Bone Marrow Core Biopsy Specimens in AL (Primary) Amyloidosis

A Morphologic and Immunohistochemical Study of 100 Cases

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Abstract

We retrospectively reviewed 100 bone marrow core biopsy specimens from patients with AL (primary) amyloidosis. The morphologic and immunohistochemical features were assessed by standard histochemical stains (H&E, periodic acid–Schiff, Congo red) and immunohistochemical stains for light chain immunoglobulins. Bone marrow core biopsy revealed a plasma cell dyscrasia in 83% (λ, 65; κ, 18) of cases. Amyloid deposits were observed in 60% of the bone marrow core biopsy specimens and, when present, were detected most often in blood vessel walls only (39/60). However, if present, interstitial amyloid deposition was significantly more associated with patients with a monoclonal κ light chain gammopathy (P = .04). Through the careful analysis of standard histochemical and immunohistochemical stains, bone marrow core biopsy provides essential diagnostic information in cases of AL amyloidosis.

AL (primary) amyloidosis is the most common form of systemic amyloidosis seen in the United States and is due to an underlying plasma cell dyscrasia that produces an amyloidogenic light chain, which deposits as amyloid fibrils within tissues.[1] The prognosis of AL amyloidosis is poor, with a median survival of 1 to 2 years but, recently, improved survival has been seen with more aggressive therapy using high-dose melphalan and autologous stem cell transplantation.[2] Once the initial histologic diagnosis of amyloid deposition is made, subsequent typing for AL amyloidosis is dependent on the identification of monoclonal light chains by serum and/or urine immunofixation electrophoresis (IFE). Bone marrow core biopsy is performed to identify the underlying plasma cell dyscrasia present and to exclude plasma cell myeloma.

Morphologic features of the bone marrow core biopsy specimen in patients with AL amyloidosis are subtle, and often the diagnosis may be missed when examining routine histochemical stains. The identification of a monoclonal population of plasma cells by using immunohistochemical techniques on bone marrow core biopsy specimens sometimes is difficult because of the low number of plasma cells present. The aim of this study was to assess the morphologic and immunohistochemical bone marrow features in a large group of cases of AL amyloidosis. In addition, we describe how bone marrow core biopsy supplements other diagnostic tests that are used in the initial evaluation of patients for AL amyloidosis.

Materials and Methods

A retrospective review of 100 bone marrow core biopsy specimens from newly diagnosed and untreated patients with

AL amyloidosis was carried out with the approval of the institutional review board of Boston University School of Medicine, Boston, MA. The diagnosis of AL amyloidosis was based on a combination of abdominal fat pad aspirates, visceral organ biopsy specimens, serum and/or urine IFE results, and bone marrow core biopsy performed at referring institutions but confirmed at the Amyloid Treatment and Research Program, Boston Medical Center. All patients were assessed for 44 months between 1995 and 1999, and, in addition to a bone marrow core biopsy, the majority of patients also underwent concurrent serum and/or urine IFE and abdominal fat pad aspirates. Clinical data were obtained from the hospital records. Cases were excluded from the review if patients had received previous chemotherapy, had plasma cell myeloma, or had another form of amyloidosis.

The bone marrow core biopsy specimens were placed in a combination of Zenker fixative and acetic acid for 24 hours, washed in water for 6 to 8 hours, and embedded in paraffin and processed routinely. Sections were stained with H&E, the periodic acid–Schiff reagent (PAS), and the Congo red stain.

