1 Introduction

Indications for bone marrow examination

Bone marrow examination, including both aspiration and biopsy sampling, can be performed on virtually any patient. However, patients with coagulation deficiencies or profound thrombocytopenia may experience prolonged bleeding, which cannot be controlled by pressure bandages. In these rare cases, specific treatment (e.g., platelet transfusion) may be indicated. Indications for performing bone marrow examination are summarized in Table 1.1. In the vast majority of cases, both a bone marrow aspiration and biopsy should be performed. Bone marrow aspiration and bone marrow biopsy are complementary (Bain, 2001a, 2001b). Bone marrow aspiration provides excellent cytologic detail; however, marrow architecture cannot be assessed. Bone marrow core biopsy allows for an accurate analysis of architecture; however, cytologic details may be lost. Table 1.2 shows the accepted indications for performing a bone marrow biopsy. This includes cases with inadequate or failed aspiration, need for accurate assessment of cellularity, cases in which the presence of focal lesions (e.g., granulomatous disease or metastatic carcinoma) is suspected, suspected bone marrow fibrosis, need to study bone marrow architecture, need to study bone structure, bone marrow stroma, or assessment of bone marrow vascularity. In general, patients with hypocellular marrows or bone marrow fibrosis are likely to need a trephine biopsy for adequate assessment. In such patients, an aspirate would probably be inadequate or even impossible. Unexplained pancytopenia and unexplained leukoerythroblastic blood pictures are further indications for a biopsy, because they are likely to indicate the presence of bone marrow metastatic disease or fibrosis.

The bone marrow biopsy specimen differs from biopsy material from most other organs, because a proper interpretation of the bone marrow requires the incorporation of a variety of specimen types and often ancillary techniques to arrive at an accurate and complete diagnosis. Bone marrow studies should be evaluated in conjunction with clinical data, peripheral blood smears, and complete blood count data as well as with bone marrow aspirate smears or imprints. Occasionally, bone marrow biopsy imprint smears may be used to assess cytology in cases with inaspirable marrows (dry taps). Many cases also benefit from cytochemical evaluation of marrow aspirate smears, flow cytometric immunophenotyping, immunohistochemical staining of bone marrow biopsy, molecular genetic and cytogenetic studies. The results of all of these should be considered in making a final diagnosis. The following chapters will emphasize this multi-factorial approach to bone marrow evaluation and attempt to highlight the diagnostic questions that require the use of ancillary techniques for accurate diagnosis. Complete clinical information is also necessary for the proper triage of the types of samples/tests required to provide the most comprehensive diagnostic information.

Obtaining the bone marrow biopsy

The technique used to obtain bone marrow aspirate smears has been described in detail previously, and the reader is referred to those publications for more detail (Brynes *et al.*, 1978; Brunning & McKenna, 1994; Perkins, 1999; Foucar, 2001). If bone marrow aspiration and bone marrow biopsy are both being performed using the same needle, it is usually preferable to obtain the biopsy first, to avoid distortion of the biopsy specimen by aspiration artifacts. Another approach uses separate needles for each procedure. This requires that the needles be placed in different sites of the bone marrow; good-quality aspirate and biopsy can be obtained in either sequence. When two needles are used,

Table 1.1. Indications for a bone marrow aspiration with or without a trephine biopsy.

Investigation and/or follow-up of
Unexplained microcytosis
Unexplained macrocytosis
Unexplained anemia
Unexplained thrombocytopenia
Pancytopenia (including suspected aplastic anemia)
Leukoerythroblastic blood smear and suspected bone marrow infiltration
Suspected acute leukemia
Assessment of remission status after treatment of acute leukemia
Suspected myelodysplastic syndrome or myelodysplastic/myeloproliferative disorder
Suspected chronic myeloproliferative disorder (chronic myelogenous leukemia, polycythemia rubra vera, essential thrombocythemia, idiopathic myelofibrosis, or systemic mastocytosis)
Suspected chronic lymphocytic leukemia and other leukemic lymphoproliferative disorders
Suspected non-Hodgkin lymphoma
Suspected hairy cell leukemia
Staging of non-Hodgkin lymphoma
Suspected multiple myeloma or other plasma cell dyscrasia
Suspected storage disease
Fever of unknown origin
Confirmation of normal bone marrow if bone marrow is being aspirated for allogeneic transplantation

Table 1.2. Indications for performing a bone marrow biopsy.

