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How I diagnose amyloidosis

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Abstract

Amyloidosis is a rare group of diseases characterized by deposition of amyloid fibrils in soft tissues. More than 28 types of amyloid have been identified. They all share common ultrastructural and chemical characteristics. Treatments are available for many types but are type specific. Therefore, confirmation and typing of amyloid is essential before initiating treatment. Monoclonal protein studies should be performed on suspected cases but the diagnosis requires a tissue biopsy. Congo red stain and electron microscopy are helpful to discriminate between amyloid and other pathologic fibrils. Once amyloid is confirmed, typing should be performed. Immunofluorescence and immunohistochemistry are frequently used and are helpful but this approach has limitations such as availability specificity and sensitivity of commercial antibodies. Genetic mutational analysis is vital for ruling in and out hereditary amyloidoses but is unhelpful in non-mutated forms. The most advanced technique of amyloid typing is laser microdissection followed by mass spectrometry (LMD-MS). Using proteomics, LMD –MS can directly identify proteins with or without mutations. Finally, imaging studies such as cardiac MRI with gadolinium and I-123 labeled SAP scintigraphy not only assist in evaluation of patients with known amyloidosis but cardiac MRI has detected amyloid in patients previously unsuspected of the disease.

Introduction

Amyloidosis is a heterogeneous group of diseases bound by the characteristic deposition of amyloid fibrils in soft tissues.¹ Other pathogenic fibrils exist but only amyloid fibrils are capable of giving off an apple-green birefringence under polarized light after binding to Congo red. To date, more than 28 proteins have been identified to be amyloidogenic.² One of the most recent additions to the amyloid family is ALECT2 (leukocyte cell derived chemotaxin-2).³ As the number of amyloidogenic proteins increases, it outgrew the capacity of the previous nomenclature which consisted of primary, secondary, hereditary, etc.² The new system uses an abbreviation of the native protein. For example: immunoglobulin light chain is AL, serum amyloid A protein is AA, transthyretin is ATTR and so on. Although the native proteins come in various sizes and tertiary structures, all amyloid fibrils have the same characteristic cross- β sheet quaternary structure.¹ Under X-ray crystallography, amyloid fibrils produce two characteristic diffraction signals at 4.7 and 10 angstroms. Fibrillogenesis begins with misfolding of the precursor protein to a lower thermodynamic state. In this state, the intermediate takes on more β -sheet structures that enable aggregation and self propagation secured by hydrogen bonds. The resultant product then undergoes a process known as steric zipper where the β -sheet regions from different molecules align themselves and interdigitate to form a dry interface.⁴ The end product is a protofibril which is more resistant to degradation. Four to six protofibrils are weaved together to form an amyloid fibril.

Mechanisms of fibrillogenesis

Amyloidogenesis can occur via a variety of mechanisms.^{1,2} Several of the more common ones will be discussed to illustrate the diversity of amyloidogenesis. Genetic mutation often with an amino acid substitution can result in an amyloidogenic protein. They represent the hereditary amyloidoses which include AFib (fibrinogen A α chain), ALys (lysozyme), AApoAI and AApoAII (apolipoprotein AI and AII), and ATTR. Amyloid can also form from wild type proteins. The best example is senile

amyloid of the wild-type transthyretin. Other senile amyloids include APro (prolactin), ACal (calcitonin) AIAPP (amylin) and AANF (atrial natriuretic factor); all are derived of wild type proteins. Some amyloids form as a result of sustained elevated concentrations of the wild type protein. This may occur either by increased production as in the case of AA amyloidosis secondary to chronic infectious, inflammatory conditions or Castleman disease, or decreased elimination as in A β 2m (β -2-microglobulin) that occurs in patients with end stage renal disease. AIns (insulin) is unique in that the injected insulin forms amyloidomas near injection sites via this process. Other ways of increasing amyloidogenicity is proteolytic remodeling. The best example of this is APP (β -amyloid precursor protein) in Alzheimer's disease. Some may involve multiple mechanisms (accumulation, mutation and proteolytic remodeling) such as AL or AH (immunoglobulin heavy chain).⁵ One unusual mechanism is seen in the familial periodic fever syndromes that include Familial Mediterranean Fever, TRAPS (TNF receptor associated periodic syndrome), Muckle-Wells syndrome and Hyper IgD syndrome.⁶ In familial periodic fever syndromes, the mutations do not code for an amyloidogenic protein but instead cause periodic fevers that lead to the overproduction of serum amyloid A protein resulting in AA amyloidosis. Amyloid can also be transmitted by prions. Prions are the only infectious agents known to contain no nucleic acids. Prions induce disease by acting as a template for the normal prion protein (PrP^c) to misfold into the pathogenic PrP^{sc} which has amyloid features.⁷ Finally, in some, the mechanism remains undetermined. The best example of this is ALECT2. No mutations, proteolytic remodeling or elevated levels have been found so far.^{8,9}

Clinical presentation

Amyloidosis presents in variety of ways and can make diagnosis difficult. Partly, this is due to the many different types of amyloid, but significant variation can exist within an individual type of amyloid. An example is ATTR. The presence of Val30Met mutation strongly predicts for peripheral neuropathy, autonomic neuropathy and abnormalities in the cerebrospinal fluid whereas patients with the Val124Ile mutation are more likely to develop cardiac symptoms especially in African Americans.^{10,11} It

is helpful to separate the presentation into two patterns: localized and systemic.¹ Localized amyloidoses are confined to one site and are generally non-lethal. Skin is a common site of deposition and can manifest as asymptomatic plaques, fissures or nodules. Examples include lichenoid or macular amyloidosis secondary to AD (keratin) deposition in the skin possibly as a result of trauma.²

