REVIEW

Amyloidosis: diagnosis and prognosis

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All patients with amyloidosis must have Congo red-stained deposits demonstrating green birefringence under polarized light. Subcutaneous fat is the easiest source for such tissue. Amyloid deposits must be characterized to establish systemic or localized disease, and all forms of systemic amyloidosis must be classified using immunohistochemistry, immunofluorescence, genetic testing for mutations known to be associated with familial amyloidosis or mass spectroscopy techniques. Where available, serum amyloid P component imaging may be used to quantify the extent of amyloid deposition. Both echocardiography and magnetic resonance imaging are important tools for assessing the extent of cardiac amyloidosis and establishing the prognosis for patients with this disease.

Amyloidosis represents all disorders characterized by the deposition of protein fibrils with an amyloid confirmation in tissue, which represents a β-pleated sheet structure. The signs and symptoms associated with amyloidosis vary among the different disorders, and the ultrastructure of the amyloid drives the decision regarding therapy [1]. The classification of amyloidosis is established by the ultrastructural subunit of the amyloid fibril protein. Immunoglobulin light-chain ultrastructure reflects primary amyloidosis (AL). Secondary amyloidosis (AA) is represented by amyloid A protein, and familial [2,3] and senile systemic amyloidosis are represented by wild-type and mutant transthyretin molecules [4]. After a diagnosis of amyloidosis is established, the extent of systemic involvement is evaluated by a functional assay, and particular attention is given to assessing myocardial involvement with amyloid because of its important impact on survival. Renal involvement has a particularly important impact on the outcome in AA, which tends to target the kidney as the first symptomatic organ. In specific instances, scintigraphic evaluation with serum amyloid P (SAP) component is also performed [5]. In this review, amyloid refers to the pathologic deposits of fibrillar protein that bind Congo red. Amyloidosis is the clinical disorder that results from the deposition of the amyloid substance in patients.

Keywords: amyloidosis, brain natriuretic peptide, Congo red, immunoglobulin free light chain, immunoglobulin light chain, monoclonal protein

The Congo red stain

The identification of amyloid deposits in a biopsy specimen is the only method of confirming the diagnosis of amyloid, and is the *sine qua non* of the disease. The affinity for the cotton-wool dye Congo red is related to the β-pleated sheet three-dimensional structure of proteins in an amyloid configuration. Congo red was reported as a specific stain for the detection of amyloidosis in 1922 [6]. The first observation of green birefringence under polarized light was first reported in the brain of Alzheimer's disease patients in 1926 [7].

The use of the Congo red stain can be challenging. Overfixation of a biopsy specimen can result in poor dye uptake, and interstitial trapping of the dye can result in false positives. Fibrin and elastin will often be congophilic, but generally do not demonstrate green birefringence. Careful interpretation of fat aspirates because of the high collagen content is necessary to avoid overinterpretation and false-positive diagnoses. Phenol Congo red stain may be superior to conventional alkaline Congo red. In one study, only seven out of ten patients were positive for alkaline Congo red, while all demonstrated reactivity to phenol Congo red [8].

There have been multiple attempts to improve the sensitivity and specificity of Congo red staining. Potassium permanganate sensitivity was used to distinguish AA from other forms of amyloidosis [9]. By incubating Congo red-stained tissues in potassium permanganate, deposits of AA will lose binding affinity for Congo red staining. AL and familial amyloidosis (AF) are permanganate resistant and retain congophilia after incubation. More sensitive immunohistochemical techniques are preferred today [10]. Performate has also been used to distinguish AA from other forms of amyloidosis, as performate extinguishes the uptake of Congo red.

Congo red has been combined with immunohistochemistry and Congo red fluorescence to enhance the diagnostic value of the technique. Congo red fluorescence, which is viewed under

ultraviolet instead of polarized light, appears to be more sensitive than conventional apple-green birefringence after Congo red staining. Minute amyloid deposits can be visualized with improved accuracy, and it appears to be a more sensitive method for a direct diagnosis from tissue sections, and does not interfere with immunohistochemical staining [11,12]. Congo red fluorescence has been applied to frozen kidney biopsy specimens. In one study of 15 patients with amyloidosis, no false-positives or -negatives were observed [13]. When 146 renal biopsy specimens previously stained with Congo red were re-evaluated using Congo red fluorescence, 87 Congo red-positive patients were confirmed, and one additional positive was identified [14]. Congo red fluorescence is simple, with high specificity and sensitivity. Amyloid deposits are pronounced and easier to evaluate under microscopy. The sulfated Alcian blue and crystal violet stains have previously been used to stain nerve biopsies and endomyocardial biopsies. These are not widely accepted as being definitive for the diagnosis of amyloidosis [15,16].

