20 Bone Marrow

Normal Histology

The marrow biopsy specimen is usually taken from the iliac bone. It consists of bony trabeculae surrounded by a mixture of fat and hematopoietic cells. The percent cellularity (non-fat) should be roughly [100 – the patient's age]. The hematopoietic cells consist of megakaryocytes, erythroid precursors, and myeloid precursors. There may also be assorted plasma cells, lymphocytes, and histiocytes. The bone marrow is considered a reflection of what is in the peripheral blood, so the *disorders of marrow affect blood counts*. Lymphomas can involve the marrow, but generally the primary malignancies of the marrow are the leukemias.

Technically, all three of the basic "trilineage hematopoiesis" lines (megakaryocytes, erythroids, and granulocytic cells) are of the *myeloid lineage*, which differentiates them from the lymphoid lineage (B and T cells). The *myelo*proliferative diseases and *myeloid* leukemias refer to this broad classification. However, the word *myeloid*, as used at the microscope, generally refers to those cells in the granulocyte/monocyte pathway only.

Megakaryocytes are most easily identified, with their lakes of pink cytoplasm and multilobated nuclei (Figure 20.1). Erythroid precursors have a distinct rim of clear cytoplasm and centrally located, perfectly round nuclei; as they mature, the nuclei become small and dense such that erythroid islands in the marrow look like handfuls of buckshot (see Figure 20.1). Myeloid cells make up almost everything else. Myeloid precursors have more open chromatin than the red cell precursors, more cytoplasm, and more convoluted nuclei as they mature. Mature neutrophils and eosinophils should be present in normal marrow. Blasts, the most primitive hematopoietic cells, can be difficult to identify on H&E stain. Lymphoid cells, especially immature, should generally not be found in the marrow, with the exception of hematogones (nonneoplastic B-cell precursors), which can be markedly increased in children.

Usually an aspirate smear will be submitted with the biopsy specimen. The aspirate is stained with the Wright-Giemsa stain, which highlights nuclear detail. Blasts, and the successive stages of maturation, are best seen on an aspirate. The blasts are large cells with a thin rim of cytoplasm and a characteristic nucleus (Figure 20.2). The blast nucleus is large and round with a very finely textured chromatin pattern and a nonstaining nucleolus that shows up as a "hole" in the chromatin. The more differentiated precursors, such as promyelocytes and myelocytes, may have a similarly immature nucleus but acquire cytoplasmic features such as granules and a "hof" (the cleared-out Golgi zone in the cytoplasm, as in a plasma cell; see Figure 20.2).

On the aspirate, a myeloblast (as seen in acute myeloid leukemia) cannot always be distinguished from a lymphoblast (as seen in acute lymphoblastic leukemia). However, the presence of granules or Auer rods identifies a blast as myeloid. Erythroblasts have royal blue cytoplasm and very round nuclei. Monocyte precursors tend to have greyer cytoplasm and a folded or creased nucleus.

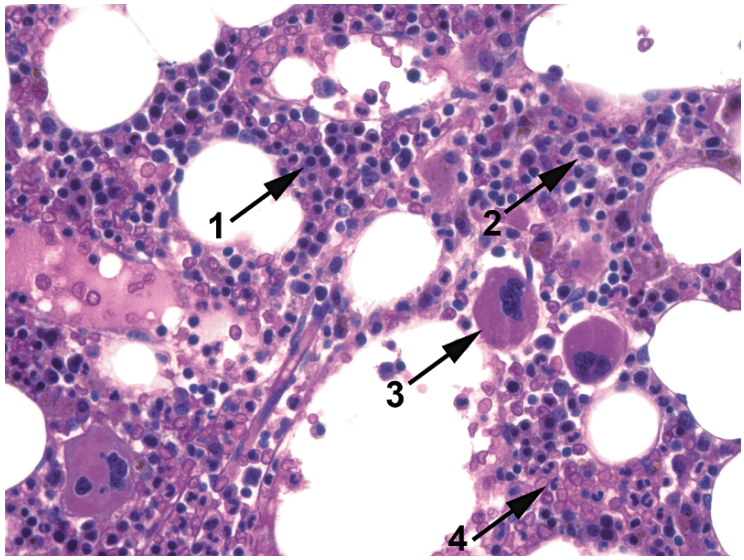

Figure 20.1. Normal megakaryocytes, erythroids, and myelocytic precursors. In this H&E stained core biopsy specimen, there are erythroid precursors (1), myeloid precursors (2), megakaryocytes (3), and maturing neutrophils (4).

