Renal amyloidosis challenges and opportunities

Maria M. Picken MD, PhD
Objectives
At the conclusion of this presentation the participants should be familiar with
1. why amyloid forms
2. what types of amyloidosis can affect the kidneys,
   - clinical manifestations
   - treatments
3. the currently available methods for amyloid diagnosis & typing
4. the challenges and opportunities in early diagnosis of amyloidoses

Disclosures - none
Amyloidoses – protein folding disorders

Amyloidoses:
- group of disorders
- all Congo red affinity and a fibrillary ultrastructure
- toxic effect of abnormally folded proteins deposit in tissue
Why amyloid forms?

SAP, Apolipoprotein E: formation & persistence of deposits; “amyloid signatures”

Intracellular protein quality control system extracellular chaperones - in vivo clearance

- direct toxicity already at the oligomeric/ protofibrillar stage…
- not mere spatial displacement/replacement
SAP scintigraphy

IHC: AP co-localizes with amyloid protein
Proteomics: “amyloid signature”

AMYLOIDOSES:

1. >31 protein types, many more variants
2. Localized, systemic or systemic or/and localized
3. Specific organs, i.e. cerebral, endocrine organs...
4. Geographic areas, i.e. Icelandic
5. Most prevalent versus rare versus exceedingly rare
6. Treatable versus not-treatable, genetics...

Kidney parenchyma ~ always systemic amyloidosis
Extra-renal-parenchymal genitourinary: systemic OR localized
AL – light chain amyloidosis
~ 85% of renal/systemic a.

Clonal proliferation of plasma cells

70% CHF
70% nephrotic syndrome renal failure
## Clonal proliferation of plasma cells

<table>
<thead>
<tr>
<th></th>
<th>“true” neoplasia: Multiple Myeloma</th>
<th>Dyscrasia/disorder: Plasma cell/B cell disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone size</td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>Manifestations due to clone</td>
<td>- many</td>
<td>- none</td>
</tr>
<tr>
<td></td>
<td>- bone lesions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- hypercalcemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- infections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- systemic symptoms</td>
<td></td>
</tr>
<tr>
<td>Manifestation due to M-component</td>
<td>- light chain cast nephropathy</td>
<td>- Amyloidosis</td>
</tr>
<tr>
<td></td>
<td>- hyper-viscosity</td>
<td>- LCDD</td>
</tr>
<tr>
<td></td>
<td>- amyloidosis 10-15%</td>
<td>- Non-organized deposits with end-organ damage:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- kidney, heart, liver failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- polyneuropathy, other</td>
</tr>
</tbody>
</table>
B-cell neoplasia

- bone lesions
- hypercalcemia
- infections
- systemic symptoms

M-component related diseases

Clone size

Clinical manifestations due to clone:
- bone lesions
- hypercalcemia
- infections
- systemic symptoms

Clinical manifestations due to M-protein:
- light chain cast nephropathy
- hyperviscosity

Progression - rare

“a small but dangerous clone”

AL, LCDD, non-organized deposits with end-organ damage:
- kidney
- heart
- liver failure
- polyneuropathy, other
Early diagnosis
Accurate typing

Cardiac stage III

ASCT: mortality >20%, now 5-10%,
most effective long-term therapy?

Early dx:
- broader range of therapeutic options
- individualized treatment ("risk-adapted")
  age, organ dysfunction, toxicities

ASCT: autologous stem cell transplantation
HDM: high-dose iv melphalan
CTD: cyclophosphamide-thalidomide-dexamethasone
MDex: melphalan-dexamethasone
Progress towards targeted therapies.... Eligibility/responses to various therapies contingent on **early diagnosis !!!**
### AA amyloidosis

**Serum amyloid A protein derived amyloidosis**

| Pathogenesis                  | - upregulated SAA (serum amyloid A protein),  
|                              | - acute phase reactant, fibril precursor  
|                              | - produced by liver  
|                              | - systemic: Kidney, GI tract, Spleen, liver  

| Sporadic                      | Chronic inflammatory conditions  
|                               | Inflammatory arthritis  
|                               | chronic infections, AIDS, etc…  
|                               | US? 5%, declining reporting?  
|                               | UK 18%  
|                               | Worldwide 45%, 2nd most common  
|                               | underdeveloped countries AA > AL  

| Familial                      | Hereditary auto-inflammatory diseases:  
|                               | - monogenic – FMF (familial Mediterranean fever),  
|                               | - other polygenic and complex – inflammatory bowel disease  

| Covert                        | no identifiable disease 6%  

Kidney, GI tract, Spleen, liver
Familial AA: 
Hereditary auto-inflammatory diseases