Biopsy diagnosis of amyloid

Systemic amyloidosis is a deposition disorder, and deposits can be located by carrying out biopsies at virtually any site. In clinical practice, biopsies have often been directed to the symptomatic organ; for example, the kidney when proteinuria is present, the liver when it is enlarged or the heart when there is heart failure. Although visceral organ biopsies are highly sensitive, endomyocardial biopsy being 100% sensitive [17], alternative methods are available. In a study of 36 renal biopsies, characteristic amyloid deposits were seen as fibrils by electron microscopy and positive reactions to Congo red or thioflavin T or thioflavin S [18]. Amyloidosis can also be diagnosed using fine-needle aspiration of the liver, reducing the risk of core-needle biopsy [19]. Endomyocardial biopsy can also establish the diagnosis of amyloidosis [20]. Endoscopic biopsy of the upper digestive tract or duodenum can be a preferred diagnosis for renal amyloidosis. The frequency of deposition in endoscopic biopsy specimens runs as high as 100% for the duodenum, 95% for the stomach, 91% for the rectum and 72% for the esophagus. Endoscopy can be used in place of renal biopsy, as it is better tolerated and safer than percutaneous renal biopsy [21]. Rectal biopsy, skin biopsy and salivary gland biopsy of the lip can also be occasionally performed as they are minimally invasive and can be particularly useful in patients who present with polyneuropathy [22]. When only small amyloid deposits are anticipated, a kidney biopsy may indeed be preferred because the tissue is then used for immunoelectron microscopy, which can then classify the type of amyloidosis with an appropriate panel of antibodies [23,24].

In most amyloidosis treatment centers, the subcutaneous fat aspiration is the primary screening technique [25–27]. The sensitivity of subcutaneous fat aspiration is 84%, and it is the diagnostic procedure of choice because it requires no specialty consultation or technical expertise and causes minimal patient discomfort. In a blinded study of the fat aspiration, the procedure performed on 82 patients and 72 disease-free adult volunteers found a sensitivity of 72%, a specificity of 99% and a diagnostic turnaround time of less than 24 h [28]. Concordance between two pathologists who were blinded is 95%. Occasional, equivocal positive stains need to be interpreted with caution because weak, nonspecific, histologic staining can be seen.

A number of different techniques have been reported that permit classification of the type of amyloidosis in a biopsy of the fat. An enzyme-linked immunosorbent assay (ELISA) successfully typed amyloidosis in 14 out of 15 positive fat biopsy specimens [29,30]. The specificity of the fat can be as high as 100%, with a positive predictive value of 100% [31]. In this study, sensitivity was only 58%, and the percentage of inadequate specimens was as high as 11%. Difficulties in confirming amyloidosis included staining variability of the specimen (pale staining) and collagen birefringence. Immunoelectron microscopy has been used to characterize amyloidosis in fat biopsy specimens in the presence of cardiac amyloidosis [32,33]. A total of 91 patients who underwent fat aspiration were positive in 22%, negative in 68%, insufficient in 9% and equivocal in 1%. Of the 62 patients with negative findings, follow-up biopsies were performed for 19, and five demonstrated amyloidosis after Congo red staining. In this case series, 55% of positive findings were seen on the first biopsy, but 31% with negative findings underwent a second biopsy with overall sensitivity and specificity of 75 and 92%. Congo red fluorescence has been used in the analysis of amyloid fat aspirates and remains useful when examining archival slides of previously stained aspirates [11].

Immunohistochemical classification of amyloid deposits in subcutaneous fat samples may be determined by western blot analysis with specific anti-

bodies directed against amyloid fibril proteins [34]. Out of a total of 120 patients, 38 with AA, 70 with AL and 12 with transthyretin amyloidosis (ATTR) had biopsies. Smears were positive in 93% of patients. Specificity was 100% in 45 control samples, and fat aspiration was far better than that for rectal biopsy. The authors concluded that subcutaneous fat aspirate was the preferred method for detecting amyloidosis. Sensitivity was 80%, but when three fat smears were examined by two observers, the sensitivity rose to 90%. The additional value of a rectal biopsy was negligible, and if the fat is negative and the diagnosis is still suspected, then a direct visceral organ biopsy is recommended. In summary, the preferred technique for the diagnosis of amyloidosis is fat biopsy. Specimens should be examined by Congo red and then antibodies can be used to classify the type.