Investigation and/or follow-up of
Diagnosis and/or staging of suspected Hodgkin lymphoma and non-Hodgkin lymphoma
Hairy cell leukemia
Chronic lymphocytic leukemia and other leukemic lymphoproliferative disorders
Diagnosis of suspected metastatic carcinoma
Diagnosis, staging, and follow-up of small cell tumors of childhood
Chronic myeloproliferative disorders (chronic myelogenous leukemia, polycythaemia rubra vera, essential thrombocythemia, idiopathic myelofibrosis, and mastocytosis)
Diagnosis of aplastic anemia
Investigation of an unexplained leukoerythroblastic blood smear
Investigation of a fever of unknown origin and/or granulomatous infection
Investigation of suspected hemophagocytic syndrome
Evaluation of any patient in whom an adequate bone marrow aspirate cannot be obtained
Suspected multiple myeloma or plasma cell dyscrasia
Suspected acute myeloid leukemia
Suspected myelodysplastic syndrome
Investigation of suspected storage disease
Suspected primary amyloidosis
Investigation of bone diseases

it is often advantageous to aspirate the marrow through a smaller needle specifically designed for that purpose. This minimizes the contamination with peripheral blood, which is often observed when the aspirate is performed through the Jamshidi needle. The skin and periosteum are infiltrated with 1% xylocaine. The bone marrow aspiration needle is introduced into the medullary cavity as for bone marrow biopsy. Once the medullary cavity has been entered, the stylus is removed and a syringe attached. The marrow is aspirated by rapidly pulling back on the syringe. Optimally, this should last less than five seconds and yields not more than 1 milliliter of bone marrow aspirate material. Any additional material which can be aspirated will be mostly peripheral blood.

The initial aspirate sample should always be used for morphology. Subsequent aspirations may be obtained for flow cytometry, cytogenetics, microbiology culture, or molecular studies. Non-anticoagulated specimens should be immediately handed to a technical assistant who will prepare various smears and bone marrow particle crush preparations. The remaining marrow aspirate material is allowed to clot and submitted for marrow clot sections. Alternatively, aspirate smears can be made in the laboratory after the procedure. To this end, bone marrow aspirate material should be immediately placed into tubes (generally coded with purple tops in the USA) containing ethylenediaminetetraacetic acid (EDTA). This metal limits the clotting of the aspirate specimen and allows material to be submitted for ancillary studies as well as particle sections. Whether the smears are made at the bedside or from the EDTA tubes, the aspirate should be grossly evaluated for the presence of bone marrow particles. The absence of particles on a smear limits its diagnostic usefulness in many cases. Such smears often show findings consistent with peripheral blood contamination. Many pediatric patients, however, will not demonstrate gross particles in the aspirate material despite numerous bone marrow elements in the smear.

Representative aspirate smears and imprints are stained with a Romanovsky type of stain. The actual stain type varies among laboratories and includes Giemsa, Wright–Giemsa, and May–Grunwald–Giemsa stains. We prefer the Wright–Giemsa stain. Rapid review of these smears helps in determining the need for ancillary studies, such as

cytochemistry, immunophenotyping, cytogenetic analysis, and molecular genetic study.

Clot biopsy sections are often made from coagulated aspirate material. This material contains predominantly blood as well as small marrow particles that can be embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) or other stains. Alternatively, when EDTA-anticoagulated aspirate specimens are used, the bone marrow particles that are left after smears are prepared can be filtered and embedded for histologic evaluation (Arber *et al.*, 1993). This method provides a more concentrated collection of marrow particles but may not yield any material, particularly in pediatric patients (Brunning *et al.*, 1975).

Trephine core biopsy is not performed for all patients, but these specimens are essential in the evaluation of patients suspected of having disease that may only focally involve the bone marrow, such as malignant lymphoma or metastatic carcinoma, and are preferred in all patients. Core biopsies allow for architectural assessment of the bone marrow and offer a number of other benefits. The incidences of bone marrow involvement by various types of malignancies are proportional to the amount of bone marrow evaluated. Review of the published findings suggests that the minimum adequate length is in the range 15 to 20 mm. One study of the relation between length of trephine and the rate of positivity for neoplasia yielded a minimum adequate length of 12 mm in section (16 mm before processing; trephine biopsies shrank by 25% during processing). The authors reported that 58% of the trephines performed in their institution were inadequate by this criterion (Bishop *et al.*, 1992). To increase the yield, bilateral bone marrow biopsies have been recommended for patients undergoing bone marrow staging by several authors (Brunning *et al.*, 1975; Wang *et al.*, 2002). The adequacy of a random iliac crest biopsy marrow sampling for detection of metastatic malignancy is, however, still controversial. Recent results have suggested that, if the diagnosis of bone marrow isolated tumor cells has clinical relevance, the preoperative assessment should be performed by rib segment resection or methods other than iliac crest aspirate and/or biopsy (Mattioli *et al.*, 2001). Further investigation is needed to determine whether isolated tumor cells have a preferential spread to bones other than the ileum.

Imprint slides from the biopsy specimens may yield positive same-day results in some of the aspirate-negative or dry-tap cases (James *et al.*, 1980). The imprints can be made either at the bedside or in the laboratory. To make them in the laboratory, the bone marrow core is submitted fresh, on saline-dampened gauze, with the imprints made immediately to allow adequate fixation of the biopsy specimen.

