bjh guideline

Guidelines on the diagnosis and investigation of AL amyloidosis

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Summary of key recommendations

The following recommendations are all Grade 1 level C evidence.

- **The National Amyloidosis Centre (NAC) provides expert support for the diagnosis, assessment and management of patients with suspected AL amyloidosis and shared care between local clinicians and the NAC is strongly recommended.**
- **AL amyloidosis is a disease with insidious onset and considerable clinical heterogeneity. A high level of clinical suspicion is essential to avoid delayed diagnosis.**
- **In suspected AL amyloidosis, a histological diagnosis is essential and, where possible, a biopsy should be taken from an apparently affected organ. Alternatively, a subcutaneous abdominal fat aspirate and bone marrow biopsy may be examined for amyloid but its absence does not exclude amyloidosis.**
- **Congo red staining with classical apple green birefringence under polarized light should be used to test for the presence of amyloid on any histological specimen.**
- **The diagnosis of amyloid requires an experienced laboratory, as false negative and false positive diagnoses on the basis of histology are not infrequent. Other (non-AL) amyloid fibril types should be excluded by using immunohistochemistry, DNA analysis, protein sequencing or mass spectrometry.**
- **Immunofixation of blood and urine and serum free light chains should be measured in all patients with suspected AL amyloidosis.**
- **Bone marrow aspirate and trephine biopsy are recommended at diagnosis. As well as estimating the plasma cell infiltrate (or confirming a diagnosis of Waldenstrom's macroglobulinaemia), a bone marrow trephine biopsy may be useful in confirming amyloidosis.**
- **Multiple tissue biopsies are not recommended and liver biopsies are best avoided, or undertaken via the transjugular route. A comprehensive assessment of the extent of organ involvement and dysfunction should be carried out by non-invasive criteria, including serum amyloid P component (SAP) scanning when feasible.**
- **Echocardiography (±Cardiac Magnetic Resonance Imaging) is recommended to assess amyloid involvement. Given the rarity of the condition, involvement may go unrecognized or not be adequately assessed.**
- **Nerve conduction studies and autonomic function tests should be performed in patients with suspected neuropathy.**
- **The cardiac biomarkers, N-terminal pro-brain natriuretic peptide (NT-proBNP) and Troponin T, and serum free light chain assessments form the basis of current validated prognostic scoring systems and can identify a particularly poor risk group of patients.**

Methodology

This guideline has been compiled by members of the Guidelines Working Group of the UK Myeloma Forum on behalf of the British Committee for Standards in Haematology (BCSH). The objective of this guideline is to provide healthcare professionals with clear guidance on the management and investigation of patients with AL amyloidosis. A Medline search for literature published between 1975 and May 2014 was performed using PubMed. The search included clinical trials in AL amyloidosis and papers or reviews where AL amyloidosis was the major focus. Abstracts from relevant meetings held between 1998 and 2014 were also included. The Cochrane database did

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not include any relevant information. Levels of evidence and grades of recommendation are based on the GRADE system (http://www.gradeworkinggroup.org/index.htm).

The draft guideline was reviewed by the UK Myeloma Forum Executive and members of the BCSH and British Society of Haematology. The guideline was then reviewed by a sounding board of approximately 50 UK haematologists and comments incorporated where appropriate. The guidance may not be appropriate to all patients with AL amyloidosis and, in all cases, individual patient circumstances may dictate an alternative approach.

National amyloidosis centre (NAC)

The NAC (www.ucl.ac.uk/medicine/amyloidosis/nac/index.html) was commissioned directly by the Department of Health to provide a diagnosis and management advisory service for the national caseload of patients with amyloidosis. The clinical unit is based in the Royal Free Hospital Campus, University College London. The Centre developed scintigraphic imaging (serum amyloid P component [SAP] scanning) of amyloid as a quantitative diagnostic procedure and provides various specialized clinical services for patients with acquired and hereditary systemic amyloidosis, including:

- Diagnosis, quantification (staging) and monitoring of amyloidosis.
- Specialized clinical chemistry service for characterization, quantification and serial monitoring of amyloid precursor proteins, including serum free monoclonal immunoglobulin light chains in patients with AL amyloidosis.
- Histological review and immunohistochemistry to determine amyloid fibril type.
- Dedicated imaging service (echocardiography, cardiac magnetic resonance imaging [MRI] and Technetium–99 m-labelled 3,3-diphosphono-1,2 propanodicarboxylic acid [Tc-DPD]) for evaluation of cardiac amyloidosis.
- Characterization and exclusion of hereditary amyloidosis and periodic fever syndromes, DNA testing and genetic counselling.
- Recommendations for treatment.
- Regular follow-up to evaluate response and requirement for further treatment.
- Specialized proteomic service to determine amyloid fibril type in selected cases.
- Amyloid fibril protein sequencing and characterization in selected cases.
- Providing information and support to amyloidosis patients.
- Systematic evaluation of existing and new treatments including clinical trials.
- Establishing and developing a UK Amyloidosis Network, consisting of physicians and healthcare professionals with a particular interest and expertise in systemic amyloidosis.

Physicians at the NAC offer telephone advice and will arrange specialist laboratory investigations that are not available locally. Some investigations may be performed on peripheral blood samples following discussion.