Amyloidomas composed of AIns are found near the injection sites of diabetics. AL amyloidosis which is often systemic also has a localized variant.^{12,13} Nodular localized cutaneous (AL) amyloidosis is likely a response to a localized inflammatory reaction often produced by polyclonal plasma cells. Patients commonly present with skin plaques (skin), microscopic or gross hematuria (bladder), and unexplained voice changes (larynx) but amyloid can be deposited in other soft tissues. Progression to systemic AL amyloidosis is rare but local recurrences are not uncommon.^{14,15}

Systemic amyloidosis implies involvement of visceral organ(s) or multiple tissues. Patients may present with symptoms limited to one organ or with multiorgan failure. The most common form of systemic amyloidosis is AL amyloidosis which outnumbers AA amyloidosis by 20:1 in United States but only 2:1 in Europe due to higher incidence of Familial Mediterranean Fever.¹⁶ Kidney is the most common organ involved in AL, AA and most forms of hereditary amyloidosis except ATTR.¹⁶⁻¹⁹ Proteinuria is present in 73% of the AL amyloidosis patients with 30% exhibiting nephrotic syndrome. Renal insufficiency is noted in nearly half of the patients which is usually associated with proteinuria but can occur without in patients with vascular limited renal amyloidosis.^{20,21} Renal involvement is nearly universal (97%) in AA amyloidosis.¹⁸ Heart is the next most common organ involved in AL amyloidosis with abnormal echocardiographic findings noted in 65% of patients.¹⁶ It is the predominate feature in senile TTR amyloidosis.^{11,19} Presentation varies from asymptomatic to a subtle decreased in exercise capacity to fatigue, dyspnea and lower extremity edema to angina, syncope, ascites and anasarca which are associated with more advanced disease.²² Overt heart failure can be seen in 24%.¹⁶ Low voltage on electrocardiogram (ECG) and concentric thickened ventricles on echocardiogram are classic signs of cardiac involvement by amyloidosis. Cardiac involvement on the other hand is rare in AA amyloidosis

occurring in only 1% of patients.¹⁸ The nervous system is a prominent feature in some forms of amyloidosis and can involve the central, peripheral nerves or both.¹⁶ Peripheral nerve involvement can present with bilateral distal progressive paraesthesia or carpal tunnel syndrome. Autonomic nervous system involvement is characterized by syncope, erectile dysfunction, gastroparesis and diarrhea. Some are quite specific as in the case of cystatin c (ACys) amyloidosis which causes the Icelandic form of hereditary cerebral amyloid angiopathy.² These patients present with cerebral hemorrhage, stroke and dementia.²

Symptoms common to most types of amyloid include fatigue and weight loss which are reported by over half of the patients.¹⁶ Weight loss however is unreliable in patients with nephrotic syndrome since many will gain weight as their edema worsens. Gastrointestinal presentation is not common and usually present with macroglossia, nausea, vomiting and pseudoobstruction which accounts for < 10% of AL amyloidosis cases. Hepatomegaly is present in 24% of AL amyloidosis patients but only 9% of AA amyloidosis. Bruising is common and often occurs after minor trauma or procedure especially around the eyes.

Treatment

It is beyond the scope of this article to discuss treatment of amyloidosis in detail. For more comprehensive review of treatment options for AL amyloidosis, interested readers should refer to the articles “How I treat amyloidosis” by Dr. Comenzo published in Blood 2009 and “I don’t know how to treat amyloidosis” by Dr. Gertz in Blood 2010.^{23,24} It is important to recognize that treatments are available for many types of amyloidosis but they are type specific. For localized amyloid, local treatment such as excision or laser removal may be appropriate once systemic disease is ruled out.¹² In AL amyloidosis which is almost always the result of a plasma cell dyscrasia (rarely lymphoproliferative disorders) anti-myeloma therapies have been effective.²⁵⁻²⁸ The treatment of AA amyloidosis is targeted toward the underlying infection or inflammatory condition.¹⁸ For patients with a periodic fever

syndrome, biologic agents such as anti-TNF or IL-1 receptor antagonists have been effective both for symptom control and improvement of the amyloidosis.^{6,29,30} Organ transplantation has been utilized in several different types of hereditary amyloidosis.^{31,32} This is done either to replace damaged organs or to eliminate the source of the mutant protein. The most common example of the latter is liver transplantation in patients with ATTR amyloidosis where the mutant transthyretin is produced primarily in the liver.³³ Liver transplantation has also been performed in combination with kidney transplantation in order to prevent recurrence in the kidney. This has been done successfully in patients with AFib and AApoAI amyloidosis.^{31,32,34} While it appears to be effective at preventing recurrence, additional risk of the liver transplantation should be considered.

