Amyloidosis:
Typing & Nomenclature

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Disclosures

- Relevant financial relationships
  - None
- Off-label usage
  - None
Outline

• Definition & Types
• Nomenclature
• Methods of Typing
  • Indirect methods
  • Direct methods
    • Antibody-based methods
    • Proteomic methods
• Conclusions
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Amyloidosis

Definition

- "amyllum" (Latin: starch)
- misfolded extracellular protein
- antiparallel ß-sheets
- fibrils
  - 7.5 - 10 nm
  - non-branching, rigid, insoluble
  - binds Congo Red + birefringe
  - characteristic cross ß-diffraction on X-ray diffraction
- 31 recognized proteins (human)


Amyloid Fibrils
Atomic-Force Microscopy

Amyloid Fibrils
Electron Microscopy
Amyloidosis
Definition

- intrinsic propensity to mis-fold
- aging
- high concentration
- mutation
- sporadic / de novo
- familial
- proteolytic remodeling of precursor protein
- biophysical / functional amyloid

Transthyretin
Ig Light Chain
Apolipoprotein A-I
Amyloidosis
Definition - Special Stains

- Congo Red
- Thioflavin T/S
- Sulfated Alcian Blue
- Crystal violet or Methyl violet
Amyloidosis
Definition - Organs Involved

Heart
Lung
Kidney
Liver
Spleen
Amyloidosis
Cardiac - Gross Features

- **Texture**
  - firm
  - waxy

- **Atria**
  - normal size or dilated
  - subendocardial deposits

- **Ventricles**
  - thick walls
  - VS: VFW ↑
Amyloidosis
Cardiac - Histologic Features

Histopathologic Findings (H&E)
Amyloidosis
Cardiac - Histologic Features

Nodular  Pericellular  Vascular
# Amyloidosis

## Types - Overall

<table>
<thead>
<tr>
<th>Protein</th>
<th>Precursor</th>
<th>Distribution</th>
<th>Type</th>
<th>Dx / Tissues</th>
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</thead>
<tbody>
<tr>
<td>Aβ</td>
<td>Aβ precursor</td>
<td>localized</td>
<td>acq / hered</td>
<td>Alzheimer</td>
</tr>
<tr>
<td>APrP</td>
<td>Prion</td>
<td>localized</td>
<td>acq / hered</td>
<td>CJD</td>
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<tr>
<td>Aβ2M</td>
<td>β2-microglobulin</td>
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<td>acquired</td>
<td>dialysis</td>
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<tr>
<td>AL</td>
<td>Ig light chain</td>
<td>sys / local</td>
<td>acquired</td>
<td>myeloma</td>
</tr>
<tr>
<td>AA</td>
<td>Amyloid A</td>
<td>systemic</td>
<td>acquired</td>
<td>infx / inflamm</td>
</tr>
<tr>
<td>ATTR</td>
<td>Transthyretin</td>
<td>systemic</td>
<td>acq / hered</td>
<td>FA / senile</td>
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<tr>
<td>AApoAI</td>
<td>Apolipoprotein A-I</td>
<td>systemic</td>
<td>hereditary</td>
<td>heart, liver</td>
</tr>
<tr>
<td>AGel</td>
<td>Gelsolin</td>
<td>systemic</td>
<td>hereditary</td>
<td>FHA</td>
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<tr>
<td>ALys</td>
<td>Lysozyme</td>
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<td>hereditary</td>
<td>kidney, liver</td>
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<tr>
<td>AFib</td>
<td>Fibrinogen</td>
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<td>hereditary</td>
<td>kidney</td>
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## Amyloidosis

**Types - Cardiac**

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<tr>
<td>AANF</td>
<td>Atrial Natriuretic Factor</td>
<td>localized</td>
<td>acquired</td>
<td>heart</td>
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<td>AApoA4</td>
<td>Apolipoprotein A-4</td>
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<tr>
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Amyloidosis
Types - Endomyocardial Biopsy

- ATTR: 62%
- AL: 36%
- Other: 2%

n = 1432
Amyloid Typing

Is Typing Important?

- AL → high-dose chemotx (+/- PBSCT)
- ATTR (hereditary) → liver/heart transplant
- ATTR (wild-type) → heart transplant / drugs
- SAA → anti-inflammatory
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Amyloidosis
Nomenclature

Naming Scheme

• “A” followed by protein abbr.
• systemic vs. localized
  (AL, AH, β2-Microglobulin)

Examples

Immunoglobulin Light Chain = AL
→ AL, systemic

Atrial Natriuretic Factor = ANF
→ AANF, localized

Amyloidosis
Nomenclature

Mutations

• Variants named according to protein

• Use “hereditary” rather than “familial”

Example

Transthyretin = TTR, V30M

ATTRV30M

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Amyloidosis
Typing - Indirect Methods

PEP + IFE

Strengths
- Inexpensive
- Non-invasive

Limitations
- Gammopathy does not 100% correlate with amyloid type
- Specificity ~75%
- Only AL-type
Amyloidosis
Typing - Indirect Methods

Serum FLC Assay

Strengths
- Inexpensive
- Non-invasive

Limitations
- Does not 100% correlate with amyloid type
- Specificity ~90%
- Only AL-type
Identification of Amyloidogenic Light Chains Requires the Combination of Serum-Free Light Chain Assay with Immunofixation of Serum and Urine


**ABSTRACT**

The diagnosis of systemic immunoglobulin light chain (AL) amyloidosis requires demonstration of amyloid deposits in tissue biopsy and amyloidogenic monoclonal light chains. The optimal strategy to identify the amyloidogenic clone has not been established. We prospectively assessed the diagnostic sensitivity of the serum free light chain (SFLC) test, a commercial serum and urine agglutination gel electrophoresis immunoturbidimetric test (SFLC-IFT) and the high-resolution agarose gel electrophoresis immunofixation (HR-IFE) developed at our referral center in patients with AL amyloidosis, in whom the amyloidogenic light chain was unequivocally identified in the amyloid deposits.

