Amyloidosis—Where Are We Now and Where Are We Heading?

Maria M. Picken, MD, PhD, FASN

Amyloidoses are disorders of diverse etiology in which deposits of abnormally folded proteins share distinctive staining properties and fibrillar ultrastructural appearance. Amyloidosis ultimately leads to destruction of tissues and progressive disease. Although they have been known since the time of Virchow in the 19th century, until relatively recently the amyloidoses were considered a medical curiosity of only academic interest rather than clinically relevant diseases. However, recent advances in the treatment of systemic amyloidoses have changed this outlook and, hence, the importance of an early diagnosis of amyloid, and a correct diagnosis of its type, has been realized.

Objective.—To summarize current recommendations for the diagnosis of amyloidosis.

Data Sources.—Presentation given at the 4th Annual Renal Pathology Society Satellite meeting in Istanbul based on discussions and recommendations formulated during an interactive diagnostic session held at the XIth International Symposium on Amyloidosis in Woods Hole, Massachusetts.

Conclusions.—Congo red stain is currently the gold standard for amyloid detection and the goal is to detect amyloid early. Diagnosis of the amyloid type must be based on the identification of amyloid protein within the deposits and not solely by reliance on clinical or DNA studies. However, the latter are recommended for confirmation of the amyloid type based on evaluation of the protein in deposits. Immunohistochemistry must be performed and interpreted with caution and inconclusive results must be evaluated further using the more sophisticated methods available in referral centers. An adequate amount and quality of tissue must be available for amyloid diagnosis and typing with emphasis on the use of fresh tissue and greater use of abdominal fat biopsy. The development of new technologies underscores the need for regular review of recommendations and standards for the clinical diagnosis of amyloidosis.

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Chemotherapy and stem cell rescue. In the second most common type of amyloidosis worldwide, AA, targeting the underlying inflammatory disease leads to a reduction of the circulating fibril precursor, serum AA protein. In patients with familial AA amyloidosis, biologic drugs appear to be effective. A subset of patients with hereditary amyloidosis will benefit from liver transplantation. A pear to be effective. A subset of patients with hereditary ATTR. In patients with dialysis associated amyloidosis, also appear to be amyloid type specific for AA and a new class of antiamyloid agents, currently in clinical trials.

Diagnosis of Amyloidosis

Diagnosis of amyloidosis is based on the detection of deposits in tissues. Thus far, no biochemical markers in body fluids, diagnostic of amyloidosis, are available. Congo red stain continues to be the gold standard for detection of amyloid deposits. In bright field, deposits of amyloid stained with Congo red typically have a salmon-pink color. However, small deposits, in particular in thinner sections, may not be apparent in bright light. Importantly, the bright field appearance in itself is not diagnostically Congo red–stained slides must be examined under polarized light and only the presence of apple-green birefringent deposits is considered diagnostic of amyloid. Caution is advised regarding “overinterpreting” collagen as amyloid. In addition to the experience of the observer, good fixation, a proper staining protocol (alkaline Congo red), and appropriate optics are required for the examination of Congo red–stained slides. Thus, a strong light source and a rotating table are recommended. Moreover, reading slides in a darkened room, after pupil acclimation, facilitates the detection of smaller deposits. Thicker sections (5–10 μm) may be helpful but are not essential if the previously listed conditions are met. Other stains, or techniques, such as fluorescence, thioflavin S and T, methyl violet, and sulphonated Alcian blue are less specific and at times also less sensitive (most notably...
sulphonated Alcian blue or methyl violet) for the detection of amyloid, and confirmation with Congo red stain is required. For example, sulphonated Alcian blue identifies glycosaminoglycans (which form scaffolding for the amyloid fibrils) rather than amyloid fibril protein per se. Immunofluorescence may increase sensitivity (especially in the case of minute deposits) but its specificity is lower. Among the stains commonly used in renal pathology, periodic acid–Schiff and silver stain may be helpful in raising a suspicion of amyloid. Deposits of amyloid are weakly periodic acid–Schiff positive and negative in a silver stain. However, Congo red stain should be examined not only to confirm a suspicion of amyloid deposits but also immunofluorescence, electron microscopy, and, if necessary, a Western blot or other molecular studies for amyloid typing. Dr G. Gallo’s personal experience in the typing of amyloid deposits in fat aspirates, with a 94% success rate, is very encouraging. Subsequent molecular analysis of extracted amyloid deposits confirmed the accuracy of the immunofluorescence method. Not infrequently, following the initial detection of amyloid in biopsy material, the amount of tissue available for further characterization of deposits is insufficient and a second biopsy should be performed. In such cases, an abdominal fat pad biopsy is preferred rather than an aspirate. As well as being a simple procedure, surgical biopsy secures more abundant tissue for additional studies.