Since treatment is specific to each type of amyloid, accurate diagnosis is essential. This is especially true when cytotoxic therapy or organ transplantation is being considered. The presence of a monoclonal protein alone is not enough to support the diagnosis of AL amyloidosis or AH amyloidosis. In a single center study of 350 patients with suspected AL amyloidosis, 34 (10%) were found to have a genetic mutation coding for one of the familial amyloidosis, most commonly AFib and ATTR.³⁵ Eight (24%) of these 34 patients had a monoclonal gammopathy. The number of misdiagnosis could be even greater since some amyloidoses are not the result of genetic mutations. Interestingly, a recent study finds ALECT2 was the most common undiagnosed type of amyloid.⁹ So far, ALECT2 does not appear to be the result of genetic mutations but there may be a predilection for homozygosity in the G allele.⁸ This also applies to senile amyloidosis which is the result of accumulation of normal transthyretin with no genetic mutations.²

Diagnostic approach

Patients presenting with multiorgan derangement should be screened for amyloidosis. The diagnosis is much more challenging when the patient presents with single organ involvement as it may mimic more common diseases. A high index of suspicion is often required. Screening should be performed in

patients with unexplained heart failure, neuropathy and nephrotic syndrome. Clues that raise suspicion include low voltage on ECG, severe hypotension without antihypertensive medications and the presence of neuropathy in combination with other organ involvement.^{16,22,36} Easy bruisability especially “raccoon eyes” after procedures or minor trauma is another hint. Major bleeding can result from factor X and vitamin K dependent clotting factors deficiency but is much less common.

Several elements of the history should alert the provider toward AA amyloidosis. Chronic inflammatory conditions (e.g. rheumatoid arthritis, ankylosing spondylitis, etc.), chronic infections (e.g. bronchiectasis, pressure ulcers, subcutaneous drug use “skin popping”, osteomyelitis, tuberculosis, etc.), inflammatory bowel disease, and rarely vasculitis are known associates of AA amyloidosis.^{18,37} A history of recurrent fevers especially in the absence of infection should prompt an evaluation for hereditary fever syndrome. While lymphoproliferative disorders are usually associated with AL, two in particular are associated with AA. First is Castleman disease, also known as angiofollicular lymph node hyperplasia.³⁸ Extensive involvement of the AA amyloid can occur even with unicentric Castleman disease. Stabilization of symptoms has been reported after surgical resection. The other is IgM monoclonal gammopathy. This is usually associated with AL amyloidosis but AA amyloidosis has also been reported.³⁹ Hepato/splenomegaly in patients with Schnitzler syndrome (IgM monoclonal gammopathy, urticarial rash, arthralgia, bone pain, lymphadenopathy) should alert one toward the possibility of AA amyloidosis.⁴⁰ AA amyloidosis has also been reported in patients with Waldenström macroglobulinemia without Schnitzler syndrome.⁴¹

Screening Tests

Monoclonal protein testing of the serum and urine is readily available and should be performed on all suspected cases. AL amyloidosis is the most common and dangerous form of amyloidosis and definitely should be ruled out.⁴² In addition to serum and urine protein electrophoresis and immunofixation, serum free light chain test can increase the sensitivity (especially in monoclonal light

chain only cases) by 10% -15%.⁴³ Bone marrow biopsy should be performed if a monoclonal protein is identified or if the amyloid is AL or AH type. Sedimentation rate (ESR) may be elevated in patients with a monoclonal protein and both ESR and c-reactive protein (CRP) may be elevated in patients with a history of chronic inflammatory disease or infection. Serum amyloid A (SAA) level can also be directly measured. Although there is a good correlation between CRP and SAA, studies found SAA is more sensitive at identifying patients at risk of development of AA and has better prognostic value.^{18,44} Unfortunately, the test is currently not approved in the U.S.¹⁸

Biopsy

While screening tests are helpful, amyloidosis is a histologic diagnosis and tissue biopsy is required.¹ Other paraprotein related diseases can mimic amyloidosis. Nephrotic syndrome with monoclonal gammopathy occurs in monoclonal immunoglobulin deposition disease (MIDD), immunotactoid glomerulonephritis, fibrillary glomerulonephritis, type I cryoglobulinemic glomerulonephritis, membranoproliferative glomerulonephritis and others.^{45,46} Neuropathy and monoclonal gammopathy is seen in POEMS syndrome, IgM associated neuropathy, MGUS associated neuropathies and anti-myelin associated glycoprotein (anti-MAG) associated neuropathies.^{47,48} It is essential that amyloid deposition in tissue is demonstrated for the diagnosis.

In general, the highest sensitivity and specificity is achieved by obtaining tissue from the dysfunctional organ. Using this approach, studies have found heart to be 100% sensitive followed by liver at 97% and kidney at 94%. While bleeding is a concern, the risk is no higher than patients without amyloidosis if standard screening procedure is followed.⁴⁹ In situations where biopsy of the organ involved is not practical or unsafe, alternative sites may be biopsied. The easiest is abdominal fat. This can be done either by fine needle aspirate or biopsy. It has a sensitivity of approximately 80%.¹⁶ Rectum is another commonly used site and has a sensitivity of 75%. It is usually performed via a flexible sigmoidoscopy. Bone marrow biopsy may also contain amyloid deposits but sensitivity is only 56%.

Amyloid can also be found in thyroid nodules but incidence is unknown and there is no data on sensitivity. Given its safety, one should not hesitate to perform a fat aspirate if the index of suspicion is moderate or higher.

