Wintrobe’s
Atlas of Clinical Hematology
SECOND EDITION

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This second edition of Wintrobe’s *Atlas of Clinical Hematology* aims to update knowledge about major aspects of blood and bone marrow disorders including new information pertaining to their pathophysiology, diagnosis, clinical features, and pathology, as the field has changed considerably since the publication of the first edition in 2007.

We are indebted to the original coeditors, Douglas C. Tkachuk and Jan V. Hirschman, and their contributing authors for the wealth of images from the first edition that provided a base from which to work. We believe that clinical and microscopic images remain an important part of learning about and practicing hematology for both the novice and the experienced hematologist and hematopathologist. In the decade since the publication of the first edition of this Atlas, much has changed in our understanding of the genetics and mechanisms of blood disorders, as well as in the development of new techniques of diagnosis, and we have attempted to incorporate as much of this information as possible. Updated discussions of each topic and additions of new figures expanded the chapters in the last edition. New chapters covering hemostasis and bleeding, benign disorders of leukocytes and spleen, and plasma cell disorders have been incorporated in this volume. We have attempted to make the reproductions of photomicrographs and other images as clear as possible despite their very large numbers, and to supply informative legends. In addition, recent references and suggested readings have been appended to the chapters.

We are aware of the tremendous progress over the last decade in the rapidly expanding power of molecular diagnostic methods. However, morphologic examination of blood, bone marrow, and other tissues continues to provide the framework for organizing this new knowledge and remains central to informed diagnoses, as well as to studies ranging from classification to therapy. As before, the proposed audience includes anyone interested in blood disorders including students, laboratory technicians, and physicians in training, as well as oncologists, hematologists, and pathologists at every level of experience. We hope that this Atlas will be a complement to the 13th edition and future editions of *Wintrobe’s Clinical Hematology*, which feature extensive discussions and references related to the topics covered in this volume.

While none of the editors or authors involved in this second edition of the Atlas can claim the privilege of direct educational lineage from Maxwell Wintrobe, all have enjoyed the benefit of his many contributions to the creation of hematology as a flourishing discipline as well as to its practice. We also believe that the textbook he created, together with many colleagues, has aided these endeavors for many decades and hope that this Atlas will also be useful to both students and professionals in this important clinical and research field.

*Babette B. Weksler*

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*Scott A. Ely*
The authors would like to acknowledge the excellent technical assistance of Kristina Oberle and her staff at Wolters Kluwer in the preparation of this second edition of the Atlas.
CHAPTER

1 Approach to the Microscopic Evaluation of Blood and Bone Marrow

Kseniya Petrova-Drus, MD, PhD and Babette B. Weksler, MD

EXAMINATION OF THE PERIPHERAL BLOOD

Slide Preparation and Staining

A hematologic evaluation begins with laboratory assessment of complete blood count parameters performed by automated hematology analyzers. Blood component indices are obtained from instruments that utilize various combinations of spectrophotometric, chemical, electrical impedance, and electro-optical analyzers. When the various cell parameters fall within a predefined normal range, the results are automatically validated and reported by the instrument. However, when suspect values outside the predetermined range are encountered, the instrument flags the sample to alert the technologist that additional investigation is required before the results can be released. In most laboratories, user-defined parameters prompt repeat or further automated testing, including automated slide making and staining for the evaluation of a peripheral blood smear. Despite increasing use of automation, manual preparation and staining of peripheral blood smears continues to be a common and important practice in many hematology laboratories.

Manual preparation of peripheral blood smears employs either the wedge or coverslip method. The wedge technique uses a spreader slide held at a 30- to 45-degree angle above a drop of blood placed near one end of a second glass slide so that the end of the spreader slide contacts the blood which extends along the entire edge between the two slides. The angled spreader slide is then pushed rapidly to generate the blood smear on the second slide, which is quickly air-dried and stained. An optimal peripheral blood smear prepared by this method contains a thick area that gradually transitions to a thin area with an even separation of red cells. The coverslip method utilizes a small drop of blood placed between two coverslips positioned so that the corners form an octagonal star. After the drop is spread, the coverslips are pulled apart in the same plane, followed by air-drying, staining, and mounting on a slide. Automated staining methods may employ similar mechanics or utilize centrifugal force to generate an even blood smear.

Aniline dyes, containing basic dyes such as methylene blue and acidic dyes such as eosin, are used to stain the smears. The most common methods used today include Wright, Giemsa, or May–Grünwald stains, or a combination of variants, based on the original method developed by Dmitri Romanowsky, a Russian protozoologist, who first used it 1890 to see malaria parasites. Eosin gives a red to orange color to the alkaline components of cells, such as hemoglobin and the granules of eosinophils, that contain an alkaline spermine derivative.
Alkaline methylene blue dye gives a bluish-purple color to the acidic cellular elements, including nucleic acids (DNA, RNA), nucleoproteins, and the granules of basophils, which contain the acid heparin.

Other stains and methods may be utilized to better visualize unique morphologic features, such as supravital brilliant cresyl blue that stains residual RNA in young red cells (reticulocytes), giving the appearance of strands of dark blue net-like material in these cells.

**Slide Examination**

Properly stained slides are usually pink to the naked eye. Bluish discoloration can arise from too thick a smear, prolonged staining time, inadequate washing, or excessively alkaline buffer in the dyes. A slide also may be blue because the blood contains abnormally high amounts of plasma proteins in such diseases as multiple myeloma. Excessively pink appearance can result from opposite problems: short staining times, prolonged washing, dye that is too acidic, or mounting the slides with coverslips before they are adequately dried. Use of unclean slides, inadequately filtered stain, and dust settling on the smear will lead to the appearance of precipitates.

Microscopic examination should begin by scanning the entire slide at low power (×10 or ×20 objective) to determine the adequacy of staining looking for uniformity of color: nuclei of leukocytes should appear purple and red cells pink, rather than brick-red or yellow. Review at this low magnification also allows detection of overt abnormalities in cell number, type, and aggregation, and permits finding the optimal areas to evaluate all the blood components. A systematic approach should include evaluation of the lateral and feather edges of blood smears prepared by the wedge method, where a disproportionate distribution in these areas of any large abnormal cells, platelet clumps, and microfilarial parasites, if present, may occur. Red cells are best evaluated in the thin areas of the smear, where they are present in a single layer, closely apposed but not overlapping, and exhibiting normal central pallor. Leukocytes may be best examined in the thick areas of the smear, where many cells are present in a single field of view, but abnormal lymphocyte morphology is best appreciated in the thin areas. Review at higher power (×40 to ×50 objective) allows for an assessment of cell size and a closer examination of the nuclear and cytoplasmic features of individual cells. Oil immersion lenses (×50 and ×100) allow for a detailed evaluation of the quality of the nuclear chromatin and presence of nucleoli, and of cytoplasmic components such as granules, vacuoles, and inclusions. This power provides confirmation of any suspicious cells or organisms seen at lower power. Depending on the procedures defined in each laboratory, the manual differential count consists of identifying and classifying 100, 200, 500, or 1,000 white blood cells and reporting each type as a percentage. Note should also be made of any atypical findings in the leukocytes including abnormal nuclear segmentation and granulation of the neutrophils, or the presence of atypical lymphocytes. The presence in a blood smear of nucleated red cells, megakaryocytes, macrophages, and immature leukocytes, which are normally not found in circulating blood, deserves special note and reporting.

The final reporting also includes an assessment of red cell morphology and an estimate of the platelet count. Evaluation of red cell morphology includes reporting of cell size, estimation of the hemoglobin content, shape, inclusions, or structural abnormalities. In the blood of a healthy individual, the red cells appear as uniform discs that range 6 to 8 μm in diameter with a slightly pale central area. Red blood cells (RBCs) smaller than 6 μm in diameter are microcytic, and those that are larger than 9 μm are macrocytic. Abnormal variation in cell size, or anisocytosis, is commonly seen in anemias and is reflected in a wider RDW (RBC distribution width) or coefficient of variation of RBC volume in automated
blood counts. The color of the red cells reflects the hemoglobin content, and increased area of central pallor is seen in hypochromic cells, whereas a denser central coloration is observed in hyperchromic cells or in spherocytes. Abnormal (increased) variation in RBC shape, or poikilocytosis, is reported by identifying the presence of specific atypical forms including elliptocytes, spherocytes, target cells, sickle cells, tear drop cells (dacrocytes), schistocytes, acanthocytes, and echinocytes, among others. Reporting of inclusions or granules in the red cells (such as Howell–Jolly bodies, basophilic stippling, or Pappenheimer bodies) or intracellular parasites (e.g., *Plasmodium* sp. or *Babesia* sp.) requires evaluation at higher magnification (∗×40 to ∗×100∗).

Areas evaluated for red cell morphology are also used to roughly estimate platelet counts on peripheral blood smears; however, automated counting is far more accurate and reliable. A normal platelet count corresponds to one platelet per every 10 to 30 RBCs, which is approximately 7 to 20 platelets per oil immersion field ∗×100∗. A small fraction of platelets may show giant morphology in a normal individual depending on how soon the blood smears were prepared after blood is drawn. However, an increase in the percent of giant platelets that exceeds the normal size range (2–4 μm in diameter) should be noted as it can be associated with neoplastic conditions, immune thrombocytopenia, macrocytic anemias, or Bernard–Soulier syndrome.

**BONE MARROW EXAMINATION**

Examination of the bone marrow begins with careful review of the peripheral blood smear and the complete blood count that were obtained on the same day. Examination of the bone marrow provides qualitative and semiquantitative information on the state of hematopoiesis, and allows for assessment of certain hereditary and acquired disorders, including neoplastic conditions.

Bone marrow examination consists of evaluation of the aspirated bone marrow, which generates a clot section and aspirate smear, and the bone marrow trephine core biopsy. The core biopsy and aspirate smear review provide complementary information, and should be performed routinely in every bone marrow evaluation. Flow cytometric and cytogenetic analyses are also performed on the aspirated marrow specimen, adding important information that aids the overall evaluation. Molecular studies for the mutational status of specific genes can be performed on nucleic acid material extracted from either the freshly aspirated marrow or, with recent advances in molecular techniques, the formalin fixed material (blood clot). Furthermore, information provided by the cytogenetic analysis and molecular mutational studies is necessary for the diagnostic classification of certain malignancies. Cytogenetic and molecular studies provide important information as rapid advances in the field continue to identify an ever-growing list of mutations associated with various diseases. However, genetic analyses add to, and do not substitute for, the clinical and morphologic evaluation. Each specimen generated for the morphologic assessment (aspirate smear, clot section, and core biopsy) differs in its processing and is characterized by unique limitations and merits that add to the overall assessment of the bone marrow. Adopting a routine approach to the evaluation of all the marrow components aids in obtaining maximum information from the morphologic examination.

**EXAMINATION OF THE BONE MARROW ASPIRATE**
Slide Preparation and Staining

As with peripheral blood smears, optimal preservation of morphology requires either air-drying of aspirates without the use of anticoagulants or the use of EDTA as the anticoagulant of choice. Heparin introduces a staining artifact that alters the morphology of the cells. Bone marrow aspirate smears are prepared by a method similar to that used for making peripheral blood smears. A drop of the aspirate containing grossly visible marrow particles, which look like gray specks to the naked eye, is placed at one end of the slide and the marrow particles are gently dragged with a spreader slide leaving behind a trail of cells. A crush/squash preparation entails placing marrow particles between two slides and applying slight pressure and rapidly pulling apart the two slides. When a bone marrow sampling fails to yield any aspirated marrow particles (dry tap), the fresh unfixed core biopsy specimen can be picked up with forceps and touched several times to a slide producing touch preparations. Smear, crush, or touch preparations should be rapidly air-dried and stained with a Romanowsky’s stain, such as May–Grünwald Giemsa or Wright–Giemsa. An optimal specimen of the marrow aspirate provides adequate morphology for a thorough evaluation of the cytologic features of the marrow components and for a manual differential count. Although touch preparations may be the only specimen available for a cytologic examination of the individual marrow elements, they are prone to artifacts, making them suboptimal for an accurate morphologic review as they may not be representative. Nonetheless, touch preparations can sometimes provide useful information and should always be attempted in a dry tap.

The presence of iron is examined by staining the aspirate smear with Prussian blue (Perl’s reaction produces a blue-green color when hemosiderin or ferritin is present) and counter-staining with safranin-O or Kernecht Red (nuclear fast red). This stain allows evaluation of storage iron present in the macrophages in the marrow particles. The iron stain to assess storage iron is most reliable when it is positive; absence of iron staining may be due to uneven distribution of iron-laden macrophages in the particles. Additionally, the iron stain is used to visualize sideroblasts, which are normoblasts containing one or more particles of iron. The presence of abnormal sideroblasts and ring sideroblasts should be noted as they are seen in various hereditary and acquired hematologic diseases.

Special cytochemical stains can be performed on the aspirate smears that aid in identifying various cell lineages in the marrow. This is especially important when evaluating an excess of immature cells. The differential reactivity with these chemical reagents among the marrow precursors served as the basis for the early neoplastic classification systems. The most common stains performed include myeloperoxidase for the myeloid lineage, and nonspecific esterases for the monocytic cells. Rarely other stains may be utilized, such as Sudan black for the myeloid lineage, periodic acid–Schiff (PAS) for the erythroid and T-cell precursors, and toluidine blue to highlight the granules of mast cells and basophils. Flow cytometry of the aspirated cells and immunohistochemical stains of the biopsy have mostly replaced the need for cytochemical stains.

In rare instances, immunohistochemical stains can be performed on the aspirate smears if flow cytometry or a biopsy/clot section is not available; however, these are technically challenging and are difficult to interpret because of artifacts. Aspirate smears can also be used for fluorescence in situ hybridization (FISH) analysis or molecular tests, though this is not done routinely.
Slide Examination

The aspirate smear is indispensable for evaluation of the cytologic details of the marrow cells. Specifically, the aspirate smear is necessary to evaluate the presence of dysplasia in the myeloid and erythroid lineages, cell inclusions, and parasites, which cannot be well appreciated on other marrow preparations. A bone marrow nucleated differential count allows assessment of the proportions of different cell lineages, and comparison to expected normal ranges. Furthermore, the manual differential count performed on an adequate aspirate smear is the gold standard for detecting, identifying, and quantifying any abnormal cells (i.e., dysmorphic cells or blasts), and establishing information needed for the classification of hematologic malignancies.

Examination of the aspirate smear begins at low power (×4 or ×10 objective) scanning for particles and for any clusters of abnormal cells such as those of extrinsic metastatic neoplasms, which tend to be more cohesive than hematopoietic cells. At this magnification, it is also possible to estimate marrow cellularity, although that is best judged on the biopsy section. To do so, the ratio of fat to cellular elements in the particles is assessed, together with the cell density around the particles. Presence of lymphoid aggregates and evaluation of megakaryocytes is also assessed at low power. A crush preparation is useful for the assessment of marrow fibrosis, focal disease (i.e., plasma cell myeloma, lymphoma, granulomas, and metastatic carcinoma), the determination of cellularity, and megakaryocyte numbers.

In a normal bone marrow, at medium to high power (×20 to ×40), the predominant marrow component should be segmented granulocytes. An evaluation at this power can give a preliminary assessment of the maturation of the myeloid and erythroid cells and can identify the best areas in which to perform a manual differential count. Bone marrow cells should be counted in cellular areas adjacent to or in the trail of the particles, where the cells are well dispersed, show good cytologic detail, and where lysed cells do not predominate. Areas with excessive air-drying artifact should be avoided, as cells in these areas show suboptimal morphology. Only intact cells should be counted, as naked nuclei lacking cytoplasmic features cannot be confidently subclassified. The differential count is performed at high (×40) or oil (×50, ×100) magnification to allow optimal identification of cell types. The count includes nucleated myeloid and erythroid cells in various stages of maturation, promonocytes, monocytes, mast cells, lymphocytes, and plasma cells. Cells that should not be included in the count include macrophages, megakaryocytes, osteoblasts, osteoclasts, stromal, or extrinsic cells. The myeloid to erythroid ratio is calculated by expressing a ratio of all the granulocytes and monocytes and their precursors to the erythroblasts in various maturation stages. Touch preparations should be examined with the same parameters as used for aspirate smears; however, these specimens tend to be characterized by suboptimal morphology and are less reliable.

EXAMINATION OF THE BONE MARROW BIOPSY AND CLOT SECTION

Processing for Histologic Sections

The trephine biopsy specimen should be taken at right angle to the cortex and be at least 1.5 cm in length. A long core biopsy increases the likelihood of finding focal lesions (i.e., lymphoma, granuloma, and metastatic disease). After the touch preparations are made, the
A bone marrow clot preparation is processed in a similar manner as the trephine core biopsy with the exception of the decalcification step. A clot section can provide additional information, especially if the trephine core biopsy is inadequate. It is generated at the time of the bone marrow aspiration, when a portion of the aspirate containing marrow particles is allowed to form a clot. Various agents may be used to facilitate clotting. The clot is then placed into an appropriate fixative without further decalcification. An important advantage of the clot preparation is that it may be used for FISH analysis or extraction of nucleic acid material suitable for molecular-based testing. Furthermore, lack of decalcification produces more reliable immunoreactivity, although interpreting these stains may be more challenging than on the trephine biopsy section.

Automated tissue processors are utilized for the bone marrow clot and trephine biopsy specimens, similar to other routine surgical biopsy material. Specimens are then embedded in paraffin, sectioned at 3 to 5 µm in thickness, and mounted on glass slides. The prepared sections are routinely stained with hematoxylin and eosin (H&E), although other stains may be used in addition. For instance, PAS helps to highlight the megakaryocytes, whereas Giemsa is helpful to identify mast cells, eosinophils, plasma cells, and to differentiate between proerythroblasts and myeloblasts. Evaluation for the presence of fibrosis is done routinely on sections by staining for reticulin by using silver impregnation, which reacts with type III collagen, whereas Masson’s trichrome is used to detect type I collagen. Other useful histochemical stains include Ziehl–Neelsen stain for acid-fast organisms, Gomori’s methenamine silver for fungi, and Congo red for amyloid. Iron stores can also be evaluated by Prussian blue on the histologic sections; however, decalcification chelates sideroblast and storage iron, so that iron evaluation is unreliable on the trephine biopsy sections.

Immunohistology is used routinely on histologic sections and aids in lineage and subtype identification of cells by probing for specific lineage-associated antigens. Testing for expression of certain proteins may also provide information regarding mutational status or proliferation, which can impact prognosis in neoplastic conditions. Although flow cytometry provides information on protein expression of individual cells and allows assessment of cell populations, immunohistology provides correlation with direct morphologic assessment. Furthermore, immunohistology is especially important if the aspirate specimen obtained for flow cytometric analysis is hemodiluted or if the cells of interest are not adequately represented in the aspirate.

**Slide Examination**

Histologic sections represent thin slices through the specimen, which preserves the overall architecture of the marrow and connective tissue elements. Compared to aspirate smears, histologic sections allow better evaluation of the marrow cellularity and number of megakaryocytes. In the clot section, however, the cellular marrow is somewhat contracted and lacks the full architecture, thus the trephine biopsy section is ideal for assessment of
cellularity. Similar to the estimate performed on bone marrow aspirate smears, cellularity is evaluated by comparing the volume of the hematopoietic cells to the adipose cells and stromal elements that make up the total marrow space. Cellularity is assessed with reference to the patient’s age, because bone marrow cellularity varies with age. The subcortical intertrabecular spaces are frequently hypocellular and therefore these areas should be excluded from the cellularity assessment.

Several histologic serial sections are examined to increase the likelihood of identifying focal diseases. A systematic approach helps to ensure that maximal information is obtained from the morphologic review. Evaluation should begin at low power (×4 to ×10) to assess the length, adequacy, and cellularity of the bone marrow specimen. This power provides an opportunity to examine the general pattern, any presence of focal lesions, abnormal cell clusters, and quality of bone structure. Megakaryocyte numbers can be appreciated at low and medium magnification, which also allow for assessment of the relative ratio of myeloid to erythroid cells. Maturation of hematopoiesis is better evaluated at higher magnification (×20 to ×40), which shows the cytology. Maturation of each cell lineage has characteristic features: including increased nuclear segmentation and cytoplasmic granularity in the myeloid lineage, and chromatin condensation and cytoplasmic eosinophilia in the erythroid lineage.

Normally, immature myeloid cells are found along the bony trabeculae without forming clusters or large aggregates. Erythroid cells are distributed interstitially in small distinct islands. Megakaryocytes are scattered as individual cells in the interstitium, and in normal individuals, one to three are typically seen per high-power field. Detection of lymphomas and metastatic neoplasms is more reliable in histologic sections than on aspirate smears, and their histologic pattern provides important clues for the specific diagnosis, which can then be confirmed by immunohistochemistry.

The bony trabeculae should always be evaluated. Osteosclerosis or thickening can be seen as part of myelofibrosis or metabolic diseases of bone, whereas trabecular thinning is seen in osteopenia. Other characteristic patterns of pronounced osteoid seams, irregular bone resorption, or “mosaic” trabeculae patterns, are seen in such conditions as osteomalacia, osteitis fibrosa, and Paget disease, respectively.

Reticulin staining to evaluate for bone marrow fibrosis is routine in most laboratories, and should be scored based on established scoring systems. Additional stains, including immunohistochemistry, are performed depending on suspicious morphologic findings or specific clinical indications. Interpretation of these studies requires understanding of common artifacts and background, use of positive controls and negative controls, common expression patterns, and comparison with morphology seen on the corresponding area of the H&E section.

<table>
<thead>
<tr>
<th>Hematology reference values in normal adults</th>
</tr>
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<tbody>
<tr>
<td>Test</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Hematocrit (volume of packed red cells)</td>
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<tr>
<td>Red cell count</td>
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</table>

19
<table>
<thead>
<tr>
<th></th>
<th>μL</th>
<th>$10^{12}$/L</th>
<th>μL</th>
<th>$10^{12}$/L</th>
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<tbody>
<tr>
<td><strong>White cell count</strong></td>
<td>$4.4–11.3 \times 10^3/\mu L$</td>
<td>$4.4–11.3 \times 10^9/L$</td>
<td>$4.4–11.3 \times 10^6/\mu L$</td>
<td>$4.4–11.3 \times 10^9/L$</td>
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<td><strong>Mean corpuscular volume (fL)</strong></td>
<td>80–96</td>
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<tr>
<td><strong>Mean corpuscular hemoglobin (pg)</strong></td>
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<td><strong>Mean corpuscular hemoglobin concentration</strong></td>
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<td><strong>Platelet count</strong></td>
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<td>$150–450 \times 10^9/L$</td>
<td>$150–450 \times 10^3/\mu L$</td>
<td>$150–450 \times 10^9/L$</td>
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<td><strong>Reticulocyte percentage</strong></td>
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<td><strong>Reticulocyte count</strong></td>
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<td>$22.5–147.5 \times 10^9/L$</td>
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<td><strong>Sedimentation rate (Westergren) &lt;50 yr of age (mm/h)</strong></td>
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</table>

**SI, Système International d’Unités.**

### Table 1.2

<table>
<thead>
<tr>
<th>Age</th>
<th>Lowest Normal Hb (g/dL)</th>
<th>Normal Red Blood Cell Size</th>
<th>Fetal Hb (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean Corpuscular Volume (fL)</td>
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<tr>
<td>Birth</td>
<td>14.0</td>
<td>100–130</td>
<td>55–90</td>
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**Hb, hemoglobin.**

### Table 1.3

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<th>Differential counts from bone marrow aspirates from 12 healthy men</th>
<th>Mean (%)</th>
<th>Observed Range (%)</th>
<th>95% Confidence Limits (%)</th>
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**Figure 1.1.** Preparation of blood smears. Blood smears may be prepared by the coverslip or slide wedge method. **A:** Coverslip smears are prepared by placing a drop of blood in the

Figure 1.2. Macroscopic appearance of peripheral blood smears. Abnormalities in the patient’s red cell concentration and the presence of marked gammopathy can be reflected in the gross appearance of the blood smears. The smear on the left, from a patient with polycythemia vera and a hemoglobin of 20 g/dL, appears noticeably redder and darker compared to the normal (center left; hemoglobin = 14 g/dL) or to the pale smear from an anemic patient (center right; hemoglobin = 7 g/dL). The blood smear on the right, from a patient with plasma cell myeloma, is visibly bluish because high levels of circulating monoclonal immunoglobulins take up the basophilic stains.
Figure 1.3. Microscopic approach to peripheral blood smears for red cell morphology. Red cells are best evaluated in areas where they are well spaced in a single layer without touching each other and exhibit central pallor (right upper panel). Conversely, when red cells are examined too close to the feathered edge of the slide, they appear misshapen, flattened, and falsely hyperchromic (left lower panel), whereas red cells in densely packed areas often appear shrunken and clumped (right lower panels). Therefore, examining cells in an optimal area is required for accurate diagnosis.
Figure 1.4. Examination of peripheral blood smears at low magnification. Abnormal aggregates, precipitated proteins, and parasites are best seen by scanning large areas of the smear at low-power magnification. The low-power views show suspicious areas to investigate, which are confirmed by the higher power insets. A: Red cell aggregation caused by a cold agglutin. B: Cryoglobulin precipitates causing red cell aggregation. C: Rouleaux (stacking of red cells) caused by the presence of a monoclonal immunoglobulin. D: Marked platelet aggregation caused by the anticoagulant EDTA, which can result in pseudothrombocytopenia. E: Aggregation of leukocytes and platelets caused by EDTA. F: Circulating microfilaria (×20; inset, ×50).
Figure 1.5. Oil immersion views of normal RBCs and various poikilocytes (i.e., abnormally shaped RBCs), which occur in different diseases. **A:** Normal red blood cells, which are quite uniform in shape and size. **B:** Target cells as seen in hemoglobin C. **C:** Acanthocytes (spur cells) seen in liver disease. **D:** Echinocytes or burr cells. **E:** Dacrocytes or teardrop RBCs (characteristic of myelofibrosis). **F:** Spherocytes seen in immune hemolysis or spherocytosis. **G:** Ovalocytes and elliptocytes. **H:** Blister cells (arrows). **I:** Bite cells. **J:** Schistocytes as in disseminated intravascular coagulation or thrombotic thrombocytopenic purpura. **K:** Sickled RBCs and target cells as in sickle cell anemia and sickle-β thalassemia. **L:** Dehydration artifact: this last effect, stemming from faulty smear preparation, should not be confused with an abnormal finding.
Figure 1.6. Abnormal inclusions in red blood cells. **A:** Howell–Jolly bodies. **B:** Nucleated RBCs (orthochromic normoblasts) seen in the peripheral blood with acute anemia, severe hemolysis, and myelophthisis. **C:** Pappenheimer bodies. **D:** Trophozoites of *Plasmodium falciparum*. **E:** Coarse basophilic stippling in a case of lead poisoning. **F:** Heinz body inclusions in a patient who has undergone a splenectomy. Heinz bodies can only be demonstrated by using the crystal violet supravital stain. Additional illustrations of these RBC inclusions can be found in Chapter 3 on Anemia.
Figure 1.7. Red blood cell morphology: size variation or anisocytosis. RBC size is best measured by automated hematology analyzers, but marked size differences can be estimated by comparing the diameters of red cells to those of lymphocyte nuclei. Normal erythrocytes are approximately the same size as the nuclei of small lymphocytes, about 8 μm in diameter. The top panel shows numerous microcytic red cells that are significantly smaller than the diameter of the lymphocyte nucleus. The microcytic cells are also hypochromic, with a large region of central pallor and just a thin peripheral rim that is hemoglobinized. The bottom panel demonstrates two macrocytic red cells (arrows), the one on the left is a polychromatophilic red cell, likely a reticulocyte. In macrocytic anemias, RBCs are often of varying size with only a small population of macroovalocytes. RDW is increased both in macrocytic and in microcytic anemias.
Normal small circulating lymphocytes are usually 10 to 15 μm (slightly bigger than normal red blood cells) and typically have high nuclear/cytoplasmic (N:C) ratios, with scant amounts of slightly basophilic staining cytoplasm. Lymphocyte nuclei are usually smoothly contoured with a mature (or “condensed”) chromatin pattern and absent nucleoli. The appearance of LGLs shows more variable morphology, but they generally have moderate N:C ratios and more abundant neutral-staining cytoplasm containing purplish granules. The nuclei of LGLs are frequently irregular and folded and the chromatin is often slightly paler or more or “open” than that of small lymphocytes.
Figure 1.9. Morphology of lymphocytes in common neoplastic lymphoid disorders: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and acute lymphoblastic leukemia (ALL). The designation ALL includes both precursor B- and T-lymphoblastic leukemias. **A:** Normal lymphocyte with mature, clumped chromatin. **B:** CLL cells are variable in appearance and typically look very much like slightly larger versions of normal small lymphocytes and can display variably clumped or “soccer ball” chromatin. **C:** The ALL cell has a high nuclear/cytoplasmic (N:C) ratio with an immature or “open” chromatin.
**Figure 1.10.** “Atypical” lymphocyte morphology in infectious mononucleosis. When referring to circulating lymphocytes, “atypical” is a confusing term that generally denotes benignity, despite the pleomorphic appearance of the cells, which can be confused with immature cells or monocytes. Characteristic features of atypical lymphocytes in infectious mononucleosis include large size, abundant basophilic and vacuolated cytoplasm, prominent nucleoli, diffuse or partially condensed chromatin, and large, often irregularly shaped nuclei. **A–E:** Samples from different patients with infectious mononucleosis. For comparison, normal lymphocytes are shown on the right-hand side of each panel. **F:** Gross image of an enlarged spleen with nodular areas in a red background, corresponding to markedly expanded white pulp follicles and red pulp sinusoids. Splenomegaly in patients with infectious mononucleosis can lead to rupture.
Figure 1.11. Appearance of circulating neoplastic cells in small and large cell lymphoproliferative disorders. Peripheral blood smears from patients with various lymphomas and leukemias that feature primarily small and large cells are shown in the top and bottom sets of panels, respectively. CLL, chronic lymphocytic leukemia/lymphoma. As described in Figure 1.9, these neoplastic cells are slightly larger than normal lymphocytes with clumped chromatin. HCL, hairy cell leukemia cells, are characterized by abundant pale blue cytoplasm with circumferential “hairy” projections and variable nuclear morphology. SMZL, splenic marginal zone lymphoma, cells have abundant pale cytoplasm that can be
characterized by short polar villi and relatively mature chromatin. NK, aggressive NK-cell leukemia cells show a range of morphologic features and can be similar to LGLs or atypical cells with irregularly folded nuclei, open chromatin, and prominent nucleoli. FL, follicular lymphoma, cells when found in the peripheral blood show cleaved nuclear morphology and scant cytoplasm. PCL, plasma cell leukemia, cells usually look similar to non-neoplastic plasma cells with eccentric nuclei, abundant blue cytoplasm with a perinuclear clear zone, and condensed “clock-face” chromatin; however, high nuclear to cytoplasmic ratio and prominent nucleoli can also be seen in these cells. ALL, acute lymphoblastic leukemia, as described in Figure 1.9 features immature chromatin and scant cytoplasm. The FAB classification, which has been superseded by the WHO classification, made morphologic distinctions of ALL blasts. L1 type blasts featured homogenous open chromatin, relatively regular nuclei, and absence of nucleoli. L2 type blasts showed heterogenous chromatin, irregular nuclear shape, prominent nucleoli, and variably abundant basophilic cytoplasm. L3 type blasts share morphologic features with leukemic Burkitt lymphoma, in that the blasts have fine chromatin with prominent nucleoli and are characterized by deeply basophilic cytoplasm with vacuoles. PLL, B-cell prolymphocytic leukemia, shows medium to large cells with a round nucleus, somewhat condensed chromatin, and a prominent central nucleolus, whereas the cytoplasm is abundant and slightly basophilic. LBCL, peripheralized diffuse large B-cell lymphoma, shows variable morphology with irregular nuclei, vesicular chromatin, and can show prominent nucleoli. ATLL, adult T-cell leukemia/lymphoma, features characteristic abnormal nuclear convolutions in the acute variant that are known as “flower cells,” and somewhat abundant basophilic cytoplasm. Although usual morphologic features are outlined pertaining to these entities, these findings alone cannot be used to establish these diagnoses.
Leukocytes are best counted by automated hematology analyzers, but an approximate estimate of white cell numbers can be done during screening of the peripheral blood smear at low magnification and by comparing the general ratio of white cells to erythrocytes (normal is approximately 1–500).
Figure 1.13. Granulocyte morphology: abnormal cytoplasmic features. A: Band showing “toxic changes,” including increased cytoplasmic granulation and numerous bluish Döhle bodies. B: Circulating band ingesting a budding yeast (*Candida albicans*). C: Monocyte showing “toxic changes,” including marked cytoplasmic granulation and vacuolization. D: May–Hegglin anomaly with large Döhle-like inclusions and giant platelets. E: Neutrophil-ingesting bacteria in a case of sepsis from *Clostridium perfringens*. (Courtesy of Dr. I. Quirt.) F: A dysplastic neutrophil with abnormal nuclear lobation and hypogranular cytoplasm (arrow) adjacent to a normal neutrophil.
Figure 1.14. Granulocyte morphology: abnormal nuclear features. 

A: Giant hypersegmented neutrophil (arrow) from a patient with vitamin B<sub>12</sub> deficiency.  
B: Hypersegmented neutrophils in a patient on antifolate chemotherapy.  
C: Dysplastic neutrophil with abnormal nuclear lobation (pseudopelgeroid) in a patient with myelodysplastic syndrome (MDS). This neutrophil with a bilobed nucleus connected by a thin filament resembles neutrophils in the hereditary Pelger–Huët anomaly. However, the Pelger–Huët-like morphology is acquired in MDS.  
D: Degenerating (apoptotic) neutrophil with marked nuclear hyperchromasia. Such apoptotic neutrophils may be an artifact of making a blood smear with old blood rather than fresh specimens, or may occur in vivo.
Figure 1.15. Abnormalities in platelet number and morphology. Platelet numbers are best measured using automated hematology analyzers, but generally microscopic visualization of 7 to 20 platelets per oil immersion field represents approximately normal platelet counts. 

A: Increased numbers of platelets in a patient with essential thrombocythemia and a platelet count of 500 × 10⁹/L (normal range is between 150 and 450 × 10⁹/L). Note several giant platelets. Elevated platelet counts also occur in reactive thrombocytosis associated with infection or postsurgery. Artifactual platelet satellitism (B) and aggregation (C) can occur from EDTA exposure in blood collection tubes and can lead to falsely low platelet counts by automated hematology analyzers. This phenomenon can be recognized by examining the peripheral blood smear and then re-drawing the blood specimen using heparin or citrate as anticoagulants in collection tubes. 

large Döhle-like inclusions in neutrophils (arrow) and giant platelets (see also Fig. 1.12).