Distinguishing between localized & systemic amyloidosis

It is important to clarify after a diagnosis of amyloidosis is established whether it is localized or systemic, since the therapies are fundamentally different between the two forms. The distinction among the various forms of systemic amyloidosis is critical, since light-chain amyloidosis requires systemic chemotherapy to suppress the plasma cell clone. Inherited forms of amyloidosis often result from mutated proteins produced in the liver and can respond to liver transplantation. Recently, the use of etanercept for amyloidosis due to AA that is not amenable to correction of an underlying cause has successfully reversed organ dysfunction, emphasizing the need for accurate classification. Typical clinical presentations for localized amyloidosis include hoarseness, stridor, hematuria, visual disturbances, cutaneous purpura or macules. Some of these can be confused with the symptoms associated with systemic forms of amyloidosis [35–37]. Presumptive suspicion of localized amyloidosis may be made based on the location of presentation. Most typically, the skin, bladder, ureter and tracheobronchial tree are target organs [38]. Most localized amyloid deposits result from plasma cells at the site of deposition that produce immunoglobulin light chains that misfold into an amyloid configuration [39,40]. Pulmonary amyloidosis may be part of a localized pulmonary process such as nodular pulmonary amyloidosis, or part of a systemic process, usually diffuse interstitial pulmonary amyloid [41]. Localized amyloidosis can involve the vocal cords, presenting as hoarseness.

Ureteral vesicular amyloid deposits are almost always localized. Patients usually present with hematuria or colic due to obstruction [42]. Cutaneous amyloidosis of the lichen or macular types is typically localized, an easily managed condition. Nodular cutaneous amyloidosis may be a component of systemic AL, and is often the first clue to an underlying, potentially life-threatening process [43]. Carpal tunnel syndrome is typically a localized form of amyloidosis [44] associated with ATTR, but is found in 15% of patients with AL amyloidosis, particularly those patients who have peripheral neuropathy [45]. Localized amyloidosis has been reported in the conjunctiva and orbits, and is usually managed symptomatically. Trace amounts of amyloid can be seen in the cartilage of the hip and knee following joint-replacement surgery [46,47].

Classification of amyloidosis on biopsy tissue specimens

After amyloidosis has been confirmed in a tissue biopsy specimen stained with Congo red and demonstrating green birefringence under polarized light, the next step is distinguishing systemic from localized amyloidosis. However, systemic amyloidosis can be AL, AF, AA, senile or dialysis-related. The clinical manifestations and the therapy are vastly different for all five forms of systemic amyloidosis, and numerous case reports exist in the literature where the amyloidosis was initially incorrectly classified. In an important study, fibrinogen A-α chain renal amyloidosis, a form of inherited amyloidosis, was confirmed by genetic testing after an initial clinical diagnosis of AL [48]. A second important study described three patients who had an incidental monoclonal gammopathy with hereditary amyloidosis, the presence of the monoclonal gammopathy suggesting AL amyloidosis, which could easily have led to the inappropriate administration of chemotherapy [49]. One case report describes a patient with presumptive renal amyloidosis who was subsequently found to have minimal-change glomerulopathy and Waldenström's macroglobulinemia [50]. Characterizing the type of amyloidosis in a tissue biopsy is often challenging because of the small amount of available tissue and the fact that only a small proportion of the biopsy contains amyloid deposits. Micro-methods have been developed for purification and sequencing of amyloid proteins in minute specimens. Amyloid deposits can be extracted and sequenced from formalin-fixed tissue specimens, and exact identification of the protein in fibrillar deposits is

possible [51]. Micro-techniques for the extraction and purification of amyloid can also be used for immunochemical characterization of the amyloid deposit [52,53].

The techniques that have been used to identify amyloid deposits include ELISA, western blot, amino acid sequencing and mass spectroscopy. Immunohistochemical assays are the most commonly used, but controversy exists with regard to their value in definitively classifying the amyloidosis. Immunoperoxidase techniques were initially reported to be extremely sensitive [54]. In one autopsy series, a panel of noncommercial antibodies was used, and identified AA in 21, ATTR in 11 and AL in 10. One patient had more than one type of systemic amyloid [55]. In AA and AL systemic amyloid, the kidney is involved most frequently. In ATTR, the heart, lungs and peripheral nerves were involved most frequently. This series demonstrated that in most patients with amyloidosis, type could be classified using specific antibodies against the five major amyloid fibril proteins – amyloid A, light chain, transthyretin, β2 microglobulin and fibrinogen. The distribution of organ involvement can sometimes be a clue to the etiology of the amyloidosis. Vitreous amyloid has only been described in familial amyloid associated with TTR. Tongue enlargement is pathognomonic of light-chain amyloidosis. Renal involvement is typical of fibrinogen inherited amyloid and light-chain amyloid. Pulmonary amyloid is overwhelmingly light chain derived. The presence of factor X deficiency is typical of light-chain amyloidosis. Polyneuropathy can occur in either inherited or light-chain amyloid, but when it appears isolated, extreme caution is required to ensure that a hidden familial amyloidosis is not present. Micro-extraction techniques of amyloid proteins from fat aspirates have been analyzed immunochemically. Concordance was seen in three of four patients with κ-light-chain AL, five out of six with λ-light-chain AL and one patient with AA [56].