Histochemistry

On light microscopy with hematoxylin and eosin (H&E) stain, amyloid appears as a light pink amorphous extracellular substance.⁵⁰ It is negative on PAS (periodic acid-Schiff) and methenamine silver stains, and blue or grey on trichrome stain. The gold standard for diagnosis of amyloid is the Congo red stain. All forms of amyloid exhibit affinity for Congo red and give off an apple-green birefringence under polarized light. It is one of the characteristics that distinguish amyloid from other fibrils. For best visualization of small amyloid deposits, the tissue sections for Congo red stain should be 6-10 μm in thickness (rather than the 2-4 μm thickness used for renal pathology and the 3-5 μm thickness for general pathology). The apple-green birefringence of amyloid should not be confused with the yellow-green birefringence of collagen.⁵¹ While the birefringence is very specific to amyloid in animals, it also occurs in cellulose. Thus it is important to avoid any cotton or paper fiber contamination on the slides. One place where deposits are often found is in vessel walls and is true for all tissues. Thioflavin T which binds to β sheet rich structures is another stain which can be used to diagnose amyloid. It is yellow but undergoes a red shift under fluorescence enhancement. Although it is sensitive, it is less specific than Congo red.

Electron microscopy

Electron microscopy (EM) is routinely used in analysis of kidney biopsy in United States and other countries. It is extremely helpful in the diagnosis because amyloid fibrils have unique EM characteristics. Amyloid fibrils are typically randomly arranged, non-branching and solid with a diameter between 7 to 12 nm.¹ In comparison, fibrils of fibrillary and immunotactoid glomerulonephritis are larger at 7-26 nm and 9-45 nm respectively.⁵⁰ Fibrils of immunotactoid are similar to those of cryoglobulins

and tend to be organized at least focally in parallel arrays. They also have a hollowed center resembling microtubules. The fibrils of these diseases typically stain for the entire immunoglobulin but rarely for a single light chain only. Most cases of immunotactoid glomerulonephritis and a minority of cases of fibrillary glomerulonephritis are associated with a monoclonal gammopathy. It is therefore not uncommon for them to be misdiagnosed as AH or ALH (immunoglobulin light and heavy chain) amyloidosis.⁵² Fibrils can also be found in hereditary nephropathies with fibronectin, and collagenofibrotic glomerulopathy, diabetic nephropathy (so called “diabetic fibrillosis”), lupus nephritis and thrombotic microangiopathy.

Amyloid Typing

Immunofluorescences and immunohistochemistry

Once amyloid has been identified, further characterization and typing must be performed. Direct immunofluorescence microscopy which is routinely performed on native kidney biopsies, is one of the most common and convenient methods of amyloid typing and should be the first step. It is most useful in cases of amyloid composed of immunoglobulin components where antibodies are widely available. AL and/or AH amyloid can be typed by immunofluorescences using FITC-labeled antibodies to IgA, IgM, IgG, kappa and lambda light chain. For AL amyloidosis, only one of the immunoglobulin light chains should stain positive in the amyloid deposits. For AH amyloidosis, the deposits will stain positive for a single subclass of immunoglobulin heavy chain without light chain while a single light chain will also stain in ALH. Standard immunofluorescence is performed on fresh frozen tissue. In the absence of frozen tissue, immunofluorescence can be performed with pronase-digested paraffin-embedded tissue. Determining the immunoglobulin component involved in amyloidogenesis identifies the target of therapy. Recent studies found reduction of the involved free light chain is more important than the intact immunoglobulin for patients with AL amyloidosis.⁵³ An alternative method is immunohistochemistry using immunoperoxidase staining of formalin-fixed paraffin embedded tissue. This works well for AL,

AH, AA, AFib, ATTR, AApoA1 and AB2m where antibodies are available commercially.

Immunohistochemistry can also aid in the diagnosis of amyloidosis. Antibodies against serum amyloid P (SAP) which co-deposits with amyloid can be used to distinguish light chain deposits of AL amyloidosis from MIDD. Immunofluorescence is superior to immunohistochemistry for the accurate typing of AL or AH amyloidosis due to the lower background staining in the former. Since frozen tissue is not usually available in non-renal samples, immunohistochemistry not immunofluorescence is typically performed on these samples.

Despite the convenience and availability, there are some drawbacks to immunofluorescence and immunohistochemistry. One limitation is the availability of antibodies. Although antibodies are generally available for the most common forms of amyloid, availability is variable for the less common forms. Sensitivity and specificity of the antibodies is another problem. Since amyloid precursors often have genetic mutations and conformational changes, antibodies produced against the wild type protein may be less reactive with the mutant form. This seems particularly problematic for AL which has the greatest variability.⁵ In renal amyloidosis, the amyloid deposits can be negative for both kappa and lambda in 14% of cases using immunofluorescence.⁵⁴ The same is true for immunohistochemistry. One study found 24 of the 76 samples suspected of AL could not be definitely diagnosed with immunohistochemistry.⁵⁵ Thus, negative or equivocal staining for immunoglobulin light and/or heavy chains does not automatically rule out AL.^{55,56} Additional testing with a panel of antibodies directed toward other amyloidogenic proteins was required to make the diagnosis. Even with that, 5 patients could not be typed.⁵⁵ On the other hand, better results were obtainable by the use of multiple antibodies. In a separate study with biopsies from patients with AL, AA, ATTR, AFib and AApoA1, the sensitivity was increased to 92% - 96% with a 100% specificity when multiple antibodies against κ and λ were used.⁵⁷ In cases where the staining of light chains is negative or equivocal, the use of antibodies from different vendors and a panel of antibodies against other amyloids may be helpful. The biggest problem with these techniques however is the requirement to perform a separate test for each type of amyloid being

considered. This becomes cumbersome once the common forms of amyloid have been ruled out and is impractical for the discovery of new type of amyloid. Finally charge interaction of the amyloid and the reagent antibody or contamination of the amyloid deposits by serum proteins can all lead to nonspecific staining for immunoglobulins in immunohistochemistry or immunofluorescence. This nonspecific staining occurs more frequently in AA than AL amyloidosis.⁵⁴⁻⁵⁶

