Should the Reporting of Bone Marrow Positivity for Amyloid Be Revised?

A Critical Assessment Based on 66 Biopsies From a Single Institution

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Amyloidoses are a heterogeneous group of disorders that are characterized by the deposition of abnormal proteins in tissues. In this process, globular, soluble proteins misfold and aggregate into insoluble fibril ("amyloid") aggregates that lead to progressive organ damage.1

By electron microscopy, amyloid deposits consist of rigid, nonbranching fibrils that typically measure 8–12 nm in diameter. The fibrils possess a cross-β-pleated structure, in which β-strands are oriented perpendicularly to the fibril axis. To date, 36 proteins have been identified to be amyloidogenic in humans.1,2 Amyloid deposits may be localized, as in Alzheimer disease or type II diabetes, or systemic, affecting various organs or tissues throughout the body. The clinical manifestations of amyloidosis are heterogeneous and may be influenced by both genetic and environmental factors.

The most common type of systemic amyloidosis is amyloid light chain (AL) amyloidosis, where fibrils are derived from the immunoglobulin light chain or its fragment.1,3-5 Approximately 2200 new cases of AL are diagnosed every year in the United States, and there has been a 2.6-fold increase between 2007 and 2015, from 15.5 to 40.5 cases per million.3 Other systemic amyloidoses include AA, ALECT2, and hereditary amyloidoses.1 AA (amyloidosis derived from serum amyloid A protein, acute-phase reactant) is typically associated with an underlying chronic inflammatory process, whereas the pathogenesis of ALECT2 (amyloidosis derived from leukocyte chemotactic factor 2) is unclear; no genetic component has emerged as yet, despite a strong association with Mexican American ethnicity. Among the hereditary amyloidoses, most types are individually rare, but collectively hereditary amyloidoses constitute approximately 10% of all systemic amyloidoses currently diagnosed. This may, however, represent under-diagnosis.6-9 Of this group, amyloidosis derived from a transthyretin variant (ATTRV) has begun to emerge as a significantly underdiagnosed cause of cardiac failure and polyneuropathy; ATTR derived from the wild type amyloid A (AA). The initial diagnosis of amyloidosis was made in a BM specimen in 21 of 66 cases (31.8%). Plasma cells ranged from 1% to 80% (mean, 13.4%; median, 8%; <10% in 44 of 66 specimens [66.6%]) and were monoclonal in 58 of 66 cases (87.8%), and in 54 of 66 cases (81.8%) amyloid deposits were documented in at least one other organ.

Conclusions.—This study demonstrates that there is significant heterogeneity in the spatial distribution of amyloid in BM biopsy specimens with medullary, extra-medullary, purely vascular, or combined involvement. Whereas stromal deposits were associated exclusively with AL, nonstromal and purely vascular deposits were seen in at least 3 types of systemic amyloidosis (AL, AA, and ATTR). We discuss the reporting of BM biopsy tissue positivity for amyloid deposits.

(ATTRwt), and causing predominantly cardiac failure, is also recognized as an underdiagnosed cause of cardiac failure in older patients, predominantly men. Although certain phenotypes may be typically associated with certain amyloid types, such as renal/cardiac/neuropathic involvement in AL, renal/gastrointestinal involvement in AA, and cardiac or cardiac/neuropathic involvement in ATTR, considerable clinical overlap exists.

Despite these phenotypic similarities, the treatment of different types of amyloidoses is markedly different. AL amyloidosis is treated with cytotoxic therapies directed against the neoplastic clonal B cells/plasma cells, whereas AA amyloidosis is treated with anti-inflammatory therapies. No specific treatments are currently available for ALECT2 amyloidosis.5,6 The treatment of several hereditary amyloidoses, where the amyloidogenic protein is exclusively (or predominantly) produced by the liver, has been, thus far, via liver transplantation. However, with regard to ATTR amyloidosis, significant advances were recently achieved consequent to the emergence of transthyretin tetramer stabilizers and gene silencers.7–9 Moreover, in cases of hereditary amyloidoses, including hereditary ATTR, genetic testing and counseling of the patient’s family are desired.