For light chain immunohistochemical analysis, 5-μm paraffin sections were cut and incubated on charged glass slides at 60°C for 1 hour, deparaffinized, and rehydrated. To remove the mercury-associated precipitate attributed to the Zenker fixative, the slides were placed in a solution of 1% iodine for 5 minutes, washed in water, placed in a solution of 5% sodium thiosulfate for 5 minutes, and washed in water again. The slides then were incubated for 10 minutes in a solution of 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Antigen retrieval was performed on positive control slides (human tonsil) only, with incubation in retrieval solution (Antigen Retrieval Citrate Plus, BioGenex, San Ramon, CA) and heated in a microwave at high power for 3 minutes and then at medium power for 8 minutes. To minimize background staining, 150 to 200 μL of Power Block (BioGenex) was added to all slides. Primary polyclonal rabbit antibodies for κ and λ immunoglobulin light chains (DAKO, Carpinteria, CA) were added at a dilution of 1:1,000 and incubated for 30 minutes at room temperature. To complete immunohistochemical staining, antirabbit peroxidase-conjugated secondary antibody (DAKO) was added to a commercially available diaminobenzidine kit (BioGenex) using the biotin-streptavidin detection system. Counterstaining was achieved with Mayer hematoxylin. Negative control samples were created by omitting the primary antibody and replacing it with phosphate-buffered saline.

Plasma cell percentages were assessed on immunoperoxidase-stained sections for κ and λ immunoglobulins and were arbitrarily divided by 1 hematopathologist (C.J.O.) into incremental groups of 5% based on a visual estimate of plasma cells relative to the total marrow cellularity (≤5%, 6%-10%, 11%-15%, 16%-20%, 21%-25%). Wright-Giemsa–stained aspirate smears also were available for evaluation of the plasma cell percentage. Plasma cell monoclonality was defined as a κ/λ ratio of more than 3 (κ clone) or less than 1 (λ clone). Amyloid deposits were graded as follows: 0, absent; 1+, blood vessels only; 2+, blood vessels and focal interstitial deposition of 2 or fewer high-power fields; or 3+, prominent interstitial deposition of more than 2 high-power fields. The definitive identification of amyloid was based on the presence of apple-green birefringence following polarization of the congophilic deposits seen on the Congo red slide. In addition, the staining characteristics of the amyloid deposits were assessed on the H&E-, PAS-, and immunoperoxidase-stained slides.

IFE of serum and/or urine samples was performed in all cases (serum, 98 cases; urine, 94 cases) using the Paragon Electrophoresis System (Beckman, Fullerton, CA). Results were interpreted as positive or negative based on the qualitative presence or absence of a restriction band on the gel indicating monoclonal protein.

Abdominal fat pad aspirates were obtained in 93 cases and, following Congo red staining, were interpreted as positive or negative based on the presence of apple-green birefringence following polarization microscopy.

For statistical analysis, the Fisher exact test was used for comparison of categorical data. A P value less than .05 indicated statistical significance.

Results

We analyzed 100 bone marrow core biopsy specimens from patients with AL amyloidosis. The median age of the patient group was 58 years (range, 29-89 years); 41 were women and 59 were men.

In each case, the monoclonal light chain gammopathy (λ, 76; κ, 24) was identified by bone marrow core biopsy, IFE, or immunohistochemical analysis of previously frozen renal biopsy tissue (2 cases). Morphologically, all 100 bone marrow core biopsy specimens showed appropriate cellularity for age with associated normal trilinear hematopoiesis and without features of abnormal maturation or myelodysplasia. Iron stores were detected in normal amounts. No overt fibrosis was observed. Other bone marrow findings included lymphoid aggregates (8 cases), a lymphoplasmacytic infiltrate (3 cases), noncaseating granulomata (2 cases), and coexistent chronic lymphocytic leukemia (1 case).

Plasma cells appeared mature **Image 1**, were seen scattered diffusely throughout the marrow but with frequent interstitial aggregates or nodules composed of 4 to 8 plasma cells, and generally were not present in a perivascular location as typically seen in reactive marrow **Image 2**. Immature or anaplastic plasma cells were not seen in any of the bone

