Amyloid deposits were detected in 100% of ankle skin biopsies and in 42% of thigh specimens in patients with amyloidosis, while it was not detected either in ONC or in NNC groups at any of the two biopsy sites. A small fiber neuropathy with reduced IENFD was encountered in 100% of amyloidosis patients and in 69% of ONC, while a large fiber neuropathy was respectively found in 57% of amyloidosis group and 92% of ONC.

Conclusions. Ankle skin biopsy can detect amyloid in polyneuropathy-associated AL and ATTR with high sensitivity and specificity. IENFD and amyloid detection in skin are reproducible, useful tools to reach a tissue diagnosis of amyloidosis

Assessing amyloid prevalence, type, and extent of burden in the ligamentum flavum of patients with spinal stenosis undergoing routine laminectomy

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Background: Identification of populations at risk of systemic amyloidosis may improve early and accurate diagnosis and lead to better patient outcomes. To this end, we previously developed an amyloidogenic light chain identification assay for patients at risk of AL amyloidosis (1). Herein, we focus on ATTR amyloidosis. Several medical conditions have been identified as early indicators of an increased risk of ATTR amyloidosis. One such indicator is lumbar spinal stenosis (LSS), which often portends the development of systemic amyloidosis, notably ATTR amyloidosis. Studies that identify amyloid in the ligamentum flavum (LF) extracted during routine lumbar laminectomy procedures have been conducted to further understand the connection between LSS and amyloidosis and to evaluate the predictive value of the analysis. Depending on the technique used for amyloid detection, reports of amyloid prevalence in the LF have varied greatly across institutions (2-4). We now report a screening assessment for LF-associated amyloid in samples obtained during routine laminectomy surgery at the University of Tennessee Medical Center using manual, alkaline Congo red (CR) staining and immunohistochemical typing.

Objective: This single-site study was designed to assess the prevalence of amyloid, using a manual Congo red staining procedure, in the LF of patients undergoing routine laminectomy surgery for spinal stenosis. Additionally, the amyloid load was assessed semi-quantitatively and amyloid type determined immunohistochemically when feasible.

Material & Methods: Males and females ≥ 35 years of age with no medical history of amyloidosis were enrolled and agreed to the use of LF tissue for this exploratory study. Tissues were fixed in formalin for 24 h, embedded in paraffin and 6-μm thick sections prepared and stained with alkaline CR solution. The tissues were examined microscopically using cross-polarized illumination for the presence of birefringent amyloid. Visual evaluation of amyloid load in the tissues was performed by an experienced reviewer and documented on a scale of 0 – 4, with 4 representing extensive amyloid infiltration. Serial tissue sections with sufficient material for immunohistochemical (IHC) evaluation (predominantly tissues with a score >2) were stained with a panel of antibodies for: transthyretin (TTR), apolipoprotein-a1 (APOA1), serum amyloid a (AA), and both free kappa and free lambda light chains (AL).

Results: Forty-two males and females (50/50; mean age 63 y) consented to participate in the study. Amyloid was detected in 86% of the tissue specimens (36/42). There was extensive amyloid (CR score = 4) present in 19% (8/42) of the samples, and ~40% (16/42) of the samples had a CR score of 1. Of the evaluable specimens (23/36), IHC typing of the amyloid revealed positivity for TTR (1/23), APOA1 (4/23), or both TTR and APOA1 deposits(11/23) often showing discrete regions of the two types throughout the tissue (Fig.1). Seven samples stained equivocally and were regarded as untypeable. No samples stained positively with either AA- or AL-specific antibodies. A significant correlation between histological amyloid load and patient age was observed (r_s = 0.57; approximate p = 0.0001, α = 0.05) (Fig 2).

Summary & Conclusion: It is not uncommon to find amyloid in the LF of patients with LSS (2,3). Our data are in concordance with those published from Europe and Asia, where >85% of cases were found to be positive; however, our population had a greater incidence of dual-amyloid, TTR and APOA1, deposition. Our data further corroborate that the amount of amyloid in the LF, based on CR visual scoring, correlated positively and significantly with age. It is unlikely that all patients with amyloid-positive LSS will develop systemic amyloidosis; however, routine screening of LF samples for amyloid, coupled with a robust scoring system, may identify patients for amyloid-targeted imaging studies (e.g. ^{124}I-AT01), leading to earlier identification of patients with presymptomatic systemic amyloidosis. Studies combining LF amyloid assessment and non-invasive amyloid imaging could be of clinical value to identify patients with a high risk of developing amyloidosis.

Figure 1

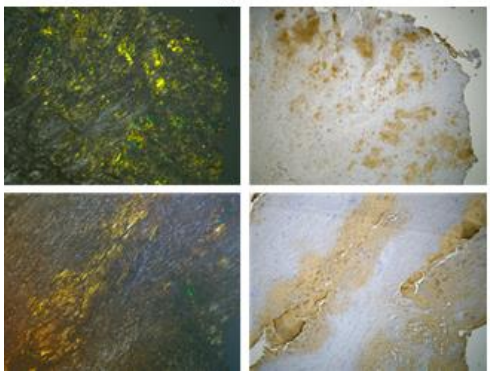

Figure 2

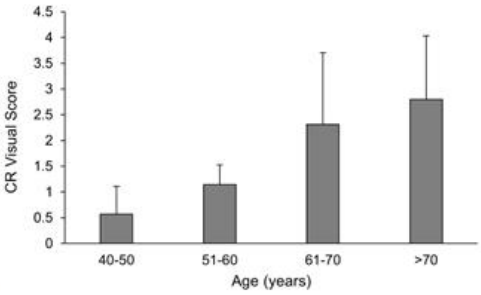

Figure 1.: Congo red staining (left panel) and immunohistochemistry (right panel) of a single representative 4+ ligamentum flavum tissue sample. The green-gold congophilic regions coincided in discrete regions immunostained with with TTR (upper)- or APOA1 (lower)-reactive antibodies, indicating the presence of both amyloid types in the tissue. Mag. 10x.

Figure 2.: Amyloid accumulation in the ligamentum flavum is greater in older subjects. Congo red positive scores in LF tissue correlate positively with subject age -there was an increase in amyloid load (higher CR visual score) in the older subjects.

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Prevalence of Localized Amyloid in Ligamentum Flavum of Patients with Lumbar Spinal Stenosis

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Background: Systemic transthyretin (ATTR) amyloidosis is characterized by progressive cardiac damage. Lumbar spinal stenosis (LSS) may precede ATTR amyloidosis by many years[1], making identification of ATTR at the time of LSS surgery a potential tool for early diagnosis.

Objective: We prospectively assessed by tissue biopsy the prevalence of ATTRwt in patients aged >50 years with LSS.

Material & Methods: Imaging assessment by MRI was performed before surgery to confirm LSS. Tissue samples from ligamentum flavum were analyzed centrally for the presence of amyloid.

Results: On a total, of 253 patients undergoing LLS decompression surgery between February 2020 and October 2021, 94 (37.1%) patients were enrolled in the study. Amyloid was found histologically in 74/94 patients (76.2%, Figure 1). Immunohistochemistry identified ATTR in 61 (64.8%) patients, whereas amyloid subtyping was inconclusive in 13 (13.8%) patients. Mean thickness of ligamentum flavum at L2-L3 reported on MRI was significantly higher in patients with amyloid deposits (4.32 ± 1.11 vs 3.00 ± 0.97 mm, p=0.009; Figure 1). Patients with amyloid deposits were older when compared to patients without (73.0 ± 9.21 vs 64.6 ± 10.1 years, p=0.019). No difference in sex, comorbidities, previous surgery for carpal tunnel syndrome or LSS was observed between patients with and without amyloid deposits.

Summary & Conclusions: Localized amyloid is present in three out of four patients undergoing interventional LSS decompression. Older age and high thickness of ligamentum flavum correlated with the presence of amyloid. Routine histological work-up of ligamentum flavum biopsies should become a new standard of care, as the detection of localized ATTR amyloid could inform clinical decision-making.

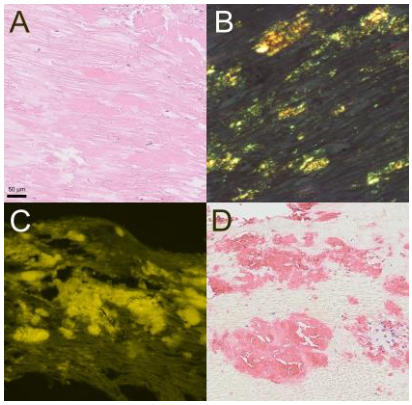

Figure 1

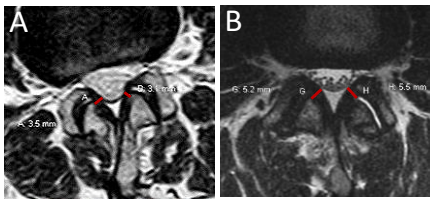

Figure 2

Figure 1: Histology of resection specimens showing homogeneous eosinophilic deposits in the ligament flavum (A). Following Congo red staining, variable amounts of amyloid were identified with a characteristic green-yellow birefringence in polarized light (B) and a typical signal in fluorescence microscopy (C). These deposits strongly and homogeneously immunoreacted with an antibody directed against transthyretin (D). Original magnifications 200-fold**.**

Figure 2: Axial T2-weighted MR images at the level L2-L3 showing measurements of ligamentum flavum thickness in a patient without amyloid deposits (A) and in another patient (B) with amyloid deposits in the tissue biopsy.

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Definition of Bone marrow biopsy involvement in amyloidosis – proposal for reporting

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Background: Amyloidoses are rare but heterogeneous disorders for which diagnosis is contingent upon the detection of deposits by Congo red stain and amyloid protein typing determines the treatment options. AL amyloidosis is associated with clonal expansion of plasma cells which reside in the bone marrow and, hence, bone marrow biopsy has been traditionally included in the diagnostic workup of these patients (1-3).

Objective: To address the reporting of bone marrow involvement by amyloid in relation to the spatial distribution of deposits and to explore whether the location of deposits may have clinical relevance. Also the objective was to evaluate the utility of bone marrow biopsy in the workup of amyloidoses beyond AL.

Material & Methods: This study is a continuation of our previously reported results on examination of **85** bone marrow biopsies positive for amyloid (1). The current study includes the period Jan 2018-March 2022 and includes 67 additional bone marrow specimens positive for amyloid. These 2 cohorts were compared. The decision to perform Congo red stain was made by the original hematopathologist and was based on the following criteria: (1) history of monoclonal gammopathy of undetermined significance or amyloidosis, (2) light-chain restriction or increased plasma cells in bone marrow biopsy, or (3) clinical suspicion of amyloidosis.

Results: During the new study period 3,857 bone marrow biopsies were evaluated of which 412 were examined by Congo red stain (9.36%). Amyloid deposits were detected in 67 specimens (16.2%). In the earlier study, among 809 cases of bone marrow biopsies that were analyzed by Congo red stain, 85 cases (10.5%) were positive. While the patients' demographics were similar (M:F ratio and age range) the mean age was currently lower with 63.5years versus 65 years in prior group.

The spatial distribution of deposits was classified as before: bone marrow stroma, vessel wall, periosteal soft tissue, and combination of the above locations, as shown in Figure 1.

The spatial distribution of deposits was similar, with amyloid deposits limited to stroma (medullary deposits) in only 8 cases, to vessel wall in 13 cases, and to periosteal soft tissue in 19 cases; 27 cases had combined deposits (vessel wall + stroma, vessel wall + periosteal soft tissue, and vessel wall + periosteal soft tissue + stroma in 8, 12, and 7 cases, respectively). Thus, there were 2 major groups: (1) 22 of 67 cases (32.8%) of amyloid with bone marrow stromal deposits (alone or in combination with other sites) and (2) 45 of 67 cases (67.2%) of amyloid deposits without a stromal component.

All cases of stromal involvement were typed as AL amyloidosis, whereas nonstromal involvement was associated with at least 3 types of amyloidosis: AL, ATTR (5 cases), and AA (2 cases).

Summary & Conclusion: bone marrow biopsy specimens continue to yield high detection rate of amyloid with an increased rate of detection in the recent cohort (from 10.5 to 16.3%) in selected cases. While there is significant heterogeneity in the spatial distribution of amyloid in BM biopsy specimens, stromal deposits appear to be associated exclusively with AL, while all other sites were involved in at least 3 types of systemic amyloidosis (AL, AA, and ATTR). Reporting of bone marrow biopsy specimen positivity for amyloid should specify structures involved and the true bone marrow positivity should be limited to cases with stromal involvement while other locations should be considered as soft tissue and/or vascular involvement (fig. 2).

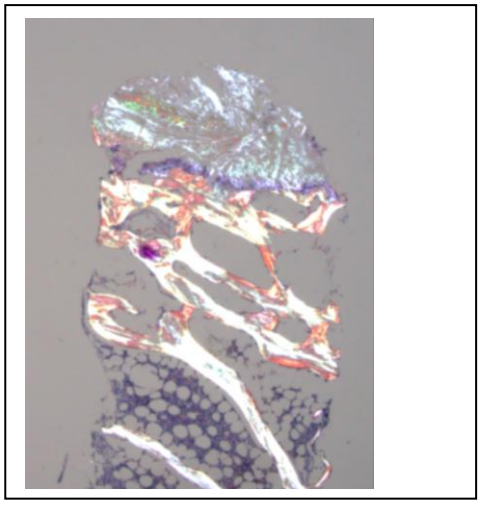

Figure 1.: Congo red stain highlighting amyloid deposits in the bone marrow stroma (top), blood vessels (middle) and in periosteal soft tissue (bottom). Congo red stained slides examined under polarized light, original magnifications ×200 (top & bottom) and 400x [middle].

Figure 2.: Focal deposits of amyloid are seen in the periosteal soft tissue while no amyloid was detected in the marrow stroma or vascular wall. Congo red stained slides examined under polarized light, original magnification x100. This example represents soft tissue amyloid and should not be considered as bone marrow amyloid.

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Detection of amyloid deposits in skin biopsies of patients with clinically suspected variant transthyretin amyloidosis

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Background: Early diagnosis of transthyretin variant (TTRv) amyloidosis (ATTRv) is critical for timely and effective initiation of disease modifying therapy. Skin biopsy is a simple, safe and minimally invasive procedure for detection of amyloid deposits by Congo-red (CR) histochemical staining in patients with clinically suspected ATTRv. However, in early disease stage amyloid deposits may be small and barely detectable by this staining. Furtheremore, amyloid typing is often required to differentiate ATTR from immunoglobulin light chain amyloidosis (AL**)** but immunohistochemical techniques empolyed are frequently equivocal due to tissue contamination by serum proteins, including immunoglobulins [1,2].

Objective: We aimed to develop a sensitive method for (1) detection and (2) characterization of amyloid deposits in skin biopsies.

Material & Methods: Skin biopsies (3.0-mm punch) were performed at the distal leg and fixed in Zamboni solution, cryoprotected, frozen and sectioned for immune-fluorescent quantification of the intraepidermal nerve fiber density. Additional 16 um thick glass mounted sections were CR stained to detect amyloid by fluorescence microscopy verified by polarizer light. Remaining tissue was subjected to protein extraction [3]. Tissue specimens were homogenized, washed, extracted using formic acid and lyophilized. The extract was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis based Western blotting. Amyloid protein type was determined by immunoreactivity of low molecular weight (MW) bands (< 25 kDa) [3-6] to one of 3 test antibodies: anti-TTR, anti-κ or anti–λ immunoglobulin light chains.

Results: Skin biopsies from 19 patients with suspected neuropathy were tested. Microscopic analysis revealed CR-positive (**CR+**) amyloid deposits in 10 cases which were all TTRv mutation carriers with clinically suspected amyloidosis. The remaining 9 cases were CR-negative (**CR-**) and had no suspected amyloidosis. In 7 of 10 CR-positive cases, 15 kDa bands showed immunoreactivity to anti-TTR (Fig.1 and 2) but not to anti-κ or anti-λ. In all Congo-red negative cases, no immunoreactivity of low MW proteins (< 25 kDa) was observed.

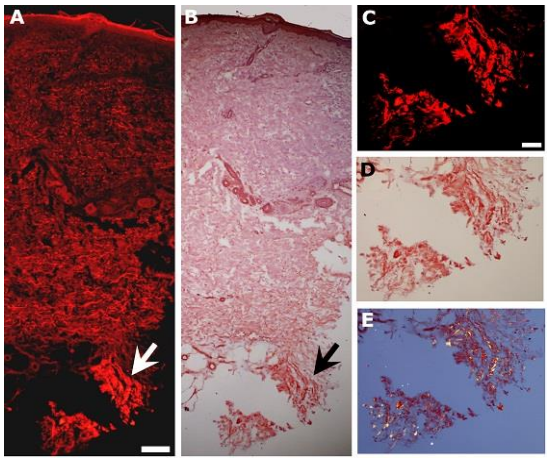

Figure 1

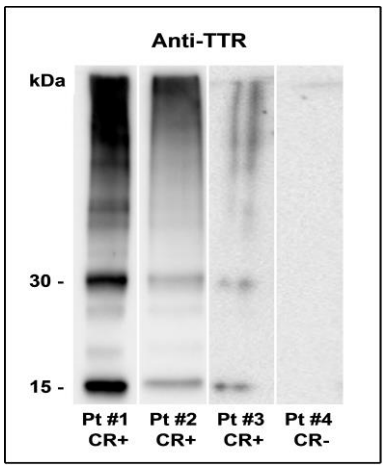

Figure 2

Figure 1: CR stained skin section of pt2. Immunofluorescence (A) and white light (B) microscopy shows congophilic deposits in the deep dermis (arrows). These deposits, enlarged in C and D show apple-green and yellow birefringence under polarized light (E), thus consistent with amyloid. Bars are 200 and 100 um in A and C, respectively.

Figure 2: Western blots of skin biopsy tissue extracts. In the **CR+** skin biopsies (patients #1-3 with ATTRv), immunoreactivity with anti-TTR antibodies is demonstrated by highlighting the 15 and 30 kDa bands which correspond to monomeric and dimeric TTR forms, respectively. No TTR immunoreactivity was observed in the **CR-** skin biopsy (pt #4, TTR carrier, unaffected).

Discussion: The major amyloid fibril subunits characterizing amyloid types are relatively small proteins [6]. In ATTRv the main fibrillar protein is the TTR monomer (about 15 kDa). In most cases of AL amyloidosis, light chain deposits contain fragments with a molecular mass varying from 12 to <25 kDa. Application of Western blotting allows (1) separation of amyloid proteins from contaminating serum proteins and immunoglobulins, and (2) identification of amyloid bands.
In our study, 7 of 10 Congo-red positive cases demonstrated TTR-immunoreactive 15 and 30 kDa protein bands which are characteristic to the monomeric and dimeric forms of this protein. No cross reaction with anti-κ and anti-λ antibodies was observed, indicating the specificity of this technique.

Summary & Conclusion: Protein extraction from fixed skin biopsies followed by Western blotting may be used for detection of TTR deposits in tissue. This allows amyloid typing, which is essential for establishing the correct diagnosis.

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Amyloid deposits are common in different spinal structures among patients with spinal stenosis referred for decompression surgery

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Background: Spinal stenosis is a commonly encountered orthopedic condition that is treatable by surgical decompression. It is now recognized that spinal stenosis can result from ligamentum flavum thickening by deposition of transthyretin amyloid fibrils (ATTR). Whether ATTR deposits are detectable in other aspects of the vertebral structure or contribute to the stenosis pathology is unknown. We sought to determine the proportion of patients with demonstrable amyloid deposits in various spinal structures.

Objective: The purpose of the study was to determine the presence of amyloid deposits in samples obtained from individuals over the age of 60 years undergoing elective lumbar or cervical spinal surgery.

Material & Methods: This is a single-center, cohort observational study. Patients over the age of 60 years without a prior history of amyloidosis were prospectively recruited prior to elective cervical (posterior cervical fusion or anterior cervical discectomy and fusion) or lumbar surgery (laminectomy or minimally invasive antepsoas surgery; MIS-ATP). Enrolled patients underwent surgery following which the resected ligamentum flavum/ and or disc tissue (nucleus and/ or annulus) was extracted and analyzed by a reviewing pathologist for the presence of amyloid deposits by Congo red staining. Subjects with positive Congo red staining for amyloid deposits were followed-up by clinical evaluation and as-needed testing, care, and treatment.

Results: A total of 54 patients (average age 67.0 +/- 5.82 years) underwent surgery yielding 94 specimens that were tested for amyloid deposits. The overall prevalence of patients with amyloid deposits was 44.4% (n=24). Table 1 illustrates the demographics of the cohort. The majority of deposits were graded 1+ (87.8%). Of 24 males, 54.2% had amyloid deposits compared to 36.7% in 30 females. In respect to self-identified race, 39.4% of White patients (n=33) tested positive, 50% Black patients (n=12) tested positive, and 50% Hispanic patients (n=4) tested positive. Patients undergoing cervical surgeries (n=32) were more likely to have amyloid deposits (46.9%) than those undergoing lumbar surgeries (n=62) (29.0%). We found 37.3% (n=83) of disc specimens tested positive while only 18.2% (n=11) of ligamentum flavum specimens were positive. Looking at 26 MIS-ATP lumbar cases and 50 samples, 48.0% of those individuals with spine instability (degenerative scoliosis or degenerative spondylolisthesis) tested positive, and 28.0% of the annulus specimens and 38.5% of the nucleus specimens were positive. Among patients with multiple specimens, 9 patients had 2 specimens with discordant results all from lumbar surgeries. Among 7 patients with a prior diagnosis of carpal tunnel syndrome or trigger finger release surgery, 5 had amyloid deposits in a spinal structure.

	Number of Samples (n = 54)	% Positivity (n = 24)	p-value
Sex			
Male	24	54.2	0.20
Female	30	36.7	
Race/ Ethnicity			
White	33	39.4	
Black	12	50.0	
Hispanic	4	50.0	0.58
NA	4	75.0	
Asian	1	0.0	
Carpal/Cubital Tunnel Syndrome and/ or Trigger Finger			
Yes	7	71.4	0.12
No	47	40.4	

Table 1 – Amyloid deposit positivity as a proportion of patients

	Number of Samples (n = 94)	% Positivity (n = 32)	p-value
Congo red Staining intensity			
1+	29	87.8	N/A
2+	2	6.25	
Both	2	6.25	
Spine Surgery Location			
Cervical	32	46.9	0.09
Lumbar	62	29.0	
Location of Specimen			
Disc	83	37.3	
Ligamentum Flavum	11	18.2	0.21
Type of Specimen from Lumbar ATP Case Only			
Annulus	24	28.0	0.49
Nucleus	26	38.5	

Table 2 – Amyloid deposit positivity as a proportion of samples

Summary & Conclusion: Amyloid deposits in the spine are common among patients over the age of 60 years of age. Amyloid was found not only in the ligamentum flavum, but also other spinal structures including the disc components, as well as in the cervical and lumbar spine. Amyloid deposits may contribute to the pathology of spinal stenosis through different locations of deposition.

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Diagnosis and typing of pulmonary amyloidosis: A series of cases

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Background: Pulmonary amyloidosis is a rare condition that may have systemic or localized presentations. The correct characterization of amyloid deposits is needed to start appropriate treatments and to establish accurate patient's prognosis. Although distinctive histopathological patterns of lung involvement have been described, a few series of cases have been reported.

Objective: This study aimed to review the histopathological patterns and amyloid typing of a series of patients with pulmonary amyloidosis.

Material & Methods: We retrospectively reviewed records of patients with pulmonary amyloidosis diagnosed at a single institution between January 2000 and March 2022. Collected data included demographics, clinical presentation, and results of pathology studies including confirmation of amyloid deposition by Congo red stain and amyloid typing by immunohistochemistry.

Results: Seventeen patients were enrolled in this study. Ten patients (59%) were women and the median age of the series was 61 years (range 31-83). For most of the patients (59%) the initial clinical suspicion was of either a primary or a metastatic pulmonary neoplasm. Diagnosis of amyloidosis was established after lung resection in 8 cases, lung biopsy in 5, and transbronchial biopsy in 4. In biopsy samples (transbronchial and lung), the diagnosis and amyloid typing were feasible in almost all cases (8 out of 9 cases (88.9%). After disease extent work-up, localized amyloidosis was diagnosed in 14 cases (82%) and systemic in 3 cases (18%). Type of amyloid was ascertained by immunohistochemistry in all systemic cases (100%) leading to the diagnosis of immunoglobulin light-chain (AL) amyloidosis of lambda type in two and amyloid A (AA) amyloidosis in one. Within the localized cases, the typification was obtained in 12 patients (86%): 8 lambda, one kappa and 3 mixed lambda and kappa AL amyloidosis. In the remaining 2 localized cases (14%), the precursor protein was not identified due to mild or unspecific positivity in more than one precursor protein or scarce deposit insufficient for the immunohistochemistry study (on case each), being the amyloid deposit categorized as not otherwise specified (NOS). Overall, three main histological patterns were observed: nodular (8 cases; 47%), diffuse (6 cases; 35%), and tracheobronchial (3 cases; 18%). All nodular and tracheobronchial patterns were observed in localized amyloidosis while, as expected, the 3 systemic amyloidosis

presented histological diffuse patterns. Interestingly, the remaining 3 cases with diffuse pattern corresponded to localized amyloid deposition in the context of a neoplasm: two metastatic colonic adenocarcinomas with NOS amyloid deposit, and one pulmonary extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) with lambda AL amyloidosis.

Summary & Conclusions: Clinical presentation of pulmonary amyloidosis can be misleading. AL amyloidosis of lambda type was the most frequent type of pulmonary amyloidosis diagnosed in both localized and systemic diseases. In our experience, correct diagnosis and amyloid typing by immunohistochemistry can also be made through transbronchial and lung biopsy. We also describe in two patients the coexistence of localized pulmonary amyloidosis with diffuse pattern and metastatic colonic adenocarcinoma, suggesting a potential role of the neoplasm in the amyloidogenesis. Although histological patterns correlate with either systemic or localized pulmonary involvement, exceptions do occur and highlight the need for an accurate histologic diagnosis.

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Concurrent light chain amyloidosis and proximal tubulopathy: insights into different aggregation behavior.

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Background: AL amyloidosis, caused by misfolded antibody fragments, may damage multiple organs and organ systems with the heart, kidneys, soft tissue, liver, peripheral and autonomic nervous system being the most commonly affected.[1] Additionally, the monoclonal light or heavy chains or the underlying B cell clone can lead to a group of rare diseases of the kidneys, known as 'monoclonal gammopathy of renal significance', MGRS.[3] MGRS is categorized into several different subclasses based on the type of deposit and whether the deposits are organized (*e.g.* light-chain proximal tubulopathy (LCPT) or light chain cast nephropathy (LCCN)) or non-organized (*e.g.* monoclonal immunoglobulin deposition disease (MIDD)).[2–4]

Objective: Due to differences in the protein folding mechanisms, it is exceedingly rare for AL amyloidosis and MIDD or LCCN to coexist. We herein report the first case of concurrent AL amyloidosis and LCPT.

Material & Methods: We have extensively characterized the patient by analyzing various different tissues such as fat, bone marrow, kidney, and gastrointestinal mucosa, and have used histochemical stains and immunohisto-chemistry, electron microscopy, and sequencing of light chains and chromosomal aberrations to further specify the plasma cell clone and the tissue deposits.

Results: We report a 53-year old female who was diagnosed with smoldering myeloma IgG *kappa* and mild proteinuria and subsequently developed a significant weight loss, new onset of neuropathic pain and bilateral periorbital ecchymoses. Fat aspiration showed substantial amyloid deposition on Congo red staining with positive green birefringence under polarized light (Figure 1). The kidney biopsy did not show amyloid deposits, but immunofluorescence demonstrated a stronger cytoplasmic signal for *kappa* within tubular epithelial cells. Electron microscopy revealed the presence of LCPT with an increased number of lysosomes as well as rhomboid and needle-shaped crystals in proximal tubular epithelial cells (Figure 2A). A repeat bone marrow aspirate and biopsy revealed smoldering myeloma and the presence of numerous Auer-rod like LC crystals in the plasma cells was noted (Figure 2B). Congo red staining of the bone marrow did not show any amyloid deposits. A neurological exam revealed a demyelinating sensory-motor polyneuropathy also affecting the autonomous nervous system in the context of suspected gastroparesis. Whole body magnetic resonance imaging showed no focal lesions. A repeat upper and lower endoscopy revealed the presence of amyloid deposits and immunohistochemical staining confirmed the presence of AL *kappa* amyloid. Sequence analysis showed that the patient-derived light chain belongs to the IGKV1/D-33 and the IGKJ3*01 family.

Summary & Conclusions: Taken together, the patient was diagnosed with systemic AL amyloidosis affecting the soft tissue as well as the peripheral and autonomic nervous and gastrointestinal system, while her proteinuria and mildly elevated creatinine were attributed to the LCPT. Hypotheses on the coexistence of AL amyloidosis and MIDD include different conformations of the same pathogenic light chain, subclonal somatic variants of the original light chain gene or the presence of biclonal gammopathy. This case of concurrent AL amyloidosis and LCPT illustrates the complex pathophysiology of protein deposition in monoclonal gammopathies and emphasizes that further studies examining the molecular determinants of light chain aggregation propensity and proteotoxicity are eagerly needed.

Figure 1

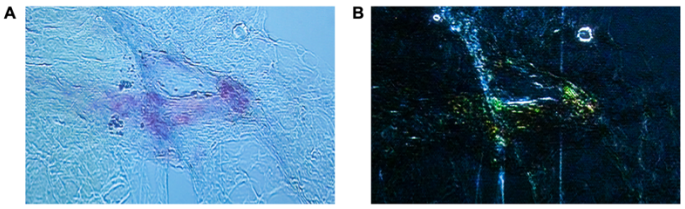

Figure 2

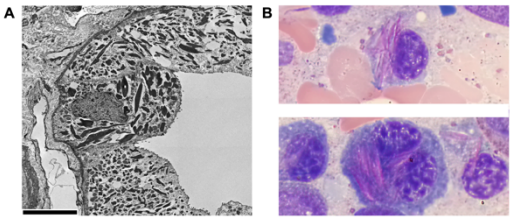

Figure 1. Amyloid deposits in fat tissue. Light microscopy. **A** Positive Congo red staining (red) and **B** positive green birefringence under polarized light (green).

Figure 2. Crystalline inclusions of monoclonal *kappa* light chains in proximal tubular epithelial cells and bone marrow plasma cells. A Electron microscopy (bar ≙ 6 µm). Granular electron-dense rhomboid and needle-shaped crystals in proximal tubular epithelial cells. **B** Light microscopy (Giemsa, oil immersion, magnification 60X). Bone marrow smear showing intracytoplasmic Auer-rod like inclusions in plasma cells.

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Clinicopathological analyses of an autopsied case with hereditary ATTR amyloidosis 22 years after liver transplantation

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Background: Long term outcome of patients with hereditary ATTR (ATTRv) amyloidosis after liver transplantation (LT) remains to be fully understood.

Objective: To analyze clinical and pathological findings of an autopsied ATTRv amyloidosis case surviving long-term after LT.

Material & Methods: We analyzed clinical course and autopsy findings of a case with ATTRv V30M amyloidosis, who survived 22 years after LT. We evaluated histopathological changes by Congo red staning and immunohistochemical stainig.

Results: A 27 years old male developed sensory disturbance in the lower limbs and autonomic disturbance and was diagnosed with hereditary ATTR Val30Met amyloidosis. He underwent cadaveric LT at 29 years old. After LT, peripheral and autonomic neuropathy did not progress significantly. However, his visual acuity declined remarkably and his eyesight was only enough to count his fingers at the time of death. Cardiac function gradually worsened, and he had been hospitalized and discharged for heart failure and infection from 1 year before his death. At 51 years old (22 years after LT), he developed cholangitis and sepsis, leading to death. He had no symptoms of central nervous system (CNS) throughout the course.
Histopathlogical analyses revealed that he had severe amyloid deposits in the tongue, heart, gastrointestinal tract, kidney, bladder, submandibular gland, and part of the aortic wall. Amyloid was mainly deposited on the walls of blood vessels, but it was deposited on salivary gland in the tongue, and on muscle fibers in the tongue and heart. In the nervous system, amyloid deposits were found in the sympathetic ganglia, spinal cord, dural and pial blood vessels, and lateral ventricular wall.

Summary & Conclusion: Ocuar and cardiac dysfunction gradually progressed even after LT. Severe leptomeningeal amyloid deposits were found, while he had no CNS symptoms. Amyloid deposits in the tongue and heart were more severe than in other tissues. The findings were similar to those in the past literature.

Clinical ApoA-IV amyloid is associated with fibrillogenic signal sequence

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Background: Apolipoprotein A-IV amyloidosis (AApoA-IV) is a rare form of the disease, mainly characterised by renal and cardiac dysfunction [1,2]. In addition to its intrinsic amyloidogenicity, ApoA-IV is also deposited along with amyloid of all protein types and is considered one of the amyloid signature proteins [3]. This can cause difficulties in distinguishing between ApoA-IV as the culprit amyloid protein and an amyloid-associated signature protein.

Objective: To identify additional proteomics criteria to type ApoA-IV amyloid. This led to a further study on the possible role of ApoA-IV signal sequence in AApoA-IV amyloidosis.

Material & Methods: 24 AApoA4 cases, among over 2000 clinical cases in the NAC proteomics database, were identified by clinical presentation, morphological, immunohistochemical and proteomics analyses. Clinical samples were analysed by proteomics [4] on a Thermo Velos Orbitrap, and on a Thermo Q-Exactive Plus. Proteomics data were processed by Mascot software using the Swiss-Prot human database with trypsin and semi-trypsin as the in silico proteolytic enzyme. ApoA-IV protein was immunoprecipitated from serum collected from 3 ApoA-IV patients and 2 healthy controls as previously described [4]. Authentic standard ApoA-IV peptides p.18-43, p.19-43, p.20-43, and p.21-43 were examined for the capacity to form amyloid fibrils in vitro at physiological pH by ThT fluorescence emission, Congo Red (CR) staining and Transmission Electron Microscopy (TEM) [5].