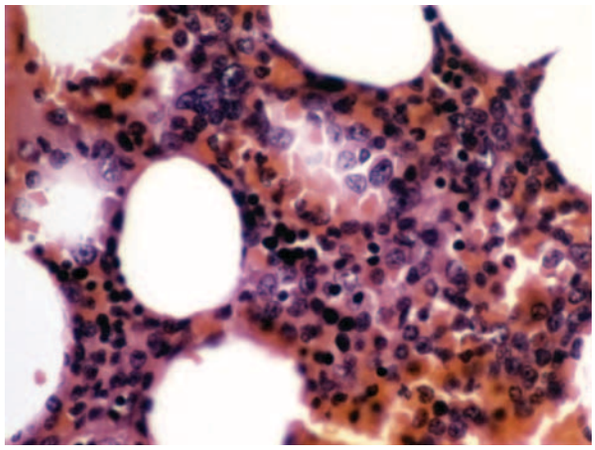

Figure 1.1. An example of a poorly prepared bone marrow core biopsy. The specimen is too thick and not well stained, preventing adequate identification of different cell types and their stages of maturation.

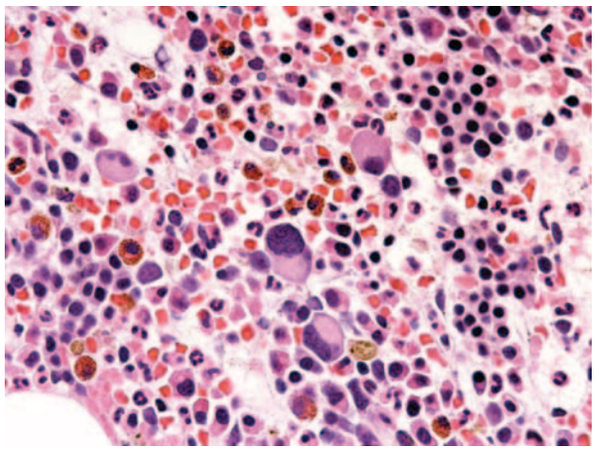

Figure 1.2. An example of a well-sectioned and well-stained core biopsy. Thin sections and a well-performed H&E stain of the core biopsy section are imperative for adequate interpretation.

Otherwise, the imprints are made at the bedside, and the biopsy specimen is submitted in fixative.

Many laboratories prefer mercuric chloride-based fixatives (e.g., B5) or Bouin's fixative for bone marrow specimens. Submission of the bone marrow biopsy material in formalin followed by EDTA or short nitric acid decalcification also provides more than acceptable results. After fixation and decalcification, the core biopsy specimen is stained with H&E and other stains. The need for well-prepared thin bone marrow biopsy sections cannot be overemphasized. Thick, poorly processed specimens (Fig. 1.1) are almost useless. A properly processed section stained with H&E is shown in Fig. 1.2.

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2

The normal bone marrow and an approach to bone marrow evaluation of neoplastic and proliferative processes

Introduction

It is often easiest to evaluate a bone marrow specimen by comparing it to what would be expected in the normal bone marrow (Brown & Gatter, 1993; Bain, 1996). The initial evaluation on low magnification includes the assessment of sample adequacy and marrow cellularity. The latter is usually based on the biopsy. Estimates of cellularity on aspirate material have been described (Fong, 1979) but may be unreliable in variably cellular marrows (Gruppo *et al.*, 1997). The normal cellularity varies with age (Table 2.1), and evaluation of cellularity must always be made in the context of the patient's age (Hartsock *et al.*, 1965) (Fig. 2.1). The marrow is approximately 100% cellular during the first three months of life, 80% cellular in children through age 10 years; it then slowly declines in cellularity until age 30 years, when it remains about 50% cellular. The usually accepted range of cellularity in normal adults is 40–70% (Hartsock *et al.*, 1965; Gulati *et al.*, 1988; Bain, 1996; Friebert *et al.*, 1998; Naeim, 1998). The marrow cellularity declines again in elderly patients to about 30% at 70 years. Because of the variation in cellularity by age, the report should clearly indicate whether the stated cellularity in a given specimen is normocellular, hypocellular, or hypercellular.

Estimates of cellularity may be inappropriately lowered by several factors. Subcortical bone marrow is normally hypocellular, and the first three subcortical trabecular spaces are usually ignored in the cellularity estimate (Fig. 2.2). Superficial core biopsies may contain only these subcortical areas, and such biopsy specimens should be considered inadequate for purpose of cellularity evaluation. Technical artifacts may also falsely lower marrow cellularity. Tears made in the section during processing and cutting as well as artifactual displacement of marrow from bony trabeculae should not be considered in the estimate. Likewise, crush artifact may falsely elevate the cellularity.

