Reactive Versus Neoplastic Bone Marrow

Problems and Pitfalls

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Examination of the bone marrow poses several unique challenges to the pathologist: it is a semisolid organ without helpful gross correlation, it exists in a dynamic state with the peripheral blood and other organs of the lymphohemopoietic system, and the diagnosis of diseases affecting bone marrow often depends heavily on ancillary special studies. The bone marrow examination ideally encompasses review of the bone marrow biopsy histology (with or without additional nondecalcified clot preparation material), bone marrow aspirate smear cytology, and the peripheral blood smear; optimal procurement and processing of these samples is critical in ensuring that a maximal level of diagnostic information can be extracted. The pathologist must be aware of the clinical context of the bone marrow and the results of ancillary tests, whether these are ordered by the pathologist or the clinician. A combination of excellent diagnostic samples, appropriate ancillary tests, and knowledge of the clinical context provides the best background to distinguish between the common reactive and neoplastic processes that involve the bone marrow and to avoid diagnostic pitfalls in making these distinctions.

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DIAGNOSTIC SAMPLES USED TO EVALUATE THE BONE MARROW

The core biopsy histology and aspirate smear cytology are complementary to one another both in terms of sample type (core tissue sample vs liquid aspirate of intertrabecular marrow material) and presentation of the microscopic pathology (histologic sections vs air-dried Wright-Giemsa–stained cytology). Both methods have strengths and weaknesses and should be reviewed in concert. If the same diagnostician does not review both biopsy and aspirate, it is critical that the individuals reviewing the aspirate and biopsy communicate and come to a consensus diagnostic rather than issuing possibly contradictory or noncommittal reports.

In addition, ancillary studies are often concurrently performed on the marrow sample at the discretion of the pathologist and/or submitting clinician. Common ancillary studies relevant to bone marrow diagnosis are listed in Table 1. These tests complement the bone marrow morphologic examination; it is critical that the pathologist reviewing the morphology and the various laboratories conducting the ancillary studies are in close communication. Such communication ensures maximal diagnostic power and accuracy, as the likelihood of achieving a specific (and cost-effective) diagnosis is increased if the pathologist and the ancillary laboratories are aware of the diagnostic considerations of one another. Ultimately, the pathologist must be able to orchestrate and synthesize the various morphologic and ancillary data into a clinically meaningful diagnosis, or at least a useful differential diagnosis.

TECHNICAL CONSIDERATIONS

Technical quality of the bone marrow sample frequently compounds difficulties in interpretation. The core biopsy may be of inadequate length, may consist predominantly of cortical bone, or may exhibit obscuring crush artifact. While the aspirate smear preparations give the pathologist a “second chance” when the core biopsy is poor, the aspirate may be inadequate due to a poor aspiration technique or a fibrotic marrow space (so-called dry tap). The latter is a more serious problem than an inadequate core biopsy, since many critical ancillary studies require unfixed smear slides or living cells in suspension. It is recommended that if a dry or poor aspirate is obtained, touch imprints should be made from the fresh, unfixed core biopsy. These touch preparation slides can be used for Wright-Giemsa staining as well as for ancillary studies, such as fluorescent in situ hybridization. If cytogenetics and/or flow cytometry are critical in a dry tap scenario, a second core biopsy may be obtained and placed in sterile saline. Using sterile needles or a mechanical disaggregation system (such as Medimachine; BD Biosciences, San Jose, Calif), viable marrow cells in suspension can be released from fibrotic tissue samples.

Even if an adequate biopsy is obtained, evaluating subtle histologic findings requires excellent fixation, processing, and sectioning of the tissue. While neutral-buffered formalin is an adequate fixative, superior nuclear detail is often produced with fixatives containing metals, such as Zenker, B-5, aceto-zinc formalin, and B-plus (BBC Biochemical, Stanwood, Wash).1 Unlike Zenker and B-5,
B-plus fixative and aceto-zinc formalin do not contain mercury, thereby circumventing problems of hazardous waste generation and providing better preservation of DNA for molecular studies.\textsuperscript{1,2} Decalcifying agents include, in order of decreasing speed of action and decreasing adverse effects on morphology, immunohistochemistry and DNA quality for fluorescent in situ hybridization and molecular studies: strong acids (hydrochloric, nitric, sulfuric), buffered acids (formic, acetic), and calcium chelators.\textsuperscript{3–5} Proprietary decalcifying agents, such as RDO (APB Engineering Products, Plainfield, Ill) and RapidCal (BBC Biochemical) may also be used. Processing and embedding bone marrow biopsies in plastic resins produces superior results. Processing and embedding bone marrow biopsies in plastic resins produces superior sections, but this procedure is time consuming and labor intensive and is not widely used.