Results: ApoA4 signal-containing peptides (p.18-43, p.19-43 and p.20-43) were surprisingly identified in 17/24 clinical biopsies from ApoA-IV amyloidosis patients either attending the NAC clinics or sent for histology review. The normal N-terminal peptide, p.21-43, was present in all cases. ApoA-IV signal was also identified in the original cardiac biopsy from a Swedish patient in which ApoA-IV amyloid was first described, and in serum from 1/3 cardiac AApoA-IV patients by targeted mass spectrometric analysis. It was not detected in controls suggesting the circulating level of signal-ApoA-IV was low. The identity of these signal-containing N-terminal peptides was confirmed by comparison with authentic standards. ApoA-IV signal was present in only 1/266 clinical biopsies where other amyloidogenic proteins were identified as the amyloid type: signature ApoA-IV did not appear to be associated with the presence of signal.
The 3 signal-containing peptides together with the normal N-terminal peptide (p.21-43) were tested for the capacity to form amyloid fibrils in vitro. The p.20-43 peptide and the normal N-terminal peptide were fibrillogenic at physiological pH generating amyloid-like CR positive fibrils, visualised also by TEM (Figure 1). Since our original report [5] we have identified a further 7 AApoA-IV cases (5 cardiac, 1 renal and 1 bladder), with 5/7 showing signal peptides. Of the remaining 2 cases, both had Mascot scores at the lower end of the AApoA-IV range: one was positive for ApoA-IV by IHC, and the other was inconclusive.

Summary & Conclusion: ApoA-IV amyloid is associated with signal-containing protein, and this is now used as an additional diagnostic criterion for discriminating ApoA-IV amyloid protein from ApoA-IV amyloid signature protein. Signal was not present in some of the ApoA-IV amyloid samples, normally those with low Mascot scores. The enhanced fibrillogenesis associated with the p.20-43 peptide suggests that the presence of signal sequence could lead to enhanced amyloid deposition in vivo and potentially influence other ApoA-IV pathologies.

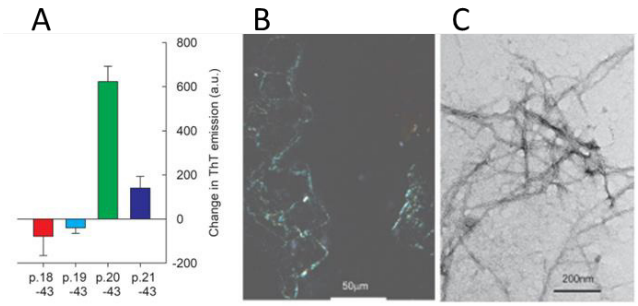

Figure 1.: Fibrillogenesis at pH 7 and 37°C of the signal-containing peptides p.18-43, p.19-43, and p.20-43, and the N-terminal peptide p.21-43. Three experiments were performed for each peptide, and the mean (SD) of the change in ThT fluorescence emission between 0.25 and 18 h is shown. Fluorescence intensity is shown in arbitrary units (a.u.) (panel A). Congo red staining with polarising filter of the p.20-43 fibrils (panel B), and the electron micrograph of the p.20-43 fibrils (after 24 h of incubation) (panel C) [5].

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Removal of Cardiac AL-Amyloid leads to Cardiomyocyte Positive Remodeling and Restrictive Pattern Reversal

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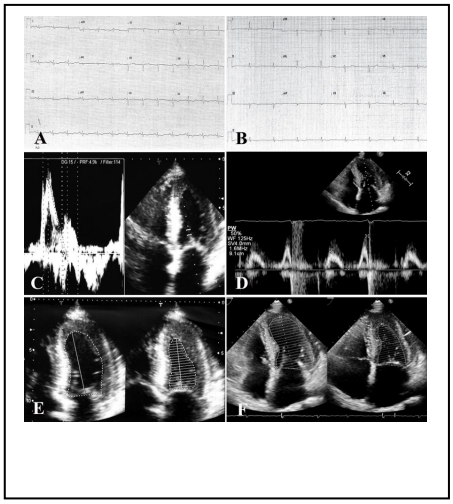
Background: Cardiac amyloid (CA) is an infiltrative myocardial disease caused by interstitial deposition of betafibrils, giving rise to a cardiomyopathy with restrictive phenotype (1). In particular, cardiac amyloid due to monoclonal gammopathy is the most common cause of restrictive cardiomyopathy in humans. Appropriately treated AL amyloid can have a survival of up to 10 years (2), however the mechanism involved, as toxic L-chain suppression or amyloid removal,is still unclear.

Objective: Our report documents for the first time a morphological removal of AL amyloid with positive remodeling of cardiomyocytes and of restrictive cardiomyopathy.

Material & Methods: A 71-year-old lady with a history of intervention for bilateral carpal tunnel syndrome, received in 2004 a diagnosis of AL CA and was submitted to several courses of chemioterapy. CA was documented with left ventricular endomyocardial biopsy and histological, ultrastructural evaluation as well as immunohistochemistry for light chain, with restrictive cardiomyopathy characterized at echocardiogram by enlarged left atrium, diffuse biventricular hypertrophy with 18.2 maximal wall thickening (MWT) at interventricular septum, diastolic disfunction with E/A ratio 2.45, preserved contractility (LVEF 55%) and NYHA class 3. Blood tests showed increased levels of alkaline phosphatase (427 U/L n.v. <279), NTproBNP 3188 ng/L (n.v. <334ng/L). After 17 years, in 2021, she was admitted because of palpitation and chest pain and documented intermittent phases of atrial fibrillation. The patient was re-evaluated with immuno-electrophoresis, echocardiogram, cardiac magnetic resonance, coronary angiography and left ventricular endomyocardial biopsy

Results: Serum AL-K monoclonal chain reduced from initial 233mg/L to 18.2mg/L, nv< 19.4 mg/L Echocardiogram showed thinning of cardiac walls with reduction of MWT from 18.2 to 14 mm, left atrial dimension and diastolic disfunction with E/A ratio shifting from 2.45 to 0.5, denoting a reversal of the restrictive pattern. LVEF remained normal to 60%. Serum NT-proBNP declined from 3188 pg/mL to 480 pg/mL (n.v.<334 pg/mL). Cardiac magnetic resonance revealed normal myocardial wall thickness, with preserved systolic function. Midventricular T1 map show normal global native myocardial T1 (952±87 ms, normal range <990ms); no areas of enhacement or typical «zebra» pattern were found on late gadolinium enhanced images. At histology and electron microscopy there were no more hypo/atrophic cardiomyocytes and the interstitium was also devoid of amyloid fibrils, that were scanty and distant from cardiac cell, suggesting a possible improvement of cell nutrition.

Summary & Conclusion: Our features strongly suggest the possibility to remove CA and the option for the related cardiomyopathy to be potentially improved along with remission of AL gammopathy. Regarding mechanism of CA reabsorption, control of amyloidogenic source by immunosuppressive agents and degradation of amyloid fibrils by interstitial macrophages and autophagy of intracellularly internalized material appear to concur



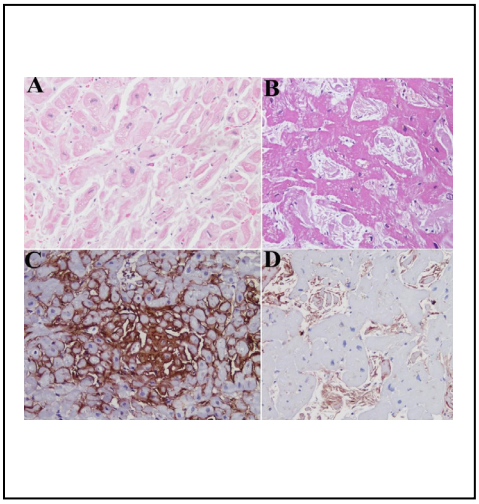


Figure 1.: Panel A: 12 leads ECG at baseline showing sinus tachycardia with diffuse low QRS voltages. Panel B: Follow-up 12 leads electrocardiogram with increased QRS voltages more evident in the peripheral leads. Panel C: 2D echocardiogram with restrictive trans-mitral flow pattern (E/A =2.5) .
Panel D: After 17 years diastolic function improved as demonstrated by *trans-mitral flow* velocity profile showing reversal of the E/A ratio (E/A= 0.5).

Figure 2.: Panel A-B: Hypotrophic cardiomyocytes surrounded by amyloid (A) becoming hypertrophied (B) after amyloid mobilization allowing cell nutrition. (EE, magnification 160x for A and B). Panel C-D: Immunohistochemistry for Al-K amyloid denoting extensive CA deposition (C) that reduce remarkably (D) after immunosuppressive therapy.

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Degree of transthyretin fragmentation in ATTR amyloidosis tissues

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Background: In most ATTR amyloidosis patients, tissue fibrils contain a mixture of full length (FL-TTR) and fragmented TTR. In some cases, however, fragments are not visible; the presence or lack of fragment bands at western blotting (WB) is the basis for the current dicotomic classification of fibrils as type A or B (1). Proteolysis was postulated to have a key pathogenetic role, leading to tetramer destabilization and amyloidogenicity (2). Complementing the qualitative (presence/absence) information with quantitative data on the extent of TTR fragmentation may provide further insights on the role and mechanisms behind this process.

Objective: To semiquantitatively characterize the fragmentation extent in deposited TTR across patients carrying distinct pathogenic variants.

Material & Methods: Consecutive individuals with a positive biopsy, referred to the Pavia Amyloidosis Center, were enrolled. Patients underwent clinical, instrumental and genetic examination. Overall, cardiac (n=2), or adipose (n=24; fine needle aspirate) tissues from 26 distinct patients with ATTR amyloidosis caused by 12 distinct variants and wt TTR were examined. Variants included Val30Met (n=3), Ala45Thr (n=2), Thr49Ala (n=2), Ser50Arg (n=1), Phe64Leu (n=4), Ile68Leu (n=3), Glu72Gly (n=1), Glu74Asp (n=1), Tyr78Phe (n=2), Glu89Gln (n=4), Glu92Lys (n=1), Val94Leu (n=1). One case had ATTRwt. No patients were consanguineous. Samples were washed and homogenized; proteins were separated on gel and WB was performed using a polyclonal rabbit antiserum raised against fragmented TTR (TTR 50–127). Band intensity was quantified by Image J; the ratio between the FL-TTR monomer (14 kDa band) over total TTR monomer (TotalTTR) (i.e. intensity of FL-TTR band plus that of the fragmented TTR monomer at 10 kDa) was calculated.

Results: Fragments were detected in 21/26 cases (81%). Among patients with type A fibrils, the fragmentation extent differed widely, with cases in which FL-TTR was predominant (FL-TTR:TotalTTR >0.5) and others in which most visible monomer was cleaved. Interestingly, when multiple unrelated individuals affected by the same mutation were evaluable, the fragmentation extent was similar across subjects, without apparent correlation with age- and gender. Specifically, in all ATTRGlu89Gln patients, fragmented TTR was predominant (59.4±12.3%), whereas in ATTRPhe64Leu and ATTRIle68Leu cases fragmented TTR was less abundant than full length (2.7±2.6% and 16.7±2.0%, respectively). Both ATTRAla45Thr patients have a similar fragmentation extent. One of the 3 ATTRPhe64Leu cases displayed type B fibrils, and the amount of fragments was very low in the 2 remaining ones. In the 3 Val30Met cases (two of whom had iatrogenic ATTR after domino liver transplant from early-onset donors, and one had early-onset phenotype), no significant amounts of fragments could be detected.

Summary & Conclusion: Wide quantitative heterogeneity exists in TTR fragmentation across patients. The qualitative dicotomization of deposits as type A and B does not account for this variability in the proportion of full length and fragmented TTR. Moving towards a quantitative evaluation of the distinct molecular species would be important to determine the factors that modulate proteolysis, and its possible correlation with clinical features. Although the number of cases herein is still limited, our data also suggest that the type of mutation and position along the sequence may influence the dynamics of fragmentation, possibly in relation to peculiar TTR structural features.

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Glomerular proteomics – unmasking sub-clinical amyloid or false positive?

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Background: Laser dissection and proteomic analysis (LDMS) is an established technique for amyloid diagnosis. Amyloid is identified by LDMS on the basis of presence of two or more 'amyloid signature' proteins, namely serum amyloid P component, (SAP), apolipoprotein E, (apoE) and/or apolipoprotein A-IV (apoA4). Glomerular LDMS, in the absence of amyloid by Congo red staining, is also used to diagnose certain other glomerulopathises such as fibrillary glomerulonephritis (FGN). However, the presence of at least two amyloid signature proteins within glomerular samples may result in a false positive diagnosis of amyloid.

Objective: To report the proteomic findings on laser captured glomeruli from twenty Congo red negative renal biopsies from four cohorts with established non-amyloid glomerular pathologies.

Material & Methods: Five renal biopsies were selected from each of four cohorts with different pathologies; 1) Focal segmental glomerulosclerosis (FSGS), 2) Diabetic neuropathy (DN), 3) Time zero transplant kidney biopsies, (T0), and 4) Membranous glomerulonephritis (MGN). Congo red staining was performed on 6µm thick tissue sections using the Puchtler et al method and viewed using brightfield and cross polarised light. On duplicate slides glomeruli were laser captured in to TEZ buffer for proteomic analysis. Each microdissection contained an area of 25 000 to 60 000 μm^2, depending on the number of glomeruli present within the sample and the amount of tissue available. Proteomic analysis was performed on the Thermo Scientific™ Q-Exactive Plus Orbitrap. Data was analysed using Mascot software and the Swiss-Prot human database. Congo red stained slides were interpreted by two independent assessors. Data obtained from proteomic analysis was analysed by an experienced panel of interpreters. Congo red results were compared with data obtained from proteomic analysis, in particular with respect to positive identification of the amyloid signature proteins.

Results: All biopsies stained negative for amyloid with Congo red. Data from the LDMS was anaylsed using the algorithm [1], which requires the presence of at least two of three signature proteins, SAP, ApoE and ApoA-IV with Mascot scores >20 to confirm amyloid, and in samples which do not exhibit Congo red staining, an increased minimum score of 50 is applied. Proteomic analysis revealed amyloid in at least 1/5 biopsies from each of the four cohorts on the basis of presence of ≥ 2 signature proteins, each with a score > 50 (Figure1). Two biopsies from the MGN cohort fulfilled criteria for amyloid and the third showed presence of SAP and ApoE with scores of 56 and 26 respectively. Overall, 25% of glomerular samples fulfilled criteria for presecence of amyloid. The two MGN biopsies indicating amyloid also revealed presence of kappa light chain suggesting AL (kappa sub-type) amyloid. (Figure 2).

Summary & Conclusion: Glomerular LDMS may indicate amyloid in patients with non-amyloid glomerular pathologies. Glomerular LDMS findings should be combined with Congo red staining and other patient characteristics in order to diagnose renal amyloidosis. This study will be expanded further to include electron micrsocpy to look further for possible presence of amyloid fibrils in patients with non-amyloid glomerulopathies.

PATHOLOGY	SAP score	ApoE score	ApoAIV score	Vitonectin score	Amyloid identified by signature proteins
FSGS	56	51		89	Amyloid
DM	202	123		203	Amyloid
MGN	106	155		327	Amyloid (AL kappa)
MGN	54	26			Equivocal
MGN	91	64	40	432	Amyloid (AL kappa)
T0	114		60	89	Amyloid

Figure 1

Protein Group	Protein	Score	Match(Sig)	Seq(Sig)	Seq(Uniq+Sig)
Signature	VTNC_HUMAN	327	9	5	5
Signature	APOE_HUMAN	155	8	5	5
Signature	SAMP_HUMAN	106	2	2	2
AL (kappa)	IGKC_HUMAN	497	12	5	5
Fibronectin	FINC_HUMAN	441	13	10	10
Heavy chain	IGHG4_HUMAN	409	12	7	5
Heavy chain	IGG1_HUMAN	213	8	5	3
AL (kappa)	KVD20_HUMAN	144	1	1	1

Figure 2

Figure 1 *showing the protein scores of the different pathology Cohorts.*

Figure 2 *showing the protein scores of a Congo red negative stained sample indicating the presence of amyloid with an AL kappa subtype*

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Complement in amyloidosis – immunohistochemical verification of activation of the complement system in renal light-chain amyloidosis

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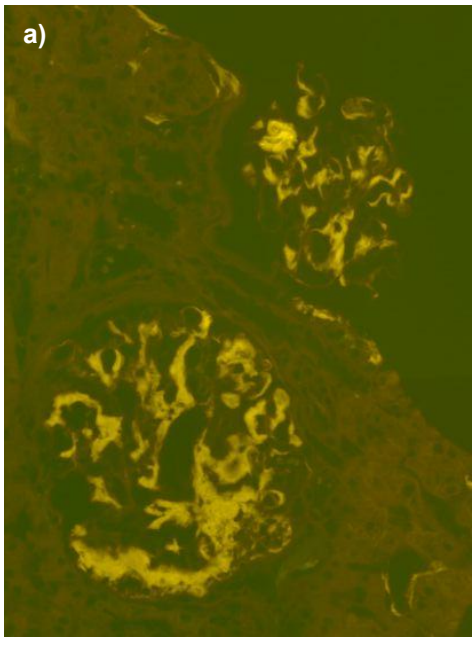
Background: Amyloidoses are a heterogeneous disease group, characterized by the misfolding and aggregation of proteins, which manifest either systemically or locally in various tissues and organs. The clinical symptoms vary and are directly linked to tissue damage and functional decrease of affected organs. In recent years, a large number of additional non-fibrillar proteins were discovered in amyloid deposits. How these non-fibrillar but seemingly disease-specific constituents affect the pathogenesis of amyloid is poorly understood.

Objective: Previous research indicates an activation of the complement system in amyloidoses. Lux et. al. were able to show an enrichment of complement component C9 (C9) as well as complement C3 (C3) in amyloid deposits of variable origin and location, but especially in renal tissue [1]. Here, we investigated the possible activation of the complement pathway in renal amyloidosis. To reach this goal we systematically explored indicators of the three different complement activation pathways in kidney biopsy specimens using immunohistochemistry.

Material & Methods: Kidney biopsy and autopsy specimens of 75 patients with immunoglobulin light-chain derived AL amyloidosis were retrieved from the archive of the Department of Nephropathology, Friedrich-Alexander University Erlangen-Nürnberg. Samples had been formalin-fixed and paraffin-embedded and were forwarded to Congo red staining and immunohistochemistry. We confirmed the presence of amyloid deposits with Congo red staining, and carried out an immunohistochemical staining with antibodies directed against lambda and kappa light-chain. Indicator components of the three complement pathways were chosen and immunohistochemical staining for those as well as MAC, T lymphocytes, and macrophages was performed (anti-complement factor D, anti-complement C5b-9, anti-CD3, anti-CD68, anti-complement C1q, anti-complement C3c, anti-MASP2). The stained tissue sections were digitalized each with the Hamamatsu Nano Zoomer S60. The digitalized scans were used to quantify the amyloid load.

Results: In all samples amyloid deposits were verified by Congo red staining and polarization microscopy. Amyloid was more abundant in glomeruli than in the interstitium. Fifty-nine cases were classified as AL amyloid lambda light-chain and nine cases as AL amyloid kappa light-chain. A positive staining of certain complement components was noted within renal amyloid deposits (Figure).

Summary & Conclusion: The histological results suggest a link between the activation of the complement system and renal AL amyloidosis. We provide further evidence of an activation of the complement system in renal amyloidosis.



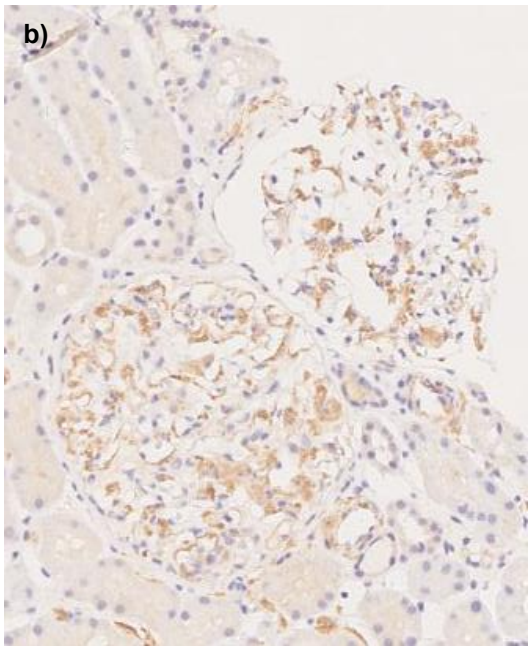


Figure: Consecutive tissue sections of renal lambda light-chain amyloid deposits. a) Congo red stained tissue section visualizes amyloid deposits in fluorescence microscopy and b) anti-Complement C5b-9 immunoreacts with amyloid deposits.
Magnification 16x.

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Trends in renal amyloidosis over a 30-year period: Data from the Norwegian Kidney Biopsy Registry 1988 – 2017

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Background: Renal amyloidosis is a fatal protein deposition disease with many possible causes (1,2). Previous studies on systemic amyloidosis have shown a stable incidence of AL amyloidosis (AL) and a decrease in AA amyloidosis (AA) in Western countries (3-5).

Objective: To give an overview of biopsy-proven renal amyloidosis in Norway and explore emerging patterns of amyloid types through a 30-year period.

Material & Methods: We present data on 476 patients with renal amyloidosis, constituting all patients 18 years or older registered with amyloid on renal biopsy in the national Norwegian Kidney Biopsy Registry (NKBR) 1988 - 2017. Through review of patients' medical records, we registered information on type and potential causes of amyloidosis. When typing data were available, this determined the type of amyloid. When typing had not been performed, clinician's diagnosis was used to categorize the amyloid. If sufficient information was not available, the case was labeled 'undetermined'.

Results: Among the 476 patients, AL was most prevalent with 226 (48%) cases, while AA constituted 206 (43%). 38 (8%) cases were grouped as 'undetermined' (UDT) and 6 (1%) cases other specified types (OT). Out of the 13495 renal biopsies registered in patients 18 years and older in NKBR in the study period, amyloid was found in 3.5% (95% CI 3.2%-3.8%). Total numbers of amyloid cases increased through the decades 1988–1997 (C1), 1998–2007 (C2), 2008–2017 (C3): from 131 (C1) to 140 (C2) and 205 (C3), parallel to an increase in total registered biopsies (Figure 1). Looking at the decades separately, amyloidosis was found in 3.8% (95% CI 3.2%-4.5%) of biopsies in C1, 3.2% (95% CI 2.7%-3.7%) in C2 and 3.6% (95% CI 3.1%-4.1%) in C3.

A shift in types (Figure 1) was demonstrated across C1-C3 (% of total amyloid biopsies/decade) with a decrease in AA: 74(57%) – 65(46%) – 67(33%) and UDT: 26(20%) – 9(6%) – 3(1%). Conversely, AL increased from 31(24%) – 66(47%) – 129(63%). All 6 cases of OT were found in C3 (3%).

Underlying causes of AA (Figure 2) were found in 194 (94%) of AA patients. Rheumatic disease decreased from 84% of AA patients in C1, to 68% in C2 and 18% in C3. Conversely, intravenous drug use (IVDU) as a cause of AA increased from 0% in C1 to 5 % (C2) and 64 % in C3. 10%–5%–5% (C1-C3) was explained by infection alone. Inflammatory bowel disease (IBD) was the only underlying disorder in 13 patients, constituting 4%- 9%- 6% of AA patients in C1-C3, while 6 patients classified otherwise had IBD as a second underlying cause.

Summary & Conclusion: Our data show a change in amyloid types over the 30-year study period while the relative frequencies of amyloidosis in renal biopsies were comparable in the first and last decade. Incidence of AA in Western countries is decreasing mainly due to low prevalence of chronic infections and improved treatment of rheumatic diseases (5, 6). As expected, our data showed a slight decrease in AA from C1-C3, reflecting a fall in AA caused by rheumatic diseases. However, this effect on the overall AA occurrence was attenuated by the rise in IVDU related AA-cases in the last decade (C3). Interestingly, AL occurrence appeared to be increasing through C1-C3. However, the UDT group showed a decline in the same period. We believe this group includes several undiagnosed AL cases. Furthermore, the increasing trend could also reflect a change in overall clinical practice, such as referral patterns and indications for biopsy.

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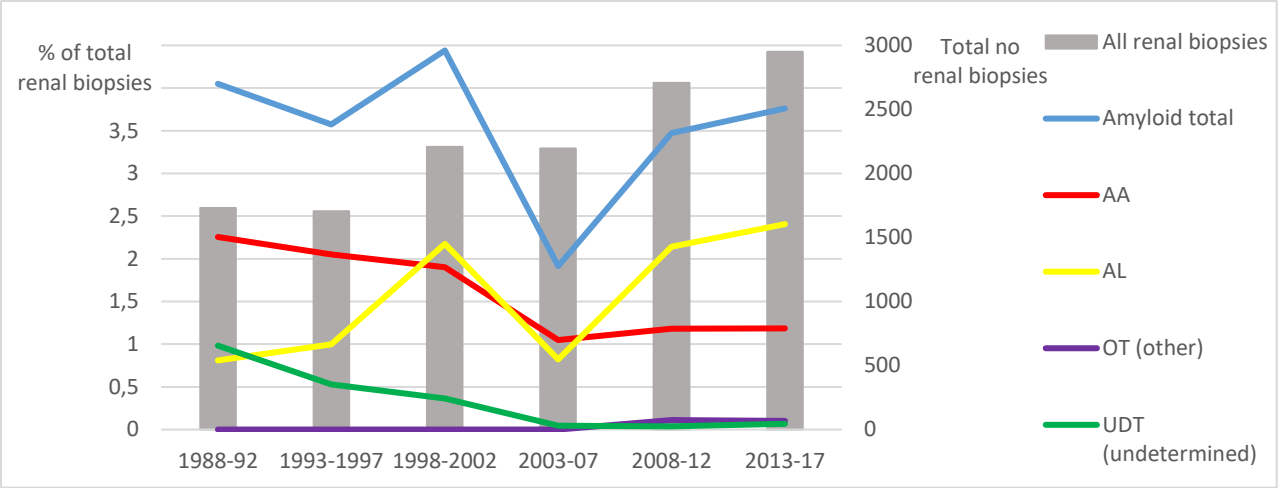

Figure 1 Amyloid type in % of total renal biopsies in Norway per 5-year period 1988-2017

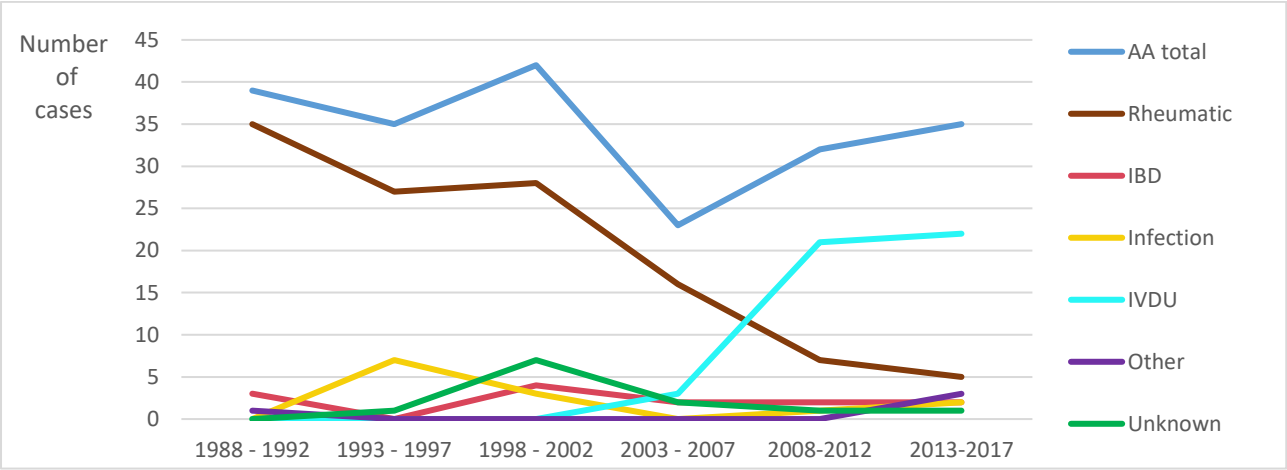

Figure 2 Causes of AA amyloid in renal AA amyloid cases in Norway per 5-year period 1988-2017

Clinical and Morphological Phenotypes of AL and ATTR Amyloidosis Detected via Gastrointestinal Biopsies

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Background: Amyloidosis refers to a group of clinical syndromes that are characterized by an abnormal deposition of misfolded proteins in end organs. Over 36 different proteins are known to cause amyloidosis with AL and ATTR constituting ~90% of the types observed in clinical populations. Amyloid typing of the protein forming the deposit determines treatment and prognosis, and therefore is important for clinical management.

Objective: To determine the distribution of amyloid types observed in gastrointestinal (GI) specimens of a clinical population.

Material & Methods: GI specimens from a total of 2,513 patients (obtained from N=43 esophagus, N=756 stomach, N=870 small bowel, and N=844 large bowel) were stained with Congo Red to detect amyloid deposits. Detected amyloid deposits were analyzed using a clinical proteomics-based amyloid typing assay at the Mayo Clinic. A pathologist scrutinized each amyloid proteomics profile and assigned a type based on the most abundant amyloidogenic protein. Clinical information was available for N=11 ATTR and N=85 AL patients. A feasible subset of N=59 AL and N=25 ATTR biopsies were subjected to morphological analysis to ascertain the involvement of muscularis mucosa (MM), submucosal vessels (SM-V) and lamina propria (LP).

Results: We detected the following amyloid types in the cohort: N=1,346 AL-lambda (53.6%), N=612 AL-kappa (24.4%), N=283 ATTR (11.3%), N=165 AA (6.6%), N= 28 AH (1.1%), N=27 AApoAIV (1.1%), N=18 EFEMP1 (0.7%), N=11 ALys (0.4%), N=9 AApoAI (0.4%), N=6 ALECT2 (0.2%), N=4 Aβ2M (0.1%), N=2 AGel (0.1%), N=2 AIAPP (0.1%), N=1 AFib (<0.1%). Of the 283 ATTR cases, we detected known amyloidogenic mutations in 69 cases (24.4%). The p.Val142Ile/Leu, p.Thr80Ala, and p.Val50Met mutations comprised 87% of the hereditary ATTR cases. Clinical assessment of N=85 AL cases and N=11 ATTR cases showed that 85% of AL cases and 54.5% of ATTR cases had a detectable M-protein by either serum or urine immunofixation electrophoresis. 83.5% of AL cases and 100% of ATTR cases had cardiac involvement via echo, troponin and NT-proBNP measures. In the majority of patients with clinical data available, amyloid was an unexpected diagnosis, and diagnosis was first made on biopsies from the GI tract (52/96, 54.2%). The most common indications for biopsy in these patients were: diarrhea (26.9%), GI bleed (21.2%), abdominal pain (19.2%), or weight loss (17.4%). Morphological analysis revealed ATTR cases had significantly less involvement of MM (p-value = 0.0019) and LP (p-value = 0.0004) when comapred to AL. There was no statistical difference of SM-V involvement between AL and ATTR cases.

Summary & Conclusion: The majority of GI biopsies in our clinical cohort were obtained for non-amyloid related medical interventions and findings of amyloid were incidental. Systemic amyloidosis types of AL and ATTR were detected in 89.2% of the cases. The majority of AL and ATTR patients had cardiac involvment, and nearly half of ATTR patients had a detectable M-protein. Morphological analysis revealed that AL had significantly more involvement of MM and LP than ATTR. However, these morphological features were not specific for AL type. In conclusion, there should be a low threshold to perform biopsy with Congo red stain in patients with unexplained GI symptoms. Clinical and histologic features are nonspecific in regard to amyloid type, and typing should be performed via a robust method such as proteomics.

Support & Funding: This study was funded by the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Amyloid deposition in granuloma of tuberculosis patients: A single-center pilot study

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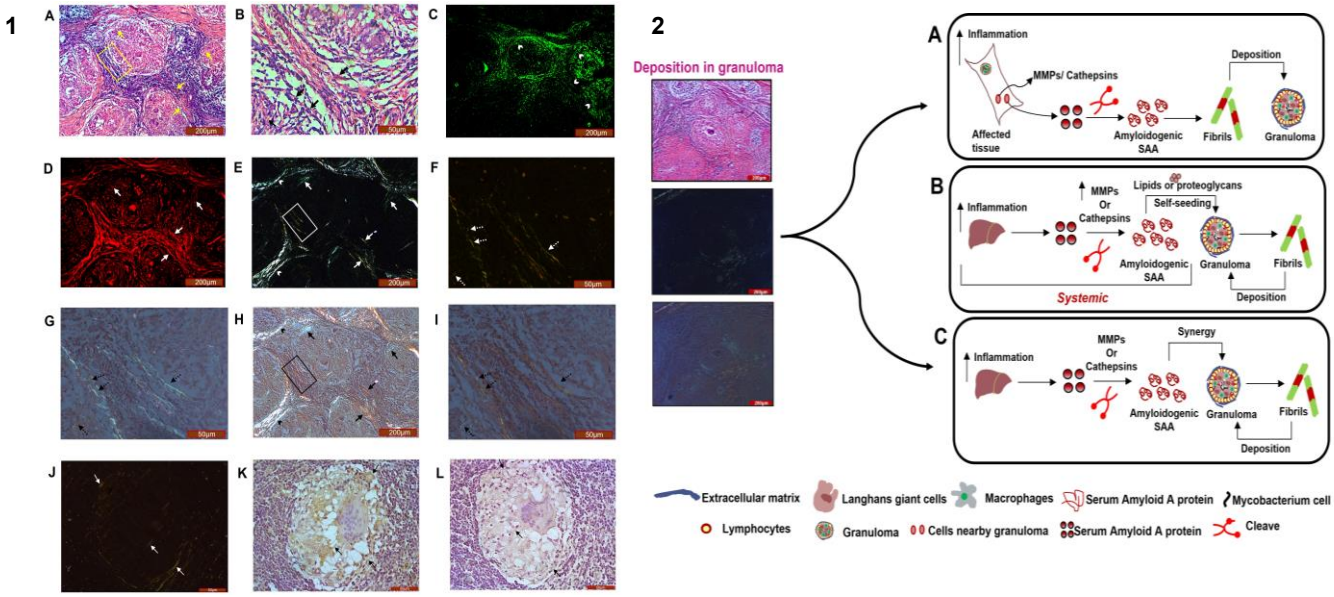
Background: The linkage between tuberculosis (TB) and AA amyloidosis is well documented[1-10]. However, SAA derived amyloid onset and deposition start sites are not well understood in tuberculosis. Formation of granuloma is one of the characteristic features of tuberculosis[11]. It is well known that SAA protein and proteases like MMPs and cathepsins, that cleaves SAA into aggregation-prone fragments are expressed in different stages of granuloma[12-20]. Hence, it was hypothesised that granuloma could be the potential site for amyloid deposition in TB patients.

Objective: To decipher the role of tuberculous granuloma in amyloid deposition and to understand the underlying mechanisms involved.

Material & Methods: Over two years, 150 tuberculosis patients were screened, and biopsies were collected from the affected organs. Biopsies showing hyaline-rich eosinophilic material in and around the granulomatous structures were screened to detect the presence of amyloid by Congo red staining. Immunohistochemical staining was performed on these amyloid-positive biopsies to trace the mechanism underlying amyloid formation.