- Mutations in genes for non-amyloid fibril proteins that play a permissive role in the development of amyloid
- An inborn error of inflammatory response of the innate immune system
- Mutations in pyrin (FMF), cryopyrin gene (CAPS)
  - tumor necrosis factor receptor gene (TRAPS)
- Young(er) age
- INFEVERS: the Registry of Hereditary Auto-inflammatory Disorders
  - Mutations [http://fmf.igh.cnrs.fr/ISSAID/infevers/](http://fmf.igh.cnrs.fr/ISSAID/infevers/)
- NIH – Dan Kastner’s laboratory
- Treatment: anti-cytokine based targeted therapies
- Genetics in sporadic AA?
Erysipeloid erythema - FMF

Age at presentation

Number of patients

TRAPS

Muckle-Wells syndrome
AA amyloidosis

Not all patients with chronically elevated SAA develop AA amyloidosis - ? genetics

Lachmann et al
ALECT2
amyloidosis derived from leukocyte chemotactic factor-2

- Mexican-American ethnicity
- etiology unknown*
- No effective treatment
- Not to be mistaken for AL!!!

- Mayo – 2.7%
- Nephropath: all – 9.6%
- South-West US – 54%

- Kidney – 3rd most common amyloidosis type**
- Liver – 2nd most common amyloidosis type

* no mutation, LECT2 G/G genotype, digenic? older age?
** kidney failure, some proteinuria

Primum non nocere – first do no harm!

avoid misdiagnosis as AL!
ALECT2
Amyloid type CANNOT be diagnosed based on morphology!!!
“Sparkly” green birefringence morphology of LECT2 amyloid
## Systemic amyloidoses and the kidneys

<table>
<thead>
<tr>
<th>Type</th>
<th>%</th>
<th>Source of Protein</th>
<th>Pathomechanism</th>
<th>Target</th>
<th>Renal Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>~85%</td>
<td>Plasma cells</td>
<td>Clonal proliferation</td>
<td>Kidneys, heart</td>
<td>Proteinuria</td>
</tr>
<tr>
<td>AA</td>
<td>~5%</td>
<td>Liver</td>
<td>Chronic inflammation: infectious, autoimmune, autoimmune-inflammatory</td>
<td>Kidneys, Liver, GI tract</td>
<td>Proteinuria</td>
</tr>
<tr>
<td>ALECT2</td>
<td>2.7%</td>
<td>Liver</td>
<td>? no mutation LECT2 G/G genotype digenic?</td>
<td>Kidneys, Liver</td>
<td>Failure, Proteinuria - some</td>
</tr>
<tr>
<td>Hereditary</td>
<td>~10%</td>
<td>Liver, Liver/sm</td>
<td>Mutation</td>
<td>AFib – Kidneys</td>
<td>Variable, dependent on protein type/mutation: proteinuria and/or progressive renal failure</td>
</tr>
<tr>
<td>Other unknown</td>
<td></td>
<td>Intestine, other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- AL: Amyloidosis of macroglobulin
- AA: Amyloidosis of albumin
- ALECT2: Amyloidosis of liver and kidney
- Hereditary: Transthyretin (ATTR), Fibrinogen (AFib), Apolipoproteins A, AII, AIV
- Other: Various other causes
AFib: mutation in Fibrinogen A α chain
most frequent hereditary amyloidosis in N. Europe
with worldwide distribution

plasma protein essential for the final phase of blood coagulation
produced exclusively by the liver
several variants, usually no effect on fibrinogen function
(exception - deletion, frame shift mutation),
some patients have decreased fibrinogen levels

median age @ presentation: 55 y

NS + HTN, massive *glomerular amyloid*, ~ no extraglomerular deposits,
- some phenotypic variability depending on mutation, with involvement of
  other organs but renal failure dominates the clinical picture
- spleen involvement may lead to anemia and rupture
AFib – typical appearance
Atypical appearance of a glomerulus with AFib amyloid
Hereditary amyloidoses – treatment of kidney failure

AVOID MISDIAGNOSIS AS AL AND CHEMOTHERAPY!!