Renal amyloidosis is usually associated with systemic amyloidosis, whereas amyloid elsewhere may be localized or part of a systemic process. Thus, the diagnosis of extrarenal amyloid requires subsequent staging to address the issue of whether we are dealing with a systemic or a localized form of amyloidosis. Again, an abdominal fat pad biopsy is the specimen of choice for amyloid staging purposes. Interestingly, amyloid deposits in other parts of the genitourinary system are most commonly localized and mimic tumors clinically.

**THE ROLE OF GENETIC TESTING IN THE EVALUATION OF PATIENTS WITH SYSTEMIC AMYLOIDOSIS**

The role of genetics in systemic amyloidoses has recently been reevaluated. Genetics may be associated with amyloidosis in several ways: either as a mutation in non-amyloid protein or as a mutation involving amyloid protein itself; a potential role for genetics in “sporadic amyloidoses” is also suspected. The first of these mechanisms occurs in patients with familial AA, which is as-

Figure 1. Abdominal fat pad aspirate with deposits birefringent under polarized light (Congo red viewed under polarized light, original magnification ×200).
associated with various periodic fevers, of which familial Mediterranean fever is the best known.25 These patients have an inborn error of inflammatory response in the innate immune system and mutations in genes for nonamyloid fibril proteins play a permissive role in the development of amyloid. The pathology of sporadic versus familial AA is similar and, therefore, diagnosis of familial AA has to be based on clinical grounds. However, the distinction between sporadic versus familial AA has implications for treatment and prognosis and should also involve genetic counseling. See also INFEVERS, the registry for familial Mediterranean fever and hereditary inflammatory disorder mutations (http://fmf.igh.cnrs.fr/ISSAID/infevers/; accessed May 7, 2009).25

Systemic amyloidoses that develop as a consequence of a mutation involving the amyloid protein itself are referred to as hereditary amyloidoses and several proteins have been shown to be amyloidogenic following mutation.1,2,18,19,26–36 Many proteins have been shown to have multiple amyloidogenic mutations and phenotypes may vary depending on the mutation.26–34 Interestingly, the kidney may be involved by all types of hereditary amyloidoses and the distribution and concentration of deposits may vary, including glomerular and/or extraglomerular deposits, some being limited to extraglomerular vasculature or to deep medulla.27–34 In certain hereditary amyloidoses, the degree of renal involvement is also greater in homozygotes than in heterozygotes (Table). Hereditary amyloidoses have traditionally been considered to be rare and believed to be associated with a positive family history. This misconception has been challenged recently. Although, in the United States, the frequency of diagnosis of hereditary amyloidoses has increased 5-fold during the last 3 decades (from 2% to 10%), they are still believed to be underdiagnosed (reviewed in reference 2). For comparison, in the United Kingdom 16% of amyloidoses were recently diagnosed as hereditary.2,33 Although hereditary amyloidoses have an autosomal dominant mode of inheritance, owing to variable penetrance, the clinical presentation can be quite diverse and the onset of clinical disease quite late.29,35 This, coupled with the pervasive lack of awareness of amyloidosis, accounts for the fact that a family history of amyloidosis is often missing.35 Clinically, hereditary amyloidoses may mimic AL, which is also the most common type of systemic amyloidosis. The biggest challenge that has emerged during recent years is the detection and correct diagnosis of these hereditary amyloidoses and their differentiation from AL. In view of the differences in treatment, the need for such distinction cannot be overemphasized.