Results: Granulomatous lesions derived from patient's affected tissues showed presence of acellular eosinophilic hyaline rich material, upon H&E staining (Fig 1A, B). Amyloid deposits are known to have an amorphous hyaline-like appearance [21]. Hence, these patients were screened further to trace the presence of amyloid in the granuloma. Congo red staining confirmed the amyloid nature of these hyaline deposits (Fig 1D-I), although the kidney function was normal in these patients. Further, immunohistochemical staining identified that these amyloid deposits (Fig 1J) were derived from SAA (Fig 1K, L). We did not get a positive signal for proteases (MMPs and cathepsins) that cleave SAA to form amyloidogenic fragments in and around the granuloma enriched with AA amyloid deposits. However, the non-amyloid areas close to the granulomatous lesions showed the presence of these proteases, especially MMP9 and Cath K, along with SAA protein.



Summary & Conclusions: Based on these observations, we speculated three possible mechanisms for the amyloid formation and deposition in tuberculous granuloma (Figure 2). We believe this is the first report showing the presence of SAA-derived amyloid deposits in the granuloma of TB patients. These findings might pave new ways for exploring the role of granuloma components and SAA in driving amyloid formation in the tuberculous granuloma. TB predisposes to renal amyloidosis several years after its onset[22, 23]. Thus, the clinical manifestations of amyloidosis are diagnosed at the later stages of tuberculosis and therefore remains undiagnosed in the initial stages. The current study would set a platform for the clinicians to diagnose the amyloid onset and progression even in the early stages of tuberculosis. This, in turn, would also aid in providing targeted therapy to TB patients with a probability of developing amyloidosis. Being a pilot study, it needs to be validated in a large number of patients in the future to attain significant clarity.

Figure 1: Histological examination showed presence of eosinophilic hyaline rich deposits mostly around the periphery of the granulomatous structure (A, B) (indicated by yellow solid arrows and marked by black solid arrows in the magnified image (B) of yellow square boxed area in A). Upon staining the same section with Thioflavin T, green fluorescence was observed corresponding to the areas of hyaline material deposits (C) (indicated by white arrow heads). Further, Congo red

staining of the same tissue section showed red fluorescence (D) (indicated by white solid arrows) along with apple green birefringence (E, F) (indicated by white solid arrows (E) and marked by white dashed arrows in the magnified image (F) of white square boxed area in E), consistent with the areas having eosinophilic deposits. A colour transition, depending on the orientation of fibrils was observed upon rotating the polarizer by 10º either in clockwise or anticlockwise direction, confirming the presence of amyloid deposits (G, H, I) (indicated by black solid arrows (H) and marked by black dashed arrows in the magnified images (G, I) of black square boxed area in H). However, this colour transition was not observed for the collagen that appeared bluish/greenish white irrespective of either crossing or uncrossing the polarizing filters (indicated by white arrow heads in E and black arrow heads in H respectively). Immunohistochemical staining showed presence of serum amyloid A protein in patches in the granulomatous structure as well as in the amyloid enriched areas respectively (J, K) (indicated by white arrows in J and black arrows in K respectively). But the antibody control (L) (indicated by black arrows) showed no positive signal for serum amyloid A protein. This depicted that the amyloid deposits are AA derived. Scale bar: 200 µm. (A, C, D, E, H); 50 µm (B, F, G, I, J, K, L).

Figure 2: Summary of probable mechanisms underlying serum amyloid A derived amyloid formation in tuberculous granuloma.

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The role of minor salivary gland biopsy in the diagnosis of systemic amyloidosis: results of a prospective study in 332 patients

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Introduction: The diagnosis of systemic amyloidosis requires a tissue biopsy with the exception of patients with cardiac involvement who do not have monoclonal components. Noninvasive approaches are usually preferred as the first step. The most commonly used is abdominal fat aspiration (AFA). Combination of AFA and bone marrow biopsy or minor salivary gland biopsy can increase the sensitivity.

Objective: to report the results of a sequential diagnostic approach including salivary gland biopsy as second step in patients with negative AFA.

Methods: All patients referred to the Pavia Amyloidosis Center between 2002 and 2020 for suspected amyloidosis, in whom amyloid deposits were not detected in the abdominal fat aspirates who underwent minor salivary gland biopsy were included. Typing was performed by immune-electron microscopy (IEM). The diagnosis of amyloidosis was eventually excluded in negative patients who were followed for at least 18 months after the salivary gland biopsy or until the cause underlying their clinical syndrome was identified.

Results: Three-hundred and thirty-two patients were included in the study. Their median age was 65 years (range 15-88 years) and 69% were males. In 237 patients (71%) a monoclonal component was detected [kappa in 106 (45%) subjects, lambda in 116 (49%), and bi-clonal in 15 (4%)]. Congo red staining was deemed positive in 75 (22%) patients. Amongst those, amyloid fibrils were detected by IEM in 41 samples, and the amyloid deposit were characterized as AL lambda in 16 cases (39%), AL kappa in 15 cases (36%), AA in 6 cases (14%) and ATTR in 4 (10%). In all the 31 AL amyloidosis cases a monoclonal component of the same type of the amyloid deposits was detected. Three of the 4 patients with ATTR amyloidosis had a mutation and a diagnosis of ATTRv was established. The remaining had ATTRwt. Systemic amyloidosis was diagnosed in 15 additional patients (5%), in whom amyloid deposits were not detected by either fine-needle aspiration of abdominal fat or salivary gland biopsy. The diagnosis was based on cardiac biopsies in 8 cases, renal biopsies in 5 cases, gastro-intestinal biopsies in 2. Interestingly, one of these subjects was found to have both AL and ATTR type deposits in the endo-myocardial biopsy. In the remaining 276 patients, the diagnosis of amyloidosis was eventually excluded. Overall, the diagnostic sensitivity of the salivary gland biopsy in patients with negative fat aspirate was 73% and the negative predictive value in this population was 95%.

Conclusions: This approach allows sparing the organ biopsy to two third of the patients with systemic amyloidosis. However, 5% of patients with suspected systemic amyloidosis can have both negative abdominal fat and salivary gland, which is not negligible given the severity of the disease and the need of an early diagnosis and the availability of effective treatments. Thus, extensive evaluation, including organ biopsy in selected cases is needed in patients with suspected systemic amyloidosis in order to reach maximum diagnostic sensitivity.

MANAGING PATIENTS WITH CARPAL TUNNEL SYNDROME AND POSITIVE CONGO RED STAIN: A WORK IN PROGRESS

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Background: Deposition of misfolded protein called amyloid can be a cause of carpal tunnel syndrome (CTS) [1]. Although amyloid deposition, both transthyretin (ATTR) and immunoglobulin light chain (AL), is a rare cause of CTS, it can be the first presenting symptom [1,2]. Systemic amyloidosis can affect multiple organ systems; patients with cardiac involvement particularly can have poor prognosis and thus early identification is crucial [2]. CTS may precede systemic amyloidosis by several years. In a study CTS leaded cardiac symptom by mean of 6.1 years and ATTR diagnosis by 6.9 years [2,3,4]. Multiple single center studies have indicated that CTS, bilateral, should be taken as early warning for clinical disease. Despite this there is a lack of clear guidelines in the management of pathological specimens from patients with CTS as well as the clinical management once amyloid is detected on specimens.

Objective: Our study aims to better understand the work up and management of patient presenting with CTS

Material & Methods: In a single institutional retrospective study, we reviewed patients who received carpal tunnel release (CTR) and underwent Congo red staining over a ten-year period between January 1st, 2012, and April 1st, 2022.

Results & Discussion: Out of 24 patients who underwent CTR, and samples sent for Congo stain, 10 were positive for the stain with positivity rate of 41.6%. This was a mostly male population (8/10) with a majority having presented with bilateral CTS (7/10). One patient presented initially with left sided symptom but interestingly later developed right sided symptoms and 2 patients had right sided symptoms only. None of the patient (3 patient with no follow up at our institution after surgery and 1 lost to follow up) had positive work up for or evidence of progression to systemic amyloidosis. Seven patients had minimum of serum paraprotein work up which was negative. One patient had both BMBX and fat pad biopsy, 2 patients had fat pad biopsy and 1 had bone marrow biopsy which were negative for amyloid involvement. Two patients were found to have cardiomyopathy without signs of systemic ATTR or AL amyloidosis. Majority of patients have been followed with repeat paraprotein and cardiac specific labs every 6 months and we did not find any evidence or progression to systemic amyloidosis within the two years follow up. In future, we plan to look at total number of CTR procedures performed over the l0 year period. Given the high positivity rate in patient who got tested for Congo red stain at our institution, we hypothesize that we are underdiagnosing patients. Patients who undergo CTR especially in the context of bilateral CTS should have material sent for evaluation to an experienced pathologist.

Summary & Conclusions: Patients who undergo evaluation for systemic amyloidosis in the context of a positive Congo red carpal tunnel pathology occasionally report this evaluation is emotionally stressful. When all the tests are negative, we discuss that we will do the tests again in the future. The patient's feel they are under the sword of Damocles, but they are not in a position of power. The optimal timing of repeat testing is not clear. This may best be individualized based on a given patient's symptoms, laboratory studies, and imaging features that are suggestive of systemic amyloidosis. There is a need of large-scale studies that are essential in understanding the role of amyloid testing in all patients undergoing carpal tunnel release and directions for long term follow up in those who test positive for amyloid.

FIGURES:

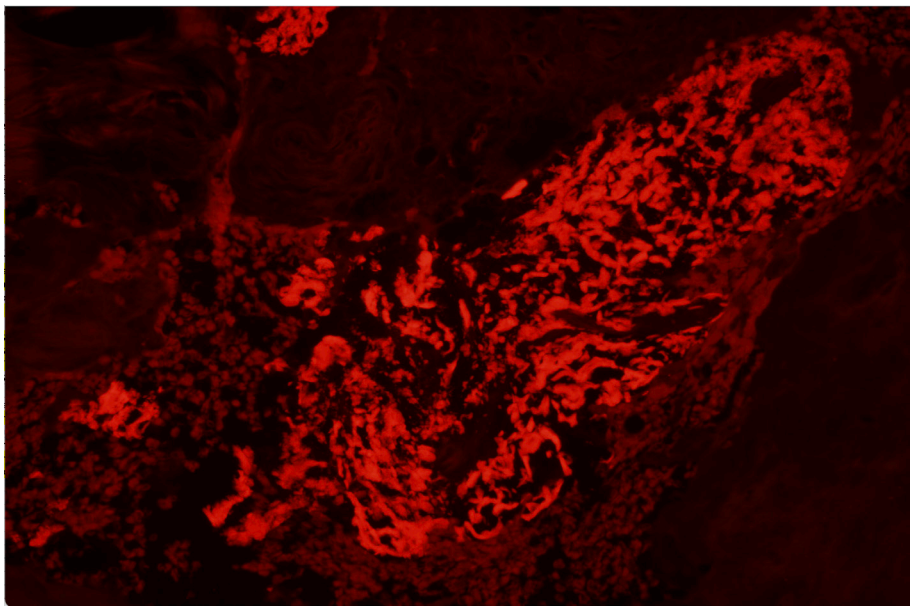

Figure 1: Carpal tunnel release specimen stained with Congo red and viewed under fluorescence light - deposits of amyloid are seen as bright red areas

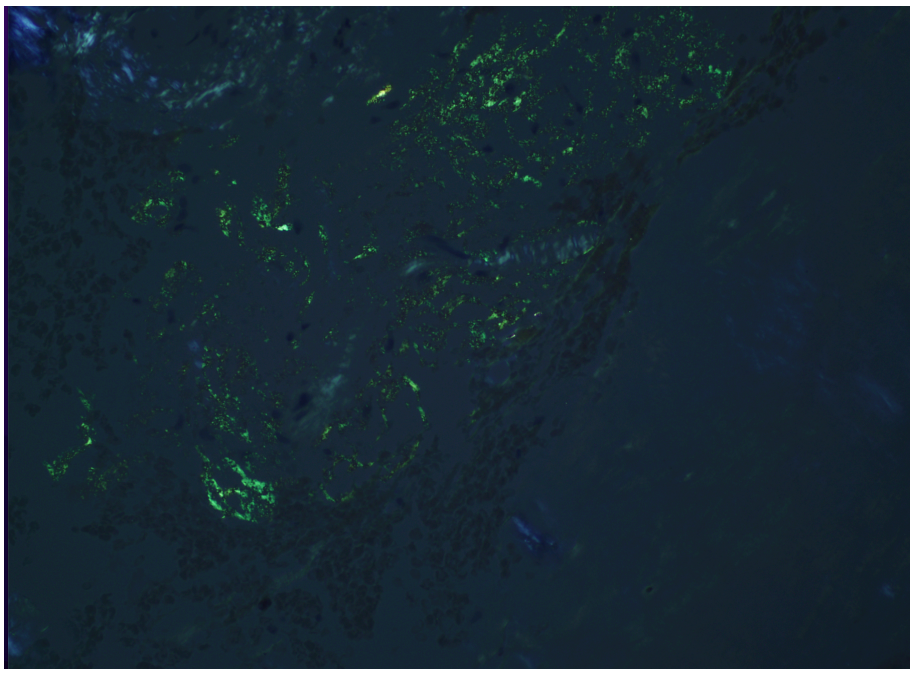

Figure 2: The same section as above, viewed under polarized light - deposits of amyloid are seen as green areas

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RNA-sequencing reveals similarity of AL amyloidosis and MGUS aberrant cells along with several potential target genes

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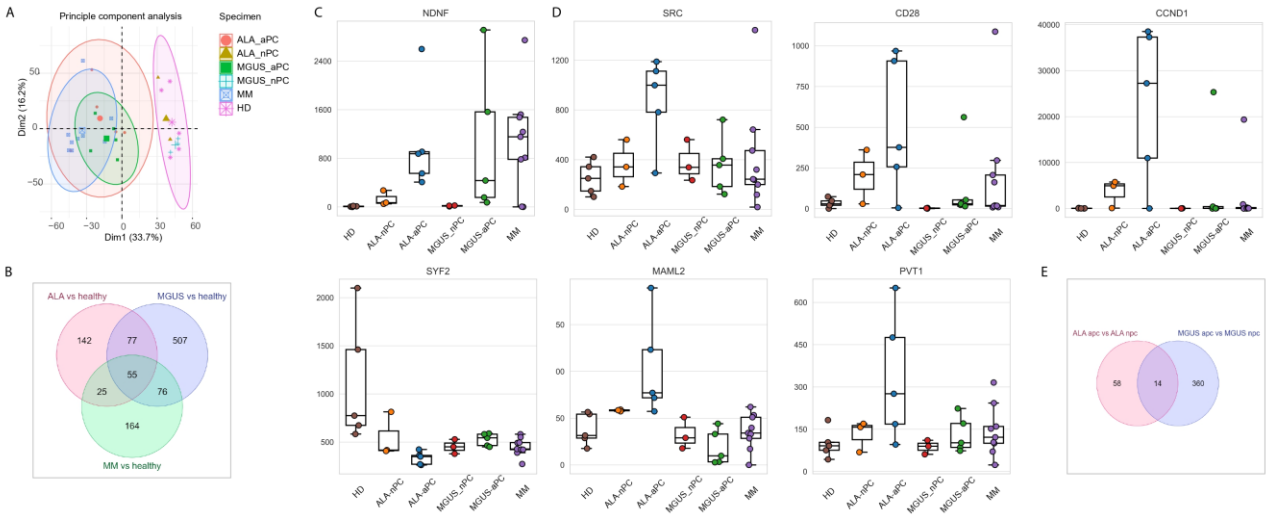
Background: Light-chain amyloidosis (ALA) is a rare and fatal monoclonal gammopathy (MG) causing organ and tissue damage resulting from the deposition of misfolded immunoglobulin free light-chains in the form of amyloid fibrils. Mutation landscape of ALA seems to be similar to monoclonal gammopathy of undetermined significance (MGUS) but expression profile by RNA-seq is still poorly studied and understood.
Objective: We investigated the gene expression profiles in clonal aberrant PC (aPC) in order to better understand ALA, MM and MGUS etiology to clarify the expression differences between individual MG diagnoses. Moreover, evaluation of expression profile of ALA can help to discover new targets for closer translational studies.
Material & Methods: Analysis of 5 newly diagnosed histologically proven ALA samples, 9 MM samples, 5 MGUS and 5 healthy donors (HD) samples was performed. Furthermore, we also added paired normal plasma cell samples from 3 ALA and 3 MGUS samples. We isolated RNA from clonal bone marrow PC sorted using CD45-PB, CD38-FITC, CD19-PECy7 and CD56-PE fluorescent antibodies. Isolated RNA served for library preparation using SMARTer Stranded Total RNA-Seq Kit v2 and sequencing aiming at 30 M pair-end reads/sample. Reads were mapped and quantified by Salmon v1.4.0. Differential gene expression was evaluated using Deseq2 v1.30.0 according p values less than 0.25 and absolute value of log 2 fold change greater than 1 were shortlisted.
Results: Principle component analysis of aberrant plasma cells (aPC) from ALA, MGUS and their polyclonal plasma cells (nPC) counterparts, MM and plasma cells from HD revealed that ALA aPC samples cluster together mainly with MGUS aPC, while all nPC and HD form a distinct non-overlaping cluster (Figure 1A). We performed differential expression analysis of each pathology group (ALA, MGUS, MM) with transcriptomes of plasma cells from HD. We identified 299 significantly deregulated genes in ALA, 715 in MGUS and 320 in MM. All diagnoses differed from HD by a set of 55 shared genes (Figure 1B), which mainly belonged to a neurogenesis pathway, probably pointing towards undifferentiated characteristics of malignant cells. One of the most deregulated and highly expressed genes in MGs was *NDNF* (Neuron Derived Neurotrophic Factor) gene (Figure 1C). Furthermore, ALA deregulated genes belong to an adhesion KEGG pathway which is activated, while oxidative phosphorylation, ribosome and spliceosome pathways that are suppressed. Among most upregulated genes were known markers as *SRC*, *CD28*, *CCND1* (and its regulator *SYF2* that is downregulated) and also gene *MAML2*, which is a prion-like transcription factor with coiled-coil protein domain that can mediate amyloid formation. We also identified a strongly overexpressed long non-coding RNA in ALA, *PVT1* lncRNA (Figure 1D), which was previously defined as candidate oncogene in breast cancer and leukemias/lymphoma. Comparison of ALA and MGUS aPC versus nPC identified 58 unique deregulated genes in ALA and 360 in MGUS (Figure 1E). ALA aPC genes belonged mainly to processes of cell cycle and cell division (*E2F1, BUB1, BUB1B, RAD51*).
Summary & Conclusion: Our study represents one of the first ALA RNA-seq projects. Comparison of ALA expression profile with other monoclonal gammopathies and nPCs showed a close relationship of ALA and MGUS. Our search for new candidates to become novel ALA markers revealed several interesting genes, and one non-coding *PVT1* RNA, which was found deregulated also in recent publication on larger ALA cohort (Alameda et al., Blood, 2021).

Figure 1.



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Presentation and outcome of patients with coexisting cardiac AL and ATTR amyloidosis

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Background: Incidence of ATTRwt amyloidosis increased in the last years, especially thanks to the possibility of a non-bioptic diagnosis. However, if a monoclonal component (MC) is detected, amyloid typing is mandatory to rule out AL amyloidosis. Differential diagnosis is even more important now that we have labelled treatment for both types of amyloidosis. In some cases, typing on endomyocardial biopsy (EMB) revealed the presence of both light chains (LC) and transthyretin (TTR) in the heart of the same patient.[1,2] We present a series of patients with coexisting AL and ATTR in the heart. In these cases, we elected to administer treatment for AL amyloidosis due to the more rapid course of this disease and because in Italy and in Germany tafamidis is not prescribable for patients with coexisting AL amyloidosis.
Objective: To evaluate the outcome of patients with coexisting AL and ATTR amyloidosis in the heart after treatment for AL amyloidosis.
Material & Methods: We identified 17 patients with coexisting AL and ATTR amyloidosis (5 in Heidelberg, 8 in Pavia and 4 in Padova). Typing was performed by immunohistochemistry with custom made antibodies (IHC; N=5) and immunoelectron microscopy (IEM; N=8). In 4 cases, EBM samples were subjected to protein extraction following a new procedure (patent n EP3417295). SDS-PAGE/Western Blot analysis was performed for the main amyloidogenic proteins and its results were validated by mass spectrometry. Cardiac responses and progression were assessed according to AL amyloidosis criteria.
Results: 3 patients were excluded because of post-mortem diagnosis and 1 because amyloid deposits were characterized on bone marrow biopsy and it was not possible to unequivocally conclude for the coexistence of LC and TTR in the heart. Thirteen patients were included in the analysis (Table 1). Four had a positive abdominal fat pad (AFP). Three underwent EMB because typing by IHC was not possible on AFP by IHC. In 1 IEM was positive for TTR only, but given the presence of an abnormal free LC ratio and albuminuria, the patient underwent EBM that was positive both for LC and TTR at IEM. Perugini score 1 was observed in 3 patients, of whom 1 was positive for a TTR mutation (Val40Ile). In 1 case, bone marrow plasma cell infiltrate was ≥60%, in absence of other multiple myeloma defining events. All patients received treatment for AL amyloidosis and 10 had response data (Table 1). None was treated for ATTR. At 6 months 9 achieved a hematologic response (HR) and 3 a cardiac response (Table 1). Six patients had a cardiac progression, despite 5 had achieved a HR (complete response [CR] in 2, very good partial response [VGPR] in 2 and partial response in 1 case). After a median follow-up of 33.8 months, 2 patients died of worsening of heart failure after 15 and 48 months from diagnosis, despite the maintenance of CR and VGPR. Both had AL cardiac progression at 6 months.
Summary & Conclusion: This is the largest series of patients with coexisting AL and ATTR amyloid deposits in the heart and the first reporting outcome to AL treatment. The AFP was commonly negative and when positive typing was not possible by IHC. Moreover, IEM did not find AL deposits in 1 case. This was unexpected and may reflect a selection bias: patients with a MC and AL amyloidosis in the AFP are unlikely to be further tested for ATTR. This finding warrants further studies and a possible revision of diagnostic algorithms. Treatment for AL amyloidosis may not be able to stop progression in these patients, who should be granted access to anti-ATTR therapies.

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Table 1. Patients' characteristics

Characteristics	N(%) – median (range)
Age, years	71 (61-83)
Sex, male	11 (85)
NYHA class: I-II / III-IV	8 (62) / 5 (38)
Atrial fibrillation/flutter	9 (69)
Tunnel carpal syndrome	5 (38)
European Mayo Staging: II / IIIa / IIIb	8 (62) / 3 (23) / 2 (15)
Gillmore Staging: I / II / III	4 (31) / 6 (46) / 3 (23)
IVS, mm	18 (11-23)
EF, %	48 (20-60)
Bone scintigraphy*	9 (69)
Perugini score 1 / 2 / 3	3 (33) / 0 (0) / 6 (67)
MC at serum/urine immunofixation and/or abnormal FLCR	13 (100)
Only MC at serum immunofixation	2 (15)
Only Bence Jones proteinuria	2 (15)
Only abnormal FLCR	2 (15)
Kappa : lambda	5 (38) : 8 (62)
dFLC, mg/L	140 (0-435)
BMPC, %	11 (2-60)
Positive abdominal fat pad aspirate	4 (31)
Amyloid typing on endomyocardial biopsy	13 (100)
Immunohistochemistry	4 (31)
Immunoelectron microscopy	8 (62)
Mass spectrometry	1 (7)
DNA analysis of TTR gene: negative / positive**	12 (93) / 1 (7)
Treatment for AL amyloidosis in 10 patients with response data	
BMDex / CyBorD / MDex	6 (60) / 3 (30) / 1 (10)
Hematologic response in 10 patients with response data	
Any hematologic response	9 (90)
CR / VGPR / PR	3 (30) / 5 (50) / 1 (10)

AL, immunoglobulin light chain amyloidosis; BMDex, bortezomib, melphalan and dexamethasone; BMPC, bone marrow plasma cells; CR, complete remission; CyBorD, cyclophosphamide, bortezomib and dexamethasone; dFLC, difference between amyloidogenic and non-amyloidogenic free light chains; EF, ejection fraction; FLCR, free light chain ratio; IVS, interventricular septum; MC, monoclonal component; MDex, melphalan and dexamethasone; NYHA, New York Heart Association; PR, partial response; TTR, transthyretin; VGPR, very good partial response

***** In 4 cases bone scintigraphy was not performed since the presence of MGUS required the histological diagnosis of amyloidosis. Bone scintigraphy was performed with ^{99m}Tc-DPD in 4 cases and and ^{99m}Tc-HMDP in 2 cases

**1 patient with the Val40Ile mutation

Validation of a mouse anti-human Serum Amyloid A antibody immunohistological test for diagnostic clinical practice.

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Background: Accurate typing of the amyloid precursor protein in patients with amyloidosis is crucial for informing effective management of the disease. We obtained a cell culture supernatum supplied by B Hazenberg and J Bijzet, University of Groningen, containing mouse IgG1 monoclonal (Reu86.1) antibodies raised against purified human Serum Amyloid A (SAA) coupled to Helix Pomatia Haemocyanine. The clone was selected for reactivity against purified amyloid fibrils from AA-amyloidosis patients and serum/plasma from patients with a high acute phase, reacting specifically with SAA-1, the major isoform of SAA in plasma. In our experience, when used for the immunohistochemical typing of amyloid, Reu86.1 is sensitive and specific, and has been shown to give true positive and true negative results in cases where commercially available IVD anti-AA antibodies have given false negative and false positive results[1].

Objective: To validate the use of the Reu86.1 antibody on histopathological specimens as a clinical immunohistochemistry (IHC) test, enabling it to be added to the scope of UKAS-accredited tests offered by the Jack O'Neil Amyloidosis Laboratory (JONAL) at the National Amyloidosis Centre. The specificity, sensitivity, accuracy and precision of the test will be assessed.

Material & Methods: Staining was performed using the Leica Bond Max platform, 'IHC protocol F + enhancer long' protocol. Formalin-fixed parrafin-embedded (FFPE) sections were stained using the 'Preparation: *Dewax' option and resin-embedded sections were stained using the 'Preparation: *- - - -' option. Optimisation was carried out by using serial dilutions of the supernatum in the protocol described above to stain AA amyloid-containing FFPE tissue sections to elucidate the optimum concentration for staining. To assess sensitivity, specificity and accuracy, a selection of archival diagnostic specimens were stained with Reu86.1. Specimens had all undergone proteomic analysis[2] and shown to contain either AA, lambda light chain (lambda), transthyretin (TTR), non-AA, kappa light chain, ApoA1 or insulin amyloid deposits. Samples chosen for the analysis were representative of the sample pool routinely analysed at the JONAL, having been referred from a large number of external centres. Samples chosen had undergone different fixation and processing procedures, and 22 different tissue types were selected including one post-mortem case. Tissues contained a range of amyloid loads and both histological and cytological preparations were used. To assess precision, 4 FFPE samples (2 containing AA amyloid, 1 containing TTR amyloid and 1 containing lambda by proteomics) were stained in triplicate over the course of 5 days. This provided data for repeatability and reproducibility. The first 3 days were performed by a different operator from the last 2 days, to assess variability introduced when different people carry out the test.

Results: Using the results from proteomic analysis as the gold standard for determining amyloid fibril type, amyloid IHC with Reu86.1 was shown to have a specificity of 100%, a sensitivity of 100% and an accuracy of 100%. The test was shown to be repeatable (100% precise on intra-run results) and reproducible (100% precise on between run results)

Summary & Conclusion: Use of the Reu86.1 antibody in IHC for amyloid typing was shown to be extremely sensitive, specific, accurate, and precise and the validation was completed successfully. After submission of the validation documents to UKAS, the test was added to the scope of accreditation of the JONAL.

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Patients with two different systemic amyloidoses – a case series

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Background: At determination of type of systemic amyloidosis by immunohistochemistry (IHC) or western blot (WB) analysis, we occasionally get signals for more than one protein. The antibodies that we use for IHC are in-house developed mouse monoclonals (MAB) for ALλ, ALλ, ATTR and AA, which give virtually no background staining. This, in addition to specific band patterns in WB analysis give solid type-determination results, indicating that the double protein signals depend on the presence of two amyloid forms in the same patients.

Objective: To study the occurrence of double systemic amyloidosis in **a consec**utive clinical material.

Material & Methods: We have gone through the data of all tissue biopsies submitted to us during the three-year period 2018-2020. The total number of specimens was 1572 with the majority, 1397, consisting of fat pad biopsies taken for primary amyloid diagnosis. From fat tissue biopsies, pressure slides, stained with Congo red were prepared. Positive samples were typed by WB analysis or, when the deposits were very sparse (particularly ATTRwt), by IHC of the original, Congo red stained slides. Some cases were studied both with WB and IHC. The non-diagnosed amyloid-containing materials were prepared for mass spectrometry.

Results: Of the 1572 received biopsies, 537 (34.2%) contained amyloid deposits. Of these, 442 were typed as ALκ (29), ALλ (165), ATTR (241) and AA (7). In addition, there were few cases of amyloid of Aβ, AMed, ASem or AIns nature. Six cases with two different systemic forms of amyloidosis were identified (Table 1). Five were a combination of ALλ and ATTR, most probably wt type. In all cases where WB was performed, the ATTR fibril type was A, i.e. a mixture of full-length and C-terminal TTR molecules. This form also has a characteristic distributen pattern in the myocardium (Figure 1). Histology indicated two separate amyloid forms rather than mixed fibrils.

Table 1. Six patients with two different systemic amyloidoses

Patient No., sex, age	Amount of amyloid	Amyloid types	Determined by
1, M, 85	+++	ALλ/ ATTR A	IHC, WB
2, F, 87	+++	ALλ/ ATTR A	IHC, WB
3, M, 81	+++	ALλ/ ATTR	IHC, WB
4, M, 80	++	ALλ/ ATTR A	WB
5, M, 78	+	ALλ/ AA	IHC, WB
6, F, 81	+++	ALλ/ ATTR	IHC

Summary & Conclusion: Combination of IHC and WB has turned is an excellent way to identify double systemic amyloidoses. Such cases are obviously rare. In most cases, ATTR, most probably of wt type constitutes one component and the other one was ALλ in all six materials. As in our index double amyloid case (1), the deposits seemed separate although it cannot be ruled out that one type participated in the pathogenesis of the other. However, combination of ALλ and ATTR occurred in 5/165 (3%) ALλ materials which seems to be a reasonable prevalence in the age group.

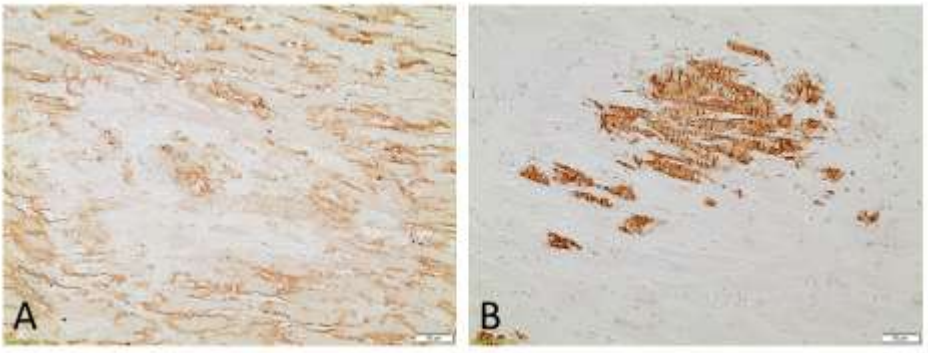

Figure 1.: Case 3, myocardium, in A immunolabelled for ALλ and in B for ATTR. DAB, bar = 50 µm

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Properties of generic monoclonal AA antibodies for classifying most vertebrate AA amyloidosis, and as probes for innate functions of SAA

Reinhold Paul Linke
Organisation(s): Domatec, Germany

Background: Systemic AA amyloidosis (AA) is usually detected by kidney biopsy for the assessment of proteinuria. Abdominal fat aspiration biopsy (FAB) can be used to detect amyloid deposits in the fat tissue and to follow them during follow-up.
Objective: To correlate FAB AA deposits with renal outcome
Material & Methods: AA amyloidosis was confirmed by immunohistochemistry. FAB was performed at the first presentation at visit 1 (V1). FAB were mounted on glass slides and stained with congo-red (CR). AA amyloid deposits were semi-quantified as CR 0 (negative), CR 1+ (<1% area), CR 2+ (1-10% area) or CR 3+ (10-60% area). Patients with CR 4+ (>60% area) were not observed in our cohort.

In order to minimize CRP and SAA the anti-inflammatory treatment was adapted according to the underlying primary disease. Patients with FMF+AA were treated with colchicine and IL1 inhibitors (IL1i). Patients with rheumatoid arthritis and idiopathic AA were treated with IL6i. Patients with Crohn's disease were treated with TNFi. A second FAB was performed about 3.5 years later during follow-up (V2). The kidney function parameters were determined at each visit until the last visit (V3).

Results & Discussion: 48 patients with AA were identified and followed for at least 3 years. The underlying primary diseases were familial mediterranean fever (FMF, n=20) and rheumatic disease (n=13) or idiopathic AA (n=15). Patients with rheumatic AA and idiopathic AA were considered as one disease spectrum and were pooled together in the Rheu+AA group (n=28). The mean age (SEM) at the start of inflammatory disease was 20.31 (4.26) years in FMF+AA and 40.73 (3.48) years in Rheu+AA (p=0.0006). The age at AA diagnosis was 41.00 (2.87) years in the FMF+AA and 58.48 (2.45) years in the Rheu+AA group (p<0.0001). The age at the end of follow-up (V3) was 52.27 (1.91) years in FMF+AA and 64.67 (2.38) years in the Rheu+AA group (p=0.0002). End-stage renal disease (ESRD) was observed in FMF+AA patients at V1, V2 and V3 in 5, 5 and 6 cases. In patients with Rheu+AA ESRD was observed in 6, 7 and 8 cases. FABs were performed at V1 and V2 (s. table 1A for results of CR staining in each group at V1 and V2) and showed a significant decrease in the FMF+AA group. Further analyses showed that a regress of the amyloid load in the fat was associated with a stable renal function and a significant decrease of the proteinuria.

Summary & Conclusions: Inhibition of pro-inflammatory cytokines (IL1i in FMF, IL6i in RA, TNFi in CED) can normalize CRP and SAA, reduce proteinuria and prevent the decline of the kidney function. We show that in repetitive FABs, AA deposits can also be reduced. Interestingly, albeit in a small cohort of AA patients, this reduction was also associated with a stable renal function and a significant decrease of the proteinuria. FAB might be a helpful tool to assess therapy response in AA amyloidosis, when our results can be confirmed in another cohort.