In the UK there is a move to develop a UK Amyloidosis Network, to include a number of regional centres throughout the UK in which there is expertise in clinical management of patients with systemic amyloidosis. The aim is to develop an expanding network of regional 'Amyloidosis Treatment Centres' characterized by a multi-disciplinary approach that includes input from a haematologist, cardiologist, nephrologist and specialist nurse with expertise in amyloidosis.

Recommendations

- **The NAC provides expert support for the diagnosis, assessment and management of patients with suspected AL amyloidosis and shared care between local clinicians and the NAC is strongly recommended.**
- **Where possible, amyloid patients should be treated in designated regional 'Amyloidosis Treatment Centres' in which there is expertise and multi-disciplinary input into management.**

Systemic AL amyloidosis background

Systemic AL amyloidosis (formerly primary amyloidosis) is a disorder of protein folding in which there is extracellular accumulation as β-pleated fibrillar deposits of monoclonal immunoglobulin light chain fragments (Falk et al. 1997) that ultimately leads to organ failure, most commonly of the kidneys, heart, liver and peripheral nervous system (Kyle & Gertz 1995).

There is considerable clinical heterogeneity in AL amyloidosis due to the variable organ involvement and poor correlation between the amount of amyloid and the degree of impairment of organ function, particularly in the kidneys. Although the natural history of AL amyloidosis is that it is fatal within 2 years in about 80% of patients (Kyle et al. 1999), treatments that reduce the supply of amyloidogenic monoclonal immunoglobulin light chains can result in stabilization or regression of existing amyloid deposits leading to preservation or improvement in the function of organs and improved survival (Gillmore et al. 1997).

Pathophysiology and relationship with other B-cell disorders

AL amyloidosis may be associated with myeloma or other B-cell malignancy, such as Waldenström macroglobulinaemia, but in most cases the underlying plasma cell dyscrasia would be classified as monoclonal gammopathy of undetermined significance (MGUS) if it were not for the presence of amyloid deposition.

A concurrent diagnosis of myeloma or other B-cell malignancy is made at diagnosis in patients with AL amyloidosis when the diagnostic criteria for these conditions are fulfilled. Importantly, the International Myeloma Working Group (2003) and, more recently, the World Health Organization (WHO), included AL amyloidosis as a symptomatic myeloma-defining condition (McKenna et al. 2008).

Although coexistent AL amyloidosis is diagnosed in approximately 10–15% of patients who present with overt myeloma, the AL amyloid deposits demonstrated histologically during the course of investigations in patients with these disorders may not be clinically significant. It is rare for AL amyloidosis to progress to overt myeloma (Rajkumar et al. 1998a), probably because of the short survival of patients with AL amyloidosis. AL amyloidosis can also complicate certain B-cell malignancies, such as Waldenstrom macroglobulinaemia, or indolent lymphomas, such as marginal zone lymphoma.

AL amyloid fibrils are derived from the *N*-terminal region of monoclonal immunoglobulin light chains and consist of the whole or, more usually, just a part of the variable (V_L) domain (molecular weight therefore varies between about 8000 and 30 000 Da). All monoclonal light chains are structurally unique with only a small proportion of monoclonal light chains being amyloidogenic; however, lambda light chains are more commonly associated with amyloid than kappa in approximately a 3:1 ratio. The propensity for certain light chains to form amyloid fibrils is an inherent property related to their particular structure. Once amyloid deposition has started, 'seeding' may occur leading to exponential amyloid accumulation (Booth et al. 1997).

The genetic abnormalities that commonly occur in multiple myeloma and MGUS, such as 14q translocations and 13q deletion, are also a feature of AL amyloidosis. However, the t(11;14) translocation is significantly more common in AL amyloidosis (39–55%) than in myeloma (15%) and, unlike in myeloma, may carry an adverse prognosis (Harrison et al. 2002, Bochtler et al. 2008, Bryce et al. 2009, Bochtler et al. 2011). Hyperdiploidy (Bochtler et al. 2011) and the poor-risk myeloma genetic abnormalities, namely the t(4;14) and deletions of 17p (*TP53*), are rare in AL amyloidosis. Increased frequency of gain of 1q21 may be associated with AL amyloidosis, usually in the context of progression to myeloma (Bochtler et al. 2008). As yet, none of the cytogenetic abnormalities are known to influence the pattern and severity of organ involvement.

Incidence and epidemiology

The incidence of AL amyloidosis, although hard to calculate, is estimated to be ~0·8/100 000 population and the cause of death in ~1 per 1500 deaths in the UK (Pinney et al. 2013). The age-adjusted incidence of AL amyloidosis in the United States is between 5·1 and 12·8 per million persons per year (Kyle et al. 1992), which is equivalent to approximately 600 new cases per year in the UK and the male:female ratio is equal. Among 474 patients seen at the Mayo Clinic (Kyle & Gertz 1995), 60% of patients were between 50 and 70 years old at diagnosis and only 10% were aged under 50 years. Similarly, among 2700 patients with AL amyloidosis who have been evaluated at the UK NAC (NAC), 28% were aged over 70 years at diagnosis, 62% were aged between 50 and 70 years at diagnosis, and 10% were aged under 50 years; 2% were aged under 40 years at diagnosis (NAC database, unpublished data). However, referrals to specialized tertiary centres under-represent the very elderly population, patients with very poor performance status and patients who are reluctant to travel.