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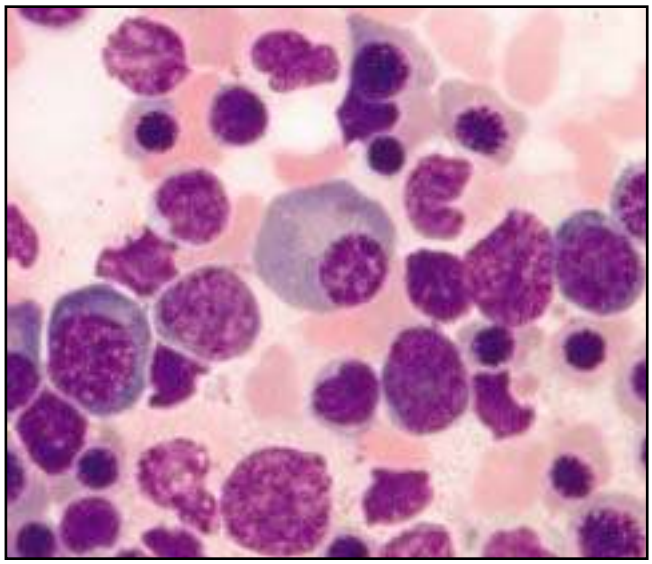

Image 1 Bone marrow aspirate in AL amyloidosis showing scattered mature plasma cells (Giemsa, ×500).

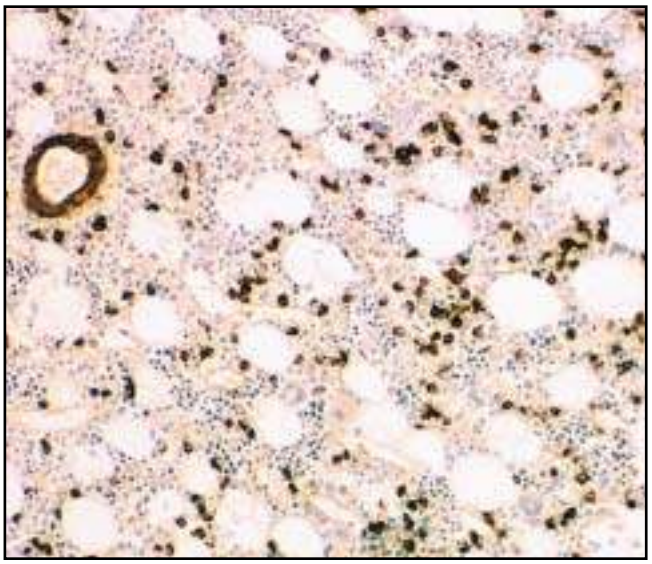

Image 2 Bone marrow core biopsy specimen with monoclonal λ light chain expression in plasma cells with scattered aggregates or nodules of monoclonal plasma cells in AL amyloidosis (immunoperoxidase with hematoxylin counterstain, ×100).

marrow core biopsy specimens. With immunohistochemical staining of the bone marrow core biopsy specimen, a plasma cell dyscrasia was diagnosed in 83 (λ, 65; κ, 18) of the 100 biopsy specimens tested **Table 1**. In the bone marrow core biopsy specimens with plasma cells exceeding or equal to 6% of the cellularity, a diagnosis of monoclonality was made in 48 (98%) of 49 biopsy specimens. In bone marrow core biopsy specimens with 5% or fewer plasma cells, monoclonality could still be discerned in 69% (35/51) of biopsy specimens (Table 1). A similar distribution of plasma cell percentages was seen in both clonal subgroups (Table 1). There was no correlation between a high plasma cell percentage (>10%) and the presence of bone marrow amyloid deposition ($P = .73$).

Serum and urine IFE detected the presence of monoclonal light chains in 60% (59/98) and 83% (78/94), respectively, and when urine and serum samples were both tested, results were positive in 91% (89/98) and in all cases were concordant with the plasma cell dyscrasia seen on the bone marrow core biopsy specimen. In the 83 bone marrow core biopsy specimens with a plasma cell dyscrasia, 92% (76/83) had a concordant positive serum IFE result. Nine patients had no detectable monoclonal immunoglobulin in the serum or urine; however, bone marrow core biopsy detected a plasma cell dyscrasia in 7 of these cases. In the remaining 2 cases, AL amyloidosis was confirmed by immunohistochemical analysis of frozen tissue from renal biopsy specimens.