1. Kidney transplantation alone, recurrence
2. Combined liver (“surgical gene therapy”) + kidney transplantation
3. Pre-emptive transplantation (liver only)?
4. Carriers?
5. Pharmacologic therapies – clinical trials for ATTR
AFib – pre-emptive liver transplantation?
Systemic damage at the pre-renal failure stage

Rare (mutation dependent?)
- Hepatic amyloidosis\(^1,4\)
- Amyloid peripheral neuropathy (T538K)\(^4\)

Variant fibrinogen
Variant fibrinogen amyloid
Endothelial cell
Autonomic neuropathy

Autonomic neuropathy
Coronary atherosclerosis
Interstitial amyloid
Atherosclerotic plaque
Glomerular amyloid
Hyperlipidemia
Gut dysmotility
?Other

Picken 2010
How to diagnose amyloidosis?

H&E NOT sensitive enough, NEED AMYLOID SPECIFIC STAIN

Congo red versus other stains
Advanced amyloidosis is suspicious on H&E while early not suspected on H&E. Include amyloidosis in the differential diagnosis.

Emphasis on early detection of amyloidosis. Congo red stain to rule out amyloidosis…
Congo red polarization - diagnostic

green
yellow or orange birefringence
aka anomalous colors
Anomalous colors

Apple green - under ideal optical conditions
Green is the most specific finding but other anomalous colors (yellow or orange) are also diagnostic
Anomalous colors appear/disappear owing to strain birefringence (yellow) and/or during uncrossing of the polarizer and analyzer (orange)
Congo red stain viewed in bright field is NOT DIAGNOSTIC

Congo red polarization is specific but relatively less sensitive due to polarization “shadow” where only a portion of deposits is exposed at any given time.

Congo red fluorescence
TRITC filter:
- increased sensitivity
- no polarization “shadow”
- useful for screening
- verification with polarization required

Requirements for the interpretation of Congo red stained slides:
- strong light source
- darkened room
- pupils accommodated
- proper optics...

- thicker sections are NOT an absolute requirement!!!

MAY NEED TO STAIN MORE THAN 1 SLIDE
Other stains?

**Thioflavin T or S**: easy to do
- more sensitive but less specific than Congo red
- not permanent
- requirement for fluorescence microscope

Other stains:
- sulfated Alcian blue – not specific, stains GAGs
- crystal violet – less sensitive
Electron microscopy – small areas examined

**Amyloid and legal issues**: delayed diagnosis!!!
Consider amyloid in the differential diagnosis!!!
Congo red stain should be examined not only to confirm suspicion of amyloid but to rule it out even when H&E is unremarkable!!!
Amyloid typing in renal pathology using frozen section immunofluorescence

- routinely used in renal pathology in North America
- 1st step in amyloid typing
- ~ 85%, expanded antibody panel
- easy & fast
- clear background, high specificity
- high sensitivity, suitable for small deposits
- correlation with Congo red stain (co-localization)
- correlation with amyloid P component (intensity)
- detection of NON-AMYLOID PATHOLOGIES!!!
Renal Amyloidosis

AL: ~85%

- IFE^ (serum, urine), sFLCh^^,
- bone marrow biopsy, cardiac assessment
- anti-plasma cell chemotherapy/ASCT^^^,
- kidney transplantation**

AA:  
- anti-inflammatory/anti-infectious  
- autoinflammatory diseases? (younger age)
- kidney transplantation**

Non-AL: ~15%

ALect2: avoid misdiagnosis as AL!
- no specific therapy
- kidney transplantation (recurrence)
- family history/renal function testing?
- regional differences in incidence

Hereditary: avoid misdiagnosis as AL!
- genetic testing, family history frequently (-)
- liver transplantation
- clinical trials (transthyretin amyloidosis)
- kidney transplantation**
- genetic counseling

Unknown/new type??? avoid misdiagnosis as AL!

^- immunofixation electrophoresis
^^ - serum Free Light Chain assay
^^^ - autologous stem cell transplantation
** - kidney transplantation (combined with amyloid type specific therapy)
IF on FS typing of ~85% of renal amyloidosis cases but NOT 100%!!!

Clearly state in your report if you cannot determine the type of amyloid state undetermined!!!

Amyloid type cannot be determined based on the distribution of deposits or clinical grounds

Amyloid type determination must be based on the tissue deposits
Collateral studies (serum free light chain assays, etc.) are done to support the diagnosis of amyloid type but NOT to make it
Patients can have both MGUS and hereditary amyloid proteins.
Avoid misdiagnosis of other types of amyloidosis as AL amyloidosis!!!