**Backgrounds**

The diagnosis of systemic immunoglobulin light chain (AL) amyloidosis requires demonstration of amyloid deposits in tissue biopsy and amyloidogenic monoclonal light chains. The optimal strategy to identify the amyloidogenic clone has not been established. We prospectively assessed the diagnostic sensitivity of the serum free light chain (SFLC) test, a commercial serum and urine agglutination gel electrophoresis immunoturbidimetric test (SFLC-IFT) and the high-resolution agarose gel electrophoresis immunofixation (HR-IFE) developed at our referral center in patients with AL amyloidosis, in whom the amyloidogenic light chain was unequivocally identified in the amyloid deposits.

**Methods**

A prospective study was conducted in 121 consecutive patients with AL amyloidosis by immunofluorescence microscopy analysis of Bioprep (3) aspirates and/or organ biopsies. We characterized the serum free light chain (SFLC) and urine immunofixation electrophoresis (IFE) of both serum and urine with the FLC x/c ratio had a 100% sensitivity.

**Results**

The identification of amyloidogenic light chains cannot rely on a single test and requires the combination of a commercially available SFLC assay with immunofixation of both serum and urine. 2009 American Association for Clinical Chemistry

**Systemic immunoglobulin light chain (AL) amyloidosis** is a progressive disease caused by the deposition of insoluble fibrils formed by the aggregation of circulating monoclonal light chains produced by a usually small-cell neoplastic plasma cell clone (1). This process causes organ dysfunction and ultimately leads to death. However, effective chemotherapy, which suppresses the production of the amyloidogenic light chain before irreversible organ damage has occurred.


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Amyloidosis
Typing - Direct Methods

Immunohistochemistry

Strengths

- Familiarity with technology
- TAT

Limitations

- Large Ab panels (site)
- High background staining
- Conformational differences in fixed vs. unfixed light chains
- Antigen masking
- Variable domains of LCs
- Protein fragmentation during amyloid formation
Amyloidosis
Typing - Direct Methods

Immunofluorescence

<table>
<thead>
<tr>
<th>Amyloid A</th>
<th>Kappa</th>
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<tr>
<td><img src="image1.png" alt="Image" /></td>
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</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
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Strengths

- Reliable
- Many labs have access to IF
- Familiarity with technology
- TAT

Limitations

- Frozen tissue
- Large Ab panels needed (depending on site)
- Ab specificity for mutant proteins not fully established

Image courtesy of Dr. James R. Stone
Massachusetts General Hospital
Amyloidosis
Typing - Direct Methods

Immuno-electron Microscopy

Strengths

- Reliable, direct

Limitations

- Cost
- Access to EM
- TAT

Light and electron microscopy
immunohistochemical characterization
of amyloid deposits

Eloisa Arneutti1, Patrizia Morbini1, Laura Verga1, Monica Concardi2, Emanuele Puro2, Andrea Pliano1, Irene Zorzoli3, Pietro Gurini1, Ernesto Arosi1 and Giampaolo Merlini1

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Key Words: amyloid, electron microscopy, immunohistochemistry

Abbreviations: IHC = immunohistochemistry; LM = light microscopy; IHC = immunohistochemistry; EM = electron microscopy; PCT = plasma cell dyscrasia; PNS = peripheral nervous system; FMD = familial Mediterranean fever; AF = abdominal fat

Abstract

The present study reports our optimized fixation and processing methods for the light and electron microscopic immunohistochemical characterization of tissue amyloid. The study involved a series of 23 abdomen fat specimens.
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Amyloidosis
Typing - Direct Methods

2D-PAGE MS

Strengths
- Reliable, direct
- Single assay, no panels req

Limitations
- Cost
- Availability of LC MS/MS
- TAT
- Low-level proteins often missed
Amyloidosis
Typing - Direct Methods

**LC MS/MS**

**Strengths**
- Reliable, direct
- Single assay, no panels req
- Reliable screen for mutations

**Limitations**
- Cost
- Availability of LC MS/MS
- TAT
Amyloidosis
Typing - Direct Methods

**LC MS/MS**

- **Strengths**
  - Reliable, direct
  - Single assay, no panels req
  - Reliable screen for mutations

- **Limitations**
  - Cost
  - Availability of LC MS/MS
  - TAT

Laser Microdissection

Proteomic Spectra
Amyloidosis
Typing - Direct Methods

Strengths

- Reliable, direct
- Single assay, no panels req
- Reliable screen for mutations

Limitations

- Cost
- Availability of LC MS/MS
- TAT

SwissProt Database Query
Amyloid
Typing - Genetics

Electropherogram
Amyloid Typing - Genetics
Amyloid Typing - MS/MS Proteomics

- **TTR, ApoA1, ApoA4, Gel**
- For known mutations
  - sensitivity 92%, specificity 100%
  - mutations at tryptic site
  - mutations not in tryptic peptides
  - synonymous Δ AA
  - some isobaric mutations
- For novel mutations
  - sensitivity 82%, specificity 99%
  - not sequencing

Tandem Mass Spectrometry
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Amyloidosis
Take Home Points

- Increasingly recognized cause of heart disease
- Nomenclature standards set by ISA (2014)
- 31 recognized types, distinguishing is important
- AL & ATTR account for ~98% of cardiac amyloid
- AL-type most common, but most likely to encounter ATTR on biopsy material
- Preferred typing method: direct (vs indirect)
  - IF, MS, ImmunoEM
Amyloidosis

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- Martha Grogan
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Amyloidosis: Typing & Nomenclature

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