Clinical features

The most common clinical features at diagnosis are (Kyle & Gertz 1995):

- Nephrotic syndrome with or without renal insufficiency
- Congestive cardiomyopathy
- Sensorimotor and/or autonomic peripheral neuropathy
- Hepatomegaly

Fatigue and weight loss are extremely common presenting symptoms but the diagnosis of amyloidosis is rarely made until symptoms referable to a particular organ appear. Although AL amyloid deposits generally affect multiple organs, dysfunction of one particular organ often predominates.

Certain patterns of organ involvement (macroglossia) may be pathognomonic of AL amyloidosis; however, the pattern of organ involvement is frequently non-diagnostic and overlaps with other (non-AL) forms of amyloid.

Renal amyloid. Nearly one half of patients have dominant renal amyloid at diagnosis, which is predominantly a glomerular lesion causing nephrotic syndrome. Substantial albuminuria in the context of myeloma, as opposed to isolated Bence Jones proteinuria, should alert the physician to the possibility of AL amyloid. Loss of renal excretory function is common in AL amyloidosis although presentation with progressive renal failure in the absence of substantial proteinuria is rare (Pinney *et al*, 2011). Symptoms include ankle swelling, fatigue, loss of energy, peripheral oedema, pleural effusions and occult pericardial effusions. Orthostatic hypotension may be a feature of autonomic neuropathy and/or cardiac involvement by amyloid but may also result from intravascular volume depletion in association with diuretic treatment for nephrotic syndrome.

Cardiac amyloidosis. About 25% of patients have dominant symptomatic cardiac amyloidosis at diagnosis, which confers a poor prognosis. Abnormalities on electrocardiography (ECG), notably reduced QRS voltages in the standard leads, may precede clinical congestive cardiac failure. The cardio-

myopathy in amyloidosis is restrictive in nature with thickened cardiac walls but often a normal cardiac silhouette on chest X-ray, and the clinical differential diagnosis may include pericardial disease or tamponade.

Clinical signs are mainly of right-sided heart failure (raised jugular venous pressure, right-sided third heart sound, peripheral oedema and hepatomegaly), arrhythmias or signs associated with a low cardiac output, including orthostatic hypotension. In severe cases atrial thrombi may be present despite sinus rhythm (Dubrey et al. 1995) and atrial fibrillation may be associated with an abrupt deterioration in cardiac performance and a high risk of thromboembolism.

Peripheral and autonomic neuropathy. AL polyneuropathy may give rise to a wide range of symptoms and there is frequently a long delay from presentation to diagnosis. Up to 15% of patients present with symptoms of an axonal length-dependent peripheral neuropathy, most commonly a peripheral symmetrical sensory neuropathy with paraesthesiae, numbness, possibly pain and muscle weakness although motor neuropathy is rare (Rajkumar et al. 1998b). Carpal tunnel syndrome is common and may predate other symptoms by over a year.

Autonomic neuropathy is a more serious feature, which typically gives rise to postural hypotension, impotence, weight loss and disturbed gastro-intestinal motility, often in association with some degree of peripheral neuropathy. The clinical manifestations of autonomic disorders are protean and should be specifically sought through enquiry about erectile failure in men, symptoms relating to poor bladder emptying, altered bowel habit, early satiety, anhidrosis or gustatory sweating, and symptoms relating to postural hypotension. The last is confirmed by demonstrating a fall in systolic blood pressure of at least 20 mmHg when a patient has been standing for 3–5 min after spending at least 5 min supine.

Gastrointestinal and hepatic involvement. Involvement of the gastrointestinal tract may be focal or diffuse and symptoms relate to its site and extent. Macroglossia occurs in about 10% of patients and is virtually pathognomonic of AL amyloidosis and can cause airway obstruction, difficulty eating and sleep apnoea. Features of gastrointestinal amyloid include early satiety, diarrhoea, chronic nausea, malabsorption, weight loss, gut perforation and frank rectal bleeding. Certain symptoms, notably early satiety and explosive postprandial diarrhoea, often reflect disturbed gastrointestinal motility due to autonomic neuropathy. Hepatomegaly is present in approximately one quarter of patients at diagnosis and, in the presence of heart failure from amyloid cardiomyopathy, it may not be possible clinically to differentiate hepatic amyloid infiltration from venous congestion.

Haemostatic abnormalities. Haemorrhage is a frequent manifestation of amyloidosis and can be a serious problem. It occurs at some stage during the course of the disease in about one-third of patients, and an abnormal clotting screen is present in about one half (Mumford et al. 2000). The mechanism is often multifactorial but may include vascular fragility as a result of endothelial amyloid deposits and/or loss of vitamin K-dependent clotting factors through binding to amyloid deposits, typically in the spleen, thus leading to a warfarin-like effect (Furie et al. 1981, Choufani et al. 2001). The most common manifestation of bleeding is purpura but life-threatening bleeding is also well described and may follow liver or renal biopsy. Peri-orbital purpura ('raccoon eyes') is particularly characteristic.

