

American Society of Hematology (ASH) 2026 Guidelines on Diagnosis of Light Chain Amyloidosis

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Abstract:

Light chain (AL) amyloidosis is a rare disease caused by the misfolding of immunoglobulin light chains produced by a plasma cell dyscrasia. Patients can present with involvement of many organs, including the heart, kidneys, liver, nerves and other sites, resulting in a high symptom burden. Timely and accurate diagnosis is important to prevent organ failure and improve patient survival. These evidence-based guidelines of the American Society of Hematology (ASH) are intended to support patients and clinicians in the timely and effective diagnosis of AL amyloidosis. ASH formed a multidisciplinary guideline panel that included 22 individuals representing various medical specialties (academic/community hematology, nephrology, neurology, cardiology, internal medicine and pathology) as well as one patient representative. The group was balanced to minimize potential bias from conflicts of interest. Kansas University Medical Center supported the guideline development process, including updating or performing systematic evidence reviews (up to 6th of March 2023). The panel prioritized clinical questions and outcomes according to their importance for clinicians and patients. The panel used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, including GRADE Evidence-to-Decision frameworks, to assess evidence and make recommendations, which were subject to public comment. The panel agreed on 12 recommendations. The use of serum immunofixation, urine immunofixation and serum free light chains enhances the clinical suspicion of AL amyloidosis. The diagnosis of AL amyloidosis can be made effectively through surrogate biopsies which require both a bone marrow biopsy and fat pad sampling. However, target organ biopsies may be favoured in certain clinical situations.

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American Society of Hematology (ASH) 2026

Guidelines on Diagnosis of Light Chain

Amyloidosis

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Abstract

Background: Light chain (AL) amyloidosis is a rare disease caused by the misfolding of immunoglobulin light chains produced by a plasma cell dyscrasia. Patients can present with involvement of many organs, including the heart, kidneys, liver, nerves and other sites, resulting in a high symptom burden. Timely and accurate diagnosis is important to prevent organ failure and improve patient survival.

Objective: These evidence-based guidelines of the American Society of Hematology (ASH) are intended to support patients and clinicians in the timely and effective diagnosis of AL amyloidosis.

Methods: ASH formed a multidisciplinary guideline panel that included 22 individuals representing various medical specialties (academic and community hematology, nephrology, neurology, cardiology, internal medicine and pathology) as well as one patient representative. The group was balanced to minimize potential bias from conflicts of interest. Kansas University Medical Center supported the guideline development process, including updating or performing systematic evidence reviews (up to 6th

of March 2023). The panel prioritized clinical questions and outcomes according to their importance for clinicians and patients. The panel used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, including GRADE Evidence-to-Decision frameworks, to assess evidence and make recommendations, which were subject to public comment.

Results: The panel agreed on 12 recommendations.

Conclusions: The use of serum immunofixation, urine immunofixation and serum free light chains enhances the clinical suspicion of AL amyloidosis. The diagnosis of AL amyloidosis can be made effectively through surrogate biopsies which require both a bone marrow biopsy and fat pad sampling. However, target organ biopsies may be favoured in certain clinical situations.

Summary of recommendations

These guidelines are based on original systematic reviews of evidence conducted under the direction of the Evidence-Based Practice and Impact Center at the University of Kansas Health System. The panel followed best practice for guideline development recommended by the Institute of Medicine and the Guidelines International Network (GIN).¹⁻³ The panel used the GRADE approach⁴⁻⁸ to assess the certainty of the evidence and formulate recommendations.

Light chain (AL) amyloidosis is a rare plasma cell dyscrasia which remains challenging to diagnose. Patients have high symptom burden and poor quality of life. It is common for AL amyloidosis to involve multiple organ systems. Goals of treatment are to prevent organ failure and improve quality of life and survival for patients. Diagnostic guidelines are essential to help clinicians make timely and effective diagnoses. The American Society of Hematology undertook evidence-based guidelines for AL amyloidosis with a focus on enhancing clinical suspicion, making a diagnosis and defining organ involvement.

Interpretation of strong and conditional recommendations

The strength of a recommendation is expressed as either strong ("the guideline panel recommends..."), or conditional ("the guideline panel suggests...") and has the following interpretation:

Strong recommendation

- For patients: most individuals in this situation would want the recommended course of action, and only a small proportion would not.
- For clinicians: most individuals should follow the recommended course of action. Formal decision aids are not likely to be needed to help individual patients make decisions consistent with their values and preferences.
- For policy makers: the recommendation can be adopted as policy in most situations. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator.
- For researchers: the recommendation is supported by credible research or other convincing judgments that make additional research unlikely to alter the recommendation. On occasion, a

strong recommendation is based on low or very low certainty of the evidence. In such instances, further research may provide important information that alters the recommendations.

Conditional recommendation

- For patients: the majority of individuals in this situation would want the suggested course of action, but many would not. Decision aids may be useful in helping patients to make decisions consistent with their individual risks, values and preferences.
- For clinicians: recognize that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences. Decision aids may be useful in helping individuals to make decisions consistent with their individual risks, values and preferences.
- For policy makers: policymaking will require substantial debate and involvement of various stakeholders. Performance measures about the suggested course of action should focus on if an appropriate decision-making process is duly documented.
- For researchers: this recommendation is likely to be strengthened (for future updates or adaptation) by additional research. An evaluation of the conditions and criteria (and the related judgments, research evidence, and additional considerations) that determined the conditional (rather than strong) recommendation will help identify possible research gaps.

Interpretation of good practice statements

As described by the GRADE Guidance Group, good practice statements endorse interventions or practices that the guideline panel agreed have unequivocal net benefit yet may not be widely recognized or used.⁹ Good practice statements in these guidelines are not based on a systematic review of available evidence. Nevertheless, they may be interpreted as strong recommendations.

Recommendations

Enhancing Clinical Suspicion

Recommendation 1. For individuals with suspected cardiac amyloidosis, the ASH Guideline Panel *recommends* the use of serum and urine immunofixation (SIFE and UIF) and serum free light chain (sFLC) assay to increase clinical suspicion of cardiac AL amyloidosis. (strong

recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○).

Remark:

- Clinical suspicion is enhanced if any one of the proposed tests (SIFE, UIFE, sFLC assay) is positive for monoclonal gammopathy.

Good practice statement. Serum and Urine protein electrophoresis should not be the screening tests or only tests for suspected or confirmed AL amyloidosis. A combination of electrophoresis and immunofixation on serum and urine along with serum immunoglobulin free light chains testing are required.

Recommendation 2. For individuals with unexplained proteinuria, the ASH Guideline Panel *suggests* performing paraprotein testing (SIFE/UIFE/sFLC) to increase clinical suspicion of AL amyloidosis (conditional recommendation based on low certainty in the evidence about effects ⊕⊕○○).

Remarks:

- Clinical suspicion is enhanced if any one of the proposed tests (SIFE, UIFE, sFLC assay) is positive for monoclonal gammopathy.
- Urine IFE should be performed on a 24-hour urine collection or a random sample, the latter preferably from an early morning first void as this would be the most concentrated specimen for detection of a urine monoclonal protein. Of note, 24-hour urine collection is needed for assessment of proteinuria/albuminuria for renal involvement from AL amyloidosis. sFLC values should be interpreted with caution in patients with renal impairment and autoimmune conditions. Monoclonal protein testing may also help to increase the suspicion of other kidney diseases associated with monoclonal gammopathy beyond AL amyloidosis.

Good practice statement. Serum and Urine protein electrophoresis should not be the screening tests or only tests for suspected or confirmed AL amyloidosis. A combination of electrophoresis and immunofixation on serum and urine along with serum immunoglobulin free light chains testing are required.

Recommendation 3. For individuals with positivity in any of the following studies SIFE, UIF, or sFLC, and abnormal cardiac biomarkers, and non-diagnostic echocardiography, the ASH Guideline Panel *suggests* performing cardiac magnetic resonance (CMR) rather than not performing CMR to increase clinical suspicion of cardiac amyloidosis. (conditional recommendation based on a moderate certainty in the evidence about effects ⊕⊕⊕○).

Remark: Diagnostic echocardiographic findings that increase clinical suspicion for cardiac amyloidosis include unexplained left ventricular hypertrophy, reduced left ventricular global longitudinal strain (especially when accompanied by an apical sparing pattern), myocardium with a sparkling appearance, worsening degrees of diastolic dysfunction, elevation in left ventricular filling pressures, elevations in right heart pressures, and presence of varying degrees of pericardial effusion.

Remark: In this clinical scenario to enhance clinical suspicion, an abnormal cardiac biomarker is defined as any one or combination of the NT pro-BNP, BNP, troponin (hs, I, or T) above the reference range for that laboratory test.

Recommendation 4. For individuals with positivity in any of the following studies SIFE, UIF, or sFLC, and abnormal cardiac biomarkers, and echocardiography consistent with amyloidosis, the ASH Guideline Panel *suggests against* performing cardiac magnetic resonance (CMR) and instead performing tissue biopsy to diagnose cardiac AL amyloidosis (conditional recommendation based on a very low certainty in the evidence about effects ⊕○○○).

Remarks:

- In individuals with high suspicion for cardiac amyloidosis, the panel advises proceeding to tissue biopsy without delay.
- Diagnostic echocardiographic findings that increase clinical suspicion for cardiac amyloidosis include unexplained left ventricular hypertrophy, reduced left ventricular global longitudinal strain (especially when accompanied by an apical sparing pattern), myocardium with a sparkling appearance, worsening degrees of diastolic dysfunction,

elevation in left ventricular filling pressures, elevations in right heart pressures, and presence of varying degrees of pericardial effusion.

- **Remark:** In this clinical scenario to enhance clinical suspicion, an abnormal cardiac biomarker is defined as any one or combination of the NT pro-BNP, BNP, troponin (hs, I, or T) above the reference range for that laboratory test.

Diagnosis

Recommendation 5. For individuals with a suspicion of AL amyloidosis, the ASH Guideline Panel *recommends against* the use of bone scintigraphy (PYP, DPD, HMDP) for the diagnosis of AL cardiac amyloidosis (strong recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○). **Remark:** Clinical suspicion for AL cardiac amyloidosis is increased in individuals with positive biomarkers (NT-proBNP, BNP, high sensitivity troponin), imaging (echocardiography/cardiac MRI compatible with cardiac amyloidosis), and immunological assays (serum free light chains, serum/urine immunofixation) indicating the presence of a monoclonal gammopathy.

Recommendation 6. For patients without evidence of a plasma cell disorder (normal serum free light chain levels and no monoclonal proteins on serum and urine immunofixation) and suspicion of cardiac amyloidosis, the ASH Guideline Panel *recommends* the use of bone scintigraphy (PYP, DPD, HMDP) for the diagnosis of Cardiac ATTR amyloidosis (strong recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○).

Remarks:

- In order to improve diagnostic accuracy, bone scintigraphy should be performed with single photon emission tomography (SPECT) rather than planar only imaging. SPECT is needed to verify that the tracer uptake is in the myocardium and not limited to the blood pool. In patients with suspected or confirmed ATTR amyloidosis, family history needs to be assessed. However, before or once diagnosis is confirmed, genetic

counselling should be offered regardless of family history since family history is frequently missing in late onset hereditary ATTRv amyloidosis.

Recommendation 7. For individuals with suspected AL cardiac amyloidosis with abnormal cardiac biomarkers, diagnostic echocardiogram, and positivity in any of the following studies: SIFE, UIFE, or sFLC, the ASH Guideline Panel *suggests either* starting with performing both fat pad sampling and bone marrow biopsy *or* with endomyocardial biopsy. (conditional recommendation based on low certainty in the evidence about effects

⊕⊕○○)

Remarks:

- The panel recognizes that a bone marrow biopsy should not be performed in isolation, without immunological assays and cardiac biomarkers. Furthermore, the panel does not suggest delaying performing other testing, including target organ biopsies if clinically indicated, while awaiting results of the bone marrow biopsy.
- In cases of suspected cardiac AL amyloidosis, it is imperative to obtain diagnostic tissue biopsy with a validated subtyping method, whether through fat pad sampling or endomyocardial biopsy.
- In certain cases, endomyocardial biopsy may be favored over fat pad sampling, such as in patients with risk factors for hereditary cardiac ATTR amyloidosis.
- Proceeding directly to an endomyocardial biopsy relies on ease of access and available expertise..
- Diagnostic echocardiographic findings that increase clinical suspicion for cardiac amyloidosis include unexplained left ventricular hypertrophy, reduced left ventricular global longitudinal strain (especially when accompanied by an apical sparing pattern), myocardium with a sparkling appearance, worsening degrees of diastolic dysfunction, elevation in left ventricular filling pressures, elevations in right heart pressures, and presence of varying degrees of pericardial effusion.

- In this clinical scenario to enhance clinical suspicion, an abnormal cardiac biomarker is defined as any one or combination of the NT pro-BNP, BNP, troponin (hs, I, or T) above the reference range for that laboratory test.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Good practice statement. The ASH panel agreed that in individuals with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be performed.

Recommendation 8. For individuals with suspected light chain renal amyloidosis and positivity in any of the following studies SIFE, UIFE, or sFLC, the ASH Guideline Panel *suggests* starting with performing both abdominal fat pad sampling and bone marrow biopsy over renal biopsy. (conditional recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○)

Remarks:

- The panel recognizes that a bone marrow biopsy should not be performed in isolation, without immunological assays and cardiac biomarkers. Furthermore, the panel does not suggest delaying performing other testing, including target organ biopsies if clinically indicated, while awaiting results of the bone marrow biops
- A positive abdominal fat pad sampling or bone marrow biopsy with subtyping makes renal biopsy unnecessary. However, in the case of a negative fat pad sampling and bone marrow biopsy, a renal biopsy is necessary to look for amyloidosis or other pathology.

- If there is a high clinical suspicion for other renal pathology which can mimic a presentation similar to renal amyloidosis or other plasma cell disorders, then an upfront renal biopsy is preferred.
- If subtyping of the abdominal fat pad is not feasible, the patient should be referred for a renal biopsy.
- The panel placed a high value on safety; however, despite the procedure-related risks, it remains important to perform a renal biopsy when appropriate.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Good practice statement. The ASH panel agreed that in individuals with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be performed.

Recommendation 9. For individuals with a monoclonal gammopathy and generalized small or large fiber peripheral neuropathy or autonomic neuropathy suspected of having AL amyloidosis, the ASH Guideline Panel *suggests* performing both fat pad sampling and bone marrow biopsy over nerve biopsy (conditional recommendation based on very low certainty in the evidence about effects ⊕○○○)

Remarks:

- The panel recognizes that a bone marrow biopsy should not be performed in isolation, without immunological assays and cardiac biomarkers. Furthermore, the panel does not suggest delaying performing other testing, including target organ biopsies if clinically indicated, while awaiting results of the bone marrow biopsy.

- In cases with high clinical suspicion, if abdominal fat pad sampling and bone marrow biopsy yield negative results for AL amyloidosis, a nerve biopsy or other organ specific biopsy should be performed.
- If there is a high clinical suspicion for other nerve pathologies which can mimic presentations similar to nerve amyloidosis or other plasma cell disorders, then an upfront nerve biopsy can be considered.
- If a nerve biopsy is performed, the panel recommends ensuring that that technique and handling is optimized to allow for an adequate diagnostic yield.
- The neuropathy observed in AL amyloidosis strongly resembles diabetic neuropathy, which is more frequent at a population level.
- A skin biopsy is undertaken for assessment of intraepidermal nerve fiber density to confirm a small fiber or autonomic neuropathy, as well as confirmation of congophilic deposits. Such skin biopsies need special handling and referral to a reference center for analysis.

Good practice statement. The ASH panel agreed that in patients with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be performed.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Recommendation 10. For individuals with suspected multiorgan AL amyloidosis, the ASH Guideline Panel *suggests* starting with surrogate biopsies (combination of fat pad sampling

and bone marrow biopsy) over target organ biopsy if surrogate biopsies can be performed expeditiously. If endomyocardial biopsy or renal biopsy are more feasible than fat pad sampling and bone marrow biopsy, these symptomatic target tissues should be preferentially biopsied (conditional recommendation based on low certainty in the evidence about effects ⊕⊕○○)

Remarks:

- Promptly obtaining a tissue biopsy with subtyping is imperative, especially when there is suspected cardiac involvement.
- On repeat biopsies:
 - If surrogate biopsies are negative and clinical suspicion is high for amyloidosis, a target organ should be biopsied.
 - Although not systematically studied, repeat fat pad sampling at an amyloidosis center with greater experience performing such tests may be helpful to reach a diagnosis.
- In patients suspected of having GI amyloid, a more generous and deep (enclosing submucosal layers) biopsy should be obtained.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Good practice statement. The ASH panel agreed that in patients with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be done.

Recommendation 11. For individuals with plasma cell dyscrasias (multiple myeloma and smoldering multiple myeloma), the ASH Guideline Panel *suggests* performing Congo red staining on bone marrow biopsies that may have already been performed (conditional recommendation based on a very low certainty in the evidence about effects ⊕○○○).

Remark:

- Patients with positive Congo Red staining should be re-evaluated for signs and symptoms of systemic AL amyloidosis.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be performed.

Good practice statement. The ASH panel agreed that in individuals with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Organ involvement

Recommendation 12. For individuals with proven AL amyloidosis and with no cardiac symptoms, the ASH Guideline Panel *recommends* performing cardiac biomarkers (high sensitivity troponin, and BNP or NT-proBNP) and cardiac imaging rather than not performing these tests to define the presence and extent of cardiac involvement at diagnosis. (strong recommendation based on low certainty in the evidence about effects ⊕⊕○○)

Remarks:

- The ASH panel recognizes that “cardiac involvement” has historically been defined by echocardiography¹⁰; however, CMR higher diagnostic accuracy with better performance, albeit often limited by cost and availability.
- Echocardiography should be performed with measurement of left ventricular global longitudinal strain to evaluate for reduction in longitudinal shortening and sparing of the apex (sensitivity 72%, may be absent in 28% of patients).

- The panel recognizes that NT-proBNP has been studied more than BNP in AL amyloidosis, but if the former is not available, BNP is an acceptable alternative cardiac biomarker.
- The panel recognizes that cardiac biomarkers (troponin, BNP, NT-pro BNP) results may be affected by kidney function and pre-existing cardiac conditions.
- The panel recognizes that most evidence in amyloidosis used hsTroponin T, but recognizes that some centers may not have access to this test, and other troponin measures that are less extensively evaluated in AL amyloidosis can be used.
- The panel recognizes that a hsTroponin T cut-off of 35 ng/L has a sensitivity of 83% and a specificity of 86% for detecting cardiac involvement. On the other hand, a cut-off of 14 ng/L has a sensitivity of 94% but a specificity of only 11%. Therefore, a 35 ng/L cut-off is more specific, while a 14 ng/L cut-off is more sensitive for cardiac involvement.¹¹
- In patients with known AL amyloidosis, for high hsTroponin T, the recommendation was based on a cutoff of 35 ng/L and a pretest probability of 40% that yields a positive predictive value (PPV) of 0.79 and a negative predictive value (NPV) of 0.88.

Good practice statements

- The ASH panel agreed that it is essential to assess for major organ involvement in patients with confirmed AL amyloidosis, as this guides further management and risk stratification.
- A multidisciplinary team is typically required for the timely and accurate diagnosis and management of AL amyloidosis.

Values and preferences

Values and preferences varied depending on the stage of the diagnostic pathway. For tests that enhanced clinical suspicion, particularly initial tests, greater emphasis was placed on the sensitivity of the test to minimize the number of patients with AL amyloidosis being missed. In these cases, specificity was valued less since subsequent tests would follow. For diagnostic tests, higher value was placed on specificity to prevent patients from receiving unnecessary treatment for conditions they do not have. Timeliness and avoiding delays in diagnosis were considered at every step of the diagnostic pathway.