Patient Number	FMF+AA 20	p	Rheu+Idio+AA 28
FAB visit 1 CR 0 | 1+|2+|3+ CR mean ± SEM	 6 | 6 | 6 | 2 1.20 ± 0.22	 0.4860	 2 | 19 | 4 | 3 1.29 ± 0.14
Time V1 to V2 (Years)	3.62 ± 0.21	0.3248	3.37 ± 0.13
FAB visit 2 CR 0 | 1+|2+|3+ CR mean ± SEM	 15 | 4 | 1 | 0 0.30 ± 0.13	 0.0001	 10 | 9 | 6 | 3 1.07 ± 0.19

Table 1A

FAB CR ≥ 1+ Patient Number	progress or stable 13	p	regress 27
FMF+AA (N)	1		13
Rheu+Idiop+AA (N)	12	0.0148	14
S-creatinine at V1 (mg/dl)	1.56 ± 0.15		1.77 ± 0.21
S-creatinine at V2 (mg/dl)	1.69 ± 0.25 p=0.2270		1.62 ± 0.14 p=0.6991
Proteinuria at V1 (g/mol crea)	311.40 ± 92.88		699.46 ± 139.77
Proteinuria at V2 (g/mol crea)	162.43 ± 59.56 p=0.3030		258.89 ± 76.54 p=0.0290

Table 1B

Renal amyloidosis in people who inject drugs in Oslo, Norway.

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Background: AA amyloidosis is a rare and heterogeneous disease known to be a potential complication to a variety of chronic inflammatory diseases and infections (1). The kidney is the organ most frequently affected in AA amyloidosis. Renal amyloidosis is often characterized by nephrotic syndrome with severe proteinuria, edema, hypoalbuminemia, and progressive loss of renal function. Over the last years, we have seen an increasing incidence of AA amyloidosis in the dialysis population in Oslo, Norway, occurring in people who inject drugs (PWID) as a complication of chronic skin and soft tissue infections (2).

Objective: We aimed to study the clinical characteristics and incidence of renal AA amyloidosis in PWID entering renal replacement therapy in Oslo, Norway.

Material & Methods: The study is a retrospective analysis of data on patients with end stage renal disease and past and/or present history of injecting drugs who started in dialysis at Oslo University Hospital/Ulleval between January 2005 and December 31st 2020. All patients starting dialysis in this time-period and fulfilling the criteria of known prior and/or present injection drug use were included and there were no specific exclusion criteria. Data from the Norwegian Renal Registry (3) and patient hospital records were reviewed.

Results: 71 patients on dialysis with a history of injection drug use were included in the study. Renal biopsy was performed in 71.8 % of patients where AA-amyloidosis was confirmed in all but one case. All patients were Caucasian and 59.2 % were male. The mean age when starting dialysis was 48.2 years (SD 7.8), with a history of injecting drugs for a mean of 28 years (SD 9.1). The whole study population had a history of repeated chronic skin infections. They all had proteinuria, all but three in nephrotic range (> 3g/L). All patients were seropositive for hepatitis C and only 2.8 % for HIV. 2.8 % had a rheumatic disease, which is the leading cause of AA-amyloidosis in other Western countries. PWID today comprise about 20% of the dialysis population at Oslo University Hospital/Ulleval. About half of the PWID (54%) were known at the clinic when starting dialysis, the remaining were crash landers (not known at the clinic for at least 4 months prior to starting dialysis). None of those patients were found eligible for renal transplantation due to chronic infections and ongoing drug use.

Summary & Conclusion: Renal AA-amyloidosis is the most common cause of end stage renal disease in people who inject drugs in Oslo, Norway and presently comprise about a 20% of the dialysis population at the university clinic. It is a challenging and complex population of patients lacking the option of renal transplantation. More focus is needed on prevention and early intervention.

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Properties of generic monoclonal AA antibodies for classifying most vertebrate AA amyloidosis, and as probes for innate functions of SAA

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Background

Among the protein storage diseases in humans and other vertebrates the amyloidoses are the most prominent. They originate from soluble precursor proteins through their proteolytic hydrophobic products which aggregate within the organism into extra cellular fibrillar protein masses with fatal pathogenic consequences.The chemical nature of the many proteins deposited as amyloid define the disease entities which enable the physician to apply, if already worked out, the amyloid class-specific therapies which today become more and more successful. The entity AA amyloidosis was first defined by Rudolph Virchow which, in his own words, was called as a "secondary" one following chronic tuberculosis, an illness which was rampant during his time. The amyloidogenic soluble precusor is the acute phase serum amyloid A protein (SAA) present in all vertebrates. After unusually prolonged inflammations with high SAA serum concentrations, SAA becomes amyloidogenic through limited fragmentation, thereby causing the normally fatal AA amyloidosis (1).

Objective

To diagnose vertebrate AA amyloidosis using monoclonal generic AA antibodies and to study their effects on SAA function.

Material & Methods

Monoclonal antibodies directed against AA were applied for the detection of AA in tissue sections from humans and SAA in human acute phase serums (2). These monoclonals have different specificities, in that some are human specific, such as mc 1 (Dako), which reacts with human AA in formalin-fixed paraffin sections and today represents the standard for the diagnosis of human AA amyloidosis. However, there are also anti-AA antibodies that detect AA amyloid independent of species specificities. The monoclonal mc 1 does not react with animals distant to humans. However, other very special monoclonals react with animal AA and are therefore in use for the diagnosis of AA amyloidosis in veterinary medicine (3).

Results & Discussion

SAA's generic epitopes: The monoclonals with generic specificities are mc 4, mc 21 and mc 29. They react with human as well with animal AA on formalin-fixed paraffin tissue sections, because they bind to species-independent epitopes of SAA. Considering the species independence of being almost unchainged in another way is the notion that these peptide stretches recognized by the three monoclonals were highly preserved during the evolution which spans from the time of the pre-vertebrates and from the lampreys to us being in existence for over 500 million years. This evolutionary stability demonstrates the outstanding significance of the SAA system for the survival of the Chordata.
SAA functions probed with generic antibodies: SAA is involved in very many innate functions for survival and blocking the evolutionary very old epitopes has elucidated various functions of SAA (4). The first example: The FMLP-induced oxidative burst of normal human neutrophils could be reduced in vivo by SAA. This concentration-dependent reduction

could be blocked by the monoclonal antibody mc 29 which binds to the synthetic peptide AA 28-40 and thus proves that this blocked area of SAA is responsible for the inhibitory effect of SAA on neutrophils during the acute phase in order to prevent the neutrophils from overreaction, which is a central function to guarantee homeostasis (5).

Summary & Conclusions

SAA is a highly complex molecule involved in multiple functions which have been analyzed using generic monoclonal antibodies for therapeutical options, particularly in sepsis.

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SARS-Cov2 infection in AA amyloidosis: a high rate of mortality

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Background:

Infection with SARS-Cov2 in patients with chronic inflammatory diseases is not completely known. Studies showed that severity of COVID-19 is correlated with pre-existing comorbidities especially diabetes, cardiovascular diseases, elevated body mass index and hypertension, immunosuppressive therapy and chronic kidney disease[1].
Previously, it was reported that patients with AL amyloidosis are extremely vulnerable and are prone to severe COVID-19 form[2]. In COVID-19, SAA is overproduced and extreme high SAA level is correlated with poor prognosis in COVID-19[3]. Several studies suggested that AA amyloidosis is a factor causing systemic complication after COVID-19[4].
To our knowledge no publication focused specifically onCOVID-19 infection amongpatients with AA Amyloidosis.

Objective: The aim of our study was to describe clinical features of SARS-Cov2 infection in a cohort of patients with AA Amyloidosis.

Material & Methods

We performed a retrospective study of COVID-19 in patients with AA amyloidosis followed up in the french national reference center for AA amyloidosis March 2020 and March 2022.

The diagnosis of SARS-Cov2 infection was retained if the patient was symptomatic with positive RT-PCR or SARS-Cov2 serology or typical chest CT scan.

Results

During the study period, 66 patients followed for AA amyloidosis were seen in our center. Eleven of them had contracted SARS-Cov2 virus (16%) with a sex ratio of 1 and a median age at diagnosis of infection of 60 years old. All patients had renal involvement secondary to AA amyloidosis: 2 were on dialysis, 7 had pre-terminal renal failure, 3 had renal localization of amyloidosis with preserved renal function and two were kidney grafted. The underlying inflammatory pathologies responsible for AA amyloidosis were respectively: Familial Mediterranean fever (n=3), mevalonate kinase deficiency (n=2), Waldenström's disease (n=1), Acne Conglobata(n=1); mutation in *PSTIP1*(n=1), unknown (n=1). Five patients were treated with interleukin-1 inhibitors (anakinra), three with colchicine, one with tofacitinib combined with steroids and one with tocilizumab combined with steroids.

Among the 11 AA patients who developed COVID infection, 50% were hospitalized in an intensive care unit (n=4); the others had a moderate (n=3) or even asymptomatic (n=2) form.

Four patients died (37%): they had at least one comorbidity for the severe COVID-19 infection with

respectively: silicosis complicated by chronic respiratory failure, BMI> 30 and corticosteroid therapy at a dose > 20mg/d. Two patients had been vaccinated, one deceased and received the vaccination very close to his infection, and he was on long-term corticosteroids; the second patient developed severe form and he had completed his vaccination 3 months prior to Sars-cov2 infection.

Summary & Conclusion

We thus report the largest cohort of SARS-cov2 infection among 11 patients with AA amyloidosis leading to death in 37% of cases. AA amyloidosis seems thus to be a risk factor for severe form for COVID-19 infection with a high mortality especially when combined with at least one other COVID19 severity factor. It is crucial to vaccinate these patients against SARS-Cov2.

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Clinicopathological characteristics of a Japanese case with fibrinogen Aα-chain amyloidosis

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Background:
Fibrinogen Aα-chain (AFib) amyloidosis is an autosomal dominant hereditary disease characterized by renal amyloid deposits caused by variants of fibrinogen Aα-chain gene (FGA). AFib amyloidosis patients have been mainly reported in Western countries. Therefore, the clinical pictures of Asian AFib amyloidosis are not full understood.

Objective: In this study, we aimed to investigate clinicopathological characteristics of a Japanese case with AFib amyloidosis associated with a rare frameshift *FGA* variant, p.Arg547GlyfsTer21.

Material & Methods: We evaluated the clinical and pathological features of a Japanese patient with renal amyloidosis by means of histological methods, proteomic analyses and DNA sequencing analysis.

Results: The case was a 38-year-old Japanese woman who was diagnosed with hypertension, proteinuria and renal dysfunction. She did not present with sensory neuropathy, muscle weakness, autonomic dysfunction, and eye manifestation. Nerve conduction study showed normal results on all tested nerves. Electrocardiogram revealed normal sinus rhythm with no abnormal findings. Echocardiography did not show concentric ventricular hypertrophy and dystelectasis with a granular sparkling pattern. Myocardial technetium-99m-pyrophosphate scintigraphy was negative. Abdominal fat aspirate and endoscopic biopsy of the stomach and duodenum demonstrated no amyloid deposits by Congo red staining. Renal biopsy revealed massive amyloid deposits in only glomeruli demonstrated by Congo red staining. Antibodies against immunoglobulin light chain λ, κ, amyloid A, and transthyretin did not react with amyloid deposits in the glomeruli by immunohistochemical staining. Antibodies against fibrinogen reacted with amyloid deposits. We performed proteomic analyses using laser microdissection and liquid chromatography-tandem mass spectrometry and tryptic peptides derived from the fibrinogen Aα-chain were detected. DNA sequencing analysis of exon 5 of *FGA* revealed a rare heterozygous frameshift variant, p.Arg547GlyfsTer21.

Summary & Conclusion: We have reported a Japanese case with AFib amyloidosis associated with a rare frameshift *FGA* variant, p.Arg547GlyfsTer21. The clinical pictures of our patient were corresponding to those of patients with AFib reported from Western countries.

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Hereditary Gelsolin amyloidosis: Clinical features from a large cohort in the US

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Background: Hereditary gelsolin (AGel) amyloidosis is a form of systemic amyloidosis, an autosomal dominant condition characterized by progressive neurologic, ophthalmologic, and dermatologic manifestations. Common manifestations include corneal lattice dystrophy type II, cranial neuropathies, and cutis laxa that present in the third and fourth decade of life. [1] In addition to cranial neuropathies, neurologic manifestations include sensory neuropathy, carpal tunnel syndrome, and autonomic dysfunction. [2-5] Polyneuropathy and carpal tunnel syndrome are described to occur in 71% and 40% of patients with AGel amyloidosis, respectively. [6] Recent studies have demonstrated that AGel amyloidosis is truly a systemic disease with soft tissue, cardiac, and renal manifestations as well. [7]

Objective: To describe the clinical manifestations and evaluate quality of life measures of patients with AGel amyloidosis in the United States.

Material & Methods: We report on 14 patients with systemic AGel amyloidosis who visited the Amyloidosis Center at Boston University between 2005 - 2018. Data collected from the prospectively maintained Amyloidosis Center database, along with electronic medical records, and patient phone interviews were used. All patients had histological evidence of AGel amyloidosis along with genetic sequencing. Health related quality of life (HRQoL) was assessed using the SF-36 health survey. The Institutional Review Board of Boston University Medical Center approved the study. All patients provided written consent for research under the approval of the Institutional Review Board and in accordance with the Declaration of Helsinki (ClinicalTrials.gov Identifier: NCT00898235).

Results: The median age at symptom onset was 40 years (range; 21-62). Of 14 patients, 2 (14%) self-identified as Finnish ethnicity and 5 (36%) as German ethnicity. Majority (79%, 11/14) had a known family history of AGel amyloidosis. Other patient characteristics are summarized in table 1.The most common presenting symptom was corneal lattice dystrophy, which was reported in 79% (11/14) patients. Cutis laxa was seen in 57% (8/14) patients, and of these 63% (5/8) required plastic surgery. Of the neurologic manifestations, 57% (8/14) had polyneuropathy. Other etiologies of neuropathy including diabetes, B12 deficiency, and thyroid abnormalities were present in only 1 out of the 8 patients with polyneuropathy. Bilateral carpal tunnel syndrome was noted in 79% (11/14) patients. Fifty- seven percent (8/14) patients had autonomic dysfunction, 35% (5/14) had orthostatic hypotension. Other symptoms are presented in table 2. Interestingly, based on this small, U.S. based sample, we did not observe meaningful deficits in generic HRQoL relative to general population.

Summary & Conclusion: We describe systemic and clinical manifestations of patients with AGel amyloidosis with a focus on neurological manifestations. Ophthalmologic symptoms, specifically corneal lattice dystrophy was the most common presenting symptom in our population. Given the symptom profile for this disease, it is possible that a generic measure of HRQoL is not sensitive enough to capture the full impact of the disease. Considering the uniqueness of this disease an AGel amyloidosis specific patient reported outcomes (PRO) tool or a constellation of symptom-specific PROs may be helpful to adequately measure concepts important to this disease area.

Table 1: Patient characteristics

Baseline characteristics	N= 14
Male (%)	3 (21)
Median age at diagnosis (range)	50 (31-78)
Median age at symptom onset (range)	40 (21-62)
Confirmed alive (%)	12 (85.7)
Abdominal fat aspirate positive	12 (85.7)
Genetic profile	
p. Asp187Asn (D187N) (%)	13 (92)
p.Tyr447His (Y36H) (%)	1 (8)
Heterozygous (%)	14 (100)
Organ involvement	
Heart %	1 (7)
Kidney %	2 (14)
PN %	8 (57)
AN %	8 (57)
Skin %	8 (57)
Eye (CLD) %	11(79)
Soft tissue %	11(79)

Abbreviations: CLD, corneal lattice dystrophy PN, peripheral neuropathy AN, autonomic neuropathy

Table 2 Common symptoms of AGel amyloidosis

Ophthalmologic manifestations
Dry Eyes (64%)
Corneal lattice dystrophy (79%)
Impaired vision (50%)
Corneal ulcer (one or more) (57%)
Neurologic manifestations
Facial nerve paresis (57%)
Polyneuropathy (57%)
Dysarthria (42%)
Dysphagia (50%)
Muscle weakness (28%)
Imbalance (42%)
Autonomic dysfunction
Dry Skin (50%)
Orthostasis (35%)
Constipation (42%)
Diarrhea (14%)
Dermatologic manifestations
Cutis laxa (57%)
Drooping eyelids (57%)
Other
Carpal Tunnel Syndrome (57%)
Breast masses (14%)
Proteinuria (14%)
Heart Failure (14%)
Impaired hearing (28%)
Drooling (50%)
Trouble chewing (21%)

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Siblings with a novel type of amyloid neuropathy, hereditary Aβ2M amyloid neuropathy: Report of the second family in the world

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Background: To date, only the two families with hereditary Aβ2M amyloidosis have been reported in the world [1, 2]. One familiy was associated with neuropathy [1] and the other was not [2]. Detail about hereditary Aβ2M amyloidosis remains unclear.

Objective: To elucidate the genetic and clinical characteristics of a family with hereditary Aβ2M amyloid neuropathy, the second family in the world, diagnosed in our department.

Patients & Material & Methods: Older brother: Finger numbness and watery diarrhea appeared in his 60s. Numbness in the toes appeared in the 70s. He visited a local hospital a few years later. Autonomic neuropathy (orthostatic hypotension, diarrhea, dysuria), numbness of limbs, and bilateral carpal tunnel syndrome were observed. Gastroduodenal mucosa biopsy revealed amyloid deposition and he was referred to our department. Immunohistochemical staining of the biopsied tissue was negative for AA, ALκ, ALλ, and ATTR. AH or Aβ2M amyloidosis was suspected in the mass spectrometry analysis. However, the amount of amyloid deposition was too small to perform further analysis. Younger brother: Alternate constipation and diarrhea appeared in his 70s. Orthostatic hypotension appeared several years later. Carpal tunnel syndrome appeared in the next year. He visited a local hospital and amyloid deposition was detected in the gastric mucosa biopsy. He was referred to our department for the diagnosis. Immunohistochemical staining of the biopsied tissue was negative for AA, ALκ, ALλ, and ATTR. Mass spectrometry analysis suggested ALκ, but no definitive diagnosis was made. Renal dysfunction appeared in the next year. A few years later, he underwent renal biopsy and was found to have amyloid deposition. His clinical course was not consistent with AL type, so that further analysis was performed.

Results: Immunostaining with major amyloid proteins revealed Aβ2M amyloid deposition in the kidney sample of the younger brother (Fig. 1 left). Aβ2M amyloid deposition was also detected in the re-evaluated gastrointestinal mucosa sample of the older brother (Fig. 1 right). A known D76N (p.D96N) variant in *B2M* gene [1] was identified in both siblings (Fig. 2) and the final diagnosis of hereditary Aβ2M amyloidosis was made.

Summary & Conclusion: Hereditary Aβ2M amyloidosis has been reported in two families (D76N and V27M) so far [1, 2]. Similar to the previously reported family with D76N variant [1], the clinical picture of our family was dominant autonomic nerve involvement, which was similar to that of hereditary ATTR amyloidosis. Hereditary Aβ2M amyloidosis is a new important form of non-ATTR amyloidosis that presents hereditary amyloid polyneuropathy.

Figure 1

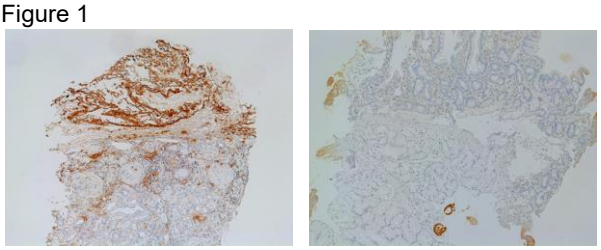

Figure 2

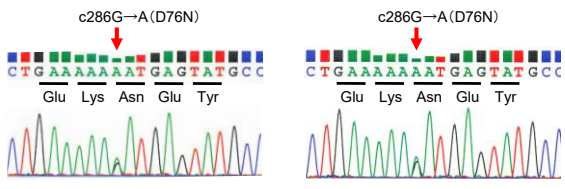

Figure 1.: The kidney sample of the younger brother (left) and the gastrointestinal mucosa sample of the older brother (right) show anti-β2M immunostaining positive amyloid deposition, respectively.

Figure 2.: A direct sequence analysis of the *B2M* gene for the variant (c.286 G>A: p. D96N) was performed and the both patients were found to be heterozygous for this variant.

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Apolipoprotein A-IV amyloidosis in a Cotton-top tamarin (*Saguinus oedipus*)

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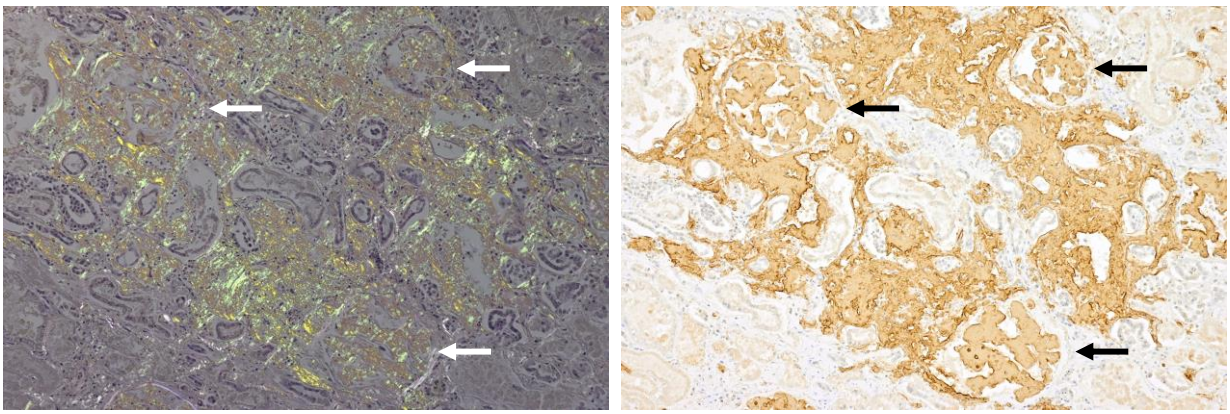
Background & Objective: In humans, apolipoprotein A-IV (APOA4) amyloidosis is an uncommon form of systemic amyloidoses that results in kidney and heart dysfunction. In animals, however, APOA4 amyloidosis has never been reported. In this study, we encountered a cottontail tamarin with systemic amyloidosis and analyzed its pathogenesis.

Material & Methods: The cottontop tamarin, which had died at the zoo, were necropsied, and the whole body tissues except for brain were formalin-fixed and paraffin-embedded for histological analyses. Amyloid deposits were confirmed by polarized light microscopy of Congo red-stained tissues. Congo red-positive regions were dissected from tissue sections of spleen, kidney, and adrenal glands, and LC/MS/MS was performed. The MS/MS data was analyzed using Mascot server with the protein database of common marmoset (*Callithrix jacchus*). Immunohistochemistry was performed using anti-APOA4, serum amyloid A, λ light chain, κ light chain and transthyretin antibody.

Results: Histologically, amyloid deposits were observed in vessel walls and perivascular stroma throughout the body. In the kidney, amyloid deposition was severe in the glomeruli and cortical interstitium and mild in the medulla. In the liver, severe deposition was observed in the space of Disse. In the spleen and adrenal glands, there was severe amyloid deposition displacing parenchyma. In the heart, moderate deposition was seen in the walls of small vessels and in the interstitium. In the lungs, thrombi derived from amyloid were frequently observed. In the intestine, moderate deposition was observed in the lamina propria. By mass spectrometry analysis, APOA4 was detected at high levels in all tissues. With the exception of ApoAIV, no amyloidogenic proteins were detected. Apolipoprotein E, clusterin, and vitronectin were also detected as amyloid signature proteins in all tissues. Immunohistochemistry showed that systemic amyloid deposits were positive for ApoAIV and negative for serum amyloid A, λ light chain, κ light chain, and transthyretin.

Summary & Conclusion: The Mayo Clinic has defined the diagnostic criteria for APOA4 amyloidosis based on mass spectrometry as a high number of APOA4 peptides detected by MS and the absence of other amyloidogenic proteins (1). According to this criteria, in this study, we made a definitive diagnosis of APOA4 amyloidosis in this case. This is the first report of APOA4 amyloidosis in animals. In human APOA4 amyloidosis, amyloid deposition in the kidney is severe in the medulla and rare in the glomeruli. On the other hand, the histological characteristics seen in the kidney of cottontop tamarins differed significantly from the distribution of amyloid deposition in humans. A more detailed search for the reason for this will be necessary in the future. In humans, genetic mutations are thought to have a small effect on the development of APOA4 amyloidosis (2). Currently, genetic analysis of this case is underway to verify the genetic mutation.



Figures: Histolgocal feature of amyloid deposition in the renal cortex. The left and right images show the same area in serial sections. Arrows indicate glomeruli. Left image: Amyloid deposits stained with Congo red show yellow to green birefringence under polarized light. Right image: Amyloid deposits are positive for APOA4 by immunohistochemistry.

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Support & Funding: This research was supported by the JSPS KAKENHI (grant no. 20K15660), the Program on Open Innovation Platform with Enterprises, Research Institute and Academia (OPERA) from JST, and the Smart-Core-Facility Promotion Organization in the Tokyo University of Agriculture and Technology.

Histological and proteomic analyses of semenogelin 1 amyloid deposits

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Background: Seminal vesicle is one of the most common organs observed with amyloid deposits in elderly men [1,2]. Seminal vesicle amyloid has been found in 21% of men over the age of 75 years [2]. Semenogelin 1 (Sg1) has been identified from seminal vesicle amyloid and considered as most common amyloid precursor protein of the amyloid fibrils [1]. However, it's difficult to identify Sg1 amyloid deposits in general hospital, due to a poor reactively of commercially available antibodies to Sg1 amyloid for immunohistochemical staining. Therefore, the pathogenesis is still not fully understood.

Objective: The purpose of this study was to investigate the components of Sg1 amyloid deposits by mass spectrometry and evalute the reactivity of antibodies to Sg1 amyloid for immunohistochemistry.

Material & Methods: For this study, seminal vesicle tissues from 5 Japanese cases with Sg1 amyloid were used. To identify proteins from Sg1 amyloid in tissue sections, LMD-MS (LMD7000; Leica Microsystems; LTQ Velos Pro; Thermo Fisher Scientific) were performed. Antibodies against N-teminus of Sg1 were produced. The reactivities of antibodies againgst Sg1 were evaluated by immunohistochemical staining.

Results: Tryptic peptides derived from near N-terminus of Sg1 were widely detected. In contrast, peptides derived from near C-terminus (p.322-462) were not detected. The number of peptides derived from midle region (p.138-321) was very small. Amyloid signature proteins, such as apolipoprotein E, serum amyloid P component, clusterin and vitronectin were high frequently identified. Newly generated antibodies against Sg1 clearly reacted with all the regions, where Congo red were positive in seminal vesicle.

Summary & Conclusion: N-terminal region of Sg1 was main component of Sg1 amyloid as previously reported [1]. As well as other types of amyloidosis, amyloid signature proteins coexisted with Sg1 amyloid. Antibodies against N-terminal region of Sg1 would be useful for immunohistochemical staining.

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Mass spectrometry-based proteomic analysis of adsorbed molecules related with dialysis-related amyloidosis in hexadecyl-immobilized cellulose beads.

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Background: Dialysis-related amyloidosis (DRA) is a serious complication in chronic kidney disease patients undergoing long-term hemodialysis (HD). β_2-microglobulin (β_2-m)-related amyloid deposition induces osteoarticular disorders including carpal tunnel syndrome while molecular interactions in the lesion is still incompletely understood. Direct hemoperfusion with a column containing hexadecyl-immobilized cellulose beads (HICB) is used to adsorb circulating β_2-m to inhibit the progression of DRA. As use of the column improves joint pain and physical functions; it is possible that the column adsorbs not only β_2-m but also other molecules associated with amyloidogenesis.

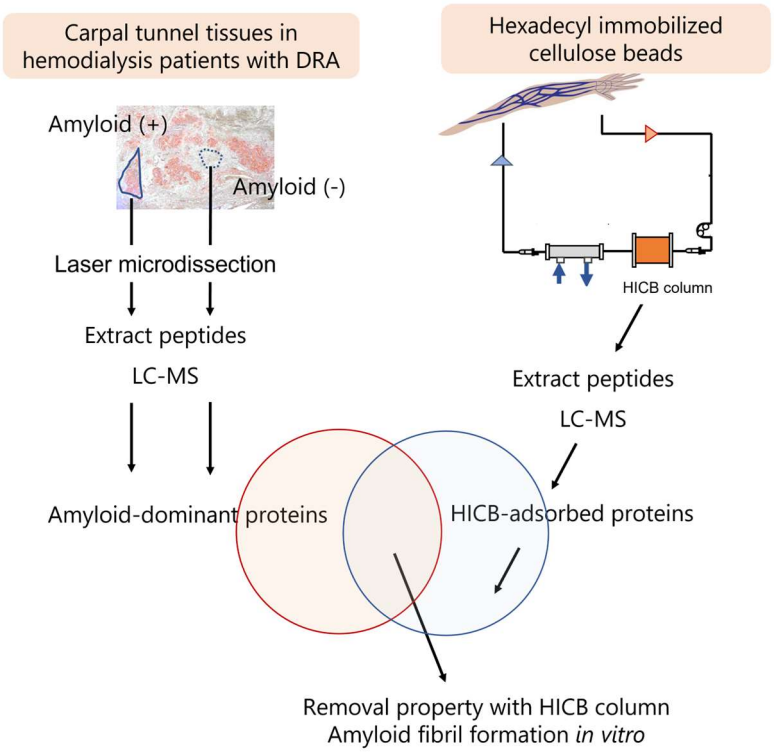
Objective: To find DRA-related proteins adsorbed with HICB column for in end-stage kidney disease patients.

Material & Methods: The amyloid tissue deposition in the carpal tunnel in the HD patients (n = 8) was corrected using laser microdissection and examined on liquid chromatography-linked mass chromatography. We included 14 HD patients with DRA. Proteins were extracted from the HICB-containing column after treatment and identified using liquid chromatography-linked mass chromatography. We measured the adsorption rate of the proteins detected by proteomics, and compared it with those in the patients undergoing HD and hemodiafiltration (HDF). The protein profiles were compared between the HICB-containing column and the amyloid regions. Among the proteins, we selected several molecules and examined immunohistochemistry in the amyloid tissue and the effect on amyloidogenesis using isothermal titration calorimetry and ultrasonic assays of amyloid fibril formation using HANABI-2000.

Results: With high confidence criteria, 52 proteins were identified ias dominant molecules in the amyloid deposited in the carpal tunnel. Supra molecular fiber organization and amyloid fiber formation were major pathways in those proteins. The number of proteins adsorbed by the HICB were identified 200 which 21 proteins were also found in the amyloid lesions in carpal tunnel tissues (e.g., β_2-m SIN, 193.8 ± 143.4; lysozyme SIN, 156.5 ± 47.8). After passing the HICB-containing column, the serum levels of several proteins were decreased as compared with those in the HD dialyzer and HDF hemofilter (e.g., adsorption rate of β_2-m, 80.5% ± 9.8% vs 38.0% ± 25.5% [HD] and 25.0% ± 14.6% [HDF], $p < 0.01$; lysozyme, 79.2% ± 10.9% vs 15.8% ± 18.8% [HD] and 10.0% ± 13.4% [HDF], $p < 0.01$). *In vitro* study showed that lysozyme interacted to β_2-m monomer and inhibited β_2-m amyloid fibril formatoin.

Summary & Conclusion: The HICB-containing column adsorbed various proteins in the HD patients with DRA, of which some were found in the lesions with amyloid deposition and associated with amyloidogenesis. The results suggest that direct hemoperfusion with the HICB-containing column contributes to the improvement of DRA by reducing the levels of related proteins.

Support & Funding: This study was conducted through collaboration between Niigata University and Kaneka Co. The authors declare no other potential competing financial interests.


Carpal tunnel tissues in hemodialysis patients with DRA
Amyloid (+)
Amyloid (-)
Laser microdissection
Extract peptides
LC-MS
Amyloid-dominant proteins
Hexadecyl immobilized cellulose beads
HICB column
Extract peptides
LC-MS
HICB-adsorbed proteins
Removal property with HICB column
Amyloid fibril formation *in vitro*

Echocardiographic findings in subjects with an amyloidogenic apolipoprotein A1 mutation

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Background: The *APOA1* gene encodes the precursor of apolipoprotein AI (ApoAI). Very small case series of patients with ApoAI amyloidosis are available. **Objective:** To characterize cardiac involvement in subjects with a novel ApoA1 mutation. **Material & Methods:** We describe clinical and echocardiographic characteristics of individuals heterozygous for the *APOA1* Leu75Pro mutation, referred to our institute for cardiac screening. **Results & Discussion:** We enrolled 189 subjects, 54% men, median age 55 years (interquartile range [IQR] 42-67), 39% with concomitant renal disease and 31% with liver disease. Median left ventricular ejection fraction was 60% (IQR, 55-66). Enrolled subjects did not show major signs of diastolic dysfunction nor LV hypertrophy. Age correlated with interventricular septal (IVS) thickness ($r=0.484$), LV mass index ($r=0.459$), E/e' ($r=0.501$), and right ventricular free wall thickness ($r=0.594$) (all $p<0.001$). Some individuals displayed red flags for cardiac amyloidosis (CA), "granular sparkling" of the IVS, 19%; pericardial effusion, 11%; apical sparing, 10%; thickened atrioventricular valves, 8%, and 14% met non-invasive criteria for CA. Twenty-nine subjects died over 5.8 years (IQR 4.1-8.0 years), with 10 deaths for cardiovascular causes; 14 out of 182 (8%) subjects had HF hospitalization and 17 (9%) had cardiovascular death or HF hospitalization. Individuals with suspected CA had a much higher risk of all-cause death ($p=0.009$), cardiovascular death ($p=0.001$), cardiovascular death or HF hospitalization ($p<0.001$), and HF hospitalization alone ($p<0.001$). Subjects with both renal and liver involvement had the greatest cardiac involvement, and shortest survival.
Summary & Conclusions: Subjects with the *APOA1* Leu75Pro mutation displayed minor echocardiographic signs of cardiac involvement, but 14% met non-invasive criteria for CA. Subjects with suspected CA had a worse outcome.