Other organ systems. These include the following:

- Skin and soft-tissue thickening
- Painful seronegative arthropathy
- Bone involvement, which is demonstrated by ^{123}I-labelled serum amyloid P component scintigraphy (SAP scan) scan in approximately 30% of patients (but, in contrast to myeloma, bone pain, lytic lesions or pathological fracture are not common). There are no characteristic radiological appearances. Lytic lesions and vertebral collapse may occur, but multiple lytic lesions are suggestive of myeloma. X-rays may be normal even when there is substantial amyloid involvement of bone
- Vocal cord infiltration may produce a hoarse voice, although this is most frequently a manifestation of localized rather than systemic AL amyloidosis or rarely, hereditary apolipoprotein A-I amyloidosis
- Adrenal gland or thyroid infiltration occasionally results in hypoadrenalism or hypothyroidism
- Lymphadenopathy and pulmonary infiltration can be features of systemic or localized AL amyloidosis
- Any organ other than the brain can be involved

Localized AL amyloidosis: background

AL amyloidosis can occur in a localized form that is most often identified in the upper respiratory, urogenital and gastrointestinal tracts, the skin and the orbit. In such circumstances the amyloidogenic light chains are produced by a subtle focal infiltrate of clonal lymphoplasmacytoid cells within the same tissue as the amyloid deposits. This type of amyloid is frequently nodular in character, but can occur quite diffusely throughout a particular tissue when it is associated with a more contiguous infiltrate of clonal cells. The AL nature of localized amyloid can often be confirmed immunohistochemically or by proteomic analysis but it may not be possible to characterize the associated clonal cells due to their scanty nature. Monoclonal immunoglobulin cannot be detected in the serum or urine of most patients with localized AL amyloidosis, even when using highly sensitive assays. The course of the disease is relatively benign in most patients, but severe damage to the affected organ can ultimately occur. Treatment is generally confined to local surgical intervention according to symptoms.

Recommendations

- **Localized AL amyloidosis does occur rarely and if problematic can usually be treated by local resection**

Diagnosis

Systemic AL amyloidosis is clinically heterogeneous with either single organ or multi-system involvement and diagnosis requires a high index of suspicion. AL amyloidosis should be considered in any patient who presents with nephrotic range proteinuria, hepatomegaly, non-dilated cardiomyopathy, peripheral or autonomic neuropathy, whether or not a paraprotein can be detected in the serum or urine. Particular vigilance for development of amyloid related organ dysfunction should be maintained in patients with multiple myeloma or known MGUS. If suspicion of the diagnosis is based on symptoms in one organ system, evidence for involvement by amyloid within other organs should be sought e.g., low voltage ECG, elevated N-terminal pro-brain natriuretic peptide (NT–proBNP) concentration, proteinuria or hepatomegaly. Multiple organ biopsies are potentially hazardous and are not generally recommended because confirmation of amyloid deposits within one organ and a careful search for dysfunction of other organs usually suffice.

Diagnostic investigations

Amyloidosis is a histological diagnosis. Whenever amyloid is identified, investigations to establish the fibril type and the extent of organ involvement should be undertaken (Table I). Immunohistochemical staining for immunoglobulin light chains in AL amyloidosis has only ~60% sensitivity and the presence of a paraprotein does not *per se* confirm a diagnosis of AL amyloidosis. Amyloidosis is therefore often diagnosed as AL type only after exclusion of AA and transthyretin (ATTR) types by immunohistochemistry and hereditary types by genetic sequencing. Importantly, both hereditary and wild-type ATTR (senile systemic) amyloidosis are more common than previously thought and may co-exist with MGUS, which can lead to misdiagnosis (Lachmann et al. 2002). Scintigraphy following injection of radiolabelled SAP or radiolabelled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) may be helpful in typing amyloid given that demonstration of bone marrow involvement by SAP scintigraphy is virtually diagnostic of AL and demonstration of a particular pattern of abnormal uptake, including in the heart, by DPD scintigraphy is strongly suggestive of cardiac ATTR amyloidosis. Immunohistochemical staining of amyloidotic tissue in conjunction with, where necessary, genetic sequencing, should be undertaken in all cases of amyloidosis but, in cases of doubt, should be followed by amyloid fibril sequencing or mass spectrometry (Vrana et al. 2009).

Histology. Amyloid is usually diagnosed by biopsy of an affected organ and staining with the dye Congo red; amyloid deposits produce pathognomonic green birefringence when viewed under cross-polarized light. Alternatively, the diagnosis may be confirmed in suspected cases with 50–80% sensitivity by staining subcutaneous abdominal fat, obtained by a low risk aspiration procedure (Libby et al. 1983), or rectal/labial salivary gland biopsies which are equally low risk (Duston et al. 1987, Kyle & Gertz 1995). Given the relatively low sensitivity of fat pad aspiration, a negative fat pad aspiration does not exclude amyloid and should be followed by biopsy of an affected organ whenever the clinical suspicion remains high. Bone marrow trephine biopsy sections should be stained with Congo red for the presence of amyloid.

Immunohistochemistry. Immunohistochemical typing of amyloid is challenging and is not yet standardized; caution is advised when interpreting results from non–specialized laboratories. Antibodies are available against most known amyloid fibril proteins but definitive results are obtained in <60% of patients with AL amyloid due to the presence of background normal immunoglobulin, and because light chain epitopes that are recognized by antisera to kappa or lambda light chains may be lost during fibril formation and tissue fixation. In contrast, immunohistochemistry in experienced hands can reliably confirm or exclude amyloidosis of AA and TTR types. Identification of the amyloid fibril protein by immunohistochemistry is rarely possible from a fat specimen and renal and/or gastrointestinal biopsies are preferable for this purpose. Immunohistochemical amyloid typing may be improved by staining the same formalin-fixed tissue slide with both antibody and Congo red (Tennent et al. 1999).