**Figure 1.16.** Routine evaluation of the bone marrow: staining and special studies. This figure shows a typical tray of slides for interpretation of a bone marrow sampling by a hematopathologist (9 slides from left to right): **A:** An unstained air-dried aspirate smear that on close inspection shows granular white-gray particles surrounded by blood that represent bone marrow particles. **B:** An unstained touch preparation of a bone marrow biopsy that shows predominantly dried drops of blood in the shape of a core. Although a touch preparation is prone to artifacts, it can provide useful information in a “dry tap.” **C** and **D:** H&E staining is routinely used for bone marrow core biopsy and clot sections after these specimens undergo routine histologic processing (see text for details). Additional studies such as immunohistology and special stains can be performed on the core or clot specimen by cutting additional sections as needed to complete the evaluation. **E:** Wright–Giemsa is used routinely to stain the aspirate smears and touch preparations for cytologic review of the marrow elements. **F:** Presence of iron stores is routinely evaluated by staining with Prussian blue, here performed on the aspirate smear. **G** and **H:** Although not routinely performed, with the use of flow cytometry, some laboratories still retain the ability to perform cytochemical staining (myeloperoxidase with counterstain and nonspecific esterase) on the aspirate smear in selected cases to further characterize blasts. **I:** Review of a peripheral blood smear, stained with Wright–Giemsa, is part of a complete bone marrow evaluation.
Figure 1.17. Microscopic approach to bone marrow aspirate smears. Selecting the correct area to examine is essential to properly assess marrow aspirate smears. A and B: The best regions are adjacent to the marrow particles (arrows) containing well-preserved clusters of cells that represent the actual cellular content of the marrow cavity. C: Areas of the aspirate where hematopoietic cells are well preserved and well spaced, almost touching each other but not overlapping are optimal (right side). Areas where the cells are stripped of cytoplasm and/or display excessive air-dry artifact should be avoided and cannot be assessed for a manual differential count (left side).
Figure 1.18. Assessing megakaryocytes in the aspirate smear. Megakaryocytes are relatively rare in normal bone marrow specimens, representing approximately 1% of all nucleated cells and are usually found in or near marrow spicules. A and B: Because of the large size of megakaryocytes, their numbers are best assessed at low magnification. C and D: The presence of morphologic atypia should be evaluated and quantified at higher power; however, megakaryocytes are normally at least twice as large as a promyelocyte and have polylobated nuclei. Presence of small megakaryocytes with nuclear hypolobation may be seen in neoplastic conditions.
**Figure 1.19.** Variations in megakaryocyte morphology. **A:** Normal megakaryocyte with multiple contiguous nuclear lobes. **B:** Markedly enlarged megakaryocyte with nuclear hyperlobation from a patient with essential thrombocythemia. **C:** An example of emperipolesis (the present of an intact leukocytes within a megakaryocyte). **D:** A stripped “naked” megakaryocytic nucleus (arrow). **E-G:** Dysplastic megakaryocytes. **E:** A megakaryocyte with a prominent nucleolus, open chromatin and cytoplasmic blabbing. **F:** A micromegakaryocyte with a monolobated nucleus (arrow). **G:** A megakaryocyte with separated nuclear lobes.
Figure 1.20. Histiocytes in bone marrow aspirate smears. **A:** Histiocytes are not usually prominent in smear aspirates, but an occasional histiocyte with abundant, pale, and granular cytoplasm, with tingible (stained) bodies can be seen in the aspirate smear, often in patients with excess iron stores. The engulfed material in such smears can be shown with appropriate staining to represent siderotic granules. **B–F:** Histiocytosis associated with infections and hematopoietic malignancies. **B:** Granulomas on aspirate smears show clusters of histiocytes with elongated nuclei, vesicular chromatin, and small inconspicuous nucleoli admixed with
lymphocytes in a patient with tuberculosis. C: An accumulation of characteristic intracellular amastigotes inside of a marrow histiocyte helps to establish this rare diagnosis in a patient with visceral leishmaniasis. D: Hemophagocytosis can be associated with a variety of neoplastic, reactive, and infectious conditions. This image of a histiocytic cell engulfing RBCs and lymphocytes is from a patient with fatal Epstein–Barr virus–associated hemophagocytic syndrome. E: Sea-blue or Gaucher-like histiocytes are often seen in patients with chronic myelogenous leukemia before treatment. F: Hemophagocytosis (arrow) associated with a case of T-cell lymphoproliferative disorder involving the bone marrow.

Figure 1.21. Bone cells in aspirate smears. A and B: Clusters of osteoblasts with the characteristic extruding or “pouting” nuclei (arrows). C: Plasma cells, shown here for comparison, are smaller than osteoblasts and do not have extruding nuclei, although their
nuclei are characteristically eccentric. Note prominent perinuclear clear areas (Golgi) in the plasma cells. D and E: Osteoclasts with numerous well-separated and uniformly sized nuclei resembling “pennies on a plate.” (Courtesy of Dr. J. Lazarchick.) F: Megakaryocytes, one shown here for comparison, have variably sized, continuous nuclear lobulations.

**GRANULOCYTIC HYPERPLASIA (POST CYTOKINE ADMINISTRATION)**

**MATURATION ARREST (POST VIRAL INFECTION)**

*Figure 1.22.* “Granulocytic pattern” in an aspirate smear may be due to diverse etiologies. The administration of cytokines or postviral infection maturation arrest may give similar morphologic findings, and clinical correlation is indispensable for the proper pathologic diagnosis. Both aspirates show predominant numbers of immature granulocytic precursors, but in the maturation arrest the early myeloid forms predominate. Granulocytic maturation arrest can also be immunologic or toxic in origin, resulting in the absence of later stages of myeloid maturation. In agranulocytosis, the most severe form, no myeloid precursors are
Figure 1.23. Morphology of erythroid maturation in bone marrow aspirates. In this composite figure, the spectrum of immature to mature erythroid precursors is designated by a series of lengthening arrows. Pronormoblasts are generally distinguished by having a scant basophilic cytoplasm and a more rounded nucleus and prominent nucleolus. As the cells mature, they become smaller but maintain a very rounded nuclear shape, the chromatin condenses, and nucleolus disappears, signaling features of basophilic normoblasts. Further decrease in size and further clumping of the chromatin, as well as changes in cytoplasmic staining, indicate transition to polychromatophilic normoblasts. Hemoglobinization of the cytoplasm and further nuclear maturation leads to orthochromatophilic normoblasts, which transition to a nucleated RBC before shedding the nucleus and finally becoming mature RBC.
Figure 1.24. Mast cells. Normally, mast cells constitute less than 1% of all the nucleated bone marrow cells. They are often found in the stroma of marrow particles, making them difficult to appreciate in standard aspirate preparations. A: A smear stained with toluidine
blue highlights metachromatic mast cell granules and shows normal numbers of mast cells in a large marrow particle. **B:** A Wright–Giemsa–stained aspirate, at high magnification, from a patient with systemic mastocytosis demonstrates numerous heavily granulated mast cells.

**Figure 1.25.** Prussian blue iron stains. Staining the bone marrow for iron is the gold standard for assessing body iron stores. Safranin is used as a counterstain to visualize the marrow elements. **A:** At low power, individual histiocytes scattered throughout the marrow particles show the normal pattern of fine granular blue staining for iron in a normal patient. **B:** Evaluation of at high power allows for detection and quantification, if any, of ring sideroblasts. **C** and **D:** In a patient with polycythemia vera, iron stores are absent.
Figure 1.26. Hematopoietic versus nonhematopoietic cells in aspirate smears. Tight clusters of cohesive large cells in aspirate smears strongly suggest marrow involvement by extrinsic neoplasms that are neither lymphoid nor hematopoietic in origin. Morphologic differences between collections of hematopoietic cells and cohesive clumps of nonhematopoietic cancer cells are illustrated. A: Acute myelomonocytic leukemia cells overlap but are clearly separated with windows between neoplastic cells. Individual cells are clearly distinguished. B: Metastatic carcinoma cluster with an indistinct border among the neoplastic cells from a patient with small cell carcinoma of the lung.
Figure 1.27. Anatomy of the bone marrow. This composite figure shows the relationships between the skeletal anatomy of the usual bone marrow biopsy site in the posterior superior iliac crest and the microscopic features of biopsy interpretation. The subcortical area is normally hypocellular and should be avoided when evaluating marrow cellularity. This illustrates how a tangential or superficial specimen may not be representative of the patient’s marrow status.
Figure 1.28. Histomorphologic architecture of the normal bone marrow biopsy. The three major hematopoietic lineages of the marrow—megakaryocytic (MEGA, black arrows), myeloid (white arrow head), and erythroid (red arrows)—all tend to intermix and are recognizable in biopsy specimens. The myeloid series tends to collect near bony trabeculae and perivascular spaces. Myeloblasts are singly scattered throughout and do not form aggregates in normal circumstances, but rather show organized progressive maturation away from the bony trabeculae. Erythroid cells, on the other hand, tend to form islands and are recognizable by their spherical and hyperchromatic nuclei. Megakaryocytes are scattered throughout. Osteoblasts (OSTEOBLASTS, white arrow) rim the bony trabeculae and responsible for bone deposition. When osteoblasts are incorporated into the bone matrix they become osteocytes (OSTEOCYTES, white arrows) and are found in bone lacunae. Osteoclasts (not shown), cells responsible for bone resorption, are usually inconspicuous, but may become more prominent when there is a high rate of bone turnover.
Figure 1.29. Megakaryocytes. Increases in megakaryocyte numbers can be best appreciated using low and medium magnification of the core biopsy. Megakaryocytic proliferation can be seen in both reactive (A) and neoplastic conditions (B–D), but is typically associated with the presence of marked atypia only in the latter. Architectural distortion (swirling and lining up of individual marrow cells) consistent with significant marrow fibrosis is present (C and D).
Figure 1.30. “Granulocytic pattern” in marrow biopsy. Relative (A) and absolute (B) increases in granulocytic precursors are readily seen. **A:** Marrow fat is maintained, and the cellular areas show granulocyte hyperplasia. **B:** The marrow shows no fat and is
hypercellular, consistent with a myeloproliferative neoplasm.

Figure 1.31. Erythropoiesis in bone marrow biopsies: increased and decreased erythropoiesis.
A: At medium magnification, an absolute increase in erythroid precursors can be appreciated that results in a low myeloid to erythroid ratio and “mononuclear pattern.” B: A case of pure red cell aplasia shows the reverse, with a severe decrease in erythroid precursors giving the biopsy a “granulocytic” appearance with a high myeloid to erythroid ratio, because of an absolute decrease in erythroid precursors in the presence of normal numbers of myeloid cells.
Figure 1.32. Hyperplastic patterns in biopsy. Two hyperplastic marrow specimens are illustrated. A: Hyperplastic marrow consisting of a heterogeneous mixture of mature hematopoietic precursors typical of the myeloproliferative syndromes. B: Expansion of a
homogenous population of immature hematopoietic cells present in sheets, replacing all the normal marrow elements, and is characteristic of acute leukemia.

Figure 1.33. Hypoplastic morphology in marrow biopsy. This biopsy from a patient with aplastic anemia shows profound hypocellularity and virtually no evidence of hematopoiesis. The hematopoietic marrow has been replaced with fat cells. The differential diagnosis of a bone marrow biopsy almost devoid of hematopoiesis includes congenital and idiopathic causes of aplastic anemia, hypocellular myelodysplastic syndromes, and paroxysmal nocturnal hemoglobinuria. Local radiation near the marrow site can produce a similar picture. Plasma cells and mast cells often appear disproportionally increased in hypoplastic specimens.
Figure 1.34. Serous atrophy. In this condition, an amorphous gelatinous ground substance with a pale eosinophilic appearance replaces fat and hematopoietic cells. Causes include chronic systemic dysfunctions, such as HIV and malnutrition.
Figure 1.35. Lymphoid aggregates of the bone marrow. 

**A:** Low power. Benign lymphoid aggregates (arrow) of the bone marrow increase with age and are more frequent in women and patients with autoimmune diseases.

**B** and **C:** High power. Benign lymphoid aggregates usually are well demarcated, small in size, few in number, perivascular in distribution and consist mostly of small mature lymphocytes with round nuclei. By immunohistology, they show a mixture of predominantly CD3-positive T cells (B) and scattered CD20-positive B cells (C).
Figure 1.36. Benign and malignant lymphoid nodules. A: As described in Figure 1.35, a benign lymphoid aggregate is well circumscribed and composed of small lymphoid cells. B: Nodules of follicular lymphoma have a characteristic paratrabecular pattern. C: Granulomas may be incidental findings, but require further workup with special stains to rule out infectious organisms. D: Diffuse large B-cell lymphoma can show a variety of patterns of involvement, and here an interstitial distribution is shown. E: CLL can show nodular or interstitial marrow involvement. F: Hodgkin lymphoma can show extensive marrow sclerosis, with a paucity of neoplastic cells.
Figure 1.37. Bone marrow granuloma, high power. This biopsy of two discrete noncaseating granulomas from a patient with sarcoidosis is a higher power view of Figure 1.36C. Causes of bone marrow granulomas include lymphoid and nonlymphoid malignancies, infectious diseases, drugs, foreign bodies, and connective tissue diseases.

Figure 1.38. Bone marrow fibrosis. Two cases of severe marrow fibrosis are shown. A and B: A case of primary myelofibrosis in which the normal marrow cells are entirely replaced by spindle-shaped fibroblasts and collagen. B: The special stain for reticulin. C: A case of secondary marrow fibrosis due to a desmoplastic reaction to breast carcinoma metastatic to the bone marrow. Note the malignant cells lining up in rows (“Indian filing”) and the swirling patterns between strands of collagenous fibrosis.
Figure 1.39. Bony trabeculae patterns in marrow biopsies. Aside from the thickened area immediately subjacent to the cortex, normal bone marrow biopsies consist of numerous, thin, bony trabeculae. Various conditions that cause increased bone resorption and formation are associated with irregular and thickened bony trabeculae and include primary and secondary bone disease (renal disease), or primary and metastatic bone tumors. In addition, thickening of bony trabeculae can be caused by non-neoplastic disorders of bone, such as: fracture (and previous biopsy) site repair, infections, circulatory disorders, osteoporosis, osteomalacia, and metabolic disorders (e.g., hyperparathyroidism). The biopsy labeled “Normal” illustrates the thickened paracortical bone that is exaggerated in the tangentially cut specimen. The biopsy labeled “Irregular” is from a patient with chronic renal disease and secondary hyperparathyroidism with increases in bony trabeculae. The biopsies labeled “Thickened” represent three cases of primary myelofibrosis showing osteosclerosis in various stages of increasing severity, most prominent on the right.
Figure 1.40. Renal osteodystrophy. This biopsy shows thickened and thinned, irregular bony trabeculae with increased numbers of osteoclasts and osteoblasts, and evidence of abnormally increased bone turnover. Irregular plates of woven and lamellar bone also signify increased bone turnover and less organized mineralization.
Figure 1.41. Metastatic adenocarcinoma in the bone marrow. Malignant gland-forming cells almost entirely replace the hematopoietic marrow in this case of metastatic prostate adenocarcinoma. Immunostains for prostate-specific antigen (PSA) were positive, supporting prostatic origin of the neoplastic infiltrate. Metastatic carcinoma in bone marrow typically forms cohesive rests of cells distributed in a sinusoidal pattern of involvement that may be associated with necrosis.
Figure 1.42. Bone marrow necrosis. Vessels and sinuses are involved by necrotic tumor metastases in this case of metastatic breast carcinoma. Cells in the necrotic areas are highly eosinophilic and lose morphologic detail. In the lower right panel is seen a blood vessel filled with tumor cells.
Figure 1.43. Clot section from normal bone marrow. Similar to the biopsy, the clot section provides information on cellularity, composition, and to some degree, architectural features of the bone marrow. This clot section shows the typical findings in a middle-aged adult with a marrow:fat ratio of approximately 1:1 that shows mixed trilineage hematopoiesis. Similar to core biopsy sections, the clot preparation can be used to perform immunohistochemical stains to further characterize the cell lineage. Additionally, the clot sections can be used for DNA-based analyses such as PCR and FISH studies, which cannot be performed on the bone marrow biopsy sections due to interfering substances in the fixatives.
Figure 1.44. Estimating cellularity and cellular composition from the clot section. A: A clot section with granulocytic hyperplasia. B: A hypoplastic specimen. As in other standard preparations, the marrow cellularity is estimated based on the ratio of the volumes of
hematopoietic marrow to fat present in the specimen.
Figure 1.45. Touch preparations, although prone to artifacts, are an important element of the bone marrow examination. **A:** Touch prep showing a normal mixture of erythroid and maturing myeloid precursors. **B:** Marrow composed exclusively of a monotonous population of primitive cells in a case of acute myeloid leukemia. Touch preps offer a “quick look” into what the biopsy will show and sometimes may give better cytologic detail than the biopsy specimen, especially if the marrow specimen shows marked fragmentation or aspiration artifact. On occasion, excessive air-drying artifact and cytoplasmic stripping in touch preps can make cells appear more immature than they actually are, and it is important to be aware of this limitation of touch preparations.

Figure 1.46. Immunohistology is routinely performed on bone marrow biopsy (or clot section) specimens to further evaluate the marrow process. Immunohistochemical stains can be used to highlight marrow elements that are not definitively obvious on H&E preparations and to better characterize infiltrative lesions based on protein expression and lineage identity. **A:** In this bone marrow biopsy from a patient with nodal-based lymphoma, a staging bone marrow examination reveals no visualization of marrow involvement. **B:** Furthermore, CD20 immunohistology for B cells shows only rare scattered positive cells, confirming that the marrow was not involved by the B-cell neoplastic process.
New techniques, platforms, and companies spring into view on a regular basis, at a seemingly exponential rate. The challenge for health care providers is to see through biotechnology industry hype and focus on clinical utility. When implementing technology in the laboratory, feasibility and cost are critical considerations. Some techniques are very powerful for research, but, for practical reasons, cannot be adapted for diagnostic use in real-life patients in a clinical setting. Over time, some techniques fall out of use altogether as new techniques are brought on board. More often, a valuable, feasible diagnostic technique will come into use, but for limited purposes and will come to exist alongside older techniques. Truly disruptive, one-size-fits-all, new techniques are rare.

A key, critical consideration affecting the use of various techniques is cost. In the United States, for a long time, laboratory testing has been billed on a fee for service basis, with little incentive for judicious use. However, bundled payment plans currently account for one-third of laboratory testing, and that fraction is growing at a steady pace. As such, a more judicious approach to testing is coming to the fore, with less redundancy and a greater demand for true clinical utility.

This chapter focuses primarily on current modalities recognized by and incorporated into the 2016 WHO classification of tumors of hematopoietic and lymphoid tissues. The mainstay techniques are multiparameter flow cytometry (MFC), and immunohistochemistry (IHC), described in relevant chapters for each type of neoplasm (see Chapters 6–10). Because multiplex IHC (mIHC) use is increasing and especially relevant, it is described in detail in this chapter. In addition, we have included short descriptions of newer techniques not currently in widespread use, but that hold great promise for clinical diagnostics. We have not included techniques that appear to hold little promise for widespread use outside of research in the near future. For example, although next-generation sequencing (NGS) for whole genome analysis has been touted as the technique of the future for several years and has become relatively cheaper, it remains prohibitively expensive and is not reimbursed by most payers. Moreover, technical analysis of the vast data generated by whole genome sequencing renders the technique of great value in the research setting, but of little or no practical use in the diagnostic setting. The typical pipeline for development of new tests has become discovery by broad genome analysis, then development of a directed test by a more feasible, cost-effective method, such as immunophenotyping, fluorescence in situ hybridization (FISH), or polymerase chain reaction (PCR) analysis of a single gene.

It is important to emphasize that there is no single, dominant, one-size-fits-all technology for diagnosis. For myelodysplasia, morphology remains the gold standard. For myeloproliferative neoplasms, a combination of clinical data and PCR is standard. For chronic lymphocytic leukemia (CLL), flow cytometry is the gold standard and typically, no
other testing is required. For myeloma, CD138⁺ magnetic bead, cell-selected FISH and mIHC are most sensitive. For each modality, its use and limitations in the diagnosis of various types of neoplasms is emphasized.

**MULTIPARAMETER FLOW CYTOMETRY**

MFC is the technique of assessing protein expression in individual cells in a liquid medium. This involves incubating cells with fluorescent-labeled antibodies that bind to specific proteins and allow detection of protein expression by a flow cytometer. Current clinical use of flow cytometry in hematology requires live cells. Because the antibodies will stick to dead cells and red blood cells in a nonspecific manner due to electrostatic forces, rather than true antigen binding, careful processing is necessary to yield a clean population of viable white cells. The viable cell population is then divided into multiple tubes and a different set of antibodies is added to each tube. Following incubation, the cells from each tube are drawn into the cytometer and analyzed, one by one, for size, cytoplasmic granularity, and degree of expression of the proteins corresponding to the antibodies that were added to an individual tube.

The data generated by this analysis can then be analyzed in any of a number of ways. Aside from the numeric assessment of lymphoid subsets, performed mainly to monitor acquired immune deficiency syndrome (AIDS) patients, there are no United States FDA-approved uses for flow cytometry. As such, each diagnostic laboratory is free to analyze samples however they see fit. The instrumentation and specimen preparation have become fairly uniform, but interpretation of data varies vastly, from one laboratory to the next. In Europe, the EuroFlow consortium has been successful in standardizing the approach across most west European countries, but EuroFlow has not yet been widely adopted elsewhere. So, unlike some other techniques, for which the methods and interpretation are fairly uniform, it is possible for an experienced user to be very comfortable interpreting flow data from his/her own institution, but be completely lost when trying to interpret data from an institution right across the street.

Nonpractitioners are often under the impression that interpretation of flow cytometry is objective or even binary. It is not. Interpretation is based on “pattern recognition.” The patterns are not evaluated by computer algorithms. They are evaluated by human eyes, just like microscopy. Interobserver variability is inherently great. There is no uniformity in interpretation of what is “positive” versus “negative.” Some centers use 10% as a cutoff, others use 30%, and still others report out without formal cutoff points. Similarly, there is no uniformity in the use of intensity reporting. What one person calls “dim positive,” another will call “negative,” or “partial.” Currently, there are some concerted efforts underway to standardize MFC assessment. In the meantime, it is incumbent on caregivers to attempt to understand MFC methodology in their own institution in order to know how results reported for their patients relate or fail to relate to published data.

The main strength of flow cytometry is the ability to isolate cell populations of interest and then assess expression of multiple proteins within each population. Increasing the number of colors in MFC is of practical use because it decreases the number of tubes, amount of reagents, cost, and technologist work time. However, going beyond the number of colors actually used to triangulate, there is no inherent added diagnostic value in increasing the number of colors. Because gating (selecting the cells of interest) is based on only two colors (e.g., for myeloma, bright coexpression of CD38 and CD138), and standard visualization of
the data is two-dimensional (x and y axes), there is no inherent value to the use of more than four colors (two colors to gate, another two colors to assess other proteins; Fig. 2.1). So, from a real-life, clinical diagnostic perspective, 10 colors are no better than eight, and eight colors are no better than six. The use of more than eight color analysis presents technical difficulties and is rare for clinical purposes.

**The Flow Cytometer**

The flow cytometer is a relatively complex device consisting of four major components (Fig. 2.2). The fluidics system begins with pumps and tubing used to introduce the specimen into the cytometer at a controlled rate. There, the specimen stream is surrounded by a pressurized stream of buffered saline known as sheath fluid, such that the cells assume a roughly single-file position due to the phenomenon of hydrodynamic focusing, which produces laminar flow. The focused stream is then propelled to the flow chamber, where the cells are illuminated by light from lasers. Ultimately, the focused stream is directed to a waste receptacle. The optics system includes: (1) the laser light source used to excite the fluorescence dyes conjugated to the antibodies; (2) the system for conveying the emitted light from the flow chamber to specific detectors; and (3) the specific detectors themselves, which are typically photomultiplier tubes (PMTs) that convert the detected photons to electrical impulses whose magnitude is proportional to the amount of light. In most flow cytometers, the emitted light is conveyed to the PMTs via a combination of dichroic mirrors (which allow light of defined wavelengths to pass, while reflecting light of other wavelengths) and optical filters (which further narrow the wavelengths reaching a PMT, and include short pass, long pass, and bandpass filters).

The electronics system measures the electrical impulses generated by the PMTs and converts these analog measurements to digital information that is gathered and interpreted by the analysis software.

The associated computer system typically consists of a personal computer that directly interfaces with the flow cytometer and controls the functions of the flow cytometer, including the acquisition and analysis of the flow cytometric data. All four of these flow cytometer components have seen major advances in recent years. Fluidics systems are managed electronically. Optical systems have progressed from using single, relatively inefficient lasers and in-air transmission of emitted light from one or two fluorochromes, to utilizing three or more, highly efficient lasers and, in some instruments, fiberoptic systems for transmitting emitted light to the PMTs. Finally, flow cytometry has benefited from the revolution in computer technology, in terms of both hardware and software for data acquisition and analysis. The net result of these technical advances has been steady progression in the number of antigens that can be measured simultaneously by flow cytometry and the quality of the data generated.

**Clinical Indications for Flow Cytometry**

Four major indications have evolved for the use of flow cytometry in the clinical laboratory for hematopoietic neoplasms. First, flow cytometry has been critical to the diagnosis and classification of hematopoietic neoplasms, especially lymphomas and acute leukemias. Second, with improved understanding of the antigen expression profiles associated with prognostic subgroups in certain diseases (e.g., CD28 and CD117 in myeloma), flow cytometry has some, limited use to identify antigens with prognostic significance in hematopoietic neoplasms. Third, with the advent of therapies targeted at specific antigens in hematopoietic neoplasms (e.g., anti-CD20 therapy in B-cell non-Hodgkin lymphomas [B-
NHL), flow cytometry can identify potential therapeutic targets, although assessment of targets is typically performed by IHC because it is typically requested ex post facto, when there are no more viable cells for flow cytometry. Fourth, flow cytometry has been extensively used to monitor response to therapy by looking for minimal residual disease (MRD). MRD assessment has become a standard to guide therapy for potentially curable diseases, like acute leukemias. Although MRD assessment is performed for other diseases, like CLL, the clinical utility is questionable in incurable diseases nearly certain to relapse regardless of MRD. MRD, defined as disease undetectable by microscopic examination, typically represents less than 1% involvement by neoplastic cells. However, if more than 500,000 cells can be evaluated, the detection limit for MRD may be as low as 0.01%.

**Specimen Requirements for Flow Cytometry**

Although flow cytometric evaluation of DNA content can be performed on isolated nuclei from fixed tissue, flow cytometric immunophenotyping requires fresh, unfixed cells to permit evaluation of surface antigens. In the clinical flow cytometry laboratory, most specimens are peripheral blood (PB), bone marrow, or finely minced lymphoid tissue (e.g., from a lymph node biopsy or splenectomy). However, body fluids, including paucicellular cerebrospinal fluid (CSF) specimens, can be evaluated by flow cytometry. Although it is technically possible to analyze disaggregated nonhematopoietic solid tumors such as carcinomas and sarcomas, analysis of solid tissues by flow cytometry is rare, even in the research setting. Regardless of the starting tissue type, it is critical that the cells be maintained in an environment that maximizes viability prior to analysis. For tissues and paucicellular fluids such as CSF, immediate immersion into an ample volume of tissue culture medium, such as RPMI supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin–streptomycin antibiotics, is a common method for enhancing and preserving cell viability and preventing bacterial overgrowth. If the specimen is likely to reach the flow cytometry laboratory in 24 hours or less, the cells may be maintained at room temperature. For tissue samples, if delivery to the flow cytometry laboratory is likely to take more than 24 hours, it is reasonable to place the sample container on wet ice prior to shipment, particularly during the summer months. Although cell suspensions should never be subjected to extremes of temperature (below 0°C or above 37°C), many laboratories analyze specimens up to 24 hours old, transported on dry ice. It is typical for 50% or more of neoplastic cells to die in overnight transit; if the specimen contains a large percentage of tumor cells, it may still be diagnostic, but if the percentage of tumor cells is low, or if they are labile (e.g., myeloma [MM] cells), especially when looking for small numbers of cells (e.g., MRD), false negatives are always a possibility. PB or bone marrow specimens, as well as other body fluids containing a large amount of PB contamination, must be anticoagulated prior to transport to the flow cytometry laboratory. The most common anticoagulants include ethylenediaminetetraacetic acid (EDTA) and heparin, both of which allow adequate flow cytometric evaluation, although EDTA may deplete myeloid cells more rapidly than heparin.

**Flow Cytometric Analysis Specimen Processing**

All clinical specimens must be handled with universal biohazard precautions. Laboratory coats and gloves should be worn by all personnel who directly manipulate specimens. Specimen processing steps that could potentially release hazardous aerosols, such as mincing lymph node or other tissues to create cell suspensions, should be performed under a hood. Sharp objects, such as scalpels for mincing tissues, must be handled with caution and disposed of properly. After specimens have been incubated with antibodies and washed, but
before they are analyzed on the flow cytometer, they should be fixed in 1% paraformaldehyde that both stabilizes antigen–antibody interactions by introducing cross-links and inactivates infectious agents. The addition of bleach to the instrument’s waste container, at a final concentration of 10%, as well as daily purging of the cytometer fluidic system with a bleach solution prior to instrument shutdown further minimize biohazard risks. A zero-tolerance policy should exist for specimen mix-ups. To minimize the chance of such mix-ups, procedures must be instituted such as the use of checklists. Perhaps most useful is advanced bar code tracking and the use of electronically generated barcode labels. Separate processing of specimens minimizes the risk of cross-contamination. Morphologic evaluation of a cytospin made after processing of each specimen is a useful mechanism for ensuring that the correct specimen is being used, in addition to providing valuable information about specimen cellularity and viability.

Red cells and dead cells must be removed from the specimen to allow accurate evaluation. This was traditionally performed by Ficoll-Hypaque density centrifugation, which is still performed in many research laboratories but is rare in the clinical laboratory setting. In this technique, the specimen is mixed with density gradient media such that, after centrifugation, red cells and dead cells lie below a clearly visible intermediate layer of medium and the viable white cells of interest are located above that layer; the viable white cells are then retrieved by a vacuum pipette. However, this technique suffers from two major shortcomings: granulocytes are not retained in the collected specimen following centrifugation and, even within the mononuclear cell component, selective cell loss may occur. Therefore, most clinical laboratories do not manipulate the leukocytes prior to adding antibodies, but simply lyse the red cells at some point during specimen processing. Bulk lysis of the red cells using either a commercially available reagent, or an ammonium chloride solution prepared in house, may be performed prior to the incubation of leukocytes with antibodies. Alternatively, antibodies may be incubated with the leukocytes in the presence of erythrocytes, and the erythrocytes lysed at the end of the preparation step (the so-called “whole blood lysis” technique), just prior to evaluation of the cell suspension in the flow cytometer. Note that erythrocyte lysis techniques, when applied to bone marrow specimens, also destroy the great majority of the nucleated erythroid precursors. Especially in specimens with a large percentage of nucleated erythroid cells, an artifactual, relative increase in the percentage of white cell subsets will result. For this reason, myeloid blast counts for diagnostic purposes are acceptable only by smear morphology (MFC blast counts are not allowed by WHO criteria).

For tissue biopsies and fine-needle aspiration (FNA) biopsies, cell suspensions should be prepared by mechanical dissociation of the tissue in nutrient medium such as RPMI, followed by removal of larger particles by filtration through mesh with 40- to 50-μm pores. Although there are proponents of enzymatic digestion for solid tissue dissociation, especially for the performance of flow cytometry on skin biopsies, we strongly advise against it. In our experience, enzymatic digestion of biopsies yields a tiny percentage of intact, viable cells available for analysis. Consequently, enzymatic digestion typically destroys tissue that might be used for histology, while providing little or no diagnostic MFC data.

Most antigens currently evaluated are cell surface associated, but cytoplasmic and nuclear antigens (e.g., terminal deoxynucleotidyl transferase, or TdT, in lymphoblast nuclei) also can be evaluated by flow cytometry by using permeabilization techniques. A number of commercially available reagents are available for fixation and permeabilization of cells prior to the addition of antibodies to cytoplasmic and nuclear antigens. When both cell surface and cytoplasmic antigens are evaluated in the same assay, the surface staining is performed first. The cells then are fixed and permeabilized, followed by staining for cytoplasmic and nuclear
antigens. It is important to note that the permeabilization process introduces some amount of artifactual, background “shift” in antigen expression. Assessment of antigen expression in permeabilized cells must be performed using the level of expression of known positive and negative cellular populations as controls.

**Instrument Configuration and Quality Control**

Deciding upon the number of antigens to simultaneously evaluate is dependent on the model of flow cytometer used. Single-laser flow cytometers typically are used to evaluate three or four antigens simultaneously, in addition to the two light scatter properties of forward scatter (proportional to cell size) and side scatter (also known as orthogonal or 90-degree light scatter, which is proportional to cytoplasmic complexity and granularity). Two-laser instruments can potentially evaluate six to eight antigens simultaneously, depending on the PMT configuration, whereas three-laser instruments generally are required to evaluate nine or more antigens simultaneously. Although most clinical flow cytometry laboratories use six- or eight-color flow cytometry for leukemia and lymphoma immunophenotyping, 10-color machines are now commercially available for clinical use. The simultaneous assessment of such a large number of antigens minimizes the number of tubes that must be set up, and therefore is of particular benefit for the analysis of paucicellular specimens, while offering the potential for cost savings. Regardless of the type of flow cytometer, quality control (QC) measures must be performed on a daily, weekly, and monthly basis to ensure optimal instrument performance. The required QC procedures are mandated by laboratory-certifying organizations, including Clinical Laboratory Improvement Amendments (CLIA), College of American Pathologists (CAP), and the appropriate arm of the Department of Health in each state. Daily QC measures typically use a stable fluorescence standard, such as brightly fluorescent microbeads, to ensure that the voltages allotted to the individual PMTs are adequate to detect the expected level of fluorescence from these microbeads. PMT voltages are typically set so that the autofluorescence of unstained lymphocytes (i.e., the innate fluorescence of the cell due to endogenous biomolecules such as flavins, in the absence of added antibodies) falls within the first decade of the logarithmic scale. On a less frequent basis, the linearity of detected fluorescence should be confirmed by using a series of microbeads with known fluorescence properties ranging from negative to very bright; in addition, acceptably low levels of cell carryover between specimens must be confirmed.

**Antibodies: Compensation and Panel Design**

Each fluorochrome has well-characterized absorption and emission spectra. Because the emission spectrum of each commonly used fluorochrome extends over a range of wavelengths (Table 2.1), the simultaneous use of multiple antibodies conjugated to different fluorochromes invariably results in some degree of spillover, in which a portion of the fluorescence from a given fluorochrome is detected by a PMT whose range of detected wavelengths is targeted for a different fluorochrome (e.g., a red fluorochrome is detected as red, but also as “orange,” possibly leading to false-positive expression of an orange-labeled antibody). As a result of this spectral overlap, the fluorescence detected by each PMT actually represents the sum of the fluorescence from multiple fluorochromes. Although most of the fluorescence typically comes from the fluorochrome, the PMT was designed to detect significant contributions may occur from other fluorochromes due to spillover. To adjust for the spillover fluorescence from other fluorochromes, a mathematical correction, known as compensation (or color compensation), is applied routinely to all MFC data. Although a detailed discussion of compensation is beyond the scope of this chapter, it is important to
recognize that proper compensation of flow cytometry data is another critical QC function.

Antibodies typically are used in defined combinations, or panels, to answer specific questions about specific cell populations. For example, most laboratories that perform leukemia and lymphoma immunophenotyping have an acute leukemia panel designed to distinguish acute myeloid leukemia from acute lymphoid leukemia and a lymphoma panel to distinguish benign from malignant lymphoid tissue. (Table 2.2 shows commonly used diagnostic panels, Table 2.3 has descriptions of relevant, commonly used antigens, and Fig. 2.3 is a graphic representation of the most useful antigens.) For the most current, regularly updated information about antigens important in the evaluation of hematopoietic cell populations, the best, most user-friendly source can be found at http://www.sciencegateway.org/resources/prow/index.html.

Although a detailed discussion of flow cytometric panel design is beyond the scope of this chapter, suffice it to say that a great deal of thought must go into panel design. At a minimum, antibody panels should measure a sufficient number of antigens to distinguish normal and benign from abnormal and neoplastic cell populations with a high degree of sensitivity and specificity. However, the quality of the flow cytometric data also depends on how antibodies are used in combination. For example, when the detection of a dimly expressed antigen—such as an aberrantly expressed lymphoid antigen on myeloid blasts—is important, conjugation of the relevant antibody to a bright fluorochrome, such as phycoerythrin (PE), can maximize the chance of detecting such aberrant antigen expression. Conversely, it may be unwise to use a bright fluorochrome to detect a strongly expressed antigen, because the bright fluorescence emission will likely create compensation problems due to spillover.

