Review Article

Bone Marrow Biopsy: Interpretive Guidelines For the Surgical Pathologist

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Summary: Ideally, the bone marrow core biopsy should be reviewed with knowledge of the clinical history, complete blood count, and findings in the peripheral blood and bone marrow aspirate smears. However, for a variety of reasons, the pathologist may receive the core biopsy and aspirate clot section without all of this information. Although this approach is not optimal, a great deal of valuable information can be generated from these specimens. Over the past 20 years, there has been considerable progress in the fields of flow cytometric analysis, immunohistochemistry, and molecular diagnostic studies that can be performed on smears or extracted DNA from paraffin embedded tissue. These modalities have augmented and refined diagnostic criteria formerly ascertained by light microscopy, cytochemistry, and cytogenetics. This is particularly true of some myeloid and lymphoreticular neoplasms where a collaborative and multidisciplinary approach to the diagnosis has become necessary. Despite this growing complexity and dependence on newer methodologies, the traditional role of histopathology in evaluating the bone marrow biopsy remains as important as it has been in the past. In this review, we focus on contemporary practices and expectations for interpreting bone marrow biopsies and clot sections. Key Words: Bone marrow core biopsy—Immunohistochemistry—Fluorescence in situ hybridization—Polymerase chain reaction.

INTRODUCTION

Assessment of changes in the bone marrow is an important prerequisite for the care and stratification of patients with disease of the hematopoietic system. Located within the labyrinth of the intertrabecular and medullary spaces of bone, this highly specialized organ with complex hematopoietic and immunologic functions provides an excellent substrate for pathologic investigation including the staging of nonhematopoietic neoplasms and the monitoring of response to therapy.

Due to variations in logistical detail, organizational experience, billing practices, and attempts at cost containment from one medical facility to another, Anatomic Pathology Services often receive only the core biopsy and clot section, while other components of the bone marrow specimen are submitted elsewhere for evaluation. Despite this fragmented and unsatisfactory approach to total specimen interpretation, the surgical pathologist can ascertain considerable clinically relevant pathologic detail by supplementing traditional histopathology on paraffin embedded tissue (PET) with state of the art immunohistochemistry (IHC) and molecular diagnostic studies.

ROLE OF THE BONE MARROW BIOPSY IN PATIENT CARE

Ever since the role of the bone marrow in hematopoiesis was described in the German literature by Neumann...
(1) and Bizzozero (2) in 1868, and the first biopsy was performed by Mosler (3) in 1876, there have been remarkable improvements in the instrumentation and techniques of specimen acquisition, analysis, and interpretation.

Before the paper by Turkel and Bethel (4) in 1943, pathologic reports were based primarily on the microscopic features of aspirated smears stained with Romanowsky dyes. Subsequently, however, there has been increasing appreciation that besides providing correlation with the cytologic features of the aspirate, the core biopsy offered a reliable means to quantitate cellularity and evaluate architectural detail in situ (5). Additionally, the core biopsy enables assessment of fibrosis, the identification of focal lesions (as in lymphoma, myeloma, metastatic tumor, and granuloma), and is a repository of material for immunohistochemical and molecular diagnostic testing. Furthermore, the biopsy provides an opportunity to report on the condition of bony trabeculae, blood vessels, and other nonhematopoietic stromal and cellular components of the intertrabecular space (5,6).

In our experience, medical facilities that support specimen acquisition, analysis, and interpretation by an organized and interactive team of health care professionals including medical technologists, allied scientists, clinicians, and pathologists working in concert, offer the advantage of conforming to operational guidelines and standards that cater to the needs of all specialties within a healthcare facility at minimum cost and patient discomfort.

To maximize yield, it is recommended that before specimen procurement, the clinical picture be correlated with the complete blood count (CBC) appearances of the peripheral blood smear and other existing laboratory data to anticipate which specimen components of the bone marrow are likely to yield information most valuable to the case under study. Such an ideal scenario is, however, the exception rather than the rule, and in most cases, the surgical pathologist has little control over the events antecedent to receiving slides on a bone marrow specimen for sign out.

**INDICATIONS FOR BONE MARROW ANALYSIS**

- To explain abnormal morphologic changes observed in the peripheral blood, such as tear drop erythrocytes, rouleaux, myeloid and lymphocytic immaturity, and leukoerythroblastosis.
- To assess marrow cellularity and to monitor the progress of therapy.
- To determine the adequacy and location of stainable bone marrow iron.
- To evaluate for bone marrow involvement by metastatic disease, lymphoma, plasma cell dyscrasia, myelofibrosis, and mast cell disease.
- As part of the workup for fever of unknown origin, to assess for bone marrow involvement in infections, granulomatous disorders, and in patients with unexplained adenopathy and hepatosplenomegaly.
- To obtain cellular bone marrow for cytochemistry, flow cytometry, immunohistochemistry, molecular genetics, cytogenetics, microbial cultures, electron microscopy, and tissue culture.
- To evaluate for bone marrow involvement in suspected storage and collagen vascular disorders.
- To define the etiology of unexplained osteosclerosis and other abnormalities of trabecular bone detected by radiologic studies.

**PREPARATION OF BONE MARROW FOR HISTOPATHOLOGIC EXAMINATION**

Information on the types of biopsy needles, and guidelines for acquisition of the aspirate and core biopsy are well documented in the literature (7–10). It is important that personnel obtaining bone marrow biopsies are familiar with the range of tests possible on each specimen component, and are aware that bilateral core biopsies are recommended for staging malignant lymphomas and metastatic carcinoma, and by some for the diagnosis of multiple myeloma at initial work-up.

The need to establish high expectations for quality histologic preparations cannot be overemphasized. In fact, the cardinal sin of misdiagnosis frequently results from erroneous interpretation of inadequate or poorly processed material.

Methodologies for processing the bone marrow core and clot section vary considerably with institutional policies and philosophical expectations. In practice, routine sections should be ready for interpretation within 24 hours after specimens are placed on the tissue processor. The choices of fixative, decalcifying agent, and stains vary from one institution to the other. A selected few, which are in general use, are described below.

Fixation of the core biopsy and clot section may be achieved with a zinc formalin fixative such as B-plus Fix.
(BBC, Stanwood, WA), B-5, or neutral buffered formalin. Thereafter, the specimen should be decalcified in a 10% aqueous solution of nitric acid. Redecal (a 12.5% aqueous solution of hydrochloric acid in EDTA, Stat Lab Medical Products Inc., Lewisville, TX) or RDO (ABP Engineering Products Corporation, Plainfield, IL), another proprietary solution of hydrochloric acid in a coal tar base ensuring good histologic sections, without damage to the microtome knife. Decalcification results in the leaching out of some storage iron from the core biopsy, and nitric acid and hydrochloric acid have been reported to diminish the acid fastness of mycobacteria (11,12) resulting in false negative results. Acid fastness is however retained when decalcification is in formic acid-sodium citrate or citric acid buffer (12). Therefore, these are the decalcifying agents of choice when the need to demonstrate mycobacteria can be anticipated. Combination fixative/decalcification preparations in use are Zenker’s solution (13), Surgipath Decalifier (a formic-acid formalin mixture, Surgipath Medical Industries, Richmond, IL), and Decal Plus (an aqueous solution of formalin, formic acid, and methanol, Stat Lab Medical products, Inc. Lewisville, TX). An advantage of Zenker’s solution and B-5 is a mordanting effect that enhances the tinctorial properties of the Giemsa and hematoxylin and eosin (H & E) stains. Additionally, glacial acetic acid, the decalcifying component in Zenker’s solution counteracts the cell shrinkage resulting from fixation. Metallic fixatives such as Zenker’s and B-5 degrade DNA and impair subsequent molecular diagnostic testing. This limitation is circumvented by fixation in a zinc-formalin fixative such as B-plus Fix. It is noteworthy that the manufacturer’s specified ratio of specimen volume to fixative/decalcifying agent and treatment times be closely followed to achieve optimum results.