After the marrow cellularity has been evaluated, the cellular elements must be considered (Table 2.2). The three main bone marrow cell lineages, erythroid, myeloid (granulocytic), and megakaryocytic, should be evaluated first. Maturing myeloid cells are the most common cell type in normal marrow, with a 2 : 1 to 4 : 1 myeloid-to-erythroid (M : E) ratio; the higher ratio is more common in women and young children. All stages of granulocyte and erythroid maturation are normally present, with blast cells usually less than 3%. The various stages of cell maturation are best evaluated from the aspirate smear material, but the distribution pattern of different cell lineages is best evaluated on the clot or core biopsy specimen (Frisch & Bartl, 1999) (Fig. 2.3). Granulopoietic precursors normally occur adjacent to bone trabeculae. Erythroblasts and megakaryocytes are predominantly found in the central regions of the marrow cavities, often adjacent to sinusoids. Erythroblasts are found in small and large clusters of cells exhibiting the full range of maturational stages from the proerythroblasts to the orthrochromatic normoblasts. Megakaryocytes are easily identifiable on smear and biopsy material in the normal marrow and should consist of predominantly mature forms (>15 μm diameter) with multilobated nuclei.

Lymphocytes normally represent 10% to 15% of cells on aspirate smears, but lymphoid precursor cells (hematogones) and mature lymphocytes may be normally increased in children and the elderly, respectively. Lymphoid precursors (Longacre *et al.*, 1989) are less obvious in the biopsy material of children, despite being evident on aspirate smears. Lymphoid aggregates are common in biopsy material of elderly patients and are non-paratrabecular in location (Fig. 2.4). The aggregates are more commonly predominantly composed of T lymphocytes. Cells that are present at a lower frequency in the bone marrow include monocytes, plasma cells,

Table 2.1. Age-related normal values in bone marrow.

Age	% Cellularity	% Granulocyte	% Erythroid	% Lymphocytes
Newborn	80–100	50	40	10
1–3 months	80–100	50–60	5–10	30–50
Child	60–80	50–60	20	20–30
Adult	40–70	50–70	20–25	10–15

Adapted from Foucar, 2001

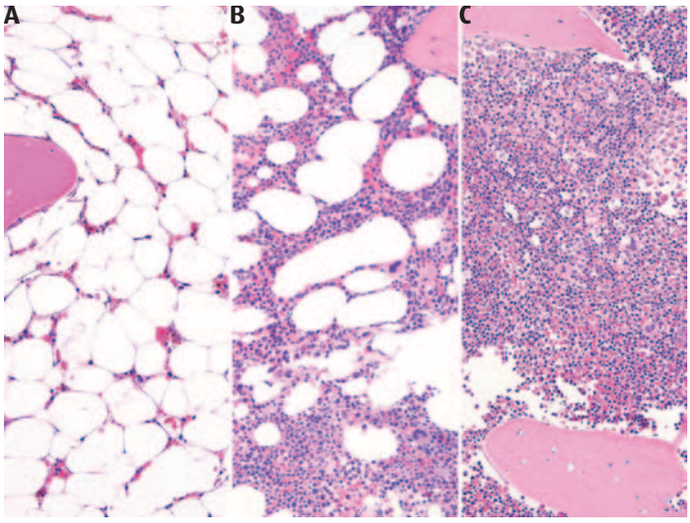

Figure 2.1. Examples of the ranges of cellularity seen in bone marrow: (A) a markedly hypocellular bone marrow (<5% cellularity), (B) approximately 40% cellularity, and (C) bone marrow with nearly 100% cellularity.

mast cells, eosinophils, basophils, and osteoblasts. These cells normally represent less than 5% of marrow cells on smears.

Cells and proliferations that do not normally occur in the marrow, including histiocyte accumulations or granulomas, fibrosis, serous atrophy, and neoplastic cells, should be systematically assessed in all specimens. The bone trabeculae should also be evaluated for evidence of osteopenia, osteoblastic proliferations, and changes of Paget's disease (Frisch & Bartl, 1999). However, detailed evaluation of metabolic bone diseases requires special techniques such as undecalcified biopsies embedded in plastic, in vitro tetracycline labeling, and histomorphometry (Teitelbaum & Bullough, 1979). A detailed discussion of bone pathology is beyond the scope of this book, which is primarily devoted to bone marrow interpretation.

Table 2.2. Normal adult values for bone marrow differential cell counts.