When a specimen is received in the flow cytometry laboratory, it is important that the pathologist or flow cytometry technologist consider the underlying clinical question in deciding which panels to use. If the clinical question is a limited one, such as whether a bone marrow aspirate from a patient with known B-NHL has any evidence of disease, then it may be appropriate to perform a limited study focused primarily on the cell population in question, such as the mature B cells (e.g., tube B1; see Table 2.2). On the other hand, when the clinical question is much broader, for example, when ruling out a hematolymphoid neoplasm in the marrow of a patient with pancytopenia and no known malignancy, then a broader evaluation of the myeloid, lymphoid, and plasmacytic lineages is likely to be appropriate (e.g., tubes B1, T1, PL1, and AML4; see Table 2.2). When clinical concern exists for a lymphoproliferative disorder, then it is prudent to evaluate both the B cells and T cells, whenever possible. However, because B-cell lymphomas are much more common than T-cell lymphomas, if analysis is limited by a paucity of cells in the specimen, it is reasonable to rule out a B-cell malignancy before evaluating the T cells.

Data Acquisition
The actual collection of flow cytometric data, while the stained cells are propelled through the flow cytometer, illuminated by the lasers, and detected by the PMTs, is known as acquisition. In flow cytometry parlance, a particle of sufficient size to be detected is typically a cell, but is referred to as an “event” (e.g., a clump of platelets may register as an event). In general, “cell” and “event” are interchangeable terms. The number of events required for evaluation depends on both the purpose of the flow cytometric evaluation and the nature of the specimen. For example, if one is evaluating a lymph node that is replaced by lymphoma, acquisition of a relatively low number of events per tube of cells and antibodies (e.g., 10,000 events) may allow adequate characterization of the neoplastic population. By contrast, if one
is looking for evidence of very low-level marrow or PB involvement by lymphoma, then a much larger number of cells (e.g., 100,000–500,000) should be evaluated. In most laboratories, if adequate cells are available, then at least 100,000 viable cells are routinely collected per tube. However, at least 500,000 cells will be collected to quantify stem cells in a specimen being evaluated for use in stem cell transplantation. Assuming that the presence of 50 cells would enable confident identification of a population, then the evaluation of 500,000 cells offers the ability to detect a population at a frequency of 1 in 10,000 cells, or 0.01%.

A potential artifact in the evaluation of flow cytometric data is carryover from one tube to the next, in which stained cells from previous specimens accumulate in the tubing of the instrument and are released in relatively small numbers as subsequent acquisition occurs. The hazard in carryover is that certain combinations of bound fluorochromes on the contaminating cells could lead to interpretation as a small abnormal cell population, suggesting the possibility of malignancy despite the fact that no such cell population truly exists in the specimen in question. Most clinical flow cytometers have manufacturer specifications guaranteeing no more than 1% carryover, and these instruments frequently demonstrate much less carryover in practice. However, if one is looking for MRD, then one should make every effort to eliminate carryover altogether. One way to accomplish this is to run plain sheath fluid through the instrument between tubes and to monitor the data generated by such a procedure to ensure that no increase occurs in the number of cellular events beyond the usual background level for the instrument.

Data Analysis

MFC data analysis requires specialized computer programs designed for this purpose, that are able to perform compensation, generate two-dimensional histograms and scatterplots (commonly referred to as “scattergrams,” also known as two-parameter dot plots) of the data that can be adjusted to define abnormal cell populations, and enumerate the various cell populations of interest for reporting purposes (Fig. 2.4).* The process of targeting the analysis to the population of interest is known as gating. In this process, greater and greater numbers of extraneous cellular events are excluded from the analysis in a sequential manner, to maximize the likelihood of identifying an abnormal cell population. The first step is exclusion of doublets (cohesive aggregates of cells and/or platelets). Cohesive cell aggregates can be excluded by displaying time of flight (TOF; the time necessary for the event to pass the detector) and gating out events with a prolonged TOF. Aggregates can also be excluded by displaying forward light scatter (FS or FSC) area by height (Fig. 2.5, left). In the latter analysis, round objects (i.e., single cells) have FS area that is proportionate to FS height. Events falling off the 45-degree FS area/FS height are excluded because they represent doublets or aggregates.

After excluding cellular aggregates, nonviable cells are excluded, which is necessary due to their nonspecific antibody binding. Nonviable cells have porous membranes that leak out cytoplasmic contents and nuclei that are shrunken, so these events are compact with low forward and side scatter. Gating out low FS/low SS cells is a simple means of excluding dead cells from the analysis (see Fig. 2.5, middle). Forward versus side scatter gating also will exclude any residual, un-lysed erythrocytes, due to their small size and lack of granules. An alternative method for removing nonviable cells from the analysis is the addition of a viability dye, such as 7-amino-actinomycin-D (7-AAD), to the cell suspension just prior to data acquisition on the flow cytometer. 7-AAD penetrates the damaged plasma membranes of nonviable cells, but is excluded from viable cells. Therefore, cells demonstrating the characteristic fluorescence of 7-AAD are excluded from further evaluation by gating.
Because 7-AAD emission complicates the process of MFC data compensation necessary for analysis, it is not used in most laboratories.

After cellular aggregates and dead cells have been excluded, the next step is separate gating of leukocyte subpopulations, which is performed by displaying CD45 expression and side scatter area SSC-A (Fig. 2.5B, right; see also Fig. 2.8). As such, a CD45 antibody must be in all tubes. WBC subpopulations typically fall into the lymphocyte gate (bright CD45, low SSC-A), monocytes (brighter CD45, higher SSC-A), neutrophils (intermediate intensity CD45 and high SSC-A), blasts (dim CD45 and low SSC-A), erythroid cells and plasma cells (PCs; negative CD45 and intermediate SSC-A), and hematogones (i.e., pre-B cells; dim CD45 and low SSC-A). After WBC are divided into these constituent groups, lineage-specific antibodies are used to delineate subgroups: CD19 is used to identify B cells, CD3 to identify T cells, CD7+/3−/5− to identify natural killer (NK) cells, bright CD38 to identify PCs, and CD34/CD117 to identify blasts.

The key to identification of a neoplastic population by MFC is finding a discrete abnormal population. This is distinguished from a continuum from normal to abnormal antigen expression (compare Figs. 2.6 and 2.7).

Aberrant antigen expression indicating a neoplastic population typically includes:

- An abnormal decrease in the expected level of expression of an antigen in the cells of interest, including complete loss of expression (e.g., aberrant loss of CD7 on the neoplastic CD4+ T cells of mycosis fungoides (MF) or Sézary syndrome; see Fig. 2.7)
- An abnormal increase in the expected level of expression (e.g., abnormally bright CD10 in B- acute lymphoblastic lymphoma [ALL] cells; Fig. 2.8)
- Abnormally homogeneous expression of antigens that normally show variable expression in a population of interest (e.g., abnormally homogeneous expression of CD34 and CD117 on myeloid blasts in acute myeloid leukemia)
- Aberrant expression of nonlineage antigens (e.g., aberrant expression of the T-cell–associated antigen, CD7, on leukemic myeloid blasts)

There are data supporting the use of asynchronous antigen expression, in which the timing of antigen expression during a maturational process is abnormal (e.g., asynchronous expression of CD13 and CD16 during neutrophil maturation in myelodysplasia), and this type of analysis is used by some practitioners for diagnostic purposes. However, assessment of maturation patterns is a highly nuanced process. Moreover, this type of analysis is not included in WHO diagnostic criteria. Lastly, all published studies show that cases diagnosed as neoplastic by this method are also diagnosed by accepted WHO criteria, making such flow analysis redundant. Lastly, no studies have shown strong data indicating that finding an “abnormal” maturation pattern is predictive of transformation to a clinical neoplasm.

**Data Reporting**

There is a great need to standardize data reporting. Although some attempts have been made, no recommendations have been widely adopted. A critical fact that must always be taken into account is that flow cytometry findings are reported to the clinical team, who typically have little or no practical experience in flow cytometry. As such, the interpretation must be clear and precise, rid of extraneous, possibly misleading information. For example, in a patient with a clinical query of CD8+ large granular lymphocyte leukemia, “r/o LGL,” reporting CD4:CD8 often is misleading, because, for example, an absolute decrease in CD4 cells will cause a ratio skewed toward CD8 cells. In this example, even if there is no expanded
population of CD8 T cells, reporting the ratio is potentially misinterpreted. Also, instead of reporting “viable, singlet events,” the best term is “all cells.” Simply reporting the percentage of cells in the population of interest that is considered positive for each of the antigens evaluated is not adequate and should be avoided. To indicate which populations were analyzed, and for billing purposes, a list of tested antigens must be included. The immunophenotype of each abnormal cell population, including the proportion of the total viable cells represented by each abnormal population should be reported in a free-text format. Descriptions of the fluorescence intensities of diagnostically relevant antigens are useful, especially considering that many reports are used as a basis for detecting residual disease after therapy. The addition of key two-dimensional histograms to the report can provide a snapshot of a neoplastic immunophenotype that can be of great help in assessing residual disease when subsequent specimens are received. The analysis and interpretation should be directed toward the clinical query or prior diagnosis. It should begin with a binary statement of positive or negative, followed by statements citing which cell populations were analyzed. If positive, the percentage of total cells should be cited along with the most salient immunophenotypic properties. What to include and exclude, and useful wording are as follows:

- **Example of negative interpretation in a known lymphoma patient:**
  - The following antibodies were used: CD2, sCD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD21, CD23, CD34, CD38, CD45, CD57, CD103, FMC7, HLA-DR, surface Kappa (sKappa), sLambda (total 20)
  - Abnormal cells were not detected. The B cells are polytypic and show no aberrant antigen expression. A discrete, abnormal T-cell population is not detected. These findings provide no evidence of the patient’s previously diagnosed lymphoma.

- **Example of positive interpretation in a known lymphoma patient:**
  - The following antibodies were used: CD2, sCD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD21, CD23, CD34, CD38, CD45, CD57, CD103, FMC7, HLA-DR, sKappa, sLambda (total 20)
  - Monotypic B cells with bright expression of surface kappa light chains, CD5+, CD23−, bright CD20+ composed 15% of all cells. These findings are consistent with involvement by the patient’s previously diagnosed mantle cell lymphoma (MCL). A discrete, abnormal T-cell population is not detected. These latter findings provide no evidence of a T-cell neoplasm.

**MFC for Nonneoplastic Disorders**

The assessment of lymphoid subsets, used to monitor patients with AIDS, is the only widely used FDA-approved indication for flow cytometry in the United States. Unlike flow cytometry for neoplasia, which is performed somewhat differently in each institution, FDA approval mandates a precise method for lymphocyte subset analysis, including software with pre-drawn gates and unchangeable algorithms. There is no interpretation; simply a reporting of percentages and absolute numbers of B cells, NK cells, CD4+ T cells, CD8+ T cells, and the CD4:CD8 (the latter calculation only including T cells, defined by expressing CD3). Although specimens are sometimes received for the evaluation of other immune abnormalities, any interpretation of the results should be performed with great caution. For example, because the etiologic diagnosis of pulmonary fibrosis can be so difficult to confirm, in many institutions, bronchoalveolar lavage specimens are sent for CD4:CD8 by flow cytometry. There are some data suggesting that CD4:CD8 falls within certain ranges in
pulmonary fibrosis, which correlate with the underlying etiology. However, because there are no normal values for CD4:CD8 in any site other than P, the ratio may be reported, but it cannot be interpreted as high or low. Similarly, there are no normal values for CD4+ or CD8+ T cell subsets in lymph nodes.

MFC for Fetomaternal Hemorrhage
Detection of fetal red cells in maternal circulation is necessary not only for the prevention of Rh D alloimmunization, but also for the diagnosis of clinically important fetomaternal hemorrhage (FMH) leading to fetal and neonatal morbidity and mortality. The rosette test is still the most widely used screening test in the United States. The most common confirmatory test remains the Kleihauer–Betke acid elution assay. Although a minority of laboratories use MFC, because the methodology employed is so different from that used for other MFC testing, technicians skilled in this analysis are rare. Also, the urgent need for results would be prohibitively disruptive to the MFC laboratory workflow. Moreover, because of the necessity of round-the-clock testing for FMH, an MFC laboratory would not serve because most are not open nights or weekends.

MFC for Paroxysmal Nocturnal Hemoglobinuria
MFC is a standard method for diagnosis of paroxysmal nocturnal hemoglobinuria (PNH). In PNH, somatic mutation of the X-linked phosphatidylinositol glycan complementation class A (PIGA) gene causes a partial or absolute inability to make glycoprophosphatidylinositol (GPI)-anchored proteins. Antigens such as CD55, CD58, CD59, CD14, CD16, and CD24 are affected. The channel-forming toxin aerolysin and its precursor, pro-aerolysin, bind selectively and with high affinity to the GPI anchor. An inactive aerolysin variant conjugated with Alexa Fluor 488 (FLAER-A) is now widely used to detect GPI-anchor-deficient cell populations. Current guidelines include a combination of CD235a-FITC and CD59-PE for detection of GPI-deficient RBC, FLAER-A/CD24-PE/CD15-PECy5/CD45-PECy7 for detection of GPI-deficient granulocytes, and FLAER-A/CD14-PE/CD64-PECy5/CD45-PECy7 for GPI-deficient monocytes (Fig. 2.9). High-resolution assays allow detection of GPI-deficient RBC at a level of 10^5 and GPI-deficient WBC at 10^4, which has been noted in patients with aplastic anemia and myelodysplastic syndrome (MDS).

MFC for Myeloid Neoplasms
WHO criteria for the diagnosis of myeloid neoplasms (described in other chapters) include morphology, genetics, and clinical features. Aside from confirming myeloid lineage of the neoplastic cells in acute myeloid leukemia (AML), immunophenotype is not included in WHO criteria for myeloid neoplasms. Still, flow cytometry can be useful in confirming that a myeloid population is neoplastic when diagnostic features are not known or are pending evaluation. Also, some WHO myeloid entities have distinctive immunophenotypic features that can be useful. Lastly, MFC is the basis of MRD detection in AML. What follows is a concise description of flow cytometry for clinically relevant purposes in myeloid neoplasms.

General Approach to Detecting Neoplastic Myeloid Cells by MFC
Unlike some lymphoma diagnoses, which can be made solely on the basis of MFC, for myeloid neoplasms, in general, MFC is used merely to confirm the immunophenotype, not as a primary diagnostic modality per se. When MFC shows a marked increase in blasts (Fig. 2.10), the percentage alone is diagnostic of acute leukemia; the remainder of the
immunophenotype confirms myeloid lineage, e.g., CD117 expression and negative for lymphoid antigens. As with detection of any neoplastic population, the key to identifying neoplastic myeloid cells is recognizing a discrete population of abnormal cells, distinct from the normal expected background populations (see Fig. 2.7 for an application of this concept to detect neoplastic T cells). Aside from subtle abnormalities like abnormally dim or abnormally bright expression of expected antigens, detection of aberrant antigen expression is useful and important. The most useful, distinctive, and common antigens aberrantly expressed on neoplastic myeloid blasts are CD7 and CD56, which can be expressed by the neoplastic blasts in MDS, myeloproliferative neoplasms (MPN), or AML (see Fig. 2.10). CD7, a pan-T–cell antigen, is rarely expressed by nonneoplastic myeloid blasts. Although dim, partial coexpression of CD7 in a small blast population is not sufficient to confirm the neoplastic nature of myeloid blasts, finding a discrete population with uniform aberrant expression can be a useful adjunct in the diagnosis of a myeloid neoplasm. CD56, an NK cell antigen, is aberrantly expressed in some myeloid neoplasms, usually on blasts, but sometimes on neoplastic monocytic cells. Aberrant expression of CD56 is acquired in the malignant transformation of some solid organ cancers and also in myeloma. Again, dim/partial expression is not significant, but uniform expression by a distinct, discrete myeloid population is typically indicative of a neoplasm.

The most important aspect of MFC in a myeloid neoplasm is in cataloging and recording the distinct immunophenotype of the neoplastic cells prior to therapy. Aside from confirming an increase in cells with blast antigen expression (CD34 and/or CD117), an adequate initial analysis, including expression of numerous antigens, can be used as a template for future specimens from the same patient. The basis of MRD detection is in finding the same, discrete, distinctive population, based on the intensity of expression of a set of antigens. The most effective way to accomplish assessment of residual disease is to display data from the original, diagnostic specimen, side by side or overlapping, with data from the post-therapy specimen, using the same tubes and display criteria (Fig. 2.11).

**MFC for Assessment of Myeloid Maturation Patterns and Mature Myeloid Cells, Including Monocytes and Granulocytes**

Although there are publications describing the use of maturation patterns in mature myeloid cells (monocytes, neutrophils) to diagnose myeloid neoplasms, this is not a well accepted practice. There are no published criteria, WHO or otherwise, that include maturation pattern data for the diagnosis of any neoplasm. Diagnostic criteria for myeloid neoplasms in which the neoplastic cells are mature (e.g., chronic myelogenous leukemia [CML], chronic eosinophilic leukemia) involve a combination of clinical and genetic features. The use of flow cytometry in such diseases is limited to the assessment of blasts in the event that a blast crisis has been diagnosed by clinical and morphologic findings.

**MFC for Diagnosis in Myelodysplasia and Myeloproliferative Neoplasms**

Although there are numerous publications over the past decades, describing the use of MFC for assessing myeloid maturation patterns, for various reasons, MFC never became an accepted method for the diagnosis of MDS or MPN. MDS is diagnosed by morphology, clinical features, and cytogenetic analysis. MPN are diagnosed by the same features and also by including specific genetic analyses. Studies have shown that some practitioners may be capable of detecting MDS or MPN by MFC pattern recognition. However, this practice is highly user dependent. The same studies have shown that MFC does not provide an increase
in sensitivity. In other words, a case thought to be MDS by MFC can be detected by standard microscopy. Also, cases with “abnormal maturation patterns” in which diagnostic criteria are not met are not found to have an increased future risk for development of a myeloid neoplasm. Moreover, when there is a need for another technique to diagnose an MPN or MDS, genetic analysis is far more specific and sensitive than MFC. In conclusion, the use of MFC in MPN and MDS is limited to the assessment of MRD, by showing the clear presence of the same, unique neoplastic population as was present at diagnosis.

**MFC in Acute Myelogenous Leukemia**

WHO criteria for the diagnosis of AML include finding at least 20% blasts that are proved to be myeloid by a combination of special stains (myeloperoxidase [MPO] and non-specific esterase [NSE]) and immunophenotypic analysis. The most useful antigens in defining the myeloid lineage are expression of CD13 and/or CD33. Other useful myeloid-associated antigens include CD11b and CD15. Also important is the general lack of lymphoid antigen expression (although some lymphoid antigens may be aberrantly expressed by neoplastic blasts). By WHO criteria, a cell is a blast as determined purely by morphology. However, in most cases, blast morphology coexists with the expression of CD34 and/or CD117. Although CD117 is expressed by pronormoblast-stage erythroid precursors and by mast cells (MCs), CD34 expression in hematopoietic cells is limited to blasts. MFC is used to confirm the lineage in an acute leukemia but is not, according to WHO criteria, used to quantify blasts. The reason for this is that, for technical reasons, the blast quantity may be over or underestimated by MFC. For example, in some cases, the marrow aspirate submitted for MFC is diluted by PB (hemodilution), causing an artfactually low number to be quantitated. Hemodilution, a marrow specimen composed mainly of PB, can be a problem in marrow evaluations, but is most commonly problematic in CSF specimens where CSF often is diluted by blood because of a traumatic spinal tap procedure (Fig. 2.12).

**MFC in Specific AML Subtypes**

Although AML subtypes must be confirmed by clinical features and cytogenetics, as MFC data typically become available within only a few hours or a day, whereas cytogenetics typically takes much longer, it is sometimes useful to provide a preliminary subtype assessment by MFC (Table 2.4).

**AML with t(8;21)(q22;q22)**

In addition to a markedly expanded myeloid blast population identified by CD45 versus SSC-A gating, this AML typically shows a relatively prominent population of maturing granulocytes, reflecting the underlying maturation characteristic of this entity. CD34 typically is expressed brightly, frequently in combination with aberrant CD15 that is normally a more mature myeloid antigen, not expressed on blasts. There is characteristic aberrant expression of the B–cell-associated antigen CD19, and CD56 and TdT also may be aberrantly expressed (see Table 2.4).

**AML with t(15;17)(q22;q12)**

This entity, also known as acute promyelocytic leukemia (APL), is a leukemic proliferation of promyelocytes. Because HLA-DR is normally expressed by myeloblasts but lost upon maturation to the promyelocyte stage, it follows that APL cells typically do not express HLA-DR (see Table 2.4). Similarly, as CD34 is expressed by normal myeloblasts but lost upon maturation to promyelocytes, it follows that CD34 is typically negative in APL. However,
because the morphology alone is typically diagnostic, especially in combination with extremely high expression of MPO, MFC is neither important nor particularly helpful in the diagnosis of most cases of APL. Moreover, WHO criteria require FISH or cytogenetic documentation of the t(15;17) or a variant translocation to confirm the diagnosis. The take-home message is that HLA-DR negativity is neither unique to APL nor particularly helpful for diagnosis in real-life practice. If APL is a diagnostic consideration, the clinical team should be alerted immediately and cytogenetic analysis expedites.

**MFC in Acute Leukemias of Ambiguous Lineage, Including Mixed Phenotype Acute Leukemia, Acute Bilineal Leukemias, and Undifferentiated Acute Leukemia**

Mixed phenotype acute leukemia is characterized by blasts that coexpress myeloid and T or B lineage-specific antigens or concurrent B and T lineage antigens. Acute biphenotypic leukemia is rare, and coexpression of markers for all three lineages is extremely rare. The WHO contrasts this entity with acute bilineal leukemias, because the latter include two or more discrete blast populations, each of which demonstrates the features of a single lineage (Table 2.5; for an example of acute bilineal leukemia, see Fig. 2.13). In such cases, IHC often is a helpful adjunct in the immunoarchitectural demonstration of separate blast populations in separate spatial areas (Figs. 2.14 and 2.15; see Table 2.5). In cases in which surface antigen evaluation is ambiguous for lineage, cytoplasmic antigen evaluation frequently is helpful. In this context, specific B-lymphoid–associated markers include cytoplasmic CD79a, cytoplasmic immunoglobulin M (IgM), and cytoplasmic CD22. The most specific T-lymphoid–associated antigens include membranous or cytoplasmic CD3 or the T-cell receptor (TCR), whereas cytoplasmic myeloperoxidase is considered the most specific myeloid-associated antigen. Because no well-defined immunophenotype exists in mixed phenotype acute leukemia, these cases must be evaluated on a case-by-case basis. Finally, both mixed phenotype acute leukemia and acute bilineal leukemia should be distinguished from undifferentiated acute leukemia. According to the WHO classification, the latter is characterized by an absence of lineage-specific antigens. Non–lineage-specific antigens expressed in undifferentiated leukemias include HLA-DR, CD34, and CD38, with a subset of cases expressing TdT and CD7. Note that CD7 is not considered to be T lymphoid specific if only expressed at low levels, in the absence of other T-cell–associated antigens.

**MFC for Precursor Lymphoid Neoplasms**

Although the official WHO terminology differs, in common parlance, these cancers are still referred to as ALL, B-ALL being implied if T-ALL is not specified. These neoplasms are defined by lymphoblast morphology and confirmed by immunophenotype.

B-lymphoblastic leukemia/lymphoma (B-ALL) has characteristic low expression of CD45 and low SSC (Fig. 2.16; see Fig. 2.8). They typically express CD10 and CD19 along with other B cell antigens (e.g., cytoplasmic CD79a and CD22). They also usually express the blast antigen, CD34, but usually lack expression of CD117. As the expression of surface immunoglobulin denotes mature B cells, B-ALL sometimes shows expression of cytoplasmic IgM, but is generally negative for surface immunoglobulin expression (see Fig. 2.8). Expression of CD20 is variable, usually low or negative. Dim coexpression of the myeloid-associated antigens CD13 and/or CD33 is common. The most helpful antigen in the diagnosis of B-ALL is TdT, which is strongly expressed in most cases, while negative in mature B cells, both reactive and neoplastic. In AML, TdT is typically negative, but some cases show
weak/partial expression of TdT

Subtypes of B-ALL are defined by the presence of cytogenetic abnormalities, not by immunophenotype.

T-lymphoblastic leukemia/lymphoma (T-ALL) also has characteristically low expression of CD45 and low SSC, like other acute leukemias. They typically express various combinations of CD2, CD4, CD5, CD7, and CD8, but are usually negative for surface CD3. Cytoplasmic CD3 is usually expressed. Unlike nonneoplastic T cells, T-ALL cells often express CD10. They also usually express the blast antigen, CD34, but usually lack expression of CD117 as well as myeloid-associated antigens, like CD13 and CD33 (Fig. 2.17). As with B-ALL, TdT is perhaps the most useful antigen in the diagnosis of T-ALL and is strongly expressed in most cases. T-ALL is relatively rare. There are no subtypes.

MFC for B-Cell Non-Hodgkin Lymphoma

Of all hematopoietic neoplasms, MFC is most straightforward for B-NHL. Whereas a reactive population of mature B cells is composed of some that express kappa surface light chains (sIgκ+) and other cells that express lambda (sIgλ+), in a ratio of approximately two or three sIgκ+ cells to each sIgλ+ cell (Fig. 2.18), a monoclonal, neoplastic population is composed of all one or the other type of surface light chain. Whereas a population of some sIgκ+ and some sIgλ+ reactive B cells is called polytypic, a neoplastic population with only sIgκ+ or sIgλ+ is called “monotypic.” Although the term monotypic often is used interchangeably with “monoclonal,” technically, clonality can only be assessed at the genotypic level. As with MFC for other types of cancer, detecting a discrete population of abnormal cells is the diagnostic key. Focusing on κ:λ ratios can be misleading. For example, an sIgκ+ lymphoma may exist in a specimen with a relatively large number of nonneoplastic B cells; because the background B cells have a normal κ:λ, the overall ratio may be skewed only slightly or not at all, depending on the size of the neoplastic population. However, in that same case, the typically discrete population of abnormal B cells may only become visible in scattergrams by the use of multiple antigen display strategies (Fig. 2.19). Also, some B-NHL do not express sIg at all; the finding of a discrete population of sIg(−) B cells in a lymph node usually indicates lymphoma and is most commonly seen in follicular lymphoma (FL) (Fig. 2.20). Also, CLL cells typically express sIg very dimly and are sometimes completely negative. In such cases, permeabilizing the cells and assessing cytoplasmic immunoglobulin light chain expression often shows clear monotypic expression.

Once a B-NHL is diagnosed on the basis of finding a discrete, abnormal, monotypic B-cell population, algorithms can be used for WHO subtyping. However, precise subtyping requires correlation with morphology. In most cases, a cytopsin preparation made from the flow specimen is adequate. In some, FNA smear morphology is required. In others, a needle or an open biopsy is required.

If the monotypic population is composed of intermediate or large cells with disbursed chromatins, it is a high-grade B-NHL. If it is composed of small cells with clumped chromatins, it is low grade.

- Most common high-grade B-NHLs:
  - Diffuse large B-cell lymphoma (DLBCL)
  - Burkitt lymphoma (BL)
- Most common low-grade B-NHLs:
  - CLL
  - Follicular lymphoma (FL)
Mantle cell lymphoma (MCL)
Marginal zone lymphoma (MZL)

Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma

The algorithm for low-grade B-NHL begins with assessment of CD5 coexpression (see Fig. 2.20B). CD5 is positive in CLL and MCL. Whereas CLL has dim CD20 and sIg, and MCL has relatively brighter CD20 and sIg, they can usually be distinguished by the expression of CD23, which is positive in CLL and negative in MCL. However, because the prognosis and therapy are vastly different for CLL and MCL, it is critical to assess the expression of cyclin D1, a nuclear antigen at the time of initial diagnosis. Cyclin D1 (formerly called bcl-1) is expressed by MCL but not CLL. Cyclin D1 expression is consequent to t(11;14), which can be detected by FISH; however, a definitive diagnosis can be established by demonstrating the standard immunophenotype (FISH is not required unless the immunophenotype is unclear). For technical reasons, principally the relatively low intensity of expression, cyclin D1 cannot be reliably detected by MFC. Cyclin D1 IHC can be performed on a cell block or tissue biopsy. Although there is a single publication describing cases of “cyclin D1–negative” MCL, the authors define it by an extensive gene panel showing similarity to MCL except for cyclin D1. Surrogate markers for the gene panel have been promulgated for the purpose of diagnosing cyclin D1–negative MCL, such as SOX11. However, published data for SOX11 expression are conflicting and published studies do not describe expression, for example, in extranodal MZL. Because MZL can rarely express CD5, this is an important gap in the published data. In summary, the existence of cyclin D1–negative MCL is neither firmly established nor well accepted.

Follicular Lymphoma and Marginal Zone Lymphoma

Once a low-grade B-NHL has been established by morphology and MFC showing monotypic sIg, if it is CD5−, the next fork in the algorithm is CD10 (Figs. 2.21 and 2.22). CD10 is positive in two-thirds of cases of FL but never in MZL. Although BCL6 is always expressed by FL and never by MZL, it is a nuclear antigen and, like cyclin D1, cannot be assessed by MFC for technical reasons, chiefly the low level of expression. Although a low-grade, CD5−, CD10+ lymphoma is usually FL, a CD5−, CD10(−) lymphoma might be FL or MZL. If tissue or a cell block is available, morphologic assessment and BCL6 immunostaining can be performed to distinguish between them. If not, the diagnosis would be descriptive, “B-NHL, CD5−, CD10− with a differential including FL and MZL.” In most patients with these findings, a conclusive subtype may be inferred based on clinical findings. It should be noted that there are rare cases of CD5+ MZL. Also, in some cases, expression of some antigens is equivocal. Also, there are rare cases of CD10+ hairy cell leukemia (HCL), which can be mistaken for FL.

Lymphoplasmacytic Lymphoma

For practical, diagnostic purposes, lymphoplasmacytic lymphoma (LPL) can be thought of as MZL limited to bone marrow: CD5−, CD10− (see Fig. 2.22). However, whereas MZL has plasmacytoid differentiation in only 30% of cases, LPL has it in 100%. LPL is usually associated with a monoclonal immunoglobulin serum spike of the IgM type. Waldenström macroglobulinemia (WM) is defined as the presence of a B-NHL and an IgM serum spike. Another helpful feature in the diagnosis of LPL is noting the presence of numerous MCs, which are accessory cells, secreting cytokines necessary for LPL growth. MCs can be found
by routine morphology on aspirate smears, or immunostaining for MC tryptase. Also, the
diagnosis of LPL/WM can be confirmed by the presence of an MYD88 gene mutation
(described below).

_Hairy Cell Leukemia_

Like MZL, HCL shows monotypic sIg and is CD5−, CD10− (see Fig. 2.22). However, a more
extensive panel shows expression of CD103, which is not seen in other B-NHL, and CD25,
which is rarely seen in other NHL (Fig. 2.23). Although expression of CD11c is fairly
nonspecific, in HCL it is extremely bright; this intensity is not seen in other lymphomas. Like
MCL, HCL cells usually show cyclin D1 expression by IHC, but it does not present a
diagnostic quandary because the HCL immunophenotype is otherwise so distinctive and
different from MCL (e.g., HCL is CD5−). Although molecular confirmation is not required
for HCL, most cases harbor a specific BRAF driver mutation, detectable by genetic
sequencing (described below).

_Diffuse Large B-Cell Lymphoma and Burkitt Lymphoma_

Once a high-grade lymphoma has been diagnosed by the combination of morphology and
MFC showing surface light chain restriction, a more complex assessment is required for
subtyping. The cornerstone is histology (reviewed elsewhere in this text). If the specimen is
limited to MFC and cytology (FNA or cyospins), it must be diagnosed descriptively, “mature
B-NHL with a differential including DLBCL and BL.” Although a discussion of therapy is
beyond the scope of this chapter, brief consideration is helpful and important when rendering
a diagnosis, as a reminder of what is at stake. B-NHL can generally be divided into low grade
and high grade (Fig. 2.24). For low-grade lymphomas, the clinical approach is “watch and
wait”, expectant management (exceptions include local therapy for extranodal MZL and
MCL may require induction chemotherapy and bone marrow transplant for MCL). By
contrast, high-grade lymphomas are treated with induction regimens.

_T-Cell Neoplasm Diagnosis by MFC_

Whereas the algorithmic approach to B-NHL diagnosis by MFC is fairly straightforward, T-
NHL diagnosis by MFC often is difficult or impossible. Also, the misdiagnosis of T-NHL by
flow cytometry is a well-known pitfall. Whereas the process of B-cell ontogenic maturation is
limited to peripheral lymphoid organs, T-cell populations are highly plastic at all sites.
Consequently, MFC results for analysis of reactive B-cell populations are predictable, but
analysis of T-cell populations is highly variable. Also, temporary “abnormal” T-cell
populations commonly arise during immune reactions and can be seen in numerous clinical
scenarios in patients with different kinds of illnesses. Consequently, whereas diagnostic
criteria for B-NHL do not require persistence of the abnormal population (i.e., finding a B-
NHL at a single point in time is diagnostic), for some T-NHLs, WHO criteria require
demonstrating persistence of the abnormal T-cell population for at least 6 months. Whereas
MFC can be diagnostic in B-NHL as a standalone test, MFC for T-NHL should be thought of
as an adjunct, not the primary means of establishing a diagnosis.

The cornerstone of T-NHL diagnosis by MFC is demonstration of a discrete population
with loss of pan-T–cell antigen expression. All peripheral (i.e., post-thymic) T cells express
CD2, CD3, CD5, and CD7. MFC for T-NHL begins with assessment of T cells, as defined by
expression of CD3, and looking for coexpression of CD2, CD5, and CD7 (Fig. 2.25; see Fig.
2.6). In addition, T cells should express either CD4 or CD8 (neither double-negative nor
double-positive). Consequent to its use in monitoring AIDS patients, most practitioners are familiar with the normal CD4:CD8, being in the range of 1.1 to 2.3 in most centers (the CD4:CD8 must be limited to T cells, CD3+, because NK cells can express CD8 and artifactually skew the ratio). It must be emphasized that this ratio only applies to PB T cells. Normal ranges are not available for any other site. Anecdotally, in a nonneoplastic marrow, the CD4:CD8 often is weighted toward CD8+ T cells and in reactive lymph nodes, it often is heavily weighted toward CD4+ T cells. “Normal” CD4:CD8 only applies to PB and must not be applied to a specimen from any other site. If, upon examination of the initial antigen panel, a T-NHL is suspected, a second panel of antigens should be performed for confirmation and subtyping (Fig. 2.25B).

Even in the PB, CD4:CD8 is not useful in the diagnosis of T-NHL. For example, in a blood sample received with the clinical query “r/o T-LGL” (a CD8+ neoplasm), reporting a CD4:CD8 can be misleading; it may be low, not because of an increase in CD8+ cells, but because of a decrease in CD4+ cells. Unlike B-NHL, in which finding even a small monotypic population is typically diagnostic of lymphoma, when assessing blood for T-NHL, absolute numbers must be reported (Table 2.6). Because any clinically relevant neoplasm is an abnormal growth, diagnosis of a neoplasm must be based upon demonstration of an expanded population. Because minor “abnormal” T-cell subsets are common, especially in sick patients, diagnosis of a T neoplasm should not be made in a blood specimen unless there is a clear, numeric expansion of a T-cell population.