Clot sections do not need to be decalcified. Bone dust and small fragments of bony trabeculae that are occasionally aspirated when acquisition of the core biopsy precedes that of the aspirate, can usually be cut through without decalcification. Agar embedding techniques (14) for processing the clot section, plastic embedding (15,16) of the undecalcified core and electron microscopy (16–20) of aspirated material are described in the literature; however, these techniques are not widely used for routine purposes.

Ideally, histologic sections of the core and clot section should be cut at 4 micron. In addition to routine hematoxylin-eosin stained sections, other stains of value include iron (preferably performed on the nondecalcified aspirate clot section), reticulin, periodic acid-Schiff stain (PAS), and Giemsa. Although difficult to perform, a well-done Giemsa preparation can be very helpful. This stain imports a purple hue to the cytoplasm of plasma cells, prominently highlights the metachromatic granules of mast cells and basophils, and enhances the tinctorial properties of neutrophilic and eosinophilic granules. Additional immunohistochemical stains may be necessary, and are further discussed below.

Samples for molecular studies require special handling. For instance, lesions for assessment by fluorescence in-situ hybridization (FISH) may need to be localized for the benefit of personnel in the molecular diagnostic laboratory. Paraffin sections for FISH are submitted on Superfrost Plus glass slides (Fisher Scientific, Pittsburgh, PA). For the polymerase chain reaction (PCR), scrolls of paraffin embedded tissue are deparaffinized before DNA isolation in accordance with local policies and practices.

EXPECTED DIAGNOSTIC YIELD FROM COMPONENTS OF THE BONE MARROW SPECIMEN

Not every item in the preceding list of indications prompting bone marrow analysis can be resolved by examination of the core biopsy and clot section. Furthermore, the optimum yield of diagnostic information from individual components of a bone marrow specimen invariably vary, and often reflect the type of disorder being investigated (21). In general, the aspirate and touch preparations provide qualitative cytologic detail, while the core biopsy and clot section provide quantitative information. Because the surgical pathologist frequently receives only PET, it is important to identify the expectations and limitations of the diagnostic yield from such material. These are enumerated and compared to those from the aspirate and touch preparations in Table 1 (5,6,21–36), and are further discussed below.

INTERPRETATION OF PARAFFIN EMBEDDED TISSUE AND DATA INTEGRATION

For most anatomic pathologists, a bone marrow sample is often one among a number of surgicals in a run requiring interpretation and sign-out. Although the patients name, age, and gender are part of the standard biographic template, information concerning the clinical picture, CBC, peripheral blood film, and bone marrow aspirate are often absent or inadequate, and need to be procured to ensure a meaningful interpretation. The guidelines used in evaluating a bone marrow specimen are understandably parochial. However, most observers integrate the microscopic features of the specimen with the ancillary data listed in Table 1 into a synopsis, un-
derscored by a diagnostic line and a comment, if necessary. To ensure that no aspect of the examination is inadvertently omitted, and to standardize reports, a checklist (5) such as the one below is recommended:

- The fat:cell ratio.
- The myeloid:erythroid ratio.
- A description of the hematopoietic cell lines (i.e., myeloipoiesis, erythropoiesis, and megakaryocytopoiesis).

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### TABLE 1. Summary of differences between aspirated and paraffin embedded bone marrow samples

<table>
<thead>
<tr>
<th>Component/parameter</th>
<th>Aspirate</th>
<th>Touch preps of clot/biopsy</th>
<th>Clot section</th>
<th>Core biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat:Cell ratio</td>
<td>Calculated from a differential count</td>
<td>Count differentials correlate with those of the aspirate</td>
<td>Normal myeloblasts and promyelocytes cannot be discriminated; however, stages thereafter, ALIP and AML blasts may be identified, but stages cannot be specifically classified</td>
<td>Working approximation possible</td>
</tr>
<tr>
<td>Myeloid: Erythroid ratio</td>
<td>Stages of maturation accurately classifiable</td>
<td>Normal megakaryoblasts not identifiable, stages thereafter classifiable generically as megakaryocytes</td>
<td>Count differentials correlate with those of the aspirate</td>
<td>Working approximation possible</td>
</tr>
<tr>
<td>Myeloid: Erythroid ratio</td>
<td>Stages of maturation can be accurately designated</td>
<td>Identifiable, but stages cannot be specifically classified</td>
<td>Normal lymphocytes can be differentiated from NRBC, T/B phenotypes can be designated by IHC</td>
<td>Can be designated in H/E stained sections, readily enumerated in IHC preps</td>
</tr>
<tr>
<td>Megakaryocytopoiesis</td>
<td>Stages of maturation accurately classifiable</td>
<td>Normal lymphocytes can be differentiated from NRBC, T/B phenotypes can be designated by IHC</td>
<td>Normal megakaryoblasts not identifiable, stages thereafter classifiable generically as megakaryocytes</td>
<td>Working approximation possible</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Small and scattered</td>
<td>Normal lymphocytes can be differentiated from NRBC, T/B phenotypes can be designated by IHC</td>
<td>Working approximation possible</td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td>Readily identified</td>
<td>Can be designated in H/E stained sections, readily enumerated in IHC preps</td>
<td>Working approximation possible</td>
<td></td>
</tr>
<tr>
<td>Metastatic tumor</td>
<td>Appearances characteristic of cell type</td>
<td>Identifiable and quantifiable.</td>
<td>Working approximation possible</td>
<td></td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>Not quantifiable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>Not quantifiable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulomata</td>
<td>Seldom observed, cannot be ruled out from these specimen components</td>
<td>Specimen components of choice to evaluate for granulomata</td>
<td>Working approximation possible</td>
<td></td>
</tr>
<tr>
<td>Iron stores</td>
<td>Quantitation reliable, best for identification of RSB</td>
<td>Quantitation unreliable, RSB identifiable</td>
<td>Quantitation reliable, RSB may be identified</td>
<td>Quantitation and identification of RSB unreliable</td>
</tr>
<tr>
<td>Monocytes/Macrophages</td>
<td>Monocytes, sea blue histocytes and other pathologic forms can be designated, and confirmed by cytochemistry</td>
<td>Monocytes not identifiable, tingible body macrophages and pathologic forms identifiable, confirmation possible with IHC</td>
<td>Working approximation possible</td>
<td></td>
</tr>
<tr>
<td>Stromal space and mesenchyme</td>
<td>Accurate assessment not possible</td>
<td>Can be identified</td>
<td>Best specimen component to assess</td>
<td></td>
</tr>
<tr>
<td>Arterial/Venous Structures</td>
<td>Capillaries may be seen</td>
<td>Not observed</td>
<td>Seldom apparent</td>
<td>Routinely observed</td>
</tr>
<tr>
<td>Trabecular sinusoids</td>
<td>Not observed</td>
<td></td>
<td>Normal, apparent, dilated in fibrosing states</td>
<td></td>
</tr>
<tr>
<td>Trabecular bone</td>
<td>Not part of specimen</td>
<td></td>
<td>Present, may offer diagnostic information</td>
<td></td>
</tr>
<tr>
<td>Cytochemistry</td>
<td>Specimen of choice</td>
<td>Can be performed</td>
<td>Selected preparations feasible</td>
<td></td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Specimen of choice</td>
<td>Not possible</td>
<td>Possible by vortex disaggregation of unfixed material</td>
<td></td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>Possible</td>
<td>Specimen components of choice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular Diagnostics</td>
<td>Possible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Specimen of choice</td>
<td>Not possible</td>
<td>Feasible on vortex disaggregated material</td>
<td></td>
</tr>
</tbody>
</table>

ALIP, Abnormally located immature precursors; AML, Acute myeloid leukemia; RSB, Ring sideroblasts; NRBC, Nucleated red blood cells; IHC, Immunohistochemistry.
● A quantitation and description of lymphocytes and plasma cells.
● Evaluation of stainable iron.
● Exclusion or description and quantitation of neoplastic infiltrates such as blast forms, lymphoma, myeloma, and metastatic tumor.
● Exclusion or description of infections and granulomatous changes.
● Exclusion or description of fibrosis.
● A statement, if necessary, to cover any miscellaneous abnormality observed in the macrophage system, bone marrow stroma, vascular structures, and trabecular bone.
● A concluding comment integrating the above with the results of any corroborating cytochemical, flow cytometric, immunohistochemical, molecular diagnostic, cytogenetic, or other contributing studies.