Explanations and other considerations

The panel used the PICO model to structure our clinical questions (P = Population, patient, or problem; I = Intervention, exposure, or prognostic factor; C = Comparison or control; O = Outcome, which is what you want to measure, improve, or affect). During PICO development, the panel prioritised questions that encompassed the diagnostic pathway of patients with AL amyloidosis, from red flag signs and symptoms to organ involvement. The panel also discussed the numerous surrogate site biopsy options available for clinicians (Table 1). The panel decided to focus on abdominal fat pad samples and bone marrow biopsies as they are the most used. Target organs in systemic amyloidosis were defined as those that are symptomatic, and the underlying condition generated a “red flag” while surrogate biopsy sites are those that, despite frequently being involved by amyloidosis, are typically asymptomatic and easily accessible. Thus, the surrogate biopsy serves as a less invasive substitute for a target organ biopsy. Target organs include the heart, kidneys, liver and peripheral nerves while gastrointestinal (GI) tract luminal involvement may be considered a target or surrogate site, depending on the severity of involvement. Rectal/GI biopsies were considered a surrogate site if patients did not present with GI symptoms. In amyloidosis, red flags are signs and symptoms that herald underlying amyloidosis and thereby may aid in early detection of amyloidosis.^{12,13}

It is important to note that in recent years, it has become apparent that tissues obtained for reasons unrelated to screening for amyloid, may harbor amyloid deposits. To this end, transverse carpal ligament (flexor retinaculum) obtained during carpal tunnel release surgery may show amyloid deposits in patients with systemic amyloidosis. Similarly, ligamentum flavum obtained during surgery for spinal stenosis or prostate or urinary bladder biopsies obtained for screening for cancer may show otherwise unsuspected deposits of amyloid. These sites may be considered fortuitous surrogates. While these specimens may aid early diagnosis of otherwise unsuspected systemic amyloidosis, the clinical significance of such findings is currently under investigation, in particular with regard to ATTR. These biopsies per se are not considered surrogate sites in the strict sense and it is not recommended to perform biopsies of these sites to screen for amyloid purposes¹⁴.

These recommendations focused mainly on AL amyloidosis. The evidence included in the systematic reviews informing the recommendations focused mainly on studies that reported the patients to have AL amyloidosis, with preference on studies that reported subtyping technique. As no limit was set to publication type, studies reporting patients to have primary amyloidosis were included. Other systemic amyloidoses (including hereditary, secondary amyloidosis, ATTR) were excluded from the data synthesis

of all questions except for those that related to cardiac involvement. Detailed inclusion and exclusion criteria can be found in the supplement (supplementary file 1). Additionally, during the evidence synthesis for recommendations on the use of SIFE, UIFE, and sFLC ratio, different studies utilized various different cutoffs to define an abnormal sFLC ratio. As a result of the lack of consensus on what constitutes an abnormal sFLC ratio, the panel took each study for its face validity and did not limit to specific cutoffs. Similarly, for recommendations relying on cardiac biomarkers, in the systematic review process, we included all cutoffs and biomarkers in the literature. Due to the inconsistencies in the reviewed literature, variation in practices, and the impact of kidney dysfunction and other comorbidities on these biomarkers, we were able to provide guidance regarding biomarkers and their cutoffs, as appropriate. In recent years, it has become apparent that tissues obtained for reasons unrelated to screening for amyloid may harbor amyloid deposits. To this end, transverse carpal ligament (flexor retinaculum) obtained during carpal tunnel release surgery may show amyloid deposits in patients with systemic amyloidosis. Similarly, ligamentum flavum obtained during surgery for spinal stenosis, or prostate or urinary bladder biopsies obtained for screening for cancer may show otherwise unsuspected deposits of amyloid. These sites may be considered fortuitous surrogates. While these specimens may aid early diagnosis of otherwise unsuspected systemic amyloidosis, the clinical significance of such findings is currently under investigation, in particular with regard to ATTR amyloidosis. These biopsies are not considered surrogate sites in the strict sense and it is not suggested to perform biopsies of these sites to screen for amyloid purposes.

Fat Pad Sampling

Types of abdominal fat biopsies may include fine needle aspiration biopsy (FNAB) with a thin needle (22 gauge), core biopsy (16-gauge needle), skin punch biopsy (6-8 mm) to include subcutaneous fat or a small surgical (excisional) biopsy; the laboratory analysis of fat pad samples should be validated.¹⁵⁻²²

The ASH panel emphasizes that abdominal fat sampling should be optimal to allow for subtyping using a validated method. Optimal sampling means that **enough** tissue is harvested to carry out amyloid subtyping. While, in some experienced centers, this can be achieved with FNAB, in most instances, fat biopsy is preferred over aspiration.¹⁵⁻²¹

Introduction

Aim of these guidelines and specific objectives

The purpose of these guidelines is to provide evidence-based recommendations of the diagnosis of AL amyloidosis. The primary goals of these guidelines are to review, critically appraise and implement evidence-based recommendations that will enhance early detection, timeliness and accuracy of diagnosis of AL amyloidosis. Through improved provider and patient education of the available evidence and creation of evidence-based recommendations, these guidelines aim to provide clinical decision support that will result in clear diagnostic decision making with known potential outcomes and enable timely diagnosis of AL amyloidosis by multidisciplinary teams.

The target audience includes patients, community and academic hematologists, general practitioners, internists, nephrologists, cardiologists, neurologists, pathologists and other clinicians as well as decision-makers. Policy makers interested in these guidelines include those involved in developing local, national or international plans with the goal to accurately diagnose AL amyloidosis in the shortest timeline. This document may also serve as the basis for adaptation by local, regional or national guideline panels.

Description of the health problem

The amyloidoses are a group of rare disorders caused by protein misfolding, leading to a complex pathway of deposition of insoluble abnormal amyloid fibrils in various organs.²³ This accumulation can lead to an altered function of the tissue involved. The term amyloid was noted in the 19th century with disease classification systems starting in the 1970s at which time the terms such as primary, secondary amyloidosis and hereditary amyloidosis began to be adopted. Amyloidosis syndromes are now much better understood, and the International Society of Amyloidosis has standardized the naming convention for amyloidosis.²⁴ Following A which stands for “amyloidosis”, current nomenclature is based on the protein deposited in the amyloid fibrils i.e. AL for light chain amyloidosis. There have been 42 different kinds of amyloid fibril proteins identified in humans; some are found in a systemic presentation while others are seen in a localized presentation. The most common types of amyloidosis are light chain amyloidosis (AL), Serum Amyloid A (SAA) protein associated amyloidosis (AA), and

transthyretin (TTR) amyloidosis (ATTR). ATTR exists in wild type and variant forms. More recently the International Consensus Classification (ICC) project has suggested that primary AL amyloidosis be referred to as Ig AL amyloidosis when systemic and an “amyloid tumor” when it exists as localized AL amyloidosis.²⁵ For this guideline, the panel has continued with the ISA nomenclature of AL amyloidosis as this terminology is most frequently used in published literature.

AL amyloidosis is a plasma cell dyscrasia which occurs when clonal plasma cells produce excess light chains that misfold and deposit in various organs as amyloid fibrils. AL amyloidosis is a rare disease, with an incidence of 5.1 to 12.8 cases per million person-years. Based on health insurance claims data, the prevalence of amyloidosis is 40.5 cases per million; this represents an increasing prevalence over time.

^{11,26} Amyloid deposits can be found in various organs but most commonly in the heart, kidneys, GI tract and peripheral nerves. Symptoms can be non-specific such as fatigue and weight loss or there can be organ related symptoms such as shortness of breath, edema, and sensory changes. This condition can be associated with significant morbidity and mortality, especially with late diagnoses.

For AL amyloidosis, the diagnosis is confirmed by tissue analysis. Patients are then staged, and risk stratified through imaging and laboratory investigations. Together this helps to prognosticate using various AL staging classification systems. After diagnosis and staging, the aim is to initiate therapy as soon as possible. First line treatment includes agents that target the plasma cell clone (proteasome inhibitors with monoclonal antibodies i.e. D-VCD, and in some cases high dose chemotherapy with autologous hematopoietic cell transplantation). There are several practice guidelines highlighting strategies for treatment of AL amyloidosis, including the need for supportive care.²⁷⁻²⁹ Clinical outcomes have improved over the decades but early death (< 6 months) in high-risk cardiac amyloidosis patients still represents a major challenge.^{30,31} Retrospective studies demonstrate that overall survival is through 43% at 5 years after diagnosis and drops to 27% at 10 years after diagnosis.³²

Delays in diagnosis, often spanning months to greater than 2 years, are common in AL amyloidosis.³³⁻³⁶ Such delays in the diagnostic journey can relate to complexity of symptoms and overlap with many other conditions. Often the diagnosis of AL amyloidosis is not considered. Patients are frequently seen by many health care providers of varying specialties before a diagnosis is made. Patient outcomes are affected negatively by diagnostic delays, particularly in patients with cardiac involvement.³⁷ This highlights the need to increase awareness of symptoms associated with AL amyloidosis and for clear diagnostic pathways for this disease.

The American Society of Hematology (ASH) has recognized the need for evidence-based guidelines for a diagnostic pathway to facilitate timely and accurate diagnosis of AL amyloidosis. There are data showing a benefit of such guidelines in rare diseases to help hematologists and other specialists who encounter such patients infrequently.^{38,39} The panel first defined the optimal diagnostic pathway of relevance for AL amyloidosis including: 1) enhancing clinical suspicion, 2) diagnosis and 3) organ involvement (Figure 1). Also “red flag” signs and symptoms which would increase the suspicion of AL amyloidosis were defined to enhance awareness and entry into a diagnostic pathway.^{12,40} The panel explored relevant questions for systematic review which were distributed throughout the phases of this pathway. As the diagnosis typically depends on multidisciplinary input with a recognition that the hematologist may not be the first person to see such patients, the panel consisted of a wide variety of medical specialists. The panel also recognized that models of care may vary from centralized systems at academic centers to decentralized systems in community practice. Recommendations were considered with these nuances in mind. At the outset, the panel developed recommendations that are more relevant to high income countries in the hope that these recommendations can then be adapted by organizations and clinicians in more resource constrained environments.

Methods

The guideline panel developed and graded the recommendations and assessed the certainty of the supporting evidence following the GRADE approach.^{4,7,41-43} The overall guideline development process, including funding of the work, panel formation, management of conflicts of interest, internal and external review, and organizational approval, was guided by ASH policies and procedures derived from the Guideline International Network (GIN)-McMaster Guideline Development Checklist^{44,45} and was intended to meet recommendations for trustworthy guidelines by the Institute of Medicine and the GIN.^{1-3,46} When limited or no published evidence was available, an expert evidence survey was utilized to collect the panel member’s unpublished observations.⁴⁶

Organization, panel composition, planning and coordination

The work of this panel was coordinated by ASH and the Evidence-Based Practice and Impact Center at the University of Kansas Health System (funded by ASH under a paid agreement). Project oversight was provided by the ASH Guideline Oversight Subcommittee, which reported to the ASH Committee on

Quality. ASH vetted and appointed individuals to the guideline panel. The Evidence-Based Practice and Impact Center at the University of Kansas Health System vetted and retained researchers to conduct systematic reviews of evidence and coordinate the guideline development process including the use of the GRADE approach. The membership of the panels and the systematic review team is described in supplement 2. The panel included hematologists, cardiologists, an internist, a neurologist, a pathologist, and a nephrologist who all had clinical and research expertise on the guideline topic, and one patient representative. One co-chair was a content expert; the other co-chair was an expert in guideline development methodology.

In addition to synthesizing evidence systematically, the Evidence-Based Practice and Impact Center at the University of Kansas Health System supported the guideline development process, including determining methods, preparing meeting materials, and facilitating panel discussions. The panel's work was performed using web-based tools (www.surveymonkey.com and www.gradeapro.org) and face-to-face and online meetings.

Guideline funding and management of conflicts of interest

Development of these guidelines was wholly funded by ASH, a nonprofit medical specialty society that represents hematologists. Most members of the guideline panel were members of ASH. ASH staff supported panel appointments and coordinated meetings but had no role in choosing the guideline questions or determining the recommendations.

Members of the guideline panel received travel reimbursement for attendance at in-person meetings. Through the Evidence-Based Practice and Impact Center at the University of Kansas Health System, some researchers who contributed to the systematic evidence reviews received salary or grant support. Other researchers participated to fulfill requirements of an academic degree or program.

Conflicts of interest of all participants were managed according to ASH policies based on recommendations of the Institute of Medicine⁴⁷ and the Guidelines International Network.³ Participants disclosed all financial and nonfinancial interests relevant to the guideline topic. ASH staff and the ASH Guideline Oversight Subcommittee reviewed the disclosures and composed the guideline panel to include a diversity of expertise and perspectives and avoid a majority of the panel having the same or similar conflicts. Greatest attention was given to direct financial conflicts with for-profit companies that could be directly affected by the guidelines. A majority of the guideline panel, including the co-chairs,

had no such conflicts. None of the Evidence-Based Practice and Impact Center at the University of Kansas Health System researchers who contributed to the systematic evidence reviews or who supported the guideline development process had any such conflicts.

Recusal was used to manage certain conflicts.⁴⁸⁻⁵¹ During deliberations about recommendations, any panel member with a current, direct financial conflict in a commercial entity that marketed any product that could be affected by a specific recommendation participated in discussions about the evidence and clinical context but was recused from making judgments or voting about individual domains (e.g., magnitude of desirable consequences) and the direction and strength of the recommendation. The Evidence-to-Decision framework for each recommendation describes which individuals were recused from making judgments about each recommendation.

Supplement 3 provides the complete disclosure-of-interests forms of all panel members. In part A of the forms, individuals disclosed direct financial interests for 2 years prior to appointment; in part B, indirect financial interests; and in part C, not mainly financial interests. Part D describes new interests disclosed by individuals after appointment. Part E summarizes ASH decisions about which interests were judged to be conflicts and how they were managed, including through recusal.

Supplement 4 provides the complete disclosure of interest forms of researchers who contributed to these guidelines.

Formulating specific clinical questions and determining outcomes of interest

The panel used the GRADEpro Guideline Development Tool (www.grade.org)⁵² and SurveyMonkey (surveymonkey.com) to brainstorm and then prioritize the questions described in Table 2.

The panel selected outcomes of interest for each question a priori, following the approach described in detail elsewhere⁶. In brief, the panel first brainstormed all possible outcomes before rating their relative importance for decision-making following the GRADE approach.⁶

Evidence review and development of recommendations

For each guideline question, the Evidence-Based Practice and Impact Center at the University of Kansas Health System prepared a GRADE “Evidence-to-Decision” (EtD) framework, using the GRADEpro Guideline Development Tool (www.grade-pro.org).⁵² The EtD table summarized the results of systematic reviews of the literature that were updated or performed for this guideline. The EtD table addressed effects of interventions, resource utilization (cost-effectiveness), values and preferences (relative importance of outcomes), equity, acceptability and feasibility. The guideline panel reviewed draft EtD tables before, during or after the guideline panel meeting and made suggestions for corrections and identified missing evidence. To ensure that recent studies were not missed, searches (presented in supplement 4) were updated 6th of March 2023, and panel members were asked to suggest any studies that may have been considered missed and fulfilled the inclusion criteria for the individual questions.

Studies that informed the evidence for the recommendations were based on a predetermined inclusion/exclusion criterion. In general, the panel included studies that included patients with AL amyloidosis that performed the test of interest (index test) and a test to confirm the diagnosis of AL amyloidosis (reference test), which would preferably be the gold standard for diagnosis AL amyloidosis. Studies addressing types of amyloidosis other than AL amyloidosis, such as ATTR localized and AA amyloidosis, were excluded. Evidence assessing amyloidosis with the type unspecified was only included for the evidence on cardiac involvement. For diagnostic tests, during PICO development, sensitivity and specificities were carefully looked at and discussed in the context of the clinical scenario of the PICO, as appropriate. The details of the inclusion/exclusion criteria for the PICO questions will be provided in separate systematic review manuscripts.

Under the direction of the Evidence-Based Practice and Impact Center at the University of Kansas Health System, researchers followed the general methods outlined in the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (handbook.cochrane.org) for conducting updated or new systematic reviews of diagnostic test accuracy. When existing reviews were used, judgments of the original authors about risk of bias were either randomly checked for accuracy and accepted or conducted de novo if they were not available or not reproducible. For new reviews, risk of bias was assessed at the health outcome level using the quality assessment of diagnostic accuracy studies (QUADAS-2) and (QUADAS-C). In addition to conducting systematic reviews of diagnostic test accuracy, the researchers searched for evidence related to baseline risks, values, preferences and costs, and

summarized findings within the EtD frameworks.^{4,8} Subsequently, the certainty of the body of evidence (also known as quality of the evidence or confidence in the estimated effects) was assessed for each effect estimate of the outcomes of interest following the GRADE approach based on the following domains: risk of bias, precision, consistency and magnitude of the estimates of effects, directness of the evidence, risk of publication bias, presence of large effects, dose-response relationship, and an assessment of the effect of residual, opposing confounding. The certainty was categorized into four levels ranging from very low to high.^{5,6,53} Within this report, these categories are represented by the symbols, as follows:

Evidence Certainty	
High Certainty	
	The panel is very confident that the true effect lies close to that of the estimate of the effect.
Moderate Certainty	
	The panel is moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low Certainty	
	Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very Low Certainty	
	The panel has very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Interested readers may find more explanation about the GRADE approach to assessing and rating certainty in a body of evidence in other publications.^{5,6,53} During a three-day in-person meeting followed by online communication and conference calls the panel developed clinical recommendations based on the evidence summarized in the EtD tables. For each recommendation, the panel took a population perspective and came to consensus on the following: the certainty of the evidence, the balance of benefits and harms of the compared test options and the assumptions about the values and preferences associated with the decision. The guideline panel also explicitly considered the extent of resource use associated with alternative management options. The panel agreed on the recommendations (including

direction and strength), remarks, and qualifications by consensus or, in rare instances, by voting (an 80% majority was required for a strong recommendation), based on the balance of all desirable and undesirable consequences. The final guidelines, including recommendations, were reviewed and approved by all members of the panel.

Interpretation of strong and conditional recommendations

The recommendations are labeled as either “strong” or “conditional” according to the GRADE approach. The words “the guideline panel recommends” are used for strong recommendations, and “the guideline panel suggests” for conditional recommendations. Table 3 provides GRADE’s interpretation of strong and conditional recommendations by patients, clinicians, health care policy makers and researchers.

Document review

Draft recommendations were reviewed by all members of the panel, revised, and then made available online on June 10, 2024 for external review by stakeholders including allied organizations, other medical professionals, patients, and the public. Eight individuals and organizations submitted comments. The document was revised to address pertinent comments, but no changes were made to recommendations. On April 9, 2025 the ASH Guideline Oversight Subcommittee and the ASH Committee on Quality approved that the defined guideline development process was followed, and on April 16, 2025 the officers of the ASH Executive Committee approved submission of the guidelines for publication under the imprimatur of ASH. The guidelines were then subjected to peer review by *Blood Advances*.

How to use these guidelines

ASH guidelines are primarily intended to help clinicians make decisions about diagnostic and treatment alternatives. Other purposes are to inform policy, education and advocacy, and to state future research needs. They may also be used by patients. These guidelines are not intended to serve or be construed as a standard of care. Clinicians must make decisions based on the clinical presentation of each individual patient, ideally through a shared process that considers the patient’s values and preferences with respect to the anticipated outcomes of the chosen option. Decisions may be constrained by the realities of a specific clinical setting and local resources, including but not limited to institutional policies, time

limitations, or availability of treatments. These guidelines may not include all appropriate methods of care for the clinical scenarios described. As science advances and new evidence becomes available, recommendations may become outdated. Following these guidelines cannot guarantee successful outcomes. ASH does not warrant or guarantee any products described in these guidelines.