DNA analysis. This is principally used to distinguish AL amyloidosis from hereditary forms of amyloidosis. Autosomal dominant hereditary systemic amyloidosis is caused by mutations in the genes for TTR (*TTR*), fibrinogen A α–chain (*FGA*), lysozyme (*LYZ*), apolipoprotein A-I (*APOA1*), apolipoprotein A2 (*APOA2*), gelsolin (*GSN*), cystatin C (*CST3*) and beta-2-microglobulin (*B2M*). A family history of amyloidosis may be absent due to incomplete penetrance. The clinical features in hereditary systemic amyloidosis may be indistinguishable from those in AL amyloidosis. Hereditary ATTR and fibrinogen A α–chain amyloidosis are much more common than previously thought, and 31 of 34 patients in whom hereditary amyloidosis was misdiagnosed as AL amyloidosis in a British series of 350 cases had amyloid of either variant TTR or fibrinogen A α– chain type (Lachmann et al. 2002). Hereditary ATTR amyloidosis presents with polyneuropathy and/or amyloid cardiomyopathy, and there should be a low threshold for sequencing the *TTR* gene in patients with this phenotype. Hereditary fibrinogen A α–chain amyloidosis should be considered in any patient with an exclusively renal presentation and has a distinctive appearance on renal biopsy with extensive glomerular involvement in the absence of significant extra-glomerular amyloid. DNA analysis is available

Table I. Investigations required in ssuspected AL Amyloidosis.

	Confirmation of amyloid	Determination of amyloid type	Evaluation of organ Involvement	Investigation of underlying clonal dyscrasia	Monitoring
Pathology	• Biopsy and Congo red staining of apparently affected organ or screening tissue (e.g. fat aspirate or rectum) • Congo red staining of marrow biopsy	• Immunohistochemical staining of tissue biopsy with a panel of antibodies against amyloid fibril proteins • Proteomic analysis of amyloidotic tissue	• Tissue biopsy of apparently affected organ • Once diagnosis of systemic amyloidosis established, organ biopsies merely to determine extent of amyloid involvement not recommended	• Bone marrow aspirate and biopsy with light chain immunophenotyping	• Follow-up tissue biopsies are not usually helpful • Bone marrow examinations for minimal residual disease may be useful
Haematology/Chemical Pathology/Immunology		• Routine electrophoresis and immunofixation of serum and urine • Quantitative serum FLC assay	• Urea, electrolytes, creatinine, albumin, 24-h urine total protein or spot urine protein/creatinine ratio, liver function tests, coagulation screen, creatinine clearance (measured or calculated), NT-proBNP high sensitivity troponin T	• FBC, urea and electrolytes, creatinine, calcium, albumin • Quantitation of serum and urine paraprotein • Serum FLC assay • Levels of normal immunoglobulins	• Paraprotein level • Serum FLC assay
Imaging	• SAP scanning	• SAP scanning (evidence of marrow involvement)	• SAP scanning • Cardiac magnetic resonance	• Skeletal survey	• SAP scanning
Other	• DPD scanning	• DNA analysis • Proteomic analysis or amyloid fibril sequencing	• ECG; Echocardiogram • Chest X-ray	• FISH, flow cytometry • Lymph node biopsy	• Organ function assessments

SAP, serum amyloid P component; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; FLC, free light chain; NT-proBNP, N-terminal pro-brain natriuretic peptide; ECG, electrocardiography; FBC, full blood count; FISH, fluorescent *in-situ* hybridization.

at the National Health Service (NHS) NAC (database available at www.amyloidosismutations.com)

Amyloid fibril protein identification by sequencing and mass spectrometry. Amyloid fibrils can be isolated from tissue biopsy samples and characterized by amino acid sequencing. This is the only uniformly definitive method for determining the amyloid fibril type and can identify genes associated with hereditary amyloidosis. Mass spectrometry can definitively identify amyloid fibril proteins from formalin–fixed tissue biopsy samples (Rodriguez et al. 2008, Vrana et al. 2009) and laser microdissection with mass spectrometry together enable precise identification of amyloid type in most cases (Vrana *et al.,* 2009). Multidimensional Protein Identification Technology (2-dimensional chromatography coupled to tandem mass spectrometry) may also enable amyloid fibril typing from fat aspirate samples (Brambilla *et al.*, 2012).

Recommendations

- **AL amyloidosis is a disease with insidious onset and considerable clinical heterogeneity. A high level of clinical suspicion is essential to avoid delayed diagnosis.**
- **In suspected AL amyloidosis, a histological diagnosis is essential and, where possible, a biopsy should be taken from an apparently affected organ. Alternatively, a subcutaneous abdominal fat aspirate and bone marrow biopsy may be examined for amyloid.**
- **Congo red staining with classical apple green birefringence under polarized light should be used to test for the presence of amyloid on any histological specimen.**
- **The diagnosis of amyloid requires an experienced laboratory as false negative and false positive diagnoses on the basis of histology are not infrequent. Other (non-AL) amyloid fibril types should be excluded by using immunohistochemistry, DNA analysis, protein sequencing or mass spectrometry.**
- **Multiple tissue biopsies are not recommended and liver biopsies are best avoided, or undertaken via the transjugular route due to the bleeding risk. The bleeding risk of renal biopsies in amyloid patients is similar to that for other renal diseases as long as clotting abnormalities are corrected beforehand.**
- **A comprehensive assessment of the extent of organ involvement and dysfunction should be carried out by non-invasive criteria including serum amyloid P component (SAP) scanning when feasible.**