For most WHO classification of T-NHLs, MFC is not a reliable mainstay for diagnosis. The remainder of this section will focus on entities for which MFC is most often used and/or most useful. It should be emphasized that the gold standard and most sensitive means for diagnosing recurrent or residual T-NHL is PCR. In particular, when TCR PCR analysis shows a clonal population in the original, diagnostic specimen, PCR is run on subsequent specimens, comparing DNA from the original sample with DNA from the new sample, a technique that can confirm the presence or absence of the neoplastic clone.

**T-Cell Large Granular Lymphocyte Leukemia**

Because T-cell large granular lymphocyte leukemia (T-LGL) is commonly associated with neutropenia, submission of a PB specimen for MFC in patients with clinically significant neutropenia is a standard part of the workup. As detailed above, the focus is on the demonstration of an abnormal T-cell population of an expanded magnitude (Fig. 2.26; see Table 2.6). WHO criteria for T-LGL diagnosis require demonstration of an expanded, abnormal T-cell LGL population in blood that persists for at least 6 months, typically between 2,000 and 20,000 cells/mm³. A method must be employed to quantify the neoplastic cells (see Fig. 2.26). As with most T-cell neoplasms, the most common MFC abnormality is loss of CD7 expression. Reactive CD7− T cells exist in most specimens. Typically, displaying CD3 versus CD7 in nonneoplastic T cells shows a teardrop-shaped scattergram, in which the CD3+ T cells include a majority population with strong coexpression, trailing down to a minority subpopulation with low/negative CD7 (see Figs. 2.6 and 2.25A). This is distinctly different from finding a normal population and a discrete, separate population with uniform low/negative CD7 (see Fig. 2.7). This same concept applies to the other pan-T–cell antigens, CD2 and CD5. The typical immunophenotype of T-LGL: CD2+, CD3+, CD4−, CD5+/−, CD7+/−, CD8+, CD16+, CD56−, CD57+. However, variations exist, including rare CD4+ cases.
**Mycosis Fungoides and Sézary Syndrome**

MF is the most common cutaneous T-cell lymphoma and is diagnosed by a skin biopsy. Sézary syndrome is the advanced, generalized form of MF, with systemic involvement. MFC is a mainstay of diagnosis for SS. Although leukemic cells can be found in the PB in many patients with MF, the presence of a small population is not of prognostic significance. The development of Sézary syndrome indicates transformation from an indolent disease (MF) to an aggressive disease with poor survival. Also, some patients present with Sézary syndrome, never having had a clinical diagnosis of MF. WHO criteria for the diagnosis of Sézary syndrome require erythroderma, generalized lymphadenopathy, and the presence of clonal T cells in skin, lymph nodes, and blood. In addition, WHO criteria require at least one of the following, as established by MFC of a blood sample: a neoplastic cell count of at least 1,000/mm$^3$ (see method for quantitation; see Fig. 2.26), an expanded CD4$^+$ T-cell population resulting in a CD4:CD8 ratio below 10, and/or loss of at least one pan-T–cell antigen.

**Adult T-Cell Leukemia/Lymphoma**

Although indolent forms have been described, adult T-cell leukemia/lymphoma (ATLL) is typically an aggressive, widely disseminated neoplasm in an acutely ill patient. Although it involves skin and marrow, because it is typically leukemic, a blood sample usually is submitted for diagnosis. The leukemic ATLL cells have distinctive, cerebriform, nuclei with highly convoluted contours, in most cases readily identifiable with routine microscopy. The immunophenotype, like most T-NHL, is typically CD2$^+$, CD3$^+$, CD5$^+$, but with loss of CD7. Like MF and Sézary syndrome, the neoplastic cells are usually CD4$^+$. Unusually strong/uniform expression of CD25 is seen in most cases. Although MFC can be diagnostic of a T-cell neoplasm in such patients, because the immunophenotype is not specific (e.g., aside from the intensity of CD25, it is nearly identical to MF and Sézary syndrome), PCR studies are mandatory. ATLL is caused by the HTLV1 virus, which is found in the neoplastic cells. PCR for HTLV1 is confirmatory. If PCR is negative, the diagnosis would be peripheral T cell lymphoma, not otherwise specified (PTCL, NOS), rather than ATLL. Serologic studies can reveal exposure to HTLV1, but are not confirmatory, because exposure is fairly common and the latency period typically lasts for decades. Also, as with all T-cell neoplasms, TCR gene PCR is a mainstay of diagnosis. Even if there is a firm diagnosis by other techniques, TCR analysis can establish that a clone was detected by PCR and the DNA extracted from the original specimen can then be used for comparative analysis on DNA from subsequent specimens, when looking for recurrent or residual disease.

**NK Cell Neoplasm Diagnosis by MFC**

NK cell neoplasms are extremely rare. NK cells are defined by the expression of CD2 and CD7, without CD3 or CD5 (Fig. 2.27). They show various combinations of CD8, CD16, CD56, and CD57. The diagnosis of the WHO entity, chronic lymphoproliferative disorders of NK cells (CLPD-NK), requires an expanded NK cell population (usually >2,000 cells/mm$^3$) that persists longer than 6 months (in PB, the NK cell normal range is 94 to 484 mm$^3$ [see Table 2.6; see Fig. 2.26 for absolute number calculation method]). Because most patients are asymptomatic, a definitive diagnosis is not usually required. However, because NK cell populations express variable KIR-family receptor proteins, it is possible to define a neoplastic population by MFC with KIR subtype analysis. Because of the rarity and lack of clinical importance of CLPD-NK, most laboratories do not perform KIR analysis. In stark contrast, the WHO entity, aggressive NK cell leukemia, typically presents with fulminant disease in a

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clinically ill patient. The diagnosis of lymphoma is typically easy to make on the basis of a large population of circulating and/or infiltrative, markedly atypical lymphoid cells. The immunophenotype is like that of reactive NK cells (CD2+, surface CD3-negative) except that they show strong/uniform expression of CD56 (an antigen typically weak/variable in NK cells). Also, aggressive NK cell leukemia is Epstein–Barr virus (EBV)-related, so in situ hybridization or immunostaining for EBV is typically confirmatory.

**Plasma Cell Neoplasm Diagnosis by MFC**

Whereas leukemias are readily diagnosable by MFC, this technique is problematic in myeloma. Leukemic cells are typically plentiful in blood and/or marrow and are readily identified by MFC. Leukemic cells typically survive well outside of the marrow and so they remain viable upon processing for MFC. By contrast, multiple myeloma (MM) cells typically cause stromal fibrosis, preventing their aspiration\(^14,15\) (Fig. 2.28). Consequently, there are, on average, 20% fewer MM cells in aspirate smears than in the corresponding marrow core biopsy. A trephine core biopsy is the gold standard specimen type for myeloma diagnosis. Also, unlike leukemia, MM cells die quickly after removal from the marrow stroma (Fig. 2.28). Also, studies have shown that the immunophenotype in the tiny population of MM cells detectable by MFC usually differs significantly from the majority, \textit{in vivo} population making the assessment of prognostic or predictive markers problematic, if performed by MFC. For example, MM cells typically show strong/uniform coexpression of CD56 and CD138, clearly demonstrable by core biopsy IHC; however, the MM cells detected by MFC often are “negative” in aspirated cells from the same patient (Fig. 2.29). Lastly, all gating strategies for MM depend on CD38 expression, which is problematic because CD38 is downregulated in patients treated with anti-CD38 drugs, which are becoming standard therapy for myeloma. All current, published MM gating strategies rely on CD38; alternatives are not known. For these reasons, bone marrow core biopsy immunostaining is the best and most accurate technique for analysis of myeloma (see below).

In MFC, PCs are typically gated by bright expression of CD38 and coexpression of CD138 or dim CD45 (see Figs. 2.1 and 2.29). Many cell types express CD38, but usually not as brightly as PCs. CD138 is specific to PCs but is technically problematic because expression can be affected by antibody clone, processing solutions, and time.\(^16\) CD138 expression decreases rapidly in PCs \textit{ex vivo} during processing for MFC.\(^17\) Also, CD138 shedding by MM cells is induced by chemotherapy, which would decrease detection of MM cells by MFC in the post-therapy setting.\(^18\) Normal PCs express dim CD19 and dim CD45 but not CD56, whereas MM cells are typically negative for CD19 and CD45 but positive for CD56. However, there are numerous, different neoplastic populations seen in the EuroFlow panel, as well as more than 50 populations of normal PCs.\(^2,19\) Also, there is great variability in antigen expression from case to case. CD45, for example, is usually negative in MM, but may be expressed at any level from case to case.\(^16\) Moreover, the immunophenotype of any cancer often changes after therapy or at relapse. Also, after intensive therapy and especially after marrow transplantation, reconstitution of the immune system gives rise to transient “abnormal” PC populations, which can easily be misinterpreted as residual disease.\(^20\) For these reasons, although MFC is the gold standard for MRD assessment in acute leukemias, it is problematic for MRD in MM.

**Figure 2.1** is composed of typical MFC data, scattergrams from a relatively straightforward MM sample. By selective gating (data not shown), dots representing the MM cells have been colored red, the background normal PC dots have been enlarged and colored blue, and background B cells are pink. MM cells represent 0.5% of the marrow, which is
relatively high for MM samples (see Fig. 2.30). The mainstay of MRD detection relies on aberrancy, such as finding CD56, CD117, or CD200 on PCs. On the CD38/CD56 and CD117/CD200 graphs, the PCs fall on the 45-degree angle between the x and y axes (see Fig. 2.1). Because “junk” and dead cells typically fall on that line, in general, populations of cells in that location would be assumed to be “background,” not representing true expression. For analysis of leukemias, this degree of scant evidence would generally not be sufficient for diagnosis, but in many centers, MM analysis gets a special dispensation because interpretation is so difficult. In this example, the most compelling scattergram (bottom right) shows monotypic kappa, but because cytoplasmic immunoglobulin analysis is so unreliable for PCs, some leaders in MFC are recommending the discontinuation of κ/λ analysis.19

MFC for Hodgkin Lymphoma
Because the Reed–Sternberg (RS) cells are so few, very large, and because T cells bind strongly to them (creating doublets and clusters that are gated out), MFC is not useful for the diagnosis of HL. Moreover, because HL is readily identifiable by IHC and morphology, even if RS could be detected by MFC, the effort would be redundant and wasteful.

MFC for Minimal Residual Disease
MRD is defined as populations of cancer cells below the limit of detection by routine microscopy (described above for each specific use). By evaluating a sufficiently large number of cells from a specimen (e.g., 500,000–1,000,000 cellular events), it is possible to detect MRD in PB or bone marrow at a level of 0.01% or less. The efficacy of MRD assessment has been shown for potentially curable diseases, like AML and ALL. However, for obvious reasons, looking for MRD in indolent, incurable diseases, like MM and CLL, is questionable. Unlike curable diseases, studies have shown that indolent/incurable diseases relapse, despite being “MRD-negative.” Regardless of the type of hematopoietic cancer, the process of MRD analysis is the same. The principle is to use MFC to identify the cancer cells and catalog their antigen expression at the time of initial diagnosis, then use that information to look for tiny numbers of the same cells after therapy (see Fig. 2.11).

An important concept that should be emphasized is that, consequent to the action of therapeutic drugs, often, the immunophenotype of the cancer cells changes after therapy. In some cases, it changes little or not at all. In others, there are subtle changes, like a decrease or increase in the level of expression of an antigen. In some cases, the changes are relatively dramatic, prompting reversion to genetic analysis, to prove the relationship to the prior cancer.

The easiest and surest way to assess MRD is, when receiving a follow-up specimen, to run an analysis that is identical to that performed upon initial diagnosis. Then, rather than referring to a written account of the original immunophenotype, to open scattergrams from the most recent prior positive sample alongside scattergrams from the current sample, then examining back and forth—the prior positive and the current data—looking for persistence of a definitive, abnormal population (see Fig. 2.11).

MFC to Detect Circulating Tumor Cells from Solid Organ Cancers
Because this technique has been so prominent in cancer news for the past several years, it bears mention here. Studies have shown that detecting circulating solid organ cancer cells (e.g., prostate carcinoma) indicates a worse prognosis. The methods described are proprietary, requiring specific, nonstandard processing machinery, reagents, and cytometers; as such, the published methods for circulating tumor cell detection cannot be performed in a
standard laboratory. In brief, MFC for circulating tumor cell detection entails a precytometry, mechanized preparation to isolate tumor cells, followed by labeling, followed by a unique form of acquisition, in which “positive events” are photographed at high speed, inside the cytometer. After the collection, the photographed events are visually examined by a technologist, who decides whether the “positive event” is actually a tumor cell. This method is highly specialized and highly labor intensive. Moreover, its use seems to be waning and there are questions about the efficacy of the information.

**MFC for Ploidy and Cell Cycle Analysis**

Although MFC is an excellent method for assessment of ploidy and cell cycle analysis, the use of such data for clinical purposes has waned greatly and is currently rare. These assays are commonly used, but only in the research setting. Consequently, a detailed description here is not warranted. In brief, the cells are permeabilized, after which a fluorescent-labeled stain is added that binds stoichiometrically to DNA. The intensity of fluorescence is then displayed for calculation of ploidy and percentages of cells in each stage of the cell cycle (Fig. 2.31).

**Laser Scanning Cytometry**

Laser scanning cytometry is similar to MFC (assessing cellular parameters such as antigen expression according to fluorescent-labeled antibody binding), but it is performed by pipetting tiny aliquots of cells in suspension, into small wells in a plastic plate. The advantages are cost savings due to the use of far less reagents and labor savings due to not having to maintain the flow cytometry apparatus. The downside is that, because the aliquots in the wells are so small, the number of cells analyzed is far less. Still, for most specimens, laser scanning cytometry works well for diagnostic purposes. Compared to MFC, laser scanning cytometry is in use in only a tiny fraction of institutions. The reasons for such limited use are not known, but may be due to resistance to change platforms and/or to a lack of effective marketing from laser scanning cytometry manufacturers. In summary, laser scanning cytometry has been commercially available for a long time, and the fact that it has not caught on at a large scale suggests that the advantages are not compelling enough for most laboratories to make the switch from MFC.

**Conclusions for MFC**

Flow cytometry is a mainstay of diagnosis for acute leukemia and for most NHLs. MFC is not useful for the diagnosis of HL. The diagnosis of MDS or MPN by MFC is not supported by WHO criteria. The value of MFC in MDS and MPN is limited to cataloging the neoplastic blast immunophenotype upon diagnosis, to use as a template, should there be a need to assess MRD in the future. Although some practitioners are adept at analysis of maturation patterns for diagnostic purposes and have supported its use since the late 1990s, this technique has never found widespread acceptance for diagnostic purposes or been included in any well accepted scheme for diagnostic criteria.

**FINE-NEEDLE ASPIRATION AND CYTOLOGY**

FNA has become a mainstay in the initial workup, primarily in well patients who present with palpable lesions, primarily lymphadenopathy, usually combining morphology with MFC. Cytology also is commonly utilized to assess CSF and difficult to biopsy internal organs
(e.g., intraabdominal and retroperitoneal). The nuances of morphologic cytology are beyond the scope of this text. In brief, if an FNA shows a polymorphous cell population with little cytologic atypia, in an otherwise well patient, with low clinical suspicion of lymphoma, no further workup is required (Fig. 2.32). By contrast, if the patient is ill, or carries a greater clinical suspicion (e.g., presents with widespread lymphadenopathy), or if the FNA shows a particularly monotonous or atypical population, it should be triaged for a lymphoma workup. This involves utilizing an unstained smear for IHC or other studies, or doing another FNA needle pass, to collect more cells and a core needle biopsy. The additional cells are submitted for MFC and worked up as described above. If a high-grade B-cell lymphoma is suspected, cells from another pass may be submitted for cytogenetic analysis, including a karyotype, FISH, and/or PCR. In addition to IHC, FISH and PCR also can be performed on the core needle biopsy. The diagnostic algorithms followed are the same as those for any other type of biopsy (see Figs. 2.20–2.22). An open biopsy is recommended when an FNA is negative in a patient with high suspicion of lymphoma. Because sampling is inherently limited in FNA, there often is a possibility that a negative FNA is a false negative due to inadequate sampling. Also, if an FNA is negative in a patient with clinical suspicion of HL, a core needle or open biopsy must be performed (HL is the lymphoma with the greatest risk of false negatives due to sampling). Lastly, there are isolated scenarios when an open biopsy must be performed, even when the FNA is positive, most commonly when the FNA shows a lymphoma but is not sufficient for subtype designation.

MULTIPLEX IMMUNOHISTOCHEMISTRY

Particular uses of IHC are detailed in the appropriate chapter for each group of entities. Unlike solid organs, in which most of the cells in a biopsy are tumor cells, in hematopoietic lesions, the cells of interest are admixed with large populations of background cells (e.g., hematopoietic marrow in the background MM cells). For this reason, mIHC provides a great advance over traditional, single staining methods.

With standard IHC, an antibody is used to identify a population on a slide. Each additional stain is performed on another, separate slide. For most purposes, a skilled practitioner can find a population of interest on one slide, then look at another slide and discern whether the second antigen is expressed by the population of interest, by inference from the spatial location in the tissue. However, if the population of interest is small, it is impossible to be sure the antigens are staining the same population (Fig. 2.33A). In mIHC, by contrast, multiple immunostaining reactions, each with a unique chromogenic color, are performed on a single piece of tissue, on the same slide. mIHC allows the user to triangulate, finding the cells of interest with one antibody/color, and determining expression of a second protein with another antibody/color (Fig. 2.33B). The most widespread use of mIHC has been for diagnosis and prognostication in plasma cell neoplasms (PCNs) (monoclonal gammopathy of undetermined significance [MGUS], amyloidosis, and MM). However, mIHC is becoming more and more prevalent, including for use as a companion diagnostic, to test whether a specific population expresses a drug target. Also, mIHC has an advantage over molecular techniques (below) in that mIHC allows assessment at the single cell level, whereas molecular methods assess nucleotides derived from an entire sample, making it impossible to discern which population contains any detected abnormality. mIHC is critical for accurate diagnosis in some cancers. For example, therapy and prognosis are different for B cell, anaplastic lymphoma kinase (ALK)+ lymphoma, and anaplastic large cell lymphoma (ALCL,
a T-cell lymphoma); to accurately differentiate between ALK+ T and B-cell lymphomas, mIHC is necessary (Fig. 2.34). A thorough discussion of current mIHC is beyond the scope of this text. However, principles from the following section on mIHC for PCNs apply to use for other cancers.

**mIHC for Diagnosis in Plasma Cell Neoplasms, Including Myeloma, MGUS, and Amyloidosis**

All PCNs harbor cytogenetic abnormalities. Various mIHC combinations can be used to identify specific cytogenetic abnormalities, at the single cell level in PCs, while excluding other cells from the analysis. Most common is hyperdiploidy (40% of all PCNs), involving increase copies of odd-number chromosomes (e.g., 5, 9, 11). Cyclin D1 resides on chromosome 11. The second most common cytogenetic abnormality in PCN is t(11;14) (20% of all PCNs). Both hyperdiploidy and t(11;14) cause overexpression of cyclin D1. Cyclin D1 is never expressed in nonneoplastic B or PCs. Therefore, finding D1 in a PC is diagnostic of neoplasia. mIHC for CD138 (red chromogen) to identify PCs and exclude non-PCs from the analysis is used with cyclin D1 (brown chromogen; Fig. 2.35). In t(11;14), the Ig heavy chain gene regulatory elements on chromosome 14 cause massive overexpression of cyclin D1 by acting on the translocated gene. By contrast, the extra copy of cyclin D1 in hyperdiploidy, due to an extra copy of chromosome 11, causes only mild overexpression by a gene-dosage effect. Consequently, mIHC for CD138/cyclin D1 can be used to specifically detect t(11;14), with a strong/uniform cyclin D1 signal (see Fig. 2.35), and distinguish it from hyperdiploidy, with a weak/focal cyclin D1 signal (data not shown). Similarly, t(6;14) (5% of PCN) causes strong/uniform aberrant expression of cyclin D3. Like cyclin D1, D3 is never expressed by nonneoplastic PCs, so finding D3+/CD138+ PCs is diagnostic of a PCN with t(6;14).

Finding cyclin D1 or D3 in PCs by mIHC is diagnostic of a PCN and the karyotypic abnormality can be inferred by the staining pattern. The precise subclassification (MGUS, MM, or amyloidosis) relies on the remainder of WHO/IMWG criteria. The pattern of D1 or D3 expression remains unchanged throughout the course of disease, so mIHC can be used for MRD assessment. Given the difficulties of cytogenetic analysis for PCN (below), the ability to use mIHC for this purpose is a welcome advance in technology.

Aside from diagnosis, mIHC also is used in MM for prognostication, by the plasma cell proliferation index (PCPI) methods. PCs can be identified by either a CD138+ membrane or a MUM1+ nucleus. mIHC is performed in one of two ways (Fig. 2.36) for this purpose (both methods are technically validated; the choice depends on the preference of the user and made on a case-by-case basis):

- **Ki67 (brown nuclei) + CD138 (red membrane) + blue nuclear counterstain** (see Fig. 2.36A)
- **Ki67 (blue nuclei) + MUM1 (red nuclei) = purple nuclei in proliferating MM cells, red nuclei in nonproliferating MM cells, and blue nuclei in proliferating background hematopoietic cells** (no counterstain; see Fig. 2.36B)

The PCPI is performed by manually counting 200 PCs (CD138+ or MUM1+) as either proliferating (coexpressing Ki67) or not. Counting can be performed with an ocular grid or by taking a photomicrograph and counting on the screen. Whereas the complex images of Ki67/CD138, nucleus/membrane mIHC cannot be recognized by image analysis systems, the red versus purple nuclei in the Ki67/MUM1 method can be counted by software programs via automated computer image analysis.
SPECIAL STAINS

Congo Red and the Workup for Amyloidosis

Amyloid is not a particular substance. It is a tertiary, molecular conformation, the β-pleated sheet, that may be composed of any of several different protein building blocks, most commonly Ig light chains. As such, diagnosis remains dependent on demonstrating amyloid by staining with Congo red, a solution containing red crystals that are of the perfect size to deposit into the deep groves of the β-pleated sheet. Congophilia, dark red deposits, denotes amyloid (Fig. 2.37). Although seeing that there are dark red, extracellular deposits by regular light microscopy generally indicates amyloid, it must be confirmed by demonstrating birefringence, the property of rotating the plane of polarized light. Positive amyloid birefringence when viewing a slide by polarized light is classically apple green, but may also be of other colors (e.g., yellow or orange). By contrast, gray–white birefringence denotes collagen.

Mass Spectrometry for Amyloid Diagnosis

The advantage of this technique is that it can identify the type of protein making up an amyloid deposit. Because therapy is markedly different for amyloidosis of different protein types, this is very important. However, it should be emphasized that, due to technical limitations, mass spectrometry cannot be used to diagnose amyloid. Mass spectrometry can only be used to determine the protein composition of amyloid once a deposit has already been identified by Congo red staining.

Acid Fast Bacteria (AFB) and Gomori Methenamine Silver (GMS) for Acid-Fast Bacteria and Fungi

Although these stains are the most specific means of identifying these microorganisms in a tissue section, it should be emphasized that the sensitivity for detection is very low. Finding an organism is diagnostic, but failing to find an organism does not exclude its presence. It is important to be aware of this and, when infection with these organisms is suspected clinically, cultures must be performed on freshly acquired tissue, prior to fixation and processing.

Reticulin and Masson Trichrome

Reticulin fibrosis is typically present around myeloma and lymphoma cells, preventing their aspiration (discussed above). Aside from making that determination, the use of these stains is generally restricted to MPNs (see Chapter 8 for grading fibrosis). Marrow fibrosis is produced by stromal fibroblasts but initiated by cytokine production and/or in a process similar to scarring, such as after chemotherapy or in reaction to infiltrating lymphoma or MM cells. In MPNs, neoplastic megakaryocytes produce platelet-derived growth factor and transforming growth factor-β, which induce fibroblasts to produce type 3 collagen. “Reticulin” is not a specific substance. In a reticulin stain, the black fibers, referred to as “reticulin,” are composed primarily of type 3 collagen (admixed with lesser amounts of other proteins). Whereas type 3 collagen (“reticulin”) fibrosis is reversible, if the stimulus for fibrosis is prolonged (e.g., primary myelofibrosis), the stroma will produce type 1 collagen (often referred to as “collagen fibrosis”). Whereas type 3 collagen is detected by a reticulin stain, type 1 collagen is detected by a Masson trichrome stain, in which type 1 collagen is
KARYOTYPING AND FLUORESCENCE IN SITU HYBRIDIZATION

The process and use of karyotypic preparation and banding is illustrated here (Fig. 2.38).

Important, diagnostic, or prognostic cytogenetic findings are included in the above sections and in other chapters relevant to each entity. Used in conjunction with FISH, and increasingly with PCR and gene sequencing, karyotypic abnormalities are the primary means of subtyping AML. In most cases, demonstration of genetic abnormalities is not necessary for lymphoma subtyping (see above Multiparameter Flow Cytometry section). However, in the case of DLBCL, FISH is used to assess MYC and BCL2 translocations. Although MYC and BCL2 translocations are somewhat nonspecific, finding both simultaneously in a DLBCL confers the designation “double hit lymphoma,” which is not a WHO subtype, but is a separate category in terms of therapy and prognosis. A list of widely utilized karyotypic/FISH abnormalities is provided (Table 2.7).

Due to the fibrosis that prevents aspiration of MM cells and due to their rapid death after aspiration, cytogenetic analysis is problematic in myeloma. To remedy this problem, a technique widely employed is the use of CD138+ magnetic beads, to select PCs and then to perform FISH only on the selected cells (Fig. 2.39). Because many of the translocations in myeloma are so close to the telomere, they often are karyotypically silent, so FISH is the mainstay of detection. The technique of cell-selected FISH is becoming widely used for myeloma, but also has applications for lymphoma and acute leukemias.

MOLECULAR APPROACHES

Although the use of molecular methods for diagnosis and prognosis is becoming broader every year, to some extent, the real life, clinical utility, and cost-effectiveness are more limited than lay and medical journals often suggest. This discussion includes molecular approaches currently in wide use for diagnostic purposes or likely to become more widely adopted. Because molecular techniques yield data in the form of graphs and gels, of limited visual use for diagnosticians and clinicians outside of the molecular laboratory, the number of figures is appropriately curtailed.

It must be noted that, aside from their limited efficacy, the main reason for the limited application of molecular methods to real life, clinical diagnostics, is cost. It is true that cost is decreasing. However, relative to other techniques (above sections), the cost of many molecular methods remains prohibitive. Moreover, in contrast to MFC and IHC, which are well reimbursed, most payers will not reimburse the cost of molecular tests. Consequently, the common pipeline has become discovery of a useful marker by genome-wide molecular methods, then translation into a means of detection by standard MFC, IHC, or FISH.

Polymerase Chain Reaction

PCR for Lymphoma Diagnosis

For B-cell lymphomas, clonality can typically be inferred by Ig light chain restriction.
However, in some cases, there are no live cells for MFC. In others, the cells of interest are too few to detect by MFC. In such cases, if needed for diagnostic purposes, PCR is performed (Figs. 2.40 and 2.41). The standard approach in most laboratories is the use of a single primer pair to amplify framework 3 (FR3) of the joining region of the heavy chain gene (Jh). The primer binding sites are too far apart in the germline configuration to yield a PCR product. However, early in B-cell ontogeny, the process of Ig heavy chain gene rearrangement brings those loci closer together, allowing a PCR product to be generated from this primer pair. So, a positive product means there is a B cell in the sample. The precise break points generated to bring the loci together are highly variable from cell to cell, so that PCR amplification in a reactive B-cell population yields numerous PCR products with somewhat different size and electrophoretic mobility, called a “smear” on the gel on which the PCR product was run. If the sample contains a B-cell clone, there will be a dominant band in addition to, or in lieu of, a smear (similar to that seen in PCR for the TCR [Fig. 2.42]). The PCR product is traditionally analyzed by gel electrophoresis, but this technique is gradually being replaced by capillary electrophoresis (see Fig. 2.41). Jh amplification yields a positive, clonal result in about 80% of B-cell lymphomas. If Jh PCR is negative in a lesion strongly suspected of being a B-cell lymphoma, reflex PCR is performed for the joining region of the Igκ light chain, which is positive in most remaining cases.

Jh and Jκ PCR can also be used for MRD detection in B-ALL. Although MFC is the standard for MRD detection in ALL, theory and some data support the idea that sensitivity and specificity would be higher using PCR. Studies are in progress to investigate and compare MFC and PCR for MRD.

The concepts described above for the use of Ig PCR in B-cell lymphoma diagnosis apply to the use of PCR for T-cell lymphoma diagnosis. The standard technique is amplification of the TCR γ chain gene (see Fig. 2.42). For the reasons depicted above (see Multiparameter Flow Cytometry section), whereas PCR is an ancillary test for B-NHL, it is employed regularly for the diagnosis of T-NHL. Also, for ATLL, as described above, PCR for HTLV-1 is standard.

Reverse Transcriptase PCR for BCR-ABL1 in Chronic Myelogenous Leukemia

Demonstration of the BCR-ABL1 translocation is a required diagnostic feature and the hallmark of CML. Although establishment of BCR-ABL1 is typically performed by a standard karyotype and/or FISH, treatment with tyrosine kinase inhibitors results in lower levels of disease, necessitating more sensitive techniques to detect response to treatment and MRD. For technical reasons, studies have shown that RT-PCR, amplifying the RNA fusion transcript, is more sensitive than standard PCR for DNA. Quantitation of BCR-ABL1 transcripts by RT-PCR has become the standard tool in therapeutic monitoring. Clinical trials have shown the prognostic significance of this technique. This is one of the few techniques in which quantitation has been standardized and implemented internationally, including guidelines for internal controls and reference materials. Monitoring is performed within specific time frames of drug administration, by comparison to curves on the International Scale. The technique involves standardized extraction of RNA from PB samples, followed by RT-PCR for BCR-ABL1 and an internal, housekeeping gene control transcript, and comparison of individual patient results to data from clinical trials.

Reverse Transcriptase PCR for PML-RARA in AML with t(15;17) Acute
**Promyelocytic Leukemia**

Because APL often is associated with bleeding, diagnosis is a medical emergency. Induction therapy is performed as in other AMLs, except for the addition of all-trans retinoic acid, which is specifically targeted at the fusion protein product of the PML-RARA translocation. A presumptive diagnosis and the initiation of therapy are typically made and instituted on the basis of the typical distinctive morphology and 4+ MPO by special stain. Confirmation is performed by karyotype and FISH. The presence of a PML-RARA detected by RT-PCR but not by FISH is exceedingly rare. However, the establishment of a PCR-detectable abnormality at initial diagnosis is useful because it allows this technique for residual disease detection or confirmation, sometimes below the detection level of FISH.

**Allele-Specific Quantitative PCR for JAK2 V617F, MPL, and CALR Mutations in the Myeloproliferative Neoplasms**

JAK2 (Janus kinase 2) V617F is the most common genetic abnormality in MPNs and is highly specific for this disease category. Of the numerous methods used for detection, allele-specific qPCR is used most commonly. It allows for quantification of the mutant as a percentage of all of the JAK2 alleles. Changes in quantity are used to monitor both JAK-directed and other therapy. Because techniques and methods vary, to detect changes in an individual patient, samples must be analyzed in the same laboratory. After JAK2 and calreticulin (CALR), MPL mutations are the next most common in MPN and also are relatively specific for this disease category. Like JAK2 testing, there are many techniques and technical variations on MPL testing, but allele-specific qPCR is most commonly used. Unlike JAK2 and MPL, there is fairly great variability in CALR mutation, necessitating PCR followed by sequencing.

**PCR Fragment Length Analysis for Prognostication and Subtyping in AML: Nucleophosmin 1, CCAAT Enhancer Binding Protein, and FMS-like Tyrosine Kinase 3**

WHO subtype categorization of AML involves assessment of clinical features, karyotype, and FISH (detailed in other chapters). WHO criteria do not require the use of advanced molecular techniques. However, the 2016 revision does include two categories that are exceptions:

- AML with mutated NPM1
- AML with biallelic mutations of CEBPA

Although not incorporated into WHO criteria, for guidance in therapy of AML with a normal karyotype, analysis typically is performed for FLT3, the most common abnormality of which is an internal tandem duplication (ITD). There are no currently used drugs for AML that are directed at specific molecular targets, with the exception of APL with t(15;17) (PML-RARA). However, the intensity of therapy is driven by prognostic subgroups. In AML with a normal karyotype, NPM1 mutation in the absence of a FLT3 ITD or isolated biallelic mutations of CEBPA is statistically correlated with favorable outcomes. Assessment of these three genetic abnormalities is most commonly performed by quantitative fragment length analysis. PCR is performed with primer pairs that amplify the gene region most commonly abnormal, revealing both the abnormality and a quantitative ratio, showing relative percentage of cells harboring the abnormal gene compared to the number of normal cells.
Although this suggests a quantitative approach to therapeutic monitoring, studies have shown that data from this technique are not precise enough for such use.

**NEXT-GENERATION SEQUENCING IN DIAGNOSIS AND MONITORING**

Although some laboratories offer NGS for other diagnostic purposes, the routine use of NGS is currently limited to myeloid neoplasms, primarily MDS. The diagnosis of AML does not require genetic analysis except in relatively rare circumstances. The diagnosis of MPN is typically confirmed by analysis of JAK2, CALR, and MPL only. The diagnosis of MDS is made by morphology and karyotype. However, in patients with a strong suspicion of MDS, but not fulfilling standard diagnostic criteria, reflex testing in the form of a 50-gene NGS panel for commonly found mutations in myeloid malignancies is becoming common.

The diagnosis of LPL/WM often is difficult because of overlapping features with MZL. MYD88 mutation has been shown to be fairly specific to LPL/WM. NGS testing for MYD88 mutation is becoming fairly common for diagnostic confirmation. However, due to insufficient supportive data, MYD88 analysis is not currently used for disease monitoring.

The diagnosis of HCL is typically straightforward by morphology and immunophenotyping. However, BRAF mutational analysis by whole exome sequencing has been shown to be fairly specific to HCL. Consequent to the development of specific BRAF-directed therapy, there will likely be a trend toward the use of BRAF mutational analysis as a companion diagnostic test.

Lastly, if trends in advancement of NGS methods continue and are paralleled by decreasing cost, the only barrier to more widespread adoption of NGS would be reimbursement. To a greater or lesser extent, it seems likely that in the near future, NGS for mutation detection will replace allele-specific PCR assays and also may be used for clonality studies.

**CELL-FREE DNA**

Numerous publications support the proof of principle that analyzing DNA in cell-free plasma can be used to detect and monitor cancer. Because of the lack of invasiveness of this approach, such techniques are being developed in many laboratories. Assessment of the fairly voluminous current data suggests that this technique may find limited clinical use. However, current published data suggest that it likely will be for solid organ cancers only. Due to the relative noninvasiveness of blood and marrow biopsy procedures for hematopoietic cancer diagnosis, the main advantage of cell-free DNA testing is far less than for solid organ cancers. Because current methods lack sufficient sensitivity and specificity, it seems unlikely that cell-free DNA analysis will be used widely for diagnostic or monitoring purposes in the near future.

**EPIGENETIC ABNORMALITIES**

Voluminous research data have conclusively shown the importance of epigenetic
abnormalities, in which gene expression is altered not due to changes in DNA sequences, but due to modifications in accessory groups bound to the DNA molecules. The prototype examples are changes in methylation of gene promoter elements and changes in histone protein–DNA binding that alters transcription. Drugs that target methylation are commonly used in hematopoietic cancers, especially MDS. However, the use of such drugs is not currently accompanied by assessment of methylation status. Moreover, there are no instances in which epigenetic analysis is currently used for clinical diagnosis or prognostication. Although assessment of epigenetic status is an interesting area of research, it seems unlikely that such techniques will be widely adopted for clinical purposes in the near future.