Statements about the underlying values and preferences as well as qualifying remarks accompanying each recommendation are its integral parts and serve to facilitate more accurate interpretation. They should never be omitted when quoting or translating recommendations from these guidelines. Implementation of the guidelines will be facilitated by the related interactive forthcoming decision aids. The use of these guidelines is also facilitated by the links to the EtD frameworks and interactive summary of findings tables in each section.

Recommendations

Overarching Good Practice Statements

1. The ASH panel agreed that it is essential to assess for major organ involvement in patients with confirmed AL amyloidosis, as this guides further management and risk stratification.
2. A multidisciplinary team is typically required for the timely and accurate diagnosis and management of AL amyloidosis.

Enhancing Clinical Suspicion

Should serum and urine immunofixation (SIFE and UIFE) and serum free light chains (sFLC) be used to increase suspicion of AL amyloidosis in individuals with cardiac symptoms?

Recommendation 1. For individuals with suspected cardiac amyloidosis, the ASH Guideline Panel *recommends* the use of serum and urine immunofixation (SIFE and UIFE) and serum free light chain (sFLC) assay to increase clinical suspicion of cardiac AL amyloidosis. (strong recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○).

Remark:

- Clinical suspicion is enhanced if any one of the proposed tests (SIFE, UIFE, sFLC assay) is positive for monoclonal gammopathy.

Good practice statement. Serum and Urine protein electrophoresis should not be the screening tests or only tests for suspected or confirmed AL amyloidosis. A combination of electrophoresis and immunofixation on serum and urine along with serum immunoglobulin free light chains testing are required.

Summary of the evidence

The ASH guideline panel found no systematic reviews that addressed this question. The panel identified 5 cohort and case control studies published after the search for the systematic review was completed. These studies measured sensitivity and specificity of SIFE, UIFE and sFLC measurements to increase suspicion of AL amyloidosis in patients suspected of having cardiac amyloidosis. The panel judged the diagnostic performance of these tests to be very accurate as these tests have high sensitivity (99%) and hence an optimal screening tool for increasing suspicion of AL amyloidosis. The specificity (77%) of these tests is low, especially when considering the general population. However, it is appropriate for differentiating AL and ATTR cardiac amyloidosis in suspected cardiac amyloidosis population. All studies demonstrate the specificity of SIFE/UIFE/sFLC ratio in a specific clinical scenario (AL vs non-AL amyloidosis). The specificity in that clinical scenario may not necessarily reflect the comparison addressed in this question. Therefore, the specificity will not play a major role in decision-making and hence is unlikely to affect the recommendation. These studies did not include the duration between the index and reference tests and not the same reference test was used in all the studies.

Benefits

The guideline panel judged that the desirable effects of performing SIFE/UIFE and sFLC measurements is large to very large. Performing SIFE, UIFE, and sFLC testing in suspected cardiac amyloidosis offers numerous benefits, including high diagnostic sensitivity/specificity, early detection, and decrease in the

time interval between establishment of diagnosis and treatment initiation. Early and accurate diagnosis can decrease mortality.⁵⁴ While false positives (detection of monoclonal protein and not having AL amyloidosis) can be a limitation of these tests, it is important for healthcare providers to carefully consider the risks and benefits of testing, as well as to communicate effectively with patients about the possibility of monoclonal gammopathy of unknown significance and the need for further evaluation if necessary.⁵⁵

Harms and burden

While immunofixation (IFE) and serum-free light chain (sFLC) assay are essential diagnostic tools in the evaluation of suspected cardiac amyloidosis, they do carry small potential harms and burdens. Physical discomfort of a venipuncture and financial costs are minimal and may not be adequately and uniformly covered by the insurance.⁵⁶ False positives can lead to unnecessary anxiety and further invasive testing, while false negatives may delay appropriate diagnosis and treatment.⁵⁷ However, given the available evidence, the guideline panel considered the risk of adverse effects most likely to be small.⁵⁸

Other EtD criteria and considerations

The panel reviewed the certainty of evidence regarding the management effects as high because SIFE/UIFE and sFLC testing directly inform the next steps in managing suspected cardiac amyloidosis. If these tests return positive, the patient would be referred to hematology and/or cardiology for further evaluation. The panel also concluded that there is probably no significant uncertainty or variability in how much key stakeholders value the outcomes of SIFE/UIFE and sFLC testing for cardiac amyloidosis. Patients suspected of having cardiac amyloidosis are often heart failure patients seeking a definitive diagnosis for their symptoms. Therefore, pursuing a diagnostic pathway that provides timely positive or negative results holds significant value for these patients.

Regarding the resources required to conduct these tests, the panel acknowledged that the cost and availability of these tests vary by country. However, they believe that in high-income settings, these tests are readily available and relatively low-cost. Although there is no direct research evidence to support this, the panel determined that implementing SIFE/UIFE and sFLC testing would probably enhance health equity. This is because Black patients with conditions such as ATTR amyloidosis, MGUS, or myeloma are likely to be identified through clinical suspicion, ensuring they receive appropriate diagnostic testing and follow-up care. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for [Recommendation 1](#).**

Conclusions and research needs for this recommendation

The guideline panel determined that there is a high certainty of evidence for a net health benefit from performing SIFE/UIFE and sFLC measurements in patients suspected of having cardiac amyloidosis.

Based on the body of available evidence, it is likely that these tests reduce the risk of delay in diagnosis of AL amyloidosis. There is very low certainty that there is an effect of performing these tests on other outcomes. However, because of low certainty of evidence or no published information about other outcomes, the fact that the panel did not find the evidence of an effect on these outcomes does not imply that such an effect does not exist.

The panel identified the following additional implementation remarks:

1. Laboratories need to be aware that complicating the use of sFLC is the heterogeneity of currently available products. Virtually all the studies on sFLC reported between 2001 and 2011 were performed using the assay from the Binding Site (The Binding Site Group, LTD, Birmingham, United Kingdom). However, in the past few years, new manufacturers have produced FLC assays with different methods, reagents, and reference intervals.

2. The panel does not recommend performing urine free light chain levels for the detection of monoclonal protein.
3. Urine IFE should be performed on a 24-hour urine collection or a random sample preferably early morning first void as this would be the most concentrated specimen for detection of a monoclonal protein in urine. Of note, 24-hour urine collection is needed for assessment of proteinuria/albuminuria for renal involvement from AL amyloidosis.
4. sFLC concentration is greatly affected by kidney function (Table 4). Using a large prospective population-based cohort of iStopMM study, new reference interval for sFLCs in persons with chronic kidney disease (CKD) have been established. Using current reference intervals, 60% and 21% had kappa and lambda sFLC values outside the normal range. The sFLC ratio was outside standard reference interval (0.26–1.65) in 9% of participants and outside current kidney reference interval (0.37–3.10) in 0.7%. Novel reference intervals (based on central 99% reference intervals) were proposed for serum kappa FLC (mg/L), serum lambda FLC (mg/L), and sFLC ratio in individuals with eGFR of 45–59, 30–44, and < 30 mL/min/1.73 m²: One caveat with these new proposed reference ranges is that these were established in a homogenous cohort of White individuals and so therefore may not be fully generalizable to a more diverse population.⁵⁹
5. Monoclonal protein can be found in conditions not related to AL amyloidosis, such as inflammatory and autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus (SLE), and inflammatory bowel diseases), chronic infections (HIV, Hepatitis C), and neurological diseases (CIDP) or in isolation (MGUS).

Discussion

Monoclonal sFLC of each individual patient is unique, which leaves sFLC quantification subject to analytical issues such as inconsistency in linear responses, imprecision or bias due to reagent lot-to-lot

variation there was a clear drift noted towards higher sFLC-ratios over the years. Consequently, in 2022 laboratories worldwide are no longer able to reproduce the defined sFLC ranges in healthy controls as they were published in the seminal paper in 2002. Laboratories may need to update their practices and reference ranges to reflect changes in sFLC distribution and ensure the validity and reliability of sFLC testing.

N-latex assays on Siemens' BNII nephelometric analyzer and assays from the Binding Site are two common approaches for the measurement of sFLCs in clinical practice. Numerous studies have shown that the analytical platform affects sFLC results and warrants the introduction of instrument specific reference ranges.

Should serum and urine immunofixation (SIFE and UIFE) and serum free light chains (sFLC) be used to increase suspicion of AL amyloidosis in individuals with unexplained proteinuria?

Recommendation 2. For individuals with unexplained proteinuria, the ASH Guideline Panel suggests performing paraprotein testing (SIFE/UIFE/sFLC) to increase clinical suspicion of AL amyloidosis (conditional recommendation based on low certainty in the evidence about effects ⊕⊕○○).

Remarks:

- Clinical suspicion is enhanced if any one of the proposed tests (SIFE, UIFE, sFLC assay) is positive for monoclonal gammopathy.
- Urine IFE should be performed on a 24-hour urine collection or a random sample preferably early morning first void as this would be the most concentrated specimen for detection of a monoclonal protein in urine. Of note, 24-hour urine collection is needed for assessment of proteinuria/albuminuria for renal involvement from AL amyloidosis. sFLC values should be interpreted with caution in patients with renal impairment and

autoimmune conditions. Monoclonal protein testing may also help to increase the suspicion of other kidney diseases associated with monoclonal gammopathy.

Good practice statement. Serum and Urine protein electrophoresis should not be the screening tests or only tests for suspected or confirmed AL amyloidosis. A combination of electrophoresis and immunofixation on serum and urine along with serum immunoglobulin free light chains testing are required.

Summary of the evidence

The panel found no systematic reviews that addressed this question. The panel identified 11 cohort and case control studies published after the search for the systematic review was completed. These studies measured sensitivity and specificity of SIFE, UIFL and sFLC measurements to increase suspicion of AL amyloidosis in patients suspected of having renal amyloidosis.

The panel judged the diagnostic performance of the test to be very accurate since the test is used for screening and the well-established high sensitivity of the test makes it an optimal screening tool for increasing suspicion of amyloidosis.⁶⁰

The panel judged that using a specificity of 25% would be appropriate to inform the PICO question. The specificity value was based on Xu et al, 2017⁶⁰ which looked at the diagnostic performance characteristics of sFLC ratio in differentiating different renal monoclonal gammopathies, which was deemed appropriate for the PICO question that is addressing the detection of AL amyloidosis in patients with renal involvement in the form of proteinuria unexplained by other causes.

The panel was aware that the studies included don't differentiate between non-nephrotic range proteinuria and nephrotic range proteinuria.

The certainty of evidence was judged as low since the panel considered the sensitivity and specificity.

The population of this PICO should be patients with unexplained proteinuria suspected to have AL amyloidosis.⁶⁰

Benefits

The panel judged that the desirable effects of performing SIFE/UIFE and sFLC measurements is large, based on the high sensitivity of SIFE, UIFE, and sFLC testing and the indirect effects of increasing clinical suspicion of AL amyloidosis and possibly decreasing the time interval between diagnosis establishment and treatment initiation.⁶¹⁻⁶⁴

While false positives (detection of monoclonal protein and not having AL amyloidosis) can be a limitation of these tests, it is important for healthcare providers to carefully consider the risks and benefits of testing, as well as to communicate effectively with patients about the possibility of monoclonal gammopathy of unknown significance and the need for further evaluation if necessary. These patients may undergo unnecessary additional possibly invasive testing, and there might be an increase in disease-related anxiety in these patients.

Harms and burden

While immunofixation electrophoresis (SIFE) and serum-free light chain (sFLC) assay are essential diagnostic tools in the evaluation of suspected renal amyloidosis, they do carry small potential harms and burdens. Physical discomfort of a venipuncture and financial costs are minimal and may not be adequately and uniformly covered by the insurance. False positives can lead to unnecessary anxiety and further invasive testing, while false negatives may delay appropriate diagnosis and treatment. However, given the available evidence, the panel considered the risk of adverse effects most likely to be small. The undesirable effects were judged to be small and not trivial based on the low specificity and the indirect effect of having patients that are incorrectly categorized to have monoclonal proteins undergoing

unnecessary additional possibly invasive testing, and the possible increase in the disease-related anxiety in these patients.

Other EtD criteria and considerations

The panel judged the certainty of evidence of management of effects to be high because the test will directly inform the next steps in management. The next steps would differ based on the result of the test. If it is positive, the panel recommends a fat pad/bone marrow biopsy over a renal biopsy. The panel mentioned that the differential diagnosis for proteinuria is very broad. The next steps would differ also based on the level of proteinuria. If nephrotic range, the patient may get renal biopsy directly, if not nephrotic range and the IF is positive, this would be followed by a fat pad sampling and bone marrow biopsy. Bone marrow biopsy will have the added benefit of ruling out other plasma cell dyscrasia such as multiple myeloma. However, most patients with unexplained proteinuria should undergo a renal biopsy.

Regarding the resources required to conduct these tests, the panel acknowledged that the cost and availability of these tests vary by country. However, they believe that in high-income settings, these tests are readily available and relatively low-cost. Although there is no direct research evidence to support this, the panel determined that implementing SIFE/UIFE and sFLC testing would probably have no impact on health equity. The panel mentioned that equity may vary across different racial groups and patients with preexisting conditions. It may be decreased in patients with diabetes, as they may have fewer investigations and may lead to missing other renal diagnoses in these patients. Also, the result of the test may affect kidney transplantation decisions. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for [Recommendation 2](#).**

Conclusions and research needs for this recommendation

The guideline panel suggests for the use of SIFE and UIFE and sFLC ratio in individuals with proteinuria unexplained by other preexisting conditions to increase clinical suspicion of AL amyloidosis if any of the three tests is positive.

The panel identified the following additional implementation remarks:

1. Urine IFE should be performed on a 24-hour urine collection or a random sample, preferably early morning first void as this would be the most concentrated specimen for detection of a monoclonal protein in urine. Of note, 24-hour urine collection is needed for assessment of proteinuria/albuminuria for renal involvement from AL amyloidosis.^{61,65-70}
2. The panel highlighted caution when interpreting sFLC values in patients with renal impairment and autoimmune conditions.
3. Monoclonal protein testing may also help to increase the suspicion of other kidney diseases associated with monoclonal gammopathy.

Should cardiac MRI (CMR) be used to diagnose cardiac AL amyloidosis in individuals suspected of having cardiac amyloidosis (positivity in any of the following studies: SIFE, UIFE, or sFLC, abnormal cardiac biomarkers, and non-diagnostic echocardiographic findings)?

Recommendation 3. For individuals with positivity in any of the following studies SIFE, UIFE, or sFLC, and abnormal cardiac biomarkers, in the setting of non-diagnostic echocardiography, the ASH Guideline Panel *suggests* performing cardiac magnetic resonance (CMR) rather than not performing CMR to increase clinical suspicion of cardiac amyloidosis. (conditional recommendation based on a moderate certainty in the evidence about effects ⊕⊕⊕○).

Remark:

- Diagnostic echocardiographic findings that increase clinical suspicion for cardiac amyloidosis include unexplained left ventricular hypertrophy, reduced left ventricular global longitudinal strain (especially when accompanied by an apical sparing pattern), myocardium with a sparkling appearance, worsening degrees of diastolic dysfunction, elevation in left ventricular filling pressures, elevations in right heart pressures, and presence of varying degrees of pericardial effusion.
- In this clinical scenario to enhance clinical suspicion, an abnormal cardiac biomarker is defined as any one or combination of the NT pro-BNP, BNP, troponin (hs, I, or T) above the reference range for that laboratory test.

Should cardiac MRI (CMR) be used to diagnose cardiac AL amyloidosis in individuals with abnormal cardiac biomarkers, echocardiography, and positivity in any of the following studies: SIFE, UIFE, or sFLC?

Recommendation 4. For individuals with positivity in any of the following studies SIFE, UIFE, or sFLC, and abnormal cardiac biomarkers, in the setting of echocardiography consistent with amyloidosis, the ASH Guideline Panel *suggests against* performing cardiac magnetic resonance (CMR) and instead performing tissue biopsy to diagnose cardiac AL amyloidosis (conditional recommendation based on a very low certainty in the evidence about effects ⊕○○○).

Remarks:

- In individuals with high suspicion for cardiac amyloidosis, the ASH guideline panel advises proceeding to tissue biopsy without delay.
- Diagnostic echocardiographic findings that increase clinical suspicion for cardiac amyloidosis include unexplained left ventricular hypertrophy, reduced left ventricular global longitudinal strain (especially when accompanied by an apical sparing pattern), myocardium with a sparkling appearance, worsening degrees of diastolic dysfunction,

elevation in left ventricular filling pressures, elevations in right heart pressures, and presence of varying degrees of pericardial effusion.

- In this clinical scenario to enhance clinical suspicion, an abnormal cardiac biomarker is defined as any one or combination of the NT pro-BNP, BNP, troponin (hs, I, or T) above the reference range for that laboratory test.

Summary of the evidence

Cardiac MRI can be helpful to evaluate the etiology of a cardiomyopathy by looking at patterns in fibrosis detected by late gadolinium enhancement (LGE) or changes in extracellular volume (ECV). Systematic review yielded 7 cohort and case-control studies that examined the diagnostic test accuracy of the finding of LGE on cardiac MRI for diagnosis of cardiac amyloidosis. These studies reported the accuracy of described LGE patterns consistent with cardiac amyloidosis; there were 2 additional studies that reported presence of LGE without specifying the pattern nor distribution, and these studies were excluded. While ECV identified that address the diagnostic test accuracy of ECV using endomyocardial biopsy as a reference. The panel found the diagnostic test accuracy of CMR to be between accurate and very accurate, with a sensitivity of 95% and specificity of 87%.⁷¹⁻⁷³

Benefits

In patients with abnormal monoclonal studies and abnormal cardiac biomarkers, echocardiography is often the initial diagnostic test to evaluate the possibility of cardiac amyloidosis. When the echocardiogram is diagnostic for cardiac amyloidosis, CMR may offer limited additional diagnostic benefit, and it may delay care, as these patients warrant expeditious tissue biopsy for disease confirmation and initiation of treatment. However, in patients who have non-diagnostic findings on echocardiography, CMR offers significant benefit in its ability to assist with risk stratification, as patients with negative scans can be reliably ruled out for cardiac amyloidosis with 87% specificity, while patients

with cardiac amyloidosis can be detected with 95% sensitivity. The high sensitivity of CMR will increase our clinical ability to discriminate disease, pursue tissue biopsy, and initiate treatment. The desirable effect of having negative CMR results is reliably ruling out cardiac amyloidosis, and thus, avoiding unnecessary invasive tissue biopsy and any associated complications. For these reasons, the desirable effects of performing CMR in patients with non-diagnostic echocardiography are deemed large.

Harms and Burden

Undesirable effects are very low in routine cardiac MRI procedures. In fact, across the studies evaluated, major complications were not reported, and the incidence of minor complications such as nausea, vomiting, and palpitations was reported at 0.04% to 0.06%.⁷¹ The indirect undesirable effects of performing CMR relate to increased rates of false positives resulting in unnecessary biopsies or false negatives reducing suspicion of disease and delaying diagnosis in patients who truly have the disease. It is also worth mentioning that there may be rare cases of positive CMR results, while the endomyocardial biopsy misses the diagnosis, particularly in cases of patchy LGE on CMR; these cases would require additional investigation.

Other EtD criteria and considerations

The panel recognizes that cardiac MRI is a resource with moderate associated costs for most patients in well-resourced health care settings. Furthermore, the panel discussed the probability of our recommendation reducing health equity, as the test is expensive and not as widely available as cardiac ultrasound. Availability of the needed equipment, technical knowledge of imaging protocols, and physician expertise for interpretation may be limited in rural areas as well as developing countries. Nonetheless, the balance of effects favors performing cardiac MRI in patients with non-diagnostic echocardiography findings in settings where these it is readily available or in locations where these barriers can be overcome. The panel determined CMR to be definitely or probably acceptable to the vast

majority of patients. Apart from its superior ability to aid in the diagnosis of cardiac amyloidosis, it is the only imaging modality known to be capable of disease quantification. Disease burden may be quantified by ECV measurements, which may be tracked for disease progression, and potentially, for response to medical intervention. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for [Recommendation 3](#) and [Recommendation 4](#).**

Conclusions and research needs for these recommendations

In patients with abnormal monoclonal studies, abnormal cardiac biomarkers, in the setting of non-diagnostic echocardiography, the panel suggests performing cardiac magnetic resonance (CMR) imaging rather than not performing CMR to increase clinical suspicion of cardiac amyloidosis (conditional recommendation based on moderate certainty of evidence).