Antibodies against synthetic amyloid peptides have been developed, corresponding to amino acids 118 to 134 in the λ-immunoglobulin light chain and position 116 to 133 of the κ immunoglobulin light chain. These synthetic peptides are used for the classification of amyloid deposits in paraffin-embedded tissue sections [57]. Anti-λ-antiserum reacts with samples from 18 of 19 patients with Aλ amyloidosis. Anti-κ-antiserum reacts with samples from nine of ten patients with Aκ amyloidosis. Immunofluorescence staining of kidney biopsy specimens for κ- and λ-light-chain proteins may be unreliable. In one study, negative immunofluorescence results for 12 patients with plasma cell dyscrasia were seen, representing over a third of all patients studied [18]. In view of the lower sensitivity of immunofluorescence microscopy for detecting AL in the kidney, additional diagnostic studies are required.

In a series of 169 biopsies from 121 patients, 12 patients with amyloidosis could not be classified immunohistochemically [58]. In 32% of biopsies, amyloid deposits did not stain diagnostically for λ or κ immunoglobulin light chains, indicative of a reduced sensitivity of immunohistochemical techniques in light-chain amyloidosis. The presumptive explanation is that some antisera will not recognize the epitopes associated with immunoglobulin light chains when they have folded into an amyloid configuration, or the proteolysis that occurs in the conversion of an intact light chain into an amyloid fragment results in the deletion of the recognizable epitopes. The authors concluded that immunohistochemical classification of amyloidosis remains a problem, particularly using commercial anti-AL antibodies. Although classification of AA and ATTR deposits is straight-forward, the classification of AL and rare forms of ATTR, those associated with fibrinogen or apolipoprotein, often cannot be made with any certainty unless there is conclusive clinical information. Senile cardiac amyloidosis can rarely be diagnosed without a cardiac biopsy. The echocardiographic features are typical of advanced amyloid, although often, the echocardiographic features are somewhat more dramatic than the clinical features. The prognosis of this form of amyloid is better than light-chain amyloid or mutant TTR amyloid. Immunohistochemical staining of the endomyocardial biopsy is required to prove TTR. Once this occurs, genetic testing is required, since one cannot clinically distinguish senile cardiac amyloidosis from familial amyloid cardiopathy presenting late in life. Senile cardiac amyloidosis requires the discovery of normal TTR or a benign polymorphism.

A recent article summarizes the mistakes that are made in immunochemical assays that can result in the misdiagnosis of amyloid [59]. The errors that are typically seen include inconsistent immunolabeling, nonspecific background staining and inconsistent reactions. A recently reported antibody against the λ-light-chain peptide has been used in immunochemical classification. This antibody specifically stains proteins in both western blot and formalin-fixed, paraffin-embedded tissue section [60]. Formic acid extraction has been

combined with immunochemical and biochemical characterization to classify amyloidosis. Constant region sequences of the immunoglobulin light chain were observed in the identification process, instead of the expected variable chain sequences.

Mass spectroscopy has been applied to the diagnosis of systemic amyloidosis. Mass spectroscopy has been used on serum samples of patients with ATTR to detect the mutation in the serum. Microextraction techniques of amyloid deposits that have been analyzed by mass spectroscopy can verify the amino acid sequence of the protein. A chemical composition is verified through amino acid sequencing or mass spectroscopy of material extracted from fibrillar deposits, and is applicable to formalin-fixed biopsy specimens [61]. Tandem mass spectrometry of material extracted from formalin-fixed, amyloid-containing tissue biopsy specimens can precisely identify the type of amyloid deposits [62]. Unique molecular profiles have been identified in light-chain amyloidosis through functional gene-expression analysis of clonal plasma cells [63]. Class prediction analysis has demonstrated a subset of 12 genes that can discriminate amyloid of the AL type from other amyloid protein. Molecular profiling of clonal plasma cells provides insights into the pathogenesis of light-chain amyloidosis.