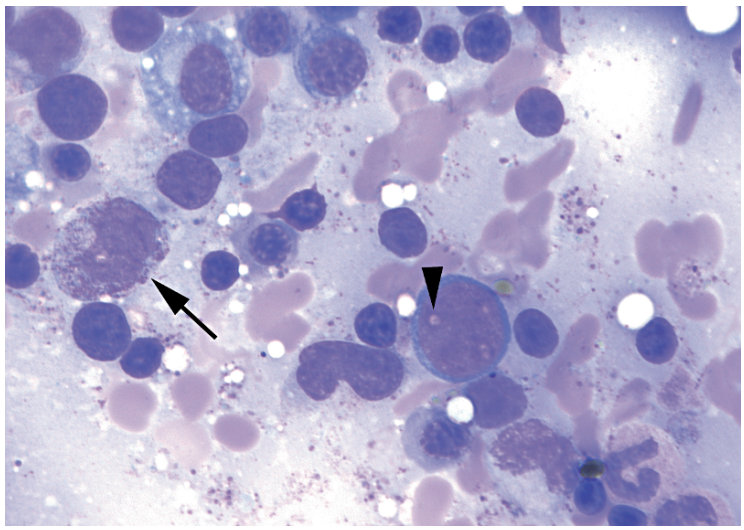

Figure 20.2. Blast on aspirate, Wright-Giemsa stain. The key to identifying a blast is the high nuclear to cytoplasm ratio and immature chromatin pattern, which consists of very finely grained, uniform chromatin with several nucleoli that show up as negative images on this stain (arrowhead). The immature cell nearby is a promyelocyte, which has the same nuclear qualities as a blast but has abundant cytoplasm with granules (arrow).

Approach to the Biopsy Specimen

A full evaluation of the specimen requires an H&E stained core biopsy, a Wright-Giemsa stained aspirate, and a peripheral smear. Beginning with the biopsy:

- On low power (4×)
  - Assess the cellularity of the marrow (Figure 20.3). A hypo- or hypercellular marrow will guide your differential diagnosis.

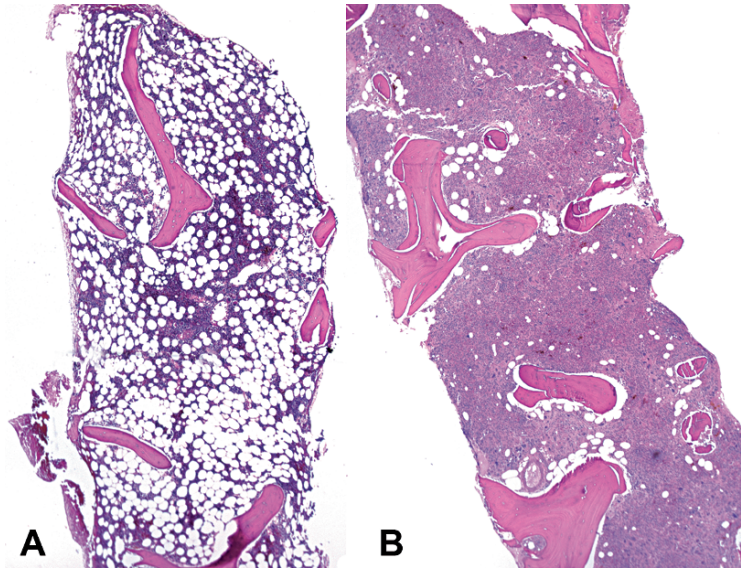

FIGURE 20.3. Marrow cellularity. **(A)** Normocellular marrow for a middle-aged adult; this cellularity is approximately 30%. **(B)** Hypercellular marrow for an adult; this cellularity is about 95% and is taken from a case of acute myeloid leukemia.

- Try to estimate the cellularity as a percentage range (i.e., 30%–40%), as clinicians sometimes follow the cellularity to monitor response to therapy.

- On medium power (10×)
  - Survey the marrow for trilineage hematopoiesis. You should see megakaryocytes, erythroid islands, and myeloid cells. Look to see if each line matures to completion: you should see mature neutrophils and red cells. Estimate the ratio of myeloid to erythroid cells (M/E ratio), which is normally about 2–4:1.
  - Look for things that do not belong in the marrow in large populations or aggregates, such as blue areas (lymphocytes), pink areas (histiocytes, plasma cells), or islands of non-heme cells (metastases). Look for fibrosis, which gives the marrow a streaming texture (Figure 20.4).

- On high power (20× and 40×)
  - Look at the individual cells, especially megakaryocytes. Small megakaryocytes with single nuclei are a feature of myelodysplasia and are also seen in chronic myeloid leukemia (Figure 20.5). Giant clustered megakaryocytes are a feature of myeloproliferative disorders. A few atypical megakaryocytes are not unusual, but a large population is significant.
  - Look for neutrophils. A packed marrow with numerous neutrophils may indicate chronic myeloid leukemia (see Figure 20.5), whereas numerous myeloid precursors with few neutrophils indicates a left-shift in maturation. Sheets of immature myeloid cells could represent anything from acute myeloid leukemia to infection; the aspirate needs to be evaluated for blasts (see next section).

Next, look at the aspirate. Hold it up to the light; an adequate aspirate will have little chunks in it (spicules) that are foci of stromal elements. Scan the slide for an optimal area of the smear. Cells should be spread out in a monolayer, with intact cytoplasm and distinct nuclei. "Naked" nuclei are not evaluable.