THE FAT:CELL RATIO

It is the best to evaluate marrow cellularity and the fat:cell ratio from the core biopsy. Although less reliable, these may also be assessed from clot sections with multiple marrow particles. Variations in the ratio of fat to cell in the bone marrow are an important index of bone marrow activity. At birth and during early childhood, the bone marrow is 100% cellular and virtually devoid of fat. By the end of the first decade, the overall fat:cell ratio is approximately 10:90, and in adults (Fig. 1), it varies between 30:70 and 70:30 with increasing age. Slight physiologic variations of the ratio from one microscopic field to another are not uncommon and review of the slide with a scanning objective can enable definition of an overall ratio. Beyond the seventh decade, bone marrow fat may be expected to increase by approximately 10% with each passing decade (37–39). Despite this increasing hypocellularity, the CBC remains within physiologic limits. However, in the presence of otherwise unexplained cytopenia, a fat content of greater than 70% reflects hypoplasia. Quantitation of the latter is usually expressed as mild, moderate, or severe.

The word “aplasia” is derived from Greek (40), and connotes total fatty replacement of the bone marrow. Aplasia may be generalized or focal (spotty). The decreased cellularity within the stromal space that results from chemotherapeutic induction of acute leukemia (Fig. 2), serous fat atrophy (Fig. 3), hypoproliferative states, and fibrosis are not accompanied by increased bone marrow fat. Therefore, these are examples of hypocellularity rather than true aplasia. Under these circumstances, it is considered more meaningful to report a ratio incorporating the relationships of fat, cell, and stroma. Accurate evaluation of the fat:cell ratio is possible only on paraffin embedded tissue, and areas with stromal hemorrhage, dropout, and aspiration artifact should be excluded. In cases with unexplained aplasia without peripheral blood cytopenia, it is advisable to recommend a contralateral core biopsy to rule out sampling error.

QUANTITATION OF CELLULAR COMPONENTS IN THE BONE MARROW

Differential counts of cellular elements in the bone marrow are traditionally expressed in percentile figures (6,27,41–50). Included herein are blast forms, myelomonocytic, erythroid, megakaryocytic, plasma cellular, and lymphoid cell lines. Macrophages, stromal cells, osteoclasts, osteoblasts, fat cells, and metastatic tumor cells are excluded. The “hematopoietic” compartment is comprised of all myelomonocytic, erythroid, and megakaryocytic elements. All other forms are classified as “nonhematopoietic.” Quantitation of the extent of involvement by tumor does convey meaning, and should be expressed as a percentage of the involved marrow space or total marrow cellularity.

MYELOID:ERYTHROID RATIO

At birth, the myeloid:erythroid (M:E) ratio is approximately 1.5:1. The myeloid (granulocytic) component gradually increases and peaks at approximately 6:1 by the end of the first week of life (51). Thereafter, values plateau at a physiologic range of 2.5:1 to 4:1, with little variation throughout life (6,20,51). Increase in either component is reported as myeloid/erythroid “predominant” in the presence of a normal fat:cell ratio, and “hyperplasia” when the cellularity of the bone marrow exceeds 70%. While an M:E ratio can be calculated from a 500 cell count down to a decimal point in the aspirate, paraffin embedded tissue provides only a coarse approximation of the same. However, paraffin embedded tissue has the advantage of providing an overview of this ratio in situ, and in different areas of the specimen. Also, eliminated herein is any bias that may be manifest in particle-depleted smears of sinusoidal blood. It is best to read M:E ratios on core biopsies, away from paratrabecular areas, which are normally myeloid predominant. Because of their characteristic cytoologic features, the myeloid component beyond the promyelocyte stage can be readily designated with a 40 x objective, right up to the neutrophil, eosinophil, and mast cell stage. Similarly, nucleated erythroid elements beyond the early normoblast stage can be differentiated from lymphocytes by their round, dense, and pyknotic nuclei. In contrast to
FIG. 1. Normocellular bone marrow biopsy. The fat:cell ratio is estimated at 60:40; H&E section (original magnification x 100).
FIG. 2. Adequate chemotherapeutic induction. Core biopsy from a patient with AML-M2 at day 14; H&E section (original magnification x 400).
FIG. 3. Serous fat atrophy. Deposits of acid mucopolysaccharide are located between fat cells. Core biopsy. (A) H&E section. (B) Alcian blue stain (original magnification x 400).
FIG. 4. Normal M:E ratio. A PAS section of the core biopsy with an M:E ratio of approximately 4:1. Intensity of PAS activity increases with granulocytic maturation. Erythroid precursors have deeply hematoxyphilic nuclei (original magnification x 400).
FIG. 5. Acute myeloid leukemia (AML-MO). Clot section at diagnosis. Most normal hematopoietic cells in the stromal space are replaced by the leukemic infiltrate; H&E section (original magnification x 400).
FIG. 6. Chronic myeloid leukemia, accelerated phase. Core biopsy. Myeloblasts are less than 20% of marrow cellularity. Myeloperoxidase immunostain (original magnification x 400).
FIG. 7. Myelodysplasia. Two morphologic features observed in core biopsies. (A) Abnormal localization of immature myeloid precursors, H&E section (Courtesy of Dr. Kathryn Foucar). (B) Myeloblasts in stromal space estimated at less than 20% of marrow cellularity; H&E section (original magnification x 400).
FIG. 8. Megaloblastic anemia. Note megaloblastic proliferation with interspersed myeloid component including giant metamyelocytes; H&E section (original magnification x 400).
FIG. 9. Erythroleukemia, AML-M6B. (A) Blast forms in an H&E section. (B) Blast forms immunostained by the immunoperoxidase technique for hemoglobin A (original magnification x 400) (Courtesy of Dr. Harold Schumacher).
FIG. 10. Dysplastic megakaryocytes including micromegakaryocytic forms from a patient with myelodysplasia. (A) H&E section. (B) FVIII RA immunostain (original magnification x 400).
FIG. 11. Essential thrombocytemia. Core biopsy demonstrating increased megakaryocytic endoreduplication and abutment; H&E section (original magnification x 400).
FIG. 12. Follicular lymphoma (grade I). Core biopsy. Tumor was located in paratrabeicular and intertrabeicular areas. (A) H&E section. (B) BCL2 preparation (original magnification x 400).
appearances in H & E preparations, cells of myeloid and erythroid lineage are easier to recognize in Giemsa and PAS stains (Fig. 4), and can be designated with certainty on myeloperoxidase (MPX) and hemoglobin peroxidase (HbPX) preparations of paraffin embedded tissue. It is noteworthy that monocytes and lymphocytes (also referred to as nongranulocytes), plasma cells, and megakaryocytes are not included in the M:E ratio.