Figures

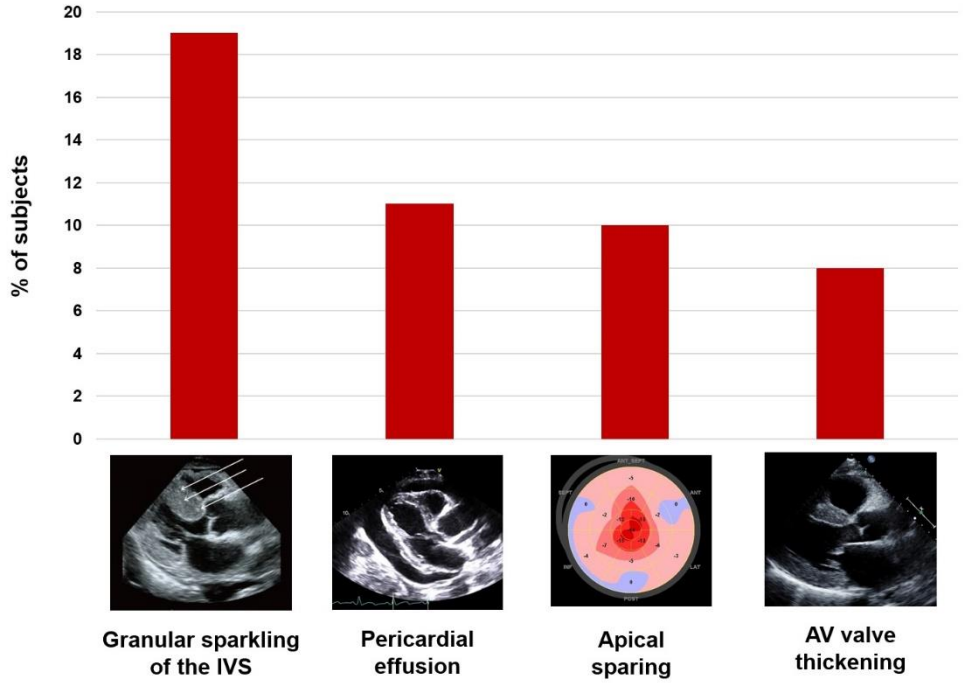

Figure 1. Prevalence of echocardiographic red flags of cardiac amyloidosis.
AV, atrioventricular; IVS, interventricular septum.

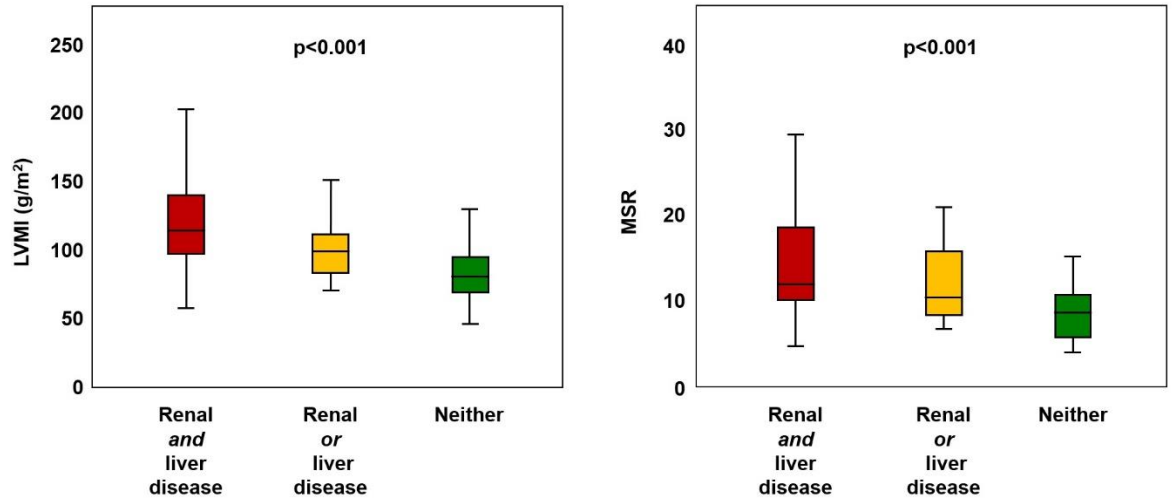

Figure 2. Renal and/or liver disease, left ventricular mass index (LVMI) and LV mass-to-strain ratio (MSR).

Support & Funding: None.

Iatrogenic cerebral amyloid angiopathy

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Background: Cerebral amyloid angiopathy (CAA) is characterized by recurrent intracerebral hemorrhages and cortical superificial siderosis and is only rarely found in patients younger than 55 years of age. Since 2015, evidence has risen of human to human transmission of beta-amyloid during (neuro-)surgical procedures and other forms. Iatrogenic CAA ermerges as a new disease entitiy, with common and distinct features from spontaneous CAA.

Objective: To describe case characteristics of three individual patients with iatrogenic CAA treated at our Neurological Department and to summerize current knowledge on iatrogenic CAA by a literature review.

Material & Methods: Review of case specific hospital archived data including detailed radiological analyses. Literature review.

Results: Three cases of young adults (two male, 37 and 42 years old, and one female, 36-year-old) have been identified, who experienced recurrent lobar intracerebral hemorrhages and who were diagnosed with cerebral amyloid angiopathy. Diagnosis was confirmed by histological examination and Aβ immunostaining of leptomeningeal biopsies. All patients had suffered from a severe traumatic brain injury (TBI) in childhood. Two of the three patients have meanwhile decased. In the patient presenting most recently, Florbetaben PET-CT showed temporal and frontal enhancement in the gray matter, in concordance with histopathological findings. Genetic testing (APP, ITM2B, and CST3) found no mutation associated with cerebral amyloidosis. ApoE allele was ε2/ε3. During the differential diagnostic work-up, in one patient the CSF was analyzed, revealing increased levels of Tau (>2200 pg/ml, norm < 450 pg/ml) and Aβ-1-42 (351 pg/ml, norm > 450 pg/ml). Between 2011 and 2020, the total number of cerebral microbleeds, all located in lobar regions, increased from 4 to 161 and continues to increase now, but at a slower pace. Literature review revealed 15 cases of assumed iatrogenic CAA. There are distinct features from sporadic CAA, including the younger age of onset, less commonly transient focal neurological episodes, and a higher rate of ICH recurrence. Also the distribution of ApoE genotypes is different, with ApoE ε3 being present in all casess of iatrogenic CAA published until today.

Summary & Conclusion: Iatrogenic CAA is an emerging new disease entity with distinct features from sporadic CAA. The real prevalence is unknown. No causal therapy exists. Avoiding transmission of Beta-Amyloid during surgery and interventions is of great importance, and preventive measures exists.

Support & Funding: No funding.

Fibrinogen A alpha-chain amyloidosis journey: dialysis and kidney transplantation interface

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Background: Fibrinogen A alpha-chain amyloidosis due to mutations in the fibrinogen A alpha-chain gene (*FGA*) is reported as one of most common type of hereditary amyloidosis with kidney involvement. Patients develop chronic kidney disease (CKD) progressing to end-stage (ESKD) usually within 5 years after diagnosis. The absence of bleeding disorders and normal clot formation suggests clotting function is not impaired. Indication for isolated kidney transplantation (KT) is controversial, as amyloid deposits may relapse in the graft leading to its failure. Because fibrinogen is produced in the liver, some authors recommend combined liver-kidney transplantation to avoid recurrence. However, the type of *FGA* mutation appears to influence the natural history, as patients with the most common one, the E526V (p.Glu545Val), have better outcomes after KT.

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Objective: The main aim of this study is to identify the clinical characteristics and limiting factors of the success of renal replacement therapy (RRT) in AFib amyloidosis, namely kidney transplantation. Our purpose is to report the comorbidity before and after RRT; to evaluate *de novo* manifestations; to identify the short and long-term complications after dialysis and kidney transplantation; to determine the survival of kidney grafts, and causes of graft losses.

Material & Methods: Retrospective study based on clinical data from the first registry (1990) to April 2022. Twelve Portuguese patients were selected. All had AFib amyloid deposits, documented E526V (p.Glu545Val) mutation, and ESKD treated by either dialysis and/or renal transplantation. We registered one homozygous and 11 heterozygous patients.

Results: All patients had hypertension and nephrotic proteinuria, with no deviations in coagulation tests (Table 1, clinical features). Fibrinogen levels were normal in the heterozygous patients, but low in the homozygous case. A misdiagnosis occurred in seven patients: two heavy chain deposition disease, three amyloid light-chain amyloidosis, one AA amyloidosis, one interstitial nephritis. *De novo* splenomegaly developed in one patient and hepatomegaly in another; the homozygous patient developed diffuse, progressing and massive splenic calcification. Intraventicular septal hypertrophy was diagnosed in two patients after diagnosis. Additionally, eight patients had left atrium (LA) enlargement at the end of follow-up, despite atrial fibrillation being detected in only two of them. RRT, in all patients hemodialysis first, was started, on average, at 58.3 (44-69) years. KT was performed, on average, 2.5 years later (N=10), with a 30% graft nephrectomy rate in the first 6 months after transplantation due to uncontrolled allograft bleeding (Table 2). Of note, all unsuccessful KT patients were previously medicated with acetylsalicylic acid and clopidogrel/heparin based on preceding thrombotic/ischemic events.

Summary & Conclusion: Our series reinforces that AFib amyloidosis is a systemic disease. In our series, it was not the relapse of amyloidosis that conditioned the success of kidney transplantation, but the difficult balance between ischemic events, need for hypocoagulation and subsequent bleeding of the grafts. AFib amyloidosis, namely E526V variant, may present a concurrent susceptibility to antigoagulation agents, leading to early graft bleeding. A better study should be developed to be a helping guide for a successful transplantation.

	Total cases (N=12)
Age, y	56.3 (44-68)
Hypertension	12 (100%)
Proteinuria, g/d	6.45[a] (4-8.9)
Serum creatinine mg/dL	3.4[b] (0.7-7.8)
Hepatomegaly	2 (16.7%)
Splenomegaly	1 (8.3%)
Sensory peripheral neuropathy	5 (41.7%)
LV hypertrophy	6[a] (60%)
LA enlargement	4[c] (44.4%)
IV septal hypertrophy	4[c] (44.4%)

.

Thrombotic/ hemorrhagic events	N. patients (11)
Acute coronary syndrome	2
Ischemic stroke[a]/TIA	5/2
Central retinal artery thrombosis	1
AV fistula /CVC thrombosis	3
Pulmonary emboly	1
Deep/superficial vein thrombosis	3
Kidney allograft vein thrombosis	1
AV fistula/prothesis hematoma	2
Kidney allograft hemorrhage	3
Gastrointestinal hemorrhage	3
Native kidney/splenic rupture	2

Table 1.: Main clinical characteristics of AFib amyloidosis patients at presentation. **Note:** Values for categorical variables give as mean; for continuous variables, as count (%). Abbreviations: AFib amyloidosis, fibrinogen A alpha-chain amyloidosis; US, ultrasound; CKD, chronic kidney disease, LV, Left ventricle; LA, left atrium; IV, Interventricular. [a]Missing data for 2 patients. [b]Missing data for 1 patient. [c]Missing data for three patients.

Table 2.: Thrombotic and hemorrhagic events in AFib amyloidosis patients during follow-up. **Note:** 6 patients reported one or more thrombotic and hemorrhagic events. Abbreviations: AV, Arteriovenous; CVC, central vein catheter; TIA, Transient ischemic attack; RV, right ventricle; CT, computed tomography; MRI, magnetic resonance imaging. [a]Ischemic stroke includes 4 lacunar strokes detected on CT/MRI.

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Support & Funding: none.

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NNC6019-0001, a humanized monoclonal antibody, in patients with transthyretin amyloid cardiomyopathy (ATTR-CM): rationale and study design of a phase 2, randomized, placebo-controlled trial

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Background: Transthyretin amyloid cardiomyopathy (ATTR-CM) is a chronic condition associated with progressive heart failure, resulting from extracellular deposition of misfolded transthyretin (TTR) protein as amyloid fibrils in the myocardium. Currently, there are few disease-modifying treatments. NNC6019-0001 is a humanized monoclonal antibody designed to deplete amyloid via antibody-mediated phagocytosis by targeting a unique epitope that is exposed only on misfolded monomeric and aggregated forms of TTR. In a phase 1, open-label, 3-month dose escalation trial, NNC6019-0001 was well tolerated at all doses tested (up to and including 30 mg/kg).[1] The maximum tolerated dose was not reached. Exploratory cardiac endpoints were stable or indicated a possible benefit.

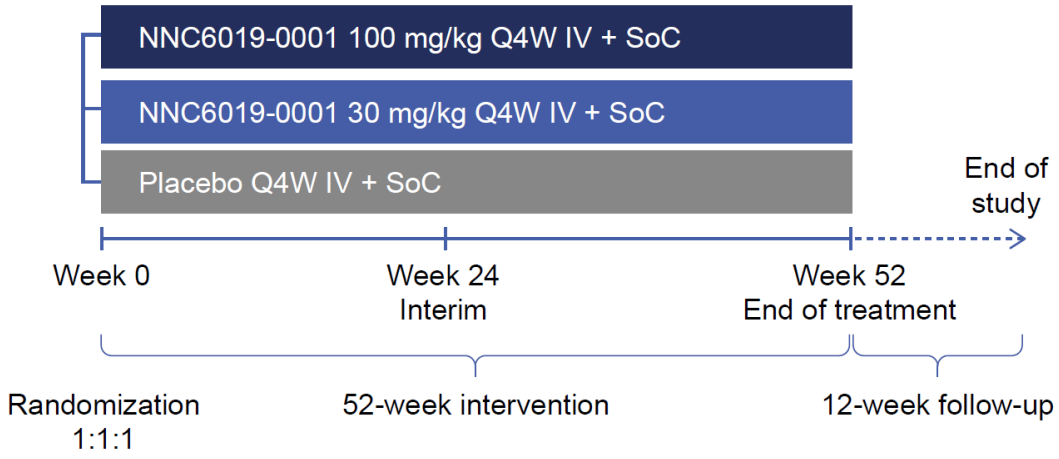
Objective: To evaluate the effect of NNC6019-0001 30 mg/kg and 100 mg/kg on cardiac functional endpoints and predictive biomarkers in patients with ATTR-CM, and to assess pharmacokinetics, safety and tolerability, to establish the optimal dose for a phase 3 trial.

Material & Methods: This is a randomized, double-blind, placebo-controlled trial recruiting 99 patients with hereditary or wild-type ATTR-CM (Figure). Inclusion criteria are New York Heart Association (NYHA) class II or III heart failure, left ventricle wall thickening (LVWT) ≥ 12 mm, N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels ≥ 650 pg/mL in sinus rhythm and > 1000 pg/mL in atrial fibrillation, and a 6-minute walk test (6MWT) distance of 150–450 m. Patients will be randomly assigned to receive intravenous NNC6019-0001 30 mg/kg or 100 mg/kg or placebo, each in addition to standard of care, every 4 weeks for 52 weeks, followed by a 12-week follow-up. In a sentinel dosing phase, three patients per arm will receive the study drug or placebo, in combination with 24-hour inpatient cardiac monitoring and 7 days of continuous cardiac (tele-) monitoring. The primary endpoints are change from baseline to week 52 in 6MWT and in NT-proBNP levels. Secondary endpoints include cardiac measures: extracellular volume on cardiac magnetic resonance imaging, global longitudinal strain, troponin T levels, hospitalization due to cardiovascular events, and urgent visits due to heart failure. Quality of life will be assessed using the Kansas City Cardiomyopathy Questionnaire (KCCQ). All-cause mortality, pharmacokinetics and treatment-emergent adverse events will also be assessed.

Results: The trial will start mid-2022 with global recruitment.

Summary & Conclusion: Disease-modifying treatments are needed for patients with ATTR-CM, where treatment is often limited to managing symptoms and best supportive care; the first disease-modifying therapies recently became available. This phase 2 trial will be used to determine the appropriate dose for the phase 3 trial of NNC6019-0001, a novel antibody therapy designed to deplete amyloid in patients with ATTR-CM.
Note: This abstract was also submitted to the European Society of Cardiology Congress 2022, 26–29 August 2022, Barcelona, Spain.

Figure 1.: Design of the phase 2 trial of NNC6019-0001 in patients with ATTR-CM.



ATTR-CM, transthyretin amyloid cardiomyopathy; IV, intravenous; Q4W, every 4 weeks; SoC, standard of care.

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Efficacy and safety of belantamab mafodotin monotherapy in patients with relapsed or refractory light chain amyloidosis: a phase 2 study by the European Myeloma Network

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Background: Managing patients (pts) with relapsed/refractory (RR) light chain (AL) amyloidosis is challenging, as there is no current standard treatment, many available options are associated with low efficacy & toxicity, and options for daratumumab (DARA)- & bortezomib-exposed pts are limited[1]. Belantamab mafodotin (belamaf), a multi-modal antibody-drug conjugate targeting BCMA, has shown efficacy & tolerability in heavily pretreated pts with RR multiple myeloma (MM), including those refractory to DARA[2]. Since clonal plasma cells in AL amyloidosis & MM are phenotypically similar, belamaf could be a novel treatment option in AL amyloidosis.

Objective: To evaluate the efficacy & safety of belamaf monotherapy off-label in pts with RR AL amyloidosis.

Material & Methods: The ongoing prospective, open-label, multinational, phase 2, EMN27 study (NCT04617925) aims to enroll 36 adult pretreated pts with AL amyloidosis who require therapy. Pts at Mayo cardiac stage 3b are excluded. Belamaf monotherapy at 2.5mg/kg is administered by intravenous infusion every 6 weeks for a maximum of 8 cycles; dosing can be reduced to 1.92mg/kg for toxicity. Per study design, a safety analysis (after 6 pts received ≥1 treatment cycle) & an efficacy analysis (after 13 pts are enrolled) were planned. The safety analysis revealed no new safety signals, and pt accrual continued to 13 pts. The efficacy analysis is currently conducted; however, already 3 pts achieved complete response, or very good partial response (VGPR), or low difference of involved to uninvolved serum free light chains (dFLC) response and enrollment is continuing to include all planned pts. This descriptive analysis included pts initiating study treatment ≥3 months before the cut-off date (15/01/2022).

Results: Of 11 pts included in the analysis, 4 (36.4%) continued treatment by the cut-off date, and 7 (63.6%) discontinued (disease progression: 5 [45.5%], death: 2 [18.2%]). The pts median age was 69.0 years (range 46.0–80.0), and most were males (7, 63.6%). At baseline, 3 (27.3%) and 8 (72.7%) pts had New York Heart Association class I & II symptoms, respectively; the median N-terminal pro-brain natriuretic peptide, high-sensitivity troponin T, and dFLC were 1,979 pg/mL (range 190.0–4,135.0), 41.6 pg/mL (range 11.0–80.8), and 34.2mg/dl (range 4.4–279.1), respectively. Except for the heart, commonly involved organs were the nervous system (4 pts, 36.4%) and the soft tissue (2 pts, 18.2%). The median number of previous AL amyloidosis treatments was 3.0 (range 1.0–7.0), including DARA. The median duration of belamaf therapy was 3.1 months (range 1.4–5.7). At a median follow up of 9.4 months (range 3.1–10.0), the overall response rate was 72.7% (8 pts; VGPR: 27.3% [3 pts] and partial response: 45.5% [5 pts]). Median time to first hematological response was 8.5 days (range 1.0–28.0) and to VGPR or better 15.0 days (range 8.0–15.0). The 3-month organ (heart, kidney, or liver) response rate was 36.4% (4 pts). All pts had ≥1 non serious adverse event (SAE). Four (36.4%) pts had ≥1 SAE, including 2 (18.2%) pts with a belamaf-related grade 2 & 4 visual impairment (1 [9.1%] pt each). Eight (72.7%) pts had ≥1 adverse event of special interest. Two (18.2%) pts had a fatal SAE (pneumonia & intestinal perforation, 1 [9.1%] pt each), both unrelated to belamaf.

Summary & Conclusions: In this prospective study, belamaf monotherapy induced rapid, clinically meaningful responses with a manageable safety profile in heavily pretreated pts with RR AL amyloidosis. As the study progresses, additional data will be generated.

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Glavonoid, a possible prophylactic supplement for ATTR amyloidosis

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Background: Transthyretin (TTR) is an amyloidogenic protein associated with hereditary and nonhereditary transthyretin amyloidoses (ATTR). Dissociation of the tetramer of TTR to the monomer induces TTR misfolding, which leads to amyloid fibril formation and triggers the onset of ATTR amyloidosis. Stabilizers of tetrameric TTR have been accepted as an effective ATTR amyloidosis treatment while effect is limited and they are too expensive. The aim of our study was to find more effective and cheep natural compound to suppress TTR amyloid formation. Glabridin, a prenylated isoflavan isolated from *Glycyrrhiza glabra* L., stabilized the TTR tetramer in vitro. The effects of licorice-derived flavonoid oil—Glavonoid, a natural substance that includes glabridin and several polyphenols—on stabilizing the TTR tetramer must still be elucidated.

Objective: To examine plasma TTR stabilization by Glavonoid in vitro and in vivo.

Material & Methods: We used plasma of healthy volunteers to analyze the TTR-stabilizing effect of glabridin and Glavonoid in vitro. We also analyzed the plasma glabridin concentration in healthy volunteers. Next, to examine the stabilization effect of Glavonoid in vivo, we administered 300 of the supplement to healthy volunteers for 12 weeks, and we evaluated changes in the forms of TTR in plasma.

Results: Glavonoid mixed with human plasma samples at 24 h incubation in vitro increased the tetramer level ($P < 0.05$) and reduced the monomer level ($P < 0.01$). Glabridin alone, a major components of Glavonoid could not show such effects. Oral Glavonoid (300 mg/ day for 12 weeks) in healthy volunteers effectively increased the plasma glabridin concentration, and increased the TTR tetramer level and reduced the monomer/tetramer ratio of TTR ($P < 0.05$) in plasma, compared to those of age matched control subjects without the supplement.

Summary & Conclusion: Our experiments suggest that oral administration of Glavonoid should effectively prevent ATTR amyloidosis. Although stabilizers of tetrameric TTR, which are widely accepted as therapeutic drugs worldwide, cannot be administered as prophylactic treatment, Glavonoid, an inexpensive and safe supplement, may be administered to asymptomatic subjects who are expected to start to manifest ATTRwt or ATTRv amyloidosis.

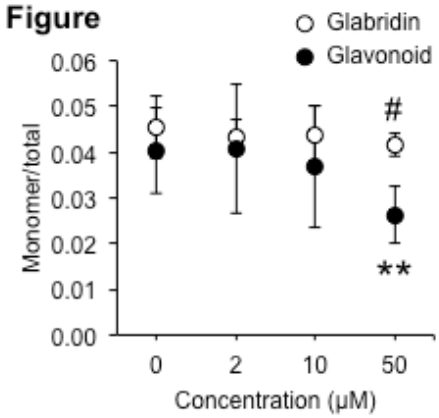

Figure: Effect of glabridin or Glavonoid on TTR stabilization. Vertical axis indicates ration of TTR monomer/ total $^{**}P < 0.01$: Glavonoid (50 μM) vs. 0 μM. # $P < 0.05$ Glavonoid (50 μM) vs. glabridin (50 μM) groups.

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Safety and tolerability of CAEL-101, an anti-amyloid monoclonal antibody, combined with anti–plasma cell dyscrasia therapy in patients with light-chain (AL) amyloidosis: results from a phase 2 study

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Background: AL amyloidosis is a rare, severe, progressive, systemic, potentially fatal disorder caused by plasma cell dyscrasia (PCD). Amyloidogenic immunoglobulin light chains misfold and aggregate into insoluble amyloid fibrils that deposit in the heart, kidneys, lungs, and liver, where they cause organ damage, dysfunction, and failure. Cardiac involvement drives morbidity and mortality in AL amyloidosis.[1,2] Current standard of care, cyclophosphamide-bortezomib-dexamethasone (CyBorD) ± daratumumab, targets PCD to prevent fibril formation.[3] CAEL-101 is an investigational first-in-class, monoclonal antibody therapy designed to eliminate deposited amyloid fibrils from organs.[4]

Objectives: To determine the long-term safety and tolerability of CAEL-101 administered in combination with anti-PCD therapy.

Material & Methods: This ongoing, open-label phase 2 study (NCT04304144) enrolled adults with a confirmed diagnosis of AL amyloidosis (Mayo Stages I-IIIa), a 6-month minimum life expectancy, and measurable serum free light chains. Patients with other forms of amyloidosis, multiple myeloma, supine systolic blood pressure <90 mm Hg, or symptomatic orthostatic hypotension were excluded.
Patients receive CAEL-101 1000 mg/m^2 every other week and CyBorD ± daratumumab (investigator decision). Treatment is continued until unacceptable toxicity, symptomatic deterioration, death, investigator decision, patient decision, or sponsor decision to terminate the study. Pharmacokinetic (PK) end points include peak and trough serum concentrations of CAEL-101. Safety assessments include treatment-emergent adverse events (TEAEs), clinical laboratory tests, electrocardiograms, vital signs, and physical examinations. Organ response is evaluated as change over time in N-terminal pro-brain natriuretic peptide (NT-proBNP; cardiomyopathy biomarker) and proteinuria (nephropathy biomarker).

Results: At baseline, the median (range) age of patients (N = 25) was 64 (48-80) years, and most (72%) were male. Patients were in Mayo Stages I (8%), II (76%), and IIIa (16%). Twenty (80%) patients had cardiac involvement at baseline (NT-proBNP ≥332 ng/L), and 20 (80%) had received prior anti-PCD therapy. At this evaluation, median CAEL-101 treatment duration was 512 days. The PK profile of CAEL-101 was unaltered by the addition of daratumumab to CyBorD.
All patients experienced ≥1 TEAE; 6 (24%) patients had possible treatment-related AEs **(Table)**. Eleven (44%) patients had ≥1 TEAE of Grade ≥3. Four (16%) patients discontinued the study including 1 death (deemed unrelated to CAEL-101 by investigator). The most common TEAEs (occurring in ≥25% of patients) included nausea (36%), constipation (32%), fatigue (32%), diarrhea (28%), and rash (28%).
Compared with baseline NT-proBNP levels, 9 (45%) patients had a cardiac response (≥30% decrease), 10 (50%) had stable disease, and 1 (5%) progressed (≥30% increase) at last evaluation. The cardiac response was sustained over the treatment duration and persisted even after anti-PCD therapy was halted **(Figure)**. Eight of 9 (89%) patients with renal impairment at baseline (single site) had ≥30% decrease in proteinuria.

Summary & Conclusion: To date, CAEL-101 administered with CyBorD ± daratumumab is generally well tolerated with mild to moderate TEAEs. Most patients responded to treatment or remained stable with organ response persisting post-cessation of anti-PCD therapy. Recruitment is underway for 2 international phase 3 clinical trials (Cardiac Amyloid Reaching for Extended Survival; CARES) that further evaluate the efficacy and safety of CAEL-101.

	CAEL-101 + anti-PCD therapy (N = 25)
Patients with ≥1 TEAE	25 (100.0)
Patients with ≥1 TEAE possibly treatment related	6 (24.0)
Patients with ≥1 TEAE of Grade ≥3	11 (44.0)
Patients with ≥1 SAE	9 (36.0)
Discontinuations, n (%)[a]	4 (16.0)
Death due to septic pneumonia	1 (4.0)
Heart transplant	1 (4.0)
Withdrawal of consent	1 (4.0)
Physician decision	1 (4.0)
MedDRA preferred term, n (%)[b]	
Nausea	9 (36.0)
Constipation	8 (32.0)
Fatigue	8 (32.0)
Diarrhea	7 (28.0)
Rash	7 (28.0)

[a]Unrelated to CAEL-101 treatment as determined by investigator.
[b]TEAEs occurring in ≥25% patients.
Abbreviations: MedDRA, Medical Dictionary for Regulatory Medical Activities; PCD, plasma cell dyscrasia; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

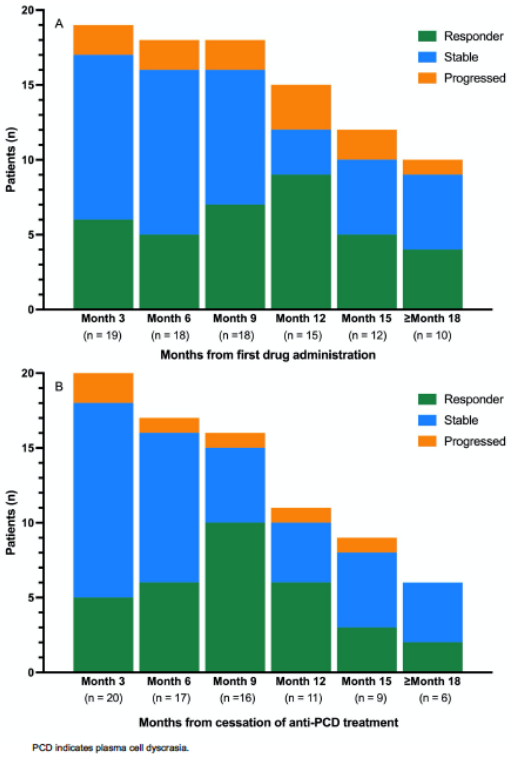

Table. Summary of treatment-emergent adverse events and discontinuations as of 2 March, 2022

Figure. Responder analysis for cardiac response over time: (A) Overall response and (B) Response after cessation of anti-PCD therapy

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Support & Funding: This study was sponsored by Caelum Biosciences, Bordentown, NJ, USA, now acquired by Alexion, AstraZeneca Rare Disease. Editorial and writing support was provided by Aparna Nori, PhD, CMPP, of rareLife solutions, Westport, CT, USA, and funded by Alexion, AstraZeneca Rare Disease.

Preclinical characterization of AT-04, a pan-amyloid-binding Fc domain-peptide fusion, to serve as an opsonin for macrophage-mediated clearance of amyloid deposits

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Background: Clearance of tissue amyloid deposits from organs and tissues, coupled with effective management of the amyloid precursor protein is anticipated to improve organ function, quality of life and survival of patients with amyloid-associated disorders. The current *modus operandi* for facilitating amyloid removal involves engaging cells of the innate immnue system, monocytes and macrophages, and induction of Fc-receptor mediated phagocytosis of the deposits (1). Given the diverse number of amyloidogenic precursor proteins and peptides that can result in amyloidosis, a pan-amyloid macrophage activating agent could have widespread clinical utility. We have previously described a murine Fc-peptide fusion reagent (peptibody) using a pan-amyloid-reactive peptide, p5, which demonstrated bifunctionalilty of the reagent – pan-amyloid reactivity and Fc-mediated phagocytosis (2). A next-generation human peptibody, comprising an IgG1 Fc and the amyloid-reactive peptide p5R (3), has now been generated with potential to serve as an opsonin to induce clearance of amyloid, regardless of the fibrillar precursor protein.

Objective: The goal of these studies was to assess the breadth of utility of the novel AT-04 peptibody. To this end, we sought to characterize it's ability to bind amyloid, of diverse types, mediated through the pan-amyloid-reactive peptide p5R and induce Fc-mediated phagocytosis of amyloid-related substrates.

Material & Methods: AT-04 was produced by either transient or stable transfection of CHO cells and purified from tissue culture medium by Protein A chromatography and characterized by SDS-PAGE electrophoresis. The binding potency (EC_{50}) for amyloid-like rVλ6WIL, Aβ, α-synuclein fibrils and tau filaments, as well as human AL and ATTR amyloid extracts was assessed by ELISA using 2-fold serial dilution of AT-04. Pan-amyloid reactivity was shown immunhistochemically with formalin-fixed tissues containing human, murine and canine systemic and localized (Aβ) amyloid deposits using 2 μg/mL biotinylated AT-04. Phagocytosis of human AL and ATTR amyloid extracts, labeled with the pH-sensitive fluorophore pHrodo red, was studied *in vitro* using phorbol myristate acetate-activated human THP-1 monocytes. Uptake of the amyloid extracts by activated THP-1 cells was quantified by segmentation of digital microscopy images. The biodistribution of radioiodinated AT-04 (^{125}I-AT-04) following IV administration was evaluated in WT mice and those with systemic AA amyloidosis by tissue measurements, SPECT/CT imaging and microautoradiography.

Results: AT-04 was produced in high yields with intact peptide at the C-terminal of the Fc domain. The potency of AT-04 binding (EC50) to AL fibrils and human amyloid extracts ranged between 0.5 – 1.8 nM. Moreover, binding to cerebral fibrillar aggregates of tau, α-synuclein and Aβ was saturable and similarly potent (0.5 – 7 nM EC50). Pan-amyloid reactivity was further demonstrated immunohistochemically with specific binding to AL amyloid in the heart, liver, and kidney, cardiac ATTR amyloid, as well as perivascular and core Aβ plaques in the brain from a patient with Alzheimer's disease (Fig. 1). Phagocytosis of synthetic rVλ6Wil fibrils, AL and ATTR extracts by PMA-activated human THP-1 cells was significantly enhanced in a dose-dependent manner as compared to a control IgG. This was further signficantly enhanced in the presence of 20% human serum as a source of complement. When injected IV into mice with systemic AA amyloidosis, ^{125}I-AT-04 accumulated rapidly in hepatosplenic (10-20% ID/g), renal (5-10% ID/g) and cardiac (~4% ID/g) amyloid deposits within 1 h post injection. Specific localization ^{124}I-AT-04 with amyloid was confirmed by microautoradiography (Fig. 2).

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Summary & Conclusion: Clearance of amyloid is a significant unmet need for patients with amyloid-related disorders. The humanized peptibody, AT-04, specifically binds systemic and cerebral amyloid with high potency. This interaction can induce phagocytosis of the material, which can be enhanced by the presence of human serum as a source of complement. AT-04 could serve as a potent opsonin to facilitate the macrophage-mediated phagocytosis of amyloid.

Figure 1

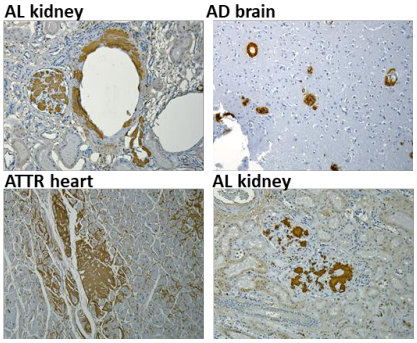

Figure 2

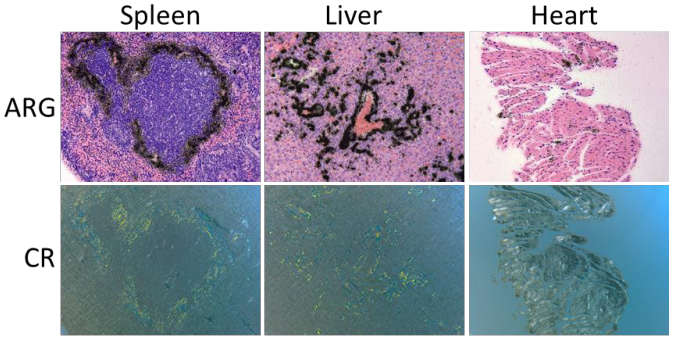

Figure 1.: AT-04 exhibits pan-amyloid reactivity. Reactivity of biotinylated AT-04 with formalin-fixed amyloid laden tissue was assessed immunohistochemically. Specific binding with AL, ATTR and Aβ-associated amyloid was evidenced by intense brown (DAB) staining which correlated with the presence of amyloid stained with Congo red in consecutive tissue sections (not shown).