You have already evaluated the megakaryocytes, so, with the aspirate, focus on erythroid and myeloid cells. On high power (20× to 100×, with oil if necessary):

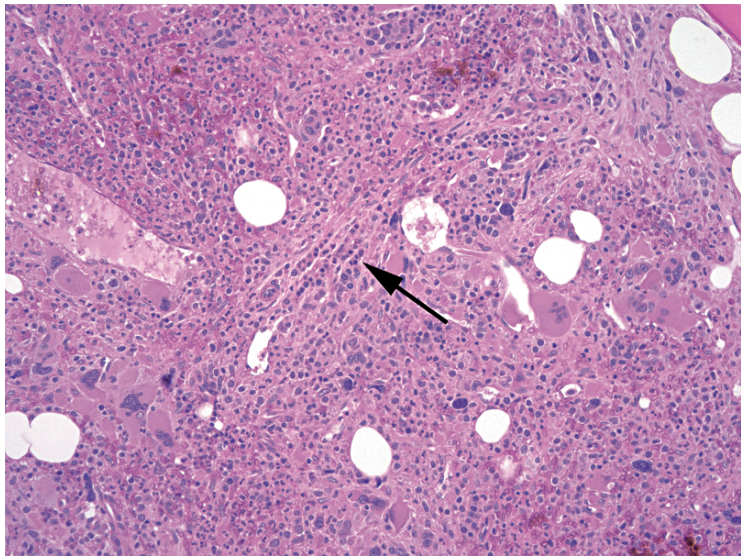

Figure 20.4. Marrow fibrosis. On hematoxylin and eosin stain, the marrow has a streaming texture (arrow), indicative of strands of collagen separating the hematopoietic cells into nests and channels.

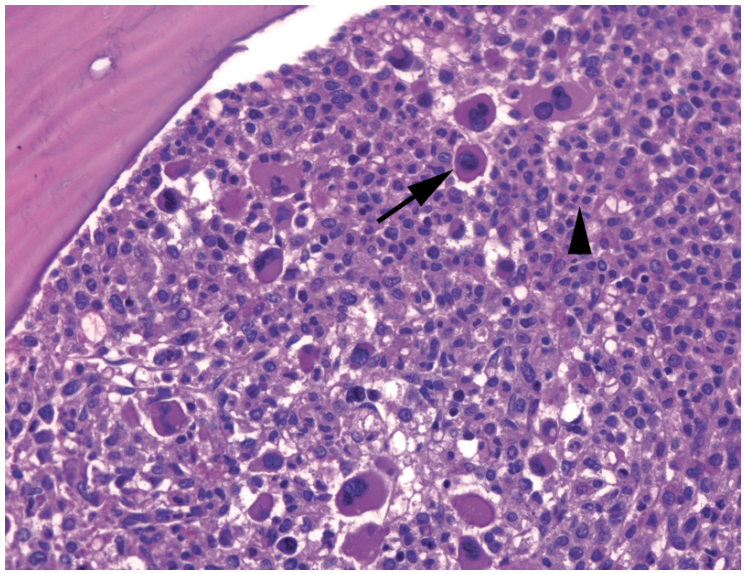

Figure 20.5. Chronic myeloid leukemia. This hypercellular marrow is full of small, hypolobated megakaryocytes (arrow) and maturing and mature neutrophils (arrowhead).

- Assess the heterogeneity of the marrow. A healthy aspirate should be a random mosaic of all different cell types at all levels of maturity. Sheets of uniform cells is a bad, bad sign (Figure 20.6).
- Look for blasts. You should be able to find scattered blasts but not clusters of them. Clumps of immature-looking cells are more commonly promyelocytes and myelocytes, as identified by their granules and cytoplasmic hofs. If you do find lots of blasts, note the shape of the nuclei, the color of the cytoplasm, and the presence of Auer rods. Presence of greater than 5% blasts is abnormal; they should be quantified by a systematic cell count of 200–500 cells. Flow cytometry will also provide a quantified percentage.

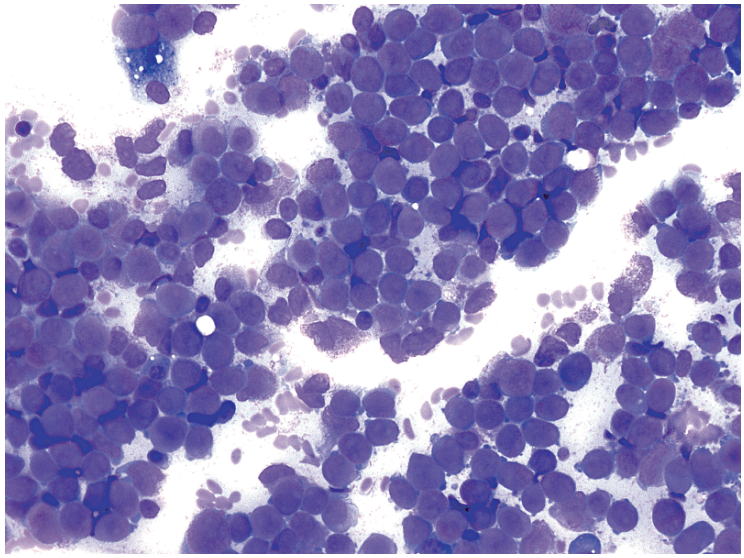

FIGURE 20.6. Monomorphic aspirate in acute leukemia. This aspirate is composed of sheets of blasts, identified by high nuclear to cytoplasmic ratios and immature chromatin.