Immuno-gold electron microscopy is a technique which combines immunohistochemistry with electron microscopy. Using gold labeled antibodies, precise localization of the amyloid in the tissue can be accomplished. This technique also insures that the antibodies are reacting with the amyloid and not other proteins in the surrounding tissue. While it can be used for diagnostics, it is mainly used for research.⁵⁸ In addition to localization of amyloid deposits it is particularly useful for the identification of chaperone proteins.⁵⁹⁻⁶¹ One study suggests it may also be used for quantification of amyloid deposits.⁵⁹⁻⁶¹ However, because it is an antibody based method, it has all of the same limitations of immunohistochemistry.

Genetic testing

Given the number of amyloid that is the result of genetic mutations, genetic testing is an important part of the typing process. Mutations have been identified in all of the hereditary amyloidosis such as ATTR amyloidosis, AApoAI amyloidosis, AApoAII, ALys amyloidosis, AFib amyloidosis, and AGel (gelsolin) amyloidosis. It is particularly important in ATTR where a senile form exists. Genetic testing is also available for periodic fever syndromes. These tests are commercially available and are relatively easy to obtain. However, it is important to understand the limitations. First is the issue of genotype versus phenotype. Variability in penetrance may result in lack of disease despite the presence of the mutation.¹ Second, the method of genetic testing plays a large role in the sensitivity and specificity. Most commercial labs use RFLP which can only detect known mutations. Complete gene sequencing may be necessary to detect novel mutations. Fortunately the cost and time involved with

DNA microarray, SNP array analysis and other modern gene sequencing techniques have both decreased substantially making complete gene analysis much more feasible. Finally, amyloid such as ALECT2 and senile systemic amyloid do not have identifiable mutations and would not be identified by genetic testing.

Proteomics

Unlike previous methods, proteomics is interested in the proteome which is composed of the entire complement of the proteins in an organism or environment. As a result, it eliminates the need to perform individual tests for each protein of interest. Limitations due to unavailable antibodies or undiscovered mutations have no effect on proteomics. Any protein that has been previously identified can be tested by this one test. Previously, proteomics was performed by N-terminal Edman degradation which involved sequencing the proteins present.⁶² Aside from being extremely time consuming, there was also a limit on the number of proteins that can be tested at any one time. Proteomics currently is performed with mass spectrometry which has the capability for high-throughput and identification of the entire proteome. Since mass spectrometry is concentration dependent, the proteins must to be separated prior to analysis. This can be accomplished by several techniques including gel electrophoresis (one or two-dimensional), but the most efficient is liquid chromatography. Most modern proteomics combined a reverse phase liquid chromatography with tandem electrospray mass spectrometer for maximum efficiency.

The gold standard in the applications of proteomics for amyloid typing today is laser microdissection followed by mass spectrometry (LMD-MS).⁶² Congo-red positive areas are dissected under a laser dissecting microscopy followed by tryptic digestion of the dissected material (Figure 1). The digested peptides are analyzed by liquid chromatography electrospray tandem mass spectrometry. The signature is queried by 3 different algorithms and is assigned a probability score. In a study using 50 biopsy samples of different tissues containing multiple types of amyloid typed by other techniques, LMD-MS was able to correctly identified 50 out of 50 samples.⁶³ In a validation test, LMD-MS identified

40/41 amyloid samples whereas immunohistochemistry only identified 15 of 36 samples. Because LMD-MS directly analyses the actual peptide, it can identify protein with or without known mutations. LMD-MS can also aid in the diagnostic process. In the kidney, the diagnosis of AL amyloidosis (lambda) requires large spectra of Ig lambda light chain C-region with or without lambda light chain V region and the absence of any significant kappa light chains. This is because immunoglobulins and immunoglobulin light chains can deposit in the kidney in other pathologic states without being amyloid. The process is more straight forward for non AL/AH amyloids, since these peptides (SAA, LECT2, ApoAI, etc.) are not found in the normal proteome of the glomerulus. For AFib, the mutated fibrinogen A α chain peptide would be present. Both senile amyloid and ATTR would show spectra for transthyretin but only ATTR would show the mutant protein. Representative mass spectrometry results from a case of AL kappa light chain amyloidosis and LECT2 amyloidosis are shown in Figure 2. In addition to typing, LMD-MS can also aid in the diagnosis. The diagnosis of amyloidosis at the proteomic level using LMD-MS is based on the presence of large spectra of SAP. In all cases, large spectra of Apolipoprotein E are also present. The typing of amyloid is then based on the presence of spectra that correspond to the specific type of amyloid. However, Congo red or EM confirmation is advised as both fibrillary and immunotactoid glomerulonephritis can have similar spectra.