REFERENCES


### Table 2.1

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<th>Ex (nm)</th>
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Em, peak emission wavelength; Ex, peak excitation wavelength; MW, molecular weight; APC, activated protein C.


### Table 2.2

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**Lymphoma and Myeloma**

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<th>B2</th>
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<td>FMC7</td>
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<td>TCR a/b</td>
<td>cKappa</td>
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<td>PE</td>
<td>Lambda</td>
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<td>cLambda</td>
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<td>Bright</td>
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<td>CD21</td>
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<td>CD3</td>
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<td>CD117</td>
<td>C</td>
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<td>APC</td>
<td>CD10</td>
<td>CD34</td>
<td>CD7</td>
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**EuroFlow Panels for Acute Leukemia**

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<td>PAC O</td>
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<tr>
<td>PAC B</td>
<td>cCD3</td>
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<td>CD34</td>
<td>CD34</td>
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<td>cTdT</td>
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<td>CD13</td>
<td>CD64</td>
<td>CD105</td>
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http://radiologyme.com/
Table 2.3
List of CD antigens most commonly used in flow cytometry immunophenotyping of hematologic samples

<table>
<thead>
<tr>
<th>CD</th>
<th>Expression in Normal Hematopoietic Cell Types</th>
<th>MW (kDa)</th>
<th>Function</th>
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<tbody>
<tr>
<td>CD1a</td>
<td>Cortical thymocytes, Langerhans cells, dendritic cells</td>
<td>49</td>
<td>Antigen presentation, w/β2m</td>
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<td>CD2</td>
<td>Thymocytes, T cells, NK cells</td>
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<td>CD58 ligand, adhesion, T-cell activation</td>
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<td>CD3</td>
<td>T cells, thymocyte subset</td>
<td></td>
<td>w/TCR, TCR surface expression/signal transduction</td>
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<tr>
<td>CD4</td>
<td>Thymocyte subset, T-cell subset, monocytes, macrophages</td>
<td>55</td>
<td>MHC class II coreceptor, HIV receptor, T-cell differentiation/activation</td>
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<tr>
<td>CD5</td>
<td>Thymocytes, T cells, B-cell subset</td>
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<td>CD72 receptor, TCR or BCR signaling, T–B interaction</td>
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<tr>
<td>CD7</td>
<td>Thymocytes, T cells, NK cells, small subset of hematopoietic progenitors</td>
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<td>T costimulation</td>
</tr>
<tr>
<td>CD8</td>
<td>Thymocyte subset, T-cell subset, NK subset</td>
<td>32–34</td>
<td>MHC class I coreceptor, receptor for some mutated HIV-1, T-cell differentiation/activation</td>
</tr>
<tr>
<td>CD9</td>
<td>Eosinophils, basophils, platelets, activated T cells</td>
<td>22–27</td>
<td>Cellular adhesion and migration</td>
</tr>
<tr>
<td>CD10</td>
<td>B precursors, germinal center B cells, thymocyte subset, neutrophils</td>
<td>100</td>
<td>Zinc-binding metalloproteinase, B-cell development</td>
</tr>
<tr>
<td>CD11a</td>
<td>Lymphocyte subsets, granulocytes, monocytes, macrophages</td>
<td>180</td>
<td>CD11a/CD18 receptor for ICAM-1, -2,-3, intercellular adhesion, T costimulation</td>
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<tr>
<td>CD11b</td>
<td>Granulopoietic cells, NK cells</td>
<td>170</td>
<td>Binds CD54, ECM, and iC3b</td>
</tr>
<tr>
<td>CD11c</td>
<td>Dendritic cells, granulopoietic cells, NK cells, and B-cell and T-cell subsets</td>
<td>150</td>
<td>Binds CD54, fibrinogen, and iC3b</td>
</tr>
<tr>
<td>CD13</td>
<td>Granulopoietic cells, monocytes</td>
<td>150–170</td>
<td>Zinc-binding metalloproteinase,</td>
</tr>
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</table>

The staining index refers to the relative brightness of the fluorochrome (Fluor). Each tube with its listed component antibodies has a title (e.g., tube B1, used for B-NHL diagnosis).

APC, activated protein C.
| CD14 | Monocytes, macrophages, Langerhans cells | 53–55 | Receptor for LPS/LBP, LPS recognition |
| CD15 | Neutrophils, eosinophils, monocytes | | Adhesion |
| CD16 | Neutrophils, macrophages, NK cells | 50–65 | Component of low-affinity Fc receptor, phagocytosis, and ADCC |
| CD19 | B cells, plasma cells | 95 | Complex w/CD21 and CD81, BCR coreceptor, B-cell activation/differentiation |
| CD20 | B cells | 33–37 | B-cell activation |
| CD21 | B cells and T-cell subsets | 145, 110 | Complement C3d and EBV receptor, complex w/CD19 and CD81, BCR coreceptor |
| CD22 | B cells | 150 | Adhesion, B-mono, B–T interactions |
| CD23 | B cells, eosinophils, platelets | 45 | CD19-CD21-CD81 receptor, IgE low-affinity receptor, signal transduction |
| CD24 | Thymocytes, erythrocytes, lymphocytes, myeloid cells | 35–45 | Binds P-selectin |
| CD25 | Activated B cells and T cells | 55 | IL-2Rα, w/IL-2Rβ, and γ to form high-affinity complex |
| CD26 | Granulopoietic cells, monocytes, dendritic cells | 67 | Adhesion |
| CD27 | Hematopoietic precursors | 105–120 | Stem cell marker, adhesion, CD62L receptor |
| CD28 | Platelets, monocytes, erythropoietic precursors | 88 | ECM receptor, adhesion, phagocytosis |
| CD29 | High expression on B-cell precursors, plasma cells and activated T cells, low on granulopoietic cells | 45 | Ecto-ADP-ribosyl cyclase, cell activation |
| CD30 | Platelets, megakaryocytes | 125/22 | w/CD61 forms GPIIb, binds fibrinogen, fibronectin, vWF, thrombospondin, platelet activation and aggregation |
| CD31 | Platelets, megakaryocytes | 22 | Complex w/CD42b, c and d, receptor for vWF and thrombin, platelet adhesion to subendothelial matrices |
| CD32 | Hematopoietic cells, multiple isoforms from alternative splicing | 180–240 | Tyrosine phosphatase, enhanced TCR and BCR signals |
| CD33 | NK subset, T-cell subset | CD175–185 | Neural cell adhesion molecule |
| CD34 | NK subset, T-cell subset | 110 | HNK 1 |
| CD35 | Ubiquitous | 18–20 | Complement regulatory protein |
| CD36 | Platelets, megakaryocytes | 105 | Integrin β3, adhesion, CD41/CD61 or CD51/CD61 mediate adhesion to ECM |
| CD37 | B cells, T-cell subsets, monocytes, granulocytes, NK cells, thymocytes | 74, 95 | CD34, GlyCAM, and MAdCAM-1 receptor, leukocyte homing, tethering, rolling |

http://radiologyme.com/
<table>
<thead>
<tr>
<th>CD64</th>
<th>Monocytes, neutrophils</th>
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<th>FCyRI, increases on neutrophils in sepsis</th>
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<tr>
<td>CD65</td>
<td>Granulopoietic cells</td>
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<td>Phagocytosis</td>
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<td>CD66</td>
<td>Neutrophils</td>
<td>90</td>
<td>Cell adhesion</td>
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<td>CD68</td>
<td>Monocytes, neutrophils, basophils, mast cells</td>
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<td>Macrosialin</td>
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<td>CD71</td>
<td>Proliferating cells, erythroid precursors, reticulocytes</td>
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<td>Transferrin receptor, iron uptake</td>
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<tr>
<td>CD79</td>
<td>B cells, plasma cells</td>
<td>33–37</td>
<td>Component of BCR, BCR surface expression, and signal transduction</td>
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<td>CD103</td>
<td>B- and T-cell subsets</td>
<td>150, 25</td>
<td>w/Integrin β7, binds E-cadherin, lymph homing/retention</td>
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<td>CD117</td>
<td>Hematopoietic progenitors, mast cells</td>
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<td>Stem cell factor receptor, hematopoietic progenitor development/differentiation</td>
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<td>CD123</td>
<td>Basophils, dendritic cell subset, hematopoietic progenitors</td>
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<td>IL-3Ra, w/CDw131</td>
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<td>Hematopoietic stem cells subset</td>
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<td>w/MHC class I HL-A-E molecules, forms heterodimer with CD94</td>
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<td>CD235a</td>
<td>Erythropoietic precursors</td>
<td>36</td>
<td>Glycophorin A</td>
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*Note: For a comprehensive list and characteristics, please see [www.hcdm.org](http://www.hcdm.org).*


### Table 2.4

**Immunophenotypic patterns associated with recurrent specific cytogenetic abnormalities in leukemia**

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<th>Type of Leukemia</th>
<th>Cytogenetic Abnormality</th>
<th>Characteristic Flow Cytometry Findings</th>
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<td>AML</td>
<td>t(8;21)(q22;q22), RUNX1-RUNX1T1</td>
<td>At least a fraction of blasts with CD34&lt;sup&gt;bright&lt;/sup&gt;, often coexpressing CD19 and TdT but not CD10 Granulocytic differentiation (CD13, CD33, MPO, and CD15), aberrant expression of CD56 common No monocytic differentiation</td>
</tr>
<tr>
<td>AML</td>
<td>Inv.(16)(p13.1q22) or t(16;16) (p13.1q22) CBF-MYH11</td>
<td>Distinct populations of blasts, granulocytic and monocytic (CD14, CD4, CD64) precursors Coexpression of CD34 and CD64 common Eosinophils can be delineated by high SS and low FS than neutrophils and CD16&lt;sup&gt;−&lt;/sup&gt; Often CD2 on blasts and precursors</td>
</tr>
<tr>
<td>AML</td>
<td>t(9;11)(p22;q23) MLLT3-MLL</td>
<td>MAb 7.1 positivity Monocytic differentiation (HLA-DR, CD4&lt;sup&gt;dim&lt;/sup&gt;, CD11b, CD13, CD15, CD36, CD33, and CD64)</td>
</tr>
<tr>
<td>AML</td>
<td>NPM1 mutated</td>
<td>Most often blasts CD34&lt;sup&gt;−&lt;/sup&gt;, often HLA-DR&lt;sup&gt;−&lt;/sup&gt;, CD117&lt;sup&gt;+&lt;/sup&gt;, CD123&lt;sup&gt;+&lt;/sup&gt;, CD33&lt;sup&gt;bright&lt;/sup&gt;, CD110&lt;sup&gt;+&lt;/sup&gt; Show granulocytic differentiation (CD15&lt;sup&gt;+&lt;/sup&gt;) Monocytic differentiation in 30% of cases Some cases only CD33&lt;sup&gt;bright&lt;/sup&gt; and MPO&lt;sup&gt;bright&lt;/sup&gt; with no differentiation</td>
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<td>AML</td>
<td>Inv.(3)(q21;q26.2) or t(3;3)(q21;q26.2)</td>
<td>Positive for CD34, CD117, CD13, CD33, HLA-DR, and MPO</td>
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### Table 2.5

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<tr>
<td>CD19</td>
<td>(−)</td>
<td>+</td>
</tr>
<tr>
<td>CD20</td>
<td>(−)</td>
<td>+</td>
</tr>
<tr>
<td>CD23</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>CD33</td>
<td>+</td>
<td>Weak +</td>
</tr>
<tr>
<td>CD34</td>
<td>+</td>
<td>(−)</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B, B cell; T, T cell.

<table>
<thead>
<tr>
<th></th>
<th>CD36</th>
<th>CD38</th>
<th>CD41</th>
<th>CD45</th>
<th>CD56</th>
<th>CD64</th>
<th>CD117</th>
<th>Glyco A</th>
<th>HLA-DR</th>
<th>Kappa</th>
<th>Lambda</th>
<th>TdT</th>
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<tbody>
<tr>
<td>Status</td>
<td>(−)</td>
<td>+</td>
<td>(−)</td>
<td>Weak</td>
<td>(−)</td>
<td>(−)</td>
<td>+</td>
<td>(−)</td>
<td>+</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
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**Immunohistochemistry**

<table>
<thead>
<tr>
<th></th>
<th>Myeloid</th>
<th>Lymphoid</th>
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<tbody>
<tr>
<td>CD117</td>
<td>+</td>
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</tr>
<tr>
<td><em>MPO</em></td>
<td>+</td>
<td>(−)</td>
</tr>
<tr>
<td><em>PAX5</em></td>
<td>(−)</td>
<td>+</td>
</tr>
<tr>
<td><em>CD79a</em></td>
<td>(−)</td>
<td>+</td>
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<tr>
<td><em>CD22</em></td>
<td>(−)</td>
<td>+</td>
</tr>
<tr>
<td><em>CD20</em></td>
<td>(−)</td>
<td>+</td>
</tr>
</tbody>
</table>

**Note:** **Bold font** indicates key antigens positive in ALL-like component, negative in AML-like and **Italic font** indicates key antigens positive in AML-like component, negative in ALL-like.

---

**Table 2.6**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Absolute Normal Values</th>
<th>Relative %</th>
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<tbody>
<tr>
<td>CD3+</td>
<td>544–2,544 mm³</td>
<td>40%–95% of lymphocytes</td>
</tr>
<tr>
<td>CD3+/CD4+</td>
<td>387–1,688 mm³</td>
<td>28%–64% of lymphocytes</td>
</tr>
<tr>
<td>CD3+/CD8+</td>
<td>157–856 mm³</td>
<td>12%–41% of lymphocytes</td>
</tr>
<tr>
<td>NK cells, CD3+/CD16+, and/or CD56+</td>
<td>94–484 mm³</td>
<td>5%–20% of lymphocytes</td>
</tr>
<tr>
<td>B cells, CD19+</td>
<td>54–610 mm³</td>
<td>5%–55% of lymphocytes</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>1,800–7,000 mm³</td>
<td>45%–75%</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>1,500–8,400 mm³</td>
<td>NA</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1,000–4,800 mm³</td>
<td>20%–50%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>200–900 mm³</td>
<td>2%–11%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0–450 mm³</td>
<td>0%–5%</td>
</tr>
<tr>
<td>Basophils</td>
<td>0–100 mm³</td>
<td>0%–1%</td>
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</table>

**Table 2.7**

<table>
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<tr>
<th>Chromosome Abnormality</th>
<th>Genes (HUGO)</th>
<th>Disease</th>
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</thead>
<tbody>
<tr>
<td>t(9;22)(q34;q11.2)</td>
<td>ABL1, BCR</td>
<td>CML, ALL</td>
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</tbody>
</table>

http://radiologyme.com/
<table>
<thead>
<tr>
<th>Chromosome Change</th>
<th>Gene</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>del(6)(q23)</td>
<td>MYB</td>
<td>CLL</td>
</tr>
<tr>
<td>del(11)(q22)</td>
<td>ATM</td>
<td>CLL</td>
</tr>
<tr>
<td>+12</td>
<td></td>
<td>CLL</td>
</tr>
<tr>
<td>del(13)(q14)</td>
<td></td>
<td>CLL, MDS, AML</td>
</tr>
<tr>
<td>del(17)(p13)</td>
<td>TP53</td>
<td>CLL</td>
</tr>
<tr>
<td>t(1;19)(q23;p13.3)</td>
<td>PBX1, TCF3</td>
<td>ALL</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>AFF1, MLL</td>
<td>ALL</td>
</tr>
<tr>
<td>t(variant;11)(variant;q23)</td>
<td>MLL</td>
<td>ALL, AML</td>
</tr>
<tr>
<td>t(12;21)(p13;q22)</td>
<td>ETV6, RUNX1</td>
<td>ALL</td>
</tr>
<tr>
<td>t(1;22)(p13;q13)</td>
<td>RBM15, MKL1</td>
<td>AML (M7)</td>
</tr>
<tr>
<td>inv(3)(q21q26.2)/t(3;3)(q21;q26.2)</td>
<td>RPN1, MECOM</td>
<td>AML</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>DEK, NUP214</td>
<td>AML</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>RUNX1T1, RUNX1</td>
<td>AML (M2)</td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td>MLLT3, MLL</td>
<td>AML</td>
</tr>
<tr>
<td>t(15;17)(q24;q21)</td>
<td>PML, RARA</td>
<td>APL (M3)</td>
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<td>inv(16)(p13.1q22)/t(16;16)(p13.1;q22)</td>
<td>MYH11, CBFB</td>
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<td>−5/del(5q)</td>
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<td>MDS, AML</td>
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<tr>
<td>−7/del(7q)</td>
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<td>MDS, AML</td>
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<td>+8</td>
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<td>del(20q)</td>
<td></td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(8;14)(q24;q32)</td>
<td>MYC, IGH@</td>
<td>BL</td>
</tr>
<tr>
<td>t(2;8)(p12;q24)</td>
<td>IGK@, MYC</td>
<td>BL</td>
</tr>
<tr>
<td>t(8;22)(q24;q11.2)</td>
<td>MYC, IGL@</td>
<td>BL</td>
</tr>
<tr>
<td>t(2;5)(p23;q35)</td>
<td>ALK, NPM1</td>
<td>ALCCL</td>
</tr>
<tr>
<td>t(2;variant)(p23;variant)</td>
<td>ALK</td>
<td>ALCCL</td>
</tr>
<tr>
<td>t(18;variant)(q21;variant)</td>
<td>BCL2</td>
<td>DLBCL</td>
</tr>
<tr>
<td>t(3;variant)(q27;variant)</td>
<td>BCL6</td>
<td>DLBCL</td>
</tr>
<tr>
<td>t(8;variant)(q24;variant)</td>
<td>MYC</td>
<td>DLBCL</td>
</tr>
<tr>
<td>t(14;18)(q32;q21)</td>
<td>IGH@, BCL2</td>
<td>FL</td>
</tr>
<tr>
<td>t(2;18)(p12;q21)</td>
<td>IGK@, BCL2</td>
<td>FL</td>
</tr>
<tr>
<td>t(18;22)(q21;q11.2)</td>
<td>BCL2, IGL@</td>
<td>FL</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>CCND1, IGH@</td>
<td>MCL</td>
</tr>
<tr>
<td>del(13)(q14)</td>
<td></td>
<td>PCM</td>
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<tr>
<td>del(17)(p13)</td>
<td>TP53</td>
<td>PCM</td>
</tr>
<tr>
<td>t(4;14)(p16.3;q32)</td>
<td>FGFR3, IGH@</td>
<td>PCM</td>
</tr>
<tr>
<td>t(14;16)(q32;q23)</td>
<td>IGH@, MAF</td>
<td>PCM</td>
</tr>
<tr>
<td>t(14;20)(q32;q12)</td>
<td>IGH@, MAFB</td>
<td>PCM</td>
</tr>
</tbody>
</table>

ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; BL, Burkitt lymphoma/B-cell lymphoma; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; PCM, plasma cell myeloma.

Figure 2.1. Myeloma (MM) and normal plasma cells. The standard gating strategy for plasma cells is finding populations with dim CD45+/bright CD38+ (upper right). All tubes for PC analysis contain CD38 and CD45 for gating, plus additional antibodies for analysis. In addition to CD38 and CD45, the first tube (top row) contained CD19, CD20, and CD56 (axes as marked). The second tube (bottom row) contained CD200, CD117, and intracytoplasmic kappa and lambda for analysis. MM cells (red), normal plasma cells (enlarged, blue), and lymphocytes (pink) are all present (all other background cells are gray). Normal plasma cells are CD38+(bright), CD19+, CD20−, CD45+, CD117−, CD200−, and express polytypic cytoplasmic light chains (i.e., subpopulations with kappa and others with lambda). The MM cells are aberrant, CD19−, CD56+, CD117+(dim), and CD200+(dim), with cytoplasmic kappa light chain restriction. The MM cells express CD38 more dimly than the normal plasma cells, but still brighter than the other hematopoietic cells.
Figure 2.2. Principles of multiparameter flow cytometry (MFC). A single cell suspension is hydrodynamically focused with sheath fluid to intersect lasers (three-laser system is shown). Fluorescence signals are collected by multiple fluorescence emission detectors, separate for every laser. Examples of fluorochromes detected by different lasers are given according to Table 2.1. Detected signals are amplified by photomultiplier tubes and converted to digital form for analysis. (From Porwit A. Clinical flow cytometry. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe's Clinical Hematology. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:19–45.)

Figure 2.3. Most useful lineage defining CDs. CD2 – CD8 (top row, red) are T cell restricted except that NK cells also express CD2 and CD7 (sometimes CD8), and monocytes express
CD4. CD3 is T cell defining, absolutely lineage restricted. CD10 and those around CD20 are B cell antigens (dark blue, 2nd and 3rd rows). PAX4 (light blue, 4th row) is a non-cluster designated, nuclear antigen that is B cell restricted. MPO (green, 4th row), is cytoplasmic and restricted to neutrophil lineage cells. LYSO (lysozyme; green, 4th row) is restricted to myeloid/monocytic lineage cells. CD79a (light blue, 5th row) is B lineage restricted, surface and cytoplasmic and expressed at all stages, from pre-B cells through plasma cells. In bone marrow specimens, CD138 (purple; 5th row) is plasma cell-restricted. MUM1 (also called IRF-4; purple, 4th row) is a relatively plasma cell restricted nuclear antigen that also can be expressed by B cell subpopulations.

Figure 2.4. Standard MFC display of leukocytes. LY, lymphocytes (green); MO, monocytes (orange); MY, mature myeloid cells (aqua blue; mainly neutrophils); OTHER, red cells, plasma cells, and any nonleukocytes (pink); BL/Baso, blasts and basophils (red), HG, hematogones (dark blue; i.e., reactive pre-B cells). y Axis displays CD45 expression; x axis displays side scatter area (SSC-A). The standard display of MFC data is log-scale, relative intensity of signal or expression on both x and y axes. In most centers, 1 log greater than background is considered “dim+,” two logs greater than background = “positive,” three logs greater = “bright.” Units are therefore arbitrary.
Figure 2.5. A: Initial gating for MFC, detection of dead cells and artifacts produced by permeabilization of cells. (Color schema same as in Fig. 2.4.) Dead cells (gray) are gated out in the first two scattergrams (upper left; see Fig. 2.4 for method), then displayed as gray (also encircled with gray ovals). Note that the dead cells nonspecifically react with antibodies because of electrostatic forces, an artifact which is not true antigen binding. As such, the artifactual “binding” of any one antibody is proportional to any other, resulting in a displayed population of dead cell distribution along a characteristic diagonal of about 45 degrees, seen in each scattergram marked “All Cells.” Note that the dead cells (gray) appear to express TdT and CD7 (bottom row left) but this is an artifact. Another artifact is the antigenic shift introduced by cell permeabilization. Analysis of TdT (a nuclear antigen) expression requires permeabilization because of its intracellular location (x axis, bottom...
scattergrams). Note that all cell populations that display cTDT after permeabilization are shifted to the right (yellow arrows) on the x axis. B: MFC for acute myeloid leukemia. Initial gating. Event data are first displayed (top row, left scattergram) as forward light scatter area (FSC-A) by height (FSC-H). Events that are relatively round have FSC-A that is proportional to FSC-H, so that they fall on a 45-degree angle. Events falling off this angle represent doublets or aggregates of cells. The singlet (i.e., single cell) population gate is drawn to exclude cellular aggregates. Next, the singlets are displayed (top row, middle scattergram) in more detail; dead cells inadvertently included in the singlet gate (gray dots, very low FSC-A and FSC-H) are gated out (i.e., excluded). These first two steps exclude all events except viable singlets; viable singlets are referred to as “all cells.” Lastly (top row, right), data for all viable cells are displayed as CD45 intensity by SSC-A, characteristics that divide cells into component WBC subgroups: lymphocytes (green, “LY,” bright CD45, low SSC-A), monocytes (yellow, “MO,” with brighter CD45, higher SSC-A), mature granulocytes (blue, “MY,” with intermediate intensity CD45 and high SSC-A), erythroid cells and plasma cells (purple, “OTHER,” with negative CD45 and intermediate SSC-A), blasts (red, “BL,” with dim CD45 and low SSC-A), and hematogones (not included in this specimen, “HG,” with dim CD45 and low SSC-A). In this peripheral blood specimen, the increase in blasts suggests acute leukemia. Further analysis is then performed (bottom row scattergram) by excluding all CD34-negative cells. The leukemic blast population is clearly seen.

Figure 2.6. Detection of a normal population of T cells. T cells are identified by expression of CD3 (x axis, first three scattergrams). Mature, post-thymic mature peripheral T cells should express all pan-T–cell antigens (i.e., CD2, CD3, CD5, and CD7). In this tube (tube T1 from Table 2.2), analysis for presence of a T-cell neoplasm begins with focusing on the CD3+ cells (x axis) and assessing their expression of CD2 (top row, left scattergram), CD5 (top row, middle), and CD7 (top row, third from left). These data show clear coexpression of CD2 and CD5. For CD7 a continuum of CD7 expression is seen so that most T cells show relatively bright CD7, but expression tapers toward lower and then to negative. Such a continuum of CD7 expression is common in reactive, normal T cells. If, rather than a continuum, a discrete, separate population with low/negative CD7 is detected, that would suggest the possibility of a T-cell neoplasm (see Fig. 2.7). Also, peripheral T cells should express CD4 or CD8, not both. A minor population of CD4+CD8+ “double-positive” T cells is visible (gray box) but these cells do not constitute a discrete population; rather, they are scattered in distribution, also a normal finding.
Figure 2.7. T-cell neoplasm in peripheral blood; discrete neoplastic population. T cells are identified by expression of CD3 (x axis, top row). According to most texts, all mature, post-thymic, peripheral T cells should express all pan-T–cell antigens (i.e., CD2, CD3, CD5, and CD7). In this case (tube T1 from Table 2.2), analysis for a T-cell neoplasm begins with focusing on the CD3+ cells (x axis) and assessing their expression of CD2 (top left), CD5 (top middle), and CD7 (top right). These data show two separate, clearly distinct populations (bottom row, left and center). The first, normal T cells (blue ovals) show uniform coexpression of CD2 and CD5. The other cell population (red ovals) is abnormal. Rather than representing a continuum, it forms a separate, discrete population with uniform coexpression of CD2 and CD5, but with dim CD3 and loss of CD7. Also, the dim CD3+ population shows uniform, restricted expression of CD4 and is CD8−, further confirmation that it is a neoplastic population. These findings are consistent with a peripheral T-cell lymphoma. Note that focusing merely on the CD4:CD8 (bottom right scattergram) would give a false-negative assessment of neoplasia as the ratio is normal.
Figure 2.8. B-cell ALL in peripheral blood with aberrant CD10 expression. Gating begins with exclusion of doublets and cell clusters (top left), followed by exclusion of nonviable cells (top row, second from left; nearly all cells in this specimen are viable), then display of all cells, CD45 by SSC-A data (top row, third from left). This shows a marked relative decrease in normal lymphoid (Ly), monocytic (Mo), and granulocytic (My) cells. There also is a marked increase in blasts (dim CD45, low SSC-A). The blasts coexpress CD19 (dark blue; all cells scattergram, top right). Whereas normal, mature B cells express polytypic kappa and lambda surface immunoglobulin (blue ovals; two bottom left scattergrams), the abnormal cells (red ovals) are negative for both kappa and lambda, consistent with immature B cells and typical of B-ALL. In contrast to the reactive, background B cells, which show dim/negative CD10 expression (blue oval; bottom third from left), the B-ALL cells contain a subpopulation positive for CD10 and a subpopulation with abnormally bright CD10 (two red ovals, same panel). The neutrophils (aqua blue; yellow arrow) show a characteristic, normal level of CD10 that can be used as an internal control. The final scattergram shows green (CD5+/CD19−) T cells and the B cells show no discrete expression of CD5.

Figure 2.9. Enumeration of blood for markers associated with paroxysmal nocturnal hemoglobinuria (PNH). Upper row: the red blood cell (RBC) assay using CD23a–FITC/CD59-PE staining. RBCs are gated on FS and SS (R1, upper left plot) and displayed on FCS versus CD235a–FITC plot (upper middle). CD235a-positive RBCs are gated (R2). RBCs from region R1 + R2 are analyzed for CD59 expression (right upper plot). Normal RBCs (CD59 bright) are in region I. RBCs with PNH-related phenotypes (i.e., with CD59dim expression or CD59 negative) are in regions II and III, respectively. Middle and
lower row: white blood cell (WBC assay) using staining with FLAER, CD24PE, CD15PECy5, and CD45PECy7. Light scatter voltages were established so that all nucleated cells are visible above the forward scatter threshold (middle left) and debris was excluded with a combination of light scatter and CD45 gating (middle plot). CD45+ events are displayed on CD15 versus SS plot (middle right plot), and granulocytes (bright CD15, high SS), monocytes (dim CD15 and intermediate SS), and lymphocytes (CD15-negative, low SS) are gated. Each of these populations is displayed on a FLAER versus CD24 plot (bottom row). PNH granulocytes (FLAER-negative, CD24-negative) are enumerated in the bottom right plot (lower left quadrant). Normal granulocytes are enumerated in the upper right quadrant. Gated monocytes are similarly displayed (bottom row middle) and the PNH monocytes (FLAER-negative, CD24-negative) are enumerated in the lower left quadrant. Gated lymphocytes (bottom row left) are assessed for PNH phenotypes in the lower left quadrant. Normal T-lymphocytes (FLAER+, CD24-negative) are visible in the lower right quadrant and normal B-lymphocytes (FLAER+, CD24+) are visible in the upper right quadrant. (Courtesy of Dr. D. Robert Sutherland, Laboratory Medicine Program, University Health Network, Toronto General Hospital, Toronto, ON, Canada. From Porwit A. Clinical flow cytometry. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe’s Clinical Hematology. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:19–45.)

Figure 2.10. Peripheral blood of AML with aberrant expression of CD7. Color schema as follows: LY, lymphocytes (green); MO, monocytes (orange); MY, mature myeloid cells (aqua blue; mainly neutrophils); OTHER, red cells, plasma cells, and any nonleukocytes (pink); BL/Baso, blasts and basophils (red), HG, hematogones (dark blue; i.e., reactive pre-B cells). CD45 by SSC-A gating shows an abnormally large blast population and relative decreases in all normal WBC subsets (top left scattergram). Computation of the percentage of blasts is performed by dividing the percentage of cells in the blast gate (red ovals) by the percentage of viable, singlets (i.e., “all cells”); in this sample, blasts compose 68% of analyzed cells (e.g., 57.6% ÷ 84.5%), far too high to represent a reactive process. The blasts (CD117+; middle row, center scattergram) show partial aberrant expression of CD7, a finding suggestive of a myeloid neoplasm. If antigen expression levels are not clear by scattergram analysis, display of cell number (y axis) by level of expression (x axis) as histograms (bottom row) sometimes is clarifying. In the lower right histogram, the level of
CD7 expression can be gauged by comparing the level in reactive T cells (green, bright) to neutrophils (aqua blue, negative). Note that the blasts (red) show partial, dim coexpression of CD7 (yellow arrow).

**Figure 2.11.** AML studied at diagnosis and after initial therapy (EuroFlow AML4). These scattergrams are all from the same patient. The first specimen (A) is from the time of initial diagnosis; the second specimen (B) was received a month later. Displaying the blast gate only (red), the initial diagnostic specimen contains an expanded blast population with uniform coexpression of CD34 and CD117 and a minor subpopulation that expresses CD117 but not CD34 (yellow oval). Comparing the initial to the post-therapy specimens shows a nearly identical population, scattergram after scattergram (blue arrow). Minor changes occur in the relative proportion of neoplastic subsets: CD117+/CD34− cells (upper yellow oval) are fewer in the initial specimen, but are relatively increased after therapy (lower yellow oval). Similarly, comparing the initial to the post-therapy specimen shows a relative increase in neoplastic myeloid blasts with aberrant coexpression of CD7 (green arrow). **C:** Overlapping display of data at two time points. A useful technique with many standard image-display software programs is to display the initial diagnostic specimen and the same tube of the post-therapy specimen simultaneously. Flipping back and forth between images of the two...
specimens is usually sufficient. However, overlapping the displays also can be useful.

**Figure 2.12.** A: Use of MFC for the assessment of CLL in dilute cerebrospinal fluid. LY, lymphocytes (green); MO, monocytes (orange); MY, mature myeloid cells (aqua blue; mainly neutrophils); OTHER, red cells, plasma cells, and any nonleukocytes (pink); BL/Baso, blasts and basophils (red), HG, hematogones (dark blue; i.e., reactive pre-B cells). The standard gating scattergram (top right) of this CSF specimen shows WBC subsets as expected for peripheral blood, which indicates hemodilution (i.e., the specimen is
contaminated with blood rather than representative of the CSF); the most likely cause is a traumatic spinal tap procedure. Although careful examination of the scattergrams in the bottom row does not conclusively reveal CLL, the possibility of a false-negative assay due to hemodilution cannot be excluded. Rather than reporting a “negative” assay, qualifying the interpretation as possibly false negative is critical. In such cases, submission of another specimen should be recommended. B: MFC in assessment of CSF for CLL where no blood contamination is present. The standard gating scattergram (top right) of this CSF specimen shows only lymphoid cells; WBC subsets expected in PB (e.g., neutrophils) are not present. This indicates a clean, nonhemodiluted CSF specimen, reliable for MFC diagnostic purposes. Standard scattergrams (middle row) show a population of B cells (CD19⁺; blue circles) that coexpress CD5 and dim monotypic lambda light chains. Selective gating on the CD19⁺ population (bottom row) confirms that the CD5⁺ B cells show very dim monotypic expression of surface lambda immunoglobulin light chains. These findings are consistent with CLL involvement of the CSF.
Figure 2.13. MFC of acute bilineal leukemia. A: In this specimen, SSC-H by CD45 gating shows two, distinct, nonoverlapping populations of blasts. The first population (red) is more AML-like, with relatively greater SSC, expression of CD33 but HLA-DR variable/dim/negative. The second population (green) is more ALL-like, with extremely low SSC, negative for CD33 but HLA-DR positive. B: Further analysis shows that the AML-like population (red) expresses CD11b and CD34 but is negative for CD10 and CD19; with respect to these antigens, the ALL-like population (green) shows the opposite. C: Still further analysis shows that the B-ALL-like population (green) expresses dim CD5 and cytoplasmic CD3, but is CD4-negative; with respect to these antigens, the AML-like population (red) shows the opposite. In summary (see Table 2.5), these findings are consistent with acute bilineal leukemia, including 42% AML-like cells, and 19% biphenotypic ALL-like cells with predominant expression of B-cell antigens, but also with cytoplasmic CD3. Considering the unusual nature of this case, IHC was performed for confirmation (see Figs. 2.14 and 2.15).
Figure 2.14. **A:** Confirmation of bilineal acute leukemia by IHC; myeloid markers. IHC of serial sections shows immunoarchitectural features consistent with spatially separate populations of myeloid blasts (CD117+, upper left; PAX5-negative, upper right; the lower images are higher power views of the outlined areas in the upper images). Arrows indicate which areas are magnified.