**MYELOID SERIES**

The myeloid component of the bone marrow arises from self-replicating stem cells, which also gives rise to the monocytic, erythroid, and megakaryocytic cell lines. Data on the physiologic range of myeloid precursors and mature forms in the bone marrow (Table 2)(41–43) have been derived from aspirate preparations where the cytologic features of each cell type are readily appreciated under an oil immersion lens. Even though quantitation of similar information on PET is in contrast largely subjective, and unlike aspirate counts lacks mathematical precision, it does have clinical utility.

In our experience, normal myeloblasts and promyelocytes are not readily differentiated from pronormoblasts and early normoblasts in PET with a high dry objective. However, mature forms beyond the promyelocyte stage and pathologic shifts in compartment size as in myeloid hyperplasia, the myelodysplastic syndromes (MDS), the acute myeloid leukemias (AML) AML-MO (Fig. 5) through M6A, some of the myeloproliferative disorders, and chronic leukemias of myeloid origin are readily differentiated. Confirmation of myeloid lineage in PET may be achieved by specific esterase (Leder), MPX (Fig. 6) and other immunostains (28–31,52–55); and a monocytic lineage may be supported by the identification of CD68, lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotrypsin activity in AML-M4, AML-M5 and chronic myelomonocytic leukemia (CMML)(56). Leisions of mast cell lineage can be specifically confirmed with mast cell tryptase and CD117 (C-Kit) preparations (57–60), and TdT activity may be apparent in cases of acute mixed lineage leukemia (61,62). Although the cytologic features of blast forms in AML on PET are not specific for the subtypes of AML according to the FAB (French-American-British) scheme, cases of AML-M3, AML-M4, and AML-M5B do tend to have “monocytic” nuclei. Also, minimal myeloid differentiation is observed in AML-M2, and an abnormal eosinophil component is apparent in AML-M4EO. Abnormal localization of immature myeloid precursors (ALIP) (Fig. 7) in the intertrabecular space is a feature of myelodysplasia (63–65). Increased expression of PCNA antigen and CD34 activity by immunostaining on PET serve to distinguish hypoplastic myelodysplastic syndromes from acquired aplastic anemia, where these parameters are decreased (66). While immunostaining with CD34 is of value in identifying immature populations of leukemic cells, it does not distinguish between a myeloid and a lymphoid lineage. Without incorporating data generated from examination of the aspirate, cytochemistry, flow cytometry, cytogenetics, and molecular diagnostic studies, it may be impossible to classify myeloid, and mixed lineage lesions, according to the recent recommendations of the Society for Hematopathology and World Health Organization (WHO) expert committee (67–69).

In cases where no aspirate is available due to a dry tap, vortex disaggregation of leukemic cells from the unfixed core biopsy and clot can serve as a resource of material for cytogenetics and flow cytometry. With the introduction of newer generations of therapy such as Mylotarg (70) (formerly known as CMA-676), which is specifically directed against the CD33 epitope in AML, and STI571, (71,72) which targets and inhibits bcr-abl tyrosine kinase in chronic myeloid leukemia (CML), there is a growing expectation that laboratory identification of such loci will become available as part of the diagnostic armamentarium. Furthermore, it has now become apparent that in patients with CML on STI571, despite microscopic restoration of marrow cellularity with normal M:E ratios, bcr-abl fusion persists (72) and may be demonstrated by FISH on the core biopsy and clot section (Table 3). This technology is therefore certain to influence therapeutic protocols for STI571 in the future. According to the WHO expert panel and the Society for Hematopathology, the threshold for transition of blast counts from myelodysplastic syndrome (MDS) (Fig. 7) to AML is set at 20% with the exception of lesions that include a t(8;21) chromosomal abnormality (67,68,73). Despite lower blast counts, this entity, which would have been formerly classified as MDS, is now, because of a

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblasts</td>
<td>0% to 2%</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>2% to 5%</td>
</tr>
<tr>
<td>Myelocytes (neutrophilic)</td>
<td>9% to 16%</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>7% to 23%</td>
</tr>
<tr>
<td>Band Forms</td>
<td>8% to 15%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4% to 10%</td>
</tr>
<tr>
<td>Myelocytes (eosinophilic)</td>
<td>0% to 2%</td>
</tr>
<tr>
<td>Band</td>
<td>0% to 2%</td>
</tr>
<tr>
<td>Mature</td>
<td>0% to 2%</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
<td>0% to 3%</td>
</tr>
<tr>
<td>Basophils</td>
<td>0% to 1%</td>
</tr>
<tr>
<td>Mast Cells</td>
<td>0% to 2%</td>
</tr>
</tbody>
</table>

**TABLE 2. Types of myeloid elements and their normal range in the bone marrow**

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superior long-term outlook in adults, diagnosed and treated earlier in its natural history as AML (69,73). The supportive role of molecular diagnostic studies, some of which are now available on PET (Table 3), is therefore of paramount importance. Additionally, it is noteworthy that the malignant progranulocytes of AML-M3 be included in the total blast count, as should the myeloblasts of erythroleukemia AML-M6A (74,75) (traditional erythroleukemia of the FAB classification).

### ERYTHROID SERIES

The erythroid component of the bone marrow normally comprises between 15% and 37% of a differential cell (6,42,43,52) count that includes granulocytes, megakaryocytes, lymphocytes, and plasma cells. Maturing erythroid precursors include pronormoblasts, early normoblasts, intermediate normoblasts, and late normoblasts. Of these, pronormoblasts and early normoblasts cannot always be distinguished with certainty from myeloblasts and promyelocytes in PET with a high dry objective. However, later stages of erythroid maturation with their characteristic round, dense and deeply basophilic nuclei can be easily identified, and distinguished from interspersed lymphocytes. In circumstances where this distinction poses a problem, IHC identification of erythroid precursors with an HbPX immunostain is definitive. This technology is also invaluable in confirming the absence of erythroid precursors in cases of total red cell aplasia. Under normal circumstances and in regenerative states, clones of normoblasts tend to cluster in the intertrabecular space. However, in florid megaloblastic hyperplasia (Fig. 8), marrow cellularity may be diffuse and resemble the neoplastic proliferations of acute leukemia and large cell lymphoma (LCL). Unlike these conditions however, myeloid elements including giant metamyelocytes are interspersed between megaloblasts. Under these circumstances, correlation of biopsy findings with other laboratory data such as the appearances of the aspirate, CBC, serum LDH, folate, and B12 can be invaluable. Arrested erythroid maturation, giant pronormoblasts, and intranuclear inclusions are manifestations of parvovirus B19 infection (76). Herein, viral material can be positively identified by in situ hybridization (ISH) in PET. Multinucleated erythroid precursors may be observed in CDA (77), where additional confirmatory hematological testing such as the Ham and Sugar Water tests become necessary. In cases of AML-M6B (74,75,78), bizarre multinuclearity of leukemic erythroid precursors may be a prominent feature and lineage identity can be established with HbPX immunostaining (Fig. 9) on PET (79). In such cases, neoplastic pronormoblasts should be included with myeloblasts in the overall blast count, and in keeping with the recommendations of the Society of Hematopathology and WHO, cases with a blast count under 20% be classified as myelodysplasia, and those above as acute leukemia. Vortex disaggregation of cellular material from the unfixed core biopsy for flow cytometric, cytogenetic, and molecular diagnostic studies can be an invaluable adjunct in patients with a dry tap (34).