Small particles of marrow are often present in blood clots submitted along with the marrow core and are usually prevalent in clots prepared from residue from the marrow aspirate syringe. These clots should be fixed and processed routinely, as they do not need to be decalcified; they often produce superior morphology to the core biopsy. At Massachusetts General Hospital, we fix bone marrow core biopsies for a minimum of 4 hours in B-plus and decalciﬁy for 1 hour in RapidCal Immuno (BBC Biochemical), which provides adequate fixation and good antigen preservation for immunohistochemistry.

Thin sectioning of paraffin-embedded sections is at least as important as good fixation in producing an interpretable slide: all bone marrow sections are cut at a thickness of 2 μm at Massachusetts General Hospital. In addition to a hematoxylin-eosin section, a well-prepared Giemsa stain is useful in differentiating myeloid and erythroid elements and in identifying plasma cells and mast cells. Iron stain may be performed on the biopsy section or clot, but it is less sensitive than an iron stain on an aspirate smear, as some iron is lost during tissue processing.\textsuperscript{6} Reticulin silver stain on the core biopsy is essential in evaluating myeloproliferative diseases and may be helpful in detecting general or focal marrow abnormalities, which can nonspecifically cause an increase in reticulin fibers (see below). Reticulin grading systems include three and four grade systems, which are reproducible if the stain is technically adequate (Table 2).\textsuperscript{7,8}

### THE CLINICAL CONTEXT IN BONE MARROW EXAMINATION

The pathologist should be aware of important clinical patient information, such as complete blood count results and the presence or absence of splenomegaly and lymphadenopathy. In evaluating samples taken after therapy, the pathologist should be aware of the details of the disease history, type of therapy, and timing of the biopsy in relation to the disease course and therapy administration. Various types of therapies may influence the appearance of both neoplastic and normal cell populations, confounding distinction between residual disease and reactive normal cells.

Knowledge of the clinical reason for bone marrow sampling is important in practical bone marrow diagnosis. The clinical scenario in which the bone marrow biopsy is performed influences the choice of ancillary studies and also places the interpretation of morphologic findings in a context; this context may allow a more specific diagnosis to be made or may help the pathologist in avoiding an erroneous diagnosis suggested by morphology. Table 3 illustrates the clinical context for bone marrow sampling performed during 1-year periods at 2 institutions. This Table illustrates that a large proportion of bone marrow biopsies are performed to follow-up or stage known disease (particularly at tertiary/referral institutions). These types of scenarios present quite a different set of problems for the pathologist than bone marrow examinations performed to evaluate a newly presenting, unknown disease. While Table 3 provides the clinical settings of bone mar-

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<th>Grade</th>
<th>Description</th>
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<tr>
<td>0</td>
<td>Scattered linear reticulin with no intersections (crossovers)</td>
</tr>
<tr>
<td>1</td>
<td>Loose network of reticulin with many intersections, especially in perivascular areas</td>
</tr>
<tr>
<td>2</td>
<td>Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis</td>
</tr>
<tr>
<td>3</td>
<td>Diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis</td>
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**Table 1.** Common Ancillary Studies That Complement Bone Marrow Morphologic Examination*  