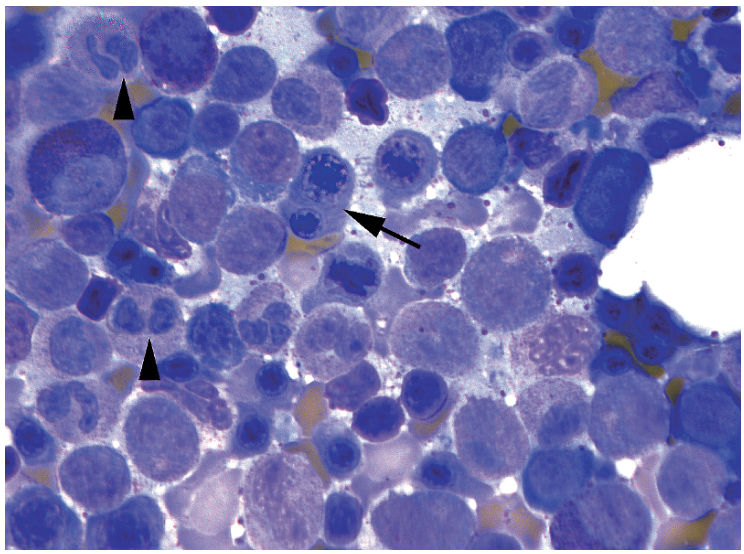

FIGURE 20.7. Dyserythropoiesis and dysgranulopoiesis. Binucleated erythroid precursors (arrow) and bilobed neutrophils without granules (arrowheads) are indicative of dysplasia.

- Evaluate the red cell precursors for dysplasia. Dyserythropoiesis shows up as binucleated red cells and red cell precursors with irregular nuclear membranes (Figure 20.7). Megaloblastoid change is a softer sign and looks like large "sliced salami" nuclei within mature (pale grey) cytoplasm.
- Dysgranulopoiesis (in neutrophils) appears as abnormalities in nuclear lobation ("Pelgeroid," which is bilobed like spectacles) and granules (absence of granules or occasionally coarse basophilic granules). Hypersegmented neutrophils can be seen in megaloblastic anemia.
- Plasma cells are easy to spot on the aspirate, with their bright blue cytoplasm and eccentric nucleus. Too many plasma cells (more than 5%–10%) may indicate a plasma cell dyscrasia.

Iron stains are performed on marrow smears to evaluate the iron stores. Hemosiderin (stainable storage iron) is found in reticuloendothelial cells and also in granules in normoblasts (developing red cell precursors). Normoblasts with iron granules in the cytoplasm are called *sideroblasts*. Sideroblasts are decreased in iron-deficiency anemia but increased in sideroblastic anemia, especially in the form of ringed sideroblasts, which have iron granules surrounding the nucleus in a ring.

After the aspirates, look at the peripheral blood film. Assess the white count, get a very rough differential, look for circulating blasts, and estimate the platelet count. (This chapter will not go into detail on peripheral smear interpretation.) Hypersegmented, hyposegmented, or hypogranular neutrophils are considered evidence of dysgranulopoiesis on the smear.

Differential Diagnosis

Once you have looked at everything, gather your facts to generate a differential diagnosis:
Hypercellular or hypocellular? (See marrow cellularity)
Dysplasia of any cell lines? (See myelodysplastic syndrome)
A prominent excess of any one cell line? (See myeloproliferative disorders)
Too many blasts? (See acute leukemia)
Lots of plasma cells? (See plasma cell dyscrasias)
Lymphocytes? (See lymphoma)

Marrow Cellularity

With hypercellular marrow, the differential diagnosis includes the following:

- Physiologic response to anemia, especially hemolytic anemia, or infection (no dysplasia, no increase in blasts)
- Ineffective hematopoiesis (such as megaloblastic anemia, human immunodeficiency virus)
- Myelodysplasia (dysplastic hematopoiesis in any cell line, <20% blasts)
- Myeloproliferative disorders (chronic myeloid leukemia, polycythemia vera (PV), essential thrombocythemia, (ET), myelofibrosis
- Acute leukemia (>20% blasts, with or without dysplasia)
- Other neoplasms (lymphoma, metastatic disease)

With hypocellular marrow, the differential diagnosis includes the following:

- Aplastic anemia
- Chemotherapy
- Infection
- Hypocellular forms of myelodysplastic syndrome (MDS)/acute myeloid leukemia (blasts are still increased)

Myelodysplastic Syndrome

Myelodysplastic syndrome includes the refractory anemia group of disorders and encompasses those diseases with *dysplasia of at least one cell line AND with blasts <20% in the marrow*. Myelodysplastic syndrome often progresses to acute myeloid leukemia, which by definition is >20% blasts. *Each dysplastic cell line has a corresponding peripheral cytopenia*, as it represents a dysfunctional hematopoiesis. Myelodysplastic syndrome tends to have an erythroid predominance in the marrow (decreased M/E ratio), as the body struggles to compensate for the anemia. The most minor disease is simple refractory anemia, which presents with anemia and shows erythroid dysplasia (see Figure 20.7). Blasts are not increased (<5%). The finding of >15% ringed sideroblasts on an iron stain indicates refractory anemia with ringed sideroblasts.

Dysplasia and cytopenias in two, or more usually three, cell lines bumps you to refractory cytopenia with multilineage dysplasia (with or without ringed sideroblasts). The dysplasia must be seen in >10% of a given cell line to be significant. Blasts are still not increased.