After amyloidosis has been diagnosed, it is essential to attempt classification of the type from tissue biopsy specimens. Abnormalities of the immunoglobulin free light chain ratio may be specific for AL, although nonreactivity of individual monoclonal free light-chain proteins has been reported, resulting in false-negative findings [64,65]. Pooled human immunoglobulin contains antibodies that recognized fibrils formed from light-chain proteins associated with AA and ATTR. A peptide purified from human immunoglobulin is reactive against all forms of amyloid deposits, including those of AA, ATTR and islet amyloidosis [66,67]. These antibodies immunostain human amyloid tissue deposits, and fibril affinity purified intravenous immunoglobulin has potential as a diagnostic agent for patients with amyloid-associated disease.

Radionuclide imaging of amyloid deposits

The use of imaging in an attempt to identify amyloid deposits has been present for some time. Technetium-99 M-pyrophosphate has been used in an attempt to diagnose amyloid. The tracer is rarely taken up, but can demonstrate when there is reduced systolic function or diastolic function [68]. Myocardial uptake of technetium-labeled pyrophosphate has been reported to be of benefit in patients with amyloid polyneuropathy [69].

Thallium-201 scintigraphic studies have been reported in patients with cardiac amyloidosis. Washout rates during rest and during delayed thallium imaging can reflect the severity of amyloidosis in the heart, and washout rates of the whole myocardium are higher in patients with amyloidosis than controls. The washout rate of thallium in four of five patients with amyloidosis is very high, and all of those died in less than a year [70]. Therefore, the washout rate in the setting of rest and delayed thallium-201 images may represent the severity of amyloid deposition in the myocardium and may be prognostic.

Gallium-67 and thallium-201 single-photon-emission tomography has been used to study dialysis amyloidosis [71]. The technique was compared with imaging with technetium-labeled methylene diphosphonate. Whole-body bone scans were able to detect active and pre-existing deposits of dialysis amyloid. Gallium and thallium scans were helpful to differentiate between active and pre-existing deposits, and when evaluating the effect of therapy.

Technetium-labeled conjugates of chrysamine-G have been used to image amyloid deposits. The agent localizes to amyloid deposits in human kidney tissue, suggesting it may be a specific targeting agent for diagnostic purposes. *In vitro* autoradiography performed in a chicken model established the usefulness of the tracer as a noninvasive, diagnostic probe for amyloid arthropathy, and the fact that it may be applicable to human amyloidosis [72]. Technetium-DPD scintigraphy was studied in eight patients with ATTR, and was found to be highly sensitive, showing retention rates of 80% in patients with FAP at 3 h, compared with 56% in controls [73]. The usefulness of the technique has been supported by other studies [74]. The increased uptake of technetium hydroxymethylene diphosphonate has been reported. The first case report described uptake in a patient with biopsy-proven cardiac amyloidosis [75]. A follow-up report demonstrated significantly increased uptake of the bone tracer within the myocardium in comparison with the highest skeletal uptake [76].

Technetium-labeled aprotinin has also been studied as a specific marker of amyloidosis, in terms of both its sensitivity and specificity as a diagnostic molecule. In a group of 23 patients, 22 had focal accumulations of technetium-labeled aprotinin in different organs; findings in 20 were confirmed by biopsy or autopsy. The accuracy for

differentiating between ATTR and AL was 100%, and uptake was absent in controls. In patients with ATTR, sensitivity and specificity were 100%. In patients with AL, sensitivity was 0% and specificity was 100%. Therefore, using technetium diphosphono propanodicarboxylic acid (aprotinin) scintigraphy was useful for differentiating AL and ATTR of the heart [77]. Aprotinin imaging may be a useful noninvasive method for the assessment of the presence and extent of extra-abdominal amyloid, particularly cardiac. The median heart:background uptake ratio was 2.0 in cardiac amyloid patients, and 1.1 in patients without cardiac amyloidosis. Myocardium uptake was noted in all five patients with a final diagnosis of cardiac amyloidosis [78]. In a study of 23 consecutive patients with known amyloidosis, aprotinin focally accumulated in 22 patients with a total of 90 lesions, of which 20 were confirmed [79].

Quantitative high-resolution microradiographic imaging of amyloid deposits has been developed in a murine model. It combines radio-iodinated SAP scanning and single-photon emission tomography imaging. The use of radiography to discern the extent of amyloid was beneficial for quantitating total-body burden of amyloid and for the evaluation of the therapeutic efficacy of pharmacologic compounds [80,81].