Cell type	Normal range (%)
Myeloblasts	0–3
Promyelocytes	2–8
Myelocytes	10–13
Metamyelocytes	10–15
Band/neutrophils	25–40
Eosinophils and precursors	1–3
Basophils and precursors	0–1
Monocytes	0–1
Erythroblasts	0–2
Other erythroid elements	15–25
Lymphocytes	10–15
Plasma cells	0–1

Adapted from Foucar, 2001

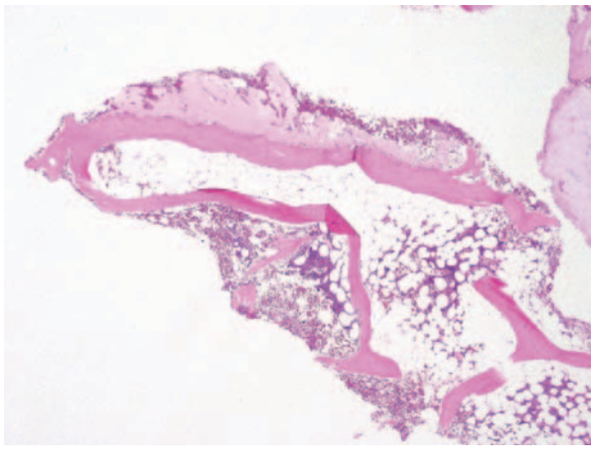

Figure 2.2. An example of a core biopsy taken in a subcortical location. Note the periosteum (upper portion of the photograph), suggesting that this is the outer cortical layer of the bone. Beneath this outer cortex of bone there is an area of hypocellular bone marrow which includes the first three subcortical trabecular spaces. Deeper in the specimen (lower right corner), the cellularity is considered to be more representative.

Evaluation of stainable iron

Marrow aspirate smears

In a normal bone marrow aspirate smear stained by Prussian blue, iron is predominantly found in histiocytes embedded inside marrow particles (Fig. 2.5). Iron incorporation in erythroid cells is also normally seen in scattered erythroblasts that usually demonstrate one or two siderotic

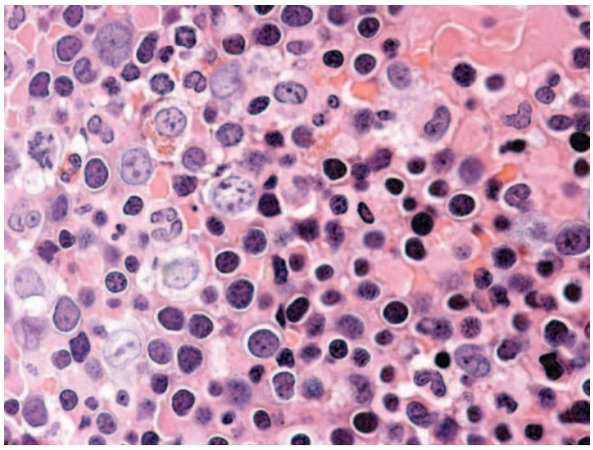

Figure 2.3. Erythroid and myeloid precursors in a clot section. Erythroid precursors typically have perfectly round nuclei with variable amounts of cytoplasm, which may appear clear.

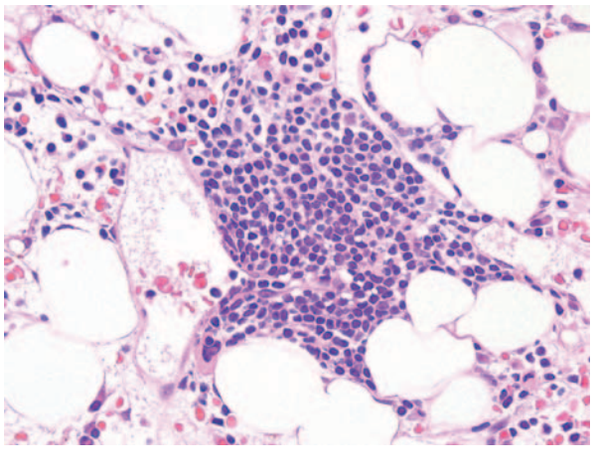

Figure 2.4. A benign lymphoid aggregate within a bone marrow biopsy. This lymphoid aggregate, which is composed predominantly of small lymphocytes, most likely represents a reactive lymphoid follicle. Note that the aggregate is in a perivascular location, which is most often associated with benign lymphoid aggregates.

granules adjacent to the nucleus (sideroblasts). Grading of marrow iron content is outlined in Table 2.3.

Bone marrow biopsy

Biopsy decalcification removes iron. Decalcified sections, therefore, underestimate iron stores and may give the misleading impression of iron deficiency. Iron stores are more accurately reflected in plastic-embedded sections (undecalcified) and in clot section preparations, if adequate marrow particles are present (Fig. 2.5). Hemosiderin may also

Table 2.3. Grading of iron storage in bone marrow aspirate material.