Amyloid and legal issues in pathology: misdiagnosis of the amyloid type
Amyloid IHC ≠ other IHC

Challenges:
(i) lack of commercially available amyloid/type-specific antibodies
(ii) the heterogeneity
(iii) serum contamination... (paraffin sections)
(iv) controls
(v) rarity

Improvements:
- “comparative IHC”, a panel instead of a single antibody
- antibodies to free light chains
Comparative IHC
...most antibodies to light chains react with whole immunoglobulins as well as free light chains, and there are generally many more whole immunoglobulins than free light chains.
IHC: antibodies to free light chains in IHC of renal biopsies

Patient with a κ paraprotein
Left: rabbit antibody to κ light chains (automated antigen retrieval)
Middle: sheep antibody to free κ light chains - selective staining of amyloid deposits
Right: free λ light chains (-)

Type II cryoglobulinaemic glomerulonephritis - IgM κ paraprotein.
Sheep antibody to free κ light chains - extensive deposition in glomeruli; no free λ light chains stain

Value of antibodies to free light chains in immunoperoxidase studies of renal biopsies.
Antibodies detecting only free light chains produced cleaner background with no “contamination” by circulating immunoglobulins

There is room for further improvements of IHC and for application of complementary methods!
LMD/MS for amyloid typing:

Rationale:
- relative abundance of amyloid protein
- frequently the *dominant* protein

Diagnosis of amyloid by proteomic methods:
- large spectra numbers for the amyloidogenic protein
- in conjunction with apolipoprotein E and SAP component (aka “amyloid signature”)
The concept of MS

41,X,-Y,add(1)(p36.3),der(1)(Y;1)(q11;p13),add(3)(q29),der(4)(4;5)(q27;q22),
del(9)(p21),psu dic(1;11)(p21;q13),+13,-14,-15,i(17)(q10),-18,-21,-22

Picken et al 2004
Proteomic techniques:

How Do Mass Spectrometers Work?

*MS* can measure only the mass of *charged* molecules in gas
Proteins in living organisms are in liquid and are usually not charged
- electrospray ionization (ESI) and matrix-assisted laser dissociation ionization (MALDI)

A charged molecule moves through an electrical or magnetic field in a precise way determined by its mass

1. Sample preparation (LMD, other)
2. Proteins are digested into peptides, charged and then separation is performed
3. Protein identification is done by MS and informatics analysis
Sample preparation:
- enrichment via laser microdissection
- entire glomerulus
- area of interest
**Fragmenting Peptides**

It is difficult to make large proteins “fly” hence proteins are fragmented (twice in MS/MS) “wings to molecular elephants”, elephants into birds

*in general amyloid proteins are relatively small*

1. proteins analyzed in the first component of the tandem MS/MS
2. peptides selected and dissociated into fragments that the second component can analyze
   (fragmenting proteins can be also done in cyberspace with a program that predicts the way that peptides fragment)

**Results:**
- displayed as *spectra* of the relative abundance of detected ions as a function of the mass-to-charge ratio
- molecules in the sample can be identified by correlating known *masses* to the identified masses or through a characteristic *fragmentation pattern* analogous to using fingerprints to identify a person

**Protein identification:**
- matching the identifying features of the peptides to a database of proteins
- more believable if based on matching mass spectra from several peptides
LCM tissue
1. 6-10µm thick sections
2. area of interest identified and laser microdissected – ‘sample’

Protein extraction
Trypsin digestion into peptides

HPLC high performance liquid chromatography
separation of peptides

ESI+++ electron spray ionization
peptides are ionized

Peptides sprayed into MS1:
- measures the parent mass of the peptide and
- selects the peptides for CID (collision-induced dissociation)

CID - upon collision with a neutral gas, the peptides are fragmented

MS2 – measures the size of each fragment derived from the parent peptide mass

These measurements are used to predict the amino acid sequence

Example of a mass spectrum

Dogan A. Classification of amyloidosis by mass spectrometry-based proteomics,

2002 Nobel Prize in chemistry John B Fenn and Koichi Tanaka:
"for the development of soft desorption ionisation methods
... for mass spectrometric analyses of biological macromolecules."
MS/MS limitations:

1. a given protein can only be identified if peptide fragments with appropriate size for MS can be generated after enzymatic digestion