Although amyloidosis may be suspected based on clinical grounds, ultimately the diagnosis of amyloidosis is based on tissue detection of deposits. Examination of tissue sections stained with Congo red under polarized light is the current gold standard for diagnosis of amyloidosis.1 A subsequent step involves the identification of the amyloid protein type, which, in turn, has direct implications for the choice of therapy.3,5,6

Bone marrow biopsy is the method of choice for the diagnosis/follow-up of patients with most hematologic disorders, including lymphomas, plasma cell dyscrasia/multiple myeloma, and abnormal serum/urine paraproteins. This latter group of patients may develop AL. In a study of 361 patients with AL amyloidosis who underwent stem cell transplantation, bone marrow amyloid deposition was relatively common, occurring in 65% of patients.10 The detection of amyloid in a bone marrow specimen can be the first evidence of amyloidosis. The majority of bone marrow pathology reports simply state the presence or absence of amyloid and do not specify the spatial distribution of the deposits. The aim of our study was to address the reporting of bone marrow involvement by amyloid in relation to the spatial distribution of deposits and to examine whether reporting the location of deposits may have clinical relevance.

MATERIALS AND METHODS

We reviewed bone marrow biopsies performed from 1995 to 2018 at Loyola University Medical Center, Maywood, Illinois, that had Congo red stain as part of their pathologic diagnostic evaluation.

Diagnosis of amyloidosis was established with Congo red stain examination under polarized light. In addition, Congo red–stained slides were examined under fluorescence (tetramethylrhodamine filter) during prescreening of sections in order to locate the areas with deposits. Representative images of Congo red stain polarization, as well as fluorescence with tetramethylrhodamine filter application, on the detection of bone marrow amyloidosis are shown in Figure 1, A and B.11 Bone marrow Congo red stains were interpreted by 2 pathologists.

The location of deposits was recorded either at initial review or upon rereview. The type of amyloid protein was determined by immunofluorescence on frozen sections or by mass spectrometry using a clinically validated protocol; in rare cases amyloid typing was done on paraffin sections using an amyloid panel.11 Examples of different amyloid deposits are illustrated in Figure 2, A through D.

Determination of the presence of amyloid in other organs was based on biopsy reports retrievable from the hospital pathology records.

In patients with multiple bone marrow biopsies, only one entry was made—that is, only one specimen positive for amyloid was included, or, in patients with multiple positive biopsies, only the first positive biopsy was included in this study. Plasma cell percentage and clonality were also documented. Repeated bone marrow biopsies were excluded.

RESULTS

The decision to perform Congo red stain was made by the original hematopathologist and was based on the following criteria: (1) history of monoclonal gammopathy of undetermined significance or amyloidosis, (2) light-chain restriction or increased plasma cells in bone marrow biopsy, or (3) clinical suspicion of amyloidosis.

Among 809 cases of bone marrow biopsies that were analyzed by Congo red stain, 85 cases (10.5%) were considered positive. The male to female ratio was 1.2:1. The mean age was 65 years; age range was from 26 to 94 years. In 66 of 85 cases, we were able to evaluate the location of amyloid deposits, whereas in 19 patients, the location of the amyloid was not originally specified and could not be retroactively determined (slides no longer available). Therefore, subsequent analysis was limited to 66 patients.

The spatial distribution of deposits was classified as follows: bone marrow stroma, vessel wall, periosteal soft tissue, and combination of the above locations, as further detailed in Figure 3. Amyloid deposits were limited to stroma (medullary deposits) in only 6 cases, to vessel wall in 13 cases, and to periosteal soft tissue in 19 cases; 28 cases had combined deposits (vessel wall + stroma, vessel wall + periosteal soft tissue, and vessel wall + periosteal soft tissue + stroma in 8, 13, and 7 cases, respectively). Thus, there were 2 major groups: (1) 21 of 66 cases (31.8%) of amyloid with bone marrow stromal deposits (alone or in combination with other sites) and (2) 45 of 66 cases (68.1%) of amyloid deposits without stromal component.

Twenty-one of 66 patients (31.8%) had a first-time diagnosis of amyloidosis based on the bone marrow specimen.