The question may arise as to how to detect these hereditary amyloidoses? Can we diagnose amyloid type based on molecular studies alone? The short and decisive answer to this question is no. The presence of a mutation does not always correlate with the amyloidosis type in some patients. Thus, patients can have AL and carry a genetic variant that is not the cause of their amyloidosis.26,36–38 On the other hand, some 25% of patients with hereditary amyloidosis may have a coincidental monoclonal gammopathy.20,33,37 Thus, currently, genetic testing should always be complementary to other diagnostic techniques that allow unequivocal identification of the amyloid protein. In cases in which DNA sequencing detects a mutant amyloid precursor protein, protein analysis must be the definitive evidence.20

However, DNA analysis is mandatory to confirm a diagnosis of hereditary amyloidosis based on identification of the protein type present in amyloid deposits (please see later). Precise identification of the mutation is important for treatment and prognosis. Only patients with hereditary amyloidoses associated with proteins produced by the liver (exclusively or predominantly) may benefit from liver transplantation as a form of a surgical gene therapy.10–13 There are also differences between phenotypes, including prognosis, associated with different mutations of the same protein. Importantly, the full spectrum of hereditary amyloidoses is still being discovered and the absence of any currently known amyloidogenic mutation does not rule out hereditary amyloidosis associated with a new, hitherto-unknown mutation or the involvement of entirely new proteins.3

ATTR, amyloidosis derived from a variant of transthyretin, is the most common hereditary amyloidosis in the United States and worldwide.27–29 Although ATTR is typically associated with polyneuropathies and cardiac involvement, some mutations show significant renal involvement as well.27 Interestingly, even the wild-type transthyretin can undergo fibrillogenesis in older individuals, targeting predominantly the heart.29 ATTR, whether hereditary or senile, continues to be underdiagnosed. AFib, amyloidosis derived from a mutant of fibrinogen, first emerged as the most common type of hereditary systemic amyloidosis in Europe, but since then its worldwide distribution is unraveling. AFib is derived from a mutant of the fibrinogen A α chain.31,32 Other hereditary amyloidoses include AApo A-I, AApo A-II, AApo AIV, ALys, and AGel (Table; recently reviewed in reference 2).

**AMYLOID TYPING: PITFALLS AND EMERGING PROSPECTS**

What issues are involved in the differential diagnosis of amyloid type? How should one approach the ever-expanding diversity of amyloidoses?

In patients not on dialysis, who are diagnosed with systemic amyloidosis, therapeutic options center on the recognition of 1 of 3 main categories of systemic amyloidosis: AL, AA, and the ever-expanding group of hereditary amyloidoses (reviewed in reference 2). Immunohistochemistry is currently the standard for amyloid typing in routine clinical practice. AA can be reliably typed in frozen and/or paraffin sections. However, immunohistochemical typing of AL is still challenging and the difficulties frequently compounded by truncation of the light (or heavy) chain. Commercial antibodies are raised against the constant regions of the respective immunoglobulin light chains. Therefore, a subset of AL, in which amyloid fibrils are derived from a truncated light chain (ie, containing only variable regions), will be expected to be nonreactive with commercial antibodies.2,19,40–45 At the same time, a limited antibody panel will also miss a number of hereditary amyloidoses as well. In such cases, the major differential diagnosis is between AL and hereditary types. Regrettably, in the past, some pathologists rushed to diagnose nonreactive cases as AL, seemingly by default.43 Given the implications for patient treatment, such an approach is a sure way of discrediting not only immunohistochemistry but also pathologists as well. The second troublesome issue is the presence in the tissue of background stain, which in paraffin sections in particular can be significant due to the ‘locking-in’ of serum proteins during fixation. However,
Figure 2. Diagnostic results of amyloid typing for λ light chain. A, Strong, 3+ stain for λ light chain; stain for κ light chain was negative (not shown). B, Stain for amyloid P component. Amyloid P component is present in all types of amyloid deposits regardless of their chemical composition. Both stains, for λ light chain and amyloid P component, correspond to areas that are Congo red positive and exhibit birefringent under polarized light (frozen sections, immunofluorescence, original magnifications ×100 [A] and [B]). Reprinted with permission from S. Karger AG, Basel, Switzerland.