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Description</th>
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<tr>
<td>1. Cytogenetics on aspirated bone marrow or peripheral blood</td>
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<td>2. FISH studies performed on aspirated bone marrow or touch preparations</td>
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<td>3. Molecular studies (typically PCR or RT-PCR) for antigen receptor gene rearrangements and/or to detect specific translocations</td>
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<td>4. Immunophenotyping of aspirated bone marrow or peripheral blood cells by flow cytometry</td>
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<td>5. Immunohistochemistry on paraffin sections</td>
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<td>6. Enzyme cytochemistry on marrow aspirate or peripheral smear slides</td>
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* FISH indicates fluorescence in situ hybridization; PCR, polymerase chain reaction; and RT-PCR, reverse transcriptase–polymerase chain reaction.
row examination at 2 example institutions, these will, of course, vary in different pathology practices. Each indication sets the stage for a typical differential diagnosis and will suggest appropriate ancillary studies. Ordering of ancillary studies inappropriate for the context is costly and may generate confusing and misleading results, which may complicate an otherwise routine diagnosis. However, the pathologist must still retain an open mind and realize that diseases may present atypically with unexpected clinical findings; he or she must be prepared to alter the ancillary testing profile in cases with unexpected morphologic findings.

### COMMON SITUATIONS THAT CHALLENGE THE PATHOLOGIST TO DETERMINE A PRIMARY NEOPLASTIC VERSUS REACTIVE PROCESSES

#### Anemia

Unexplained anemia is a common indication for bone marrow examination (and is the most common indication at many institutions). Generally, the anemia is chronic in duration and the hematologist has typically already excluded iron, vitamin B$_{12}$, and folate deficiencies as well as possible effects of a drug or known infection, although this cannot always be assumed. The degree and duration of anemias that elicit a bone marrow examination vary among different hematologists. As mentioned above, examination of the bone marrow in this context should include knowledge of the complete blood count results and examination of the peripheral smear.

Typically, the main clinical suspicion for bone marrow biopsy in anemia workup is a myelodysplastic syndrome (MDS). In this setting, MDS should be considered a diagnosis of exclusion: striking morphologic dysplasia may be seen in a variety of reactive conditions, such as effects of alcohol, drug or toxin, folate, or vitamin B$_{12}$ deficiencies.

Morphologic dysplasia may also occur in inherited conditions, such as hemoglobinopathies (Figure 1, A through D). In general, the pathologist should strive to find an “excuse” for the dysplasia and should be particularly cautious of making a diagnosis of MDS when only unilineage dysplasia is present. Unlike MDS, which usually exhibits architectural disorganization of hemopoiesis, reactive marrow processes typically maintain relative segregation of erythropoietic and myelopoietic “islands” (Figure 1, E and F). A cytogenetic abnormality characteristic of MDS confirms the diagnosis, but cytogenetic abnormalities are only seen in about 50% of MDS cases. An increase in blasts in the context of morphologic dysplasia and prolonged anemia in the absence of growth factor therapy is also a very strong indicator of MDS. Other less definitive features that increase the suspicion of MDS in the anemic patient are macrocytosis (if folate and vitamin B$_{12}$ deficiency have been excluded), other cytopenias, and the presence of ringed sideroblasts. Flow cytometric identification of aberrant antigen expression can also be helpful in raising the suspicion of MDS. However, unless the diagnosis of MDS is clear cut, it is most prudent to raise the possibility of MDS without rendering a definitive diagnosis: clinical follow-up will often eventually confirm a diagnosis of “true” MDS by establishing the chronicity of the abnormality and by allowing time for exclusion of possible reactive/reversible causes.

Aside from MDS, other neoplasms presenting with anemia include lymphoma and multiple myeloma. Hairy cell leukemia is an important consideration, as the interstitial infiltration pattern may be subtle in early stages of the disease, and hairy cells may be rare in the peripheral blood and the often poor or dry aspirate. CD20 and/or DBA.44 immunostaining of the bone marrow core is very helpful in these situations (Figure 2, A and B). It is important to keep in mind that marrow lymphomas (particularly hairy cell leukemia and also multiple myeloma) can elicit a “sympathetic” morphologic dysplasia in the hematopoietic elements and may lead to misdiagnosis as MDS. Helpful information to avoid this pitfall includes correlation with clinical features (splenomegaly, for example, is uncommon in MDS), flow cytometry, and the typical limitation of such reactive morphologic dysplasia to the erythroid lineage.