SAP component scanning

The SAP scan uses radio-iodinated SAP component. SAP is a pentraxin plasma protein that specifically undergoes calcium-dependent binding to amyloid fibrils and is present in all forms of amyloid deposits. The injection of I-123 labeled SAP component can accurately diagnose, locate and monitor the extent of systemic amyloid deposits [5]. The uptake of SAP is proportional to the quantity of amyloid deposited in various tissues, and 24-h retention levels are abnormal in all patients with AL [82]. SAP scanning can demonstrate regression of visceral amyloid deposits after liver transplantation in patients with ATTR [83]. Hepatic amyloid deposits are identified in 54% of AL patients, 18% of AA patients and 2% of ATTR patients using this technique [84]. The SAP scan be used as a noninvasive method to monitor patients after renal transplantation for amyloid end-stage renal disease [85]. I-131 has also been used for radiolabeling, but its imaging characteristics are unfavorable compared with I-123 [86]. Amyloidosis is characterized by an accelerated initial clearance of SAP from the plasma, increased interstitial exchange rate and extravascular retention. This would suggest that the binding of radiolabeled SAP is reversible and can be used to monitor the effects of therapy [1]. The scan can demonstrate the overall distribution of amyloid in organs, can identify amyloidosis in sites that cannot be biopsied and can monitor progression or regression of amyloid deposits [87].

Echocardiographic assessment of amyloidosis

Echocardiography has been used to establish the diagnosis of cardiac amyloidosis and has differentiated amyloidosis from hypertrophic cardiomyopathy [88,89]. Echocardiographic features in amyloidosis include left ventricular fractional shortening reduction, a transmitral flow velocity compatible with abnormal relaxation, right ventricular systolic dysfunction and ventricular wall thickening [90]. Doppler imaging adds value to two-dimensional imaging studies for evaluation of systolic and diastolic left ventricular function [91,92]. Right ventricular dysfunction is commonly recognized in patients with cardiac amyloidosis [93]. Amyloidosis can be diagnosed using the velocity profile in the hypertrophied left ventricular wall. The myocardial velocity profile in the ventricular septum and left ventricular posterior wall shows a distinctive serrated pattern that may be related to amyloid deposition in the myocardium. Low-voltage pattern, pseudo-infarction patterns on electrocardiography and increased myocardial thickness, and a speckled appearance of the myocardium on echocardiography, are associated with cardiac amyloidosis. The most useful model in predicting survival is a combination of low voltage and measures of myocardial thickness [94]. Tissue Doppler imaging is a more reliable method for early detection of cardiac functional abnormalities. The recent development of strain rate imaging to assess myocardial function has added to tissue Doppler evaluation for patients with cardiac amyloidosis. Strain rate imaging can detect the earliest degree of cardiac dysfunction and is now routine in the assessment of patients with cardiac amyloidosis [95,96].

MRI in amyloid

MRI has a role in the diagnosis of cardiac amyloidosis. It can be used to identify morphology and suggests the presence of infiltration [97,98]. Two types of MRI lesions are typically noted:

- Thickened hypointense lesions that are enhanced by gadolinium on T1-weighted images and hyperintense on T2-weighted images

- Tumor-forming lesions hypointense on T1- and T2-weighted imaging, not enhanced by gadolinium

Patients with cardiac amyloidosis have a characteristic pattern of global subendocardial, late enhancement coupled with abnormal gadolinium kinetics in the myocardium and blood pools. These findings are in concordance with the transmural histologic distribution of amyloid. Other MRI findings in amyloidosis include atrial septal thickening significantly greater than controls and left ventricular end-diastolic volume smaller than controls.

Prognosis

The outcome of patients with amyloidosis is driven by the extent of cardiac involvement. In the 1960s and 70s, this meant clinical evaluation for heart failure as manifest by cardiomegaly, pulmonary vascular redistribution on a chest radiograph and the clinical signs of orthopnea and lower extremity edema. These insensitive methods for assessing cardiac amyloid were replaced in the 1970s through 1990s by echocardiography where infiltration of the myocardial wall, Doppler studies to indicate restriction to inflow and strain rate imaging have all resulted in increasingly sensitive methods to detect cardiac involvement [99,100]. Both brain natriuretic peptide (BNP) or N-terminal prohormone BNP (NT-proBNP) and serum troponin have been shown to be elevated in patients with cardiac amyloidosis. Patients with a troponin T level of more than 0.035 µg/ml have a median overall survival of only 3.7 months. Patients with a NT-proBNP level of greater than 332 pg/ml have a median survival of 5.8 months, compared with those patients who have an NT-proBNP level of less than 332 pg/ml, who have a median survival of 20 months. The BNP and the troponin can be combined to result in a useful staging system that classifies patients into groups of approximately equal size, with overall survivals of 26, 10 and 4 months [101].