### MEGAKARYOCYTES

Megakaryocytes are the largest of the hematopoietic cells, and comprise between 0.5% and 2.0% of all nucleated marrow elements (6,52,80). The earliest cell of

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**TABLE 3. Lineage specific molecular cytogenetic probes** currently in use to identify myeloid lesions by fluorescence in situ hybridization (FISH) on core biopsies of bone marrow

<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Chromosome abnormality</th>
<th>FISH Signal color</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML-M0/M1, MDS</td>
<td>EGR1</td>
<td>5q Deletions-Interstitial (5q31)</td>
<td>5p15.2 (control) 5q31-EGR1 N/A</td>
</tr>
<tr>
<td>AML-M0/M1, MDS</td>
<td>CSF1R</td>
<td>5q Deletions-telomeric (5q33-34)</td>
<td>5p15.2 (control) 5q33-CSF1R N/A</td>
</tr>
<tr>
<td>AML/MDS</td>
<td>D2S108 (20q12)</td>
<td>Deletions in 20q</td>
<td>20q12 N/A</td>
</tr>
<tr>
<td>AML/MDS</td>
<td>D7S486 (7q31)</td>
<td>Deletions in 7q, t(7q)</td>
<td>Alpha-satellite 7 D7S486 (7q) N/A</td>
</tr>
<tr>
<td>AML/MDS</td>
<td>8p11.1-q11.1</td>
<td>Trisomy 8</td>
<td>N/A</td>
</tr>
<tr>
<td>AML-M2</td>
<td>AML1/ETO</td>
<td>t(8;21)(q22;q22)</td>
<td>Abnormal-1 green (split signal) 21q-AML1 (RUNX1) 8;21 fusion signal Dual color (normal)</td>
</tr>
<tr>
<td>AML-M3 (variant)</td>
<td>RARα</td>
<td>t(11;17)(q23;q21) and related X/RARA</td>
<td>17q-RARA Abnormal-1 orange (split signal) 15q-PML 15;17 fusion signal Dual color (normal)</td>
</tr>
<tr>
<td>AML-M3</td>
<td>PML/RARα</td>
<td>t(15;17)(q22;q21)</td>
<td>Abnormal-1 orange (split signal) 15q-PML 15;17 fusion signal Dual color (normal)</td>
</tr>
<tr>
<td>AML-M4EO</td>
<td>CBFβ</td>
<td>inv(16)(p13q22) and t(16;16)(p13q22)</td>
<td>Abnormal-1 green (split signal) Abnormal-1 orange (split signal) Dual color (normal)</td>
</tr>
<tr>
<td>AML-M5 and Mixed lineage</td>
<td>MLL(ALL-1, HRX)</td>
<td>11q23 rearrangements</td>
<td>9q22-BCR 9q-ABL 9;22 fusion signal</td>
</tr>
</tbody>
</table>

* Vysis Inc. Downers Grove, IL. Tabulated by Mary L. Nordberg PhD.
megakaryocytic lineage discernable microscopically is the megakaryoblast, a mononucleated cell with scant deeply basophilic cytoplasm rich in RNA. Megakaryoblasts are slightly larger than myeloblasts and pronormoblasts, from which they are not readily distinguished in PET with a high dry objective. Subsequent stages of maturation are characterized by increased cell size, progressive nuclear lobulation (endoreduplication), loss of cytoplasmic basophilia, and increase in “cotton candy” like granules. Normal megakaryocytes are randomly distributed among other hematopoietic cells, occasionally reveal mature hematopoietic cells within their cytoplasm (emperipolesis), and only seldom make physical contact with other megakaryocytes. Because megakaryocytes normally comprise about 1:200 nucleated marrow cells, increase in the megakaryocytic compartment as in idiopathic thrombocytopenic purpura and other reactive states can be readily appreciated with a low power objective. Megakaryocytes are PAS positive, and in PET, megakaryoblasts and megakaryocytes are FVIII related antigen (von Willebrand factor), FXIII, CD41, and CD61 positive by immunohistochemistry (81,82,83). This technology is invaluable in establishing lineage identity in acute megakaryocytic leukemia (AML M-7), where blast forms may be indistinguishable from those of some other forms of AML on H & E stains of PET (84,85). Immunohistochemistry also helps in the identification of dysmorphic, dysplastic, and micromegakaryocytic forms that may be observed in the myeloproliferative and myelodysplastic disorders (Fig. 10). It is noteworthy that megakaryocytes abut, cluster, sheet out, and reveal increased endoreduplication in essential thrombocythemia (Fig. 11). These features may be variably present in the other myeloproliferative disorders, but are less pronounced. Increased numbers of mature mononuclear megakaryocytic forms may be observed in chronic myeloid leukemia and the 5q− syndrome (86–89), and loss of nuclear lobulation with development of small discrete nuclei (“pawn ball” forms) may be apparent in myelodysplasia on H&E sections in PET.

**LYMPHOCYTES AND LYMPHORETICULAR LESIONS**

In the bone marrow of normal adults, lymphocytes are small, randomly interspersed between hematopoietic cells, and comprise between 8% and 24% of all nucleated elements (44–47). The ratio of T-cells to B-cells is approximately 3:1, and the CD4:CD8 ratio is approximately 2:1. In pediatric samples, the total lymphocyte count is higher, and normal values up to 40% may be observed (90). Labeled antibodies are now available commercially, facilitating the evaluation of lymphocyte subsets in PET by IHC. Beyond the third decade, benign nonparatrabecular lymphoid aggregates may be observed and rarely may contain reactive germinal centers (91). In aspirate smears, it is not uncommon to observe benign polyclonal lymphoid aggregates as a monolayer of mature lymphocytes.

In PET, monoclonal lymphoid infiltrates are usually characteristic of involvement by a chronic lymphoid leukemia (5) or a low or intermediate grade nonHodgkin lymphoma. Rarely, follicle center cell lymphoma (FCL) first presents within the bone marrow in patients with cytopenia but without organomegaly or adenopathy. The aspirate smears in these patients are commonly negative (92) but paratrabecular aggregates diagnostic of lymphoma are found in the core (Fig. 12) and are CD20 and BCL2 positive (93). Such infiltrates may be accompanied by some reactive CD3-positive T-cells. In patients with treated FCL, minimal residual (and occasionally intertrabecular) deposits of tumor can be identified by similar IHC procedures. This may correlate with the presence of a combined CD19, CD10, and BCL2 population of lymphocytes on flow cytometric analysis. Large cell lymphoma has appearances in the bone marrow comparable to those seen in node-based biopsies (Fig. 13). The presence of BCL6 activity in LCL serves to distinguish this lesion from blastic mantle cell lymphoma.

Hodgkin lymphoma in the bone marrow (Fig. 14) is associated with fibrosis, accounting for the rarity with which Reed-Sternberg cells are observed in aspirate preparations (94–98). As with the nonHodgkin lymphomas, bilateral core biopsies are recommended for staging of Hodgkin lymphoma (21). The true identity of mononuclear Reed-Sternberg cells in the bone marrow can be established with antibodies specific for CD15 and CD30 and other related immunostains (69).

In cases of bone marrow involvement by acute lymphoblastic leukemia (Fig. 15) and the high-grade lymphomas (i.e., lymphoblastic lymphoma and Burkitt lymphoma) (Fig. 15), the pattern of infiltration is invariably interstitial and diffuse (6). Correlation of the morphology with immunophenotypic, cytochemical, and cytogenetic data is invaluable (99–101). The identification of a minimal residual population of acute lymphoblastic leukemia (ALL) in PET can be achieved by immunostaining for CD34 (Fig. 16), TdT, CD20, CD3, CD45RO, and additionally with molecular diagnostic studies for IgH, TCR gene rearrangement, and the identification of abnormal genetic loci by PCR or FISH (Table 4). Also of great importance is the distinction of acute lymphoblastic leukemia from hematogones (102). The latter are B-
FIG. 13. Large cell lymphoma. Core biopsy. Diffuse involvement of the intertrabecular space. (A) H&E section. (B) CD20 preparation (original magnification x 400).