Figure 2.: ^{125}I-AT-04, administered IV, specifically binds AA amyloid deposits in a murine model. Microautoradiography demonstrated highly-specific and intense accumulation of the radiolabeled AT-04 in amyloid deposits in the liver, spleen and heart (and other organs, shown), as evidenced by the presence of black silver grain deposits in amyloid which stained positively with Congo red in consecutive tissue sections.

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Characteristics of patients with hereditary transthyretin amyloidosis-polyneuropathy (ATTRv-PN) in NEURO-TTRansform, a phase 3 study of eplontersen

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Background: Hereditary transthyretin (TTR) amyloidosis (ATTRv) is a rare, severe, progressive, debilitating, and ultimately fatal disease caused by systemic deposition of TTR amyloid fibrils, leading to multiorgan failure.[1] The investigational drug eplontersen (ION-682884) is a ligand-conjugated antisense drug designed to degrade *TTR* mRNA in the liver, inhibiting TTR protein synthesis.[2] Eplontersen is being studied in the phase 3, international, multicenter, open-label, randomized, controlled NEURO-TTRansform study (NCT04136184) for the treatment of ATTRv with polyneuropathy (ATTRv-PN).

Objective: To report baseline characteristics of patients enrolled in the NEURO-TTRansform study.

Material & Methods: As reported by Coelho et al in 2021,[2] adult patients aged 18–82 years diagnosed with ATTRv-PN as defined by Familial Amyloid Polyneuropathy (FAP or Coutinho) stage 1–2, a documented TTR sequence variant, and signs/symptoms consistent with polyneuropathy (Neuropathy Impairment Score [NIS] ≥10 and ≤130) were enrolled in the study. Baseline demographics and disease characteristics were analyzed descriptively.

Results: The NEURO-TTRansform study enrolled 168 patients across 15 countries, of whom 26 (15.5%) were recruited in North America, 64 (38.1%) in Europe, and 78 (46.4%) in South America/Australia/Asia. Patients have a mean (±SD) age of 52.8 (±14.9) years; the majority of patients are male (69% [116/168]) and white (79% [131/168]). The most common TTR variant is the V30M sequence variant, occurring in 60% of patients. Approximately 57% (95/168) and 17% (29/168) of patients received previous treatment with tafamidis or diflunisal, respectively. Most patients have FAP stage 1 neuropathy (79% [133/168]) and an early disease onset (53% [89/168]), defined as disease symptoms occurring before or at age 50. The mean (±SD) time from diagnosis to enrollment was 46.6 (±57.4) months. Polyneuropathy Disability scores are I for 41% (68/167) of patients, II for 41% (69/167), IIIa for 11% (19/167), and IIIb for 7% (11/167).The mean (±SD) modified NIS plus 7 (mNIS+7) score was 79.0 (±42.4).

Summary & Conclusion: The baseline characteristics of the NEURO-TTRansform study population are overall representative of the trial eligibility criteria, the enrollment of patients across multiple countries, and contemporary clinical practice. The results of this study will provide important information on clinical and health-related quality-of-life outcomes to better inform future treatment choices for patients with ATTRv-PN.

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AT-03 Demonstrated Pan-amyloid Binding and Stimulated the Removal of Amyloid Deposits through Macrophage-Mediated Phagocytosis

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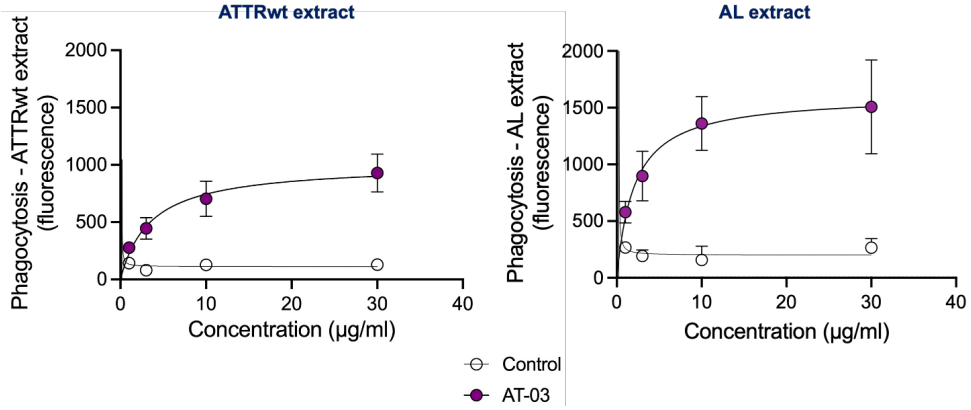
Background: There is an urgent unmet need for novel therapies which effectively and safely promote amyloid removal and rapidly restore organ function across all subtypes of systemic amyloidosis. AT-03 is a novel fusion protein consisting of serum amyloid P component (SAP) linked to a single chain human immunoglobulin G1 (IgG1) Fc domain. SAP is a naturally occurring pentameric serum protein that binds with high avidity to all forms of amyloid, regardless of the amyloidogenic precursor protein. The pentameric conformation of SAP is essential to bind to amyloid, and a single chain Fc (scFc) is fused to each SAP molecule. The scFc has two Fc chains connected through a glycine-serine (G-S) linker region. Once bound, the IgG1 Fc domain actively engages the immune system to signal macrophages to engulf and remove amyloid through phagocytosis.

Objective: The goal of the study was to characterize the preclinical profile of AT-03, including the binding affinity to human amyloid extracts, the biodistribution in murine models of amyloidosis, and validate the mechanism of action by studying the promotion of macrophage-mediated phagocytosis.

Material & Methods: Binding of AT-03 to ATTR and AL amyloid extracts was assessed by ELISA. Phagocytosis of amyloid substrates was studied using activated human THP-1 cells. Complement-enhanced phagocytosis was also assessed. We determined *in vivo* phagocytosis of human AL amyloid extracts with and without exposure to AT-03. The biodistribution of AT-03 in murine models of AA, AApoA2 and AL amyloidosis was evaluated immunohistochemically or by SPECT imaging and microautoradiography.

Results: The binding of AT-03 was evaluated using synthetic ALλ fibrils (rVλ6Wil), as well as human ATTR and AL extracts using a europium-linked immunosorbent assay (ELISA). AT-03 demonstrated potent low nanomolar (nM) binding, with the estimated half-maximal effective concentration (EC_{50}) values ranging from 0.27 to 0.63 nanomolar (nM) for all amyloid substrates tested. Intravenous (IV) administration of AT-03 resulted in specific binding to diverse forms of amyloid in the heart, kidney, liver, and spleen in murine models of amyloidosis. AT-03 co-localization was also observed with cardiac amyloid deposits in a novel model of murine AL amyloidosis. Macrophage-mediated phagocytosis of human ATTR and AL amyloid extracts and amyloid-like fibrils was observed *in vitro* through visualization of a pH sensitive fluorophore. AT-03 also demonstrated complement-enhanced opsonization and phagocytosis. *In vivo* recruitment of macrophages and stimulation of phagocytosis by AT-03 was observed in an AL amyloidoma model.

Summary & Conclusion: AT-03 shows promise at being able to bridge the gap in current amyloid therapies, with the potential for pan-amyloid removal, regardless of the underlying amyloidogenic precursor protein.



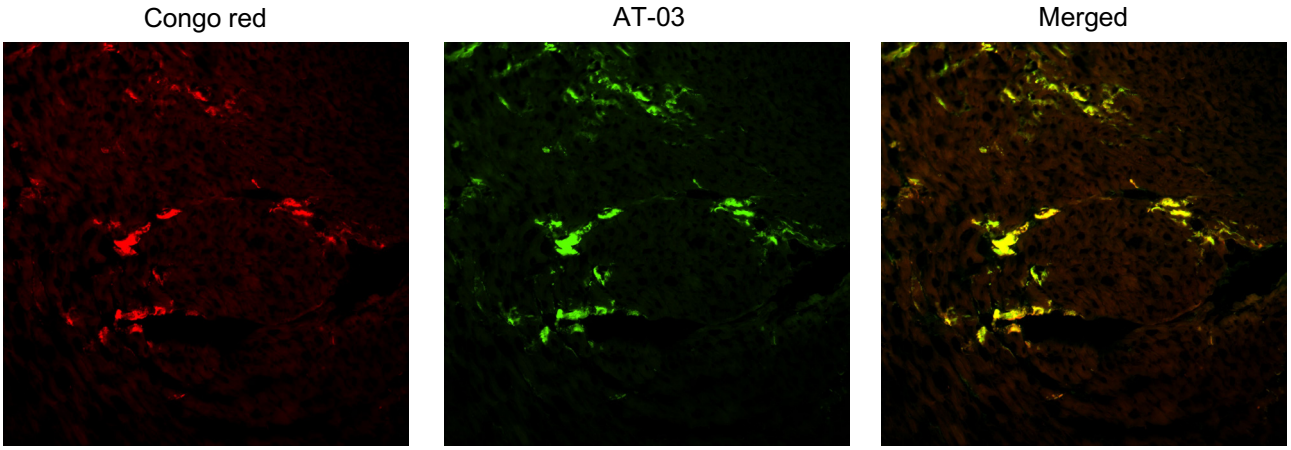


Figure 1.: Phagocytosis of AT-03 by PMA-activated THP1 cells. Dose dependent changes in phagocytosis of ATTRwt and AL (ALκ) amyloid extract.

Figure 2.: Heart tissue from AL amyloid mouse model injected with 0.5 mg of AT-03. The heart tissue sections were stained with Congo red and anti-human IgG-FITC to detect AT-03 bound to heart tissue.

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Support & Funding: Support for this work was provided by Attralus. GMR is funded by Region Nouvelle Aquitaine and French Ministry of Research. CS was supported by grants from Fondation Française pour la Recherche sur le Myelome et les Gammapathies (FFRMG) and Agence National de la Recherche # ANR-21-CE17-0040-01.

Design and Evaluation of shRNA Polyplex with Cyclodextrin-modified Dendrimer for Treatment of Systemic and Localized Amyloidosis

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Background: Systemic and localized amyloidosis, such as hereditary transthyretin (ATTR) amyloidosis and familial Alzheimer's disease (AD), generally develop through 3 critical pathological steps: 1) production of amyloid precursor proteins, 2) amyloid formation, and 3) amyloid deposition. Because of difficulty in targeting and treating each step of the pathological process independently, the combination of single therapeutic targeted agents may provide effective amyloidosis treatment, and novel strategies and therapeutic agents with multi-step mechanisms for the treatment of amyloidosis are urgently needed. Previously, we reported that the polyamidoamine dendrimer (dendrimer, generation 2) exhibited the therapeutic effects on ATTR amyloidosis through inhibiting and disrupting ATTR amyloid formation [1]. In addition, we also found that the dendrimer conjugate with branched β-cyclodextrin (CDE) served as an advanced carrier for gene and RNA interference (RNAi) drugs [2].

Objective: In this study, we fabricated a novel CDE comlex with plasmid DNA encoding short hairpin RNA (shRNA), and evaluated the utility of CDE/shRNA complex as a multifunctional therapeutic agent for systemic (ATTR) and localized (AD) amyloidosis.

Material & Methods: **Preparation of CDE/shRNA.** CDE, synthesized according to our previous report [2], was complexed with shRNA (OriGene, Maryland, USA) by mixing in aqueous solution. ***In vitro* RNAi effects.** The TTR and BACE1 mRNA expression levels were evalulated by real-time PCR method in human hepatoma HepG2 and mouse neuroblastoma Neuro-2a cells transfected by CDE/shRNA, respectively. ***In vitro* thioflavin-T (Th-T) assay.** Recombinant TTR V30M protein or human Aβ42 peptide was incubated, respectively, with or without CDE/shRNA. The amyloid fibrils were detected by Th-T assay. ***In vivo* experiments.** CDE/shRNA was administrated to human ATTR V30M transgenic (Tg) rats, as an ATTR amyloidosis model, and App$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ knock-in mice, as an AD model [3], respectively. Deposited TTR in the colon of ATTR amyloidosis model and Aβ in the brain of AD model, were detected by immunohistochemical staining, respectevely.

Results & Discussion: **Preparation of CDE/shRNA.** CDE formed a submicron-sized cationic polyplex with shRNA. ***In vitro* RNAi effects.** CDE/shRNA (charge ratio =100) treatment showed 56% knock-down efficacy against the human *TTR* mRNA in HepG2 cells, and also significantly suppressed mouse *BACE1* mRNA levels compared with CDE/scrambled shRNA in Neuro-2a cells. ***In vitro* Th-T assay.** CDE/shRNA treatment significantly inhibited amyloid formation of both TTR V30M and human Aβ42. Moreover, the amyloid fibrils of both TTR V30M and Aβ42 were significantly decreased by CDE/shRNA, respectively. ***In vivo* experiments.** CDE/shRNA treatment significantly reduced the TTR deosition in the gastrointestinal tracts of ATTR amyloidosis model, and also inhibited the Aβ depsition in the brain of AD model. It should be noted that CDE/shRNA treatment did not exhibit any significant changes of the safety profiles in both ATTR amyloidosis and AD model.

Summary & Conclusion: We successfully developed the CDE/shRNA polyplex, with multifunctional therapeutic efficacy, such as, suppression of amyloidgenic proteins production, inhibition of amyloid formation, and disruption of amyloid fibrils, in both systemic and localized amyloidosis. These findings suggest the possibility for the development of novel therapeutic agents against various amyloidosis (Figure 1).

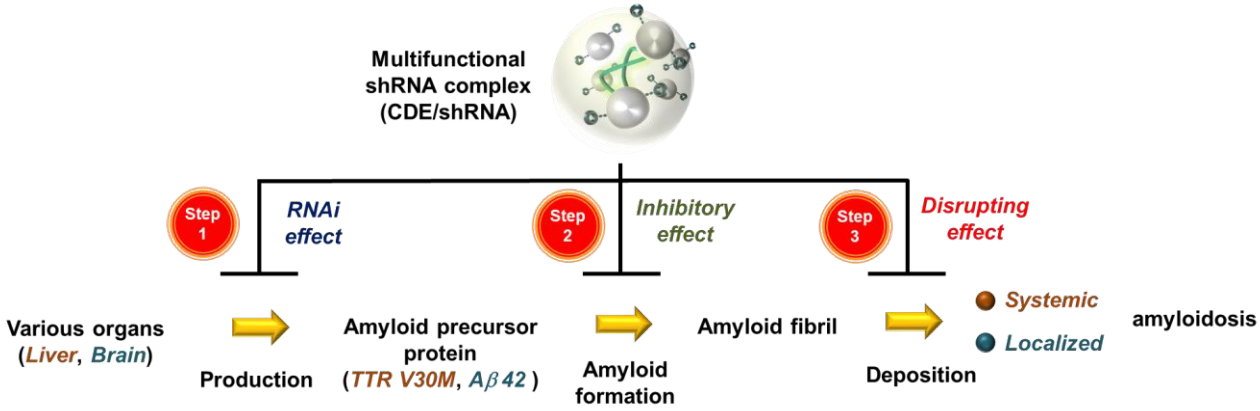

Figure 1.: Therapeutic strategy using CDE/shRNA for systemic (ATTR) and localized (AD) amyloidosis.

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Rational design of AML therapeutic agents that specifically bind and clear diverse amyloid aggregates

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Background: Ig-fusion to the specific g3p N1N2 domain from M13 has been demonstrated to target and remodel a large variety of amyloid fibers. This binding is mediated by N1N2 hydrophobic and polar residues docking specifically the cross-beta sheet assembly common of amyloid fibers (Krishnan *et al*, 2014). First generation molecules have been developed for treating Alzheimer disease (Levenson *et al*, 2016). Preclinical studies in models for light chain (LC) amyloidosis showed the co-localization of injected radio-iodinated of Ig-fusions with subdermal and gut amyloid deposits, demonstrating the expected biodistribution of systemically administrated molecules (Proschitsky *et al*, 2018). Open stabilized variants have been generated, exhibiting improved binding potency, inhibition of amyloid assembly and blocking cell to cell propagation of pathologic proteins (Asp *et al, 2019)*.

Objective: This study compares new AML therapeutic candidates designed for reducing amyloid loads in Amyloidosis patients. Focusing firstly to improve LC *in vitro* and *ex vivo* binding, specificity and immunogenicity, we are also generating evidences aiming to further confirm the mode of action of our candidates.

Material & Methods: N1N2 binding domains fused to the IgG1 Fc are produced and purified as described in Asp *et al*, 2019. Recombinant Lambda LC (J.To and J.Wil) have been produced in *E. coli* , purified and buffered in acidic conditions for 7 days at 37 °C. Fibers structure is assayed by ThT fluorescence and TEM. ELISA optimized from protocols developed in Asp *et al* (2019) allow to estimate EC50 and confirm binding potency. Specificity is *in vitro* assessed in a dedicated ELISA to confirm the absence of "off target binding". For *in vitro* phagocytosis, PHrodo™ dye conjugates are non-fluorescent outside the cell, but fluoresce brightly red in phagosomes. After coupling of the fibres/soluble forms of LC with the pHrodo™ dye, they are placed in the presence of the candidates for 30 min at 37° C. before depositing the solution of coupled proteins/candidates on the cells.

Results: In-depth analysis of data describing the performances of ~100 different mutants has been done. New sequences have been identified and created aiming at the best balance between the binding performances, specificity, half life and reduced immunogenicity. A total of 6 lead candidates were selected and tested in different *in vitro* settings. *In silico* immunogenicity confirms the optimization of the immuno profile. Preliminary ELISA data developed with recombinant LC fibers demonstrate a significantly improved binding potency to LC fibers of the new candidates as compared to previously developed molecules (see Fig 1).The specificity profile determined by ELISA using different human and animal tissues confirm the absence of off-target binding. Initial results of *in vitro* phagocytosis show an increase in fluorescence when the THP1s are incubated with the LC fibers-pHrodo-candidate complexes and not with the solubles LC-pHrodo-candidate. Moreover, we collaborate with different hospitals having access to tissues extrated from confirmed LC/ TTR amyloidosis patients, aiming to confirm the *ex vivo* binding to this specific material.These results will be available for poster presentation.

Summary & Conclusion: Current data with optimized AML fusions candidates are promising and confirm the highly specific binding to LC fibers. Together with initial inhibition & phagocytosis data, this data will be confirmed by *ex vivo* evaluation, allowing lead candidate selection.

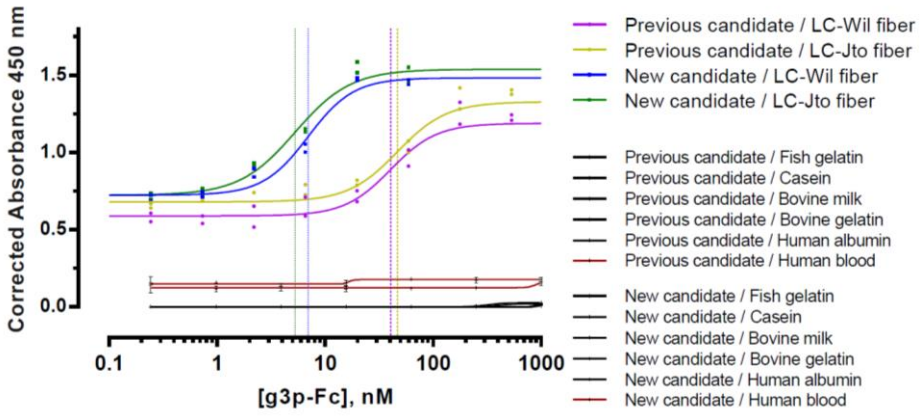

Figure
1.: New candidates strongly and specifiacally bind to LC fibers.

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DEVELOPMENT OF NEW ANTIBODY-BASED THERAPIES AGAINST SYSTEMIC AMYLOIDOSES

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Background: It is increasingly recognized that post-translational modifications of amyloid proteins play an important role in the development and progression of human pathophysiological processes [1]. Therefore, a particular focus is the investigation of the influence of post-translational peptide modifications. The modified proteins show higher toxicity and the proteins show greater aggregation potential due to increased hydrophobicity. Forms of modification are i.e. pyroglutamate formation, isoaspartate formation, phosphorylation, nitration or glycosylation [2].

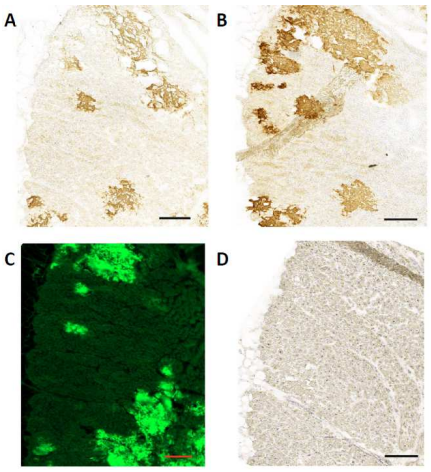
Objective: Since the cause of protein degeneration is known to vary from patient to patient, the aim is to develop personalized therapies in the form of monoclonal antibodies directed against the described modifications. In our study, we focused on the transthyretin amyloidosis (ATTR), which is characterized by the deposition of the protein transthyretin (TTR) as amyloid fibrils inside various organs, leading to progressive organ failure. We identified an post-translational-modified TTR present in TTR fibrils and developed monoclonal antibodies, that selectively bind this misfolded, non-native amyloidogenic form of TTR.

Material & Methods: Monoclonal antibodies were produced by immunization of mice with antigen peptide, subsequent hybridoma generation and screened for selective binding to post-translational-modified TTR. Monoclonal antibodies were characterized by ELISA, surface plasmon resonance, transmission electron microscopy and immunohistochemistry of human cardiac tissue of ATTR patients. We further analyzed the ability of the monoclonal antibodies for antibody-dependent phagocytic uptake of TTR fibrils.

Results: The two selective monoclonal antibodies 2F2 and 4D4 were generated and characterized. These antibodies selectively bound post-translational-modified TTR in non-native misfolded forms of TTR with a high specificity as shown in staining of human cardiac ATTR tissue. They promoted the phagocytic uptake of TTR fibrils by macrophage-like THP-1 cells.

Summary & Conclusion: Post-translational modification-specific monoclonal TTR antibodies selectively bind amyloidogenic, but not native TTR forms. Both antibodies 2F2 and 4D4 detect modified amyloid peptides in cardiac samples from patients with sporadic or hereditary ATTR and can induce antibody-dependent phagocytic uptake of TTR fibrils *in vitro*. Hence, we propose that these antibodies may have diagnostic and therapeutic potential to label deposits of TTR protein for clearance in patients.

1



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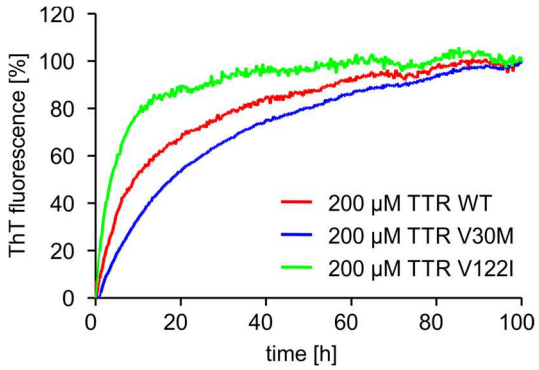

Figure 1.: Staining of human cardiac tissue of an ATTR-V30M patient. A) DAB staining with anti-PTM-TTR-AK 2F2. B) DAB staining with anti-PTM-TTR-AK 4D4. C) Thioflavin S staining. D) DAB/nickel staining with commercial polyclonal TTR-AK, counterstaining with hematoxylin. Scale bar 200 µm.

Figure 2.: Aggregation kinetics of three different recombinant TTR proteins monitored by Thioflavin-T assay [3]. The samples were incubated with 50 mM NaH_2PO_4, pH 4 and 20 µM ThT at 37°C.

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Exploring the inhibitory effect of a natural product, Siderol, on the aggregation of Amyloid-β peptide.

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Background: Nowadays, fifty million people are affected by the devastating impacts of the most common neurodegenerative disease, according to the World Health Organization, called Alzheimer's disease (AD). It is widely accepted that one of the pathological hallmarks of AD is senile plaques which mainly consist of amyloid fibrils. The major component of these extracellular deposits is the neurotoxic Amyloid-β (Aβ) peptide which derives from the degradation of amyloid precursor protein (APP) by β- and γ-secretase.(1) APP is a transmembrane protein that is involved in synaptogenesis, neurite development, and neuronal adhesion. According to the amyloid cascade hypothesis, Aβ peptides self-assemble to form oligomers and then amyloid fibrils. Recently, oligomers are considered to be a more toxic structure than the amyloid fibrils.(2) Regarding the therapeutic approach for AD, only two drug categories exist, the cholinesterase inhibitors and the N-methyl D-aspartate (NMDA) antagonists. In spite of the increasing number of studies, the available drugs are aiming to treat the symptoms and not to prevent or cure AD. Recently, natural extracts have been evaluated for their neuroprotective effects.(3)

Objective: Taking into consideration that natural products seem to have therapeutic potential, we attempted to elucidate the role of a natural product, Siderol, in the formation of $A\beta_{1-42}$ amyloid fibrils in vitro. The kaurene diterpene, Siderol, was isolated from a famous mountain Greek tea and mixed with $A\beta_{1-42}$ peptide in order to examine its potential inhibitory effect.

Material & Methods: Specifically, the $A\beta_{1-42}$ peptide was chemically synthesized and the ability of Siderol to inhibit its aggregation was studied *in vitro* in three different molar ratios, 1 $A\beta_{1-42}$ peptide:1 Siderol, 1 $A\beta_{1-42}$ peptide:5 Siderol, 1 $A\beta_{1-42}$ peptide:10 Siderol. We utilized biophysical methods to inspect the amyloidogenicity of $A\beta_{1-42}$ after the incubation with the natural product, such as Congo Red staining and polarized light microscopy, negative staining and Transmission Electron Microscopy, and Thioflavin T (ThT) kinetic assays. Also, computational simulations monitored the interaction of the natural product with the $A\beta_{1-42}$ peptide over the course of time.

Results: Overall, the findings of our experiments suggested that the natural product Siderol is able only to delay the formation of amyloid fibrils in one ratio. TEM micrographs showed that the fibrils co-existed with amorphous aggregates in the ratio 1:1. Interestingly, we observed that the dominant structure in the other two mixes were wide, tape-like ribbons. In the ratio 1:5, fibrils were found together with wide, tape-like ribbons and amorphous aggregates, as well. On the other hand, in the ratio 1:10, we only detected the distinctive ribbons and some fibrils. In addition, the characteristic apple-green birefringence was not detected. Last but not least, the ThT signal was reduced only in the ratio 1:5 by 60.61%.

Summary & Conclusion: The aim of the present work was to shed light on the effects of a kaurene diterpene, extracted from native Greek plants, on the aggregation of Aβ peptide *in vitro*. After the incubation of $A\beta_{1-42}$ with Siderol in three ratios, we concluded that the natural product is efficient at delaying the self-assembly of $A\beta_{1-42}$. Even though a complete inhibition of the $A\beta_{1-42}$ peptide's aggregation was not clearly observed, further investigation could lead to the exploitation of Siderol as a drug candidate.

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A Phase 1/2 Multi-Center, Dose-Finding Study to Assess Safety, Tolerability, PK and Efficacy of ZN-d5, a Novel BCL-2 Inhibitor, for Treatment of Subjects with Relapsed/Refractory AL Amyloidosis

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Background: AL amyloidosis arises from a plasma cell clone that overproduces immunoglobulin light chains. The light chains aggregate and deposit within tissues and organs, interfering with normal function, most commonly manifesting with cardiac, hepatic and renal involvement. t(11;14) is the most common cytogenetic aberration in patients with AL amyloidosis observed in approximately 60% of cases. At present, treatment of AL amyloidosis aims at the elimination of the plasma cell clone to rapidly reduce the level of circulating light chains. Stem cell transplant remains an effective yet toxic treatment for eligible patients; bortezomib-based regimens are the most used primary treatment, however patients that harbour t(11;14) have a less favourable response. Addition of subcutaneous daratumumab to standard bortezomib, cyclophosphamide and dexamethasone (CyBor/VCd) combination improves complete hematologic and organ response rates even among those with t(11;14), but the management of patients with relapsed or refractory disease remains challenging. Pre-clinical and clinical data have shown that BCL-2 inhibition is an effective mechanism of treatment for t(11;14) plasma cells or those overexpressing BCL-2. Interestingly, small, retrospective studies have shown that BCL-2 inhibition may be an effective treatment option for patients with AL amyloidosis, particularly among those who harbour t(11;14). ZN-d5 is a novel, highly BCL-2-selective BH3 mimetic in clinical development for hematologic malignancies, and has shown activity against multiple myeloma in preclinical models and which may be useful in the treatment of AL amyloidosis.

Objective: ZN-d5-003 is a multicenter, international Phase 1/2 clinical trial evaluating single-agent ZN-d5 in relapsed or refractory AL amyloidosis subjects. In the Phase 1 portion, the primary objectives are to determine the safety, tolerability and maximum tolerated dose of ZN-d5, and to determine the recommended Phase 2 dose. In Phase 2, the primary objective is to assess the hematological response rate (HRR) among subjects with or without t(11;14) translocation.

Material & Methods: Cohorts of increasing doses (200mg; 400mg; 800mg; 1200mg; 1600mg; PO) will enroll subjects based on Bayesian Optimum Interval (BOIN) design parameters. Because ZN-d5 absorption and exposure is influenced by food, the study was recently amended to switch dosing from an empty stomach to taking study drug with food. Key criteria for eligibility include the following: subjects must have a biopsy-confirmed diagnosis of AL amyloidosis, at least 1 and no more than 3 prior lines of therapy (including HSCT), adequate organ function and ECOG performance status of 0-2. All subjects must have measurable disease defined as dFLC of at least 20 mg/L; those considered for the trial will undergo bone marrow aspiration (or biopsy) for assessment of t(11;14) status by FISH. Subjects must have a history of organ involvement (current measurable organ disease is not required). Exclusion criteria include non-AL amyloidosis, diagnosis of multiple myeloma per 2014 IMWG criteria, or Mayo 2012 Stage IV disease.

Results: The first study subject was treated in March 2022 and enrollment is on-going. Once DLTs have been assessed and the Recommended Phase 2 Dose is determined, the study will commence Phase 2.

Summary & Conclusion: This study will evaluate the scientific basis for treatment of light-chain (AL) amyloidosis utilizing BCL-2 inhibition and will test the hypothesis of whether t(11;14) is a relevant prognostic biomarker of response. Clinical trial: NCT05199337.

Support & Funding: Funding for this study comes from K-Group Alpha & Zentalis Pharmaceuticals, Inc.

Venetoclax and simvastatin synergize to enhance responses in AL amyloidosis harboring t(11;14)

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Background: Despite recent advances, cure of AL remains elusive. t(11;14), often accompanied by overexpression of Cyclin D1 and Bcl-2, is encountered in about 50% of AL patients and predicts poor outcomes.[1,2] Venetoclax has emerged as an effective tool in the management of this subset of patients, yiedling response rates of up to 68% in the relapsed/refractory (RR) setting.[3] However, safety concerns remain a significant challenge,[4] and beg discovery of novel combinations that can enhance venetoclax efficacy while limiting treatment-related toxicity. HMG coA-reductase inhibitors are relatively safe agents, effective in various hematologic malignancies,[5] and present a rational choice for combination therapy in AL.[6]

Objective: We sought to evaluate synergy between venetoclax and simvastatin in human myeloma cell lines (HMCLs) in *in vitro* studies and investigated clinical outcomes of patients treated with this combination at our institution.

Material & Methods: KMS-12-PE and U266 cells were selected as t(11;14)-positive HMCLs of interest. Synchronized cells were incubated with venetoclax, simvastatin, or the combination for 48 hours. Cell viability was assessed using CellTiter-Glo (Promega, Madison, WI), and synergy was assessed using SynergyFinder2.0 (Helsinki, Finland). Intracellular flow cytometry for BCL-2 family members was also done. In AL patients, venetoclax was started at 200 mg daily and escalated to 400 mg daily after 2 weeks, if tolerated. Atorvastatin 10-40 mg daily or simvastatin 40 mg daily was started either concurrently or for a lack of response to venetoclax. Bone marrow (BM) assessment was performed prior to starting therapy, and response assessment was performed monthly thereafter.

Results: KMS-12-PE cells showed exquisite sensitivity to both venetoclax (IC_{50} 5.3 nM) and simvastatin (IC_{50} 1.3 μM); whereas U266 were resistant to venetoclax and only moderately sensitive to simvastatin (IC_{50} 2.8 μM). Combination of the two drugs exhibited strong synergism in KMS-12-PE across a wide range of doses (Fig 1A), when compared to U266 (Fig 1B). Simvastatin-treated KMS-12-PE cells were successfully rescued after co-incubation with intermediates of the mevalonate pathway but no changes in BIM and PUMA expression were noted.

Twenty-three patients with relapsed AL were treated with venetoclax, of whom 19 also received a statin. Median age was 70, 15 were male, and 16 had λ-type AL (6–κ, 1–AH). Cardiac involvement was most common (74%), while 48% had ≥2 organs involved. Median baseline iFLC was 235.4 mg/L, with 17.5% median BM plasmacytosis. FISH for t(11;14) and/or Cyclin D1 overexpression by IHC was seen in 91%. Median treatment duration was 39 weeks. At a median follow-up of 60 weeks, overall hematologic response rate was 78%, with ≥VGPR rate of 65%. Seven patients were able to achieve deep hematologic reponses (iFLC <10 mg/L), of whom 6 received the combination therapy. Median time to best response was 8 weeks. One patient with a 33-year history of MGUS, 9-year history of heavily-treated AL, and Cyclin D1 overexpression (Fig 2B) was able to promptly achieve iFLC <10 mg/L within 8 weeks of venetoclax and atorvastatin initiation. Statin dose was reduced in 1 patient due to grade 1 myalgia. All responders continue on treatment at the most recent follow-up.