When echocardiographic findings are non-diagnostic, cardiac MRI may serve to increase or reduce suspicion for cardiac light amyloidosis in patients who may have systemic AL amyloidosis. Cardiac MRI may, therefore, serve as a key test to assist clinicians in deciding whether to pursue tissue biopsy in patients with suspected cardiac amyloidosis.

Further studies are needed to validate CMR findings other than LGE that assist in distinguishing the diagnosis of cardiac amyloidosis.

In patients with suspected AL amyloidosis with abnormal cardiac biomarkers and echocardiography consistent with cardiac amyloidosis, the panel suggests against performing cardiac magnetic resonance (CMR) imaging and instead recommends proceeding to tissue biopsy without delay (conditional recommendation based on a very low certainty of evidence).

In the setting of a diagnostic echocardiogram, consistent with cardiac amyloidosis, the diagnostic performance of CMR will bear unnecessary costs, and delay definitive diagnosis and initiation of treatment.

Diagnosis

Should Bone Scintigraphy with technetium 99m – pyrophosphate (PYP), technetium 99 m – 3, 3 diphosphono –1,2 propranodicarboxylic (DPD) and technetium 99 m- hydroxymethylene Diphosphonate (HMDP) be used to diagnose Amyloidosis in suspected individuals?

Recommendation 5. For individuals with a suspicion of AL amyloidosis, the ASH Guideline Panel *recommends against* the use of bone scintigraphy (PYP, DPD, HMDP) for the diagnosis of AL cardiac amyloidosis (strong recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○).

Remark:

- Clinical suspicion for light chain cardiac amyloidosis is increased in individuals with positive biomarkers (NT-proBNP, BNP, high sensitivity troponin), imaging (echocardiography/cardiac MRI compatible with cardiac amyloidosis), and immunological assays (serum free light chains, serum/urine immunofixation) indicating the presence of a monoclonal gammopathy.

Summary of the Evidence

The technetium 99mtracers PYP, DPD, and HMDP that were initially used to evaluate bone may also be taken up by the heart if there is amyloid deposition. There is commonly differential tracer uptake in the heart depending on the type of amyloidosis. In patients with AL cardiac amyloidosis there is typically no or limited tracer uptake while in ATTR cardiac amyloidosis there may be more pronounced uptake. Bone

scintigraphy should be performed with single photon emission tomography (SPECT) and not with planar imaging only to confirm tracer uptake in the myocardium and avoid false positives.⁸⁵ The tracer uptake in the heart is visually compared to bone or the heart uptake is compared to the contralateral lung to generate a ratio (H/CL). Patients with AL amyloidosis and cardiac involvement may have no myocardial uptake (visual score of 0), myocardial uptake less than bone (visual score of 1), or grade 2 uptake (myocardial uptake equal to bone) with a H/CL is <1.5 whereas patients with ATTR cardiac amyloidosis often have grade 2 (myocardial uptake equal to bone) or grade 3 uptake (myocardial uptake greater than bone) that is confirmed on single photon emission tomography (SPECT) and a H/CL ≥ 1.5 .^{74,75} The panel evaluated 7 studies with 869 patients to evaluate the use of visual grades 1 and 2 to diagnose AL amyloidosis.^{74,76-81} The pretest probability of cardiac AL amyloidosis was estimated to be 1%. Using a visual score of 1 the sensitivity was 43% and specificity 12%. When a visual score of 2 was used to diagnose AL amyloidosis the sensitivity was 17% and specificity 18%. There was concern for bias of the data because the studies did not specify the time between the bone marrow biopsy and scintigraphy.

Benefits

Bone scintigraphy with SPECT is a non-invasive test that can be useful to diagnosis cardiac involvement in some forms of cardiac amyloidosis. This test appears to be most useful to diagnosis cardiac ATTR amyloidosis in the absence of a monoclonal protein. In patients with a monoclonal protein, bone scintigraphy with visual uptake less than or equal to 2 is not sufficient to diagnosis cardiac AL amyloidosis because of its low sensitivity and specificity. However, visual uptake less than or equal to 1 may be useful to rule out ATTR cardiac amyloidosis in select patients with advanced cardiac disease. (See figure 2)

Harms and burden

Bone scintigraphy has a low sensitivity and specificity to diagnose AL amyloidosis using a visual score of 1 or 2 and is not accurate for diagnosing AL cardiac amyloidosis. Patients with false negatives will have a delay of diagnosis and will need to undergo additional testing to reach a diagnosis. Additionally, these patients will have been exposed to radiation and may still require a tissue biopsy. Patients with a false positive diagnosis of AL cardiac amyloidosis may receive inappropriate treatment.

Other EtD criteria and considerations

In patients with suspected cardiac amyloidosis, it is imperative to rule out a plasma cell dyscrasia with serum free light chains, serum IFE and urine IFE before bone scintigraphy is considered. In patients with evidence of a plasma cell dyscrasia, tissue biopsy of a surrogate or target organ should not be delayed, and bone scintigraphy should not be performed as this will result in diagnostic delays. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for**

[Recommendation 5](#)

Conclusions and research needs for this recommendation

Bone scintigraphy has a low sensitivity and specificity to diagnose AL amyloidosis and should not be used to confirm a diagnosis of AL cardiac amyloidosis and may lead to delays in diagnosis or misdiagnosis. In certain cases, bone scintigraphy with visual uptake less than or equal to 1 may be used to rule out ATTR cardiac amyloidosis in patients without a monoclonal protein.

Should Bone Scintigraphy (PYP, DPD, HMDP) be used to diagnose ATTR Amyloidosis in suspected individuals?

Recommendation 6. For individuals without evidence of a plasma cell disorder (normal serum free light chain levels and no monoclonal proteins on serum and urine

immunofixation) and suspicion of cardiac amyloidosis, the ASH Guideline Panel *recommends* the use of bone scintigraphy (PYP, DPD, HMDP) for the diagnosis of Cardiac ATTR amyloidosis (strong recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○).

Remarks:

- Bone scintigraphy should be performed with single photon emission tomography (SPECT) rather than planar only imaging to verify myocardial uptake to improve diagnostic accuracy.
- In patients with suspected or confirmed ATTR amyloidosis, family history needs to be assessed and once diagnosis is confirmed genetic counselling offered.

Summary of the evidence

More uptake of the bone tracers in the heart is seen in transthyretin amyloidosis compared with other forms of cardiac amyloidosis.⁷⁴ The panel estimated the pre-test probability of ATTR to be between 15-30%. The quality of evidence was graded as moderate and possibly affected by bias due to an unspecified time duration between the bone scintigraphy and endomyocardial biopsy. The sensitivity of diagnosing ATTR was 96% and 97% and specificity 90% and 88% using a visual score and heart to contralateral lung ratio, respectively.^{74,76,78,80,82-85} The panel looked at different bone tracers and found that both DPD and PYP had sensitivities and specificities > 90%. The panel also analyzed one study with 21 participants that utilized HMDP and found the sensitivity was 79% and specificity 100%.⁷⁴ However, due to the limited number of patients analyzed utilizing HMDP the panel was not able to determine the reproducibility of this data.

Benefits

Bone scintigraphy with SPECT allows for the non-invasive diagnosis of ATTR amyloidosis without the need for a tissue biopsy. Accurate diagnosis of ATTR amyloidosis by bone scintigraphy with SPECT can lead to initiation of amyloid specific therapy and appropriate follow-up.

Ruiz-Negrón et al found that 99mTc-PYP SPECT is cost-effective for the diagnosis of ATTR compared to heart biopsy from a US payer perspective.⁸⁶ In another model-based analysis comparing different diagnostic modalities (cardiac biopsy, cardiac MRI, PYP scan), PYP was found to be the most cost-effective strategy at a willingness-to-pay threshold of \$100 000/quality-adjusted life year (Figure 2).⁸⁷

Harms and burden

The test utilizes a radioactive tracer. The small amount of radiation used is unlikely to have significant direct adverse biological consequences. Cumulative exposure to ionizing radiation, however, including from diagnostic imaging, may increase the risk of cancer. Einstein, et al. estimated that 99mTc-PYP testing resulted in <1% excess risk of future cancers.⁸⁸ Additionally, extravasation of the radiopharmaceutical at the injection site can lead to local tissue irritation. Rare allergic reactions to the radiopharmaceutical tracers are also possible.⁸⁹ According to the 2018 EANM practice guidelines for bone scintigraphy, bone scintigraphy may not be appropriate in patients who are pregnant or had a prior allergic reaction to the radioactive tracers unless the benefits of the tests outweigh the risks.⁹⁰

Other EtD criteria and considerations

AL amyloidosis should be ruled out with serum free light chains and serum and urine immunofixation before performing bone scintigraphy to evaluate for ATTR amyloidosis. The following grading system is used when evaluating 99mTc-PYP scans: grade 0=no cardiac uptake; grade 1=cardiac uptake less than bone; grade 2= cardiac uptake equal to bone; and grade 3 =cardiac uptake greater than bone. In

addition, the heart-to-contralateral lung (H/CL) ratio is calculated as the fraction of the heart region of interest (ROI) mean counts to contralateral chest ROI mean counts. Grade 2 or 3 uptake is considered a positive test. When myocardial uptake is visually present on single-photon emission computerized tomography images (SPECT images), H/CL ratios of ≥ 1.5 at one hour are classified as ATTR positive and ratios of <1.5 ATTR negative.⁹¹ False positives can occur using planar only imaging due to blood pool uptake, areas of prior myocardial infarction or rib/sternal fractures.⁹² Therefore, to improve diagnostic accuracy, bone scintigraphy should be performed with SPECT to confirm tracer uptake is in the myocardium and not limited to the blood pool or related to a rib/sternal fractures.⁸⁵ The H/L ratio ≥ 1.5 at 1 hour should not be used to make a positive diagnosis of ATTR amyloidosis unless there is also evidence of myocardial uptake on SPECT imaging. The cost of the test is moderate as shown in the ETD

Bone scintigraphy does have some limitations, and additional testing may be indicated to confirm or exclude a diagnosis of ATTR cardiac amyloidosis when clinical uncertainty exists. For example, in certain TTR mutations with full length fibrils only, such as early onset V30M, there appears to be less uptake of the bone tracers, and this may result in false negative bone scintigraphy.⁹⁰⁻⁹² Additionally in patients with early cardiac involvement the amyloid burden may not be sufficient to result a positive test. Thus, a single negative test does not exclude the diagnosis of transthyretin amyloidosis. Grade 1 cardiac uptake may also be seen in patients such with other forms of amyloidosis such as AL amyloidosis, apolipoprotein A1, and Amyloid A (AA) amyloidosis.⁹² Therefore, in patients with grade 1 uptake (positive scan and without a mutation in TTR), other genes may need to be sequenced, or other diagnostic tests performed.^{74,92,93}

PYP is commonly used in the US, while DPD and HMDP are used in other countries. Of note, there have been supply shortages of PYP in the US and HMDP has been available as an alternative.⁹⁴ The sensitivity of HMDP, may be less than the other tracers, however there is limited data for this tracer.

The health equity for bone scintigraphy with SPECT is felt to be comparable to other diagnostic tests. Bone scintigraphy with SPECT utilizes medical equipment commonly used for nuclear imaging and may be more readily available than endomyocardial biopsy. The 2–4-hour time commitment required to complete the test is similar to other nuclear cardiac imaging tests, however, the expertise required to accurately interpret bone scintigraphy for the diagnosis of amyloidosis, may vary at different institutions. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for [Recommendation 6](#).**

Conclusions and research needs for this recommendation

Bone scintigraphy with SPECT is an accurate non-invasive test for cardiac ATTR in patients who do not have monoclonal gammopathies and provides significant benefit to patients.

The panel determined that there is a moderate certainty of evidence for a net health benefit from using bone scintigraphy with SPECT.

Research priorities include defining the role of bone scintigraphy with SPECT in asymptomatic patients with risk factors or family history of ATTR cardiomyopathy, to monitor response to treatment, and utility of bone scintigraphy with SPECT in patients with monoclonal gammopathy of undetermined significance (MGUS). More data may also be needed about the accuracy of using HMDP to diagnose ATTR amyloidosis.

Should surrogate biopsy vs. cardiac biopsy be used to diagnose AL amyloidosis in individuals suspected to have cardiac amyloidosis?

Recommendation 7. For individuals with suspected AL cardiac amyloidosis with abnormal cardiac biomarkers, diagnostic echocardiography, and positivity in any of the following studies: SIFE, UIFE, or sFLC, the ASH Guideline Panel *suggests either* starting with performing both fat pad sampling and bone marrow biopsy *or* with endomyocardial biopsy.

(conditional recommendation based on low certainty in the evidence about effects

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Remarks:

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- The panel recognizes that a bone marrow biopsy should not be performed in isolation, without immunological assays and cardiac biomarkers. Furthermore, the panel does not suggest delaying performing other testing, including target organ biopsies if clinically indicated, while awaiting results of the bone marrow biopsy.

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- In cases of suspected cardiac AL amyloidosis, it is imperative to obtain diagnostic tissue biopsy with a validated subtyping method, whether through fat pad sampling or endomyocardial biopsy.
- In certain cases, endomyocardial biopsy may be favored over fat pad sampling, such as in patients with risk factors for hereditary cardiac ATTR amyloidosis.
- Proceeding directly to an endomyocardial biopsy relies on ease of access and available expertise..
- Diagnostic echocardiographic findings that increase clinical suspicion for cardiac amyloidosis include unexplained left ventricular hypertrophy, reduced left ventricular global longitudinal strain (especially when accompanied by an apical sparing pattern), myocardium with a sparkling appearance, worsening degrees of diastolic dysfunction, elevation in left ventricular filling pressures, elevations in right heart pressures, and presence of varying degrees of pericardial effusion.
- In this clinical scenario to enhance clinical suspicion, an abnormal cardiac biomarker is defined as any one or combination of the NT pro-BNP, BNP, troponin (hs, I, or T) above the reference range for that laboratory test.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Good practice statement. The ASH panel agreed that in individuals with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be done.

Red Flag Signs and Symptoms for Cardiac Involvement

1. HFpEF (heart failure with preserved ejection fraction)
2. Moderate or Severe LVH in absence of a significant history of untreated hypertension on imaging.
3. Echocardiogram: Severe left ventricular hypertrophy, advanced diastolic dysfunction, reduced left ventricular global longitudinal strain with an apical sparing pattern
4. EKG/Arrhythmia: Low voltage and/or discordance between voltage on EKG and left ventricular wall thickness on imaging, pseudo-infarct pattern, and arrhythmias including atrial fibrillation, heart block, and ventricular tachycardia/ventricular fibrillation
5. Elevated biomarkers (Troponin and NT-Pro BNP) in absence of CAD
6. Constellation of symptoms suggesting cardiac, renal, and peripheral nervous system disease
7. Low Flow, Low Gradient Aortic Stenosis

Summary of the evidence

After the search for the systematic review was completed, the panel found 21 studies reporting on bone marrow biopsy, 25 on fat pad sampling, 1 on combined bone marrow biopsy and fat pad sampling and 5 on endomyocardial biopsy. These studies measured sensitivity and specificity of fat pad sampling, bone marrow biopsy alone, combined fat pad sampling and bone marrow biopsy, and endomyocardial biopsy to diagnose AL amyloidosis in suspected patients. All studies included subjects suspected of AL

amyloidosis based on the clinical, laboratory and/or imaging (echocardiogram, etc.). The panel judged the certainty of evidence as moderate for fat pad sampling, low for bone marrow biopsy, very low for combined fat pad sampling and bone marrow biopsy and low for endomyocardial biopsy.^{15,19,95-142}

Fat pad sampling and bone marrow biopsy combined were noted to have high sensitivity of 89% but when performed individually, they had low sensitivity of 76.6% and 55.1%, respectively. Endomyocardial biopsy also had high test sensitivity with 93.5%. (See Supplement 5)

Benefits

The panel judged that the desirable effects of performing fat pad sampling, combined fat pad sampling and bone marrow biopsy and endomyocardial biopsy are large while that of bone marrow biopsy is moderate. This was primarily based on the sensitivity of these tests. Performing these tests in patients with suspected AL amyloidosis has numerous benefits, including accurate diagnosis, avoiding an organ biopsy that may have higher risks, early detection, and decrease in the time interval between establishment of diagnosis and treatment initiation. Early and accurate diagnosis can decrease mortality. It is important for healthcare providers to carefully consider the risks and benefits of testing, as well as to communicate effectively with the patients about the limitations of these tests, including the false negatives. (Figure 2)

Harms and burden

Generally, biopsies come with risks depending on the site. Overall, the undesirable effects of fat pad sampling, bone marrow and endomyocardial biopsies were considered trivial to small by the panel. Complications of a fat pad biopsy are rare and include bleeding, hematoma, infection, allergic reactions, and aesthetically suboptimal scar formation.^{143,144} Similarly, bone marrow biopsies have a small risk of complications, which include biopsy site infection, bleeding and transient site discomfort. Bain et al conducted a survey among members of the British Society for Hematology for adverse events related to

bone marrow aspirate with or without trephine biopsy. Twenty-six adverse events were reported among an estimated 54,890 biopsies (0.0047%), with the most common being hemorrhage (14 events).¹⁴⁵

Endomyocardial biopsies should be performed by experienced operators in experienced centres.

Endomyocardial biopsies have a risk of bleeding, and the need to wait for anticoagulation washout may delay biopsy in patients who would benefit from prompt diagnosis and treatment institution.

Perforation leading to death can be observed in 1% of endomyocardial biopsies but is lower in patients with thickened myocardium compared to patients with thinner myocardia. , (^{146,147}). Hence, it is plausible that for patients with light chain cardiac amyloidosis (who usually have left ventricular hypertrophy) there is a lower risk of perforation.

Other EtD criteria and considerations

The required resources and associated cost vary for fat pad and marrow biopsy. Fat pad aspiration is a relatively low-cost and non-invasive method for obtaining tissue.¹⁸ The subcutaneous adipose tissue in the abdomen may be a less expensive and less invasive source of tissue for diagnosis and amyloid typing.¹⁶ Regarding cost, it is negligible if performed at the bedside but becomes moderate if executed in the operating room. Similarly, the cost of a bone marrow biopsy ranges from negligible to moderate when performed by hematology/oncology versus interventional radiology; patient charges of \$5254 vs \$413 were reported by a study conducted at Bassett Medical Center, NY.¹⁴⁸ Generally, endomyocardial biopsy carries a moderate cost. In a study looking at its cost in the records at 8,170 records (6,385 commercial payers and 1,785 Medicare) of heart transplant patients with evidence of EMB procedures, the median cost was US \$9,049.¹⁴⁹ The panel mentioned that the cost can occasionally reach up to \$50,000 depending on the center. All biopsies were believed to have no impact on equity except for endomyocardial biopsy which probably reduces equity due to variability in ease of access and available expertise. The feasibility of fat pad biopsy varies depending on access to the proceduralist and availability of subtyping by mass spectrometry. Appropriate sampling by fat pad biopsy (or aspirate, if

amenable to process with mass spectrometry) that allows for subtyping by mass spectrometry must be ensured. Bone marrow biopsy is considered feasible; however, access may differ if it needs to be performed by interventional radiology. The feasibility of endomyocardial biopsy varies depending on access to centers with the required expertise and the inability of patients with advanced heart failure related to amyloidosis to lie flat. **A multi-comparison ETD framework is in the supplement for Recommendations 7,8,9,10.**

Conclusions and research needs for this recommendation

The panel determined that the balance of desirable and undesirable effects favored the combination of fat pad and bone marrow biopsy for patients with suspected cardiac amyloidosis but agrees that there is a very low certainty of evidence for a net benefit/harm from using this approach. Similarly, the panel also determined that the balance of desirable and undesirable effects favored endomyocardial biopsy for patients with suspected cardiac amyloidosis and the certainty of the evidence for a net benefit/harm from using this approach is low. The panel recognizes that proceeding with one approach versus the other relies on several factors including access, available expertise, clinical presentation, and cost.