FIG. 14. Hodgkin lymphoma. Core biopsy. Note solitary Reed-Sternberg cell and fibrosis (original magnification x 400).

FIG. 15. Acute lymphoblastic leukemia (A) and Burkitt lymphoma (B). H&E stained sections of core biopsies (original magnification x 400).

FIG. 16. Relapsed acute lymphoblastic leukemia. Clot section; CD34 immunostain (original magnification x 400).

FIG. 17. Chronic lymphocytic leukemia. Core biopsy. Interstitial pattern of infiltration. (A) H&E section and Waldenstrom macroglobulinemia. Core biopsy. Note Dutcher body. (B) PAS section (original magnification x 400).

FIG. 18. Adult T-cell leukemia/lymphoma. Core biopsy. Note erosion of bony trabecula adjacent to tumor; H&E section (original magnification x 400).

FIG. 19. Anaplastic myeloma. Core biopsy; H&E section (original magnification x 400).

FIG. 20. Anaplastic myeloma. Core biopsy with kappa light chain restriction; Kappa immunostain (original magnification x 400).

FIG. 21. Metastatic carcinoma of breast. Core biopsy; H&E section (original magnification x 400).

FIG. 22. Kaposi sarcoma. Core biopsy; H&E section (original magnification x 400).

FIG. 23. Reticulin and collagen fibrosis in bone marrow core biopsies. (A) Gordon and Sweet’s reticulin stain with markedly increased reticulin. (B) H&E section with total effacement of hematopoietic tissue by collagenous fibrosis. (C) Trichrome stain demonstrating total collagenous fibrosis (original magnification x 400).

FIG. 24. Granulomatous changes in core biopsy of bone marrow. (A) H&E stain of epithelioid granuloma from patient infected with Mycobacterium tuberculosis. (B) H&E stain of “ring granuloma” in patient with Q-fever (original magnification x 400).
lymphocytic precursors that express CD10, CD19, and TdT, variably express CD20, usually do not appear in the peripheral blood, lack sIg, do not reveal any cytogenetic or molecular abnormality, and show a characteristic pattern of antigen expression in flow cytometry histograms. Hematogones cannot be positively differentiated from lymphocytes and lymphoblasts on PET. The list of locus probes that may specifically identify the molecular basis of lymphoid lesions in PET (Table 4) is growing. While it is unnecessary to apply such technology to cases where histologic appearances and the corroboratory data support a diagnosis, these molecular genetic probes are invaluable when such data is either sparse or unavailable.

By complimenting light microscopy with the modalities mentioned in Table 4, this differential and the features characteristic of the indolent and intermediate grade lesions, i.e., chronic lymphocytic leukemia (Fig. 17), Waldenström's macroglobulinemia (Fig. 17), prolymphocytic leukemia, hairy cell leukemia, malignant lymphoma-leukemic phase, splenic lymphoma with villous lymphocytes, follicular lymphoma, mantle cell lymphoma, marginal cell lymphoma, malignant lymphoma originating from mucosa associated lymphoid tissue (MALT), LCL, malignant lymphoma with intravascular infiltration, T-cell rich B-cell lymphoma, adult T-cell leukemia/lymphoma (Fig. 18), large granular lymphocyte disorders, peripheral T-cell lymphoma, mycosis fungoides, anaplastic large cell lymphoma, and hepatosplenic gamma/delta T-cell lymphoma can now be definitively ascertained (103–132). However, because of deficiencies in standardization and the known false negative and false positive rate for PCR-based immunoglobulin gene and T-cell receptor assays to determine clonality, it seems unlikely that PCR will replace microscopy to detect lymphoreticular lesions of the bone marrow in the near future (133–135).

### PLASMA CELLS

Plasma cells are absent in the bone marrow at birth, but begin to appear during the first few years of infancy. In the adult, plasma cells are rare, polyclonal, comprise 3% to 6% of all nucleated cells, and are occasionally perivascular in distribution. The normal ratio of kappa:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Chromosome abnormality</th>
<th>FISH signal color</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL TEL/ETV6/AML1</td>
<td>t(12;21)(p13;q22)</td>
<td>TEL Green</td>
<td>Abnormal-1 green (split signal)</td>
</tr>
<tr>
<td>ALL BCR/ABL</td>
<td>t(9;22)(q34;q11.2)</td>
<td>BCR Orange</td>
<td>Abnormal-1 orange (split signal)</td>
</tr>
<tr>
<td>ALL p16</td>
<td>del(16)(p21)</td>
<td>AML1 Yellow</td>
<td>t(12;21) Fusion</td>
</tr>
<tr>
<td>Burkitt Leukemia/ Lymphoma MYC</td>
<td>t(8;14), t(2;8), t(8;22)</td>
<td>Normal-1 Green</td>
<td>8:14 Fusion signal</td>
</tr>
<tr>
<td>Burkitt Leukemia/ Lymphoma IgH/MYC</td>
<td>t(8;14)(q24;q32)</td>
<td>IgH Orange</td>
<td>11:14 Fusion signal</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>IgH/CCND1</td>
<td>t(11;14)(q13;q32)</td>
<td>IgH Green</td>
</tr>
<tr>
<td>Follicle center cell lymphoma</td>
<td>IgH/BCL2</td>
<td>t(14;18)(q22;q21)</td>
<td>IgH Orange</td>
</tr>
<tr>
<td>Extramedullary marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)</td>
<td>D3Z1</td>
<td>+3</td>
<td>Alpha-satellite 3</td>
</tr>
<tr>
<td>Large cell lymphoma</td>
<td>BCL6</td>
<td>t(6;9)(q27)</td>
<td>N/A</td>
</tr>
<tr>
<td>CLL, Multiple myeloma</td>
<td>p53</td>
<td>del(16)(p13.1)</td>
<td>p53 Green</td>
</tr>
<tr>
<td>CLL, Multiple myeloma</td>
<td>D12Z3</td>
<td>+12</td>
<td>N/A</td>
</tr>
<tr>
<td>CLL, Multiple myeloma</td>
<td>RB1</td>
<td>del(13)(q14.3)</td>
<td>RB1 Green</td>
</tr>
<tr>
<td>B-cell lymphoproliferative disorders, including nodal marginal zone B-cell lymphoma and monocytoid B-celllymphoma</td>
<td>IgH</td>
<td>t(7;14)(q32)</td>
<td>N/A</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma</td>
<td>ALK1</td>
<td>t(2;5)(p23.2;q35)</td>
<td>Abnormal-1 Green (split signal)</td>
</tr>
</tbody>
</table>

* Vysis Inc. Downers Grove, IL.
# Most T-cell monoclonalities are best determined by alternate molecular genetic techniques. Tabulated by Mary L. Nordberg, PhD.