Summary & Conclusions: We provide preclincial evidence for synergism between venetoclax and statins in t(11;14) AL/MM and also provide support for the efficacy and safety of this regimen in management of RR AL, highlighting the need to validate this approach in larger clinical trials.

Figure 1

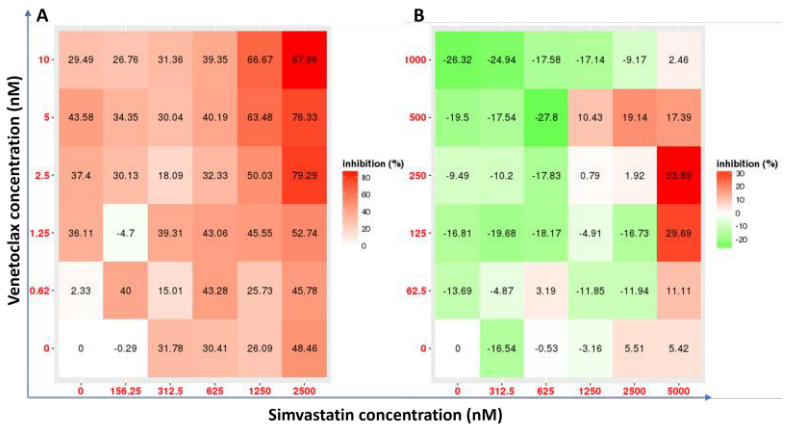

Figure 2

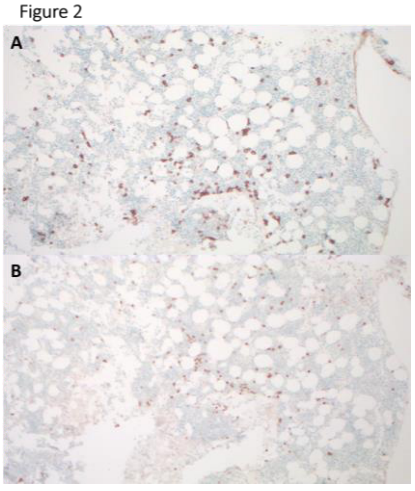

Figure 1.: Dose-response matrices demonstrate synergy between venetoclax and simvastatin in KMS-12-PE (A) and its lack in U266 (B) cells.
Figure 2.: Immunohistochemical examination of bone marrow specimen in a representative case identifying plasma cells

using CD138 (A; 100X) and Cyclin D1 (B; 100X) stains.

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Exploiting existing drugs against Alzheimer's disease: a protein-drug interaction network approach

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Background: Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects an increasing number of individuals. The pathological hallmarks of this disease are amyloid plaques and neurofibrillary tangles, composed of the amyloidogenic proteins amyloid-β (Aβ) peptide and Tau protein, respectively [1]. Despite immense effort to develop drugs for AD over the past decade there is still no available effective treatment [2]. Drug development is a slow and costly process with high attrition rates. To mitigate these challenges, a strategy called drug repurposing is used. It centers around investigating existing drugs for uses beyond their original scope [3].

Objective: The aim of this work was to explore drugs with the potential of repurposing for use in AD. The primary focus was the proteins found in amyloid deposits associated with AD and the drugs that target them. A network of protein-protein and protein-drug interactions was constructed to identify promising drugs and drug targets for AD.

Material & Methods: The Amyloidoses Collection (AmyCo) [4] was used to collect AD-related proteins, including the Aβ peptide, Tau protein and other co-deposited proteins. These proteins and the drugs that target them, collected from the DrugBank database [5], were the seed dataset for the constructed protein interaction network. Protein-protein interactions were extracted from the IntAct database of experimentally verified molecular interactions [6]. Lastly, drugs currently in use for AD were collected from the literature and their protein targets were found in DrugBank.

Results: A total of 12 AD-related proteins were collected from AmyCo, 2 amyloidogenic and 10 co-deposited proteins. These proteins interacted with almost 900 other proteins, with the network composed of their interactions having more than 3500 edges. In addition to those interactors, the AD-related proteins were targets of 62 drugs, excluding elements, with a total of 205 protein-drug interactions. Furthermore, these drugs also target 109 other proteins, 31 of which were among the interactors. Separately, 6 drugs used for AD were found that target 58 proteins, 5 of which were in the aforementioned protein interaction network. Drugs and potential drug targets with a strong connection to the network were the primary focus. These drugs can be studied for their impact on AD-related processes, such as the drug Deferoxamine which was shown to decrease Aβ peptide production [7].

Summary & Conclusion: The increasing prevalence of AD means that finding an appropriate treatment is imperative, but drug development is a long painstaking endeavor. A strategy that can help circumvent this problem without compromising on safety concerns is therefore necessary; one such strategy is drug repurposing. In this work, we investigated proteins related to AD and the drugs that target them that could be used for the treatment of AD. Promising drugs are showcased, including both drugs under investigation for their role in AD and not yet explored, that could hold the key to the elusive treatment for AD.

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Support & Funding: This research has been co-financed by the European Union and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "RESEARCH – CREATE – INNOVATE" (project code: T1EDK-00353).

Inhibition of antibody light chain amyloid formation by small molecules

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Background: AL amyloidosis remains incurable for most patients and new therapies are needed. Hematological responses do not always translate into rapid organ responses, due to pre-existing tissue damage and the potential for even low levels of monoclonal free antibody light chains (LC) to extend fibrils. Therapies that suppress LC aggregation or enhance clearance of amyloid deposits could complement cytotoxic therapies and benefit patients. The clinical success of tafamidis, diflunisal and other small molecule "kinetic stabilizers" of transthyretin encouraged us to seek analogous molecules that could stabilize LCs [1]. We have previously identified molecules that stabilize LCs by high-throughput screening [2], then used structure-guided medicinal chemistry to extend and optimize the binding interface [3]. This yielded small molecules with high affinity for full-length LCs.

Objective: A successful LC kinetic stabilizer needs to bind tightly and specifically to free LCs in order to prevent their unfolding, proteolysis and/or aggregation. It also needs to be sufficiently bioavailable to stabilize the majority of free LC in circulation, and well-tolerated so that patients can take it for prolonged periods, potentially as a maintenance therapy. Our work so far has focused on identifying molecules that can prevent unfolding and subsequent proteolysis of full-length LCs. However, LC fragments including variable domains form the core of patient amyloid fibrils and are much more prone to aggregation under native-like conditions in vitro. Here, we investigated whether binding of small molecules to LC variable domains can prevent their unfolding and aggregation.

Material & Methods: We measured the ability of stabilizer molecules to suppress aggregation of recombinant LC variable domains by measuring the rates of amyloid formation by binding of the fluorescent dye thioflavin T. We measured the stability of recombinant LCs as a function of protein and stabilizer concentration using urea titrations followed by fluorescence spectroscopy.

Results: Binding of small molecules suppressed unfolding and subsequent proteolysis of multiple full length LCs with sequences derived from amyloidosis patients. Our initial hit molecules were not sufficient to suppress aggregation of isolated variable domains, which form amyloid much more readily than full-length LCs. In contrast, high-affinity stabilizers were able to delay aggregation. Although small molecules were selected for their ability to protect full-length LCs from proteolysis, this protection from aggregation represents an additional mechanism by which stabilization could suppress amyloid deposition. These data support the strong association between unfolding and aggregation that characterizes amyloid formation by LCs.

Summary & Conclusion: These data are consistent with our hypothesis that stabilization of natively folded LCs can suppress aggregation directly, as well as via prevention of proteolysis that could accelerate amyloid formation. We have demonstrated that stabilizers with high affinity and protective efficacy can be created. Further work will be needed to identify molecules with desirable drug-like properties in order to identify clinical candidates.

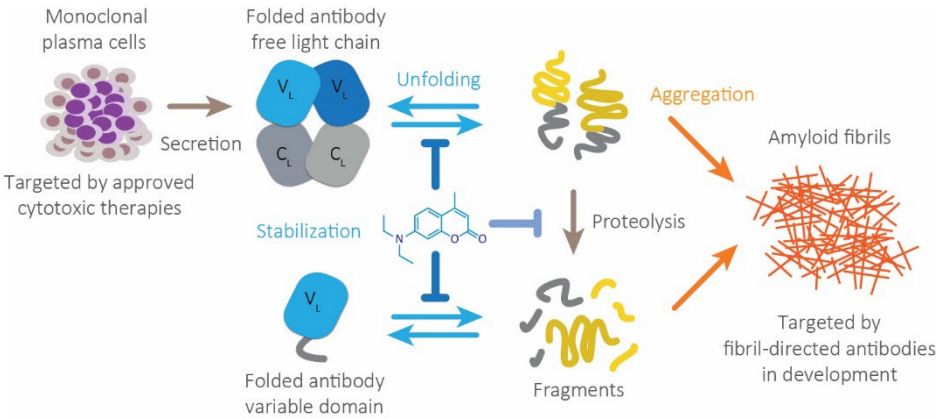

Figure 1.: Stabilization of amyloidogenic LCs could prevent unfolding that is required for aggregation with or without concomitant proteolysis, complementing existing cytotoxic therapies and emerging anti-amyloid therapies.

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Cell-Penetrating Peptides with Unexpected Anti-Amyloid Properties

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Background: Self-assembly of proteins and peptides into soluble and toxic oligomers, and then into insoluble fibrillar aggregates enriched in β-sheet secondary structure, is a hallmark in amyloid diseases such as Alzheimer's and Parkinson's. Creating suitable molecules that can interfere with the harmful amyloid aggregation process is imperative for combating these diseases.
Objective: To test the anti-amyloid efficiency of designed cell-penetrating peptides consisting of an N-terminal hydrophobic signal sequence followed by a C-terminal highly positively charged hexapeptide segment.
Material & Methods: The effect of the designed cell-penetrating peptides on amyloid aggregation were tested both in cell cultures and in vitro, using techniques such as fluorescence spectroscopy and AFM imaging.
Results & Discussion: The cell-penetrating peptides were found to interfere with the amyloid aggregation processes of various amyloid-forming proteins and peptides, such as the prion protein (PrP) and the amyloid-β (Aβ) peptide in Alzheimer's disease. The cell experiments show that the toxicity of the amyloid aggregates is significantly reduced in presence of our cell-penetrating peptides.
Summary & Conclusions: Our designed cell-penetrating peptides are promising drug candidates against amyloid diseases, where the signal sequence appears to help the peptides penetrate cell membranes and then target the right intra-cellular locations.

Discovering Potent Small Molecule Kinetic Stabilizers of Amyloidogenic Immunoglobulin Light Chains

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Background: In immunoglobulin light-chain (LC) amyloidosis (AL), transient unfolding, or unfolding and proteolysis enable aggregation of LC proteins, causing potentially fatal organ damage. Existing treatments such as the subcutaneous formulation of daratumumab, dosed in combination with bortezomib, cyclophosphamide and dexamethasone focus on eradicating the aberrant plasma cells secreting misfolding-prone LCs; however, not all patients respond to this treatment, possibly due to residual circulating non-native LCs (it is difficult to kill all the slowly proliferating clonal plasma cells).

A drug that directly binds and stabilizes full-length LC (FLC) dimers could suppress LC aggregation, reduce toxicity from circulating LCs, and be complementary to treatments that eradicate clonal plasma cells[1]. FLC kinetic stabilizers offer the potential to ameliorate non-native LC-induced organ toxicity and improve the condition of particularly sick patients to be able to tolerate existing chemotherapy regimens. In addition, kinetic stabilizers could improve the organ responses of patients not fully responding to anti-plasma cell therapies. Finally, stabilizers could be introduced after chemotherapy to minimize the consequences of relapse, by kinetically stabilizing secreted FLCs. FLC sequences are variable and have no natural ligands. We previously identified high-throughput screening hits that bind to a conserved site at the interface between the two variable domains of the FLC homodimer[2]. We hypothesized that extending the stabilizers beyond this initially characterized binding site would improve their affinity and more completely stabilize FLC. Stabilizers with high binding affinity and selectivity for most FLC sequences over other blood proteins are needed for clinical efficacy.

Objective: Develop LC stabilizers with improved binding affinity and FLC binding selectivity compared to the original high-throughput screening hits.

Materials & Methods: We used structure-based design, employing X-ray crystallographic data, medicinal chemistry, and computational docking to improve the potency of FLC stabilizers using the original high-throughput screening hits as starting design templates. To improve the ability of one stabilizer to tightly bind multiple FLC sequences, we focused stabilizer design to target highly conserved side chains found in most FLC sequences. We used protease sensitivity assays to assess candidate stabilizers. We calculated EC_{50} values to measure potency, which is the concentration of kinetic stabilizer that provides 50% of maximal protease protection.

Results: We identified FLC stabilizers with nanomolar EC_{50} values–a 3,000-fold enhancement over the screening hits. Crystal structures indicate that the stabilizers utilize highly conserved residues for FLC binding[3]. To assess stabilizer generality, we tested protease sensitivity of a panel of recombinant LCs from germlines common in AL, and found that the stabilizers were effective on most of these sequences.

Summary & Conclusion: We identififed potent FLC stabilizers that are effective at stabilizing multiple LC sequences. The structure-based design and assay pipeline we descibe will be used to optimize the binding affinity, selectivity and drug-like properties of future stabilizers. Optimized stabilizers offer the potential to be useful for the treatment of AL and benefit patients whose needs are unmet by current standard of care.

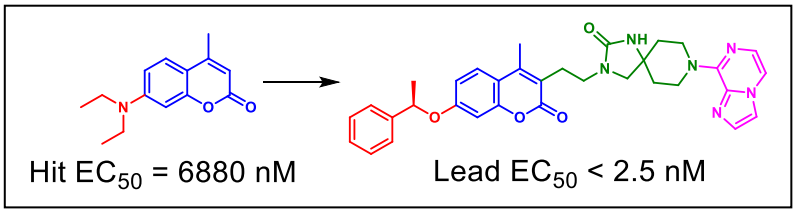

Figure 1. Compared to a high-throughput screening hit (left), an optimized LC stabilizer (right) has more than a 3,000-fold improvement in potency (EC_{50}).

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Long Term Survival of AL Patients Treated on Phase 1 trial of CAEL-101

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Background: Patients with advanced AL Amyloidosis continue to have high rates of early mortality and the organ responses achieved with anti-plasma cell therapies are insufficient and delayed (1,2). CAEL-101 is a monoclonal IgG1 antibody that directly binds to a conformational epitope present on both human kappa and lambda light-chain amyloid fibrils. We have previously reported results of an open-label phase 1a/1b clinical trial of the CAEL-101 and showed that the drug was overall well tolerated and organ response were seen in about 63% of patients (3). Here we are reporting long term survival of patients enrolled on the trial in comparison to a control group of patients.

Objective: To compare the long term survival of patients with AL Amyloidosis treated on phase 1 trial with CAEL-101 with control population.

Materials and Methods: We performed a retrospective analysis of patients with AL amyloidosis treated at Columbia University Medical Center (CUMC) between 1/1/2012 to 12/30/2019. After identifying the patients, we performed a retrospective chart review to obtain demographics, various disease characteristics including organ involvement. Patients were then divided into three groups for comparison 1. Patients treated with CAEL-101, 2. Control patients not treated with CAEL-101, 3. Control patients with cardiac involvement not treated with CAEL-101. Given that the patients enrolled on the trial were previously treated and patients with this disorder tend to have the highest risk of mortality during the first year after diagnosis, patients who survived for at least one year after diagnosis were included for analysis in the control group.

Results: A total of 108 patients were identified, 22 patients treated with the CAEL-101 and 86 controls. Further we decided to evaluate the third group of control patients with cardiac involvement (N=62). The baseline characteristics (Table 1) were comparable except that the cardiac control patients had a higher baseline NT-pro-BNP and the control patients had a higher percentage of lambda type AL Amyloidosis as compared to the CAEL-101 group. The median time from diagnosis to enrollment on the trial among the CAEL-101 patients was 1.6 years.

For the patients treated with CAEL-101 long-term survival data is available for 19/22 patients. With a median follow-up of 56 months (range 12-75), the median survival has not been reached and (16/19) of patients are alive and 3 patients have died from progressive AL Amyloidosis. Among the patients who have died 2/3 had cardiac involvement and did not have a cardiac response to CAEL-101 therapy. Among the evaluable patients 10/17 have experienced hematological progression and 5/17 patients have experienced organ progression, 11/17 patients have received further anti-plasma cell therapy after the treatment on the clinical trial. The survival of patients treated on the study was compared to patients in the two control groups and in both the comparisons we found a trend towards improved long term survival with HR 2.4 (95% CI 0.55-10.63) P = 0.23 in control group (figure 1) and HR 5.08 (95% CI 0.86-29.9) P = 0.07 in cardiac control group (figure 2).

Conclusion: Patients treated with CAEL-101 for AL Amyloidosis have excellent long term durability of organ responses and long term survival. These preliminary findings should be tested further in larger prospective clinical trial.

Table 1: Patient Characteristics

	CAEL-101 (N=22)	Control (N=86)	Cardiac Control (CC) (N=62)
Age	66	63	62
Gender (M/F)	14/8	46/40	32/30
Cardiac involvement	54%	72%	100%
Renal involvement	45%	61%	51%
Baseline NT-proBNP	1377 (662-13131)	1040 (89-17000)	2289 (191-17000)
Baseline Creatinine (median)	1.03 (0.8-1.3)	1.07 (0.53-5.4)	1.01 (0.59-5.4)
Light chain type	K 11 (50%) L 11 (50%)	K 19 (22%) L 67 (78%)	K 14 (22%) L 48 (78%)
24 hour urine protein (mg)	3062 (1200-7260)	2346 (600-23,000)	3700 (330-23,000)

Figure 1: Comparison of CAEL-101 (all patients) with Control (1Y)

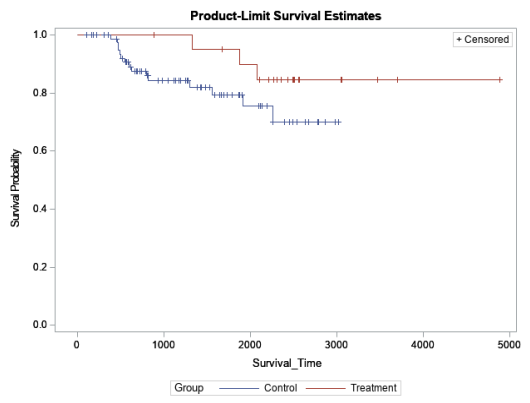
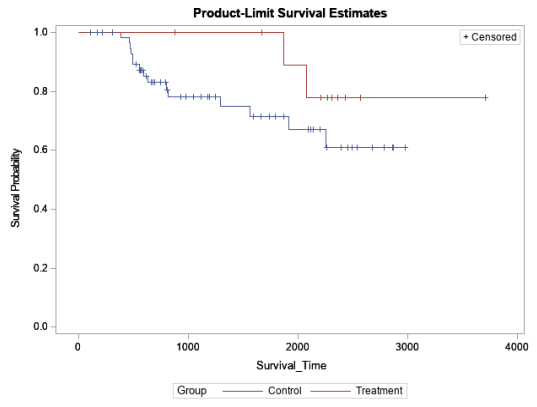


Figure 2: Comparison of CAEL-101 (cardiac patients) with Cardiac Control (C1Y)



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Limited cardiomyocytes' growth response to stimulation with human plasma and phenylephrine predicts poor outcome in ATTR cardiomyopathy in an in-vitro model.

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Background:
In ATTR cardiomyopathy misfolded protein is deposited in the myocardium leading to myocardial dysfunction. However, direct effects of mutant or misfoldet transthyretin (TTR) on cardiomyocytes and cardiomyocytes' hypertrophic growth response are not yet fully elucidated [1]. Furthermore no clinical outcome data of the predictive value of hypertrophic capacitiy in ATTR cardiomyopathy is available.

Objective:
We therefore analyzed cellular hypertrophy of neonatal rat cardiomyocytes (NRCMs) after phenylephrine stimulation in vitro in the presence of ATTR patients' plasma and correlated hypertrophic response with clincial parameters and prognosis in a monocentric prospective study.

Material & Methods:
Patients presenting at our tertial referral center for amyloidosis were ask to participate in the study, donated blood for in-vitro experiments and agreed to attend follow-up visits. Plasma samples were stored at -80° degrees until the experiments were conducted. Human plasma was added to NRCM cell culture with and without additional phenylephrine (PE) stimulation. After 48 hours, cells were fixed and immunohistochemically stained for nuclear DNA and Desmin. After immunhistological staining, fully automated high throughput microscopy was used to acquired images from the cardiomyocytes and its nuclei (see Figure 1A). Using Cell Profiler software and CMORE, a custom written R package, cell size analysis was attained automatically [2].

Results:
Hypertrophic response was evaluated in vitro in 105 individuals. 89 suffered from hereditary or wildtype transthyretin amyloidosis (ATTR). Furthermore 16 healthy individuals were included in the study to serve as a control group. In order to integrate information of untreated and PE-stimulated conditions to one parameter, we created a new metric – the "Hypertrophic Index (HI)". It is defined as increase in cell size upon PE stimulation normalized to cell size under the unstimulated condition (Figure 1B).
Its prognostic value was assessed for different cardiac endpoints (HTX: death/heart transplantation; DMP: cardiac decompensation and MACE: a combined endpoint) using Cox proportional hazard models (Figure 1C). Cells treated with plasma from healthy controls and hereditary transthyretin amyloidosis with polyneuropathy showed an elevation in HI, whereas stimulation after treatment with hereditary cardiac amyloidosis or wild-type transthyretin patient plasma showed a significantly reduced response. In univariate analyses HI was associated with HTX (hazard ratio (HR) high vs low: 0.12 [0.02-0.58], $p = 0.004$), DMP: (HR 0.26 [0.11-0.62], $p = 0.003$) and MACE (HR 0.24 [0.11-0.55], $p < 0.001$). Its prognostic value was independent of established risk factors, cardiac TroponinT or N-terminal prohormone brain natriuretic peptide (NTproBNP) in the

multivariate analysis.

Summary & Conclusion:
Reduced HI is an independent risk predictor of poor outcome in ATTR patients. Underlying mechanisms need to be addressed in further studies.

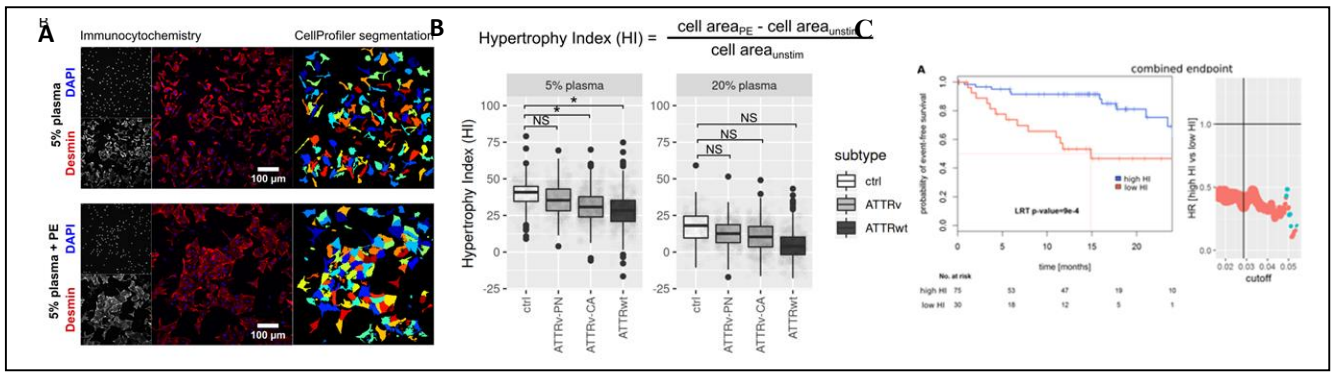

Figure 1.: A Immunhistochemical images (left and middle): Upper row stimulation solely with plasma, lower row: stimulation with plasma and PE, cardiomyocytes stained with Desmin (red) and Dapi (blue), automated area recognition by the software (right). **B** Hypertrophy Index in ATTR patients and controls, * indicates significantly different values, p<0.05, NS: not significant. **C** Kaplan-Meier-curves for high and low HI for the combined endpoint.

Support & Funding:
This study was sponsored by Pfizer Aspire Grant.

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DETAILED PROGRAM

SUNDAY, 4TH – THURSDAY, 8TH

DETAILED PROGRAM – SUNDAY, 4TH

4:30 PM – 5:00 PM | SOCIAL EVENT | Neue Aula/Main Lecture Hall

Opening Ceremony

Musical accompaniment

Kathrin Christians, *Flute*, Sonia Akchar, *Piano*

Ute Hegenbart, *host ISA 2022, Amyloidosis Center of the University Hospital, Heidelberg, Germany*

Giovanni Palladini, *ISA president, Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Christian Schaaf, *Representative of the Center of Rare Diseases, University Hospital, Heidelberg, Germany*

5:00 PM – 7:00 PM | SCIENTIFIC SESSION | Neue Aula/Main Lecture Hall

Keynote Lecture & Opening Lectures

Chairs

Stefan Schönland, *host ISA 2022, Amyloidosis Center of the University Hospital, Heidelberg, Germany*

Giovanni Palladini, *ISA president, Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Introduction — Stefan Schönland

Keynote Lecture — Aging (of the Immune System)

Cornelia Weyand, *Stanford University, Stanford, and Mayo Clinic, Rochester, USA*

Opening Lecture — AL Amyloidosis model – leading the way to cure

Giampaolo Merlini, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Introduction — Giovanni Palladini

Opening Lectures — Turning swords into plowshares: the case of functional amyloid

Daniel Otzen, *Aarhus University, Aarhus, Denmark*

New horizons of gene targeting therapy in ATTRv amyloidosis

Julian Gillmore, *National Amyloidosis Centre, London, United Kingdom*

7:00 PM – 8:00 PM | SOCIAL EVENT | ground floor/2nd floor

Welcome Reception

Get Together with served snacks and beverages

9:30 AM – 5:00 PM | EXHIBITION | ground floor/2nd floor

Exhibition on-site and virtual

7:30 AM – 8:30 AM | SATELLITE SYMPOSIUM | H14

Sponsored breakfast starts at 7:00 AM

Global Bridges: Building Diagnostic Capacity Worldwide

Amyloidosis Grantee Presentations

Chairs

Morie Gertz, *Mayo Clinic, Rochester, USA*

Martha Grogan, *Mayo Clinic, Rochester, USA*

Introduction

Morie Gertz

Presentations

Building Awareness and Improving the Quality of Diagnosis

Sharmila Dorbala, *Brigham and Women's Hospital, USA*

Firas Al Badarin, *Cleveland Clinic Abu Dhabi, UAE*

Bruno Bueno, *Heart Institute, Hospital Das Clinicas, U Sao Paolo, Brazil*

Isabel Carvajal Juárez, *ASNC Latin America, Mexico*

Special patient populations/leveraging technology

Saurabh Malhotra, *Cook County Health, Illinois, USA*

Paolo Milani, *Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Establishing Regional Centers of Excellence

Ramzi Tabbalat, *Abdali Medical Center, Jordan*

Silvia Lupu, *Emergency Institute for Cardiovascular Disease and Transplant, Romania*

Kwaku Appiah-Kubi, C.K. Tedam University of Technology and Applied Science, Ghana

Summary/Close

Martha Grogan

7:15 AM – 8:30 AM | SCIENTIFIC SESSION | Neue Aula/Main Lecture Hall

IKMG/ISA – Challenges in Monoclonal Gammopathy of Renal Significance

Joint Session

Chairs

Ute Hegenbart, *host ISA 2022, Amyloidosis Center of the University Hospital, Heidelberg, Germany*

Nelson Leung, *Mayo Clinic, Rochester, USA*

DETAILED PROGRAM – MONDAY, 5TH

Lectures	Differential diagnosis of MGRS Guillermo Herrera, *University of South Alabama, USA* Treatment endpoints in MGRS Angela Dispenzieri, *Mayo Clinic, Rochester, USA* MGRS: directions for future research Frank Bridoux, *Hôpital Jean Bernard, Poitiers, France*

8:30 AM – 10:05 AM	SCIENTIFIC SESSION	Neue Aula/Main Lecture Hall
	Basic research – light chains including cardiotoxicity	
Chairs	Stefano Ricagno, *Università di Milano, Milano, Italy* Marina Ramirez-Alvarado, *Mayo Clinic, Rochester, USA*	
State-of-the-art lecture	Stefano Ricagno	
OP001	Next Generation Sequencing Identifies AL-related IGLV Genes in Patients with λ-isotype MGUS or Smoldering Multiple Myeloma Ray Comenzo, *Tufts Medical Center, Boston, USA*	
OP002	Elucidation of the cardiotoxicity of full-length light chains derived from patients with cardiac light chain amyloidosis in comparison to other plasma cell dyscrasias Panagiota-Efstathia Nikolaou, *National and Kapodistrian University, Athens, Greece*	
OP003	Investigation of IGLV2-14 AL amyloidosis and multiple myeloma light chain sequences Natalie Berghaus, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*	
OP004	Sequence diversity of the kappa light chains from patients with AL amyloidosis and multiple myeloma Sarah Schreiner, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*	
OP005	Single Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq): toward personalized medicine approaches in monoclonal gammopathies Mario Nuvolone, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*	
OP006	An N-glycosylation hotspot in immunoglobulin κ light chains is associated with AL amyloidosis Alice Nevone, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*	

OP007	Amyloidogenesis by mesangial cells involves active participation of lysosomes Guillermo A. Herrera, *University of South Alabama, Alabama, USA*
OP008	Mapping and modelling the molecular mechanisms that drive amyloidogenic light chain induced cardiotoxicity Camille Vanessa Edwards, *Boston University, Boston, USA*

10:05 AM – 10:30 AM COFFEE BREAK  New University, ground floor/2nd floor/courtyard

10:05 AM – 10:30 AM EXHIBITION Virtual Venue

Virtual Speaker Corner

Talk with industry partners

10:30 AM -12:05 PM SCIENTIFIC SESSION Neue Aula/Main Lecture Hall

ATTRwt – Clinical aspects

Chairs:
Thibaud Damy, *Referral Center for Cardiac Amyloidois, Henri Mondor Hospital, Creteil, France*
Matthew Maurer, *Columbia University, New York, USA*

State-of-the-art lecture Thibaud Damy

OP009	Long-term tafamidis treatment reduces the decline in quality of life among patients with transthyretin amyloid cardiomyopathy Martha Grogan, *Mayo Clinic, Rochester, USA*
OP010	Atrial fibrillation as a prognostic factor for all-cause mortality in patients with transthyretin amyloid cardiomyopathy Ronald Witteles, *Stanford University, Stanford, USA*
OP011	Long-term safety and tolerability of acoramidis (AG10) in symptomatic transthyretin amyloid cardiomyopathy: Updated analysis from an ongoing phase 2 open-label extension study Ahmad Masri, *OHSU, Portland, USA*
OP012	Chronic Intravenous Inotropic Therapy in Cardiac Amyloidosis Johana Rocio Fajardo, *Medstar Heart & Vascular Institute, Washington, USA*
OP013	Tolerability and side-effects of therapy in an open-label trial of inotersen for transthyretin amyloid cardiomyopathy Leo C. Samuels, *Brigham and Women's Hospital, Boston, USA*

OP014 Looking over your shoulder to catch amyloidosis earlier: shoulder pathologies are significantly more prevalent in patients with transthyretin cardiac amyloidosis
Alyssa Basdavanos, *Cleveland Clinic Foundation, Cleveland, USA*

OP015 SARS-CoV-2 infection in systemic amyloidosis: the International Society of Amyloidosis' survey
Paolo Milani, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

OP016 Cardiac Transplantation in Transthyretin Amyloid Cardiomyopathy: Outcomes from Three Decades of Tertiary Centre Experience
Yousuf Razvi, *National Amyloidosis Centre, London, United Kingdom*

12:05 PM – 1:20 PM LUNCH  New University, ground floor/courtyard & Triplex Canteen

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12:05 PM – 1:20 PM POSTER PRESENTATIONS Triplex Canteen, 1st floor

Poster Numbers: P001 – P121, P208: AL and ATTR amyloidosis
For the virtual audience the poster and an audio summary by the presenting authors will be provided in the Virtual Venue.