Common reactive causes of anemia are often diagnosed clinically by the hematologist through a detailed history, physical examination, and laboratory tests, and these patients do not undergo bone marrow biopsy. Evolving aplastic anemia or paroxysmal nocturnal hemoglobinuria may present with progressive anemia; the latter can be excluded by peripheral blood immunophenotyping for the glycosylphosphatidylinositol-anchored proteins CD55 and CD59. Rare disorders, such as Gaucher and other storage diseases, bone marrow involvement by sarcoidosis, and systemic mastocytosis, may also present with anemia or pancytopenia. Anemia and pancytopenia in infants present a different differential diagnosis; primary MDS is less likely, whereas aplastic anemia, hereditary bone marrow failure syndromes, and hemophagocytic syndromes must be considered. Unfortunately, no specific diagnosis can be rendered in many bone marrow biopsies performed to evaluate anemia. Such cases likely represent a mixture of anemia of chronic disease, examples of reactive disorders described above that were not diagnosed prior to the bone marrow biopsy, or early cases of MDS in which morp-
logic changes are insufficiently well developed to warrant a definitive diagnosis.

**Thrombocytopenia**

By far the most common cause for thrombocytopenia eliciting a bone marrow examination is idiopathic thrombocytopenic purpura, which is mediated by antiplatelet autoantibodies. Isolated thrombocytopenia is less commonly a presenting manifestation of a myelodysplastic syndrome, acute leukemia, or a bone marrow failure syndrome. The pathologist’s role in evaluating the bone marrow in this setting is in establishing a normal or increased

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**Figure 1.** Examples of bone marrow findings in myelodysplastic syndromes and nonneoplastic mimics. A, Erythroid lineage morphologic dysplasia in β-thalassemia (bone marrow aspirate; Wright-Giemsa, original magnification ×1000). B, Erythroid lineage morphologic dysplasia in human immunodeficiency virus infection (bone marrow aspirate; Wright-Giemsa, original magnification ×1000). C, Parvovirus infection with giant pro-normoblast (arrow; bone marrow biopsy; hematoxylin-eosin, original magnification ×400). D, Marked nuclear irregularities of erythroid forms in vitamin B12 deficiency (bone marrow biopsy; hematoxylin-eosin, original magnification ×400). E, Hypercellular bone marrow biopsy section in myelodysplastic syndrome (refractory anemia with excess blasts 1) showing marked architectural disorganization of hemopoiesis (hematoxylin-eosin, original magnification ×200). F, Hypercellular bone marrow biopsy section from a patient with autoimmune hemolytic anemia, showing preserved marrow architecture and well-defined areas of erythropoiesis (hematoxylin-eosin, original magnification ×200).
Figure 2. Examples of bone marrow biopsy findings in lymphoma, human immunodeficiency virus (HIV) infection, and plasma cell proliferations. A, Patient with mild anemia and leukopenia with no obvious lymphoid infiltrate (hematoxylin-eosin, original magnification ×200). B, DBA-44 immunohistochemical stain reveals numerous interstitial lymphoid cells with abundant cytoplasm, representing early hairy cell leukemia (original magnification ×200). C, Autopsy bone marrow from HIV-infected patient showing serous fat atrophy (gelatinous transformation; hematoxylin-eosin, original magnification ×200). D, Bone marrow from HIV-infected patient with bare, hypolobated megakaryocyte nuclei (hematoxylin-eosin, original magnification ×200). E, Plasma cell infiltrate in patient with multiple myeloma, showing large plasma cell clusters (CD138 immunohistochemical stain, original magnification ×200). F, Nonneoplastic perivascular plasma cells in HIV-infected patient; these cells were polytypic by κ and λ immunohistochemistry (CD138 immunohistochemical stain, original magnification ×200).
number of megakaryocytes and in excluding dysplastic changes diagnostic of MDS; however, isolated thrombocytopenia with minimal morphologic dysplasia may occur in indolent forms of MDS, particularly in those with a del(20q) cytogenetic abnormality.