TABLE 4. Lineage specific molecular cytogenetic probes* currently in use to identify lymphoid lesions by fluorescence in situ hybridization(FISH) on core biopsies of bone marrow

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<th>FISH signal color</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL TEL/ETV6/AML1</td>
<td>t(12;21)(p13;q22)</td>
<td>TEL Green</td>
<td>Abnormal-1 green (split signal)</td>
</tr>
<tr>
<td>ALL BCR/ABL</td>
<td>t(9;22)(q34;q11.2)</td>
<td>BCR Orange</td>
<td>Abnormal-1 orange (split signal)</td>
</tr>
<tr>
<td>ALL p16</td>
<td>del(16)(p21)</td>
<td>AML1 Yellow</td>
<td>t(12;21) Fusion</td>
</tr>
<tr>
<td>Burkitt Leukemia/ Lymphoma MYC</td>
<td>t(8;14), t(2;8), t(8;22)</td>
<td>Normal-1 Green</td>
<td>8:14 Fusion signal</td>
</tr>
<tr>
<td>Burkitt Leukemia/ Lymphoma IgH/MYC</td>
<td>t(8;14)(q24;q32)</td>
<td>IgH Orange</td>
<td>11:14 Fusion signal</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>IgH/CCND1</td>
<td>t(11;14)(q13;q32)</td>
<td>IgH Green</td>
</tr>
<tr>
<td>Follicle center cell lymphoma</td>
<td>IgH/BCL2</td>
<td>t(14;18)(q22;q21)</td>
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</tr>
<tr>
<td>Extramedullary marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)</td>
<td>D3Z1</td>
<td>+3</td>
<td>Alpha-satellite 3</td>
</tr>
<tr>
<td>Large cell lymphoma</td>
<td>BCL6</td>
<td>t(6;9)(q27)</td>
<td>N/A</td>
</tr>
<tr>
<td>CLL, Multiple myeloma</td>
<td>p53</td>
<td>del(16)(p13.1)</td>
<td>p53 Green</td>
</tr>
<tr>
<td>CLL, Multiple myeloma</td>
<td>D12Z3</td>
<td>+12</td>
<td>N/A</td>
</tr>
<tr>
<td>CLL, Multiple myeloma</td>
<td>RB1</td>
<td>del(13)(q14.3)</td>
<td>RB1 Green</td>
</tr>
<tr>
<td>B-cell lymphoproliferative disorders, including nodal marginal zone B-cell lymphoma and monocytoid B-celllymphoma</td>
<td>IgH</td>
<td>t(7;14)(q32)</td>
<td>N/A</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma#</td>
<td>ALK1</td>
<td>t(2;5)(p23.2;q35)</td>
<td>Abnormal-1 Green (split signal)</td>
</tr>
</tbody>
</table>

* Vysis Inc. Downers Grove, IL.
# Most T-cell monoclonalities are best determined by alternate molecular genetic techniques. Tabulated by Mary L. Nordberg, PhD.
BONE MARROW BIOPSY

lambda light chain producing cells is approximately 4:1 as evaluated by flow cytometry and IHC (136). Plasma cells characteristically reveal intracytoplasmic immunoglobulin and are CD38 and CD138 positive and CD20 negative. Increased numbers of polyclonal plasma cells are observed in reactive states. However, when immunoglobulin light chain restriction is observed, a plasma cell dyscrasia such as monoclonal gammopathy of undetermined significance (MGUS) or multiple myeloma (Fig. 19) should be considered (136). In such cases, plasma cells often cluster and demonstrate cytologic atypia and light chain restriction on kappa and lambda immunostains in PET (Fig. 20). Several locus probes for the prognostication of multiple myeloma in PET are now available (Table 4). However, these may be of practical value only in controversial or research settings.

Correlation of plasma cell counts with clinical, radiologic, and biochemical parameters is of considerable value. In patients with abnormal lymphoplasmyacytic infiltrates, Waldenström macroglobulinemia, heavy chain disease, and amyloidosis additionally enter the differential diagnosis (107,108,137). Plasma cells may occasionally reveal flame-like cytoplasmic transformation, binuclearity, and crystalline inclusions (Snapper-Schneid bodies), features that are better appreciated on aspirate smears (138–140). These changes may be observed in both benign and malignant plasma cells. Because the lesions of multiple myeloma are focal in the bone marrow, some authorities recommend bilateral core biopsies at the time of initial investigation.

METASTATIC DISEASE

A large number of epithelial (Fig. 21) and mesenchymal (Fig. 22) tumors metastasize to the bone marrow (141,142). There is, however, considerable institutional, biologic, and specimen related variation in the incidence of these lesions. Tumors that commonly metastasize to the bone marrow include carcinomas of the breast, prostate, lung, and gastrointestinal tract in adults, and neuroblastoma in children. Metastatic tumor deposits in the bone marrow may be detected in one or all of the specimen components submitted for review (21). Occasionally, tumor deposits in the core biopsy are necrotic. Nevertheless, tumor cells variably retain their protein epitopes and their true nature may be confirmed by their IHC profile. Based on the architectural and cytologic pattern of the tumor in question, a recommended battery of IHC stains should include but not be limited to cytokeratin, epithelial membrane antigen, carcinoembryonic antigen, alpha-fetoprotein, prostate specific antigen, prostatic acid phosphatase, HMB-45, S-100, leukocyte common antigen (CD45), chromogranin, synaptophysin, CD99, neuron-specific enolase, Leu-7, smooth muscle actin, desmin, vimentin, and FVIII related-antigen (143–146).

Semiquestitation of tumor burden as a percentage of bone marrow cellularity or medullary space involved conveys additional meaning in the pathologic report.

RETICULIN AND COLLAGEN PROLIFERATION

Normally, a delicate filigree of reticulin (type III collagen) is found to pervade the stroma of the bone marrow, encircle fat cells and vascular structures, and separate cellular bone marrow from adjoining bony trabeculae. In core biopsy and aspirate clot sections, reticulin can be demonstrated with a good Wilder or Jones stain, and is reported as normal or increased. The latter may be graded as mild, moderate, or marked (Fig. 23). Reticulin proliferation is usually antecedent to collagen (type I collagen) deposition. The latter may be suspected by its coarse fiber pattern in H&E sections (Fig. 23), but should be confirmed with a trichrome (Fig. 23) or other collagen stain. Because of its inaspirable nature, collagen fibrosis is not accurately quantifiable in aspirate clot sections and should be evaluated only in the core biopsy.

Myelofibrosis may be acute or chronic, and idiopathic or secondary to myeloproliferative, lymphoproliferative, metastatic, or granulomatous disease (147–150). Slowly progressive fibrosis, as evident in the early stages of agnogenic myeloid metaplasia and established polycythemia vera, causes sinusoids in the bone marrow to permanently dilate and develop intravascular hematopoiesis. These changes may also be observed in the other myeloproliferative disorders. In contrast, rapidly progressive fibrosis replaces hematopoietic tissue, vascular elements, stroma, and fat, resulting in obliteration of the marrow space.

Grading of bone marrow reticulin and collagen may be quantitated according to the Bauermeister Scale (151) (Table 5). The limits of normality lie between grades 0 and 2.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Morphologic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No reticulin fibers demonstrable.</td>
</tr>
<tr>
<td>1</td>
<td>Occasional fine individual fibers and foci of a fine fiber network.</td>
</tr>
<tr>
<td>2</td>
<td>Fine fiber network throughout most of the section. No coarse fibers present.</td>
</tr>
<tr>
<td>3</td>
<td>Diffuse fiber network with scattered thick coarse fibers. No mature collagen present.</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse and often coarse fiber network with areas of collagenization.</td>
</tr>
</tbody>
</table>
INFECTIONS AND GRANULOMATOUS CHANGES

A wide range of infections localize to the bone marrow (152), and may result in an inflammatory, necrotizing, or granulomatous response. Intact granulomas are seldom observed in aspirate preparations, and their presence cannot be excluded if the aspirate is the only specimen available for evaluation. Granulomatous changes (Fig. 24) may be specific or nonspecific (150,153), and are best defined in the core biopsy and clot section. It is necessary to work up all epithelioid granulomas with acid fast and fungus stains, and correlate changes with the results of microbial cultures (154). In immunocompromised patients, well-defined granulomas may fail to develop. Under these circumstances, proliferation of microbial-laden macrophages (as in *Mycobacterium avium–intracellulare* infection) may be evident (155) (Fig. 25). The most frequently encountered granuloma in clinical practice is the nonspecific lipid granuloma (156). The latter are not associated with hematologic disease, and do not require work up with microbial stains. It is noteworthy that the amastigote forms of leishmaniasis and the intranuclear viral inclusions of cytomegalovirus (Fig. 26), herpes simplex and parvovirus B19 infection (Fig. 27) are readily detected with a high dry lens and may be confirmed by in situ hybridization if necessary (157).