**B:** Confirmation of bilineal acute leukemia by IHC; lymphoid markers. IHC of serial sections shows immunoarchitectural features consistent with spatially separate populations of lymphoid blasts (CD117-negative, upper left; PAX5+, upper right; the lower images are higher power views of the outlined areas in the upper images). Arrows indicate which areas are magnified.
Figure 2.15. After therapy for the acute bilineal leukemia (AML/ALL) represented in Figures 2.13 and 2.14, a repeat specimen showed no evidence of the ALL-like component, but a residual AML-like component was demonstrated by multiplex IHC. Staining for CD117 (blue) and glycophorin C (red) showed the persistence of AML-like blasts (blue, CD117+ only membranes, negative for glycophorin C); the background marrow shows red, glycophorin+ only erythroid precursors. There are rare reactive, erythroid pronormoblasts (coexpression of CD117-blue with glycophorin-red yields blasts with blue + red = purple membranes).
Figure 2.16. MFC of peripheral blood B-ALL with typical antigen expression. Color schema as in Figure 2.4 and as below. Both B- and T-ALL cells characteristically express low CD45 (sometimes completely negative) and low SSC-A. The B-ALL cells (red, top row) typically show bright CD10 and are CD34+ but CD117− and are also negative for myeloid antigens, such as CD11b. B-ALL cells (black, middle row) coexpress B cell antigens CD9 and CD22, with CD34 and TdT, but are CD7−. Further analysis for cytoplasmic antigens (black, bottom row) shows that the B-ALL cells express cCD79a, but not MPO. Lastly, B-ALL cells are negative both for surface CD3 (smCD3, lower right) and for cytoplasmic CD3 (cCD3); in this scattergram, smCD3 is clearly negative, but there is an artifactual background shift seen for cytoplasmic CD3; the internal negative control population is neutrophils (aqua blue); the internal positive control population is T cells (green).

Figure 2.17. MFC of cerebrospinal fluid that shows presence of T-ALL. Color schema as follows: T-ALL cells, black; green, lymphocytes; aqua, neutrophils; orange, monocytes; red, reactive hematopoietic blasts. The CSF specimen mainly contains reactive T cells (green, cCD3 bright, last scattergram), but also contains an abnormal a population of dim CD45+ cells with low SSC-A (black; gating not shown). They coexpress CD34, TdT, and relatively dim cCD3, consistent with T lymphoblasts. They also show dim/partial aberrant expression of CD13 (third scattergram from left). Side-by-side comparison to the patient’s prior, diagnostic T-ALL specimen shows the same, abnormal immunophenotype. These findings are consistent with CSF involvement by T-ALL.

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Figure 2.18. MFC of normal B cells. Reactive, nonneoplastic B cell populations are mainly mature, sIg+, but polytypic, mainly sIgk+, but also including sIg-lambda+ population (sIgk:sIgl typically is about 2:1). Reactive B cells are mainly CD10−, but typically include a CD10− subpopulation that displays as a teardrop-shaped continuum (yellow outline), mostly CD10−, but trailing off with a minor CD10+ subpopulation. Similarly, a minor number of scattered, CD5+, nonneoplastic B cells may be seen (far right scattergram), but no tightly clustered, discrete aberrant populations are present.
Figure 2.19. MFC of lymph node showing involvement with lymphoma. Examination of scattergrams showing all cells (top row); the CD19\(^+\) B cell population (blue) appear polytypic, with only slight abnormalities. Evaluating the cells using CD20 (middle row) shows a clearer population of monotypic B cells within the polytypic background. However, examining only the CD19\(^+\) B cells versus expression of CD10 (bottom row) shows a clear, monotypic, lambda-restricted population with coexpression of CD10 (green circle) in a polytypic background (red circles). These findings are consistent with partial involvement by follicular lymphoma (FL).
Figure 2.20. **A:** Demonstrating an sIg-negative B-cell neoplasm by MFC. T cells, green; neutrophils, aqua; monocytes, orange; reactive B cells (normal), blue; neoplastic B cells (abnormal), red. These scattergrams show a reactive population of polytypic B cells (blue). However, there also is a clearly abnormal B-cell population, negative for surface Ig, but with bright expression of CD10 (red). The differential diagnosis for an sIg-negative, bright CD10⁺ B-cell neoplasm includes follicular lymphoma (FL), but is more commonly encountered in B-ALL. **B:** Diagnostic algorithm for low-grade B-NHL, subtype CLL. Once the diagnosis of low-grade B-NHL is established by a combination of morphology and surface immunoglobulin light chain expression, or by morphology and immunostaining, subtyping begins with assessment of CD5. CD5 is positive in CLL and MCL. If CD5 is positive, the next step is assessment of cyclin D1, a nuclear antigen, aberrantly expressed in MCL, but negative in CLL. For technical reasons, cyclin D1 expression cannot be assessed by MFC. Though cyclin D1 assessment is necessary to exclude MCL, another means of distinguishing CLL from MCL is CD23, which is typically positive in CLL but negative in MCL. In this example, MFC showed a monotypic sIg-restricted B-NHL that is morphologically low grade. It is CD5⁺ and CD23⁺. The presumptive diagnosis is CLL, but it should be confirmed by IHC assessment of cyclin D1 expression, or by FISH, showing the absence of t(11;14).
Figure 2.21. Diagnostic algorithm for low-grade B-NHL, subtype FL. Once the diagnosis of low-grade B-NHL is established by a combination of morphology and surface immunoglobulin light chain expression, or by morphology and immunostaining, subtyping begins with assessment of CD5. CD5 is negative in FL and MZL. If CD5 is negative, the next step is assessment of BCL6, a nuclear antigen, always expressed by FL, but never by MZL. For technical reasons, BCL6 expression cannot be assessed by MFC. Another means of distinguishing FL from MZL is CD10, which is typically positive in FL and always negative in MZL. Because CD10 is only expressed in about two-thirds of FL cases, negative expression does not exclude FL. In CD5−, CD10− low-grade B-NHL, subtyping requires the addition of conclusive morphology and/or cytogenetic analysis, showing t(14;18) in FL (negative in MZL). In this example, MFC shows a monotypic sIg-restricted B-NHL that is morphologically low grade. Morphology shows cytologically variable, small mature cells, some with cleaved nuclei. It is CD5− and CD10+. The presumptive diagnosis is FL, but it should be confirmed by histology and/or IHC or by FISH, showing t(14;18).

Figure 2.22. Diagnostic algorithm for low-grade B-NHL, subtype MZL. Once the diagnosis of low-grade B-NHL is established by a combination of morphology and surface immunoglobulin light chain expression, or by morphology and immunostaining, subtyping begins with assessment of CD5. CD5 is negative in FL and MZL. If CD5 is negative, the next step is assessment of BCL6, a nuclear antigen, always expressed by FL, but never by
MZL. For technical reasons, BCL6 expression cannot be assessed by MFC. Another means of distinguishing FL from MZL is CD10, which is typically positive in FL and always negative in MZL. Because CD10 is only expressed in about two-thirds of FL cases, negative expression does not exclude FL. In CD5−, CD10− low-grade B-NHL, subtyping requires the addition of conclusive morphology and/or cytogenetic analysis, showing absent t(14;18) in MZL. In this example, MFC shows a monotypic sIg-restricted B-NHL that is morphologically low grade. Morphology shows a uniform population of small, mature lymphocytes with monocytoid morphology (relatively abundant cytoplasm). It is CD5− and CD10−. The presumptive diagnosis is MZL, but it should be confirmed by histology and/or IHC; FISH should demonstrate the absence of t(14;18). Lastly, other CD5−, CD10− lymphomas include LPL/WM and HCL (bottom right rectangle).

Figure 2.23. MFC in diagnosis of hairy cell leukemia. Initial gating (upper right) shows monocytopenia, which is typical in HCL. Selective gating on CD19+ B cells shows monotypic expression of lambda light chains in cells that are CD5− (upper left) and CD10− (middle row). Analysis also shows bright coexpression of CD22 and CD103, exceptionally bright expression of CD11c, along with coexpression of CD25 (bottom row). These findings are typical of HCL.

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Figure 2.24. Precise typing of B-NHL affects therapeutic decisions. Although a discussion of therapy is beyond the scope of this chapter, B-NHL can generally be divided into low grade (left of dashed line) and high grade (right of dashed line). For low-grade B-NHL, the clinical approach is “watch and wait,” expectant management (exceptions include local therapy for extranodal MZL, and MCL may require intensive induction chemotherapy and bone marrow transplant depending on the proliferation index). By contrast, high-grade lymphomas are treated with induction regimens.
Figure 2.25. A: Evaluation by MFC of reactive rather than neoplastic T and NK cells. T cells are defined by expression of CD3, either surface or cytoplasmic (cCD3). Initial assessment of T-NHL is with CD2 through CD7, CD8, and CD57 (e.g., tube T1 in Table 2.2, represented here). The most common, reliable finding suggestive of a T-NHL is discrete loss of a pan-T–cell antigen, usually CD7. Note that the CD3
+ T cells show clear coexpression of CD2 and CD5 (top left scattergrams). Although T cells lacking CD7 are present, they exist as a part of a continuous population with CD7
+ T cells, trailing off in a teardrop shape (black outlined teardrop, upper right). This situation is not suggestive of a T-NHL because there is no discrete population with loss of CD7 that is separate from the background T cells. NK cells also are identified in this tube (blue balloons).

B: Advanced analysis of T cell populations. (Color schema as in A.) Initial assessment for T-NHL by CD2 through CD7, CD8, and CD57 (e.g., tube T1 in Table 2.2, pictured here) can sometimes establish or exclude T-NHL. Then, for T-NHL subtyping, a second tube should be analyzed (e.g., tube T2 from Table 2.2) can sometimes establish or exclude T-NHL. In most reactive specimens and most T-NHL, the T cells express α/β-type T-cell receptors (TCR A/B; top left scattergram); TCR γ/δ-type receptors (TCR G/D, bottom middle scattergram) are expressed by rare reactive subsets and usually by hepatosplenic-type T-NHL. Most reactive T cells are negative for CD16 and CD56 (antigens more commonly expressed by NK cells; dark green). This ancillary panel includes CD25 and CD30 for subtype diagnosis of ATLL and ALCL, respectively. Also, assessment of CD30 is important because it is a target of available, selective drugs. Lastly, this ancillary tube can be used to assess NK cells (green balloons).
Figure 2.26. T-cell large granular lymphocyte leukemia. Although these scattergrams show no clear, discrete, abnormal T-cell population, using the calculation shown, there is an absolute expansion of CD3+/CD8+ T cells (green boxes) with partial expression of CD57. Note that to calculate the absolute number of CD3+/CD8+ cells, their “%Total” displayed by the MFC software must be divided by “All Cells,” to exclude dead cells and doublets. In conjunction with neutropenia (note low number of aqua blue neutrophils in upper scattergram; reported Absolute Neutrophil Count (ANC) was low at 844 neutrophils/mL [reference range 1800 - 7000 mm3]) and the remainder of the clinical workup, this suggests the possibility of T-LGL. For confirmation, T-cell PCR should be performed for evaluation of a monoclonal T-cell γ receptor mutation and a repeat specimen should be analyzed after an interval of at least 6 months.

Figure 2.27. Immunophenotype differentiating NK cells from T cells.
Figure 2.28. Reticulin fibrosis in multiple myeloma. In normal hematopoietic marrow, reticulin fibrosis is limited to blood vessels. The left image of a normal marrow shows reticulin fibers (stained black) only incorporated in blood vessel walls. The right image shows marrow from a patient with an intense myeloma infiltrate accompanied by a marked increase in reticulin fibers. The neoplastic plasma cells in a myeloma infiltrate typically cause focal fibrosis in the areas of involved marrow (uninvolved marrow, not shown here, is not fibrotic). Thus, myeloma cells are underrepresented in aspirates in half of the patients.  

Figure 2.29. MPF for myeloma (MM) cells. LY, lymphocytes (green); MO, monocytes (orange); MY, mature myeloid cells (aqua blue; mainly neutrophils); OTHER, red cells, plasma cells (PC), and any nonleukocytes (pink); BL/Baso, blasts and basophils (red), HG, hematogones (dark blue; i.e., reactive pre-B cells). In this patient’s marrow, the upper left image shows the core biopsy packed with CD138+ MM cells. By contrast, MM cells are not
detected by MFC with CD45 by SSC-A gating with the usual number of cells collected (upper right). Use of an increased number of cells reveals 0.2% PCs (pink; bright CD38/dim CD45, upper middle image). In the middle row of scattergrams, the left image shows that the pink cells have bright restricted cytoplasmic kappa ( Clyk/bright CD38 (the central image shows negative cytoplasmic lambda/bright CD38), and the right image shows apparently negative MFC for CD138 and CD56 (pink balloon), both of which had shown strong, uniform coexpression by IHC in the biopsy (upper left image). In the bottom row, the left image shows restricted bright kappa in the pink MM population, again with false-negative CD138; the middle panel shows the lack of lambda staining. The calculations in the bottom right table show that MFC detected only 0.1% Clyk-restricted MM cells and there was false-negative expression of both CD56 and CD138 (pink and black rectangles). In summary, core biopsy IHC showed 90% MM cells, whereas MFC detected only 0.1%.

**Figure 2.30.** The percentage of plasma cells (PCs) from different specimen types from patients with myeloma. The image shows the retrospective analysis of 73 myeloma patients, including manual MM cell counts from biopsies stained for CD138, marrow aspirates, cytopsins after processing for MPF, and quantitation by MFC. The percentage of PCs was typically highest in the CD138 IHC-stained core biopsy, lower in in aspirate smears, dropped precipitously after processing for MFC, and was lowest (sometimes undetectable) by MFC. Focal fibrosis typically accounts for the underrepresentation of plasma cells in aspirate smears, and poor survival ex vivo seen after processing for MFC is due to the absence of stromal cytokines and other support.

Figure 2.32. Analysis of lymph node cytology. The key to exclusion of lymphoma in a cytology specimen is the assessment of cytologic variability versus monotony. This image of a normal lymph node shows the marked cytologic variability typical in a reactive lymphoid infiltrate. Also, reactive populations are composed mainly of T cells, recognizable by their small size, scant cytoplasm, and round nuclei with hyperchromatic chromatin. By contrast, a lymphoma usually shows a population with morphologic monotony because it is a clonal, and, as most lymphomas are B-cell lymphomas, the relative number of T cells is greatly diminished.
Figure 2.33. **A:** Standard immunohistochemistry (IHC) technique. In standard IHC, each immunostain is performed on a separate slide. In the example shown, a membrane stain is performed to identify a cancer population (upper slide). To test for expression of a nuclear antigen, a second immunostain is performed on a second slide. Although sufficient for analysis of solid organ cancers, standard IHC is often inadequate in marrow and lymph nodes biopsies as these specimens contain an admixture of reactive hematopoietic cells and cancer cells. In the example shown, one may not be able to tell whether the second antibody is expressed by the cells of interest identified on the first slide. **B:** Multiplex immunohistochemistry technique (mIHC) provides a precise diagnosis for hematologic tumors. With mIHC, multiple immunostain reactions are performed on a single slide, each with a unique color chromogen. In this example, a red CD138 membrane stain is performed to identify myeloma cells. A brown Ki67 nuclear stain is performed to assess proliferation along with a nuclear counterstain (blue). To compute the myeloma cell-specific proliferation index, myeloma cells (red, CD138+ membrane) are counted as either proliferating (brown, Ki67+ nucleus) or nonproliferating (blue, negative counterstained nucleus). By this mIHC method, bystander cells are excluded from the analysis.
Figure 2.34. A: Standard IHC for anaplastic large cell lymphoma (ALCL) may be erroneous. In this example, standard IHC is performed on serial sections (ALK1 IHC on the left panel and CD20 IHC on the right panel). Neoplastic cells are identified by ALK1; an area containing ALK1+ cells (red circle, lower left) is compared (yellow arrow) to the same area on the CD20 section (red circle, right); upon analysis at high magnification (insets), it appears that the ALK1+ cells identified in the left panel coexpress CD20 in the right panel. As such, the rendered (incorrect) diagnosis would be ALK+ diffuse large B-cell lymphoma. However, as shown in B, this is not correct. B: Multiparameter IHC for ALCL. mIHC performed on a single slide from the same case clearly shows that the large B cells (CD20+, brown) do not coexpress ALK1 (red). Further mIHC evaluation (data not shown) demonstrated T-cell antigen expression in the ALK1+ cells. In summary, the correct diagnosis is ALCL (a T-cell lymphoma), but an incorrect diagnosis (i.e., ALK+ diffuse large B-cell lymphoma) would have been rendered by standard IHC.
Figure 2.35. miHC for CD138/cyclin D1 in myeloma. Cyclin D1 is never expressed by nonneoplastic plasma cells (PC). In this marrow miHC image, PCs are identified by red CD138$^+$ staining of cell membranes. Coexpression of cyclin D1 (brown nuclei; brown arrows) indicates either t(11;14) translocation or hyperdiploidy in a particular PC, both of which are diagnostic of neoplasia. Cyclin D1-negative, nonneoplastic PCs have blue, counterstained nuclei (blue arrows). By standard, single stain IHC, it would not be possible to tell with certainty whether the cyclin D1$^+$ nuclei were in PCs or in other cells (e.g., stroma or histiocytes; brown circles).
Figure 2.36. A: mIHC for plasma cell proliferation index. By this method, the plasma cell proliferation index (PCPI) is performed by counting PCs (red, CD138+, membrane staining) as either proliferating (containing a brown, Ki67+ nucleus) or nonproliferating (blue counterstained nucleus). The index is performed by counting 200 cells and reported as a percentage of proliferating PCs. B: mIHC for myeloma cell proliferation index. By this method, the PCPI is performed by counting PCs (red, MUM1+ nucleus) as either proliferating (blue Ki67+ signal, combined with red, MUM1+ signal = purple nucleus; purple arrow) or nonproliferating (red, MUM1+/Ki67(−) nucleus; red arrow). Blue, Ki67+/MUM1(−) cells are background proliferating hematopoietic cells, not included in the analysis. Because a counterstain is not used, double-negative cells are invisible. The index is performed by counting 200 PCs and reported as a percentage of proliferating PCs.
Figure 2.37. Congophilia (dark red areas seen on a Congo red slide by standard light microscopy; left) indicates amyloid. Confirmation requires non–gray/white birefringence when viewed by polarized light (right). Classically, birefringence is apple green, but it may also be yellow or orange.
Figure 2.39. Magnetic bead column cell separation for FISH analysis. Because of the difficulties of analyzing plasma cells (PCs) described above, prior to FISH, cell selection is performed using CD138, an antigen uniquely expressed by PCs. Anti-CD138 antibodies are bound to magnetic beads (upper left, schematic representation), then placed into a column (lower left) that is inserted into a magnet (middle panel). The cellular specimen is pipetted into the bead column where only CD138⁺ PCs (yellow cells, lower right) bind to the beads and in turn are retained by the column walls (lower right), whereas all other cell types (green cells, CD138-negative; right middle) flow through the column. Subsequently, the column is removed from the magnet and the bead-bound PCs are eluted and then used for FISH. (Adapted from Miltenyi Biotec. http://www.miltenyibiotech.com/en.aspx.)
Figure 2.40. Polymerase chain reaction (PCR). **A:** A three-stage conventional PCR, with denaturation, annealing, and extension steps. Components of the typical PCR are illustrated including a DNA template (e.g., target gene), unlabeled nucleotides (dNTPs), a DNA polymerase to copy the templates and forward (F) and reverse (R) DNA primers, one of which is fluorescently labeled (asterisk). **B:** Fluorescent products from the above PCR are then detected by capillary electrophoresis. Shown is a trace with a normally sized 167 base pair (bp) NPM1 gene product and an abnormal copy with a 4bp insertion (171bp) characteristic of acute myeloid leukemia. **C:** Quantitative PCR using the TaqMan method with four samples showing differing amounts of the target gene as indicated by Cts ranging from 23 to 39 cycles (arrows). A graph showing 10-fold dilutions of a reference sample is plotted below, which are used to convert Ct in patient sample into copy number. **D:** Design of a TaqMan qPCR assay for detection of the JAK2 V617F mutation, with identical F and R primers but two different fluorescent probes; the red one detecting the normal JAK2 sequence (“G” at that position) and a green probe recognizing the mutated “T” sequence. The black 3’ moiety on the probes represents the quencher dye. (From Jones D. Molecular diagnosis in hematology. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe’s Clinical Hematology. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:58–64.)
Figure 2.41. DNA sequencing. Steps in the dideoxy chain termination (Sanger) method follow. Step 1: Standard polymerase chain reaction (PCR) to produce large amounts of a gene-specific template, detected by slab electrophoresis followed by ethidium bromide staining of the gel. Step 2: Unidirectional (or asymmetric) PCR using the template from the first PCR along with either a forward or reverse primer in a reaction containing normal nucleotides mixed with chain terminating A, C, G, and T bases. Step 3: The range of products from the asymmetric PCR that are terminated at every possible base in the PCR amplicon are then separated by capillary electrophoresis and detected by a laser recognizing the fluorochrome/nucleotide present at the end of products. Base-calling is performed using software which normalizes the peak heights to produce the depicted electropherogram. (From Jones D. Molecular diagnosis in hematology. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe’s Clinical Hematology. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:58–64.)
Figure 2.42. PCR detection of a monoclonal band in a polyclonal background. PCR is performed for the TCR-γ chain and the TCR-γ PCR product is run on a polyacrylamide gel. In a normal, reactive lymphoid population, a polyclonal smear is detected, representing many different PCR products of different sizes. Here, such smears are seen for three patients (patient samples run in duplicate, Pt 1, Pt 2, and Pt3), as well as in the negative control (NC). However, the sample from Pt 1 also contains a monoclonal band (red arrow), clearly visible in the polyclonal background (red box). The band is the same size as that seen in the positive control (PC) sample (green arrow). In the proper context, this would confirm the diagnosis of a peripheral T-cell lymphoma (i.e., a clonal product).

* Unless otherwise indicated, the color schema in all MFC examples in the figures in this chapter is as follows: LY = lymphocytes (green); MO = monocytes (orange); MY = mature myeloid cells (aqua blue; mainly neutrophils); OTHER = red cells, plasma cells, and any non-leukocytes (pink); BL/Baso = blasts and basophils (red), HG = hematogones (dark blue; i.e., reactive pre-B cells). The standard display of MFC data is log-scale, relative intensity of signal or expression. Therefore, scale numbers are relative, inconsequential, and often not displayed. In most centers, 1 log greater than background is considered “dim+,” another log greater = “positive,” another log greater = “bright.”
The criteria for anemia depend upon age, gender, ethnicity, and pregnancy status. The World Health Organization (WHO) has defined anemia as a hemoglobin level below 13 g/dL (hematocrit [Hct] <39) in adult males and below 12 g/dL (Hct <36) in adult females.\(^1\) For persons of African origin, the lower limit of normal hemoglobin is about 0.5 g/dL less.\(^2\) In newborns, hemoglobin level averages 16.5 g/dL, falling to 12 g/dL between age 6 months and 2 years, then rising to normal adult levels by age 6 years. For accurate diagnosis of anemia, hemoglobin levels must be assessed with a reliable method, such as the cyanmethemoglobin method.\(^3\) Maintenance of a normal hemoglobin level requires good nutrition, normal gastrointestinal (GI) function, and absence of chronic diseases. Thus, although anemia is common in the elderly, in most cases it is not caused by normal aging; rather anemia is secondary to the effects of other diseases, especially chronic renal or inflammatory disease.\(^4\) Whether normal aging may be the cause of some anemia in the elderly is under active research.

Physical signs and symptoms caused by anemia depend upon rate of onset, exercise tolerance, and degree of anemia, and may include weakness, pallor, exertional dyspnea, tachycardia, dizziness, or poor concentration. Usually, tissue oxygen supply is not limiting for normal activity in anemia if the hematocrit is above 20%, unless the subject has underlying heart disease. In patients with coronary artery disease, angina may develop or worsen if anemia is present. Diminished tissue oxygenation may provoke the compensatory mechanisms of tachycardia and increased force of ventricular contraction, which patients sometimes detect as palpitations.

Physical examination may be unremarkable, or may show pallor of the conjunctiva, palms, and face, or in the presence of a hemolytic anemia, jaundice as well as pallor. A smooth or painful tongue may occur, especially with iron deficiency or macrocytic anemias. Flat, spoon-shaped nails (koilonychia) are seen in severe iron deficiency. Systolic heart murmurs, usually in the pulmonic area, can develop in anemia, probably resulting from a combination of lowered blood viscosity and increased blood flow across the heart valves. Pulmonary rales (“crackles”) or peripheral edema may reflect heart failure. Retinal examination in severe anemia may reveal hemorrhages that are white-centered (Roth spots), flame-shaped, or round. Some may be preretinal. The retinal veins are sometimes tortuous, and cotton wool spots, representing infarction of the nerve fiber layer, may occur. Ischemia of the vessels can lead to leakage of proteinaceous material, causing “hard” exudates.

Anemias are classified either morphologically (by erythrocyte size or shape) or by mechanism of hemoglobin reduction (decreased erythrocyte [RBC] production or increased RBC loss, either through increased RBC destruction or increased RBC loss as in bleeding). Normal RBCs are flexible biconcave discs about 8 μm in diameter with little variation in size or shape. Any deviation from normal morphology is associated with shortened RBC lifespan. The morphologic schema divides anemia into three groups, based on RBC mean corpuscular
volume (MCV): (1) normocytic (MCV 90–100 fL); (2) macrocytic (MCV >100 fL); and (3) microcytic (MCV <80 fL). In some anemias, the RBCs may vary considerably in size and shape and result in mixed morphologies. In hypothyroidism, for example, the RBCs may be normocytic or macrocytic. A valuable aspect of morphologic classification of anemia is that the measurement of RBC size is rapidly available from automated blood counts, and the differential diagnosis of microcytic and macrocytic anemias is limited. In contrast, the causes of normocytic, normochromic anemias are more numerous and complex (see below).

RBC production can be estimated from the abundance of erythroid precursors seen on bone marrow examination, but the simplest measure is by evaluating the number of newly released RBCs, i.e., reticulocytes, in the peripheral blood. Reticulocytes actively synthesize hemoglobin, and detection of their ribosomal RNA requires staining of RBCs with RNA-binding dyes, such as new methylene blue, revealing blue granules or networks of RNA, hence the name “reticulocyte.” Automated cell counters similarly use a fluorescing RNA-binding dye like acridine orange to detect reticulocytes; the reticulocyte count is expressed as an absolute number per volume of blood or as a percentage of the red cells. The percentage of reticulocytes needs to be corrected for degree of anemia by multiplying it by the patient’s hemoglobin (or hematocrit) divided by the normal value. For a hematocrit of 20% and a reticulocyte count of 6%, for example, the corrected reticulocyte count would be 6% × (20/45) = 2.6%. When the anemia is severe and polychromatophilia (a bluish tinge to the RBC cytoplasm) is prominent on the smear, a second correction is necessary because polychromatophilic RBCs circulate for 2 to 3 days, rather than for 1 day. The reticulocyte percentage that emerges from these corrections is called the reticulocyte index.

The reticulocyte count is important in distinguishing hypoproliferative anemias from ineffective erythropoiesis or increased bone marrow response to acute hemorrhage or hemolysis. The reticulocyte index, the bone marrow findings, and measures of hemolysis such as serum bilirubin and lactate dehydrogenase (LDH) permit an accurate designation of the type of anemia.

MICROCYTIC ANEMIA

The major causes of microcytic anemia are iron deficiency, the thalassemias, and anemia of chronic disease (ACD; in which microcytosis occurs in about 30% of cases). Less common causes include certain microcytic hemoglobinopathies such as hemoglobin C or hemoglobin E, in which latter microcytic anemia is accompanied by ineffective erythropoiesis with excess destruction of the developing, abnormal RBC within the bone marrow and sideroblastic anemias characterized by deficiencies in heme or porphyrin synthesis (either acquired, as in lead poisoning, or genetic). In iron deficiency, thalassemias, and microcytic hemoglobinopathies, the MCV is often smaller than in ACD, and the blood smear often discloses dramatic variations in RBC size and shape. This anisocytosis is captured in the RBC distribution width (or RDW) parameter.

Anemia of Iron Deficiency

It is estimated that there are more than 2 billion cases of iron deficiency anemia in the world making iron deficiency the commonest cause of microcytic anemia worldwide. Iron deficiency is caused by blood loss, inadequate dietary iron, rapid growth without iron supplementation, or poor iron absorption. Iron is normally the most abundant trace metal in the human body, totaling 3 to 4 g, mainly contained in hemoglobin, myoglobin, and enzymes;
only a minor fraction, about 1 g, is stored as a reserve for production of new hemoglobin. Normal iron balance is tightly maintained, with daily needs of about 1 mg of absorbed iron, reflecting a dietary intake of about 20 mg, to balance obligatory excretion of about 1 mg of iron in sweat, shed skin or shed mucosal cells. Little absorbable iron is present in many foods, including most fruits and vegetables and grains, where iron is tightly complexed to phytates. Good iron sources are meat, poultry, fish, eggs, and legumes. Because daily iron loss is slight in adult males, men need little dietary iron, and deficiency from inadequate dietary intake is uncommon in men. When iron utilization is increased as in infancy, during growth spurts, and in pregnancy, or when concurrent blood loss occurs, as in menstruation, dietary iron intake may be insufficient, especially in women and children who tend to consume less than the recommended minimal daily requirement. The problem increases during pregnancy, when maternal iron is diverted to the fetus for hematopoiesis and with breastfeeding, as iron is lost in the milk.

Plasma iron levels are closely regulated by the hepatic peptide hormone hepcidin, which limits the release of newly absorbed iron from both enterocytes in the GI tract and from macrophages in the bone marrow and reticuloendothelial organs by binding to the membrane iron transporter, ferroportin, which promotes degradation of both proteins and thus limits the amount of iron bound to transferrin available to maturing erythroblasts for hemoglobin synthesis. Inflammation, chronic disease, and cancer, as well as high plasma iron levels, all favor increases in hepcidin, resulting in decreased iron availability, and thus, decreased erythropoiesis associated with these disease states. Conversely, in iron deficiency, hepcidin falls, promoting iron trafficking. Erythroferrone present on developing erythroblasts is a natural inhibitor of hepcidin. Numerous other proteins including hemojuvelin, bone morphogenic protein-2, SMADs, furin, and TMPRSS6 (transmembrane protease serine member 6) affect hepcidin transcription. During infection, stimulation of toll receptors can directly depress ferroportin levels independently of hepcidin and thus lower plasma iron levels; in addition, activated neutrophils release the iron-binding protein lactoferrin, which further limits the availability of iron required for bacterial growth.

The reduction of body iron in developing iron deficiency anemia follows a standard pattern. Well prior to any anemia, iron stores (i.e., bone marrow ferritin) disappear, followed by a decrease in plasma iron levels and increase in unbound transferrin. Next, a normocytic anemia develops, which only with increasing iron deficiency becomes microcytic and then hypochromic. The reticulocyte count decreases. Thus, with mild and recent iron deficiency, the red cell indices (MCV, mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]) and the blood smear remain normal, but over time, increasingly severe anemia develops and the MCV and MCHC fall. Anisocytosis, resulting in an increase in RDW, occurs. Later morphologic changes in iron deficiency anemia include poikilocytosis, microcytosis, and hypochromia. Tiny microcytes, elongated pale elliptical RBCs (pencil cells), and target cells may be visible on the blood smear, but some of the erythrocytes may appear normal. Often, the platelet count is elevated, with characteristically very small, single platelets. In overt iron deficiency anemia, the serum iron is markedly decreased, whereas the total iron-binding capacity (i.e., transferrin level) is elevated, and the transferrin saturation is below 10%. The serum ferritin is markedly decreased (<20 ng/mL). Soluble transferrin receptors increase in iron deficiency but remain normal in ACD. Usually, the diagnosis of iron deficiency anemia is established by these tests, but occasionally, obtaining a bone marrow sample to verify the absence of iron stores or a trial of iron therapy may be necessary to confirm the presence of iron deficiency.

The clinical features of iron deficiency are generally similar to other anemias, e.g., fatigue,
weakness, pallor, poor exercise tolerance, but three distinctive findings are only seen in iron deficiency anemia: pica, koilonychia, and blue sclera. Pica is the craving for, and ingestion of, certain unusual substances, such as starch, dirt, clay, cardboard, and ice (pagophagia). Koilonychia, marked by fingernails that become thin, brittle, and concave (spoon-shaped), is virtually pathognomonic of iron deficiency. Thinning of the sclera from impaired epithelial growth causes a blue tint because of the more visible choroid beneath.

Iron deficiency anemia in adults frequently results from chronic blood loss. The major cause of blood loss in women of childbearing age is heavy menstruation or repeated pregnancies. In non-menstruating women and in men, the most common reason for iron deficiency is GI hemorrhage. However, in resource-poor countries, lack of dietary iron or parasitic infestation with GI or genitourinary bleeding are common causes of iron deficiency. Infants, children, and pregnant women develop iron deficiency because of poor dietary intake of iron in the face of increased demands such as growth spurts or later stages of pregnancy (a developing fetus takes about 400 mg of iron from the mother). Other less common reasons for iron deficiency include chronic hematuria, epistaxes, hemoptysis, or intrapulmonary hemorrhage in such disorders as idiopathic pulmonary hemosiderosis, microscopic polyangiitis, or Goodpasture syndrome. In hereditary hemorrhagic telangiectasia, repeated mucosal bleeding from the vascular malformations may result in severe chronic iron deficiency anemia only remediable by parenteral iron repletion. Rare causes of iron deficiency include intravascular hemolysis from such diseases as paroxysmal nocturnal hemoglobinuria (PNH) or mechanical heart valve–related fragmentation of RBC, conditions which result in chronic intravascular hemolysis that leads to chronic urinary excretion of iron as hemoglobinuria (for PNH) or hemosiderinuria (for both).

Iron deficiency occasionally develops from iron malabsorption related to chronic malabsorptive GI disease such as inflammatory bowel disorders or sprue, or following gastric bypass surgery. Iron is absorbed throughout the GI tract, but best in the upper duodenum. In the presence of small intestinal disease, such as celiac sprue, or after gastric resection or bariatric surgery, which may accelerate the movement of intestinal materials through the duodenum and thereby diminish absorption time, iron deficiency may develop. Indeed, iron deficiency anemia, often refractory to oral iron, frequently develops within a year in the majority of patients who have undergone gastric bypass procedures and may require parenteral iron for correction.

A rare variant of iron deficiency anemia, iron-refractory iron deficiency anemia (IRIDA), representing a mutation in the TMPRSS6 gene, has improved our understanding of susceptibility to iron deficiency. In IRIDA, the TMPRSS6 mutation interrupts the normal pathway that inhibits hepcidin transcription, so that hepcidin levels remain high, leading to blocked GI absorption of iron, and a microcytic, hypochromic anemia develops that is refractory to oral iron. Anemia severe enough to require parenteral iron may be required in some menstruating women. However, as normal release of macrophage iron is also blocked, IRIDA patients will show increased iron stores when treated with parenteral iron. Studying other variants in the TMPRSS6 gene has revealed that some variants are associated with low hepcidin levels which have been described in blood donors with a relative resistance to development of iron deficiency, whereas carriers of variants with high hepcidin levels are more susceptible to iron deficiency anemia after blood donations. Similarly, male blood donors who carry a common polymorphism for the HIF-1 α gene that affects protein stabilization and hypoxia have higher hemoglobin and ferritin levels after blood donation than persons who have the wild-type allele. In the future, therapies that alter hepcidin concentration may become important in managing both iron deficiency and iron overload.
**Sideroblastic Anemia**

Sideroblastic anemia results from impaired synthesis of heme. This impairment results in the presence of ring sideroblasts in the bone marrow. Ring sideroblasts are erythroblasts which contain a perinuclear ring of at least five blue granules representing iron-loaded mitochondria covering at least one-third of the nuclear circumference upon staining with Prussian blue. Sideroblastic anemia can be a genetic or acquired condition. The most common inherited form is X-linked sideroblastic anemia, which is caused by a germline mutation in the erythroid-specific aminolevulinic acid (ALA) synthase gene (ALAS2). ALAS2 catalyzes an early step in the synthesis of heme and mutation of this gene leads to impaired production. Because ALAS2 is an early step in the synthesis of heme, there is no buildup of toxic porphyrin metabolites or the associated symptoms seen with the porphyrias. Recent studies have identified mutations in a range of additional genes which can cause sideroblastic anemia. These include mutations in the glutaredoxin 5 (GLRX5) and the SLC25A38 genes, which encode mitochondrial proteins. Although X-linked sideroblastic anemia generally only affects males, there are documented cases of affected females as a result of autosomal dominance and lyonization. Acquired sideroblastic anemia is most often caused by myelodysplastic syndromes (MDS) or lead poisoning. MDS are a group of hematopoietic stem cell diseases with dysplasia in one or all of the major myeloid cell lineages; patients with MDS have a 15% chance of developing acute leukemia. The WHO defines three distinct entities for MDS with sideroblastic anemia: refractory anemia with ringed sideroblasts (RARS), refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T), and refractory cytopenia with multilineage dysplasia with at least 15% ring sideroblasts [RCMD(+RS)].