Grade	Characteristic
0 or negative	No iron identified under oil immersion
1+	Small iron-positive particles visible only under oil immersion
2+	Small, sparsely distributed iron particles usually visible under low magnification
3+	Numerous small particles present in histiocytes throughout the marrow particles
4+	Larger particles throughout the marrow with tendency to aggregate into clumps
5+	Dense, large clumps of iron throughout the marrow
6+	Large deposits of iron, both intracellular and extracellular, that obscure cellular detail in the marrow particles

Adapted from Gale *et al.*, 1963

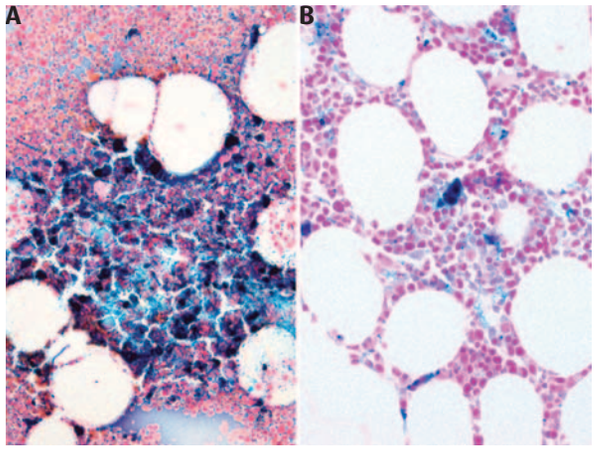

Figure 2.5. Iron stains on bone marrows. (A) An iron stain on an aspirate smear shows a markedly increased amount of stainable iron. (B) Iron staining on a bone marrow core biopsy, which typically under-represents the amount of iron present. This is due to the iron solubilization effect that occurs during histologic tissue processing. The loss can be partially prevented by using non-acid decalcification methods.

be visible in H&E-stained sections of marrow as coarse golden-brown granules in macrophages. The presence of visible hemosiderin in H&E-stained marrow sections usually indicates increased iron stores (Strauchen, 1996).

Evaluation of bone marrow extracellular stroma, marrow fibrosis, and mesenchymal cells

The extracellular matrix is demonstrable in routine preparations by reticulin (Gomori) silver stain, which stains most

Table 2.4. Grading of bone marrow fibrosis.

1+	Focal fine fibers with only rare coarse fibers
2+	A diffuse fine fiber network with an increase in scattered coarse fibers
3+	A diffuse coarse fiber network with no collagenization (negative trichrome stain)
4+	A diffuse fiber network with collagenization (positive trichrome stain)

Modified from Manoharan *et al.*, 1979

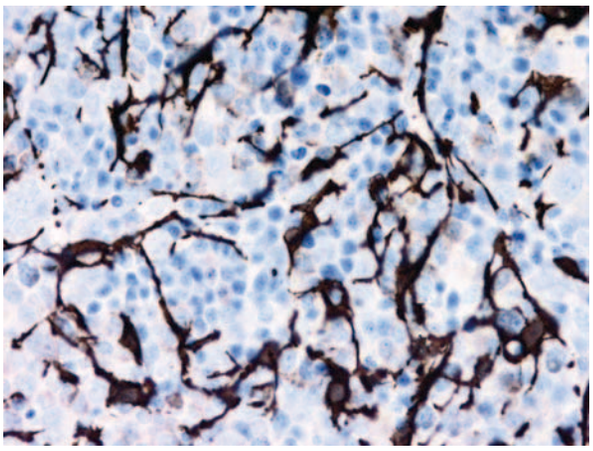

Figure 2.6. An immunohistochemical stain for low-affinity nerve growth factor receptor (LNGFR), highlighting bone marrow adventitial reticular cells (ARC). Note the characteristic dendritic morphology of the ARC.

types of collagen including collagen III and collagen IV, and by collagen IV immunostain, which stains the basal membrane collagen type. Bone marrow reticulum cells, also termed adventitial reticular cells, can be identified by their nerve growth factor receptor positivity (NGFR) (Cattoretti *et al.*, 1993). In most cases, the amount of NGFR staining roughly parallels the degree of reticulin fibrosis (Fig. 2.6).

Evaluation of fibrosis

Assessment for the presence and degree of marrow fibrosis is usually done by staining marrow sections with the Gomori silver stain technique. The degree of fibrosis can be estimated by using the approach proposed by Manoharan *et al.* (1979) (see Table 2.4). Trichrome stain (e.g., Masson's) is commonly used to demonstrate the presence of "mature" collagen, which can occur in advanced stages of marrow fibrosis (i.e., osteomyelosclerosis). The grading of fibrosis, as a general rule, should be performed taking into account only areas of active hematopoiesis (fatty areas are excluded). In pathological bone marrow, areas of prominent stroma alterations, such as those with marked edema or extensive fibrosclerosis, should also be included in the overall grading of the myelofibrosis.

Ancillary techniques useful in bone marrow evaluation

Ancillary techniques are essential for the proper diagnosis of many bone marrow neoplasms. Because therapy is now often specific for the exact type of the neoplastic cells and prognosis is often directly related to genetic changes associated with various types of neoplasm, the tests described here are often vital for a proper evaluation of the patient. Despite remarkable advances in immunophenotyping and cancer genetics, morphologic evaluation still remains a crucial step in the assessment of the bone marrow. In most cases, morphologic findings guide the pathologist in the selection of appropriate additional studies, to identify clinically significant immunophenotypic and genetic findings. These morphologic features are discussed with the specific diseases, as are the specific findings of the various ancillary tests. The general utility and applications of ancillary testing, however, are discussed here.