STAINABLE IRON STORES

Quantitation of stainable iron in the bone marrow is most accurately interpreted in Prussian blue preparations of the aspirate smear and clot section. The choice between manual and automated stains (BioGenex Laboratories, San Ramon, CA) is largely parochial and workload related. Decalcification, a prerequisite for preparation of the core biopsy, results in leaching out of some storage iron. Despite this drawback, evaluation of iron in the core biopsy is informative in cases where the aspirate and clot sections were not procured or are dilute and devoid of particles. Assessment on a scale of 0 to 4 plus is both simple and reproducible (158,159). In this scale, stainable iron is designated as follows: 0 = absent, 1 = trace/decreased, 2 to 3 = adequate, and 4 = increased (Fig. 28). In the bone marrow, elemental iron can be visualized in several compartments, including the cytoplasm of macrophages, in developing normoblasts (sideroblasts), and as free hemosiderin deposits within the bone marrow stroma. Reports should include descriptions of both the quantity and the location of iron, and also the results of a search for pathologic sideroblasts including ring forms.

DISORDERS OF THE MACROPHAGE SYSTEM

Normally, monocytes and macrophages comprise up to 3% of cellular elements in the marrow (Table 2). In H&E stained sections of PET, monocytes cannot be positively identified. Nevertheless, by immunostaining for CD68, lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotripsin, infiltrates of this lineage, as in AML–M4, AML–M5, and CMML, can be designated (56). Occasional tingible body macrophages can be encountered in cellular marrow, and do not connote a pathologic process. However, an abnormal macrophage component may be observed in Gaucher disease (160) (Fig. 29), CML (161,162) (pseudo-Gaucher cells), the hemophagocytic syndromes (163), oxalosis (164–166) (Fig. 30), with thorotrast deposits (167) (Figure 31), sea-blue histocytosis, and in the acquired and inherited lipidosis (168). In each of these conditions, correlation with the clinical picture and related laboratory data is invaluable.

STROMAL AND VASCULAR ABNORMALITIES

Stromal cells and the mesenchyme of the extracellular matrix are located between fat cells, and surround both nonhematopoietic and hematopoietic tissue (169). The latter are decreased in hypoproliferative states, and are virtually effaced by chemotherapy, an index of adequate therapeutic induction in acute leukemia. Replacement of hematopoietic tissue by mucopolysaccharide with commensurate atrophy of fat cells is observed in serous fat atrophy (170). Herein, the mucopolysaccharide can be delineated with an Alcian Blue stain (Fig. 3). It is important that the patterns of aplasia, serous fat atrophy, and adequate therapeutic induction of the acute leukemias be so designated.

Nutrient arteries, veins, and intervening sinusoids are also located in the stromal compartment. Lesions that affect larger vessels, which may be incidentally observed, are those of amyloid infiltration (171) (Fig. 32), vasculitis, (172) and thrombotic thrombocytopenic purpura (TTP) (6). It is noteworthy that sinusoidal dilatation is associated with increased stromal reticulin and collagen as in the cellular phase of agnogenic myeloid metaplasia, and is occasionally accompanied by intrasinusoidal hematopoiesis (6,173,174) (Fig. 33).

DISORDERS OF TRABECULAR BONE

Although a core biopsy may have been procured to evaluate hematologic disease, the bony component of

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FIG. 25. *Mycobacterium avium-intracellulare* in the core biopsy of a terminal patient with the acquired immunodeficiency syndrome. (A) H&E section with marked histiocytic proliferation. (B) Fite stain with numerous acid-fast bacteria within histiocytes (original magnification x 400).

FIG. 26. Cytomegalovirus inclusion. Core biopsy in patient with acquired immunodeficiency syndrome. Nature of inclusion serologically and immunohistochemically confirmed; H&E section (original magnification x 400) (Courtesy of Dr. Diana Veillon).

FIG. 27. Parvovirus B19 inclusions in early erythroid precursors. Core biopsy. Patient with acquired immunodeficiency syndrome. Nature of inclusions confirmed by serology and in-situ hybridization; H&E section (original magnification x 400).

FIG. 28. Increased (+) iron stores. Core biopsy. Patient with anemia of chronic disease; Prussian blue stain (original magnification x 400).

FIG. 29. Gaucher disease. Core biopsy; H&E section (original magnification x 400).

FIG. 30. Oxalosis. Core biopsy; H&E section (original magnification x 400) (Courtesy of Dr. Richard Brunning).

FIG. 31. Intrasinusoidal hematopoiesis. Core biopsy. Patient with anogenetic myeloid metaplasia; H&E stain (original magnification x 400).

FIG. 32. Amyloid deposits. Core biopsy. (A) Congo-red preparation. (B) Birefringent appearances of amyloid with polarized light (original magnification x 400).

FIG. 33. Increased osteoblastic activity in the vicinity of metastatic carcinoma of the prostate. Core biopsy; H&E section (original magnification x 400).

FIG. 34. Renal osteodystrophy. Core biopsy; H&E section (original magnification x 400).

FIG. 35. Paget disease. Core biopsy; H&E section (original magnification x 400) (Courtesy of Dr. Tuyethoa Vinh).
this specimen should be routinely assessed. It has been estimated that the volume of cortical and trabecular bone in an iliac crest bone biopsy comprises greater than 20% of the specimen (175); and despite physiologic variances related to gender, race, and age, the mean trabecular width is approximately 200 microns (175,176). Cancellous bone of the iliac crest is in dynamic equilibrium with the rest of the skeleton, and offers protection to the tissues within the labyrinthine marrow space. Trabecular bone is lamellar, and is variably bordered by a slender osteoid seam. Osteoblasts (Fig. 34) and osteoclasts are located along the margins of trabeculae. These cells effectively maintain the structural and physiologic integrity of bone when dispersed in a ratio of approximately 100:1. Variations in this ratio along with changes in the structure of trabecular bone may be observed in previous biopsy sites, renal osteodystrophy (177,178) (Fig. 35), osteopenia, osteomalacia, (179) osteoporosis, (180) Paget disease, (181) (Fig. 36) and osteopetrosis (182). These lesions should be appropriately described and designated in the pathology report.

SUMMARY

Reporting takes many forms, and is understandably parochial. Whatever the choice, accuracy, clarity, brevity, and timeliness are paramount. A summary comment following the microscopic description and diagnostic line which ties together other ancillary aspirate related, cytotoxic, flow cytometric, molecular diagnostic, and other clinicopathologic data gleaned during the work up conveys depth to the report. This is invaluable for purposes of prognostication, teaching, coding, retrieval, and research in keeping with local needs and philosophies. It is our practice to promptly appraise clinical personnel by telephone of lesions and changes that are unexpected or have immediate therapeutic impact. This brings excellence in communication and service and also keeps the pathologist abreast of clinical events.

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