Once the blast percentage begins to increase, you get to refractory anemia with excess blasts (RAEB)-1 or -2 for marrow blasts 5%–9% and 10%–19%, respectively. Refractory anemia with excess blasts is otherwise known as high-grade MDS and usually progresses to acute myeloid leukemia. The presence of an Auer rod in the setting of MDS, regardless of the percentage of blasts, indicates RAEB-2. Similarly, the presence of >5% circulating or peripheral blasts indicates RAEB-2.

Myeloproliferative Disorders

The myeloproliferative disorders (MPDs), in contrast to myelodysplasia, show a superhematopoiesis. *There is little or no dysplasia, and the peripheral counts of the involved cell line(s) are high*. In chronic myeloid leukemia, the most common MPD, the polys are pushed out of the marrow so fast that they are not fully mature, so there are immature as well as mature granulocytes in circulation. Unlike MDS, there is an increased M/E ratio in the marrow. Like MDS, though, the marrow is hypercellular and the blast percentage is low (at least until it reaches a blast crisis). The gradual replacement of the bone marrow usually leads to cytopenias of the uninvolved cell lines (although chronic myeloid leukemia usually comes with a thrombocytosis), as well as extramedullary hematopoiesis and organomegaly.

Myeloproliferative disorders can affect any of the myeloid (nonlymphoid) cell lines: granulocytic (chronic myeloid leukemia), megakaryocytic (essential thrombocythemia), and erythroid (polycythemia vera). Chronic idiopathic myelofibrosis involves both the megakaryocytic and granulocytic lines. The MPDs can have overlapping features and be difficult to separate, with the exception of chronic myeloid leukemia, which is defined by unique cytogenetics (t 9;22, *bcr-abl*, the Philadelphia chromosome).

Myelodysplastic/Myeloproliferative Disorders

Of course there would be an overlap among the myelodysplastic and myeloproliferative disorders. The MDS/MPD category has features of both. The most common disorder is chronic myelomonocytic leukemia. It has the dysplasia, anemia, and thrombocytopenia of MDS, a proliferation of monocytes (monocytosis), <20% blasts, and no Philadelphia chromosome.

Acute Leukemia

Acute leukemia is defined as >20% blasts in the blood or marrow, but it is not uncommon to see >90% blasts in an initial presentation (Figure 20.8). *Acute lymphoblastic leukemia* (*ALL*) is the leukemic counterpart to acute lymphoblastic lymphoma and is primarily a disease of children in which immature lymphocyte precursors fill the marrow. Most commonly the cells are B-cell precursors (pre-B ALL), although T-cell ALL can also involve the marrow. Acute lymphoblastic leukemia can be very difficult to distinguish from acute myeloid leukemia on routine stains, as the lymphoblasts look very similar to myeloblasts. However, the diagnosis is easily confirmed by immunostains or flow cytometry.

Acute myeloid leukemia (*AML*) spans a wide category of diseases, which are roughly grouped by the type of blast that has gone bad. The classification of AML was by the FAB system for many years, which subgrouped the acute myeloid leukemias into the M0 to M7 categories (Table 20.1). The classification system used since 2001 is the World Health Organization (WHO) classification, which relies more on cytogenetic, molecular, and immunophenotypic features. In this system, some acute myeloid leukemias are defined by their cytogenetics, such as t(8;21) (ETO), t(15;17) (acute promyelocytic leukemia), and inv(16) (acute myelomonocytic leukemia with abnormal eosinophils). These are more likely to arise de novo and have a better prognosis. AML arising from MDS, however, has a poor prognosis and often deletions of chromosomes 5 and 7. Similar cytogenetics may be found in chemotherapy-related acute myeloid leukemia. The remaining AML subtypes include the leukemias previously defined in the FAB system, such as AML and acute megakaryoblastic leukemia.

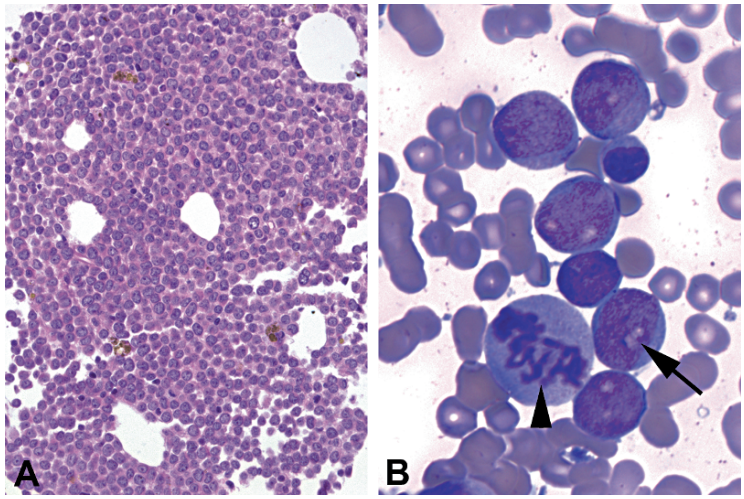

Figure 20.8. Acute myeloid leukemia. **(A)** The marrow biopsy material shows sheets of immature cells, with little or no background hematopoietic elements. Nucleoli appear dark on hematoxylin and eosin stain. **(B)** The aspirate shows clusters of myeloblasts with clear nucleoli (arrow). A large mitosis is visible (arrowhead); this in itself is not an unusual finding in the marrow.