In multivariate analysis, cardiac troponin T and the number of organ systems involved with amyloid are highly predictive of outcome. We now use the troponin T as an exclusion criterion for stem-cell transplant. If the troponin T is 0.06 or greater at the time of evaluation, we consider the treatment-related mortality risk excessive and are no longer using high-dose therapy with these patients [102]. It is important to note that cardiac biomarkers are useful only for establishing the prognosis of light-chain amyloidosis. It has not as yet been determined whether it is of value in determining the presence of cardiac involvement in a patient with biopsy-proven amyloidosis. Other prognostic features include the severity of baseline proteinuria, which consistently predicts renal response after autologous stem-cell transplantation. In univariate analysis, the serum creatinine, serum alkaline phosphatase and neurodisability score have all been shown to be prognostic for survival in amyloidosis. However, with the advent of dialysis and the high frequency of cardiac involvement in multivariate analysis, cardiac involvement eliminates these other variables from a statistical model. Therefore, although hepatic, renal and pulmonary involvement are important in amyloidosis, they independently predict survival only when patients with cardiac amyloid are excluded from analysis. Achievement of a renal response is associated with improved survival [103]. The absolute value of the immunoglobulin free light chain is also prognostic in patients with AL. Normalization of the free light chain level after stem-cell transplants predicts for both organ response and complete hematologic response, and it is a better predictor of survival than achievement of a complete hematologic response using non-free light chain criteria [104]. Recently, serum uric acid has been shown to be a prognostic factor in systemic amyloidosis, and elevated serum uric acid levels have been associated with cardiovascular mortality in population studies. In amyloidosis, patients with a uric acid level greater than 8 mg/dl have a median overall survival of 9 months compared with 20.3 months for all other patients. Uric acid provided additional survival prognostic information with the troponin T and the NT-proBNP value. Uric acid was predictive of survival in patients undergoing stem-cell transplant for AL [105].

The prognosis and overall survival for patients with hereditary amyloidosis is far better than patients with light-chain amyloidosis. Patients who present with primary peripheral neuropathy often have a course that will evolve slowly over the period of a decade. Even when the myocardium is involved in inherited amyloidosis, the rate of progression and the degree of cardiac dysfunction for a given degree of infiltration measured by echocardiography is less, and therefore, the survival, even with cardiac involvement, is superior to light-chain amyloid. Senile cardiac amyloidosis has a better prognosis than

Executive summary

Requirement for the diagnosis of amyloidosis

- Biopsy of skin, rectum or abdominal fat
  - The abdominal fat biopsy is the simplest of the techniques.
- Congo red staining
  - Congo red immunofluorescence may be used to enhance sensitivity.
- Immunofluorescent/immunohistochemical staining may be used to classify the amyloidosis
  - Requires specific antibodies to κλ immunoglobulin light chain, amyloid A, transthyretin (TTR) amyloidosis and A fibrinogen proteins.

Clarifying the nature of the amyloidosis

- Distinguish localized from systemic
  - Skin, bladder and tracheobronchial tend to be localized
- If systemic amyloid, must distinguish primary amyloidosis (AL), secondary amyloidosis (AA), familial amyloidosis (AF) and β2 microglobulin (dialysis amyloidosis)
  - Immunohistochemistry, mass spectroscopy useful
- Senile and familial amyloid are due to TTR. The former is wild-type TTR, the latter demonstrates mutations in the *TTR* gene.
- Rare forms of familial amyloidosis include fibrinogen, apolipoprotein and lysozyme.

Approach to the diagnosis of amyloidosis

- All patients with amyloidosis must have Congo red stain deposits showing green birefringence under polarized light.
- Subcutaneous fat is the easiest source of obtaining such tissue.
- All deposits must be characterized to establish systemic or localized disease.
- All forms of systemic amyloidosis must be classified as type.
- Serum amyloid P imaging can be used to quantify the extent of amyloid.
- MRI and echocardiography are important tools for assessing the extent of cardiac amyloid involvement.