Evaluation of plasma cell dyscrasia

Relevant investigations are as follows:

Serum free light chain estimation. The free light chain (FLC) assay (Bradwell et al. 2001) gives a positive result (raised level of either kappa or lambda together with an altered ratio of free kappa to free lambda light chain) in 98% of patients with systemic AL amyloidosis, including those in whom a monoclonal immunoglobulin cannot be demonstrated by conventional means (Lachmann et al. 2003). This assay is not specific for AL amyloidosis in that monoclonal FLCs are found in patients with other B-cell malignancies, particularly in the majority of myeloma patients and approximately 50% of MGUS patients. In patients with chronic kidney disease (CKD) the half-life of both kappa and lambda FLCs is substantially prolonged with the absolute FLC concentration increasing 20–fold and, importantly, the range for a normal FLC ratio alters with progressive renal failure (Hutchison et al. 2008). The difference between the amyloidogenic and uninvolved FLC concentration (dFLC) has become recognized as being useful in estimating the 'monoclonal' component and is applicable to patients with renal failure (Dispenzieri et al. 2008, Pinney et al, 2011). It is important to note that 10–15% of AL patients have only minimally abnormal FLC and for these patients FLC cannot be used for accurate monitoring. A difference between the involved and uninvolved FLC of ≥50 mg/l at diagnosis has been defined as being necessary for using changes in dFLC as a disease marker (Palladini et al. 2010a) and this includes about 85% of patients with newly diagnosed systemic AL amyloidosis. The current serum FLC assay is well established and recognized to have some variability. Newer emerging assays for FLC analysis have not been validated. For 15% of patients with minimally abnormal FLC, monitoring the haematological response relies on there being a measurable M-protein, which has been defined as >5 g/l (Palladini et al. 2010a). A minority of patients lack an adequate measurable marker of haematological response.

Serum and urinary protein electrophoresis and immunofixation. A paraprotein is detectable in the serum or urine by routine electrophoresis in approximately 50% of patients with AL amyloidosis. When an intact whole monoclonal immunoglobulin is present in serum the concentration is <10 g/l in 30% of patients, <20 g/l in over 70% of patients and very rarely above 30 g/l (Kyle & Gertz 1995). When patients with myeloma are excluded, fewer than 10% AL amyloidosis patients have a serum paraprotein of >10 g/l (NAC, unpublished data). It is therefore essential to perform immunofixation because the level of paraprotein in AL amyloidosis is usually very low and routine electrophoresis is often negative. However, even on immunofixation, no paraprotein is detectable in serum or urine in ~20% of cases. Urine immunofixation is recommended when screening for AL amyloidosis because rare clonal dyscrasias may be missed using serum tests alone.

Bone marrow aspirate and trephine biopsy.

- Bone marrow aspirate and trephine biopsy in patients with AL amyloidosis are usually reported to be normal or

Table II. Updated non-invasive consensus diagnostic criteria for amyloid–related organ involvement (Gertz et al. 2005, Gertz & Merlini, 2010)*.

Organ involvement	Criteria
Heart	Mean left ventricular wall thickness on echocardiography >12 mm, no other cause found NT-proBNP >39 pmol/l in the absence of renal failure or atrial fibrillation
Kidney	Non-Bence-Jones proteinuria of >0·5 g/24 h
Liver	Hepatomegaly with total liver span >15 cm in the absence of heart failure Or Alkaline phosphatase >1·5 times upper limit of normal
Nerve	Peripheral neuropathy; symmetrical lower extremity sensorimotor 'axonal' peripheral neuropathy Autonomic neuropathy; gastric emptying disorder, pseudo-obstruction, postural hypotension, erectile dysfunction (males), voiding dysfunction unrelated to direct organ infiltration
Gastrointestinal tract	Direct biopsy verification with symptoms
Lung	Direct biopsy verification with symptoms Or Radiographic pattern of interstitial infiltration
Soft tissue	Macroglossia, arthropathy, skin changes, myopathy by biopsy or pseudohypertrophy of muscle, lymphadenopathy, carpal tunnel syndrome

NT-proBNP, N-terminal pro-brain natriuretic peptide.

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*Non-invasive diagnostic criteria of organ involvement are used only in patients in whom a diagnosis of systemic amyloidosis has been made by tissue biopsy.

to show only a small increase in the percentage of plasma cells, unless the patient has overt myeloma. Immunophenotyping should be undertaken to establish clonality whenever small numbers of plasma cells are present as monoclonal plasma cells are detectable in 97% of patients by flow cytometry/immunophenotyping (Paiva *et al.*, 2011). A recent retrospective study of fluorescent *in-situ* hybridization (FISH) cytogenetic profiling on bone marrow biopsy specimens from patients with AL amyloidosis showed abnormal FISH among 81%. Presence of any abnormality on FISH, most frequently a translocation of chromosome 14, was associated with a better prognosis than normal FISH (Warsame et al., 2014). These data require prospective validation. Routine staining of the trephine for amyloid is recommended.