Table 20.1. Acute myeloid leukemia subtypes according to the old FAB and the newer WHO classifications.

FAB classification	WHO classification	Blast or equivalent	Histologic hallmark
M0	AML minimally differentiated	Blast	
M1	AML without maturation	Blast	
M2	AML with maturation	Myeloblast	Granules, Auer rods, or myeloid markers
M2-ETO	AML with t(8;21)	Myeloblast with neutrophilic differentiation	Blasts with blue cytoplasm and granules, plus the immature "sunset" cells
M3	Acute promyelocytic leukemia, t(15;17)	Promyelocyte	Promyelocytes with blue granules and Auer rods
M4	AMML	Blast with myelo and mono features	Features of monocytic differentiation (grey cytoplasm, folded nuclei)
M4-Eo	AMML with inv(16)	Blast with myelo and mono features	Features of monocytic differentiation plus lots of eosinophils
M5	Acute monocytic leukemia	Monoblast	
M6	Acute erythroid leukemia	Erythroblast	
M7	Acute megakaryoblastic leukemia	Megakaryoblast	

Note: AML, acute myeloid leukemia; AMML, acute myelomonocytic leukemia; eo, eosinophils.

Histologically, acute myeloid leukemia usually presents as a hypercellular marrow mostly replaced by blasts. The differentiation of the blasts (and therefore the subclassification of the AML) is identified by morphology on the aspirate, flow cytometry, and cytogenetics. Some unique findings of the different subtypes include the following:

- t(8;21), acute myeloid leukemia–ETO: This subtype consists of a population of blasts with blue cytoplasm and granules, plus "sunset" cells, which are immature myeloid cells with salmon-pink granules, a pink cytoplasm with hof, and a peripheral blue rim. These look like a little sunset to some observers (Figure 20.9).
- inv(16): This subtype is acute myeloid leukemia with both granulocytic and monocytic differentiation (monocytic differentiation is bluish grey cytoplasm and folded or convoluted

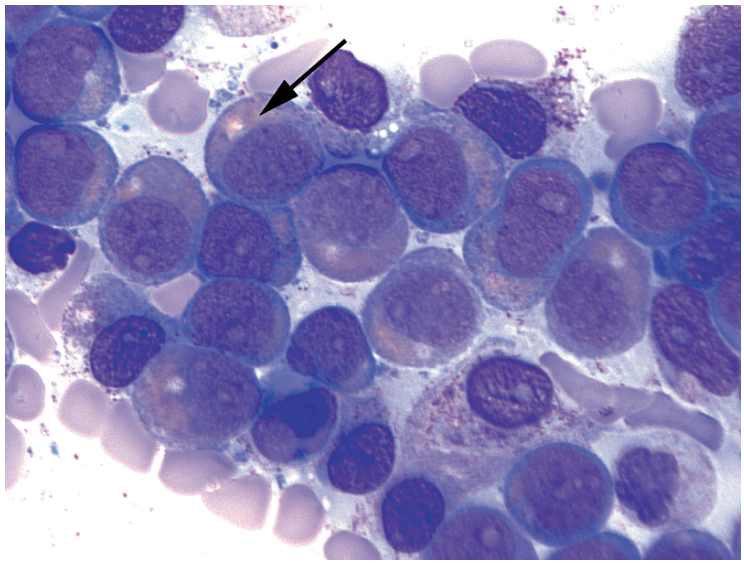

FIGURE 20.9. Acute myeloid leukemia–ETO. A population of the blasts have pink cytoplasm with pale hof, a peripheral blue rim, and pink granules (arrow).

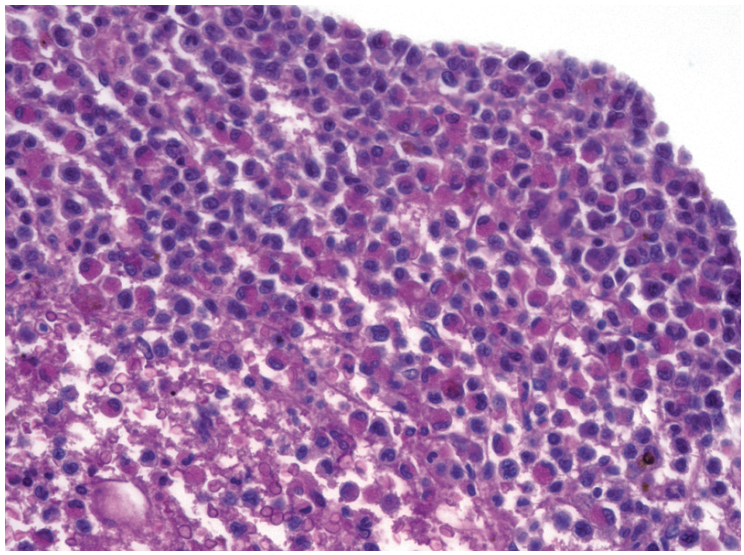

FIGURE 20.10. Acute myeloid leukemia inv (16). The marrow shows sheets of immature myeloid cells (the aspirate showed blasts) and numerous eosinophils.

nuclei) plus a high number of eosinophils in the marrow (Figure 20.10). Some of the eosinophils may have large abnormal blue granules (like basophils).