Cytochemistry

Despite the widespread use of immunophenotyping in the diagnosis of hematopoietic neoplasms, cytochemical studies are still of diagnostic importance (Scott, 1993). This is particularly true of the acute leukemias, although a large panel of cytochemical tests is probably not necessary in most cases. In rare patients with inconclusive flow cytometry results, cytochemical stains may provide information which can confirm a diagnosis (Mhawech *et al.*, 2001). Myeloperoxidase or Sudan black B cytochemical stains remains the hallmark of a diagnosis of acute myeloid leukemia (AML) in most cases. Some cases, such as minimally differentiated AML and monoblastic leukemias, are myeloperoxidase-negative. The use of non-specific esterase cytochemistry, such as a α-naphthyl butyrate esterase, is still the primary means of identifying monocytic differentiation for classification purposes.

Cytochemistry is of limited value in the diagnosis of acute lymphoblastic leukemia (ALL). Whereas negative results of peroxidase cytochemical studies are expected in ALL, they do not sufficiently exclude a myeloid leukemia and should not be used as the sole evidence of lymphoid lineage. Periodic acid-Schiff staining, frequently showing "block" positivity in lymphoblast cytoplasm, is also not sufficiently specific to confirm a diagnosis.

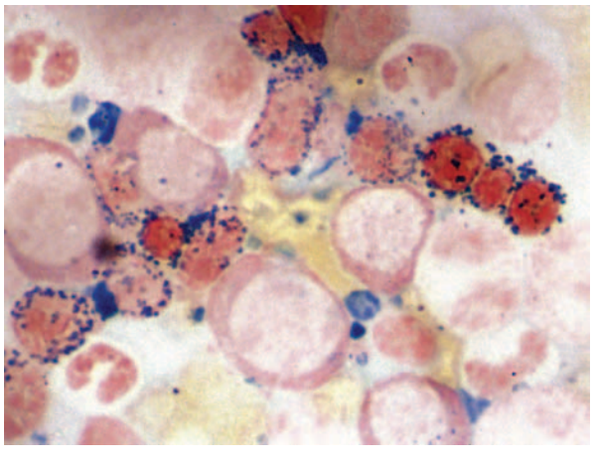

Figure 2.7. An iron stain, illustrating numerous ringed sideroblasts (iron is highlighted as blue granules) in a case of refractory anemia with ringed sideroblasts. Note the perinuclear location of the granules in the erythroid precursors.

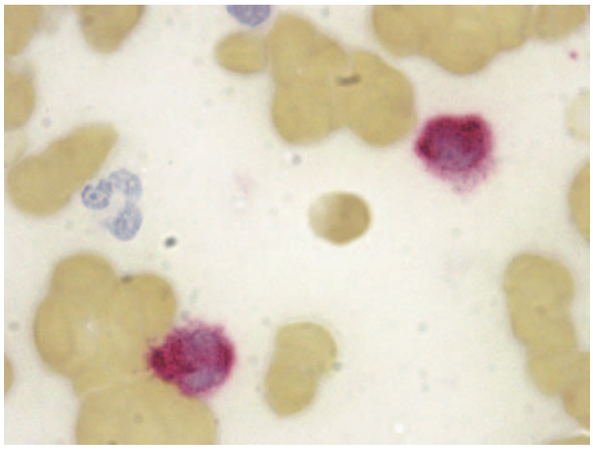

Figure 2.8. Cytochemical stains for tartrate-resistant acid phosphatase (TRAP), demonstrating positivity in circulating hairy cells (hairy cell leukemia).

The Prussian blue stain for iron is a histochemical stain that is commonly employed on bone marrow specimens (Sundberg & Broman, 1955). Although it may be used for clot or biopsy material, it is most reliable and useful for bone marrow aspirate smears, as long as sufficient particles are present on the smear. Iron staining is useful in identifying reticuloendothelial iron stores in the evaluation of a patient for iron deficiency or overload, but it also helps in the evaluation of red blood cell iron incorporation. Iron stores are often graded from 0 to 6+ (Gale *et al.*, 1963), as summarized in Table 2.3, and such grading correlates well with other chemical measures of iron. The identification of increased iron within erythroid precursors, particularly in the form of ringed sideroblasts, helps in the diagnosis of sideroblastic anemias, myelodysplastic syndromes with ringed sideroblasts, and AML with associated multilineage dysplasia (Fig. 2.7).

The other cytochemical test that is commonly used on bone marrow (and peripheral blood) smears is the detection of tartrate-resistant acid phosphatase (Fig. 2.8) in hairy cell leukemia (HCL) (Yam *et al.*, 1971). At present this cytochemical stain should be used in conjunction with immunophenotypic studies for a complete diagnostic characterization of HCL.