The panel identified the following additional research questions:

1. Studies to evaluate the utility of repeat surrogate biopsy in comparison to an organ biopsy
2. Longitudinal studies for patients with amyloid deposits on bone marrow assessing the natural history and comparing the risk of progression with vascular versus interstitial amyloid deposits
3. Evaluation and comparison of minimally invasive surrogate biopsies
4. Utility of evaluation of heart biopsies using electron microscopy on the early detection and outcomes of AL amyloidosis

Should surrogate biopsy vs renal biopsy be used to diagnose AL amyloidosis in individuals suspected to have renal amyloidosis?

Recommendation 8. For individuals with suspected AL renal amyloidosis and positivity in any of the following studies SIFE, UIFE, or sFLC, the ASH Guideline Panel *suggests* starting with performing both abdominal fat pad sampling and bone marrow biopsy over renal biopsy. (conditional recommendation based on moderate certainty in the evidence about effects ⊕⊕⊕○)

Remarks:

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- The panel recognizes that a bone marrow biopsy should not be performed in isolation, without immunological assays and cardiac biomarkers. Furthermore, the panel does not suggest delaying performing other testing, including target organ biopsies if clinically indicated, while awaiting results of the bone marrow biopsy.
-
- A positive abdominal fat pad sampling or bone marrow biopsy with subtyping makes renal biopsy unnecessary. However, in the case of a negative fat pad sampling and bone marrow biopsy, a renal biopsy is necessary to look for amyloidosis or other pathology.
- If there is a high clinical suspicion for other renal pathology which can mimic a presentation similar to renal amyloidosis or other plasma cell disorders, then an upfront renal biopsy is preferred.
- If subtyping of the abdominal fat pad is not feasible, the patient should be referred for a renal biopsy.
- The panel placed a high value on safety; however, despite the procedure-related risks, it remains important to perform a renal biopsy when appropriate.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Good practice statement. The ASH panel agreed that in individuals with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be done.

Red Flag Signs and Symptoms for Renal Involvement

1. Inability to tolerate ACE/ARB
2. Change in hypertension status unexplained by medication i.e. intolerance/hypotension on previously tolerated therapy.
3. Unexplained proteinuria (albumin predominant) without diabetes (or not thought to be related to be diabetes or any other reason) and positive laboratory tests (monoclonal proteins and/or abnormal light chains (must be clonal or above renal limits)
4. Proteinuria in diabetic with positive monoclonal protein

Summary of the evidence

The ASH guideline panel found 21 studies on bone marrow (BM) biopsy, 25 on fat pad sampling, and 6 studies on renal biopsies for amyloid detection. For the composite analysis of fat pad and BM the panel utilized a single study supplemented by modeling based on calculated values from the individual fat pad and BM studies.

Renal biopsy demonstrated the highest sensitivity for detecting amyloidosis at 95.5%. In contrast, combined fat pad sampling and bone marrow biopsy had a sensitivity of 89%. When performed individually, fat pad sampling and bone marrow biopsy had sensitivities of 76.6% and 55.1%,

respectively. Due to the lack of RCTs, panel assessed the certainty of evidence as follows: moderate for fat pad sampling, low for bone marrow biopsy, very low for combined fat pad sampling and bone marrow biopsy and moderate for renal biopsy.^{113,122,124,127,137,142} (See Supplement 5)

Benefits

The panel found that the desirable effects of performing fat pad sampling, combined fat pad sampling and bone marrow biopsy and, renal biopsy are large while that of bone marrow biopsy alone are moderate. This assessment is primarily based on the sensitivity of these tests.

Although there are no randomized trials available comparing renal biopsy versus surrogate site biopsy, combining fat pad and bone marrow biopsy offers numerous benefits, including timely diagnosis and advantage of avoiding renal biopsy. Bone marrow biopsy provides the added benefit of diagnosing a plasma cell neoplasm and assessing plasma cell burden in the bone marrow.

Renal biopsy, with a higher sensitivity of 95.5% compared to the combined fat pad and bone marrow biopsy at 89%, determines the definite involvement of AL amyloidosis in the kidney and can rule out other renal pathologies. However, surrogate biopsies present a lower risk of complications, and access to fat pad and bone marrow biopsy is often more readily available, facilitating a timelier diagnosis. In instances, where combined fat pad sampling and bone marrow biopsy are inconclusive, renal biopsies can confirm the presence of the amyloidosis and rule out other renal pathologies.

Harms and burden

The panel found that the undesirable effects of fat pad sampling and bone marrow biopsies were considered trivial to small. Complications of a fat pad biopsy are rare and include bleeding, hematoma, infection, allergic reactions, and aesthetically suboptimal scar formation.^{143 144} Bone marrow biopsies

also have a small risk of complications, which include biopsy site infection, bleeding and transient site discomfort.

The undesirable effects of renal biopsy are moderate. Renal biopsy carries a risk of bleeding, and the need to wait for anticoagulation washout may delay biopsy in patients who would benefit from prompt diagnosis and treatment. In a study of 101 patients with amyloidosis undergoing percutaneous renal biopsy, post-renal biopsy bleeding was observed in 9.9% of patients with amyloidosis, with bleeding classified as major (requiring blood transfusions and selective renal angiography) in 4% of the patients.¹⁵⁰ A review of large clinical trials provided an estimate of the frequency of complications after renal biopsy with required blood transfusions from 0.3% to 10% and death due to biopsy in less than 0.1%.¹⁵¹ Transjugular renal biopsies have a lower rate of complications, however, they are rarely performed in the U.S due to a lack of appropriate equipment.¹⁵⁰

Other EtD criteria and considerations

Fat pad aspiration is a relatively low-cost and non-invasive method for obtaining tissue.¹⁸ Abdominal subcutaneous adipose tissue is a less expensive and less invasive source of tissue for diagnosis and amyloid typing.¹⁶ The cost of this procedure is negligible if performed at the bedside but becomes moderate if conducted in the operating room. Similarly, the cost of a bone marrow biopsy ranges from negligible to moderate, depending on whether is performed by hematology/oncology versus interventional radiology. A study conducted at Bassett Medical Center, NY reported patient charges of \$413 if performed by Hematology Oncology vs \$5254 performed by Interventional radiology.¹⁴⁸ Renal biopsy generally incurs moderate cost. The estimated national average cost of renal biopsy ranges from \$4,470- 5,368\$.^{152,153} All biopsies were considered to have no impact on equity, except for renal biopsy which may reduce equity in Medicaid patients requiring admission to perform the renal biopsy, patients

with obese body habitus, solitary kidney and patients requiring anticoagulation (e.g., coronary artery disease with recent stent placement).

The panel assessed the feasibility of fat pad biopsy, which varies depending on access to the proceduralist and availability of mass spectrometry for subtyping of amyloidosis. It is crucial to ensure appropriate sampling by fat pad biopsy (or aspirate, if compatible with mass spectrometry processing), ensuring accurate subtyping by mass spectrometry. Bone marrow biopsy is generally feasible, though access may vary if performed by interventional radiology. Renal biopsy is widely available in health centers; however, in amyloidosis patients, it is associated with an increased risk of bleeding, which can impact the feasibility of this procedure. **A multi-comparison ETD framework is in the supplement for Recommendations 7,8,9,10.**

Conclusions and research needs for this recommendation

The panel agreed that the balance of desirable and undesirable effects favored the combination of fat pad and bone marrow biopsy for suspected AL amyloidosis over renal biopsy despite the very low certainty of evidence. The panel determined the moderate certainty of evidence for a benefits or harms for renal biopsy. Emphasizing the importance of timely diagnosis, the decision to proceed with one approach over the other depends on several factors such as available expertise, clinical presentation, complications, access, equity and cost. Therefore, the panel opted for a conditional recommendation for performing both fat pad and bone marrow biopsy over renal biopsy.

The panel prioritized the following research needs:

1. Randomized controlled trials comparing surrogate biopsy to renal biopsy for accurate and timely diagnosis of AL amyloidosis.
2. Randomized controlled trials to evaluate the utility of repeat surrogate biopsy in comparison to an organ biopsy.

Should surrogate biopsy vs. peripheral nerve biopsy be used to diagnose AL amyloidosis in individuals suspected to have neurological amyloidosis?

Recommendation 9. For individuals with a monoclonal gammopathy and generalized small or large fiber peripheral neuropathy or autonomic neuropathy suspected of having AL amyloidosis, the ASH Guideline Panel *suggests* performing both fat pad sampling and bone marrow biopsy over nerve biopsy (conditional recommendation based on very low certainty in the evidence about effects ⊕○○○)

Remarks:

The panel recognizes that a bone marrow biopsy should not be performed in isolation, without immunological assays and cardiac biomarkers. Furthermore, the panel does not suggest delaying performing other testing, including target organ biopsies if clinically indicated, while awaiting results of the bone marrow biopsy.

-
- In cases with high clinical suspicion, if abdominal fat pad sampling and bone marrow biopsy yield negative results for AL amyloidosis, a nerve biopsy or other organ specific biopsy should be performed.
- If there is a high clinical suspicion for other nerve pathologies which can mimic presentations similar to nerve amyloidosis or other plasma cell disorders, then an upfront nerve biopsy can be considered.
- If a nerve biopsy is performed, the panel recommends ensuring that that technique and handling is optimized to allow for an adequate diagnostic yield.
- The neuropathy observed in AL amyloidosis strongly resembles diabetic neuropathy, which is more frequent at a population level.
- A skin biopsy is undertaken for assessment of intraepidermal nerve fiber density to confirm a small fiber or autonomic neuropathy, as well as confirmation of congophilic

deposits. Such skin biopsies need special handling and referral to a reference center for analysis.

Good practice statement. The ASH panel agreed that in patients with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be done.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Red Flag Signs and Symptoms for Neurological Involvement

1. Small fiber neuropathy: pain and temperature impairments, allodynia and hyperalgesia
2. Autonomic neuropathy: orthostatic hypotension, erectile dysfunction, diarrhea and/or constipation, gastroparesis, urinary dysfunction, sweating abnormalities, pupillary dysfunction and dry eye and mouth.
3. Sensorimotor neuropathy: numbness, paresthesia, imbalance, weakness and atrophy.
4. Autonomic, Small fiber or sensorimotor neuropathy unexplained by other causes, including diabetes.
5. Abnormal nerve conduction studies/ electromyography, typically a sensory or sensorimotor neuropathy with axonal pattern.
6. Carpal Tunnel Syndrome – bilateral, particularly with heart disease and a monoclonal protein
7. Peripheral neuropathy with positive monoclonal gammopathy

8. Rapid progression of neuropathy
9. Constellation of symptoms suggesting cardiac, renal, and peripheral nervous system disease

Summary of the evidence

The ASH guideline panel found 21 studies on bone marrow (BM) biopsy, 25 on fat pad sampling, 5 studies on nerve biopsy and 1 study on skin biopsy for intraepidermal nerve fiber density (IENFD). For the composite analysis of fat pad and BM the panel utilized a single study supplemented by modeling based on calculated values from the individual fat pad and BM studies. All studies included subjects appropriately diagnosed with AL based on amyloid detection and subtyping and measured the sensitivity and specificity of either nerve biopsy or skin biopsy to diagnose AL amyloidosis in patients with AL amyloidosis.

Four studies reported the risks and undesirable effects of nerve biopsy, and one study reported on the risks and undesirable effects of skin biopsy.

When combined, fat pad sampling and bone marrow biopsy were noted to have high sensitivity of 89% but when performed individually, they had low sensitivities of 76.6% and 55.1%, respectively. Whereas nerve biopsy and skin biopsy were found to have lower sensitivities of 66.3% and 60.2%, respectively.

The panel assessed the certainty of evidence as follows: moderate for fat pad sampling, low for bone marrow biopsy and very low for combined fat pad sampling and bone marrow biopsy, nerve biopsy and skin biopsy.^{15,100,101,103,106-108,111,113,114,116-118,121,122,127-129,133,136,137,141,154-159} (See Supplement 5)

Benefits

The panel judged that the desirable effects of performing fat pad sampling and combined fat pad sampling and bone marrow biopsy were large, while the desirable effects of bone marrow biopsy, nerve

biopsy and skin biopsy were moderate. This judgement was primarily based on the sensitivity of these tests. Although no randomized trials were available comparing nerve or skin biopsies with surrogate site biopsy, the diagnostic performance of a fat pad and bone marrow biopsy in patients with suspected AL amyloidosis has numerous benefits, including accurate diagnosis, avoidance of a target organ biopsy that may have higher risks, early detection of disease, and the potential decrease in the time interval between establishment of diagnosis and treatment initiation. In AL amyloidosis, early, accurate diagnosis and treatment initiation results in decreased mortality. Bone marrow biopsy has an additional benefit of diagnosing an underlying plasma cell dyscrasia and assessing plasma cell burden in the bone marrow.

Although the available data is limited, both nerve (66.3%) and skin biopsy for intraepidermal nerve fibre density (60.2%) have lower sensitivity when compared to the combined fat pad and bone marrow biopsy (89%). Furthermore, these surrogate tests are judged to be less invasive, have a lower risk of complications, are less expensive and more readily available than the target organ biopsies (see EtD in Supplement 5), which may allow a timelier diagnosis. However, in instances where combined fat pad sampling and bone marrow biopsy are inconclusive, nerve or skin biopsy can be used to confirm the presence of amyloidosis and rule out other nerve pathologies. It is important for healthcare providers to carefully consider the risks and benefits of testing, as well as to communicate effectively with the patients about the limitations of these tests, including the potential for false negatives.

Harms and burden

The panel judged that the undesirable effects of fat pad sampling were trivial and bone marrow biopsies were small. Complications of a fat pad biopsy are rare and include bleeding, hematoma, infection, allergic reactions, and aesthetically suboptimal scar formation.^{143,144} Bone marrow biopsies also have a small risk of complications, which include biopsy site infection, bleeding and transient site discomfort

(0.0047%).¹⁴⁵ The small undesirable effect of bone marrow biopsy was judged to be predominantly driven by the false negative rates of this test, rather than the risks associated with the biopsy. However, the panel judged that Individuals with a high index of suspicion for AL amyloidosis would proceed to having a target organ biopsy if a fat pad or bone marrow biopsy was negative.

The undesirable effects of nerve and skin biopsies were judged by the panel to be moderate and small, respectively. Nerve biopsy can result in complications including perioperative and chronic pain, dysaesthesia, paraesthesia, poor wound healing and infection.¹⁶⁰⁻¹⁶² In studies of sural nerve biopsy, long-lasting sensory deficits were reported in 93%, dysaesthesia in 19%, and chronic pain in 19-33% of patients.^{161,163} In contrast, skin biopsies (for the evaluation of intraepidermal nerve fibre density and Congo red) were judged by the panel to be safe and painless.¹⁶⁴ The small undesirable effects of skin biopsy were felt to be predominantly driven by the high false negative rates, rather than the risks associated with this biopsy. For both nerve and skin biopsies a false negative result may lead to a delay in diagnosis, need for repetition of an invasive organ biopsy, and may incorrectly rule out AL amyloidosis. Amyloid deposition in the nerve and skin is frequently patchy in deposition, making a false negative result not uncommon.

Other EtD criteria and considerations

Overall, the balance of desirable and undesirable effects of all biopsies favoured the intervention for fat pad and combined fat pad and bone marrow biopsies and probably favoured the intervention for bone marrow biopsy alone, nerve and skin biopsies. Of note, the certainty of the evidence was low for bone marrow biopsy, and very low for combined fat pad and bone marrow biopsy, nerve biopsy and skin biopsy, and further research is required to improve the quality of this evidence.

Fat pad aspiration is a relatively low-cost and non-invasive method for obtaining tissue.¹⁸ Subcutaneous adipose tissue in the abdomen a less invasive source of tissue for diagnosis and amyloid typing¹⁶ and is

less expensive. In terms of cost, the procedure is negligible if performed at the bedside but becomes moderate if conducted in the operating room. Similarly, the cost of a bone marrow biopsy ranges from negligible to moderate, depending on whether is performed by hematology/oncology versus interventional radiology. A study conducted at Bassett Medical Center, NY reported patient charges of \$413 if performed by Hematology/Oncology when compared with \$5254 if performed by Interventional radiology.¹⁴⁸ Of note, bone marrow biopsy will frequently need to be performed for delineation of the underlying plasma cell dyscrasia, and hence the addition of Congo red testing requires minimal additional a cost.

Limited information is available for the cost of nerve biopsies. According to US Medicare, a nerve biopsy costs USD997 in an ambulatory surgical center and USD 1920 in a hospital outpatient department.¹⁶⁵ However, these estimates do not consider additional costs relating to the surgical procedure, anaesthetic requirements and neuropathological testing. No evidence was available on the cost of skin biopsy for small fibre neuropathy assessment. The price of skin biopsies is thought by the panel to likely be variable depending on how the biopsy was collected and evaluated. It is approximated that skin biopsies of the distal and proximal leg with IENFD and Congo red staining will cost USD 6000 (personal communication). Of note, the handling of skin biopsy for IENFD differs than normal skin biopsy, which reduces availability and equity of access.

All biopsies were considered to have probably no impact on equity, except for nerve and skin biopsy which may reduce equity due to the costs of performing these studies which may prevent access for some individuals. These procedures may be covered by some insurance policies.

It should be noted that skin biopsy for intraepidermal nerve fiber density is the gold standard test for evaluation of small fiber neuropathy, with the additional benefit of identifying amyloid deposits if stained for Congo red, however alternative assessments for small and autonomic neuropathy include quantitative sensory testing (QST), quantitative sudomotor axon reflex test (QSART), Thermoregulatory

sweat tests, Sudoscan, postural blood pressure assessments and tilt table testing, formal autonomic function testing and nuclear medicine studies including gastric-emptying and Cardiac 123I-metaiodobenzylguanidine (123I-MIBG) scintigraphy. These tests can be utilised to confirm the presence of a small or autonomic neuropathy, and to monitor disease, however, are unable to distinguish between different aetiologies of these neuropathies. It should also be noted that the neuropathy observed in AL amyloidosis, strongly resembles diabetic neuropathy, which is more frequent at a population level. Clinicians should evaluate for other causes of neuropathy, including diabetes, recognizing that amyloidosis can occur in the setting of other causes. Further testing for AL amyloidosis should occur if the neuropathy is felt to be unexplained by other factors, for example, progression of the neuropathy despite stable diabetic parameters.