Amyloid deposits were identified in 60 bone marrow core biopsy specimens with the majority (39/60) showing vascular deposition only **Table 2**. Interstitial amyloid deposits **Image 3** were noted in 38% (9/24) of the bone

Table 1
Bone Marrow Core Biopsy Specimen Analysis in 100 Cases of AL Amyloidosis*

Plasma Cell Percentage	λ (n = 76)	κ (n = 24)
0-5	41 (30)	10 (5)
6-10	18 (18)	8 (7)
11-15	7 (7)	4 (4)
16-20	6 (6)	2 (2)
21-25	4 (4)	0 (0)

* Numbers in parentheses indicate the number of cases with detectable plasma cell dyscrasia.

Table 2
Summary of Amyloid Deposition in Cases of AL Amyloidosis

	λ	κ
Bone marrow core biopsy result*	(n = 76)	(n = 24)
0	33	7
1+	31	8
2+	4	3
3+	8	6
Fat pad aspirate result	(n = 71)	(n =22)
Positive	61	12
Negative	10	10

* Scored as follows: 0, absent; 1+, blood vessels only; 2+, blood vessels and focal interstitial deposition of ≤2 high-power fields; 3+, prominent interstitial deposition of >2 high-power fields.

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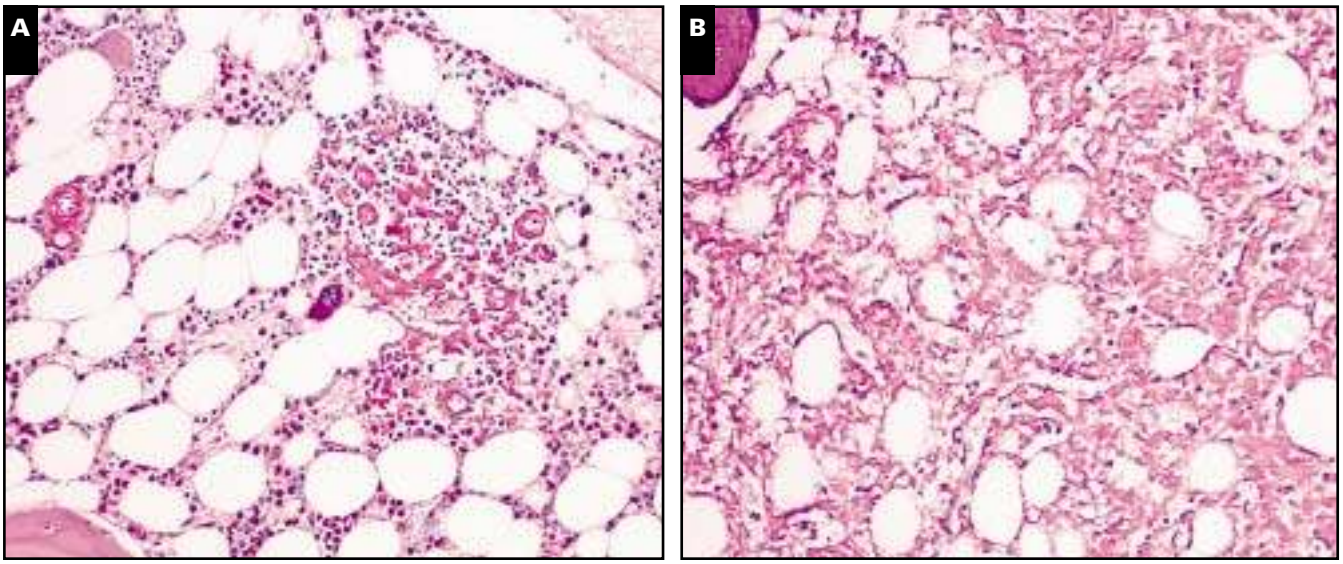