Distinction between idiopathic thrombocytopenic purpura and other causes of platelet consumption (splenic sequestration, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura) cannot be made on the basis of the bone marrow findings alone. Decreased or absent megakaryocytes in the setting of thrombocytopenia suggest suppression of megakaryopoiesis, usually due to a drug or toxin. It is important to note that in rare cases of idiopathic thrombocytopenic purpura there may be antibodies directed against megakaryocytes, with resulting decrease or absence of bone marrow megakaryocytes.

Leukopenia or Pancytopenia

In the absence of any comorbid condition, pancytopenia is often an indicator of a primary bone marrow disease. In particular, bone marrow sampling is often done for isolated leukopenia or pancytopenia in patients with autoimmune diseases, HIV infection, or other chronic diseases. In these situations, the underlying disease itself may be the cause of the cytopenias and may produce an abnormal-appearing bone marrow.

Special Scenario: Cytopenias in HIV-Infected Patients—Disseminated infections, such as histoplasmosis, leishmaniasis, and mycobacteria, may manifest in the bone marrow of HIV-infected patients with cytopenias. Pan- cytopenic presentation due to primary bone marrow non-Hodgkin or Hodgkin lymphoma is also not uncommon in the setting of HIV infection. In the absence of a specific identifiable infectious agent or neoplasm, cytopenias may be due to autoimmune destruction or direct bone marrow effects due to the virus itself as well as the effects of antiviral drugs. Various abnormalities described in the bone marrow of HIV-infected patients are listed in Table 4 and are illustrated in Figure 2, C and D. In particular, morphologic dysplasia may be present and may mimic a primary myelodysplastic syndrome.

Special Scenario: Cytopenias in Autoimmune Diseases—Systemic lupus erythematosus and other autoimmune diseases may cause marked pancytopenia. Histologic changes in the bone marrow include serous atrophy/gelatinous transformation, varying degrees of reticulin fibrosis, lymphocytosis, plasmacytosis, and morphologic dysplasia. A diagnosis of a primary myelodysplastic syndrome should be made with utmost caution in the setting of known autoimmune disease (or HIV infection).

Special Scenario: Bone Marrow Granulomas—Gran-
effective targeted therapies (imatinib mesylate and related tyrosine kinase inhibitors), CML should always be excluded by molecular and/or cytogenetic testing in any fibrotic marrow of unknown cause.

**Lymphoid Aggregates**

Benign lymphoid aggregates may occur in the bone marrow in a variety of reactive conditions and are also more likely to be found in older individuals. Conditions associated with the presence of benign lymphoid aggregates include autoimmune diseases and HIV infection, as well as myeloid neoplasms, such as MDS and myeloproliferative diseases; in the latter cases, the problem may arise whether the aggregates are reacting to a myeloid neoplasm or whether there is reactive marrow “dysplasia” to lymphoma involving bone marrow (see above)! In contrast to neoplastic lymphoid aggregates, reactive aggregates tend to be few in number, are nonparatrabecular (although many lymphomas may have exclusively non-paratrabecular aggregates), are small with good circumscription and minimal infiltration of surrounding hematopoietic marrow, have a polymorphous lymphoid population, and may contain reactive germinal centers. However, there is considerable morphologic overlap between active and neoplastic lymphoid aggregates. Unfortunately, immunohistochemistry is often not helpful, as both reactive and neoplastic lymphoid aggregates may have a variable admixture of B and T cells. Certainly, a vast excess of aggregated B cells or phenotypic aberrancy, such as abnormal coexpression of CD5 or CD43 in B cells, helps in confirming a neoplastic lymphoid population. Immunohistochemistry and/or in situ hybridization for κ and λ light chains can also be helpful in establishing a clonal B-cell population in lymphomas with a plasmacytic component (lymphoplasmacytic lymphoma and marginal zone lymphomas). Flow cytometry is a critical adjunct to morphology in establishing lymphoid aggregates as neoplastic or reactive. Immunophenotypic demonstration of a clonal B-cell population or markedly aberrant T-cell population can confirm lymphoma involving bone marrow, but it may not provide definitive classification. Conversely, failure to identify an abnormal lymphoid population by flow cytometry is reassuring, but it does not necessarily exclude marrow lymphoma. Typical pitfalls producing a false-negative flow cytometry result in a bone marrow involved by lymphoma include sampling artifact due to hemodilution, inadequate sampling of paratrabecular aggregates due to associated reticulin fibrosis, and preferential loss of lymphoma cells; plasma cells and large cell neoplasms are particularly vulnerable to the latter effect. Many lymphomas, such as Hodgkin lymphomas and some T-cell lymphomas, do not have a diagnostically abnormal immunophenotype.