Recommendations

- **Immunofixation of blood and urine and serum FLC should be measured in all patients with suspected AL amyloidosis.**
- **Bone marrow aspirate and trephine biopsy are recommended at diagnosis. As well as estimating the plasma cell infiltrate (or confirming a diagnosis of Waldenstrom macroglobulinaemia), a bone marrow trephine biopsy may be useful in confirming amyloidosis.**

Differential diagnosis

The possibility of the following alternative diagnoses should be considered in all patients with biopsy-proven amyloidosis:

- Systemic non-AL amyloidosis including senile systemic amyloidosis (wild-type ATTR amyloidosis), hereditary amyloidosis and AA amyloidosis. Note that patients with AA amyloidosis may not have an overt inflammatory disorder, and that non-AL amyloidosis may co-exist with MGUS.
- Localized AL amyloidosis

The possibility of the following alternative diagnoses should be considered in all patients with suspected amyloidosis who have a proven plasma cell dyscrasia:

- Other paraprotein-associated diseases including peripheral neuropathy and monoclonal immunoglobulin deposition diseases

Evaluation of organ involvement

Once a diagnosis of AL amyloidosis has been established, investigations are required to evaluate the extent and severity of organ involvement, along with further evaluation of the underlying monoclonal plasma cell dyscrasia to exclude a diagnosis of myeloma or other lymphoid malignancy. Uniform criteria for amyloid organ involvement were defined in 2005 and now form the basis for data collection and reporting, including clinical trials (Gertz et al. 2005). The criteria were recently updated and the updated criteria are shown in Table II (Gertz & Merlini 2010).

Serological and urinary investigations

Frequent laboratory findings in patients with AL amyloidosis include glomerular proteinuria (predominantly albuminuria)

in ~75% of patients and impairment of renal excretory function. Liver function test abnormalities are unusual until liver amyloidosis is substantial, and are most commonly obstructive in nature. Right–sided heart failure due to amyloid cardiomyopathy may cause obstructive liver function tests in the absence of liver involvement by amyloid. Anaemia is uncommon unless amyloidosis is associated with myeloma, bleeding or chronic kidney disease. An abnormal clotting screen is relatively common; a prolonged thrombin time is the most frequent abnormality, but is of no clinical consequence. A prolonged prothrombin time is the only coagulation abnormality associated with bleeding (Mumford et al. 2000).

Elevation of NT–proBNP and cardiac troponin T (TnT) concentrations are seen in a wide variety of cardiac conditions and in chronic kidney disease. However, significant myocardial AL amyloidosis is almost completely excluded by an NT–proBNP concentration of <30 pmol/l (NAC, unpublished data; Palladini et al. 2003).

SAP scintigraphy

This investigation is available at the NHS NAC, and is performed routinely in patients who are referred for evaluation of proven or suspected amyloidosis. Amyloid fibrils associate *in vivo* with the normal plasma protein SAP, and this phenomenon is the basis for the use of SAP scintigraphy for imaging and monitoring amyloid deposits (Hawkins et al. 1990). Radiolabelled SAP localizes rapidly and specifically to amyloid deposits in proportion to the quantity of amyloid present. This allows diagnosis and quantification of deposits by whole body scintigraphy although cardiac and nerve amyloid is poorly visualized (Hawkins et al. 1990, Hawkins 2002). It is useful in assessing the extent and distribution of organ involvement by amyloid, and for evaluating the effects of treatment and it is recommended that it be performed in all patients when feasible. It can also be used as supporting evidence for a diagnosis of amyloidosis when tissue biopsy is not possible.

Cardiac involvement

ECG and echocardiography. Cardiac amyloid is poorly visualized by SAP scintigraphy but ECG and echocardiography provide essential information about the extent of involvement, cardiac function and prognosis. Accurate interpretation of echocardiography is very operator-dependent in this context given the rarity of AL amyloidosis. Characteristic features of cardiac AL amyloidosis on ECG include reduced QRS voltages and a pattern suggestive of myocardial infarction without evidence of a regional wall motion abnormality on echocardiography. The echocardiographic features of amyloid include concentrically thickened ventricular walls with normal or small ventricular cavities, thickened valves and dilated atria. The ejection fraction is frequently normal, although Doppler flow studies and strain imaging may show a reduction in systolic strain in early stage cardiac amyloidosis (Porciani et al. 2009). There is a poor correlation between echocardiographic and ECG findings, one or other of which may sometimes appear normal in the presence of clinically significant cardiac amyloidosis. Mild diastolic dysfunction is common in the elderly population and in advanced chronic kidney disease however, and it may be difficult to determine whether a patient with AL amyloidosis has cardiac involvement. The value of the use of NT–proBNP and cardiac TnT in conjunction with echocardiography in this context has not been determined. The consensus criteria for defining cardiac involvement in AL amyloidosis in the absence of an endomyocardial biopsy are a mean wall thickness on echocardiography of >12 mm with no other cardiac cause (Gertz et al. 2005). The New York Heart Association (NYHA) functional classification for patients with cardiac disease may be applicable to patients with cardiac amyloidosis.

Cardiac magnetic resonance imaging (CMR). CMR provides functional and morphological information on cardiac amyloid in a similar way to echocardiography, though the latter is superior for evaluating and quantifying diastolic abnormalities. An advantage of CMR is in myocardial tissue characterization. Amyloidotic myocardium reveals subtle pre-contrast abnormalities (T1, T2) (Hosch et al. 2007, Sparrow et al. 2009), but extravascular contrast agents based on chelated gadolinium provide key information. The appearance of subendocardial late gadolinium enhancement is highly characteristic of cardiac amyloid (Maceira et al. 2005) and correlates with prognosis (Maceira et al. 2008). CMR is especially useful in patients with other causes of left ventricle thickening/hypertrophy because it can differentiate amyloidosis from the effects of hypertension, which may not be possible by routine echocardiography.