Image 3 Interstitial amyloid deposits in bone marrow core biopsy specimens with AL amyloidosis. **A**, Focal (2+) deposition (periodic acid–Schiff, ×100). **B**, Extensive (3+) deposition (H&E, ×100).

marrow core biopsy specimens associated with a κ monoclonal gammopathy and in only 16% (12/76) of those with λ monoclonal light chains (P = .04). Amyloid deposits were identified readily by using routine stains, as well as special tissue stains. With PAS, the deposits had a distinct "smudgy" appearance **Image 4A**. The amyloid deposits appeared less vibrant with Congo red staining of Zenker-fixed decalcified specimens **Image 4B** than in tissues that had been formalin-fixed. Immunohistochemical stains revealed positive staining of the amyloid deposits for the corresponding circulating monoclonal immunoglobulin in 67% (40/60) of the biopsy specimens **Image 4C** and **Image 4D**, but the remaining 33% (20/60) of the specimens showed equal staining of the deposits for both κ and λ light chains.

Abdominal fat pad aspirates were positive in 78% (73/93) of cases (Table 2), and in 10 cases in which the fat pad aspirate was negative, bone marrow core biopsy revealed amyloid deposition. Combining these test results thus yielded a detection rate of 96% (89/93). Interestingly, cases with a λ light chain monoclonal gammopathy had a significantly higher positive fat pad aspirate rate compared with κ-positive cases (P = .006). No association was observed between a high plasma cell percentage (>10%) and a positive fat pad aspirate (P = .251).

Discussion

The key to establishing a diagnosis of AL amyloidosis is the recognition that the primary source of the amyloid protein is a monoclonal population of plasma cells in the bone marrow (plasma cell dyscrasia) that produce fibrillogenic light chains. Given the recent success in treating AL amyloidosis with high-dose intravenous melphalan and peripheral stem cell support,[2] it is imperative that a plasma cell dyscrasia be identified before treatment is started. Despite being the source of the subsequent systemic amyloid deposition, the bone marrow core biopsy specimen in AL amyloidosis most often is morphologically unremarkable. The marrow typically is normocellular with appropriate trilinear hematopoiesis, normal iron stores, and no overt fibrosis. The plasma cell percentage typically is only slightly increased, and the plasma cells appear mature and lack the atypia or anaplasia usually observed in plasma cell myeloma. In addition, the distribution of the neoplastic plasma cells in AL amyloidosis is subtle, occurring singly or in small clusters, unlike the sheet-like pattern usually seen in plasma cell myeloma.

Detection and enumeration of the plasma cells in the bone marrow core biopsy specimen most often is accomplished by immunohistochemical stains for light chain immunoglobulins. By using this method, previous studies analyzing the bone marrow core biopsy specimen in AL amyloidosis have shown mean plasma cell percentages ranging from 7% to 14%.[3,4] The present study findings support this observation; the majority of cases had low plasma cell percentages (<10% of the total marrow cellularity). Given these low numbers, the detection of a plasma cell dyscrasia in AL amyloidosis by immunohistochemical analysis of bone marrow core biopsy specimens can be challenging; reported positive rates range from 60% to 69%.[4,5]

Other methods have been used to try to increase the detection rate of plasma cell dyscrasias. A study combining