The advantage of LMD-MS over conventional methods of amyloid typing is that LMD-MS is a single test that can identify the amyloid protein in question versus others which test the biopsy material for amyloid proteins individually. LMD-MS can be performed on all tissue including fat aspirate as long as amyloid deposits are present. Also, LMD-MS is performed on paraffin-embedded material and does not require frozen material. Thus, studies can be performed on archival material. Others have described the use tryptic digest of unfixed whole fat for LMD-MS with equally impressive results which potentially further simplifying the methodology of amyloid typing.⁶⁴ Finally, with the current software and database, it is possible to identify the genetic variants of amyloidogenic proteins, such as variants of SAA or mutated fibrinogen- α protein.

Imaging modalities

Finally, there are two imaging modalities that deserve recognition for their ability to detect amyloid in the body. While they are generally not used for diagnostic purpose, they are quite useful in detecting amyloid in the body once the diagnosis is made. The first is magnetic resonance imaging (MRI). It is most useful for cardiac amyloid and cases of amyloidosis have been identified in unsuspected patients undergoing the test for cardiomyopathy. Global delayed post-gadolinium enhancement of the subendocardial myocardium is characteristic of amyloid infiltration and can be used to distinguish amyloid from other types of cardiomyopathies.²² Together with concentric ventricular hypertrophy and blood-pool gadolinium kinetics, the accuracy cardiac MRI can be as high as 97%. Unfortunately, these findings are associated with advanced disease and sensitivity may be lower in early amyloid cases.²² Demonstration of abnormal strain rate by pulsed tissue Doppler imaging on echocardiography is more sensitive for early amyloid involvement but it is not specific.^{65,66} Also, while advanced findings are prognostic, milder cardiac involvement is more difficult to correlate with patient outcomes. Cardiac biomarkers have been extremely helpful in prognostication of these patients.⁶⁷ The other technique is I-123 labeled SAP scintigraphy. This technique uses radiolabeled serum amyloid P component to locate amyloid deposits in the body.^{68,69} Since SAP binds to all amyloid, it can be used in every type. Deposits can be quantified from the scintigrams and the results can be used to monitor amyloid regression after treatment or recurrence after organ transplantation.^{70,71} One area where SAP scintigraphy does not image well is the heart. Other techniques are required for assessment if heart involvement is suspected. Cardiac MRI and echocardiography can often be used. Recently 99mTc-DPD scintigraphy was thought to be superior to I-123 label SAP scintigraphy but this may be limited to the ATTR patients.⁷² I-123 labeled SAP scintigraphy is currently available in Europe but has not been approved in the United States.

While amyloidosis remains a serious disease with fatal consequences, treatment is now available for many types.^{26,31,73} Because treatments are type specific and quite intensive or invasive, it is essential the diagnosis is confirmed before treatment is initiated. Confirming the fibrils are amyloid is the first step

because other pathologic fibrils exist. Once amyloid is confirmed, typing must be performed so the proper treatment can be prescribed. An algorithm for diagnosis and typing of amyloidosis is shown in figure 3. Circumstantial evidence such as circulating monoclonal protein or monoclonal plasma cells cannot be used for typing. Direct testing of the amyloid protein is ideal. Immunotyping by direct immunofluorescence or immunohistochemistry is widely available and should be the first step in typing. But antibodies are not available for all types of amyloid, and sensitivity can also be an issue. The current gold standard is proteomics via LMD-MS. It accurately identifies known amyloid protein and has the capability of characterizing novel ones.⁷⁴ If unavailable, genetic testing can be performed to look for known hereditary amyloidoses in cases where immunotyping is equivocal or negative. For patients with AA, it is important to rule out familial periodic fever syndromes even in patients without characteristic symptoms as cases of TRAPS have been reported in patients without fever.⁷⁵ Cardiac MRI can be used to determine cardiac involvement without the need for a cardiac biopsy if amyloid has been detected elsewhere in the body. Regression and progression can be monitored with I-123 SAP scintigraphy. Hopefully, future advances will make the diagnosis and typing of amyloid easier and more accurate which will insure better treatment for these patients.