Beyond MDS, lead poisoning is a major cause of acquired sideroblastic anemia. Lead can inhibit porphobilinogen synthase, which leads to impaired heme synthesis. In addition, lead inhibits ferrochelatase, which catalyzes the insertion of iron into the completed porphyrin ring to produce heme. This inhibition results in the formation of zinc protoporphyrin (containing zinc instead of iron). Measurement of the zinc protoporphyrin:heme ratio can be used to detect lead poisoning. Beyond this, routine measurements of blood lead levels are possible using either atomic absorption spectroscopy or inductively coupled plasma mass spectrometry (ICP-MS).

**MACROCYTIC ANEMIAS**

Macrocytic anemias result from several mechanisms including slowed DNA synthesis in the bone marrow (leading to megaloblastic changes in RBC precursors), MDS, increased reticulocytosis, alcoholism, liver disease, hypothyroidism, and hemolysis or hemorrhage resulting in early marrow release of immature, large RBCs. In general, the macrocytosis in disorders where DNA synthesis is normal is mild (MCV 100–110 fL) and the enlarged RBCs are round, rather than oval, and often show polychromasia as they represent reticulocytes. Hypersegmented neutrophils, a feature of nutritional megaloblastic anemias, are not present in the nonmegaloblastic macrocytic anemias, except with myelodysplastic disorders.

**Megaloblastic Anemia**

Megaloblastic anemia is most often caused by nutritional deficiencies (especially deficiencies of folic acid or vitamin B₁₂, vitamins essential for normal DNA synthesis) or medications
that impair DNA synthesis or block folic acid metabolism, such as cytotoxic agents used in
cancer chemotherapy or immunosuppression (e.g., cyclophosphamide, azathioprine,
hydroxyurea, methotrexate, trimethoprim). In either instance, DNA synthesis is slowed
whereas protein synthesis continues normally, resulting in abnormally large erythrocyte
precursors (megaloblasts) in the bone marrow and large well-hemoglobinized RBCs
(macrocytes) in the blood. In addition to the macrocytic anemia, macrocytic leukopenia and
giant platelets are often present, and other nonhematopoietic cells such as skin cells and
cervical mucosal cells are also macrocytic.

Nuclear maturation of hematopoietic cells in the bone marrow involves rapid cell division,
which requires high levels of DNA synthesis. In situations where these high levels of DNA
synthesis cannot be supported, as in deficiencies of essential nutrients, there is defective
nuclear maturation of hematopoietic cells in the bone marrow. The defective nuclear
maturation is primarily due to inadequate production of thymidine, an essential nucleic acid
specific for DNA. Folic acid and vitamin B_{12} are essential in the production of thymidine
from deoxyuridine, participating in methylation of the latter nucleotide. Tetramethyl folate is
a methyl carrier, whereas vitamin B_{12} is the cofactor for the methyl transfer reaction.
Whereas thymidine deficiency slows nuclear maturation and cell division, cytoplasmic
maturation continues normally, as it is regulated by RNA (which does not require thymidine).
As a consequence, hematopoietic precursor cells become megaloblastic, i.e., unusually large
with relatively immature nuclei at each growth stage. This nuclear-cytoplasmic asynchrony is
seen not only in erythroblasts but also in granulocyte precursors and
megakaryocytes/platelets. The bone marrow is markedly hypercellular, but many of these
megaloblastic precursors are destroyed in the marrow before release into the circulation,
termed ineffective erythropoiesis. This intramedullary cell wastage is reflected by peripheral
leukopenia and thrombocytopenia as well as anemia, and by elevated levels of serum iron,
 unconjugated bilirubin, and LDH. Early in megaloblastic anemias, mild macrocytosis may
be the only abnormality in the peripheral blood, where only a small percentage of RBCs are
actually macroovalocytes. As anemia worsens, the peripheral blood smear typically shows
many RBC anomalies including anisocytosis, poikilocytosis, teardrop cells, schistocytes, and
basophilic stippling. Polychromasia is uncommon, and the reticulocyte count is
inappropriately low. Leukopenia and thrombocytopenia may occur. The presence of oval-
shaped macrocytes, giant platelets, and hypersegmented neutrophils (>5% of cells with five
lobes or any with six or more lobes) strongly suggests a megaloblastic process.

The clinical features of folic acid and vitamin B_{12} deficiency include signs of a hemolytic
anemia such as pallor, mild jaundice, and exercise intolerance. The anemia may develop very
slowly so that exercise intolerance is minimal even at very low hemoglobin levels. In
addition, vitamin B_{12} deficiency can result in GI complaints, such as diarrhea and weight
loss, and episodes of glossitis, leading to erythema, soreness, and, eventually, to loss of
papillae, causing a smooth, beefy red tongue. Most importantly, vitamin B_{12} deficiency
impairs nerve myelination, leading to degeneration of white matter in the brain and in both
the dorsal and lateral columns of the spinal cord (subacute combined degeneration). Dorsal
column involvement causes diminished vibratory sensation, creating numbness and tingling
in the feet and hands (stocking–glove neuropathy), and decreased proprioception, producing
gait difficulties and a positive Romberg sign. Lateral column damage causes limb weakness,
spasticity, hyperactive reflexes, and a positive Babinski sign. Evidence of cerebral
involvement includes depression, dementia, confusion, delusions, and hallucinations. With
regard to folic acid deficiency, the symptoms of anemia are similar to those of B_{12}
deficiency, but the neurologic changes do not occur, as folic acid is not involved in myelination in adults.

Folic acid deficiency is usually caused by an inadequate diet. Rich sources are fruits, vegetables, and animal protein, but cooking easily destroys folate. Furthermore, the body stores of folate are small, and only a few months of poor intake, caused by food fads, ignorance, poverty, or alcoholism, are necessary before anemia develops. A major concern for folate deficiency is in pregnancy where the folate requirement is increased and inadequate folic acid intake results in low birth rates and neural tube defects.

Alcohol intake compounds the problem by increasing urinary folate excretion, impeding liver storage of folate, and decreasing folate absorption, which occurs primarily in the duodenum and jejunum. Disorders affecting these portions of the intestine, such as sprue, lymphoma, amyloidosis, and Crohn’s disease, can cause folate malabsorption. Folic acid deficiency also can occur when the body’s demand for it increases, not only in pregnancy but also in any condition associated with increased cell turnover, such as chronic hemolytic anemias, acute exacerbations of hemolytic anemia, leukemia, and exfoliative dermatitis. Some medications, such as methotrexate and trimethoprim, cause folate deficiency by altering its metabolism. The measurement of serum folate level is very sensitive to recent folate intake and thus testing should be performed on fasting samples. Although some have advocated for the use of red cell folate as a more accurate indicator of long-term folate storage, this test has a number of analytical issues (including high coefficient of variation) that make it of limited value; published studies have shown that red cell folate performs no better than fasting serum folate measurements. Although folic acid deficiency is possible, recent studies have shown that the introduction of mandatory folic acid supplementation in cereal grain in the United States has drastically reduced the prevalence of folic acid deficiency in the population and has been associated with a documented reduction in neural tube defects and as well as macrocytic anemia due to folate deficiency.

Vitamin B\textsubscript{12} is the common name for either cyanocobalamin or hydroxycobalamin; these compounds are cobalmins, which include a group of molecules with a central cobalt atom bound by tetraphyrrole rings. In serum, methylcobalamin is the major form, whereas deoxyadenosylcobalamin is the primary form found in the cytosol. Cobalmins are not synthesized by humans but must be acquired in the diet, mainly meat, poultry, seafood, and dairy. The recommended daily allowance of cobalmins is 5 μg, and the total body content is about 2 to 5 mg, about 1 mg being present in the liver. Because the daily losses are minute, cobalamin deficiency from diet alone takes years and occurs almost exclusively in strict vegetarians, e.g., vegans. Therefore, in adults, the main cause of vitamin B\textsubscript{12} deficiency is impaired absorption. Food cobalamin binds to a substance in gastric juice called R protein (haptocorrin) and is released by pancreatic enzymes when it reaches the second portion of the duodenum. Vitamin B\textsubscript{12} then binds to intrinsic factor, a glycoprotein produced by the parietal cells in the fundus and cardia of the stomach, and the complex then travels to the distal ileum. Intrinsic factor receptors are present on the ileal mucosa, especially in the terminal ileum, where cobalamin is specifically absorbed from the cobalamin-intrinsic factor complex in a receptor-mediated process.

A major cause of cobalamin deficiency is from reduced intrinsic factor activity. This can occur from destruction of parietal cells by gastric resection or from chronic gastric inflammation due to autoimmune mechanisms that lead to mucosal atrophy in the stomach’s fundus and body. The latter disorder, pernicious anemia, occurs primarily in older adults, often with a family history of the disease; northern European descent; or concurrent
autoimmune disorders, such as Graves disease, vitiligo, or Hashimoto thyroiditis. About 90% of patients with pernicious anemia have antibodies to parietal cells, compared with 5% in the general population, and approximately 60% have antibodies to intrinsic factor, which are rare in healthy people.

Disorders of the ileum, such as Crohn’s disease or lymphoma, can also cause cobalamin deficiency because of vitamin B\textsubscript{12} malabsorption. Malabsorption of cobalamin also can occur with pancreatic insufficiency, when inadequate pancreatic enzymes fail to release cobalamin from the R proteins. Another cause of cobalamin deficiency is its consumption in the small intestine by a fish tapeworm, \textit{Diphyllobothrium latum}, found mostly in fish from Canada, Alaska, and the Baltic Sea, and acquired by humans by eating undercooked fish or fish roe. Excessive intestinal bacteria in diseases associated with impaired motility, or intestinal stasis, such as systemic sclerosis, extensive diverticula, or surgical blind loops, also can consume enough cobalamin to cause vitamin B\textsubscript{12}–related macrocytic anemia.

Assessment of vitamin B\textsubscript{12} levels is more complicated than folic acid levels. The Schilling test had previously been used to help distinguish among the causes of cobalamin deficiency. Unfortunately, because of technical difficulties producing and measuring the cobalt radioisotopes required, this test is no longer available in the United States. The most common tests offered clinically are quantitative immunoassays. These assays typically first convert serum cobalamins to cyanocobalamin and then quantify this species. Recently, approximation of vitamin B\textsubscript{12} levels has been accomplished by measuring serum homocysteine and methylmalonic acid (MMA). Homocysteine is increased in both vitamin B\textsubscript{12} and folate deficiency because methionine synthesis is impaired by deficiency of either. This testing has higher sensitivity because the increase occurs earlier in nutritional deficiency. A cobalamin-dependent, but folate-independent, enzymatic reaction leads to increased serum levels of MMA in cases of vitamin B\textsubscript{12} deficiency. This finding also tends to precede changes in serum cobalamin. Accordingly, measurement of both homocysteine and MMA can reliably detect, and distinguish between, folate and cobalamin deficiencies. When both are elevated, cobalamin deficiency is confirmed, although concurrent folate deficiency is possible. If homocysteine is elevated and MMA is normal, folate deficiency is likely. If both are normal, deficiency of either is highly improbable. If cobalamin deficiency is present, the presence of antibody against intrinsic factor confirms the diagnosis of pernicious anemia.

Nonmegaloblastic Macrocytic Anemia
Macrocytic anemia also occurs frequently in MDS because of abnormal erythrocyte maturation and ineffective erythropoiesis caused by a clonal expansion of abnormal hematopoietic stem cells. Another source of mild macrocytosis is reticulocytosis, an increase in young erythrocytes released early from the marrow, in hemolysis. Reticulocytes may be presumptively identified on peripheral blood smears because they still contain residual blue-staining nuclear RNA, as well as red-staining hemoglobin, producing a purplish color with the Romanowsky stains ordinarily used for peripheral blood films. The presence of a few reticulocytes is common on normal smears, but numerous polychromatophilic cells, representing a stress erythropoiesis, can result in mild macrocytosis. Even more immature normoblasts that have not yet shed their nuclei (nucleated RBC) can be detected in severe anemias, especially in chronic severe hemolytic anemias. Macrocytosis, usually with an MCV of 100 to 110, but typically without anemia, is present in about 60% of alcoholics. The cause may be deficiency of folic acid or vitamin B\textsubscript{12} due to poor nutrition, but this anemia
can also be a direct effect of ethanol byproducts (acetaldehyde) to depress hematopoiesis. In addition, there are a number of inherited dyserythropoietic anemias or marrow failure states, which are characterized by macrocytosis, such as Fanconi syndrome, dyskeratosis congenita, or congenital dyserythropoietic anemia.

**NORMOCYTIC ANEMIAS**

Normocytic anemias have many diverse causes. In response to acute hemorrhage or hemolysis, the bone marrow responds maximally by increasing RBC production and releasing young erythrocytes prematurely. However, marrow erythropoiesis may be inadequate in normocytic anemias due to intrinsic bone marrow disease, insufficient iron availability, or inadequate erythropoietin effect. Reasons for anemia resulting from poor bone marrow response include (1) diminished numbers of erythroid precursors, as in aplastic anemia or following irradiation or cancer chemotherapy; (2) infiltration of the marrow by fibrosis, leukemia, cancer, or tuberculosis; and (3) myelodysplastic disorders, in which abnormalities in RBC maturation result in excessive RBC death in the marrow, i.e., ineffective erythropoiesis. Insufficient iron availability results from either absence of iron (iron deficiency) or from poor iron utilization (such as during inflammatory states). Low erythropoietin levels can result from (1) impaired renal production because of renal disease; (2) reduced erythropoietin target effect, as in endocrine disorders such as hypothyroidism and hypogonadism or antibodies to erythropoietin; or (3) interference with erythropoietin production or with its bone marrow effects, caused by inflammatory cytokines, contributing to the pathogenesis of the ACD. Rarer causes are pure red cell aplasia and aplastic anemia.

**Anemia of Chronic Renal Disease**

Anemia typically occurs with chronic renal disease only after the creatinine clearance decreases below 40 mL/min, which corresponds to a serum creatinine of about 2.5 mg/mL. Studies have shown that anemia of chronic renal disease can be more accurately assessed with cystatin C compared to creatinine. The anemia tends to worsen as the renal function decreases, but it usually stabilizes at a hematocrit of 15% to 30%. The cause of the kidney disease is not usually important in determining the severity of anemia, but anemia is typically less severe with polycystic kidney disease possibly due to erythropoietin production by the renal cyst epithelial cells. Several factors contribute to the anemia of chronic renal disease, the most important, however, being inadequate renal production of erythropoietin, a glycoprotein hormone synthesized in the kidney and responsible for the proliferation, maturation, and differentiation of erythrocytes in the bone marrow. Erythropoietin is also an erythroblast survival factor. In addition, red cell survival is shortened in uremia, and various toxins ordinarily excreted by the kidney accumulate in the serum and appear to depress erythropoiesis. Replacement by recombinant erythropoietin or erythropoietin-stimulating agents has become central to the management of anemia in chronic renal disease.

The anemia is normochromic, normocytic, and most RBCs are unremarkable on the peripheral smear. Burr cells (echinocytes), however, may form via unknown mechanisms, and sometimes schistocytes appear. Both burr cells and schistocytes have shortened survival.

**Anemia of Endocrine Disorders**

Anemia, usually normocytic, occurs in several endocrine disorders. About 30% of patients
with hypothyroidism have anemia, and about one-third of these are macrocytic. The anemia, usually mild, seems to relate to the hormone deficiency itself, and its severity is related to the duration and degree of hypothyroidism. Approximately 10% to 25% of patients with hyperthyroidism, usually with severe, prolonged disease, are anemic. The mechanism is uncertain.

Most patients with adrenal insufficiency have anemia, usually normocytic, normochromic. In those with autoimmune causes, pernicious anemia, producing a macrocytic anemia, is present in about 10%. Androgen deficiency also is a cause of normochromic, normocytic anemia. Hypopituitarism causes anemia through deficiencies of the previously mentioned thyroid, adrenal, and androgenic hormones.

A small number of patients with hyperparathyroidism have a normocytic, normochromic anemia, with bone marrow examinations typically demonstrating fibrosis. The increased parathyroid hormone may also decrease erythropoiesis.

**Anemia of Chronic Disease**

ACD is quite common with infections, noninfectious inflammatory states that last more than 1 or 2 months, or malignancy. In about 25% of cases of ACD, anemia may be associated with congestive heart failure, hypertension, or diabetes mellitus. The anemia is mild to moderate, usually with a hemoglobin of about 10 mg/dL, but, in about 20% of patients, it can be more severe. The RBCs are typically normocytic, normochromic, but in about 30% they are mildly microcytic (usually 70–79 fL), and in about 50% they are hypochromic (MCHC 26–32). On peripheral smear, the RBC may display mild poikilocytosis and anisocytosis, but markedly small and thin cells, often seen in iron deficiency, are absent.

In ACD, both serum iron and iron-binding capacity are low, unlike iron deficiency in which low serum iron and elevated iron-binding capacity coexist. As in iron deficiency, the iron saturation may be below 10%. The serum ferritin, which is characteristically less than 15 μg/L in iron deficiency, is at least 100 μg/L in ACD and usually much higher unless there is coexisting iron deficiency. Circulating transferrin receptor levels are normal in ACD, but elevated in iron deficiency. Bone marrow iron is present in ACD, and a trial of oral iron therapy does not correct the anemia.

The factors linking different anemias of chronic disease are inflammatory cytokines that negatively affect erythropoiesis, in particular interferon and interleukin-6. They impair the proliferation and differentiation of erythroid precursors, diminish erythropoietin production, and decrease the bone marrow response to erythropoietin. Cytokines affect iron metabolism by increasing iron retention in the bone marrow and in macrophages by stimulating hepcidin synthesis, thus decreasing iron availability for production of hemoglobin in maturing erythroid precursors. In addition, RBC lifespan is moderately decreased in ACD.

**Pure Red Cell Aplasia**

In this rare disorder, a normocytic anemia with diminished reticulocytes (<1%) and absence of polychromasia on the peripheral blood smear is accompanied by a marked diminution to absence of erythroblasts in the bone marrow (<0.5% of the marrow differential count), despite the presence of normal numbers of megakaryocytes and myeloid precursors and normal overall marrow cellularity. Pure red cell aplasia may develop without apparent cause or be associated with a wide variety of systemic diseases. It may be associated with viral infections such as parvovirus B19, in which case giant erythroblasts containing viral inclusions may be seen in the bone marrow. Pure red cell aplasia is also associated with numerous conditions characterized by disturbed immunity: in about 5% of patients with...
thymoma (accounting for approximately 10% of cases of pure red cell aplasia), hematologic malignancies, especially chronic lymphocytic and large granular lymphocytic leukemias; rheumatologic diseases (such as Sjögren syndrome and systemic lupus erythematosus), and even solid tumors. Numerous medications have been implicated, including phenytoin, azathioprine, and isoniazid. Sometimes, pure red cell aplasia occurs during pregnancy without any apparent explanation and typically disappears following delivery. In many patients, no cause is found but an immunoglobulin G (IgG) that inhibits erythropoiesis is present in the serum.

Aplastic Anemia
In aplastic anemia, pancytopenia in the peripheral blood and hypocellularity of the bone marrow accompany the anemia. Clinical presentations usually are for anemia or bleeding; infections are less common until the late stages. Certain types, such as Fanconi anemia, are hereditary, whereas some acquired aplastic anemias may have identifiable causes, such as exposure to medications, (chloramphenicol) or chemicals (benzene), or infections with certain viruses (hepatitis). Aplastic anemia may develop in patients with PNH or in other bone marrow failure states (e.g., dyskeratosis congenita) or as a complication of certain rheumatic diseases, such as eosinophilic fasciitis, systemic lupus erythematosus, or Sjögren syndrome. In the hemophagocytic syndrome, most commonly associated with viral infections or certain malignancies, pancytopenia, fever, hepatosplenomegaly, and lymph node enlargement occur, and the bone marrow, often hypocellular, shows macrophages ingesting erythrocytes.

Acquired aplastic anemia in the majority of cases appears to result from an immunologic reaction against an unknown target that damages bone marrow stem cells. The use of intensive immunosuppressive therapy during the past 30 years has resulted in marked improvement in survival, which has further improved since the introduction of the thrombopoietin agonist eltrombopag which has been able to stimulate growth of megakaryocytic and erythroid precursors. Clonal hematopoiesis is common in aplastic anemia (documented in 30%–40% of cases) as is acquired mutations associated with MDS/acute myeloid leukemia, which, as well as PNH occur in increased frequency in patients with preceding aplastic anemia.

HEMOLYTIC ANEMIAS
Hemolytic anemias are characterized by increased RBC destruction, so that the average lifespan of RBC may be shortened from the normal 120 days to a few days or even hours. Hemolytic anemias are classified either by site of destruction (extravascular vs. intravascular) or by mechanism (destruction that is intrinsic or extrinsic to the RBC). In extravascular hemolysis, which is common, the red cells are destroyed by macrophages in the spleen, liver, or bone marrow, whereas in intravascular hemolysis, which is rare, RBCs are destroyed within the bloodstream. The differential diagnosis includes (1) an abnormal RBC environment because of infections, medications, or immunologic processes; (2) RBC membrane abnormalities; (3) RBC metabolic defects (including glucose-6-phosphate dehydrogenase [G6PDH] deficiency and pyruvate kinase deficiency); and (4) abnormalities in hemoglobin structure or synthesis.

During intravascular hemolysis, released hemoglobin first binds to haptoglobin, and then to hemopexin. When free hemoglobin exceeds the binding capacity of haptoglobin, it turns
the plasma pink. Free hemoglobin is filtered in the kidneys, and the urine may appear red. The dipstick testing of urine for blood is positive, but the urine microscopy is negative for RBC. This discrepancy is an important distinction between hemoglobinuria and hematuria. The renal tubular epithelium cells take up some of the hemoglobin, transforming it into hemosiderin, which is visible on iron stains of cells in the urinary sediment. Evidence of recent or ongoing intravascular hemolysis, thus, includes a reduced serum haptoglobin level (which also occurs in extravascular hemolysis), the presence of plasma or urine hemoglobin, and detection of hemosiderin in renal tubular cells in the urinary sediment. Intravascular hemolysis is typically severe and has disparate causes, including (1) mechanical damage to the red cell caused by fibrin strands within the vessel lumen during disseminated intravascular coagulation, vasculitis, or thrombotic thrombocytopenic purpura; (2) physical trauma from red cells passing through dysfunctional prosthetic valves or through small vessels of the feet during hard marching; (3) thermal injury from burns; (4) RBC infection by malaria parasites or toxic exposure to snake venoms; (5) complement-mediated damage to erythrocytes caused by cold agglutinins, incompatible red cell transfusions, and PNH; and (6) hemolytic uremic syndrome from shiga toxin–producing microorganisms like Escherichia coli.

The other major classification of hemolytic anemias differentiates disorders intrinsic to the red cell, which are typically hereditary, and those extrinsic to the red cell, usually acquired. The intrinsic disorders include abnormal hemoglobins, enzyme defects, and membrane abnormalities. The extrinsic disorders are immunologic disorders, mechanical factors, infections and toxins, liver disease (spur cell anemia), and hypersplenism.

Immunologic hemolytic anemias are a collection of relatively rare disorders all characterized by the production of autoantibodies that target RBCs for destruction. Immune hemolytic anemia can arise spontaneously, be associated with disturbed immunity (e.g., in immunodeficiency state, in chronic lymphocytic leukemia, in lupus erythematosus, or after bone marrow transplantation), or follow transfusion with incompatible or incompletely matched blood products. The three major categories of autoimmune hemolytic anemia are (1) warm antibody (most cases and typically involves an IgG antibody), (2) cold antibody (antibodies that react at temperatures below body temperature and are usually IgM), and (3) mixed cold and warm type. Drugs such as penicillin and cephalosporins can evoke a warm antibody type autoimmune hemolytic anemia by functioning as haptens that attach to the RBC membrane.29 The direct antiglobulin test (or Coombs test) is used to detect autoimmune hemolytic anemias; in this test, the patient’s RBCs are incubated with antibodies to human IgG and complement component 3 (C3).30 If autoantibodies are present, they will have coated the patient’s RBCs and the addition of the anti-IgG and anti-C3 will result in RBC agglutination. The indirect Coombs test is used to detect the presence of anti-RBC antibodies in the patient’s serum, even when the sensitized RBCs have all been destroyed.

The Coombs test is negative in cases of PNH (where destruction of the RBCs is due to deficiency of complement inhibitors on them, occurs sporadically and often at night, but is not immunologically mediated).31

Diagnostic abnormalities in RBC morphology associated with hemolytic anemias may be detected on peripheral blood smear, such as sickle cells, bite cells, schistocytes, and spherocytes. The presence of relatively uniform spherocytes may suggest an underlying RBC membrane abnormality such as hereditary spherocytosis (in which abnormal cytoskeletal proteins result in spherical rather than discoid RBC forms), hereditary elliptocytosis (an autosomal dominant genetic defect characterized by a destabilized cytoskeleton and thus an elliptical shape), and hereditary stomatocytoses (autosomal dominant genetic defect
impairing the ability of the RBC to maintain proper cationic balance). Other findings may include red cell agglutination denoting the presence of a cold antibody, organisms such as malarial parasites, and ingestion of erythrocytes by macrophages (erythrophagocytosis), which especially suggests immune hemolytic anemias, but also can occur with infections or toxins. The peripheral smear in immune hemolytic anemias typically shows spherocytes of varied size, substantial polychromatophilia caused by the increased release of immature red cells from the bone marrow, and if anemia is severe, nucleated RBCs. The reticulocyte index is above 3 and the absolute reticulocyte count is over 100,000/mm³. The indirect bilirubin is increased and represents over 80% of the total bilirubin. The serum LDH is often increased and the serum haptoglobin low or absent. Coombs tests detect immunoglobulin and/or complement on the red cell surface, indicating an immune hemolysis. For suspected intravascular hemolysis, urine and plasma hemoglobin measurements are useful, and if hemolysis is chronic, iron stains of urinary sediment may be positive. A hemoglobin electrophoresis is indicated for suspected hemoglobinopathies.

Microangiopathic hemolytic anemias are caused by intravascular fibrin formation or by mechanical valve dysfunction and will be discussed in the chapter on hemostasis.

The most frequent hemolytic anemia caused by an intrinsic RBC enzyme defect is G6PDH deficiency. This involves a mutation in the G6PDH gene, which is active in the pentose monophosphate shunt. The pentose monophosphate shunt is the sole source of nicotinamide adenine dinucleotide phosphate (NADPH), the main antioxidant in RBCs. NADPH is essential for preventing oxidative damage to the RBCs and lack of this important reductant can lead to increased RBC hemolysis, especially in situations that exacerbate oxidative damage as treatment with certain drugs (antimalarials, methylene blue, and benzocaine) or consumption of foods like fava beans. G6PDH deficiency is an X-linked disorder most common in Africa, the Mediterranean and Southeast Asia, and affects over 400 million persons worldwide.

HEMOGLOBINOPATHIES AND THALASSEMIAS

Hemoglobin A (Hb A), which constitutes more than 90% of the adult hemoglobin, consists of four polypeptide chains, two α and two β (α₂β₂). Hemoglobin A₂, composed of two α and two δ (α₂δ₂), is present in small quantities. Hemoglobin F (α₂γ₂), the main hemoglobin during fetal life, comprises two α and two γ chains, and usually comprises <1% of the normal adult’s hemoglobin. As β-chain production begins before birth, the level of Hb F represents about 75% of the hemoglobin at birth. By 6 months of age, Hb F has normally diminished to 5%. Defects in globin gene synthesis can result in two distinct categories of mutations: qualitative defects (the hemoglobinopathies) and quantitative defects (thalassemias). Qualitative defects involve globin gene mutations which lead to altered hemoglobin structure. It is worth noting that the vast majority of hemoglobin variants are clinically silent. Major clinically important abnormal hemoglobins include Hb S, Hb C-Harlem, and Hb C. Hb S and the rare Hb C-Harlem both have an essential glutamic acid residue at position 6 mutated to a valine (Hb C-Harlem has an additional mutation). This single amino acid substitution in Hb S arising from a missense mutation drastically alters the behavior of the Hb, especially at low oxygen conditions. The presence of valine results in the formation of Hb S polymers within the RBCs that cause the RBCs to deform into the characteristic sickle shape.

In the individual with homozygous Hb S, “sickle cell disease,” sickling leads to chronic
hemolysis and, more importantly, intermittent vaso-occlusion and severe pain crises, which over time result in organ dysfunction, including autosplenectomy, strokes, renal dysfunction, cardiac failure, and pulmonary hypertension. Hb C also has a point mutation at position 6 but in this case it is the substitution of a lysine. The result is that Hb C does not sickle but rather forms insoluble precipitates due to higher intracellular hemoglobin concentration so that hemoglobin crystals can be visualized on the peripheral smear as brick-like, dense RBCs in individuals with homozygous Hb C. The different hemoglobinopathies can be diagnosed by a variety of techniques including gel electrophoresis, HPLC, and capillary electrophoresis. Capillary electrophoresis has become increasingly popular for its ability to separate and quantify hemoglobin variants in the diagnosis of hemoglobinopathies as well as the thalassemias. This technique relies on passing the hemoglobins through a narrow capillary. The hemoglobins are separated by electro-osmotic force and measured with absorbance at a wavelength characteristic of the heme group (450 nm).

To date, treatment for sickle cell disease and other hemoglobinopathies has been mainly supportive including early immunizations and penicillin from infancy to combat splenic dysfunction, hydration, supplementary folic acid, analgesics, and RBC transfusions. These simple measures have improved survival of both children and adults. Bone marrow transplant is curative but impractical. The single drug that has had an important impact on clinical course is hydroxyurea, which decreases crises and acute chest syndrome, diminishes stroke rates in children, and modestly raises hemoglobin levels through stimulating Hb F production, which decreases sickling and moderates the chronic inflammatory state. In the future, gene editing may offer definite curative therapy for hemoglobinopathies like sickle cell disease.

The thalassemias are inherited disorders of globin chain synthesis, involving mutations causing reduced or absent synthesis of one or more globin chains. Many different α or β chain mutations have been detected that cause the clinical syndromes. Two major consequences occur: reduced production of functioning hemoglobin, leading to hypochromic, microcytic erythrocytes; and continued production of the unaffected globin chains, which accumulate, precipitate, and damage RBCs and erythropoietic precursors, leading to ineffective erythropoiesis and contributing to hemolysis. Thus, thalassemias involve both diminished hemoglobin production and a chronic hemolytic state that involves not only ineffective erythropoiesis but also poor survival of circulating RBCs. The thalassemias are named by the globin chain that shows impaired production. In β-thalassemia, β-chains are absent or diminished, whereas in α-thalassemias, α-chain production is impaired. These are the two most important thalassemias, although others exist. Precise diagnosis of thalassemias requires evaluating globin chain ratios as well as DNA structure, because the genetics are so varied.

The β-thalassemias are common in the Mediterranean basin (“thalassos” in Greek means “sea”), the Middle East, India, and Southeast Asia. The clinical spectrum includes severe (thalassemia major), moderate (thalassemia intermedia), and mild (thalassemia minor) cases. β-Thalassemia major, or homozygous disease, is caused by the inheritance of two β-thalassemia alleles, resulting in little or no β-chain production, although α-chain synthesis remains normal. The abnormal β-globin synthesis depresses Hb A production, resulting in severe anemia with RBCs containing a diminished concentration of hemoglobin, making them very hypochromic. Accumulated excess free α-chains deposit in and damage erythroid precursors, resulting in ineffective erythropoiesis. RBCs containing these precipitates that do reach the peripheral blood are prematurely destroyed by macrophages in the liver, spleen, and bone marrow. Because Hb F is present in substantial quantities at birth, anemia appears only
as γ-chain synthesis diminishes, and children who are adequately transfused grow normally until iron overload problems begin to develop. In untreated or insufficiently transfused children, growth is subnormal and many health problems develop in addition to severe anemia. Increased erythropoiesis in response to the anemia leads to expanded marrow cavities that can eventuate in long-bone fractures and expanded marrow areas in the skull and maxilla, causing abnormal contours of the face and head. Increased erythrocyte destruction in the spleen causes splenomegaly, which eventuates in hypersplenism with thrombocytopenia and leukopenia. In all thalassemias, iron absorption is excessive, and this problem is worsened by chronic transfusion needed to maintain life in β-thalassemia major. Deposition of excess iron in the heart and endocrine organs leads to fibrosis, cardiac failure, diabetes, and delayed puberty, and other endocrinopathies. The blood smear shows marked anisocytosis and poikilocytosis, with microcytosis, elliptocytes, teardrop cells, and other bizarrely shaped RBCs. Target cells and nucleated red cells are typically numerous, and basophilic stippling is common. RBC inclusions, representing excess α-chains, may be apparent. Findings on bone marrow examination include erythroid hyperplasia, basophilic stippling, and diminished hemoglobin in the red cell precursors, which also show inclusions. Iron content is markedly increased.

Thalassemia intermedia is usually the result of the inheritance of two different β-thalassemia mutations: both mild or one mild, one severe. Although the anemia is severe in the 8 to 9 g/dL hemoglobin range, transfusions may not be necessary. The blood smear resembles that of thalassemia major. In contrast, thalassemia minor represents a single β-thalassemia mutation and is associated with mildly microcytic, hypochromic blood smear and either no or minimal anemia. Serum iron is normal. No clinical problems result, whereas there may be clinical protection from severe malaria. Poikilocytosis, target cells, and basophilic stippling are variably present. Hemoglobin electrophoresis demonstrates elevated Hb A₂ (about twice normal) and Hb F is often increased.

In α thalassemia, prevalent in Southeast Asia, India, and to a lesser degree in Africa, clinical severity depends on how many mutations are present in the four α globin genes. If one gene is mutated, there are no hematologic abnormalities. In α-thalassemia-1, two genes are mutated, leading to a microcytic, hypochromic smear with mild or no anemia. Three mutated α-globin genes produce chronic microcytic anemia, with Hct 20% to 30%, called Hb H disease, in which the excess β-chains form soluble tetramers in the circulating RBC produce inclusion bodies as the RBC age, thus resulting in extravascular hemolysis and splenomegaly. The blood smear shows substantial hypochromia, microcytosis, basophilic stippling, polychromasia, target cells, teardrop cells, and nucleated and fragmented cells. Heinz body preparations disclose precipitated Hb H, visible as multiple small erythrocyte inclusions. On hemoglobin electrophoresis, Hb H comprises about 3% to 30% of the total.

Mutations in all four α globin genes results in an absence of α-chains, so that tetramers of γ-chains (Hb Bart) are produced in the fetus. Hb Bart carries oxygen poorly and is unstable, resulting in tissue hypoxia, hemolysis, severe anemia, and causing fetal liver failure, heart failure and massive edema (hydrops fetalis), and intrauterine death.