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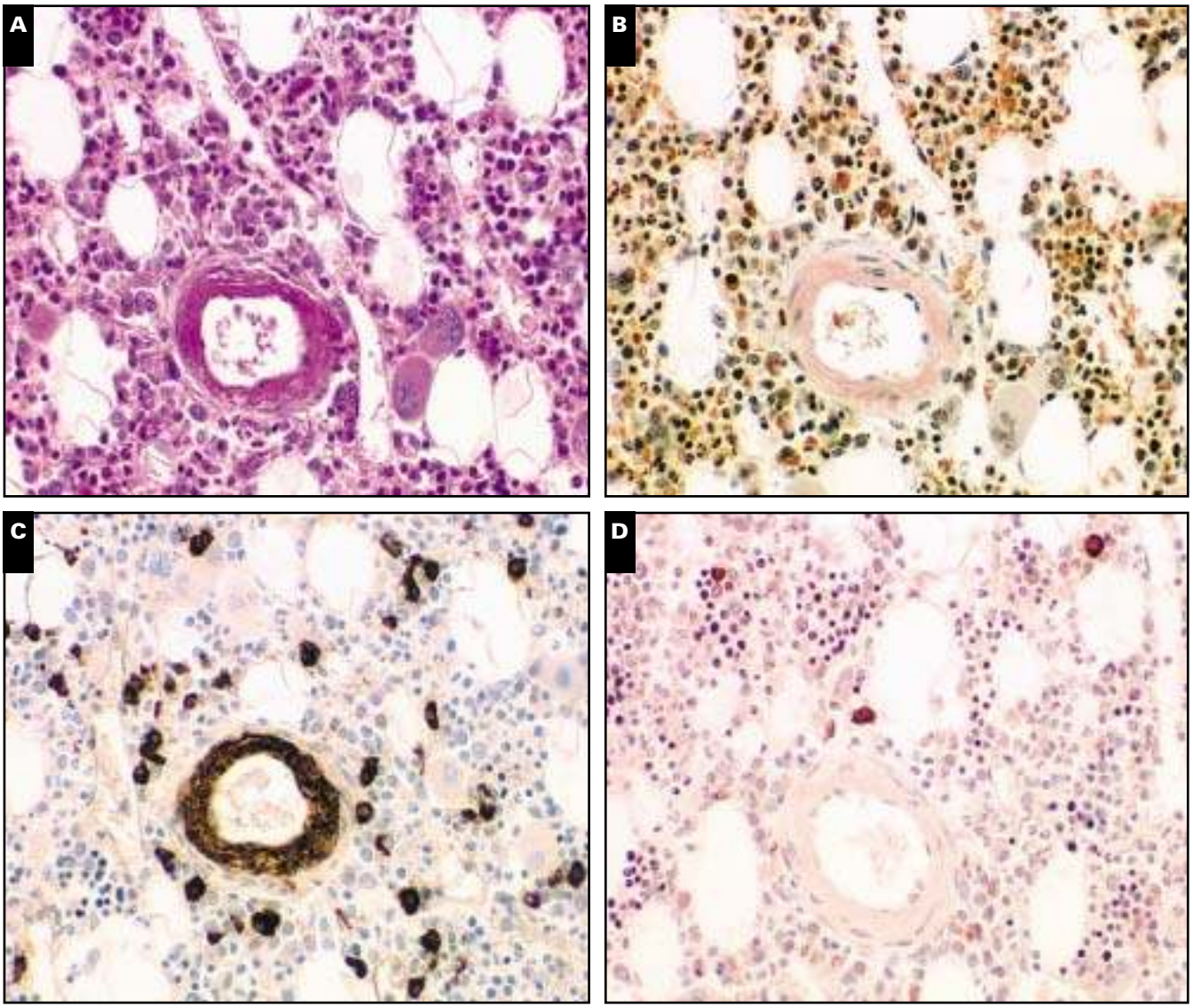

Image 4 Zenker-fixed bone marrow core biopsy specimen showing the staining characteristics of vascular (1+) amyloid deposits in a patient with λ AL amyloidosis. **A**, "Smudgy" positive staining (periodic acid–Schiff, ×400). **B**, Weak congophilic staining (Congo red, ×400). **C**, Strong positive staining with λ light chain immunoglobulin antibody (immunoperoxidase with hematoxylin counterstain, ×400). **D**, No staining with κ light chain immunoglobulin antibody (immunoperoxidase with hematoxylin counterstain, ×400).

immunofluorescent staining of bone marrow aspirate slides with immunohistochemical analysis of bone marrow core biopsy specimens in 26 patients with an occult plasma cell dyscrasia (plasma cells <10%) showed an overall detection rate for light chain restriction of 84% of cases, but the immunofluorescent technique alone did not show significantly superior sensitivity over bone marrow core biopsy.[6] In a study involving 52 patients with AL amyloidosis, immunofluorescent staining of bone marrow aspirate slides alone identified plasma cell clonality in 44 patients (85%).[7] Although the present study showed lower sensitivity with smaller plasma cell percentages, the overall sensitivity of 83% supports the contention that immunohistochemical analysis of bone marrow core biopsy specimens in AL amyloidosis is comparable to immunofluorescence of bone marrow aspirates and avoids all the disadvantages inherent in immunofluorescent technology.