Information regarding the type of amyloid protein was retrievable in 36 of 66 cases (54.5%), among which 32 cases (88.8%) were typed as AL and 4 cases (11.1%) were typed as non-AL. Of the latter, 2 cases were typed as AA and 2 cases were typed as ATTR (Figure 4, A). Amyloid typing was performed on bone marrow deposits in only 2 cases; in all remaining cases, other tissues were used, as listed in the Table. Specifically, AA cases were diagnosed based on gastrointestinal and kidney specimens and ATTR was diagnosed based on bone marrow and abdominal fat biopsies. The AL amyloidosis cases included 26 cases of λ-restricted AL amyloidosis and 6 cases of κ-restricted AL amyloidosis. In 2 patients, amyloid typing was performed on 2 biopsies: in one case on bone marrow and muscle (AL-κ) and in the second case on colon and kidney (AL-λ), with concurrence of amyloid type in both sites.

Thirty of the 66 patients had no record of amyloid typing in our institutional electronic medical records, and among

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Figure 1. Use of a tetramethylrhodamine red filter on a Congo red–stained bone marrow specimen highlights the area of amyloidosis (A, arrow). The same specimen under polarized light shows characteristic amyloid apple green birefringence (B, arrow) (original magnification ×200).

Figure 2. Congo red stain highlighting amyloid deposits in blood vessels (A) and bone marrow stroma (B), small deposits in periosteal soft tissue (C), and abundant deposits in periosteal soft tissue (D) (original magnifications ×400 [A and C] and ×200 [B and D]).
Figure 3. Distribution of amyloid deposits in bone marrow specimens.

Figure 4. A, Detailed distribution and type of amyloid deposits in bone marrow. B, Summary of distribution and type of amyloid deposits. Abbreviations: AA, amyloid A; AL, amyloid light chain; ATTR, amyloid transthyretin.
them, 22 cases were suspected to have been AL amyloidosis based on the other pathologic/clinical findings (history of multiple myeloma, monoclonal plasma cells in the bone marrow, etc). These cases are listed as presumed AL in Figure 4, A and B. However, among the remaining 8 cases, the possibility of non-AL cannot be ruled out in at least 5 patients, including a patient with 4% κ-restricted lymphoplasmacytic lymphoma with clinical suspicion of ATTR. Of note, all 5 cases had amyloid deposits in the periosteal soft tissue.

Figure 4, A and B, shows details and summary data on the typing and distribution of amyloid deposits in bone marrow specimens. Plasma cell percentage ranged from 1% to 80%, with a mean of 13.46% and a median of 8%. Forty-four of 66 bone marrow biopsy specimens (66.66%) had less than 10% plasma cells; 8 of these were posttherapy bone marrow biopsy specimens. Twenty-two of the 66 cases showed 10% or more plasma cells; 17 of these cases met the World Health Organization12 criteria for plasma cell myeloma. Plasma cells were polyclonal in 8 of 66 cases (12.1%).

In this study, 4 cases of AL amyloidosis were associated with near-normal counts (<5% plasma cells) and polyclonal plasma cells prior to receiving treatment. The 2 cases with AA amyloidosis had 6% and 11% polyclonal plasma cells and the 2 cases with ATTR amyloidosis had 1% and 5% polyclonal plasma cells.

In 54 of 66 cases (81%) there were amyloid deposits in at least one other organ, mostly in kidneys (n = 18) and abdominal fat (n = 16). Other organs (n = 31) included heart, gastrointestinal tract and liver, salivary gland, thyroid, lung, bone, prostate, urinary bladder, spleen, lymph node, and skin. Ten patients were shown to have involvement of more than one organ.

**DISCUSSION**

In this study, of 809 consecutive bone marrow biopsies where Congo red stain was performed, deposits of amyloid were detected in 85 cases (10.5%). In 66 of 85 cases, we were able to review the spatial distribution of deposits. The study demonstrated that there was significant heterogeneity of amyloid spatial distribution in bone marrow biopsy specimens with medullary, extramedullary, purely vascular, or combined involvement. Whereas stromal deposits were associated exclusively with AL, nonstromal and purely vascular deposits were seen in at least 3 types of systemic amyloidoses (AL, AA, and ATTR). We postulate that reporting the location of amyloid deposits may have clinical relevance.