The panel assessed that the feasibility of fat pad biopsy varies depending on access to the proceduralist, and availability of mass spectrometry for subtyping amyloidosis. It is crucial to ensure appropriate sampling methods occur for fat pad biopsy (or aspirate), so that mass spectrometry processing is possible, thereby ensuring accurate. Bone marrow biopsy is generally feasible, though access may vary if performed by interventional radiology. Nerve biopsy requires surgical and anaesthetic support and skin biopsy of IENFD is only performed in specific academic centres, as a result there is relative difficulties in either performing the biopsy or organising and interpreting the biopsy, respectively. **A multi-comparison ETD framework is in the supplement for Recommendations 7,8,9,10.**

Conclusions and research needs for this recommendation

The panel determined that the combination of fat pad and bone marrow biopsy was favoured over either a nerve or skin biopsy for the diagnosis of suspected AL amyloidosis, despite very low certainty of evidence. The quality of evidence to support performing nerve and skin biopsies is poor, with low numbers of included patients and studies, increased risk of bias, inconsistency in data collection and

availability of only indirect evidence. Furthermore, due to the patchy distribution of amyloid deposition in the skin and nerves, the sensitivity of these biopsies is reduced compared to other biopsy sites. Emphasizing the importance of timely diagnosis, the decision to proceed with one approach over the other depends on several factors such as available expertise, clinical presentation, complications, access, equity and cost. Given the higher false negative rates, small to moderate risks and moderate costs the panel opted for conditional recommendation for performing both fat pad and bone marrow biopsy over either a nerve or skin biopsy for the diagnosis of AL Amyloidosis. However, in cases with high clinical suspicion, if abdominal fat pad or bone marrow biopsies yield negative results for AL amyloidosis, a nerve biopsy or other involved organ biopsy should be performed. The choice of which surrogate or target biopsy to perform should be influenced by center experience, time to obtain the biopsy, and target organ involvement, including the sensitivity and risks associated with the biopsy. Furthermore, if other nerve pathologies are considered (e.g. vasculitis, neurolymphomatosis), a nerve biopsy may be required to exclude alternate causes.

The panel identified the following additional research questions:

1. Randomized controlled trials comparing surrogate biopsy to nerve or skin biopsy for accurate and timely diagnosis.
2. Further evaluation of the sensitivity and specificity of nerve and skin biopsy to diagnose AL amyloidosis, when compared to a reference standard, such as biopsy of a target organ.
3. Randomized controlled trials to evaluate the utility of repeat surrogate biopsy in comparison to an organ biopsy.
4. Studies investigating the optimal strategies for diagnosing AL amyloidosis in patients with diabetes mellitus, in particular assessing the rate of neuropathy progression and sensitivity and specificity of biopsy in this cohort.

Should surrogate biopsy vs target organ biopsy be used to diagnose AL amyloidosis in individuals suspected to have AL amyloidosis with multiorgan presentation?

Recommendation 10. For individuals with suspected multiorgan AL amyloidosis, the ASH Guideline Panel *suggests* starting with surrogate biopsies (combination of fat pad sampling and bone marrow biopsy) over target organ biopsy if surrogate biopsies can be performed expeditiously. If endomyocardial biopsy or renal biopsy are more feasible than fat pad sampling and bone marrow biopsy, these symptomatic target tissues should be preferentially biopsied (conditional recommendation based on low certainty in the evidence about effects ⊕⊕○○)

Remarks:

- Promptly obtaining a tissue biopsy with subtyping is imperative, especially when there is suspected cardiac involvement.
- On repeat biopsies:
 - If surrogate biopsies are negative and suspicion is high for amyloidosis, a target organ should be biopsied.
 - Although not systematically studied, repeat fat pad sampling at an amyloidosis center with greater experience performing such tests may be helpful to reach a diagnosis.
- In patients suspected of having GI amyloid, a more generous and deeper (enclosing submucosal layers) biopsy should be obtained.

Good practice statement. The ASH panel agreed that the technique for abdominal fat pad sampling should be optimal to allow for subtyping using a validated method.

Good practice statement. The ASH panel agreed that in patients with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Good practice statement. The ASH panel agreed that for individuals highly suspected of having AL amyloidosis with absence of amyloid deposits in the fat pad and bone marrow biopsy, consider performing biopsy of an affected organ.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be done.

Red Flag Signs and Symptoms

Constellation of symptoms suggesting cardiac, renal or nerve involvement (see red flags described for cardiac, renal and neurological presentations)

Liver Presentation

1. Cholestatic (associated with raised serum alkaline phosphatase) not related to other disorders.
2. Hepatomegaly not related to other disorders.

Gastrointestinal tract Presentation

3. Unexplained diarrhea and/or constipation, weight loss, loss of appetite, early satiety, nausea, vomiting, abdominal pain, gastrointestinal bleeding

Other

4. Easy Bleeding and bruising (associated with acquired factor X deficiency)

Summary of the evidence

Performed individually, fat pad sampling and bone marrow biopsy have sensitivities of 76.6% and 55.1%, respectively but have a combined sensitivity of 89%. These data are derived from 25 studies reporting on fat pad sampling,^{100,103,106,108,111,113,114,116,122,128,133,155,157} 21 on bone marrow biopsy.^{100,106-108,112,113,122,127-}

^{129,132,135-137,141,155,156,159,166,167} The panel judged the certainty of evidence as moderate for fat pad sampling, low for bone marrow biopsy, very low for combined fat pad sampling and bone marrow

biopsy, largely due to the absence of studies addressing specificity. The risks of performing a biopsy at these sites were deemed trivial based on a review of the literature and personal experience of the panel members, so in aggregate there was a very positive desirable effect, assuming these tests are readily available to the treating physician. The panel noted that other sites have also been utilized as a surrogate tissue to diagnose systemic amyloidosis including lip (minor salivary glands), gingiva, skin biopsy (performed for non-neuropathic indications) and GI tract biopsies in asymptomatic patients.^{168,169} A formal analysis of these other sites was beyond the scope of this formal review, please see also below under Other EtD criteria. (See Supplement 5)

Among patients with hepatic involvement (approximately 15% of patients determined in clinical series and 70% in autopsy series),^{106,107,113,127,135,154,156} the sensitivity of liver biopsy is 87%, based on data from 57 patients from 5 studies,^{108,127,156,170,171} but the associated risk of liver biopsy in general¹⁷² and amyloid specifically,¹⁰⁸ makes the composite fat and bone marrow biopsy preferred initial step towards tissue diagnosis. The certainty of evidence was very low based on limited data. For patients with gastrointestinal symptoms, gastrointestinal biopsy has a sensitivity of 67% based on data from 522 patients from 6 studies,^{106,111,113,127,137,142} but once again the certainty of the evidence was characterized as low. Importantly, a more generous sampling of to include submucosal layers has been recommended.¹⁷³⁻¹⁷⁵

The merits and disadvantages of renal, heart, peripheral nerve are discussed in other sections.

Benefits and Harms

Fat sampling and bone marrow biopsy as surrogate biopsies

The panel judged that the desirable effects of performing surrogate fat sampling and bone marrow biopsy in suspected multi-organ AL amyloidosis are moderate based on the sensitivity of these tests, their safety, and relatively lower cost compared to target organ biopsies. The benefit, however, is only

present if these tests are accessible; if not accessible and target organ (especially heart) is more accessible, the target organ biopsy may be preferred to expedite the diagnosis. Bone marrow biopsy has the added benefit of ruling out other plasma cell dyscrasia such as multiple myeloma.

Complications of a fat pad biopsy are rare and include physical discomfort, bleeding, hematoma, infection, allergic reactions, and aesthetically suboptimal scar formation.^{143,144} Similarly, bone marrow biopsies have a small risk of complications, which include biopsy site infection, bleeding and transient site discomfort (0.0047%).¹⁴⁵ Bain et al conducted a survey among members of the British Society of Hematology for adverse events related to bone marrow aspirate with or without trephine biopsy. Twenty-six adverse events were reported among an estimated 54,890 biopsies (0.0047%), with the most common being hemorrhage (14 events).¹⁴⁵ Overall, a comparison of balance of effects favors or probably favors the intervention

Overall, the certainty of these estimated effects is moderate for fat sampling, low for bone marrow biopsy and very low for combined fat and bone marrow biopsy owing to variable sensitivity in the studies and imprecision of the estimates (See Supplement 5).

Liver biopsy as a target organ biopsy

The benefit of liver biopsy is the high sensitivity of this diagnostic procedure, but the harms include hemorrhage in as many as 11% of the liver biopsies in contemporary hepatology practice (Supplement 5).^{171,172} In one study, major complications were reported in approximately 0.6 to 2.4% of patients biopsied.¹⁷⁴ The rates in patients with AL are harder to estimate, with early reports citing hemorrhage in 1 in 18 patients, but a subsequent larger study in which 57 AL amyloidosis patients underwent percutaneous liver biopsy reported serious bleeding in 3.5% of patients and death in 1.7%.¹⁰⁸ If tissue biopsy cannot be made via other means, transvenous biopsy can be considered over percutaneous biopsy.¹⁷⁶ Overall, the certainty of these estimated effects are very low to low.

Gastrointestinal biopsy as a target organ biopsy

GI tract biopsy is less invasive and likely more accessible than many other biopsy sites (e.g. heart, liver, kidney, and nerve), but the aggregate sensitivity is only 67% (see EtD in Supplement 5).

^{106,111,113,127,137,142} This relatively low sensitivity may in part be due to the complexity of distinguishing symptoms due to overt GI involvement from GI symptoms due to autonomic involvement. Based on the available literature, the balance of effects (between desirable and undesirable effects) favored the use of gastrointestinal biopsy over liver biopsy in the appropriate multiorgan situations. Overall, the certainty of these estimated effects are very low to low.^{169,173,177,178}

Other EtD criteria and considerations

Feasibility and reliability of performing different tests will vary from practice to practice and level of expertise, but the panel emphasizes that speed and accuracy of diagnosis are imperative, especially if there is suspected cardiac involvement. Any biopsy performed should be adequate for subtyping should the result prove positive for amyloid. For example, the utility of fat pad biopsy varies depending on access to the proceduralist and availability of subtyping by validated methods, preferably by mass spectrometry. Although not systematically studied, there are retrospective data demonstrating the potential value of a historical biopsy as a surrogate biopsy tool to aid in the diagnosis of systemic amyloidosis.^{179,180}

Typical and most widely utilized surrogate biopsy sites include abdominal fat and bone marrow biopsies, and these were subjected to systematic review in this study. Other surrogate sites that have been utilized (lip [minor salivary glands], gingiva, skin [for non-neuropathic indications] and GI tract biopsy [typically rectum but increasingly also upper/lower GI sites] were beyond the scope of this formal review. It should also be noted that, in patients with dominant GI tract involvement, GI tract biopsy serves as a target biopsy.^{106,111,113,115,127,137,142,181-186}

Obtaining sufficient biopsy material is essential. Types of abdominal fat biopsies may include fine needle aspiration biopsy (FNAB) with a thin needle (22 gauge), core biopsy (16-gauge needle), and skin punch biopsy (6-8mm) to include subcutaneous fat (“telescoping”) or a small surgical (excisional) biopsy. The laboratory analysis of fat pad samples should be optimal to allow for subtyping using a validated method.¹⁵⁻²¹ Optimal sampling means that enough tissue is harvested to carry out amyloid subtyping. Although this can be achieved with fat FNAB in some experienced centers, in most instances, fat biopsy is preferred over aspiration. In the case of GI biopsies, a more generous sampling, which includes submucosal layers, has been recommended.¹⁷⁵ Similarly, for skin biopsy, a more generous biopsy (aka “telescoping” biopsy), which includes deeper subcutaneous adipose tissue, is advisable.^{19,169,173,177,178}

The required resources and associated cost vary for fat pad and marrow biopsy. Fat pad aspiration is a relatively low-cost and non-invasive method for obtaining tissue.¹⁸ The subcutaneous adipose tissue in the abdomen may be a less expensive and less invasive source of tissue for diagnosis and amyloid typing.¹⁶ Regarding cost, it is negligible if performed at the bedside but becomes moderate if executed in the operating room. Similarly, the cost of a bone marrow biopsy ranges from negligible to moderate when performed by hematology/oncology versus interventional radiology; patient charges of \$5254 vs \$413 were reported by a study conducted at Bassett Medical Center, NY.¹⁴⁸

Additionally, during the systematic reviews the panel looked for evidence regarding repeating surrogate site biopsy versus target organ biopsy. No evidence was found that investigated the diagnostic test accuracy of repeating a surrogate biopsy versus target organ biopsy after performing both fat pad and bone marrow biopsy in combination.

Patient acceptability of undergoing fat sampling and bone marrow biopsy was deemed as high. It should be noted that the risk of bias for all recommendations in this section was judged to be high due to concerns about flow time (the majority of studies do not specify duration between surrogate and

reference biopsy). **A multi-comparison ETD framework is in the supplement for Recommendations 7,8,9,10.**

Conclusions and research needs for this recommendation

The panel determined that the balance of desirable and undesirable effects favored the combination of fat pad sampling and bone marrow biopsy for patients with suspected multiorgan amyloidosis. However, the panel agrees that there is a very low certainty of evidence for a net benefit/harm from using this approach. Similarly, the panel determined that the balance of desirable and undesirable effects favored endomyocardial biopsy for patients requiring an urgent diagnosis with suspected cardiac amyloidosis and negative or not readily available surrogate biopsies. For this, the certainty of the evidence for a net benefit/harm from using this approach is low. The panel recognizes that proceeding with one approach versus the other relies on several factors including access, available expertise, clinical presentation, and cost.

The panel identified the following additional research needs:

1. Evaluation and comparison of minimally invasive surrogate biopsies
2. Studies to evaluate the utility of repeat surrogate biopsy in comparison to a target/involved organ biopsy
3. Improvements in fat sampling performance and Congo red stain interpretation.
4. Systematic evaluation of historical specimens suitable for early diagnosis (e.g. luminal GI, genitourinary)

Should Congo Red Staining on bone marrow biopsy that has already been performed be used to diagnose AL amyloidosis in individuals with Multiple Myeloma and Smoldering Myeloma?

Recommendation 11. For individuals with plasma cell dyscrasias (multiple myeloma and smoldering multiple myeloma), the ASH Guideline Panel *suggests* performing Congo red staining on bone marrow biopsies that may have already been performed (conditional recommendation based on a very low certainty in the evidence about effects ⊕○○○).

Remark:

- Patients with positive Congo Red staining should be re-evaluated for signs and symptoms of systemic AL amyloidosis.

Good practice statement. In individuals with suspected AL amyloidosis, testing of historical pathology samples (within last 3 years) for amyloidosis could be done.

Good practice statement. The ASH panel agreed that in individuals with proven amyloid deposits on histology, amyloid subtyping using a validated method must be performed in a timely fashion.

Summary of the evidence

Systemic AL Amyloidosis can coexist in patients with plasma cell dyscrasias including newly diagnosed multiple myeloma and smoldering multiple myeloma; AL amyloidosis can also be detected in some patients with low clonal plasma cell burden and MGUS. Bone marrow biopsies are routinely performed for the diagnosis of multiple myeloma and smoldering myeloma. Congo red staining on previously performed bone marrow biopsy for the detection of amyloid presents a potential method for detecting concurrent AL amyloidosis in these patients.

There is a paucity of literature on the prevalence of amyloid deposits in the bone marrow biopsy specimens in patients with MGUS such that this population could not be evaluated by a systematic review.

The ASH guideline panel found no systematic reviews that addressed this question. The panel identified 21 relevant cohort and case-control studies. These studies measured the incidence of amyloidosis as

detected by Congo red stain of bone marrow samples. Samples were from patients who already had bone marrow biopsies performed as standard of care in patients with plasma cell dyscrasias (multiple myeloma, smoldering multiple myeloma). The panel judged the diagnostic performance of this testing to be accurate, with a sensitivity of 55% (95% CI: 45% to 64%). The panel would like to highlight that test diagnostic accuracy is heavily dependent on user skill; experience and expertise in the interpretation of Congo red staining for amyloidosis diagnosis is required. In the literature, the specificity estimates described do not necessarily reflect clinical scenarios of light chain amyloidosis as other subtypes of amyloidosis were included within the disease-negative population. Therefore, the specificity did not play a role in decision-making. Another limitation in the literature is that these studies did not include the duration between the index and reference tests and not the same reference test(s) were used.

Benefits

The panel judged that the desirable effects of performing Congo red staining on bone marrow biopsies already performed as standard of care in patients with multiple myeloma and smoldering multiple myeloma as moderate. Performing Congo red stain on such samples has the benefit of early diagnosis and decrease in the time interval between establishment of AL amyloidosis diagnosis and amyloid directed therapy. Early and accurate diagnosis may also decrease mortality. In cases with false negative testing, i.e., amyloid deposits were present but not detected, these patients may have a delayed diagnosis with significant impact on prognosis. However, there is discordance regarding the impact of finding Congo red positive amyloid deposits on prognosis of these patients. Petruzzello et al¹⁸⁷ showed limited impact on prognosis while Veda-Ojeda et al¹⁸⁸ showed it as an independent high risk prognostic factor. Nonetheless, the panel judged this as moderate for desirable effects favoring the early detection of systemic AL amyloidosis.

While false positives (positive Congo red staining on bone marrow biopsy and not having AL amyloidosis, i.e., bone marrow only AL amyloid deposits) can be a limitation of this test, it is important for healthcare providers to carefully consider the risks and benefits of testing, as well as to communicate effectively with patients about the possibility of amyloidosis and the need for further evaluation if necessary. The panel would also like to highlight cases of inconclusive results from bone marrow biopsy test and the possibility for additional testing which would otherwise not be required.

Harms and burden

Congo red staining on such bone marrow biopsy samples can increase diagnosis of amyloidosis. However, they do carry small potential harms and burden. False positives can lead to unnecessary anxiety and further invasive testing, while false negatives may delay appropriate diagnosis and treatment. There is also possible harm due to inconclusive results from bone marrow biopsy typing. The panel does recognize the possibility for additional testing which would otherwise not be required. In one study¹⁸⁷, after a mean follow-up of around 2 years range: 2- 99 months, 0 out of 51 patients has developed overt signs attributable to systemic amyloidosis while being observed with no plasma cell directed therapy. In another patient cohort,¹⁸⁹ reported 2 patients with asymptomatic amyloid deposits did not develop systemic amyloidosis after 7 years of follow up. In the cohort from ¹⁹⁰, 3 out of 68 with positive bone marrow developed systemic amyloidosis. Among these 3 populations 3/121 (2.4%) of patients with amyloid deposits developed systemic amyloidosis. However, the impact of treatment for those patients was not evaluated. According to estimates from centers of the panel members, the percentage of inconclusive results of mass spectrometry on bone marrow biopsy is less than 5%. ¹⁹¹ The panel believes that there is no direct harm from the test as the bone marrow biopsy was already performed for the diagnosis of multiple myeloma/smoldering myeloma but understand potential for

possible harm due to inconclusive results from bone marrow biopsy typing. Expert members of the panel emphasized that the performing an amyloid directed review of systems was likely as important. The panel strongly emphasizes that amyloid-directed treatment should never be initiated based on bone marrow Congo red staining alone.

Other EtD criteria and considerations

The panel reviewed the certainty of evidence of management of effect to be very low due to the lack of data to inform this decision. The panel judged that there is effect to management in patients such as changes in initial therapy, transplant eligibility, early involvement of cardiology, need for initial and subsequent cardiac monitoring (both biomarkers and imaging). The panel recognizes the potential for false positive test results and emphasizes on need for subtyping. If test results are negative (false negative and true negative), the panel does not expect change in management.

The panel determined that there was possibly important uncertainty or variability in how much the main stakeholders value the outcomes of the Congo red staining. A false positive would lead to further testing (IHC or Mass Spec) which may accurately diagnose the patient. The time delay in results can also lead to additional stress to the patient. A false negative may lead to delay in diagnosis, impacting treatment and possibly prognosis. Furthermore, the panel judged that there would be no direct harm from the test as the bone marrow biopsy was performed for the diagnosis of multiple myeloma/smoldering myeloma. As there is no extra procedure being performed, the test is deemed to be acceptable to the stakeholders.

As per estimate from selected panel members, cost for Congo red testing is approximately \$200, which the panel considered to be a reasonable cost. The panel would again like to emphasize that the recommendation is to stain samples already obtained as standard of care practice. There was no research evidence to suggest impact on cost effectiveness and health equity. Congo red staining is

readily available in most centers. The panel again emphasizes the need for experienced and expert pathologists' availability in interpretation of the staining.