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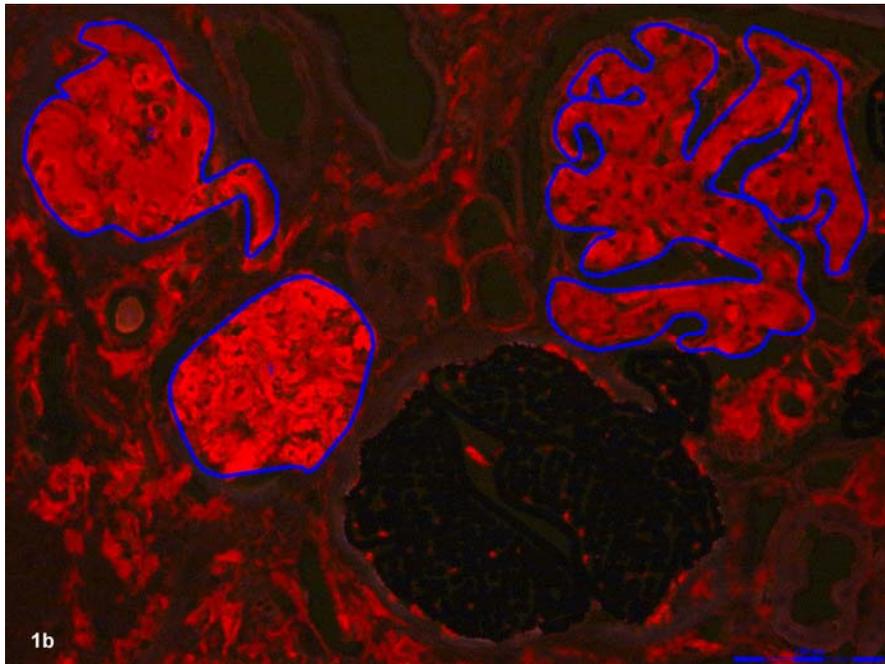
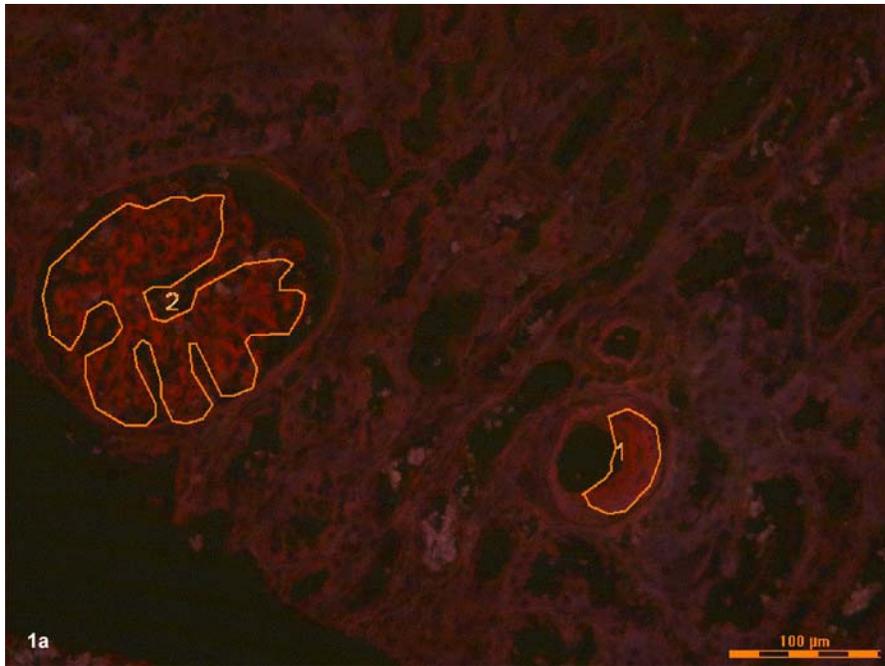
All authors contributed to the writing and design of the manuscript.

The authors have no conflict of interest to declare.

Figure 1. Laser microdissection. Congo red positive (A) glomeruli and vessels marked for microdissection from case 1 (data shown below) and (B) glomeruli marked for microdissection from case 2 (data shown below).

Figure 2. Representative mass spectrometry data: (A). AL kappa light chain amyloidosis showing the top 15 spectra. Mass spectrometry shows large spectra for serum amyloid P-component, Apolipoprotein E and immunoglobulin kappa V-I region and kappa constant region, and. (B) LECT-2 amyloidosis showing large spectra for serum amyloid P-component, Apolipoprotein E and leukocyte cell derived chemotaxin-2. The probability number (>95% is highlighted by green, 80-94% by yellow) indicates essentially the percent homology between peptides detected in the specimens and the published amino acid sequences of their corresponding proteins.

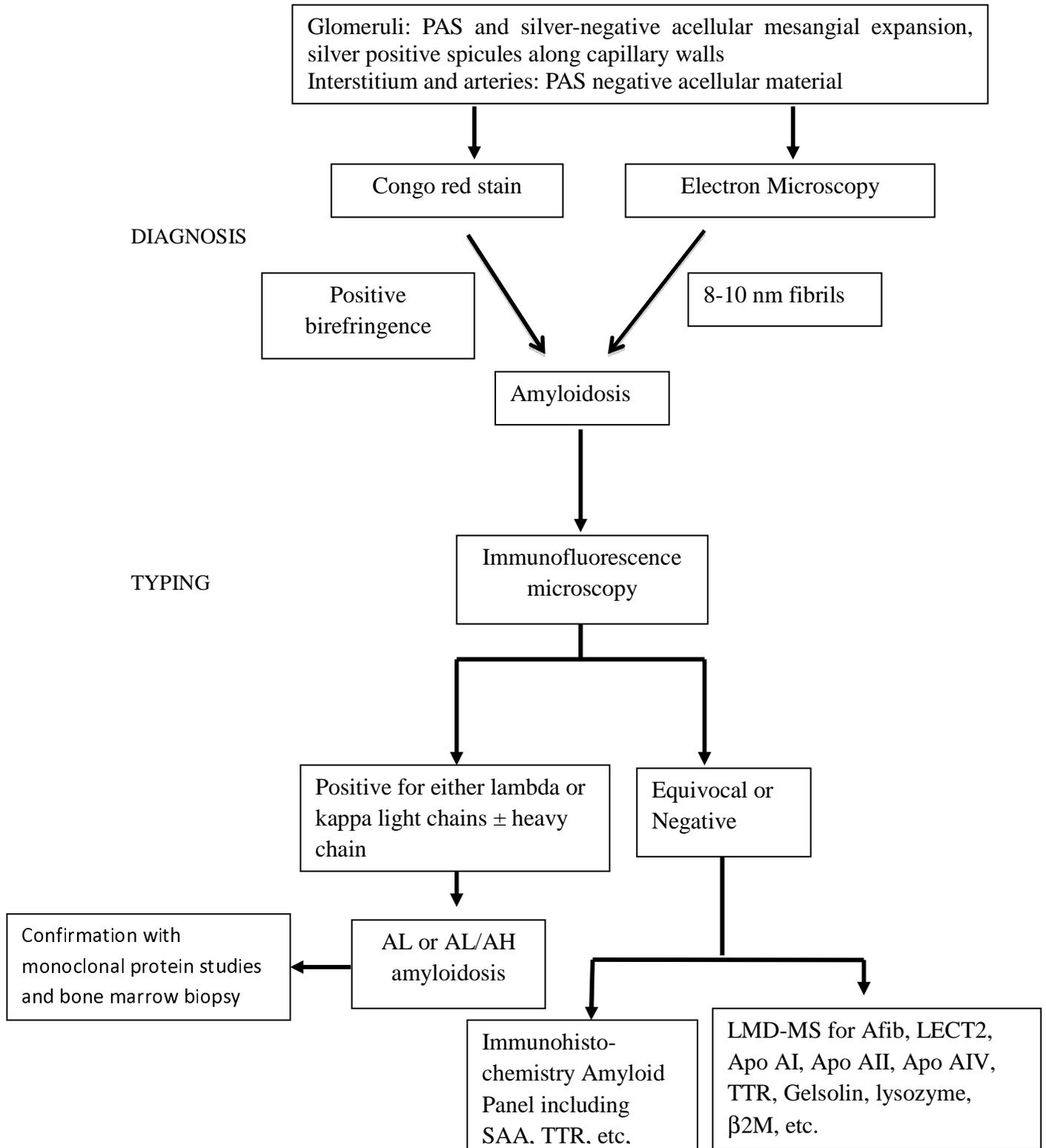
Figure 3. Algorithm for diagnosis and typing of renal amyloidosis used at the Mayo Clinic.