First, our results showed a rather high rate (>10%) of positivity for amyloid in bone marrow biopsy specimens. Because amyloidoses are relatively rare, the detection of amyloid deposits requires the screening of large numbers of patients.1,3,12,13 Based on our experience, we postulate that bone marrow biopsies represent an excellent source of tissue for the diagnosis of systemic amyloidoses, including also non-AL, and that Congo red stain should be examined proactively in these biopsies. In this series, 21 of 66 patients (31.8%) had a first-time diagnosis of amyloidosis based on bone marrow biopsy.

Whereas the detection of small deposits by Congo red stain alone may be challenging, combining Congo red stain with fluorescence greatly facilitates and enhances the sensitivity of detection.11 Currently Congo red stain is recommended for amyloid detection by the International Society of Amyloidosis; however, other stains, in particular thioflavin, may merit consideration.1,14

In this study, the majority of patients with amyloid in their bone marrow biopsy had evidence of systemic amyloidosis. Thus, in this series, in at least 54 of 66 cases (81%), amyloid deposits were reported in at least one other organ, predominantly in fat and kidney biopsies, whereas no data were retrievable in the remaining patients. Therefore, we consider, similarly to Muchtar et al,15 that bone marrow biopsy together with fat pad biopsy may obviate the need for more invasive target organ biopsies in a significant number of patients.

In systemic AL, the criteria for the definition of organ involvement in kidney, heart, liver, nerve, gastrointestinal, lung, and soft tissue were decided more than a decade ago.4 Proposals for the definition of organ involvement in ATTR have been under consideration for some time.16 However, thus far, the situation with regard to bone marrow has not been conclusively addressed.

The majority of bone marrow pathology reports simply describe the presence or absence of amyloid deposits in the biopsy specimen and do not specify their spatial location.10,14,17-21 In rare studies,10,14,18-21 where the presence of amyloid deposits in the bone marrow, vessels, or stroma was described, vascular amyloid deposits were most frequently encountered.

In this series, amyloid deposits were seen in the vessel wall, bone marrow stroma, periosseal soft tissue, or a combination thereof. Vascular amyloid (alone or in combination with other sites) was also frequently encountered (in 41 of 66 cases; 62.1%). However, although medullary deposits (limited to marrow stroma or in combination with other sites) were seen in only 21 patients (31.8%), extramedullary and/or purely vascular deposits were seen in 45 patients (68.1%). Thus, nonstromal deposits are more than 2.25 times more common than stromal deposits. Hence, it is important to look for amyloid in periosteal soft tissue in bone marrow biopsy specimens. These results are in agreement with a recent study20 of 233 patients, which showed that only 12% of biopsies had interstitial (stromal) deposits in bone marrow biopsies.

A scoring system for bone marrow involvement in AL was previously devised in an effort to predict outcomes of bone marrow transplantation.14,22 The following grades were included: amyloid deposits limited to the blood vessels (1+), blood vessels with focal infiltrate into interstitium (2+), and diffuse amyloid deposits in the bone marrow intersti-
tium (3+). By these criteria, the majority of cases in this study had a 1+ score (ie, involvement limited to the blood vessels). However, no evaluation of extramedullary tissue was included, and no consideration was given to bone marrow biopsy in other types of amyloidosis.

Indeed, little is known about bone marrow findings in non-AL patients. Sungur et al. reported that 79% (31 of 39) of patients with AA amyloidosis secondary to familial Mediterranean fever had amyloid deposits in their bone marrow biopsies. Despite being at different stages of their disease, all patients had deposits of amyloid limited to the vascular wall.

In our study, of 36 cases where information regarding the amyloid type was retrievable, 32 cases (88.8%) were AL amyloidosis. Despite selection bias (bone marrow biopsies primarily done for diagnosis/staging of plasma cell disorders), our data show that other types of amyloidosis, at least AA and ATTR, can be present in bone marrow biopsy specimens (in this study in 11.1%). In our study, in the 2 patients with AA amyloidosis, deposits were limited to the vessel wall; similar findings have been reported in earlier publications. Of our 2 cases of bone marrow biopsy tissue involvement in ATTR, in one case deposits were limited to the vessel wall, whereas in the other case, deposits were limited to perivascular soft tissue; similar observations were also reported by Sidiqi et al. and Wolf et al. Our limited experience with 3 cases of ATTR autopsy bone marrow samples (M.M.P., unpublished data, 2019) also showed no evidence of stromal deposits. Unfortunately, in the largest series of bone marrow biopsies in patients with ATTR reported to date, the location of amyloid deposits was not specified.