AL amyloidosis

Chairs
Vaishali Sanchorawala, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*
Paolo Milani, *Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*
Maite Cibeira, *Amyloidosis and Myeloma Unit, Barcelona, Spain*
Timon Hansen, *Haematology-Oncology Clinic, Altona, Hamburg, Germany*
Hermine Agis, *Department of Medicine, Medical University of Vienna, Vienna, Austria*
Angela Dispenzieri, *Mayo Clinic, Rochester, USA*
Jeffrey Zonder, *Karmanos Cancer Institute, Detroit, USA*

ATTRwt amyloidosis

Chairs
Thibaud Damy, *Referral Center for Cardiac Amyloidois, Henri Mondor Hospital, Creteil, France*
Matthew Maurer, *Columbia University, New York, USA*
Pablo Garcia-Pavia, *Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain*
Maria Papathanasiou, *University Hospital Essen, Germany*

ATTRv amyloidosis

Chairs
Ernst Hund, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*
Katrin Hahn, *Amyloidosis Center, Charité, Berlin, Germany*
Isabel Maria Conceicao, *Centro Hospitalar Universitário Lisboa Norte – Hospital de Santa Maria, Lisboa, Portugal*
Markus Weiler, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*

1:20 PM – 3:00 PM | SCIENTIFIC SESSION | Neue Aula/Main Lecture Hall

Imaging in amyloidosis

Chairs
Ashutosh Wechalekar, *National Amyloidosis Centre, London, United Kingdom*
Sharmila Dorbala, *Brigham and Women's Hospital, Boston, USA*

State-of-the-art lecture — Amyloid Imaging
Ashutosh Wechalekar

State-of-the-art lecture — Cardiac Imaging
Sharmila Dorbala

OP017 — Results of the first-in-human PET/CT imaging study of the amyloid-reactive peptide 124I-AT-01 (124I-p5+14) for the detection of systemic amyloidosis
Jonathan Wall, *University of Tennessee, Knoxville, USA*

OP018 — Pan-amyloid reactivity of radioiodinated peptide 124I-AT-01 in patients with systemic amyloidosis demonstrated by PET/CT imaging
Emily Martin, *University of Tennessee, Knoxville, USA*

OP019 — Tracking multi-organ treatment response in systemic AL-amyloidosis with cardiac magnetic resonance derived extracellular volume mapping
Adam Ioannou, *Royal Free Hospital, London, United Kingdom*

OP020 — Regression of Cardiac Bone-Tracer Uptake in Patients with Hereditary Transthyretin Amyloidosis after One Year Treatment with Patisiran. An early Marker of Treatment Response?
Hendrea S.A. Tingen, *University Medical Center, Groningen, Netherlands*

OP021 — Real World Experience of Tc-DPD scintigraphy as a diagnostic imaging tool in amyloidosis
Muhammad Umaid Rauf, *National Amyloidosis Centre, London, United Kingdom*

OP022	Assessing left ventricular strain in cardiac amyloidosis: the importance of accurate measurement technique Rodney H. Falk, *Brigham and Women's Hospital, Boston, USA*
OP023	Quantitative magnetic resonance neurography biomarkers: Cross-sectional results from a single center study in 80 subjects with symptomatic or asymptomatic hereditary transthyretin amyloidosis Jennifer Hayes, *Amyloidosis Center of the University Hospital, Heidelberg*
OP024	Assessment of the clinical value of whole-body MRI in untreated patients with systemic light chain amyloidosis Simone Christine Brandelik, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*

3:05 PM – 4:10 PM	SCIENTIFIC SESSION — Neue Aula/Main Lecture Hall **Preclinical models of systemic amyloidosis**
Chairs	Alexander Carpinteiro, *Amyloidosis Center of the University Hospital, Essen, Germany* Gunilla Westermark, *Uppsala University, Uppsala, Sweden*
Introduction	Alexander Carpinteiro
Lectures	A mouse model of AL amyloidosis Christophe Sirac, *University of Limoges, Limoges, France* Insights into ATTR amyloidosis from a new transgenic mouse model Paul Simons, *National Amyloidosis Centre, London, United Kingdom* AL amyloidosis modelled in Caenorhabditis elegans Luisa Diomede, *Mario Negri IRCCS, Milano, Italy*
Debate	Christophe Sirac, Paul Simons, Luisa Diomede

4:10 PM – 4:30 PM	COFFEE BREAK — New University, ground floor/2nd floor/courtyard

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4:30 PM – 6:00 PM	SATELLITE SYMPOSIUM — Neue Aula/Main Lecture Hall **Janssen: Navigating the patient journey in AL amyloidosis: a multidisciplinary approach**
Chairs	Giovanni Palladini, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy* Marianna Fontana, *National Amyloidosis Centre, London, United Kingdom*

Introduction	Giovanni Palladini
Lecture	Diagnostic pit-falls and risk stratification in AL amyloidosis Marianna Fontana
Panel Discussion	Frank Bridoux, *Hôpital Jean Bernard, Poitiers, France* Darren Foard, *National Amyloidosis Centre, London, United Kingdom* Christoph Röcken, *University Hospital, Kiel, German*
Lecture	Key considerations when selecting treatment for patients with AL amyloidosis Giovanni Palladini
Panel Discussion	Frank Bridoux, Darren Foard, Christoph Röcken
Summary/Close	Giovanni Palladini

6:00 PM – 7:00 PM SATELLITE SYMPOSIUM Neue Aula/Main Lecture Hall

Pfizer: How can one solution solve the multifaceted challenges of ATTR amyloidosis?

Chairs	Arnt Kristen, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*
Introduction	Arnt Kristen
Lectures	Transthyretin in health and disease Laura Obici, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy* Improving patient outcomes in ATTR-PN Laura Obici Addressing cardiac involvement in patients with ATTR amyloidosis Arnt Kristen Clinical clues to cardiac involvement in ATTR amyloidosis – how to identify patients early Arnt Kristen, Rachele Bonfiglioli, *Azienda Ospedaliero-Universitaria, Bologna, Italy*
Panel Discussion/Close	Arnt Kristen, Laura Obici, Rachele Bonfiglioli

DETAILED PROGRAM – MONDAY, 5TH

7:05 PM – 8:05 PM	SOCIAL EVENT **Merlini Award Ceremony** Musical accompaniment Kathrin Christians, *Flute*, Sonia Akchar, *Piano*	Alte Aula/Building "Alte Universität"
Laudatio	Giovanni Palladini, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*	
Lecture	Amyloidosis: Reflections on passed and coming times Per Westermark, *University Hospital, Uppsala, Sweden* Merlini Award Winner 2022	

DETAILED PROGRAM – TUESDAY, 6TH

9:30 AM – 5:00 PM	EXHIBITION Exhibition on-site and virtual	ground floor/2nd floor

7:30 AM – 8:30 AM	SATELLITE SYMPOSIUM **Prothena: Addressing the Unmet Need in Advanced AL Amyloidosis: Key Insights from a Panel of Experts**	Neue Aula/ Main Lecture Hall Sponsored breakfast starts at 7:00 AM
Chair	Morie Gertz, *Mayo Clinic, Rochester, USA*	
Introduction	Morie Gertz	
Lectures	Epidemiology and Unmet Need in Advanced AL Amyloidosis Maria Papathanasiou, *University Hospital Essen, Germany* Diagnosis and Current Treatment Approaches in AL Amyloidosis Giovanni Palladini, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy* Emerging Therapies in Advanced AL Amyloidosis Morie Gertz	
Panel Discussion	Morie Gertz, Maria Papathanasiou, Giovanni Palladini	

8:30 AM – 10:05 AM	SCIENTIFIC SESSION **AL – Clinical aspects**	Neue Aula/Main Lecture Hall
Chairs	Shaji Kumar, *Mayo Clinic, Rochester, Minnesota, USA* Vaishali Sanchorawala, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*	
State-of-the-art lecture	Shaji Kumar	
OP025	Predictors of hematologic response and survival with stem cell transplantation in AL amyloidosis: a 25-year longitudinal study Joshua Gustine, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*	
OP026	The prognostic importance of flow cytometry-based measurable residual disease (MRD) in patients with systemic light chain amyloidosis Andrew Staron, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*	

OP027	Prognostic Impact of Translocation t(11;14) and of other cytogenetic abnormalities in patients with AL amyloidosis in the era of contemporary therapies Despina Fotiou, *National and Kapodistrian University, Athens, Greece*
OP028	Incidence and risk factors of sudden death in patients with cardiacamyloidosis Fernando de Frutos, *Hospital Universitario Puerta de Hierro, Majadahonda, Spain*
OP029	Clonal features affect survival of patients with non-cardiac light chain (AL) amyloidosis: a European study of 386 patients Paolo Milani, *Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*
OP030	Light chain deposition disease: an international study in 523 patients Paolo Milani, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*
OP031	Elotuzumab in combination with IMIDS for AL amyloidosis patients with relapsed/refractory plasma cell dyscrasia and advanced organ involvement Tobias Dittrich, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*
OP032	First Report on Outcome of Patients with Newly Diagnosed Stage IIIb Cardiac Light Chain Amyloidosis Treated with Daratumumab-based Frontline Therapy Rajshekhar Chakraborty, *Irving Medical Center, Columbia, USA*

10:05 AM – 10:30 AM COFFEE BREAK New University, ground floor/2nd floor/courtyard

10:30 AM – 12:05 PM SCIENTIFIC SESSION Neue Aula/Main Lecture Hall

Basic Research – New treatment targets and biomarkers

Chairs

Gareth Morgan, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*

Carlos Fernández de Larrea, *Amyloidosis and Myeloma Unit, Barcelona, Spain*

State-of-the-art lecture	Gareth Morgan
OP033	Collagen associated with AL amyloid inhibits fibril phagocytosis - Collagen degradation renders amyloid sensitive to uptake by the innate immune system Joseph W. Jackson, *University of Tennessee, Knoxville, USA*
OP034	In-vitro ultrasonic assay indicates importance of extracellular chaperon-like effect of serum albumin to protect dialysis patients from dialysis-related amyloidosis Kichitaro Nakajima, *Osaka University, Osaka, Japan*
OP035	Dissecting FAM46C-dependent tuning of antibody secretion in systemic AL amyloidosis Enrico Milan, *San Raffaele Scientific Institute, Milano, Italy*
OP036	Development of novel human chimeric antigen receptor-macrophages (CAR-M) as a potential therapeutic for amyloid clearance Manasi Balachandran, *University of Tennessee, Knoxville, USA*
OP037	Preclinical characterization of AT-02, a pan-amyloid-binding immunoglobulin-peptide fusion protein capable of inducing enhanced phagocytosis of amyloid Jonathan Wall, *University of Tennessee, Knoxville, USA*
OP038	Regulation of BCL2 family members by microRNA-9 and microRNA-181a in AL amyloidosis Oshrat Hershkovitz-Rokah, *Assuta Medical Center, Tel Aviv, Israel*
OP065	AA amyloid-containing diet potentiates Aβ42 induced effects in transgenic Drosophila melanogaster Gunilla Westermark, *Uppsala University, Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden*
OP040	Targeting protein secretion as a therapeutic strategy in AL amyloidosis Maria Moscvin, *Brigham and Women's Hospital, Boston, USA*

12:05 PM – 1:15 PM LUNCH New University, ground floor/courtyard & Triplex Canteen

DETAILED PROGRAM – TUESDAY, 6TH

12:05 PM – 1:15 PM | POSTER PRESENTATIONS | Triplex Canteen, 1st floor

Poster Numbers: P122 – P190: Basic science and Imaging

For the virtual audience the poster and an audio summary by the presenting authors will be provided in the Virtual Venue.

Fibril and Amyloid Formation

Chairs
Mario Nuvolone, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*
Christoph Röcken, *University Hospital, Kiel, Germany*
Joost Schymkowitz, *Leuven Center for Brain & Disease Research, Leuven, Belgium*

Mechanism of organ dysfunction and damage

Chairs
Marina Ramirez-Alvarado, *Mayo Clinic, Rochester, USA*
Stefano Ricagno, *Università di Milano, Milano, Italy*

Pre-clinical disease models

Chairs
Gunilla Westermark, *Uppsala University, Uppsala, Sweden*
Alexander Carpinteiro, *University Hospital Essen, Germany*

Imaging

Chairs
Simon Gibbs, *The Victorian and Tasmanian Amyloidosis Service, Australian Amyloidosis Network, Australia*
Jennifer Hayes, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*
Jonathan Wall, *University of Tennessee, Knoxville, USA*
Martha Grogan, *Mayo Clinic, Rochester, USA*

12:05 PM – 1:15 PM | EXHIBITION | Virtual Venue

Virtual Speaker Corner

Talk with industry partners

1:15 PM – 2:30 PM | SCIENTIFIC SESSION | Neue Aula/Main Lecture Hall

AA – Clinical aspects

Chairs
Norbert Blank, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*
Sophie Georgin-Lavialle, *Sorbonne University, Paris, France*

State-of-the-art lecture
Norbert Blank

Lectures
AA Amyloidosis in Europe, Perspectives and Challenges
Sophie Georgin-Lavialle
AA Amyloidosis in Africa, Perspectives and Challenges
Ghalia Khellaf, *Centre Hospitalo-Universitaire, Alger, Algeria*

OP041
Tocilizumab can prevent the progression of renal AA amyloidosis to end stage renal disease
Peter Kvacskay, Amyloidosis Center of the University Hospital, *Heidelberg, Germany*

OP042
Natural history and risk stratification of AA amyloidosis based on a 40-year experience in the United States
Tracy Joshi, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*

OP043
Long-term transplant outcomes in recipients with renal amyloidosis
Tale Norbye Wien, *Bærum Hospital Vestre Viken, Drammen, Norway*

2:30 PM – 3:30 PM | SATELLITE SYMPOSIUM | Neue Aula/Main Lecture Hall

BridgeBio: Improving patient outcomes in ATTR-CM

Chairs
Julian Gillmore, *National Amyloidosis Centre, London, United Kingdom*
Matthew S. Maurer, *Columbia University, New York, USA*

Introduction
Julian Gillmore, Mathew S. Maurer

Lectures
ATTR-CM consensus – what's new?
Claudio Rapezzi, *University of Ferrara, Ferrara, Italy*
Present and future in ATTR-CM
Pablo Garcia-Pavia, *Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain*

Q&A/Close
Julian Gillmore, Mathew S. Maurer

DETAILED PROGRAM – TUESDAY, 6TH

3:30 PM – 5:00 PM	SATELLITE SYMPOSIUM **Ionis/AstraZeneca: Optimizing Multidisciplinary Care in Patients With ATTR**	Neue Aula/Main Lecture Hall
Chair	Arnt Kristen, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*	
Introduction	Arnt Kristen	
Patient Journey Video	Arnt Kristen	
	ATTR: Disease Overview Arnt Kristen	
Lecture	Optimizing Patient Care: A Neurologist's Perspective Teresa Coelho, *Centro Hospitalar Universitário, Porto, Portugal*	
	Interactive Case Studies: Neurology	
Panel Discussion	Arnt Kristen, Teresa Coelho, Pablo Garcia-Pavia, *Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain*	
Lecture	Optimizing Patient Care: A Cardiologist's Perspective Pablo Garcia-Pavia	
	Interactive Case Studies: Cardiology	
Panel Discussion/Q&A	Arnt Kristen, Teresa Coelho, Pablo Garcia-Pavia	
Close	Arnt Kristen	

5:00 PM – 7:00 PM	**Free Time/Explore Heidelberg** We have posted some nice options for sightseeing and leisure tours on our ISA 2022 homepage.	Heidelberg

7:00 PM – 10:00 PM | SOCIAL EVENT | Triplex Canteen Foyer/1st floor/Courtyard

Get Together

Get Together with served snacks and beverages

07:00 PM – 07:30 PM | POSTER PRESENTATIONS | Triplex Canteen, 1st floor

Poster Numbers: PLB001 – 017: Late breaking abstracts

For the virtual audience the poster and an audio summary by the presenting authors will be provided in the Virtual Venue.

Chairs

Suzanne Lentzsch, *Columbia University, New York, USA*
Per Westermark, *Uppsala University, Uppsala, Sweden*

7:30 PM – 8:15 PM | SCIENTIFIC SESSION | Triplex Canteen, Foyer

Challenging Cases

Three cases of diagnostic and therapeutic challenges will be presented during this session. Panelists and audience will participate to solve these difficult cases.

Chairs

Vaishali Sanchorawala, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*
Maite Cibeira, *Amyloidosis and Myeloma Unit, Barcelona, Spain*
Paolo Milani, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Panel of Experts

Angela Dispenzieri, *Mayo Clinic, Rochester, USA*
Julian Gillmore, *National Amyloidosis Centre, London, United Kingdom*
Pablo Garcia-Pavia, *Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain*

DETAILED PROGRAM – TUESDAY, 6TH

8:15 PM – 9:15 PM

SOCIAL EVENT

Triplex Canteen, Foyer

Junior Meets Senior
Round Table

The path of a scientific career is often long and sometimes difficult. The goal of this session is to provide junior physicians and scientists in the audience a chance to interact with experts in the field of Amyloidosis and beyond. Both, young and established colleagues might profit from sharing their experience in diverse areas, including their academic careers, approach to research and publishing, and also more personal aspects like work-life balance.

Chairs

Fabian aus dem Siepen, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*
Eloisa Riva, *Hospital de Clínicas "Dr. Manuel Quintela", Montevideo, Uruguay*

Senior Experts

Per Westermark, *Uppsala University, Uppsala, Sweden*
Suzanne Lentzsch, *Columbia University, New York, USA*
Cornelia Weyand, *Stanford University, Stanford, and Mayo Clinic, Rochester, USA*
Jörg Goronzy, *Stanford University, Stanford and Mayo Clinic, Rochester, USA*
Martha Grogan, *Mayo Clinic, Rochester, USA*
Carsten Müller-Tidow, *University Hospital, Heidelberg, Germany*
Norbert Frey, *University Hospital, Heidelberg, Germany*


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DETAILED PROGRAM – WEDNESDAY, 7TH

9:30 AM – 5:00 PM	EXHIBITION **Exhibition on-site and virtual**	ground floor/2nd floor

7:45 AM – 8:30 AM	SATELLITE SYMPOSIUM **Sobi™: Expert Discussion: Is Disease Remission in hATTR a Realistic Goal?**	Neue Aula/Main Lecture Hall Sponsored coffee starts at 7:00 AM
Chair	Teresa Coelho, *Centro Hospitalar Universitário, Porto, Portugal*	
Introduction	Teresa Coelho	
Lectures	Evolution of diagnosis and management of hATTR Katrin Hahn, *Amyloidosis Center, Charité, Berlin, Germany* "Flipping the pyramid": lessons learned from MS Gavin Giovannoni, *Queen Mary University, London, United Kingdom* Are we primed to make remission a reality? Marco Luigetti, *Università Cattolica del Sacro Cuore, Rome, Italy*	
Panel Discussion	Teresa Coelho, Katrin Hahn, Gavin Giovannoni, Marco Luigetti	
Conclusion	Teresa Coelho	

8:30 AM – 10:05 AM	SCIENTIFIC SESSION **ATTRv – Clinical aspects**	Neue Aula/Main Lecture Hall
Chairs	David Adams, *Université Paris Saclay, Paris, France* Marcia Waddington-Cruz, *Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*	
State-of-the-art lecture	David Adams	
OP044	Patisiran Global Open-label Extension Study at 36 Months: Effect of Long-term Treatment on Mortality and Ambulatory Function in Patients with hATTR Amyloidosis with Polyneuropathy Jonas Wixner, *Umeå University, Umeå, Sweden*	
OP045	Progression and distribution pattern of cerebral amyloid angiopathy in hereditary ATTR amyloidosis patients visualized by 11C-PiB-PET imaging Yusuke Takahashi, *Shinshu University, Matsumoto, Japan*	

OP046	An international Delphi survey for the definition of a multidisciplinary holistic approach to the care of hereditary ATTR amyloidosis Laura Obici, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*
OP047	Eplontersen in ATTR-polyneuropathy: results from the 35-week interim analysis of NEURO-TTRansform Teresa Coelho, *Centro Hospitalar Universitário, Porto, Portugal*
OP048	Diflunisal treatment for hereditary transthyretin amyloidosis – the Swedish DFNS-02 trial Jonas Wixner, *Umeå University, Umeå, Sweden*
OP049	Kidney phenotype and immune activation in hereditary ATTR amyloidosis during inotersen therapy Joana Tavares, *Centro Hospitalar Universitário do Porto, Porto, Portugal*
OP050	Association of hereditary V122I amyloidogenic transthyretin variant with heart failure: A systematic review and meta-analysis Kwaku Appiah-Kubi, *C. K. Tedam University, Navrongo, Ghana*
OP051	Tafamidis reduces skin denervation and amyloid in skin biopsies of very early symptomatic hATTRv patients after one year of treatment Alejandra Gonzalez-Duarte, *INCMNSZ, Ciudad de México,Mexico*

9:00 AM – 10:00 AM	SCIENTIFIC SESSION **EHA/ISA – Better understanding and targeting the clone in AL amyloidosis**	H14
Chairs	Efstathios Kastritis, *National and Kapodistrian University, Athens, Greece* Monique Minnema, *University Medical Center, Utrecht, Netherlands*	
Lectures	Future Myeloma therapies suitable for AL amyloidosis Marc Raab, *Myeloma Center, University Heidelberg, Germany* Marginal zone lymphoma and M. Waldenström as underlying disease in AL amyloidosis Davide Rossi, *Oncology Institute of Southern Switzerland, Bellinzona, Switzerland*	

10:05 AM – 10:30 AM	COFFEE BREAK	New University, ground floor/2nd floor/courtyard

10:30 AM – 12:05 PM SCIENTIFIC SESSION Neue Aula/Main Lecture Hall

Pathways to diagnosis

Chairs
Hironobu Naiki, *University of Fukui, Fukui, Japan*
Kerstin Amann, *University Hospital, Erlangen, Germany*

State-of-the-art lecture Hironobu Naiki

OP052 Automated cardiac amyloidosis risk detection on whole body bone scintigraphy using deep-learning approach
Marc-Antoine Delbarre, *University Hospital, Amiens, France*

OP053 Establishment of the nation-wide pathology consultation system for the typing diagnosis of amyloidosis in Japan: Steep increase in the number of transthyretin-positive cardiac biopsy cases
Hironobu Naiki

OP054 The amyloid proteome: a two-way approach for a protein classification system
Juliane Gottwald, *University Hospital, Kiel, Germany*

OP055 Fat aspiration as a screening tool for symptomatic systemic amyloidosis - sensitivity and specificity analysis with better sensitivity results for females with all major subtypes
Christoph Richard Kimmich, *University Hospital, Oldenburg, Germany*

OP056 Sequence of diagnostic testing in cardiac amyloidosis patients: early monoclonal protein study is associated with better outcomes in AL amyloidosis
Francesca Fabris, *Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

OP057 Artificial intelligence modeling for earlier identification of cardiac amyloidosis
Surendra Dasari, *Mayo Clinic, Rochester, USA*

OP058 A novel mass spectrometry-based method for the identification of subtype specific amyloidogenic proteins from fat aspirates
Hans Christian Beck, *University Hospital, Odense, Denmark*

OP059 Pre-symptomatic diagnosis of systemic AL amyloidosis by biomarker-based screening in patients with MGUS
Silvia Mangiacavalli, *Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

DETAILED PROGRAM – WEDNESDAY, 7TH

12:05 PM – 1:20 PM LUNCH New University, ground floor/courtyard & Triplex Canteen

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12:05 PM – 1:20 PM POSTER PRESENTATIONS Triplex Canteen, 1st floor

Poster Numbers: P191 – P295 (except P208, moved to Monday):
Pathway to diagnosis, Innovative drugs and non-AL/ non-ATTR amyloidosis
For the virtual audience the poster and an audio summary by the presenting authors will be provided in the Virtual Venue.

Pathway to Diagnosis

Chairs
Eli Muchtar, *Mayo Clinic, Rochester, USA*
Rahel Schwotzer, *University Hospital, Zurich, Switzerland*
Fabian aus dem Siepen, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*
Eloisa Riva, *Hospital de Clínicas "Dr. Manuel Quintela", Montevideo, Uruguay*
Sandra Ihne, *Amyloidosis Center, University Hospital Würzburg, Germany*
Hans Nienhuis, *Amyloidosis Centre of Expertise, University Medical Center, Groningen, Netherlands*
Kerstin Amann, *University Hospital, Erlangen, Germany*
Hironobu Naiki, *University of Fukui, Fukui, Japan*
Arnaud Jaccard, *CHU, Limoges, France*

AA Amyloidosis

Chair
Norbert Blank, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*

Biology, Clinics and Therapeutics in other types of amyloidosis

Chairs
Laura Obici, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Innovative Therapies

Chairs
Efstathios Kastritis, *National and Kapodistrian University, Athens, Greece*
Gareth Morgan, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*
Peter Mollee, *Princess Alexandra Hospital, Brisbane, Queensland, Australia*

1:20 PM – 3:00 PM | SCIENTIFIC SESSION | Neue Aula/Main Lecture Hall

Basic research – Amyloid fibril formation including proteolysis and tissue interactions

Chairs

Matthias Schmidt, *University Ulm, Ulm, Germany*
Christoph Röcken, *University Hospital, Kiel, Germany*
Stefan Schönland, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*

State-of-the-art lecture — Matthias Schmidt, Christoph Röcken, Stefan Schönland

OP060 Kinetics of the aggregation process of the human λ-III immunoglobulin light chain FOR005 involved in AL amyloidosis at atomic resolution
Tejaswini Pradhan, *Technical University, Munich, Germany*

OP061 The ex vivo cryo-EM structure of AA amyloid from a domestic short hair cat
Tim Schulte, *Gruppo San Donato, San Donato Milanese, Italy*

OP062 The interplay between protein dynamics and proteolysis in LC amyloid aggregation
Stefan Ricagno, *Università di Milano, Milano, Italy*

OP063 Time-resolved nano-spectroscopy with single-molecule sensitivity for blood-based non-immune diagnosis of amyloid diseases
Ann Tiiman, *Center for Molecular Medicine (CMM), Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden*

OP064 In vitro and in vivo effects of SerpinA1 on the modulation of transthyretin proteolysis
Maria Rosario Almeida, *Universidade do Porto, Molecular Biology Department, Porto, Portugal*

OP039 Elevated fibrosis associated biomarkers in ATTR amyloidosis patients are associated with impaired cardiovascular outcome
Selina Julia Hein, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*

OP066 Dichotomy of Circulating Non-native TTR (NNTTR) Levels in Polyneuropathy and Cardiomyopathy Patients Provides a Glimpse to ATTR Tissue Specificity
Xin Jiang, *Protego Biopharma, San Diego, USA*

OP067 Heterotypic amyloid interactions and their effect on amyloid assembly
Frederic Rousseau, *VIB-KU Leuven Center for Brain and Disease Research, Leuven, Belgium*

DETAILED PROGRAM – WEDNESDAY, 7TH

3:00 PM – 4:30 PM	SATELLITE SYMPOSIUM	Neue Aula/Main Lecture Hall
	Alexion: Beyond Survival: Unmet Medical Needs in AL Amyloidosis	
Chair	Vaishali Sanchorawala, *Amyloidosis Center at Boston University School of Medicine, Boston, USA*	
Introduction	Vaishali Sanchorawala	
Lecture	Looking Beyond Plasma Cell Dyscrasia in AL Amyloidosis Rodney Falk, *Brigham and Women's Hospital, Boston, USA*	
Panelists	Julian Gillmore, *National Amyloidosis Centre, London, United Kingdom* Giovanni Palladini, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy* Vaishali Sanchorawala Ashutosh Wechalekar, *National Amyloidosis Centre, London, United Kingdom*	
Lecture	Unmet Medical Needs in Managing Tissue Toxicity and Organ Dysfunction Giovanni Palladini	
Panelists	Rodney Falk, Julian Gillmore, Vaishali Sanchorawala, Ashutosh Wechalekar	
Lecture	Beyond Survival - Assessing and Monotoring Organ and Tissues Ashutosh Wechalekar	
Panelists	Rodney Falk, Julian Gillmore, Giovanni Palladini, Vaishali Sanchorawala	
Lecture	Continuing Conversation to Learn from Patient Insights Giovanni Palladini	
Panelists	Deborah Boedicker, *Amyloidosis Speakers Bureau, Washington, DC, USA* Kristen Hsu, *Amyloidosis Research Consortium, Newton, USA*	
Q&A	Vaishali Sanchorawala Rodney Falk, Giovanni Palladini, Ashutosh Wechalekar	
Close	Vaishali Sanchorawala	

4:30 PM – 5:00 PM	COFFEE BREAK	New University, ground floor/2nd floor/courtyard
4:30 PM – 5:00 PM	EXHIBITION **Virtual Speaker Corner** Talk with industry partners	Virtual Venue
5:00 PM – 6:30 PM	SATELLITE SYMPOSIUM **Alnylam: Meeting the needs of patients with hATTR amyloidosis: innovation in practice**	Neue Aula/Main Lecture Hall
Chair	Thomas Skripuletz, *Department of Neurology, Hannover Medical School, Hannover, Germany*	
Introduction	Thomas Skripuletz	
	The origin and evolution of treatment Thomas Skripuletz	
	Evolving experience with RNAi therapies in a multisystem disease – Experience of managing hATTR amyloidosis at a reference centre Marcus Anthony Urey, *Division of Cardiology, Department of Medicine, University of California, San Diego, USA*	
	Evolving experience with RNAi therapies in a multisystem disease – A cardiologist's perspective on hATTR amyloidosis Andoni Echaniz-Laguna, *APHP, CHU de Bicêtre, Le Kremlin-Bicêtre, France*	
	Neurofilament Light Chain (NfL) as a biomarker in hATTR amyloidosis Hans Nienhuis, *Amyloidosis Centre of Expertise, University Medical Center, Groningen, Netherlands*	
	Redefining standards of care Laura Obici, *Amyloidosis Research and Treatment Center, University of Pavia and IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*	

DETAILED PROGRAM – WEDNESDAY, 7TH

7:00/7:30 PM – open end

SOCIAL EVENT

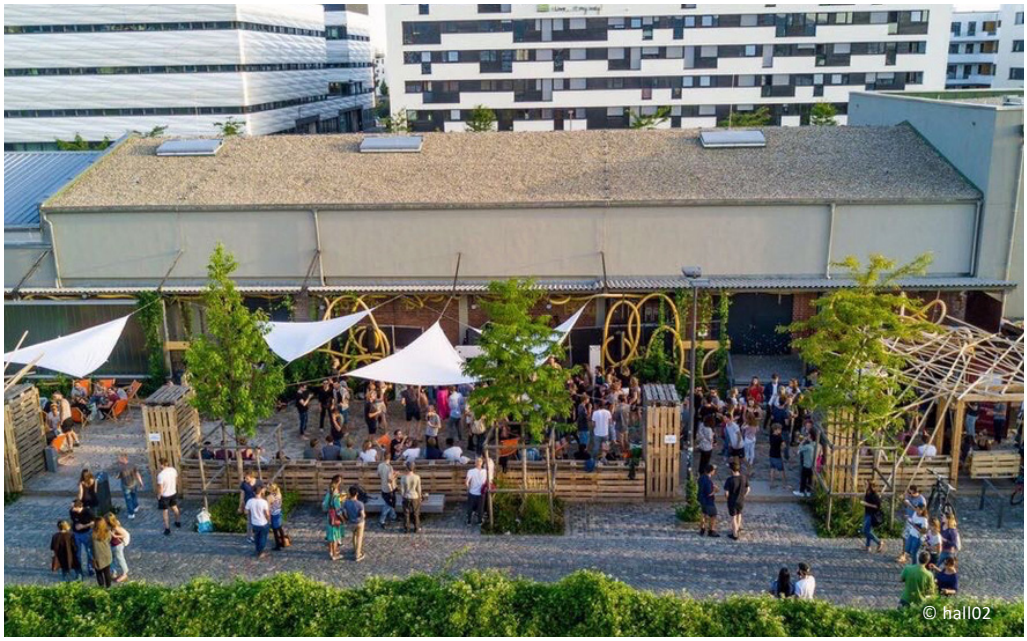
"halle02" Heidelberg

Conference Dinner/Award Ceremony

The Conference Dinner is held in the largest event venue in Heidelberg located in the building of the former freight station. It offers conference participants an excellent chance to enjoy delicious food in a unique industry ambience while getting to know each other and making new connections outside the main conference environment.
Finally, the winner of the ISA 2022 presidential awards and poster awards will be presented.

The halle02 offers more than 1,500 sqm of inspiring rooms and 1,200 sqm of outdoor space for events of all kinds and is exclusively available to conference participants for this event.

How to get there?
Please see the information distributed at the congress



9:30 AM – 1:00 PM | EXHIBITION | ground floor/2ndfloor

Exhibition on-site and virtual

7:45 AM – 8:30 AM | SATELLITE SYMPOSIUM | H14

Sponsored coffee starts at 7:00 AM

Attralus: Unmet Need and Rationale for Anti-Amyloid Therapy in Systemic Amyloidosis

Chair: Ashutosh Wechalekar, *National Amyloidosis Centre, London, United Kingdom*

Introduction: Ashutosh Wechalekar

Lectures:

Unmet need in Systemic Amyloidosis
Morie Gertz, *Mayo Clinic, Rochester, USA*

Preclinical Rationale for Anti-Amyloid therapy
Marina Ramirez-Alvarado, *Mayo Clinic, Rochester, USA*

Review of Efficacy with Anti-Amyloid therapy
Ashutosh Wechalekar

Safety of Anti-Amyloid Approach
Arnt Kristen, *Amyloidosis Center of the University Hospital, Heidelberg, Germany*

Discussion/Close: Ashutosh Wechalekar, Morie Gertz, Marina Ramirez-Alvarado, Arnt Kristen

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8:30 AM – 10:15 AM | SCIENTIFIC SESSION | Neue Aula/Main Lecture Hall

Best abstracts (including late breaking)

Chairs: Markus Weiler, *Amyloidosis Center Heidelberg, Germany*
Peter Mollee, University of Queensland, Australi

OP068: Feasibility of a novel academic BCMA-CART (HBI0101) for the treatment of relapsed and refractory AL amyloidosis
Moshe Gatt, *Hadassah Medical Center, Jerusalem, Israel*

OP069	A Proteomic Atlas of Renal Amyloid Plaques Provides Insights Into Disease Pathogenesis Charalampos Charalampous, *Mayo Clinic, Rochester, USA*
OP070	Cryo-EM study of cardiac ATTR fibrils and structure-based development of detection probes and anti-seeding inhibitors Lorena Saelices, *UT Southwestern, Dallas, USA*
OP071	An European collaborative study on 476 patients with AA amyloidosis: identification and validation of survival and renal staging systems Marco Basset, *Amyloid Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*
OPLB001	Primary Results From APOLLO-B, A Phase 3 Study Of Patisiran In Patients With Transthyretin-Mediated Amyloidosis With Cardiomyopathy Matthew Maurer, *Columbia University, New York, USA*
OPLB002	Venetoclax targeted therapy in t(11;14) AL amyloidosis patients: a retrospective analysis from the French Amyloidosis Network Murielle Roussel, *CHU Duputyren, Limoges, France*
OPLB003	Amyloidosis-related orthopedic events, low plasma transthyretin, and risk of cardiac events Anders Moller Greve, *Rigshospitalet, Copenhagen, Denmark*

10:15 AM – 10:45 AM COFFEE BREAK  New University, ground floor/2nd floor/courtyard

10:45 AM – 11:45 AM SCIENTIFIC SESSION Neue Aula/ Main Lecture Hall

Translation – How to close the gap of basic and clinical research in systemic amyloidosis

Round Table

Chairs

Stefan Schönland, *host ISA 2022, Amyloidosis Center of the University Hospital, Heidelberg, Germany*

Vittorio Bellotti, *IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Panel of Experts

Marina Ramirez-Alvarado, *Mayo Clinic, Rochester, USA*

Joost Schymkowitz, *Leuven Center for Brain & Disease Research, Leuven, Belgium*

Jonathan Wall, *University of Tennessee, Knoxville, USA*

Arnaud Jaccard, *CHU, Limoges, France*

Gunilla Westermark, *Uppsala University, Uppsala, Sweden*

11:45 AM – 12:30 PM SOCIAL EVENT Neue Aula/Main Lecture Hall

ISA Membership Meeting

The outgoing President, Giovanni Palladini, will report on the scientific and organisational activities of ISA during the last 2 years.
Shaji Kumar, treasurer will give his financial report.
Per Westermark, editor in chief of Amyloid, will give a summary on the recent developments of our journal.
Vaishali Sanchorawala, secretary, will present the election results (board and bylaws).
Stefan Schönland, President elect, will acknowledge the outgoing Board members, welcome and introduce the new Board members and give his prospects for the next 2 years.

12:30 PM – 1:00 PM SOCIAL EVENT Neue Aula/Main Lecture Hall

Closing Ceremony

Chairs ISA 2022 Organizing Committee

Summary ISA 2022 Heidelberg
Bouke Hazenberg, *University Medical Center, Groningen, Netherlands*
Outlook ISA 2024
Morie Gertz, Martha Grogan, *Angela Dispenzieri, Mayo Clinic, Rochester, USA*

LUNCH BOXES 

Thank you for your participation and have a safe trip back home!