- t(15;17), acute promyelocytic leukemia: Technically there is not an increase in blasts but in promyelocytes. The nuclei are large with blast-like chromatin but folded (kidney-shaped), and the cytoplasm has large and numerous blue granules or Auer rods.

Plasma Cell Dyscrasias

A normal marrow should have 1%–2% plasma cells, scattered in a perivascular distribution. Aggregates of plasma cells and atypical forms (large nucleoli, binucleate forms) are abnormal. The diagnosis of a plasma cell myeloma requires a marrow plasmacytosis with a monoclonal gammopathy. The marrow plasmacytosis is typically >30% but can be as little

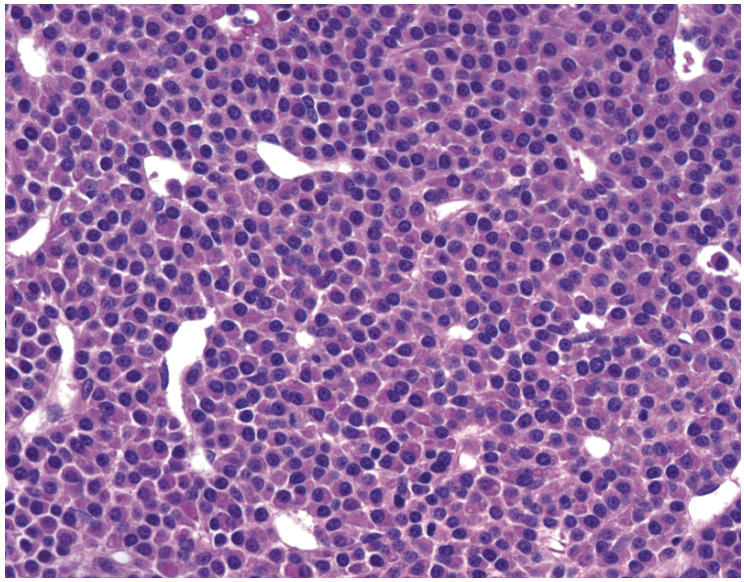

Figure 20.11. Myeloma. The marrow is replaced by sheets of plasma cells. Although the section is thick and the chromatin appears very dark, some nuclei show the distinct soccer-ball chromatin of plasma cells. The eccentric nucleus and abundant pink cytoplasm are also characteristic.

as 10% if there are also lytic bone lesions or certain other criteria (see the WHO hematopathology book for the exact diagnostic criteria and variants). A softer call is the plasma cell dyscrasia, which implies that there is a plasma cell problem but does not meet the diagnostic criteria for myeloma. A localized plasma cell lesion without systemic gammopathy is a plasmacytoma.

Sheets of plasma cells are recognizable on hematoxylin and eosin stain (Figure 20.11), but it is difficult to pick out a subtle interstitial plasmacytosis. CD138 can be used to help estimate the percentage. Immunostains for light chains kappa and lambda can identify an abnormally restricted (all kappa or all lambda) population, implying a monoclonal process. Flow cytometry tends to underestimate the number of plasma cells.

Lymphoma

Lymphocytes in the bone marrow can be a normal or reactive finding. They can also be an indication of lymphoma involvement. History is important, as the marrow is an unlikely place for a presentation of occult lymphoma (i.e., without lymphadenopathy). However, non-Hodgkin lymphoma has a 30%–50% chance of involving the marrow *at the time of diagnosis*. The subtypes most likely to go to marrow are follicular, small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), mantle cell, lymphoblastic, Burkitt's, and peripheral T-cell lymphomas. Diffuse large B cell is less likely.

Lymphoid infiltrates can come in four basic patterns:

- Paratrabecular: This is a collection of lymphocytes that hugs the bony trabeculae. This pattern favors follicular lymphoma (Figure 20.12).
- Nonparatrabecular: This is a lymphoid aggregate that is not closely associated with a trabeculum. Benign lymphoid aggregates (discussed further below) are common in elderly patients, but small lymphocytic and chronic lymphocytic lymphomas can also have this pattern (Figure 20.13).
- Interstitial: This implies a scattered collection of lymphocytes in and among the marrow elements. It can be hard to pick out on H&E stain, because single lymphocytes will blend into the hematopoietic soup. Mantle cell and SLL/CLL tend to have this pattern, often in conjunction with nonparatrabecular aggregates.