The value of the bone marrow biopsy for detecting amyloid is strongly enhanced when periosteal tissue is present. Interstitial stromal and/or vascular amyloid deposits were common in AL cases but were absent/rare in ATTR. Exclusive involvement of the periosteal soft tissue was more common in ATTR than AL. The sensitivity of detecting amyloid goes up if periosteal tissue is also examined.^{191,192} A formal analysis of pathologic interpretation for periosteal/stromal or vascular amyloid deposition was beyond the scope of this review. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for [Recommendation 11](#).**

Conclusions and research needs for this recommendation

The panel determined that in patients with multiple myeloma and smoldering multiple myeloma, Congo red staining should be performed on bone marrow biopsies already performed, even in the absence of amyloidosis related symptoms or elevated biomarkers. The panel does not suggest performing bone marrow biopsies in patients with plasma cell dyscrasia (e.g. Low risk MGUS and without clinical suspicion of systemic amyloidosis) for the sole purpose of Congo red staining.

The panel determined that there is low certainty evidence for a net health benefit from performing Congo red staining on bone marrow biopsies already performed in patients with multiple myeloma and smoldering multiple myeloma). Based on available evidence, it is likely that this test could increase clinical suspicion for AL amyloidosis. The panel recognizes that there is very low certainty that there is an effect of performing these tests on other outcomes. However, because of low certainty of evidence or no published information about other outcomes, the absence of evidence of an effect on these outcomes does not imply that such an effect does not exist.

The panel also recognized that in some cases performing Congo red staining on previously performed bone marrow would affect management. Hence, it is highly recommended that these management changes be made along with expert consultation, after accurate subtyping of the amyloid protein. The panel recognizes the potential for false positive test results and additionally emphasizes the need for further amyloid subtyping. If test results are discordant, expert pathological consultation should be sought. In the panel discussion, we believe performing Congo red staining would be a cost-effective way to screen for amyloidosis. The panel also recommends performing Congo red staining on historical biopsies (within the last 3 years) if possible. While Congo red staining on these samples may help confirm diagnosis, these should not delay testing for diagnosis of amyloidosis. Moreover, if historical Congo red stain positive samples were obtained from different organ/tissue this may support systemic nature of amyloid deposits.

The panel identified the following additional implementation remarks:

1. When Congo red staining is positive on the bone marrow, the panel strongly encourages subtyping the amyloid using validated methods.
2. The interpretation of the Congo red staining may vary based on the experience and expertise of the pathologist. If there is difficulty in interpretation, the panel suggest evaluating for second opinion pathology at an academic medical center.
3. The panel does not suggest performing bone marrow biopsies in patients with plasma cell dyscrasia (e.g. Low risk MGUS and without clinical suspicion of systemic amyloidosis) for the sole purpose of Congo red staining. However, if there is a clinical suspicion of amyloidosis, bone marrow biopsy combined with fat sampling can be utilized in the diagnosis of amyloid with Congo red stain (refer to Supplement 5).

The panel identified the following additional research questions:

1. The panel recognized that MGUS encompasses a heterogeneous group of patients and research to develop further stratification of MGUS should be prioritized. Signs and symptoms of target organ damage attributable to amyloid deposits (heart, kidney, and peripheral nervous system) are incompatible with MGUS.
2. The members of the panel judged that the management of plasma cell disorders must be considered in the context of the growing recognition of monoclonal gammopathy of renal significance (MGRS) and emergence of monoclonal gammopathy of clinical significance (MGCS).
3. The panel members considered cases of “silent” marrow amyloidosis, i.e., Congo red positive amyloid deposits without signs or symptoms of systemic amyloidosis. There is a lack of data regarding prognosis and management of such cases. Further investigation is required in these cases. The panel strongly emphasizes that amyloid-directed treatment should never be initiated based on bone marrow Congo red staining alone.

Organ involvement

In individuals with AL amyloidosis with no cardiac symptoms, should clinicians use cardiac biomarkers/investigations [BNP, NT-proBNP, troponin (I,C,T, Highly Sensitive), 2D Echo with strain, Cardiac MRI] or not to evaluate for cardiac involvement?

Recommendation 12. For individuals with proven AL amyloidosis and with no cardiac symptoms, the ASH Guideline Panel *recommends* performing cardiac biomarkers (high sensitivity troponin, and BNP or NT-proBNP) and cardiac imaging rather than not performing these tests to define the presence and extent of cardiac involvement at diagnosis. (strong recommendation based on low certainty in the evidence about effects

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Remarks:

- The ASH panel recognizes that “cardiac involvement” has historically been defined by echocardiography¹⁰; however, CMR is a more accurate test with better diagnostic performance, albeit often limited by cost and availability.
- Echocardiography should be performed with measurement of left ventricular global longitudinal strain to evaluate for reduction in longitudinal shortening and sparing of the apex (sensitivity 72%, may be absent in 28% of patients).
- The panel recognizes that NT-proBNP has been studied more than BNP in AL amyloidosis, but if not available, BNP is an acceptable alternative cardiac biomarker.
- The panel recognizes that cardiac biomarkers (troponin, BNP, NT-pro BNP) results may be affected by kidney function and pre-existing cardiac conditions.
- The panel recognizes that most evidence in amyloidosis is using hsTroponin T, but also recognizes that some centers may not have access to it and other troponin measures that are less extensively evaluated in AL amyloidosis can be used.
- The panel recognizes that a hsTroponin T cut-off of 35 ng/L has a sensitivity of 83% and a specificity of 86% for detecting cardiac involvement. On the other hand, a cut-off of 14 ng/L has a sensitivity of 94% but a specificity of only 11%. Therefore, a 35 ng/L cut-off is more specific, while a 14 ng/L cut-off is more sensitive for cardiac involvement.¹¹
- In patients with known AL amyloidosis, for high hsTroponin T, the recommendation was based on a cutoff of 35 ng/L and a pretest probability of 40% that yields a positive predictive value (PPV) of 0.79 and a negative predictive value (NPV) of 0.88.

Summary of the evidence – Echocardiography

Echocardiography is a widely available non-invasive diagnostic test that can evaluate for structural and functional changes associated with cardiac amyloidosis.⁹¹ The panel reviewed 36 studies with 4,956 patients that evaluated echocardiographic features seen in cardiac amyloidosis. Echocardiographic features with high sensitivity and specificity were prioritized to provide incremental diagnostic information and included left ventricular hypertrophy, diastolic dysfunction, and reduced global

longitudinal strain with an apical sparing pattern. While in clinical practice integration of multiple echocardiographic features may be useful to increase clinical suspicion for amyloidosis, the panel could not make recommendations about the possible additive benefit of combining these parameters because of the bias that occurs when evaluating different components of the same test.

Left ventricular wall thickness on echo is typically obtained by measuring the interventricular septum (IVS) and posterior wall (PW).¹⁹³ A left ventricular wall thickness (IVS and/or PW) ≥ 12 mm unexplained by other causes may be useful to screen for cardiac involvement in patients with AL amyloidosis (IVS: sensitivity 77%, specificity 71% and PW: sensitivity 76%-100%, specificity 84-89%). Diastolic function is assessed by echocardiography by integrating multiple features.¹⁹⁴ One technique to evaluate diastolic function compares the flow across the mitral valve in early diastole from early filling (E wave) to late diastole due to atrial contraction (A wave) to generate a ratio of early versus late mitral valve flow known as the E/A ratio. The E/A ratio is typically greater than or equal to 2 in patients with more advanced diastolic dysfunction due to high filling pressures. An increased E/A ratio has a sensitivity of 45-65% and specificity of 85-98%. Two studies were excluded from the analysis with an E/A ratio near 1 as this may be close to normal in an older population. The E/A ratio combined with other echocardiographic parameter is used to assess for the stage of diastolic dysfunction from I-III, with higher grades indicating more severe impairment. Stage II and III diastolic dysfunction are often seen in cardiac amyloidosis and combined have a sensitivity of 71% and specificity of 78%. Grade III diastolic dysfunction, also known as a restrictive diastolic filling pattern, has a sensitivity of 42% and specificity of 89%.

Global longitudinal strain (GLS) is a semi-automated technique that can evaluate subclinical changes in mechanical deformation of the left ventricle.^{195,196} GLS values are typically negative when myocardial segments shorten, and a higher absolute value indicates more shortening/contraction. For example, GLS of -14 is reduced or worse compared to a GLS of -17 . There are no established normal ranges for

GLS as they may vary with the vendor and software package, however for reference the American Society of Echocardiography states that a value in the range of -20 would be expected in a healthy person.¹⁸² Reduced GLS $\leq -17\%$ has a high sensitivity (86%) and specificity (78%) to identify cardiac amyloidosis. In cardiac amyloidosis the commonly observed pattern in cardiac amyloidosis is that the strain is preserved/normal in the left ventricular apex compared to the basal and mid segments where it is impaired and is referred to as an apical sparing pattern.⁷⁵ The reduced contractile function of the basal and mid segments leads to an overall reduction in global longitudinal strain when the segments are averaged. The pooled sensitivity and specificity of an apical sparing pattern was 72% and 78% respectively. Therefore, in 28% of patients, an apical sparing pattern may not be present, so cardiac amyloidosis should not be excluded in patients with a high clinical suspicion that lack an apical sparing pattern.

Summary of the evidence – Cardiac MRI

Pooled analysis suggests the diagnostic test accuracy of CMR to be between accurate and very accurate, with a sensitivity of 95% and specificity of 87%. This determination is a product of systematic review across 7 cohort and case-control studies that examined the diagnostic test accuracy of the finding of late gadolinium enhancement (LGE) on cardiac MRI for diagnosis of cardiac amyloidosis.

Summary of the evidence – Biomarkers

Cardiac biomarkers NT-proBNP, BNP and troponin are an essential part of cardiac involvement assessment in patients with AL amyloidosis, cardiac involvement would have significant impact patient's prognosis and management, and there also part of initial staging in any newly diagnosed AL amyloidosis patient.^{197,198}

The ASH guideline panel analyzed 8 studies that included 645 patients, using NT-proBNP at a 332ng/l cut off the panel found it was highly sensitive (0.99) and had low specificity (0.53). The panel also

analyzed the data for BNP in 6 studies including 257 patients with AL amyloidosis using 3 different cutoffs and the data was heterogeneous. The panel also looked at High Sensitivity troponin at a cut-off of 35ng/L showed high sensitivity (0.83) specificity (0.86).

Benefits – Echocardiography with LV GLS strain

Given that echocardiography is a low cost, widely available diagnostic modality, and the fact that cardiac involvement in patients with AL amyloidosis is an important prognostic factor used in staging the disease and guiding treatment options, echocardiography in asymptomatic patients offers significant benefit and may help direct patient care.

Benefits – Cardiac MRI

In comparison to echocardiography, cardiac MRI offers greater levels of sensitivity and specificity for presence of cardiac amyloidosis in patients with AL amyloidosis who may not have cardiac symptoms. As mentioned, cardiac involvement impacts treatment choice and prognosis, and thus, ascertaining presence or absence of amyloid within the myocardium is an important step in management of this systemic disease. Patients with negative scans can be reliably ruled out for cardiac amyloidosis with 87% specificity, while patients with cardiac amyloidosis can be detected with 95% sensitivity. Apart from disease discrimination, cardiac MRI offers the added benefit of disease quantification with measurement of extracellular volume (ECV). Quantification of disease burden may assist with greater levels of risk stratification, assessment of disease progression, and monitoring disease response to various therapeutic strategies.

Benefits – Biomarkers

The panel found there is a moderate desirable effect of performing cardiac biomarkers (NT-proBNP, BNP, and troponin), ¹⁹⁹ found NT-proBNP was found to be cost effective in patients that were assessed

for heart failure. The panel believes the same could be considered in AL amyloidosis patients due to its sensitivity, biomarkers can be reported in minutes making them feasible, all these factors favor in balance of effects the intervention.

Harms and burden – Echocardiography

Echocardiography is a safe a non-invasive test with no major adverse events. Minor side effects include possible patient discomfort and mild bruising. The price of echocardiography varies by country, health system, and the type of medical insurance. The panel found the cost in the United States varies from \$204 to \$3,000 in the United States and is 88 euros in the United Kingdom. The costs may be affected by the presence or absence of medical insurance and the location of where the test is performed.^{199,200}

There is no research evidence about the cost effectiveness of performing echocardiography in patients with AL amyloidosis. The panel did not think it was appropriate to extrapolate cost effectiveness data from other populations.

The panel recommends that global longitudinal strain be performed to evaluate for cardiac involvement in patients with AL amyloidosis. The technique is semi-automated and requires the use of special analysis software to existing echo machines. Although, additional software may be required, the panel thought this would be feasible to perform during standard echocardiograms to improve detection of cardiac involvement in patients with amyloidosis.

The parameters described to screen for cardiac involvement by echocardiography may be less sensitive in early cardiac disease and could lead to false negative diagnoses. In patients with early cardiac involvement with a non-diagnostic echo, cardiac MRI may be more sensitive to detect cardiac involvement if available and not cost prohibitive.

Harms and burden – Cardiac MRI

Risk of harm from CMR is very low. Across the studies evaluated, major complications were not reported, and the incidence of minor complications such as nausea, vomiting, and palpitations was reported at 0.04% to 0.06%.

Harms and burden – Biomarkers

There is no direct undesirable effects related to cardiac biomarkers as the test is safe and simple, and part of routine blood test. And the panel concluded that the undesirable effects of performing biomarkers would be small.

The panel recognizes that institutional level of experience is needed as some centers may not have access and routine do hsTroponin and there are several manufactures that can lead to variability in the results. The panel recognizes that BNP may be more readily available in most centers and is an alternative to NT-proBNP.

Other EtD criteria and considerations – Echocardiography

Other conditions besides amyloidosis, such as hypertension, can also be associated with left ventricular hypertrophy. In many of the studies the panel evaluated the patients also had hypertension so the specificity of IVS/PW thickness may be impacted. Hypertension is more prevalent in African Americans and may affect the specificity of left ventricular wall thickness (IVS/PW) in this population. Assessment of left ventricular hypertrophy may also be impacted by other factors such as sex, body habitus, and image quality. A left ventricular wall thickness up to 11 mm is often considered the upper limits of normal. However, the reference ranges used to evaluate for left ventricular hypertrophy may not be appropriate in female patients and patients with a smaller body habitus as values <12 mm may represent left ventricular hypertrophy.¹⁹³ Additionally, in obese patients or with other factors that

affect image quality (ex. Breast implants) echocardiographic image quality may be suboptimal and non-diagnostic. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for** Echocardiography Considerations - [Recommendation 12](#).

Other EtD criteria and considerations – Cardiac MRI

Cost and availability remain limiting barriers to greater utilization of cardiac MRI. Apart from these factors, institutions must further consider their physician expertise for interpretation, as advanced imaging training is often needed for appropriate evaluation of scan findings. One other consideration is patient comfort, given the required scan time within a confined space. Despite these considerations, the panel determined CMR to be acceptable to the vast majority of patients, and its superior diagnostic performance profile renders it the most sensitive and specific imaging examination for evaluation of cardiac amyloidosis. **Evidence profiles with the characteristics of all included studies and the complete ETD framework are online for** [Cardiac MRI Considerations - Recommendation 12](#).

Other EtD criteria and considerations – Biomarkers

The panel recognizes that BNP results may be affected by kidney function or pre-existing cardiac disease. Due to the inconsistencies in the reviewed literature, and variation in clinical practices, the panel does not specify either specific cardiac biomarkers nor cutoffs of these biomarkers. Furthermore, the panel recognizes that the cardiac biomarker and its cutoff may be different depending on the presence or absence of symptoms.

Cardiac biomarkers should be used in conjunction with cardiac imaging to rule out cardiac involvement, and most patients have both cardiac biomarkers and cardiac imaging performed at the same time, as these may avoid delays.

The panel recognizes there are limited data to support their conclusion and highlights the need for further research in this area. **Evidence profiles with the characteristics of all included studies and the**

complete ETD framework are online for [Troponin Considerations - Recommendation 12](#), [BNP Considerations - Recommendation 12](#), [NT-PROBNP Considerations - Recommendation 12](#).

Conclusions and research needs for these recommendations

Echocardiography can be helpful to identify cardiac involvement in AL amyloidosis in patients with advanced cardiac disease. Other techniques such as cardiac MRI or biopsy may be required in patients with early disease or nondiagnostic echocardiograms to confirm a diagnosis. More research is needed to determine appropriate thresholds to diagnose identify left ventricular hypertrophy in women as well as on techniques to distinguish left ventricular hypertrophy from cardiac amyloidosis from other causes. Artificial intelligence is being increasingly used in medicine and could potentially be leveraged to identify cardiac amyloidosis on echocardiography.

The guideline panel determined that there is low certainty of evidence of test accuracy of performing hsTroponin on patients with known AL amyloidosis and without any cardiac symptoms but strongly recommend performing BNP or NT-proBNP and troponin rather than not performing it, as it is also essential for staging of patients with AL amyloidosis and will increase the clinical suspicion of cardiac involvement.

What are others saying and what is new in these guidelines?

1. The College of American Pathologists (CAP) is in the process of publishing a practice guideline on the workup of amyloidosis. This guideline is targeted towards pathologists and laboratories regarding amyloidosis workup and diagnosis.²⁰¹ In contrast to the more clinically oriented recommendations included in the current ASH guideline, the CAP guideline is primarily focused on identifying optimal laboratory approaches and techniques. The CAP guideline asks one

question that does have relevance outside of laboratory medicine: “In patients with suspected systemic amyloidosis (amyloid transthyretin protein [ATTR], amyloid serum A protein [AA], and amyloid light chain [AL]), does fat pad aspiration/biopsy, salivary gland biopsy, and rectal biopsy all provide adequate diagnostic sensitivity?” The ASH guideline panel views this question as complementary to those addressed in the current ASH guideline. ASH looks forward to the final publication of the CAP guideline.

2. In 2023, the American College of Cardiology (ACC) published an Expert Consensus document on Cardiac Amyloidosis. [2023 ACC Expert Consensus Decision Pathway on Comprehensive Multidisciplinary Care for the Patient with Cardiac Amyloidosis: A Report of the American College of Cardiology Solution Set Oversight Committee | Journal of the American College of Cardiology (jacc.org)].²⁰² The ACC guideline provides a clinical pathway for clinicians faced with patients with suspected cardiac amyloidosis, including advice on both diagnosis and treatment of cardiac amyloidosis. Importantly, the ACC recommendations emphasize the importance of a collaborative management approach between cardiologists and amyloidosis specialists. The ACC recommendations were not formulated according to a structured guideline approach such as GRADE or another established framework.²⁰³
3. In 2014, guidelines on the diagnosis and investigation of AL amyloidosis were published by the British Committee for Standards in Haematology (BCSH). This guideline was based on a Pubmed literature search for relevant publications between 1975 and 2014. The BCSH guideline highlights the importance of obtaining a tissue biopsy for the diagnosis of AL amyloidosis, a point with which the ASH guideline strongly agrees. The BCSH guideline recommends that the extent of organ involvement and should be assessed by non-invasive criteria including serum amyloid P component (SAP) scanning. The current ASH guideline does not assess the role of SAP scanning in the management of AL amyloidosis. SAP scanning is not routinely available in North

America, and the ASH guideline panel elected to exclude this test from their systematic review and recommendations. The BCSH recommends the use of cardiac MRI as an adjunctive diagnostic test for cardiac amyloidosis, highlighting its advantages over echocardiography with respect to differentiating amyloidosis from other causes of left ventricular thickening. The current ASH guideline states that MRI is a particularly useful test when echocardiography is non-diagnostic.