Tc-DPD scintigraphy

Whole body scintigraphy 3 h after intravenous injection of Technetium–99 m-labelled 3,3-diphosphono-1,2 propanodicarboxylic acid (^{99}Tc-DPD), a licensed bone tracer which continues to be used in certain European countries although not in the UK, appears to be exquisitely sensitive for detecting cardiac ATTR amyloid deposits (Rapezzi et al. 2008, Rapezzi et al. 2011a, Rapezzi et al. 2011b). Its precise role in the diagnostic algorithm of suspected or proven amyloid remains to be determined.

Chest X-ray

Chest X-ray in patients with pulmonary amyloidosis may show reticulo-nodular shadowing and there may be impaired CO diffusion on pulmonary function testing.

Nerve conduction studies and autonomic function tests

These may be indicated where neuropathy is suspected or present and indicate an axonal sensorimotor neuropathy.

Peripheral nerve biopsy may be required to establish the diagnosis.

Recommendations

- **Multiple tissue biopsies are not recommended and a comprehensive assessment of the extent of organ involvement and dysfunction should be carried out by non-invasive criteria including serum amyloid P component (SAP) scanning when feasible (ideally at NAC). This includes investigations listed in Table I.**
- **Echocardiography (± Cardiac Magnetic Resonance Imaging (CMR)) is recommended to assess amyloid involvement. Given the rarity of the condition, involvement may go unrecognized or not be adequately assessed.**
- **Nerve conduction studies and autonomic function tests should be performed in patients with suspected neuropathy.**

Prognostic factors and staging systems

Prognosis is variable but is generally poor if AL amyloidosis is untreated. Patient survival has steadily improved from a median of 1–2 years in the 1980s and 1990s to more than 5 years in recent series (Kyle & Gertz 1995, Skinner et al. 2004, Wechalekar et al. 2008), probably reflecting a combination of better supportive care and more successful chemotherapeutic strategies. The natural history varies with the extent and nature of organ involvement but only 5–30% of all AL amyloidosis patients survive 10 or more years from the time of diagnosis (Kyle et al. 1999, Merlini & Palladini 2008).

Staging systems and prognostic markers have predominantly focused on cardiac markers or FLC (Dispenzieri et al. 2004, Palladini et al. 2010b, Kumar et al. 2011). The most widely used staging system for AL amyloidosis to date is based on the biomarkers NT-proBNP and TnT (Dispenzieri et al. 2004).

The stages of disease and relation to prognosis are as follows:

- Stage I: Both NT-proBNP <39 pmol/l AND TnT <0·035 μg/l. Median survival 26·4 months
- Stage II: Either NT-proBNP >39 pmol/l OR TnT >0·035 μg/l. Median survival 10·5 months
- Stage III: Both NT-proBNP >39 pmol/l AND TnT >0·035 μg/l. Median survival 3·5 months

More recently, a staging system incorporating both cardiac biomarkers and dFLCin a cohort of over 750 patients, identified 4 prognostic stages based on a scoring system that scored 1 for the presence of each of the following three prognostic variables (TnT >0·025 μg/l, NT-proBNP >213 pmol/l and dFLC >180 mg/l)(Kumar et al. 2012). There is a need to standardize and prospectively validate novel scoring systems internationally, particularly in defining cardiac biomarker cut-offs. Nonetheless, the staging system proposed by Dispenzieri *et al.* (2004), detailed above, remains the validated 'gold standard' at present.

As well as the above serum markers, a poor prognosis is associated with:

- Symptomatic or substantial echocardiographic evidence of cardiac amyloid; this is associated with a median survival of only ~6 months (Kyle et al. 1986)
- A large whole body amyloid load on SAP scintigraphy and evidence of accumulation of amyloid on serial SAP scans (Lachmann et al. 2003)
- Autonomic neuropathy (Rajkumar et al. 1998b)
- Liver involvement with hyperbilirubinaemia (Lovat et al. 1998)
- Lack of suppression of underlying clonal disease by chemotherapy (Lachmann et al. 2003)
- Associated multiple myeloma (Abraham et al. 2003)
- Normal FISH cytogenetic profile on bone marrow biopsy specimen (Warsame et al., 2014)

A better prognosis is associated with:

- Proteinuria or peripheral neuropathy (without autonomic neuropathy) as the dominant clinical feature (Kyle & Gertz 1995)
- Substantial suppression of underlying clonal disease by chemotherapy (Lachmann et al. 2003, Dispenzieri et al. 2004, Pinney et al, 2011)
- Regression of amyloid deposits on serial SAP scintigraphy (Lachmann et al. 2003)

Selection of appropriate chemotherapeutic treatment regimens in AL amyloidosis depends on age, general wellbeing and extent of amyloidotic organ involvement. Details are provided in the 2014 AL amyloidosis Treatment Guideline (Wechalekar *et al.* 2014).

Clinical presentation and outcome in AL amyloidosis is influenced by the specific immunoglobulin light chain variable region germline gene usage of the clonal B cells (Comenzo et al. 2001, Abraham et al. 2003).

Recommendations

- **The cardiac biomarkers, NT-proBNP and Troponin T and serum FLC assessments form the basis of current prognostic scoring systems and can identify a particularly poor risk group of patients.**

Disclaimer

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, the British Society of Haematology, the UK Myeloma Forum nor the publishers accept any legal responsibility for the content of these guidelines.

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