More recently, automated colorimetric in situ hybridization has been used to detect the expression of immunoglobulin light chain messenger RNA in plasma cell dyscrasias.[8] While that study showed superior detection of light chain restriction by colorimetric in situ hybridization compared with immunohistochemical staining, more experience with AL amyloidosis is needed

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because the study primarily analyzed bone marrow core biopsy specimens in plasma cell myeloma, with only 1 case of amyloidosis included.

All bone marrow core biopsy specimens in the present study were processed using Zenker solution, which permitted crisp clear staining of plasma cells for light chain immunoglobulins. This was in contrast with the staining frequently observed in formalin-fixed biopsy specimens that often are hampered by background staining. However, advances in antigen-retrieval methods[9] have improved this situation, which is especially important given the universal use of formalin. As found in previous studies, λ-positive cases exceeded κ-positive cases, although in the present study, there was a 3:1 ratio of λ to κ, which is higher than the 2:1 ratio reported by Kyle and Gertz.[10] A consistent and useful morphologic feature that predicted a plasma cell dyscrasia in the bone marrow specimens of cases in the present study was the presence of clustered clonal plasma cells (plasma cell aggregates or nodules), which is particularly useful in the bone marrow core biopsy specimens with 5% or fewer plasma cells.

IFE analysis of serum and/or urine samples has been reported to confirm a monoclonal gammopathy in 90% of patients with AL amyloidosis.[11] The present study showed a similar overall IFE positive rate (91% [89/98]). With improving IFE technology, the detection of smaller concentrations of circulating monoclonal light chains has resulted in greater sensitivity by this method.[12]

In addition to the analysis of plasma cells, the bone marrow core biopsy also may demonstrate amyloid deposition. Amyloid was detected in 60% of cases in this study (60/100), a yield similar to previously published data.[10] Although the Congo red stain remains the "gold standard" for diagnosing amyloid, it can be very well supported by other stains, including PAS, which gives amyloid a distinctive smudgy appearance. In fact, it was an observation in the present study that PAS often was more reliable for demonstrating amyloid deposits than the Congo red stain, for which results often were, at best, only faintly congophilic. This may be a nuance of Zenker fixation, as it was not particularly observed in formalin-fixed tissue. When present, amyloid deposition in the bone marrow core biopsy specimen most often is observed in blood vessel walls. It also might be detected in the interstitium, focally or, in some cases, extensively. Interestingly, there was a significantly greater likelihood for κ-positive cases to be associated with interstitial deposition.

In the present study, abdominal fat pad aspirates were positive in 78% of cases (73/93), a rate similar to that previously reported.[13,14] The combined yield from fat pad aspirates and bone marrow core biopsy specimens was sufficient in most cases to confirm the diagnosis of amyloidosis, obviating the need for further invasive procedures. An additional previously unreported finding, however, was that cases with a monoclonal λ gammopathy were more likely to have a positive fat pad aspirate than cases with monoclonal κ light chains.

Conclusion

This comprehensive review of bone marrow core biopsy specimens from 100 patients with untreated AL amyloidosis confirms that, despite being an occult plasma cell dyscrasia, careful morphologic, histochemical, and immunohistochemical analysis of the bone marrow core biopsy specimen will provide diagnostic information for the majority of patients with AL amyloidosis. In conjunction with IFE and abdominal fat pad aspiration, bone marrow core biopsy is an essential component in the evaluation of any patient with suspected amyloidosis.

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