#	Starred?	Bio View: Identified Proteins (595)	Accession Number	Molecular Weight	2a		
					Sample 1	Sample 2	Sample 3
1	★	Serum amyloid P-component	SAMP_HUMAN	25 kDa	18	18	20
2	★	Apolipoprotein E	APOE_HUMAN	36 kDa	14	25	17
3	★	Ig kappa chain V-I region	KV117_HUMAN	12 kDa	13	12	14
4	★	Ig kappa chain C region	IGKC_HUMAN	12 kDa	6	9	8
5	☆	Keratin, type I cytoskeletal 9	K1C9_HUMAN	62 kDa	38	8	63
6	☆	Vimentin	VIME_HUMAN	54 kDa	27	32	34
7	☆	Actin, cytoplasmic 1	ACTB_HUMAN	42 kDa	22	32	28
8	☆	Vitronectin	VTNC_HUMAN	54 kDa	23	25	30
9	☆	Lactotransferrin	TRFL_HUMAN	78 kDa			56
10	☆	Apolipoprotein D	APOD_HUMAN	21 kDa			41
11	☆	Apolipoprotein A-IV	APOA4_HUMAN	45 kDa	15	11	14
12	☆	Zinc-alpha-2-glycoprotein	ZA2G_HUMAN	34 kDa			26
13	☆	Keratin, type II cytoskeletal 2	K22E_HUMAN	65 kDa	9		27
14	☆	Collagen alpha-1(I) chain	CO1A1_HUMAN	139 kDa	6	7	6
15	☆	Actin, alpha cardiac muscle 1	ACTC_HUMAN	42 kDa	19	26	30

#	Starred?	Bio View: Identified Proteins (102)	Accession Number	Molecular Weight	2b		
					Sample 1	Sample 2	Sample 3
1	★	Apolipoprotein E	APOE_HUMAN	36 kDa	31	19	10
2	★	Serum amyloid P-component	SAMP_HUMAN	25 kDa	16	12	11
3	★	Leukocyte cell-derived chemotaxin-2	LECT2_HUMAN	16 kDa	8	11	6
4	☆	Vitronectin	VTNC_HUMAN	54 kDa	43	32	23
5	☆	Keratin, type II cytoskeletal 1	K2C1_HUMAN	66 kDa	5	7	76
6	☆	Keratin, type I cytoskeletal 9	K1C9_HUMAN	62 kDa			69
7	☆	Keratin, type I cytoskeletal 10	K1C10_HUMAN	59 kDa	4	6	39
8	☆	Actin, cytoplasmic 1	ACTB_HUMAN...	42 kDa	20	18	6
9	☆	Collagen alpha-2(I) chain	CO1A2_HUMAN	129 kDa	10	12	7
10	☆	Complement C3	CO3_HUMAN	187 kDa	13	10	4
11	☆	Katanin p60 ATPase-containing subunit	KATL1_HUMAN	55 kDa		1	2
12	☆	Collagen alpha-1(I) chain	CO1A1_HUMAN	139 kDa	12	7	6
13	☆	Clusterin	CLUS_HUMAN	52 kDa	14	10	2
14	☆	Complement factor H-related protein 1	FHR1_HUMAN	38 kDa	9	7	6
15	☆	Alpha-1-antitrypsin	A1AT_HUMAN	47 kDa	12	5	

Figure 3. Diagnosis and typing in renal amyloidosis



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