In the situation of morphologically suspicious lymphoid aggregates with negative flow cytometry (or no available flow cytometry) and ambiguous immunohistochemistry, the pathologist should step back and consider the whole picture: Is there an appropriate clinical context for reactive aggregates, such as HIV infection or autoimmune disease? Is there lymphadenopathy or organomegaly? Is there peripheral lymphocytosis? Do the aggregates occupy sufficient marrow space to explain any cytopenias? Biopsy of any clinically involved extramedullary site is the best way to accurately diagnose and classify most lymphomas. If this proves to be impractical or difficult, flow cytometry on peripheral blood may yield a diagnosis even if there is no lymphocytosis; most lymphomas that primarily involve the bone marrow manifest some degree of peripheral blood involvement. Finally, a fresh bone marrow biopsy may be obtained to include flow cytometry and molecular studies to demonstrate clonality. Communication with the clinician is important in determining the appropriate handling of these situations.

**Paraprotein**

A paraprotein, discovered either incidentally or during the workup of anemia or other symptoms, frequently elicits a bone marrow examination. Bone marrow examination may also be done as a follow-up response to therapy for known multiple myeloma, although this can also be done by assessing paraprotein levels. In either scenario, obtaining an accurate plasma cell count is critical. Fortunately, sensitive and specific plasma cell markers, such as CD138 and CD38, are available for immunohistochemistry on paraffin sections, which allow for a reasonably accurate plasma cell count in tissue sections to correlate with the plasma cell count in the aspirate smear. While plasma cells are rare in normal bone marrow, they may increase to striking levels in reactive disorders such as infection and autoimmune disease that may also have associated paraproteins. The plasma cell pattern is helpful in these situations, as plasma cells in reactive conditions usually occur around blood vessels and in small clusters rather than large aggregates or sheets away from vascular structures (Figure 2, E and F). Nevertheless, unless the plasma cells are overtly malignant, it is recommended that plasma cell clonality be proven by light chain immunohistochemistry and/or flow cytometry immunophenotyping in all new diagnoses of multiple myeloma.

It is important that the diagnosis rendered fulfill the diagnostic criteria of the World Health Organization Classification: a plasma cell percentage of 10% fulfills only a minor criterion for the diagnosis of plasma cell myeloma, and additional features are required for diagnosis. It is reasonable to render a diagnosis of “plasma cell neoplasm” in such cases if the full clinical information allowing a diagnosis of myeloma is not available. New or untreated cases with less than 10% bone marrow plasma cells, even if they are shown to be monotypic, must be placed into the category of monoclonal gammopathy of undetermined significance.

Non-Hodgkin lymphomas (including T-cell lymphomas) may present with paraproteins. An immunoglobulin M (IgM) paraprotein is almost always indicative of lymphoma rather than myeloma, but it may be present in any type of B-cell lymphoma. Although a high IgM paraprotein usually correlates with lymphoplasmacytic lymphoma, classification of lymphomas in bone marrow samples should rest on morphology, immunophenotype, and genetic features as well as the clinical context.

**Special Scenario: Bone Marrow Sampling in Amyloidosis Patients.**—In the setting of systemic amyloidosis, bone marrow examination should focus on finding any monoclonal plasma cell population, regardless of whether the numbers fulfill criteria for plasma cell myeloma. Identifying any clonal plasma cell population establishes a diagnosis of systemic amyloid light chain amyloidosis, with significant treatment implications. Amyloid light chain amyloidosis patients often have very small numbers of clonal plasma cells; immunohistochemistry and/or in...
situ hybridization for light chains is recommended to dis- close these subtle populations (which may produce highly significant amounts of amyloid protein), even if plasma cells do not appear to be increased on biopsy and aspirate morphologic review.45

References