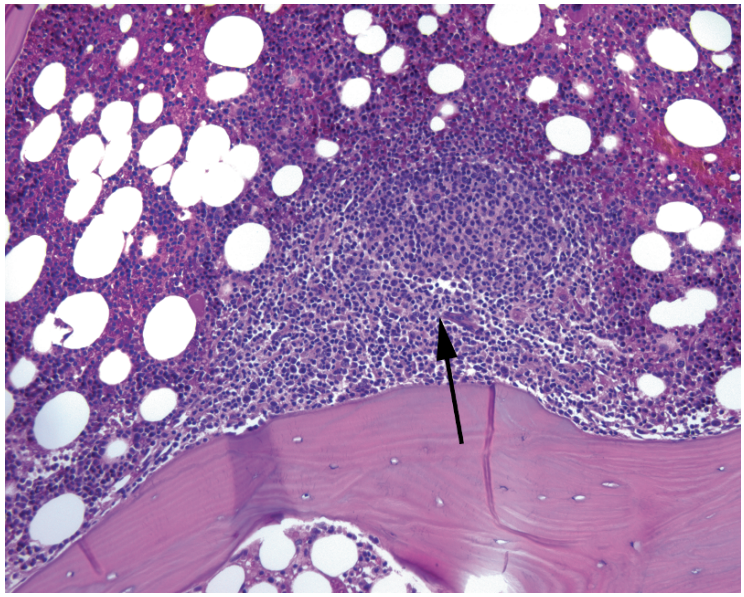

FIGURE 20.12. Paratrabecular aggregate. The arrow points to the center of this lymphoid aggregate, which appears more blue, relatively, than the surrounding marrow. One surface of the aggregate is plastered down to the bony trabeculum.

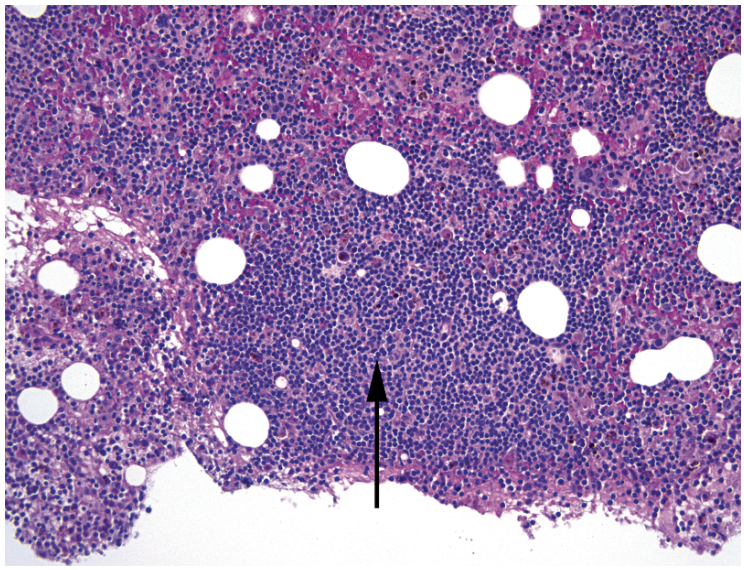

FIGURE 20.13. Nonparatrabecular aggregate in chronic lymphocytic leukemia. The arrow here points to the center of a free-floating, rounded lymphoid aggregate.

- Diffuse: This means sheets of lymphocytes replacing the marrow (Figure 20.14) and is more typical of aggressive lymphomas such as Burkitt's lymphoma, diffuse large B-cell lymphoma, or advanced SLL/CLL.

Features of a *benign lymphoid aggregate* include a nonparatrabecular site; heterogeneous mixtures of lymphocytes, plasma cells, and histiocytes; well-demarcated borders; germinal centers; and older patient age. Immunostains can also help. A benign aggregate should be a mixture of B and T cells (CD20 and CD3). Beware the CD20 stain in a patient who is taking

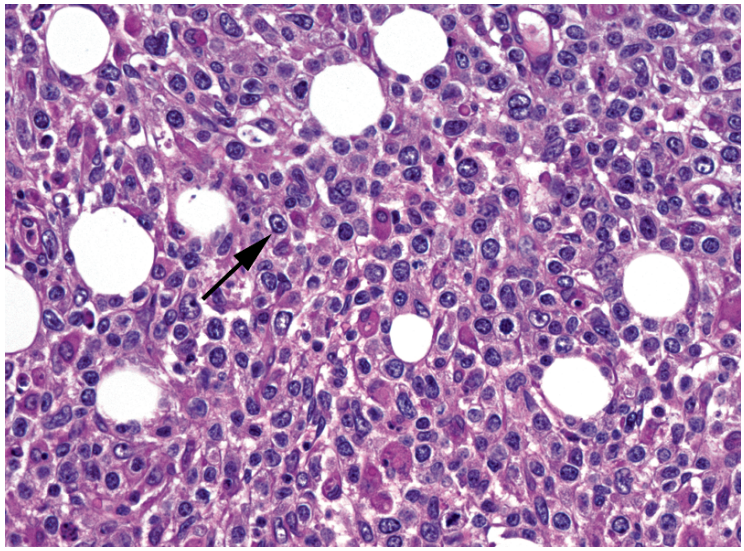

FIGURE 20.14. Diffuse large B-cell lymphoma in the marrow. The marrow is infiltrated by large atypical cells with prominent nucleoli, thick nuclear rims, abundant cytoplasm, and irregular nuclear membranes (arrow). This is suggestive of involvement by diffuse large B-cell lymphoma or carcinoma.

Rituximab, though, as this drug targets and eradicates CD20 expression. Use another B-cell marker such as CD22, CD79a, or PAX5.

Hodgkin's lymphoma in the marrow can be extremely subtle, as the defining trait of Hodgkin's is a mixed infiltrate with scattered neoplastic (Reed-Sternberg) cells. Focal fibrosis or granulomatous inflammation may be all that is seen initially; immunostains can often pick out the rare neoplastic cells.