In AL amyloidosis, bone marrow is the site of the amyloidogenic plasma cell clone and light-chain production. Hence, not surprisingly, in our series, bone marrow stromal involvement was seen only in the AL cases, and we found no report of bone marrow stromal involvement in the non-AL amyloidoses in the literature. However, whereas bone marrow stromal involvement (alone or in combination with other sites) appears to be specific for AL, extramedullary and pure vascular deposits can also occur in AL. Importantly, however, pure vascular and extramedullary periosteal soft tissue involvement was also seen in non-AL amyloidoses.

The question thus arises: what does it mean when a bone marrow biopsy is positive for amyloid and how should it be reported? In AL, in other organs, not only the presence but also the location of deposits and evidence of organ impairment have been included in the definition of organ involvement. Thus, only interstitial deposits in gastrointestinal and liver tissue were considered to be relevant for the purpose of counting the number of organs involved; pure vascular involvement was not sufficient. Similarly, we propose that pure vascular amyloid in bone marrow specimens, including intramedullary vasculature, should be reported as vascular amyloid and that bone marrow positivity for amyloid should be limited to stromal involvement; periosteal soft tissue deposits of amyloid should be listed as soft tissue involvement. We consider that the reporting of bone marrow positivity for amyloid based on the presence of amyloid anywhere in the biopsy tissue is potentially misleading because it suggests a marrow origin of amyloid protein and may lead to a presumed diagnosis of AL. Our proposed modified means of reporting would remove this ambiguity and provide improved clarity.

Only 20% of AL amyloidosis patients present with an accompanying multiple myeloma; the majority are diagnosed with plasma cell dyscrasia. This was also the case in the current series, with 44 of 66 patients (66.6%) showing a bone marrow plasma cell count of 10% or lower.

Importantly, AL amyloidosis may be associated with near-normal counts and polyclonal plasma cells, as seen in 4 of our 32 cases (12.5%). Concurrently, however, non-AL amyloidosis may be associated with monoclonal gammapathy of undetermined significance and an increased percentage of plasma cells in bone marrow biopsies, which may be monoclonal. To this end, it was earlier recognized that a “small fraction of patients with familial, secondary (AA), and senile systemic amyloidosis will have an incidental monoclonal gammapathy.” However, more recent studies showed that a substantial proportion of patients with ATTR also have monoclonal gammapathy of undetermined significance. A recent study from Boston University showed that 39% of patients with cardiac wild-type ATTR amyloidosis and 49% of patients with mutated ATTR had concurrent monoclonal gammapathy of undetermined significance.

One important limitation of our study is its retrospective nature and consequent lack of complete data concerning the amyloid type present in a subset of patients as well as incomplete clinical data. Although, in the majority of our patients (81%), amyloid deposits were also detected in other organs, the question of whether the detection of amyloid in bone marrow biopsy specimens is always associated with systemic amyloidosis remains open. It is feasible that some ATTR perivascular deposits may represent localized amyloid. Although rare patients with more than one type of amyloid deposit have been reported in the literature, thus far we have not encountered such patients.

The present study emphasizes the importance of bone marrow biopsy in the management of all patients with systemic amyloidosis, not just those with AL. Bone marrow biopsy is important not only for the determination of the precursor protein but also as a source of potentially diagnostic tissue. With recently expanded options for the treatment of systemic amyloidoses, it is important that pathology reporting of bone marrow biopsies be accurate and appropriate to the definition of other organ involvement. Hence, we propose that reporting of bone marrow involvement by amyloid should include a description of its spatial distribution. Whether bone marrow stromal deposits, which have invariably been associated with systemic amyloidosis of AL type, are type specific requires further study for confirmation.

References


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