4. The International Society for Amyloidosis (ISA) published a review of diagnostic approaches for amyloidosis in 2021, providing expert advice on choice timing of a selection of modalities.¹⁷ This narrative review was not formulated according to a structured guideline approach such as GRADE or another established framework.²⁰³

Limitations of these guidelines

In the realm of rare diseases, evidence generation is limited by difficulty in performing properly sized studies, limitations in dissemination of results, and are often heterogeneous. An inherent limitation in the evidence informing the recommendations is the rarity of AL Amyloidosis leading to limited evidence. Despite this, utilization of the limited evidence in conjunction with the panel's experience in diagnosis of AL Amyloidosis in an open discussion within the prioritized clinical scenarios allowed for the construction of detailed recommendations. This allowed for the discussion regarding the impact of different healthcare resources and geographic variations in the ETD considerations. Nevertheless, we acknowledge that due to the complexity of the diagnostic consideration of AL Amyloidosis, that the single comparative nature of the PICO questions may not fully capture diagnostic nuances or contextual factors

The limitations of these guidelines are inherent in the low or very low certainty of the evidence the panel identified for many of the questions. Low and very low certainty evidence was mainly due to risk of bias in the included studies as well as imprecision. Different references and different cutoffs for some of the evidence used to address certain PICO questions; the panel relied on the face validity of the studies for what a normal or abnormal test is. The availability of diagnostic tests and the expertise to interpret the results is variable and may affect how these guidelines will be used.

Revision or adaptation of the guidelines

Plans for updating these guidelines

After publication of these guidelines, ASH will maintain them through surveillance for new evidence, ongoing review by experts, and regular revisions.

Updating or adapting recommendations locally

Adaptation of these guidelines will be necessary in many circumstances. These adaptations should be based on the associated EtD frameworks.²⁰⁴

Priorities for research

Based on gaps in evidence identified during guideline development, the panel makes the following recommendations for further research:

- 1. Earlier detection of Amyloidosis/Enhancing clinical suspicion**
 - a. Impact of earlier detection of amyloidosis with respect to quality of life, patient experience, overall survival
 - b. Evaluating the generalizability of the iSTOPMM normal ranges for serum FLCs
 - c. Utility of validated tools for screening for AL amyloidosis
 - d. Choice and frequency of follow-up tests.

- e. Evaluation of the effect of cardiac biopsy using EM on early detection and outcomes
 - f. Utility of different biopsy/subtyping techniques on the diagnosis of AL amyloidosis with a focus on time to diagnosis
 - g. Accuracy of new techniques for monoclonal gammopathy detection and its effect on early diagnosis
 - h. Evaluating the utility and test accuracy of CMR versus ECHO in AL amyloidosis for diagnosis and staging
 - i. Evaluating the utility and test accuracy of CMR versus ECHO in AL amyloidosis for diagnosis and staging
- 2. Predictive Models for likelihood of AL amyloidosis or of organ involvement**
- a. Predictive modelling for the development of AL amyloidosis in patient pre-existing plasma cell dyscrasia and laboratory studies looking at the amyloidogenic propensity of proteins and the early detection of amyloid
 - b. Models of care impact on the diagnosis and outcome i.e. expert centre centralized model versus distributed model across a multidisciplinary team
 - c. Predictive modelling in imaging for likelihood of suspicion i.e. cardiac MRI
 - d. Disease heterogeneity - Diagnosing AL amyloidosis in patients with diabetes, the difficulties they face, the duration of time; comparing patients with and without neuropathy and nephropathy
 - e. Sex, race and gender differences in the clinical presentation of AL amyloidosis
 - f. Patients with renal failure - How does renal failure effect the staging predictability of existing cardiac biomarkers?
 - g. The prevalence of cardiac involvement in asymptomatic patients and their outcomes
- 3. Optimizing Diagnostic pathways**
- a. Studies to evaluate the utility of repeat surrogate biopsy in comparison to a target organ biopsy
 - b. Studies addressing optimal diagnostic pathways for GI, Liver, Nerve, Skin in AL amyloidosis
 - c. Cost effectiveness research on diagnostic pathways for amyloidosis i.e. use of various biomarkers and imaging such as cardiac MRI

- d. Optimal diagnostic pathway for patients who have both SIFE/UIFE/sFLC and ATTR
- e. Studies addressing the cutoffs for cardiac biomarkers for increasing suspicion and diagnosis of AL amyloidosis, with distinction between the cutoff for symptomatic and asymptomatic individuals.

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Authorship contributions

VK wrote the first draft of this manuscript and revised the manuscript based on authors' suggestions. Guideline panel members critically reviewed the manuscript and provided suggestions for improvement. Members of the knowledge synthesis team (MA, HA, RM, JN, QH, AA, TK, MMA, HKA, IA, MAA, NJ) contributed evidence summaries to the guidelines. All authors approved of the content. VK chaired the panel and led panel meetings. MS was the ex-officio representative from the ASH clinical practice guidelines oversight committee.

Disclosures of conflicts of interest

All authors were members of the guideline panel or members of the systematic review team or both. As such, they completed a disclosure of interest form, which was reviewed by ASH and is available as supplements 3 and 4.

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List of Abbreviations:

AA – Amyloid A protein

ACC – American College of Cardiology

ACE/ARB – Angiotensin converting enzyme/Angiotensin receptor blocker

AL – Amyloid light chain

ASH – American Society of Hematology

ATTR – Transthyretin amyloidosis

BCSH – British Committee for Standards in Haematology

BM – Bone marrow

BNP – Brain natriuretic peptide

CAD – Coronary artery disease

CAP – The College of American Pathologists

CMR – Cardiac magnetic resonance

CKD – Chronic kidney disease

CIDP – Chronic Inflammatory Demyelinating Polyneuropathy

D-VCD – Daratumumab, bortezomib, cyclophosphamide, and dexamethasone

DPD – 3,3-diphosphono-1,2-propanodicarboxylic acid

EANM – European Association of Nuclear Medicine

ECHO – Echocardiography

ECV – Extracellular volume

eGFR – Estimated glomerular filtration rate

EKG – Electrocardiogram

EM – Electron microscopy

EMB – Endomyocardial Biopsy

EtD – Evidence to Decision

FNAB – Fine needle aspiration biopsy

GIN – Guidelines International Network

GI – Gastrointestinal

GLS – Global longitudinal strain

GRADE – Grading of Recommendations Assessment, Development and Evaluation

HFpEF – Heart failure with preserved ejection fraction

HIV – Human Immunodeficiency Virus

HMDP – Hydroxymethylene Diphosphonate

H/CL ratio – Heart-to-contralateral ratio

hsT – High sensitivity Troponin

ICD – Implantable cardioverter-defibrillator

ICC – International Consensus Classification

IENFD – Intraepidermal nerve fiber density

Ig – Immunoglobulin

IHC – Immunohistochemistry

ISA – The International Society for Amyloidosis

iStopMM – Iceland screens, treats or prevents multiple myeloma

IVS – Interventricular septum

LGE – Late gadolinium enhancement

LVH – Left ventricular hypertrophy

MGRS – Monoclonal gammopathy of renal significance

MGCS – Monoclonal gammopathy of clinical significance

MGUS – Monoclonal gammopathy of undetermined significance

MIBG – Metaiodobenzylguanidine

MRI – Magnetic resonance imaging

NPV – Negative predictive value

NT-proBNP – N-terminal pro-B-type natriuretic peptide

NY – New York

PICO – Patient or problem, Intervention, Comparison, and Outcome

PPV – Positive predictive value

PW – Posterior wall

PYP – Pyrophosphate

QUADAS – Quality Assessment of Diagnostic Accuracy Studies

QST – Quantitative sensory testing

QSART – Quantitative sudomotor axon reflex test

RCT – Randomized controlled trials

ROI – Region of interest

SAA – Serum amyloid A protein

SAP – Serum amyloid P component

sFLC – Serum free light chain values with ratio

SIFE – Serum immunofixation

SLE – Systemic Lupus Erythematosus

SPECT – Single Photon Emission Computed Tomography

TTR – Transthyretin

Tc – Technetium

UIFE – Urine immunofixation

US – United States

USD – US Dollar

Table 1: Types of Surrogate Site Biopsies

Surrogate Site Biopsies	
<u>In-Scope</u>	<u>Out of Scope</u>
Abdominal fat pad	Buccal/Gingival Mucosa/Lip/minor Salivary Gland
Bone marrow	Rectal
	Spinal surgical specimens (i.e. ligamentum flavum)
	Carpal Tunnel release Surgical Samples (Flexor retinaculum)
	Prostate
	Bladder

Table 2: Summary of PICO questions and methodological approach

PICO Number	PICO Question	Population of Interest	Index Test	Reference Test
1	Should serum and urine immunofixation (SIFE and UIFE) and serum free light chains (sFLC) be used to increase suspicion of AL amyloidosis in individuals with cardiac symptoms?	Suspicion of AL amyloidosis with cardiac symptoms	SIFE/UIFE/ sFLC	Surrogate or Target Organ Biopsy
2	Should serum and urine immunofixation (SIFE and UIFE) and serum free light chains (sFLC) be used to increase suspicion of AL amyloidosis in individuals with unexplained proteinuria?	Suspicion of AL amyloidosis with unexplained proteinuria	SIFE/UIFE/ sFLC	Surrogate or Target Organ Biopsy
3	Should cardiac MRI (CMR) be used to diagnose cardiac AL amyloidosis in individuals suspected of having cardiac amyloidosis (positivity in any of the following studies: SIFE, UIFE, or sFLC, abnormal cardiac biomarkers, and non-diagnostic	Individuals with positivity in any of the following studies: SIFE, UIFE, or sFLC, abnormal cardiac biomarkers, and non-diagnostic echocardiographic findings	MRI	EMB

	echocardiographic findings)?			
4	Should cardiac MRI (CMR) be used to diagnose cardiac AL amyloidosis in individuals with abnormal cardiac biomarkers, echocardiography, and positivity in any of the following studies: SIFE, UIFE, or sFLC?	Individuals with abnormal cardiac biomarkers, echocardiography, and positivity in any of the following studies: SIFE, UIFE, or sFLC	MRI	EMB
5	Should Bone Scintigraphy with technetium 99m – pyrophosphate (PYP), technetium 99 m – 3, 3 diphosphono –1,2 propranodicarboxylic (DPD) and technetium 99 m- hydroxymethylene diphosphonate (HMDP) be used to diagnose AL Amyloidosis in suspected individuals?	Suspicion of AL amyloidosis with cardiac symptoms	Bone Scintigraphy	EMB
6	Should Bone Scintigraphy (PYP, DPD, HMDP) be used to diagnose ATTR Amyloidosis in suspected individuals?	Suspicion of ATTR amyloidosis	Bone Scintigraphy	EMB
7	Should surrogate biopsy vs cardiac biopsy be used to	Suspicion of cardiac AL amyloidosis	Fat Pad Sample or	EMB

	diagnose AL amyloidosis in individuals suspected to have cardiac amyloidosis?		Bone Marrow Biopsy	
8	Should surrogate biopsy vs renal biopsy be used to diagnose AL amyloidosis in individuals suspected to have renal amyloidosis?	Suspicion of renal AL amyloidosis	Fat Pad Sample or Bone Marrow Biopsy	Renal Biopsy
9	Should surrogate biopsy vs peripheral nerve biopsy be used to diagnose AL amyloidosis in suspected patients?	Suspicion of neurological AL amyloidosis	Fat Pad Sample or Bone Marrow Biopsy	Peripheral Nerve Biopsy
10	Should surrogate biopsy vs Skin Biopsy (for intraepidermal nerve fibre density) biopsy be used to diagnose AL amyloidosis in individuals suspected to have neurological amyloidosis?	Suspicion of neurological AL amyloidosis	Fat Pad Sample or Bone Marrow Biopsy	Skin Biopsy (for intraepidermal nerve fibre density)
11	Should surrogate biopsy vs target organ biopsy be used to diagnose AL amyloidosis in individuals suspected to have AL amyloidosis with multiorgan presentation?	Suspicion of multisystem AL amyloidosis	Fat Pad Sample or Bone Marrow Biopsy	EMB Renal Biopsy Peripheral Nerve Skin Gastrointestinal (GI) Tract Liver

12	Should Congo Red Staining on bone marrow biopsy already performed be used to diagnose AL amyloidosis in individuals with Multiple Myeloma and Smoldering Myeloma?	Multiple Myeloma and Smoldering Myeloma	Congo Red Staining on Bone Marrow Biopsy	Organ Biopsy
13	In individuals with AL amyloidosis with no cardiac symptoms, should clinicians use cardiac biomarkers/investigations [BNP, NT-proBNP, troponin (I,C,T, Highly Sensitive), 2D Echo with strain, Cardiac MRI] or not to evaluate for cardiac involvement?	AL amyloidosis	ECHO	Composite reference including cardiac imaging (ECHO or MRI) and surrogate biopsy or EMB

Table 3. Interpretation of Strong and Conditional Recommendations

Recommendation Strength				
	“Recommends...”	“Recommends against...”	“Suggests...”	“Suggests against...”
				
	Interpretation of Strong Recommendations		Interpretation of Conditional Recommendations	
Patients	Most individuals in this situation would want the recommended course of action, and only a small proportion would not.		Most individuals in this situation would want the suggested course of action, but many would not. Decision aids may be useful in helping patients to make decisions consistent with their individual risks, values, and preferences.	
Clinicians	Most individuals should follow the recommended course of action. Formal decision aids are not likely to be needed to help individual patients make decisions consistent with their values and preferences.		Different choices will be appropriate for individual patients; clinicians must help each patient arrive at a management decision consistent with the patient’s values and preferences. Decision aids may be useful in helping individuals to make decisions consistent with their individual risks, values, and preferences.	
Policymakers	The recommendation can be adopted as policy in most situations. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator.		Policymaking will require substantial debate and involvement of various stakeholders. Performance measures should assess if decision making is appropriate.	
Researchers	The recommendation is supported by credible research or other convincing judgments that make additional research unlikely to alter the recommendation. On occasion, a strong recommendation is based on low or very low certainty in the evidence. In such instances, further research may provide important information that alters the recommendations.		The recommendation is likely to be strengthened (for future updates or adaptation) by additional research. An evaluation of the conditions and criteria (and the related judgments, research evidence, and additional considerations) that determined the conditional (rather than strong) recommendation will help identify possible research gaps.	

Table 4: Renal Function and Light Chain Values

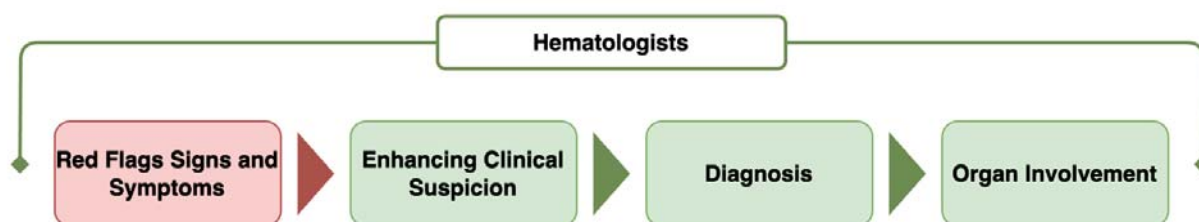
Kidney function mL/min/1.73 m ²	Kappa (mg/L)	Lambda (mg/L)	sFLC ratio
eGFR 45–59	7.8–83.6	7.3–65.1	0.46–2.62
eGFR 30–44	8.8–103.3	8.2–73.2	0.48–3.38
eGFR <30	11.7–265.1	12.6–150.9	0.54–3.30

Figure Legends

Figure 1: Diagnostic Pathway for Light Chain (AL) Amyloidosis

Diagnosis pathway for AL amyloidosis including 1) Red Flag Signs and Symptoms which are used to increase clinician awareness of the possibility of AL amyloidosis; 2) Enhancing Clinical Suspicion in patients who may have a diagnosis of AL amyloidosis; 3) Diagnosis – how to make a timely and effective diagnosis; 4) Organ Involvement – how to confirm if an organ is involved in patients with a biopsy proven diagnosis of AL amyloidosis.

Figure 2: Diagnostic Algorithm for the Evaluation of Cardiac Amyloidosis

Figure 1: Diagnostic Pathway for Light Chain (AL) Amyloidosis

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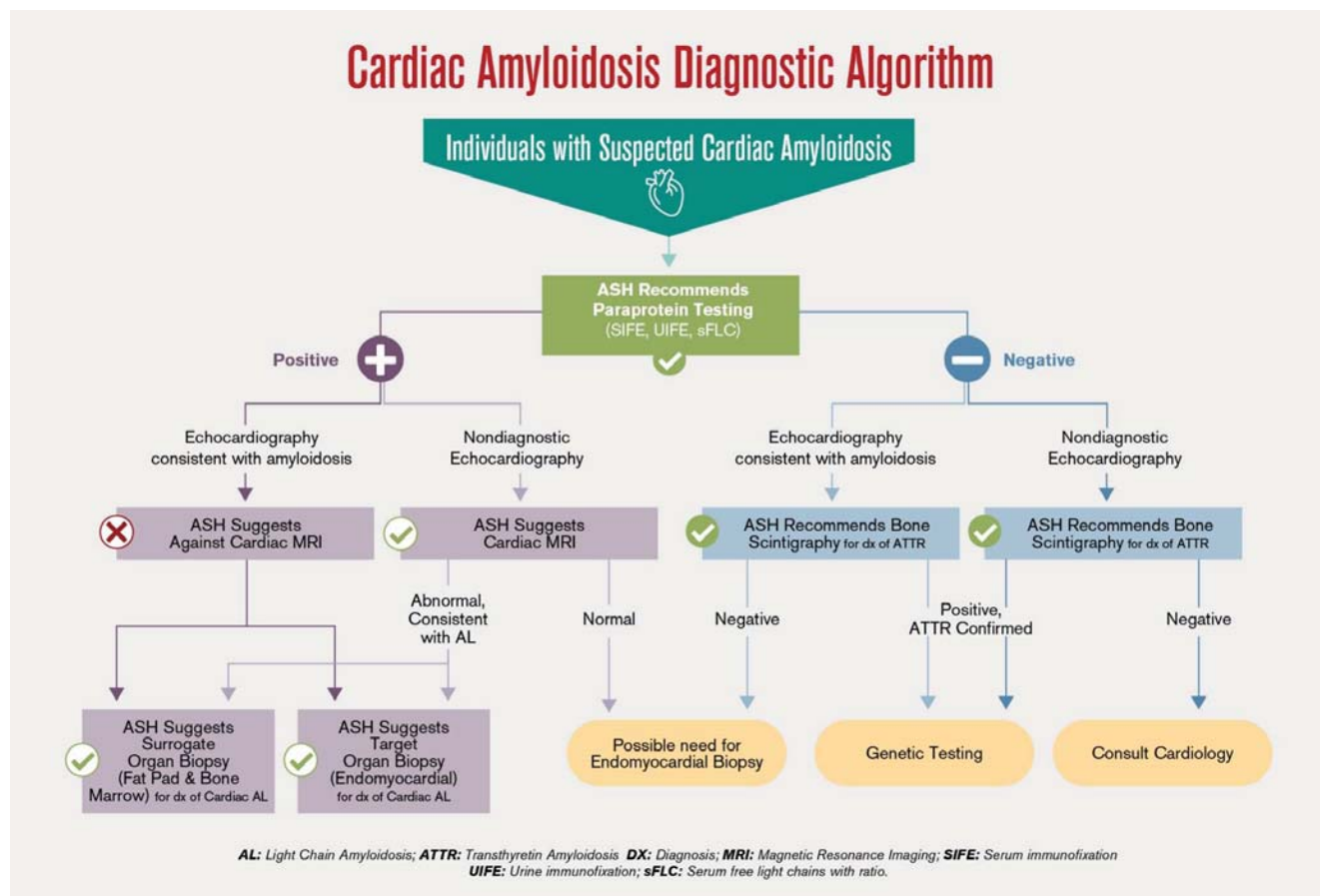


Figure 2: Diagnostic Algorithm for the Evaluation of Cardiac Amyloidosis