2. It may be difficult to detect low abundance proteins/peptides as signals from these peptides may be buried among massive amount of information obtained from more abundant proteins, and MS simply may not be able to scan them

3. Reliance on computational predictive algorithms to a reference human genome obtained from publicly available databases

MS/MS advantage:
1. Global identification of proteins
2. Discovery of unsuspected proteins/biomarkers
IgD HCDD:

(A, B) Glomerular and tubular deposits of IgD
(C) diabetic nodular glomerulosclerosis (-) for IgD
(D) Plasma cells (+) for IgD – bone marrow biopsy

IgD HCDD is difficult to diagnose, because routinely IgD is not tested by IF MS results validated with IHC!

Royal V, JASN 2014
Amyloid typing by mass spectrometry (MS)

Current application:

- routine IF/IHC equivocal or (-)
- confirmation of type
- detection of less common/unusual types
- inadequate sample for IF typing
Amyloid detection and typing - summary

1. **awareness** of the amyloidoses among pathologists/clinicians & diagnostic and treatment options

2. **expertise** in the diagnosis – opportunities for renal pathologist!!!

3. **early** detection:
   a) higher sensitivity methods for screening
   b) second opinion to establish/or confirm the diagnosis

Reporting - pathology report (applies to all body sites):
(i) anatomic site(s)
(ii) histologic structure(s) involved by amyloid/patterns of involvement
(iii) scoring where applicable
(iv) method (stain) by which amyloid was diagnosed
(v) results of amyloid typing and method used
Amyloid detection and typing:

opportunities for renal pathologists to provide leadership in surgical pathology in amyloid diagnosis and typing
Clinical suspicion of amyloidosis - biopsy of a “surrogate” organ abdominal fat
- aspiration or
- surgical biopsy

Renal amyloidosis – usually clinically not suspected
Also look at the perirenal fat!!!

Surgical fat biopsy – enough tissue available for typing!!!
Save frozen tissue!!!
The big picture - where does amyloid fit?
**Amyloid:**
- role in vertebrate and invertebrate biology, as “functional amyloid”
- amyloid fibrils also have applications in the fields of nanotechnology and bioengineering

“**Functional fold**”:
Amyloid-based natural adhesives, biomimetic adhesives...

*Schematic model of the mechanical manipulation of single intermolecular beta sheets of an amyloid fibril by an AFM tip. The sequential unfolding of individual molecules corresponds to the repetitive sawtooth peaks in force-extension curves.*

(b) *Representative force-extension curve sawtooth from probing the adhesive of the aeroterrestrial green alga, Prasiola linearis. The regularly spaced, highly-ordered peaks have been fitted to the worm-like chain model.*

(c) *AFM image of functional amyloid fibrils in the adhesive extruded from the marine parasite, Entobdella soleae, which attaches to the skin of the common sole (Solea solea) in the marine environment.*
Thank you

Clinical suspicion...
Pathologic suspicion...
Patients’ perspective...
Awareness and education

Patients Support Group

ISA
INTERNATIONAL SOCIETY OF AMYLOIDOSIS
OPPORTUNITIES....

Gauge 16 or thicker needle
Fat aspiration procedure

Fat biopsy for screening, staging (localized vs systemic amyloidosis)
ASO: anti-sense nucleotides, siRNA: small interfering RNAs, CPHPC – inhibition of SAP binding to amyloid fibrils, IDOX – doxorubicin interferes with amyloid fibril growth and promotes amyloid clearance.
MS-based proteomics:

to replace, or complement, the existing methods?

Complementary approach !!! MS findings confirmed by IHC

Discovery of new amyloid types by MS techniques validated by IHC

IHC better for the detection of very small deposits

MS-based proteomics limited by the abundance of amyloid in the tissue examined

Both approaches require experience & expertise
Proteomic techniques:

1. Sample preparation (LMD, other)
2. Proteins are digested into peptides and then separation is performed
3. Protein identification is done by MS and informatics analysis

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*MS* can measure only the mass of *charged* molecules in gas.
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A charged molecule moves through an electrical or magnetic field in a precise way determined by its mass.
Outline

- introduction to amyloidoses
- pathogenesis and changing concept of pathogenicity
- major types of renal amyloidoses and their therapies
- generic diagnosis and amyloid typing
- legal issues
- opportunities for renal pathologists to provide leadership in amyloid diagnosis