Figure 3. Amyloid typing in paraffin sections. A, Strong, 3+ stain for λ light chain. B, Stain for κ light chain with only focal positivity, corresponding to serum proteins (arrows) and only a blush stain in areas corresponding to amyloid (asterisks) (immunoperoxidase, original magnifications ×150 [A and B]). Reprinted with permission from S. Karger AG, Basel, Switzerland.

this issue can be alleviated by the use of frozen sections and immunofluorescence stains, which provide a cleaner background (Figure 2). Moreover, immunofluorescence stains on frozen sections have a higher sensitivity when compared with immunoperoxidase stains on paraffin sections (reviewed in reference 2). Therefore, in inconclusive cases, acquisition of an additional sample of fresh tissue should be considered. Abdominal fat surgical biopsy, being essentially a noninvasive procedure, is a clinically acceptable source of additional diagnostic material for amyloid typing, which has hitherto been largely used only for screening purposes.20

Interpretation of amyloid typing must be done in the context of Congo red positivity, in which areas that are positive for amyloid by Congo red stain are correlated with immunohistochemistry. To address this issue, an elegant “overlay technique” has been developed, in which a Congo red stain and immunohistochemistry are performed on the same slide—at least in the case of AA amyloidosis.22 Permanganate pretreatment of Congo red-stained slides for amyloid typing is completely obsolete and should not be used or reported.20 The interpretation of immunohistochemistry performed in paraffin sections and immunofluorescence in frozen sections is not a simple matter and also depends on the experience and expertise of the operator. It is important to use an antibody panel and a built-in control for evaluation of these studies, as well as positive and negative controls (Figure 3). In addition to antigenic preservation and sensitivity of detection, stringency of diagnostic criteria and technical issues...
also play a role (recently reviewed in reference 2). Good results for amyloid typing using amyloid-specific antibodies are reported with other antibody-based techniques, such as immunoelectronmicroscopy and Western blotting. Recently, antibodies raised against recombinant peptides corresponding to the variable region of immunoglobulin light chains were also tested for their potential utility in amyloid typing.

To conclude, immunohistochemistry, in particular immunofluorescence on frozen sections, is a fast and valid methodology for amyloid typing but should be done with caution and with a full awareness of its limitations and pitfalls. In cases that are inconclusive or negative, evaluation by a reference laboratory, using more sophisticated methods, should be pursued.

Direct typing of amyloid protein extracted from formalin-fixed, paraffin-embedded specimens has also been reported. Using proteomics techniques, amyloid typing can be successful in small samples, including biopsies. Again, even though such studies are feasible in paraffin-embedded biopsies, fresh tissue is preferable and fat may be an excellent source of such samples. Although these technologies, which are currently available only in highly specialized laboratories, have not yet been validated in large numbers of samples at multiple centers, their development is a welcome advancement in the diagnosis of amyloidosis. In current pathology practice the molecular characterization of amyloid proteins represents a valuable complement to immunohistochemistry.

Finally, processing and interpretation of all amyloid specimens is best relegated to renal pathologists who are more familiar than general pathologists with polarization microscopy, immunofluorescence microscopy, and electron microscopy.

The current recommendations regarding amyloid diagnosis can be summarized as follows:

1. Congo red stain is currently the gold standard for amyloid detection and the goal is to detect amyloid early.
2. Diagnosis of the amyloid type must be based on the identification of amyloid protein within the deposits and not solely by reliance on clinical or DNA studies. However, the latter are recommended for confirmation of the amyloid type based on evaluation of the protein in deposits.
3. Immunohistochemistry must be performed and interpreted with caution and inconclusive results must be evaluated further using the more sophisticated methods available in referral centers.
4. An adequate amount and quality of tissue must be available for amyloid diagnosis and typing with emphasis on the use of fresh tissue and greater use of abdominal fat biopsy.
5. The development of new technologies underscores the need for regular review of recommendations and standards for the clinical diagnosis of amyloidosis and the need to address the regulatory (accreditation, licensing, validation, analyte-specific reagent status) and reimbursement issues connected with these emerging technologies.

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References

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