Immunophenotyping

Immunophenotyping studies are essential for the proper diagnosis of lymphoblastic malignant neoplasms, and they help in the classification of mature lymphoid neoplasms and some myeloid neoplasms. In addition, these studies can provide a characteristic immunologic "fingerprint" of an acute leukemia that may be useful in the subsequent evaluation of residual disease.

Some antibodies that are useful in the immunophenotypic evaluation of blastic proliferations by flow cytometry and immunocytochemistry are listed in Table 2.5. The best markers for investigating lymphomas are discussed in Chapter 11.

Both flow cytometry and immunocytochemistry primarily detect surface antigens, although some cytoplasmic and nuclear antigens may also be detected (e.g., CD3 and TdT).

Flow cytometry

Flow cytometry has the advantage of allowing the evaluation of several thousand cells in a rapid manner, and it has the ability to assess the expression of multiple antigens on a single cell. Also, the use of CD45 versus side-scatter gating strategies allows cells with specific characteristics (such as blast cells or lymphoid cells) to be evaluated, and this method greatly increases the ability to detect residual disease in a specimen (Borowitz *et al.*, 1993). Flow cytometry is also the best technique for assessment of clonality in B-cell lymphoid neoplasms (by surface immunoglobulin light chain analysis).

Several consensus reports and reviews regarding the use of flow cytometric immunophenotyping in hematological malignant neoplasms offer guidelines on the use of this methodology on peripheral blood and bone marrow specimens (Rothe & Schmitz, 1996; Borowitz *et al.*, 1997; Braylan *et al.*, 1997; Davis *et al.*, 1997; Jennings & Foon, 1997; Stelzer *et al.*, 1997; Stewart *et al.*, 1997).

Table 2.5. Selected useful flow cytometry and immunocytochemistry markers in acute leukemia.

General
CD45
Myeloid
CD11c
CD13
CD15
CD33
CD65
CD117
Cytoplasmic myeloperoxidase
Myelomonocytic
CD14
CD36
CD64
Megakaryocyte
CD41
CD61
Immature B lineage
CD10
CD19
CD22
TdT
Mature B lineage
CD19
CD20
κ and λ light chains
T lineage
CD2
CD5
CD7
CD4/CD8
TdT
Cytoplasmic CD3
Others
CD34
CD56
HLA-DR

Immunohistochemistry

The majority of antibodies that are available for flow cytometry can also be used for immunostaining of paraffin sections of core biopsy or clot material. The main advantages of immunocytochemistry include the direct visualization of the marker on the tumor cell and that it does not require the instrumentation needed for flow cytometry. Disadvantages are a relative lack of standardization, both of the technique and of the reactivity analysis, which frequently limits the reproducibility of the results obtained in different laboratories, and lack of sensitivity with several antigens.

Immunohistochemistry is ideal for the assessment of lesions that are seen in the biopsy but which, due to sampling differences or dry taps, may not be present in the aspirated material submitted for flow cytometry. This includes, in particular, focal marrow involvement by malignant lymphoma and leukemic conditions associated with marrow fibrosis. Immunohistochemistry is also particularly useful for the characterization of tumors that are not routinely assessed by flow cytometry, such as Hodgkin lymphoma, metastatic carcinomas, and small round cell tumors of childhood.

Because of limitations in the detection of some antigens by paraffin section immunohistochemistry and its lesser degree of reproducibility, flow cytometric immunophenotyping is preferred for the evaluation of chronic lymphoproliferative disorders (e.g., chronic lymphocytic leukemia) and acute leukemias. When such material is not available, immunohistochemistry may still provide diagnostic information (Kurec *et al.*, 1990; Arber & Jenkins, 1996; Chuang & Li, 1997; Manaloor *et al.*, 2000). Paraffin section antibodies useful for the evaluation of hematologic malignancies are listed in Table 2.6.

As with all immunophenotyping studies, pertinent positive and negative findings should be obtained with a panel of antibodies because the detection of a single antigen is usually not sufficiently lineage-specific. For example, whereas terminal deoxynucleotidyl transferase (TdT) is usually detectable in lymphoblastic malignant neoplasms (Orazi, 1994), it is also present in a subgroup of myeloid leukemias.

Molecular genetic and cytogenetic analysis

Molecular genetic and cytogenetic studies on bone marrow specimens offer valuable information in certain clinical situations, and the prognostic significance of karyotypic changes in acute leukemia are now well established. In general, routine karyotype analysis is the preferred first-line test in a newly diagnosed case of leukemia or aggressive myelodysplastic syndrome, because a multitude of acquired genetic abnormalities may be detected by this method. When cryptic or masked translocations are suspected, when a precise genetic breakpoint with prognostic implications needs to be confirmed, or when residual disease testing is needed, molecular genetic tests are useful. This testing also helps in identifying gene rearrangements in lymphomas that are not readily identifiable by karyotype analysis and in detecting some lymphoma translocations that may not be consistently found by karyotype analysis. Details about the specific molecular genetic abnormalities