Prognosis of amyloid is driven by the extent of cardiac involvement

- This is best assessed by troponin T, N-terminal prohormone brain natriuretic peptide or brain natriuretic peptide and serum uric acid.
- Immunoglobulin free light-chain levels are prognostic in amyloidosis.

familial amyloid cardiomyopathy. When matched for age and gender, the progression of senile cardiac amyloidosis is slower and outcomes are superior.

Conclusion

Amyloidosis should be suspected in all patients presenting with nephrotic syndrome, cardiomyopathy, hepatomegaly, peripheral or autonomic neuropathy, or atypical multiple myeloma. Screening should include serum and urine immunofixation and an immunoglobulin free light-chain assay. The diagnosis is generally confirmed with a subcutaneous fat aspirate. Immunohistochemistry of deposits of amyloid to ensure the protein subunit and genetic analysis can be used to confirm the type of amyloid. Echocardiography, MRI and SAP scanning, when available, provide useful adjunctive data. Prognosis is determined by the extent of cardiac involvement. Useful parameters include troponin T, BNP, uric acid, the number of organs involved, β2 microglobulin and baseline serum immunoglobulin free light chain level. Figure 1 provides an algorithm for the diagnosis, classification and prognosis of amyloidosis.

Future perspective

It is anticipated that over the next 10 years, ease of diagnosis will be enhanced by microextraction techniques followed by mass spectroscopic analysis to allow absolute confirmation of the protein subunit of an amyloid deposit. It is anticipated that new biomarkers will become available to further define the prognosis of patients and attempt to use these prognostic indicators to assign rational therapy.

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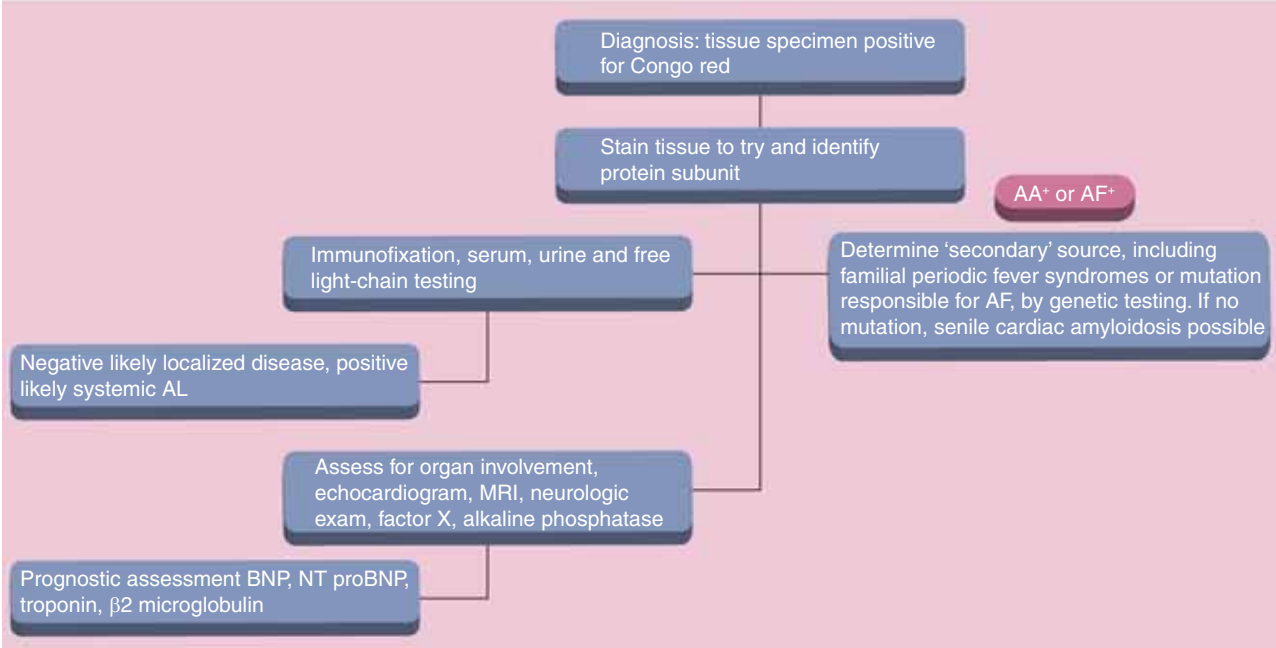
Figure 1. Algorithm for amyloidosis.



AA: Secondary amyloidosis; AF: Familial amyloidosis; AL: Primary amyloidosis; BNP: Brain natriuretic peptide; NT-proBNP: N-terminal prohormone BNP.

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