Management of the severe thalassemias has been mainly supportive using transfusions, iron chelation, prevention of infection, and management of endocrine and cardiac failure. Curative bone marrow transplantation is often impractical. Gene editing strategies may become useful in the future.

REFERENCES
Figure 3.1. Approach to anemia.


Figure 3.3. Automated hemocytometer report. Red blood cell (RBC) volume curves demonstrating, from left to right: a normal, unimodal RBC population; a small macrocytic
population from a reactive increase in reticulocytes following therapy in iron deficiency; and a dual population of RBCs in sideroblastic anemia.

**Figure 3.4.** Automated hemocytometer reticulocyte counts. Fluorochromes are used to bind to the RNA of reticulocytes, which then fluoresce and can be counted by flow cytometry. The degree of fluorescence gauges the maturity of reticulocytes, with more immature reticulocytes demonstrating more fluorescence. Mature RBCs are red, and reticulocytes are green. The histogram on the left demonstrates a very low reticulocyte count, and the histogram on the right shows a high reticulocyte count. Automated reticulocyte counts allow more reticulocytes to be counted than manual reticulocyte counts and provide more precise and rapid measurement.
Figure 3.5. Peripheral blood smear with Romanowsky stain demonstrating polychromatophilic cells. The cytoplasm of polychromatophilic cells stains basophilic (bluish) because of increased RNA content. Not all reticulocytes are polychromatophilic on Romanowsky stains, and assigning the number of polychromatophilic cells alone as a surrogate marker for reticulocytosis underestimates the reticulocyte count. The cells are usually larger than normocytic RBCs.

Figure 3.6. RBC inclusions. In Wright–Giemsa–stained blood films Pappenheimer bodies, which stain positive for iron, are usually multiple, angular, and vary in size. Usually located in the cell periphery, Pappenheimer bodies represent retained siderotic mitochondria and may be seen in sideroblastic and hemolytic anemias, and post splenectomy. Howell–Jolly bodies are typically round, smooth, and single, and represent nuclear remnants of dense chromatin. They are frequently found in smears of asplenic individuals or in megaloblastic anemia.

<table>
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<td>RNA</td>
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<td>• Pappenheimer body</td>
<td>Iron</td>
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<td>β-Globin tetramers (β₄)</td>
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<td>• Heinz body (supravital only)</td>
<td>Denatured hemoglobin</td>
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<td>Hb C homozygous</td>
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<tr>
<td>• Cabot rings</td>
<td>Mitotic spindle remnants</td>
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<tr>
<td>• Nucleus</td>
<td>DNA</td>
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Figure 3.7. Approach to microcytic anemia.
Figure 3.8. Supravital stain of reticulocytes with brilliant cresyl blue. The blue-stained net-like inclusions in the RBCs represent ribosomes that are precipitated when exposed to brilliant cresyl blue. The more immature the reticulocyte, the more reticulin precipitation occurs. The National Committee for Clinical Laboratory Standards (NCCLS) definition of reticulocyte is “any nonnucleated red blood cell containing ≥2 particles of blue-staining material corresponding to ribosomal RNA.” Howell–Jolly bodies, nuclear DNA remnants, Pappenheimer bodies (precipitated iron inclusions), and Heinz bodies (precipitated globin chains) can occasionally be mistaken for reticulin precipitation in the manual reticulocyte stain.

Figure 3.9. Sideroblastic anemia. Peripheral blood smear of a dual population of predominantly normal sized, well-hemoglobinized RBCs and a minor population of microcytic markedly hypochromic, possessing a thin rim hemoglobin. This pattern is seen particularly in X-linked sideroblastic anemia. Occasional teardrop cells are visible.
Pappenheimer bodies, target cells, and basophilic stippling occur in some cases.

### Table 3.2

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<td>• Megaloblastic anemia</td>
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<td>• Pyrimidine 5’ nucleotidase deficiency</td>
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<tr>
<td>• Heavy metal poisoning (coarse basophilic stippling)</td>
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<td>• Lead, zinc, arsenic, silver, mercury</td>
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**Figure 3.10.** Sideroblastic anemia. Numerous ringed sideroblasts are seen in this marrow aspirate smear stained for iron. They are normoblasts with 10 iron-containing granules in the cytoplasm encircling at least one-third of the nucleus. Often, focusing up and down on the cell will more clearly demonstrate the iron-containing granules.
Figure 3.11. Morphologic features of sideroblastic anemia. **A:** Electron micrograph of an erythroblast with iron-laden mitochondria. **B:** Bone marrow smear (Prussian blue stain) with ring sideroblasts. **C** and **D:** Blood smears (Wright stain) of severe and mild sideroblastic anemia. **E:** Siderocytes (Wright stain). **F:** Electron micrograph of a Pappenheimer body in a peripheral RBC. (From Bottomley S. Sideroblastic anemias. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe’s *Clinical Hematology*. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:643–661.)
Figure 3.12. Smooth tongue and koilonychia in iron deficiency. **A:** Iron deficiency can result in a usually painless, smooth, shiny, and reddened tongue. (Courtesy of Dr. P. Galbraith.) **B:** Koilonychia, a condition also referred to as “spoon-shaped nails,” is associated with long-
standing iron deficiency in which the fingernails are thin, brittle, and concave with raised edges. The image is from a child with chronic iron deficiency. This condition is now rarely seen in the United States.

Figure 3.13. Iron deficiency blood smears. **A:** Microcytic, hypochromic blood film typical of severe iron deficiency. RBC size can be visually estimated by comparing RBC size with that of the nucleus of a small lymphocyte. Microcytic RBCs are smaller than the condensed nucleus of a mature lymphocyte. **B:** Poikilocytosis and microcytosis that include numerous pencil cells are shown in this iron deficiency blood smear. The degree of poikilocytosis has been observed to correlate with the degree of iron deficiency anemia.
Figure 3.14. Iron deficiency blood films. **A:** This peripheral blood film demonstrates at low power severe iron deficiency with microcytosis, hypochromia, and multiple morphologic changes: pencil cells, target cells, teardrops, and rare fragments. In contrast, in early iron deficiency, RBCs may be normocytic with no significant morphologic changes. Once the hemoglobin drops below 10 or 11 g/dL, RBC changes appear. Thrombocytosis is also common, but thrombocytopenia occasionally develops in severe iron deficiency. The morphologic changes in early iron deficiency may be indistinguishable from α- or β-thalassemia trait. A low MCV/MCH combined with a high RDW suggests iron deficiency, but plasma iron studies are essential to distinguish iron deficiency from thalassemia trait. If iron studies are normal, hemoglobin electrophoresis to quantitate Hb A₂ can distinguish between β-thalassemia and α-thalassemia trait. If iron deficiency is present, and it is necessary to test for α and β thalassemia carrier status, DNA studies or globin chain analysis will be necessary to differentiate these processes accurately. **B:** Peripheral blood smear of dual RBC population after transfusion in iron deficiency. Many RBCs are hypochromic and microcytic (patient’s own iron-deficient RBCs) interspersed with normochromic, normocytic...
RBCs (transfused RBCs). This combination also occurs during the response to treatment of iron deficiency with iron, as the pretreatment RBCs continue to circulate together with the newly produced iron-replete RBCs. It differs from sideroblastic anemia with a dual population in which most RBCs are normochromic and the minority hypochromic.

Figure 3.15. Iron deficiency and malabsorption. Peripheral blood smear demonstrating hyposplenic changes in a patient with celiac disease causing iron deficiency due to malabsorption of iron. Target cells, acanthocytes, and Howell–Jolly bodies (arrows) are seen. This patient has not had splenectomy, but shows functional hyposplenism related to the celiac disease.

Figure 3.16. Lead poisoning. Peripheral shows hypochromic microcytic cells with prominent

Figure 3.17. Bone marrow iron stores. Bone marrow aspirate particle stained with Prussian blue stores with no visible blue staining consistent with absent iron stores. The finding is consistent with iron deficiency if the particle is representative of the marrow particles. Serum ferritin studies are necessary to confirm iron-deficient stores. Absence of bone marrow iron is one of the earliest findings in iron deficiency.
Figure 3.18. Bone marrow iron stores. Low and higher magnification views (left and right, respectively) of bone marrow aspirate smears stained with Prussian blue showing varying degrees of iron staining in histiocytes.
Figure 3.19. Hereditary hemorrhagic telangiectasia. Vascular malformations are present on the face, lips, and hands in this patient with hereditary telangiectasia. This patient presented with iron deficiency anemia caused by recurrent gastrointestinal bleeding from gastrointestinal tract telangiectasia. (Courtesy of Dr. J. Crookston.)
Approach to normocytic anemia

Reticulocyte count

Decreased or not increased

See Figure 3.20B

History, physical examination, medications, chronic illness, laboratory testing: ESR, C-reactive protein, blood film, iron studies, renal, hepatic, endocrine testing, serum immunoelectrophoresis

Positive for:

- Decreased iron
  - Renal, hepatic, endocrine dysfunction

- Iron deficiency (early)

History of chronic disease, ↑CRP, ↑ESR, increased iron

Medications

Peripheral blood film for leukoerythroblastic picture

Negative

Iron deficiency (early)

- Renal disease
- Liver disease
- Hyper/hypothyroidism
- Hypoadrenal function
- Hypopituitarism
- Hypoparathyroidism
- Low-androgen state
- Vitamin D intoxication

Anemia of chronic disease

Anemia 2 to medications

- Pure red cell aplasia
- Myelodysplastic syndrome
- Bone marrow infiltrate

Bone marrow examination
Figure 3.20. Approach to normocytic anemia.
Figure 3.21. Macrocytic RBCs and hypersegmented neutrophils. **A:** Peripheral blood smear demonstrating oval macrocytes and one very large oval macrocyte. **B:** Blood smear showing a very large round macrocyte. The rule of thumb for both oval and round macrocyte is that they should be larger than the condensed nucleus of a mature lymphocyte, and should not be polychromatophilic (blue/purple staining), which differentiates them from reticulocytes. Because the MCV is an average of cell size, macrocytes can be found in a smear from a sample with a normal MCV (<100 fl). Oval macrocytes usually occur in megaloblastic states. When oval macrocytes are noted, the examiner should search for neutrophil abnormalities, including hypersegmented and hyposegmented nuclei or hypogranular cytoplasm. Round macrocytes occur in several disorders including hyper- and hyposegmented nuclei or hypogranulated cytoplasm. Round macrocytes also are larger than the condensed nucleus of a mature lymphocyte. The cells should not be polychromatophilic (blue/purple staining), which differentiates them from reticulocytes. **C:** Peripheral blood film demonstrating hypersegmented neutrophils with many oval macrocytes (*arrows*) with marked heterogeneity in overall RBC size in a patient with megaloblastic anemia due to vitamin B$_{12}$ deficiency. Neutrophils are considered hypersegmented when over 5% of neutrophils have five lobes or more, or occasional neutrophils have six lobes or more.
Figure 3.22. Anatomic changes associated with pernicious anemia. A: Smooth tongue. The loss of papillae in pernicious anemia causes the tongue to be smooth, usually most marked along the edges. The tongue also may be red and painful. Occasionally, red patches are observed on the edges of the dorsum of the tongue. B: Atrophic gastritis of pernicious anemia. Gross pathologic sample of stomach from a patient with pernicious anemia and vitamin B\textsubscript{12} deficiency. Atrophy of the fundus is present. Classic pernicious anemia is caused by the failure of gastric parietal cells to produce intrinsic factor to permit the absorption of vitamin B\textsubscript{12}. This disease is associated with gastric atrophy and achlorhydria. Coexistent iron deficiency is common because achlorhydria decreases iron absorption. Pernicious anemia is associated with an increased risk of gastric carcinoma. C: Normal
gastric mucosa for comparison.

Figure 3.23. Demyelination in pernicious anemia. A: Head magnetic resonance imaging (MRI) showing a T2-weighted image with multiple high signal foci in the periventricular white matter. This is nonspecific but compatible with demyelination associated with vitamin B₁₂ deficiency. B: T2-weighted image of the cervical spine displaying high signal in the posterior aspect of the cervical cord (arrow). This appearance is consistent with demyelination. C: Subacute combined degeneration of spinal cord in vitamin B₁₂ deficiency. Cross section of autopsy spinal cord showing spongiform changes and myelin and axonal destruction in the posterior and lateral columns (arrows). This neurologic complication of vitamin B₁₂ deficiency may precede any hematologic abnormality. Elevated serum or urinary levels of methylmalonic acid and homocysteine are sensitive for the diagnosis of vitamin B₁₂ deficiency and usually precede the development of hematologic abnormalities and reductions in the serum vitamin B₁₂ level. (Courtesy of Dr. J. Bilbao.)
Figure 3.24. Bone marrow in megaloblastic anemia. Megaloblastic erythroid and granulocytic precursors are demonstrated in this marrow aspirate smear. Giant bands, twice the size of a normal neutrophil band form, and a giant hypersegmented neutrophil are shown. Hypersegmented neutrophils may be seen in megaloblastic states including vitamin B₁₂ and folic acid deficiencies, myeloproliferative disorders, following granulocyte colony-stimulating factor (G-CSF) administration, and in chronic infections.
Figure 3.25. Megaloblastic versus dysplastic changes in marrow precursors. Composite figure from several cases comparing normal, megaloblastic, and dysplastic findings in various stages of differentiation of erythroid precursors.
Figure 3.26. Macrocytic anemia in MDS. Macrocytic anemia and thrombocytosis are seen in this case of MDS with isolated del(5q) chromosome abnormality. Macrocytic RBCs (arrows) are larger than the nucleus of small mature lymphocytes, as shown here.
Figure 3.27. Vitamin C deficiency (scurvy) in a patient with megaloblastic anemia. The gums demonstrate gingival hemorrhage (A); corkscrew hairs with perifollicular hemorrhages are visible on a close-up view of skin (B) in this patient suffering from vitamin C deficiency. (Courtesy of Drs. J. Crookston and D. Amato.)
Figure 3.28. Macrocytosis of liver disease. Patients with liver disease and obstructive jaundice have macrocytosis and target cells caused from increased cholesterol and/or phospholipid deposition on the RBC membranes. This blood smear, from a case of alcoholic liver disease, shows prominent numbers of macrocytic target cells. The macrocytosis associated with alcoholism may be multifactorial and include direct toxicity of alcohol to the marrow, cirrhosis, and in some cases poor nutrition with folate deficiency (this is less common in countries where food is fortified with folic acid). In alcoholic liver disease, thrombocytopenia and leukopenia are also present.
Figure 3.29. Approach to macrocytic anemia.
Figure 3.30. Anemia of chronic disease. Increased marrow iron stores. A: Bone marrow aspirate demonstrating increased iron staining present in a patient with anemia of chronic disease. Increased iron stores also occur in frequently transfused patients. B: Normal iron staining in bone marrow histiocytes is shown for comparison.
Figure 3.31. Echinocytes or burr cells seen in patients with severe liver or renal disease. The cells have central pallor and a diffuse, even distribution of short, bumpy projections with rounded edges around the surface of RBCs, resembling a bottle cap when viewed from above. This blood smear can be seen in the anemia of chronic disease but usually that type of anemia is normocytic and normochromic. Mild microcytosis and hypochromia can develop as the severity of the underlying condition causing the anemia of chronic disease worsens and iron availability markedly declines. The RBC may display features secondary to the underlying cause (e.g., rouleaux due to increased fibrinogen or to increased immunoglobulin levels as in myeloma). Neutrophilia and thrombocytosis may occur.

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<td>- Hyperlipidemia</td>
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Figure 3.32. Peripheral blood smear of patient recently transfused with RBCs. The small population of echinocytes may represent the recently transfused RBCs.

Table 3.4

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Figure 3.33. Bone marrow biopsy from a patient with pure red cell aplasia before and after treatment with immunosuppressive agents. **A:** Although the pretreatment marrow appears normocellular at first inspection, there is a noticeable absence of maturing erythroblasts on closer evaluation. **B:** Normal erythroid maturation is detected following successful treatment.
Figure 3.34. Parvovirus B19-induced pure red cell aplasia. **A:** Bone marrow aspirate smear shows a giant erythroblast with intranuclear viral inclusion. This inclusion can resemble a large nucleolus, and the erythroblast cytoplasm may appear dark blue and contain vacuoles.  
**B:** Bone marrow biopsy with early erythroid precursors showing “glassy” intranuclear inclusions (so-called lantern cells; arrows). Peripheral blood will show very low to absent reticulocytes.

Figure 3.35. **A:** Normal bone marrow biopsy. **B:** Empty bone marrow in aplastic anemia. A few residual lymphocytes and mast cells are present. Marrow space is mainly fat. (From McInnis M, Mehta S, Lewis C. Hematology. In: Step-Up to USMLE Step 1 2015.
Figure 3.36. Fanconi syndrome. A: Hands in a patient with Fanconi syndrome. Bilateral thumb hypoplasia is present. Other congenital anomalies may occur: skin pigmentation changes, short stature, upper limb abnormalities, renal malformations, ophthalmologic problems, hypogonadism, and cardiac malformations. Thrombocytopenia and leukopenia precede macrocytic anemia. Eventually, aplastic anemia develops, and there is an increased risk of progression to myelodysplastic syndrome and myeloid leukemia. (Courtesy of Dr. I. Quirt.) B: Spontaneous chromosomal breakage in Fanconi syndrome. Chromosomal karyotyping of peripheral blood lymphocytes in a patient with Fanconi anemia. Increased spontaneous chromosomal breakage may be seen, but on exposure to mitomycin C or diepoxynbutane (right side), increased chromosomal breaks and radial chromosomal fusions occur. (Courtesy of M. Chago.)
Figure 3.37. Bone marrow findings in hemolytic anemia. A: Normoblastic erythroid hyperplasia is present with a predominance of erythroid precursors. The normal myeloid to erythroid ratio in a bone marrow aspirate is 3 to 5:1. In ongoing hemolysis, this ratio reverses as much as 1:4. Erythroid hyperplasia can also occur after acute hemorrhage. If the hemolytic process is severe, immature RBCs (nucleated RBCs) may be released into the blood. Occasional mild dysplastic erythroid precursors may be present. B: Bone marrow section shows erythroid hyperplasia in an erythroid colony. (From Chai CC. Erythrocytes. In: Fauri DC, ed. Pathology of Bone Marrow and Blood Cells. Philadelphia, PA: Lippincott Williams & Wilkins; 2004:56–80.)
Figure 3.39. Infectious organisms in the peripheral blood that can cause hemolysis. **A:** RBC parasitic inclusions of *Falciparum malaria* are seen. **B:** *Babesiosis* is seen within the RBCs. *Bartonellosis* hemolysis is also associated with inclusions of the bacilli in the red cells.
Figure 3.40. Peripheral blood film of microspherocytes seen in *Clostridium perfringens* sepsis. Although regular spherocytes are usually smaller than normocytic RBCs, microspherocytes are even smaller than that. This finding is usually seen in critically ill, septic patients with severe *C. perfringens* infection.

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<th>Table 3.5</th>
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<tr>
<td>Red blood cell agglutination</td>
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<tr>
<td>• Cold agglutinins</td>
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<tr>
<td>• Cold autoimmune hemolytic anemia</td>
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<tr>
<td>• Paroxysmal cold hemoglobinuria</td>
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<tr>
<td>• IgM paraproteinemias</td>
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**Figure 3.41.** Autoimmune hemolytic anemia. Smear shows numerous spherocytes, small round RBCs lacking central pallor, as well as many polychromatic RBCs (reticulocytes) in a case of Coombs-positive hemolytic anemia. Note that spherocytes are of varied sizes.

**Figure 3.42.** Hereditary spherocytosis. Peripheral blood smear of spherocytic hemolysis due to an inherited membrane abnormality. Spherocytes are round, are slightly smaller than normal RBCs, and lack central pallor. They are more homogeneous in size than in the autoimmune hemolytic anemia smear. Note the nucleated RBCs and polychromatophilic cells. To identify spherocytes properly, it is important to look in the area of the slide where RBCs are nearly touching each other but not stretched out. Normal RBCs when stretched, as at the tail or thin end of the blood smear, have an artifactual spherical appearance.

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<th>Table 3.6</th>
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<tr>
<td><strong>Spherocytosis</strong></td>
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<tr>
<td>• Warm autoimmune hemolytic anemia</td>
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<tr>
<td>• Acute and delayed hemolytic transfusion reactions</td>
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<tr>
<td>• ABO hemolytic disease of newborn/Rh hemolytic disease of newborn</td>
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<tr>
<td>• Hereditary spherocytosis</td>
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<tr>
<td>• Clostridium sepsis</td>
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<tr>
<td>• Intravenous water infusion or drowning (fresh water)</td>
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<tr>
<td>• Hypophosphatemia</td>
</tr>
<tr>
<td>• Bartonellosis</td>
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<tr>
<td>• Snake bite</td>
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<tr>
<td>• Cold autoimmune hemolytic anemia/paroxysmal cold hemoglobinuria</td>
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<tr>
<td>• Hyposplenism</td>
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<td>• Rh-null phenotype</td>
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Figure 3.43. Osmotic fragility curves of normal and hereditary spherocytosis RBCs. RBCs are exposed to decreasing strengths of hypotonic saline solutions, and the degree of hemolysis (%) is measured. Increased fragility is indicated by a shift of the curve to the left, and is seen in conditions associated with spherocytosis. **A:** In the fresh sample, a tail of HS cells occurs with increased sensitivity. **B:** Incubation of the sample for 24 hours prior accentuates the osmotic fragility of spherocytes, whereas normal cells only become more slightly fragile. The osmotic fragility of unincubated blood may be normal in some patients with hereditary spherocytosis (HS); therefore, incubated testing should be performed as well.

Figure 3.44. Hemolytic anemia due to enzyme deficiency. Peripheral blood smear demonstrating spiculated spheroid cells in a patient with pyruvate kinase deficiency hemolytic anemia. In some cases, elliptocytes are present. After splenectomy, spiculated cells increase.
Figure 3.45. Hereditary elliptocytosis. Elliptocytes and ovalocytes are present in this relatively common hereditary common membrane defect. Elliptocytes are elongated with rounded edges (as opposed to sharp edges in sickle cells). A mild hemolytic anemia is the only clinical manifestation, although rare patients may have more severe hemolysis.

Figure 3.46. Hereditary stomatocytosis. The RBCs in this blood smear demonstrate slit-like central pallor, creating the appearance of a mouth (stoma in Greek), from which the name stomatocytes derives. Hereditary stomatocytosis disorders are due to membrane defects associated with altered sodium permeability and may demonstrate 10% to 50% stomatocytes on the peripheral blood film. Ovalocytes and macrocytes also may be present.

Table 3.7

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<th>Red blood cell fragmentation</th>
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<td></td>
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<tr>
<td>With thrombocytopenia</td>
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<td>-----------------------</td>
<td></td>
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<tr>
<td>• Disseminated intravascular coagulopathy</td>
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<tr>
<td>• Thrombotic thrombocytopenic purpura</td>
<td></td>
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<tr>
<td>• Hemolytic uremic syndrome</td>
<td></td>
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<tr>
<td>• HELLP syndrome</td>
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<tr>
<td>• Preeclampsia/eclampsia</td>
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<tr>
<td>• Malignant hypertension</td>
<td></td>
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<tr>
<td>• Systemic lupus erythematosus</td>
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<tr>
<td>• Vasculitis</td>
<td></td>
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<tr>
<td>• Scleroderma crisis</td>
<td></td>
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<tr>
<td>• Antiphospholipid antibody crisis</td>
<td></td>
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<tr>
<td>• Drugs (cyclosporine, tacrolimus, mitomycin C, gemcitabine)</td>
<td></td>
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<tr>
<td>• Sepsis</td>
<td></td>
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<tr>
<td>• Disseminated carcinoma (mucin secreting)</td>
<td></td>
</tr>
<tr>
<td>• Extracorporeal circulation devices</td>
<td></td>
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<tr>
<td>• Vascular malformations</td>
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<table>
<thead>
<tr>
<th>Without thrombocytopenia</th>
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<tbody>
<tr>
<td>• Damaged native and prosthetic heart valves</td>
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<tr>
<td>• Malignant hypertension</td>
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<tr>
<td>• Acute glomerulonephritis</td>
</tr>
<tr>
<td>• Rejection of transplanted kidney</td>
</tr>
<tr>
<td>• Renal cortical necrosis</td>
</tr>
<tr>
<td>• Drugs (cyclosporine, tacrolimus)</td>
</tr>
<tr>
<td>• Vasculitis</td>
</tr>
<tr>
<td>• Systemic lupus erythematosus</td>
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Figure 3.47. Hereditary pyropoikilocytosis. Peripheral blood smear from a patient with hereditary pyropoikilocytosis, which is a variant of hereditary elliptocytosis. Significant variations in size and shape are present: teardrops, fragments, microspherocytes, elliptocytes, and small pieces and buds of RBCs. Heating the specimen increases this effect. These morphologic changes occur at a lower temperature than with samples from people without this disorder.
Figure 3.48. Peripheral blood smear of thermal injury. This slide, from a patient with severe burns, demonstrates microspherocytes and pieces of RBCs that can appear as barbell shapes, rod-like cells, or budding erythrocytes. Even these small pieces may retain a central pallor. Similar morphologic changes occur in hereditary pyropoikilocytosis. Clinical history will differentiate these two processes.

Figure 3.49. Paroxysmal cold hemoglobinuria (PCH). A: Blood film demonstrates small
RBC agglutinates in a patient with paroxysmal cold hemoglobinuria. B: Neutrophil in a patient with erythropagocytosis, which is related to acquisition of anti-P IgG antibodies, which cause binding of complement to RBCs and resulting in intravascular hemolysis after exposure to cold. Most commonly, PCH is a transient post-infection disorder occurring in children and only rarely presents as a chronic idiopathic disorder.

Figure 3.50. Oxidative hemolysis in glucose-6-phosphate dehydrogenase (G6PDH) deficiency. Peripheral blood film demonstrating blister cells in a patient with G6PDH deficiency undergoing a hemolytic episode. The blister appears as a vacuole in the erythrocyte’s hemoglobin at the edge of the RBC surface. A thin rim of RBC membrane seems to enclose this vacuole. This cell is usually a precursor to a bite cell.
Figure 3.51. Oxidative hemolysis. Irregularly contracted cells (indicated by the arrows) are smaller than normocytic RBCs in this blood film from a case of oxidative hemolysis. Here the RBCs do not have central pallor but, in contrast to spherocytes, they are irregular in shape and not spherical. The increased density of the irregularly contracted cells may give the hemoglobin a dark copper color. Occasional bite cells are present. Causes of oxidative hemolysis besides inherited causes such as G6PDH deficiency include drugs and toxins.

Figure 3.52. Bite cells (indicated by the arrows). The RBCs in this peripheral smear appear
bitten. The erythrocyte may retain or lose central pallor, depending on the size and numbers of bites. In some cases, the bite cell may be mistaken for helmet cells, a type of fragmented erythrocyte. The examiner, therefore, should consider the company that this cell keeps (i.e., the predominant morphology of the surrounding RBCs) before deciding if the process is one of oxidation or fragmentation. A double bite cell is displayed in the center of the figure. Besides factors that produce oxidative hemolysis, bite cells also occur in inherited unstable hemoglobins due to the formation of denatured hemoglobin (Heinz bodies), which are phagocytized in the spleen.

Table 3.8

<table>
<thead>
<tr>
<th>Rouleaux</th>
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<tbody>
<tr>
<td>• Increased plasma proteins (polyclonal or other proteins)</td>
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<tr>
<td>• Inflammatory state</td>
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<tr>
<td>• Infection</td>
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<tr>
<td>• Increased plasma proteins (monoclonal)</td>
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<tr>
<td>• MGUS (monoclonal gammopathy of unknown significance)</td>
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<tr>
<td>• Myeloma</td>
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<td>• Amyloidosis</td>
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<td>• Lymphoma</td>
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Figure 3.53. Peripheral blood smear of rouleaux and background staining. Excessive
amounts of acidic proteins (often immunoglobulins produced by myeloma cells) cause an increase in background bluish staining in blood smears. **A:** Top slide is normal and bottom slide shows background staining. **B:** The abnormal proteins interact with the sialic acid on the RBCs causing them to stack and line up in columns. Rouleaux have been defined as linear patterns of at least four RBCs. As opposed to agglutinates, the borders of the RBCs still can be distinguished. It is important to look in the area of the slide (center) where RBCs nearly touch each other to identify rouleaux indicating hyperproteinemia. RBCs normally may form rouleaux of no diagnostic significance in the thick, and sometimes thin, ends of peripheral blood films.

![Image of blood smear]

**Figure 3.54.** Cold agglutinin syndrome. Peripheral blood smear of RBC aggregates and cold agglutinin disorder. The RBCs form clumps for which distinguishing the borders of individual erythrocytes is difficult. In cold agglutinin disease, polychromasia, nucleated RBCs, and spherocytes also may be present. Agglutination of RBCs and hemolysis are due to IgM antibodies, usually of anti-I or anti-i specificity, that bind to RBCs at low temperature and fix complement on the RBCs. The IgM dissociates from the RBCs at normal body temperature, leaving the RBCs coated with complement, usually C3d. Because of the polyvalent nature of IgM, the RBCs in cold agglutinin disease tend to form clumps or grape-like clusters that are different from rouleaux, in which RBCs are arranged linearly in chains. The borders of erythrocytes are more easily distinguishable in rouleaux. When blood is warmed and the blood smear prepared on a warmed slide, the RBC clumps disappear or diminish. Hemolysis is mainly extravascular in this condition.

<table>
<thead>
<tr>
<th>Causes of intravascular hemolysis</th>
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<tbody>
<tr>
<td>• Fragments</td>
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<tr>
<td>• Hemolytic transfusion reaction</td>
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<tr>
<td>• Paroxysmal nocturnal hemoglobinuria</td>
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<tr>
<td>• Paroxysmal cold hemoglobinuria</td>
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<tr>
<td>• Infection: malaria, clostridia</td>
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<tr>
<td>• Chemical: arsenic, intravascular distilled water, venom</td>
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<tr>
<td>• Thermal injury</td>
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<tr>
<td>• Glucose-6-phosphate dehydrogenase deficiency</td>
</tr>
<tr>
<td>• Occasional overwhelming autoimmune hemolytic anemia</td>
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Figure 3.55. Chronic cold agglutinin disease. **A:** The hand on the right in this picture is warmed in a patient with chronic cold agglutinin disease. **B:** Macroscopic appearance of RBC agglutination at 37°C in a tube of blood from a patient with cold agglutinin hemolytic anemia secondary to infection with *Mycoplasma pneumoniae*. **C:** Serum in capillary tubes from a cold agglutinin specimen at room temperature (top) and 4°C (bottom) illustrating RBC agglutination at cold temperature that is reversible with warming. The temperature at which agglutination occurs is called the thermal amplitude. (Courtesy of Dr. I. Quirt.)
Figure 3.56. Microangiopathic hemolytic anemia. Formation of intravascular fibrin strands that “guillotine” circulated RBC lead to production of RBC fragments of variable shapes and sizes that lack central pallor as seen in this blood smear. RBC fragments have received specific names because of their shape; for example, comet cell, triangular cell, and schistocyte. The presence or absence of any of these cells does not have an impact on diagnosis or prognosis. In the process of red cell fragmentation, an occasional irregularly contracted erythrocyte, bite cells, or spherocytes can be seen, but the diagnosis of microangiopathic hemolytic anemia should be made by the morphology of most of the cells, which in this case are fragments. Note the severe thrombocytopenia related to peripheral destruction of platelets in microthrombi indicating that the diagnosis for this patient is thrombotic thrombocytopenic purpura or disseminated intravascular coagulation.
Figure 3.57. Thrombotic thrombocytopenic purpura. A: Coronal section through the brain at autopsy showing multiple small hemorrhages (arrows). B: Histologic section from the same patient shows diffuse microvascular occlusion of arterioles and capillaries. C: Blood smear from the same patient displays the microangiopathic picture with RBC fragments and profound thrombocytopenia. (Courtesy of Dr. J. Bilbao.)
Figure 3.58. Acanthocytes and liver disease. **A:** Peripheral blood film demonstrating acanthocytes, alternatively called *spur cells*. The acanthocytes do not have central pallor and are smaller than normocytic RBCs. They have long thin projections, some with bulbous or pointy edges, which are unevenly distributed around the surface of the RBC. Note target cells as well. **B:** This peripheral blood film demonstrates many acanthocytes in a patient with severe liver disease and hemolysis. This disorder is known as spur cell hemolytic anemia.

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<tr>
<td><strong>Acanthocytes</strong></td>
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<tr>
<td><strong>Large numbers of acanthocytes</strong></td>
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<tr>
<td>• Advanced liver disease</td>
</tr>
</tbody>
</table>
- Spur cell hemolytic anemia
- Abetalipoproteinemia
- Hypobetalipoproteinemia, homozygous
- McLeod phenotype
- In(Lu) phenotype
- Choreoacanthycytosis

**Small numbers of acanthocytes**

- Postsplenectomy
- Hypothyroidism
- Panhypopituitarism
- Vitamin E deficiency
- Malnutrition
- Thalassemia
- Iron deficiency
- Psoriasis

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**Figure 3.59.** Peripheral blood film demonstrating prominent teardrop RBC in primary myelofibrosis. One side of the teardrop RBC is tapered and ends in a blunt tip.
Figure 3.60. Paroxysmal nocturnal hemoglobinuria. The RBCs in this disorder may show anisocytosis, some macrocytes, and even nucleated RBCs reflecting ongoing hemolysis. However, the diagnosis is based on the clinical picture and detection of the loss of the glycophasphaditylinositol (GPI) anchor for membrane proteins, which leads to the RBC sensitivity to activated complement. Leukocytes and platelets, as well as RBCs, are affected in PNH by a lack of PGI-anchored membrane proteins. Flow cytometry histogram of WBC marked for fluorescent aerolysin (FLAER) is shown. The aerolysin stains positive those cells that possess GPI anchors. PNH cells lack these structures and will stain negative. The green cells are granulocytes, the blue cells monocytes, and the red cells lymphocytes (A). A large population of granulocytes and monocytes stain negative for FLAER (B and C) consistent with PNH, whereas the lymphocytes show positive staining with FLAER (D). (Courtesy of J. Davidson and R. Sutherland.)
Figure 3.61. Jaundice, anemia, and hemoglobinemia from intravascular hemolysis. A: Scleral icterus and pallor from autoimmune hemolysis. B: The clear sample on the left is normal, and the sample on the right is from a patient with hemolysis. The red color is from free hemoglobin released from lysed RBCs in the serum. C: Normal control without scleral icterus and pallor.
Figure 3.62. Hemoglobinuria resulting from intravascular hemolysis. A: The color of the urine sample on the left is normal. The sample to the right demonstrates the red appearance of urine due to hemoglobinuria during acute hemolysis. B: Urine positive for hemosiderin in a patient with intravascular hemolysis. This urine sample is stained for iron with Perl’s stain. Hemosiderin deposition (blue) in sloughed epithelial cells of renal tubules occurs in patients who have had intravascular hemolysis within the last 7 days. This test may be useful in detecting a recent episode of hemolysis that has resolved.
Figure 3.63. **A:** Hemoglobinuria in paroxysmal nocturnal hemoglobinuria (PNH). Urine taken over the course of days in a patient with PNH. The varying color depends on the degree of hemolysis at any one time. First morning urines tend to be darker, as well as urines taken during intercurrent illnesses, because of increased intravascular hemolysis. (Courtesy of Dr. J. Crookston.) **B:** Flow cytometry histogram of granulocytes marked for CD59. CD59 is a protein anchored by a GPI anchor that is deficient in individuals with PNH. This histogram demonstrates a population of 23.9% negative CD59 granulocytes (PNH cells also called PNH type I cells) and 63.2% positive CD59 granulocytes (normal cells called PNH type III cells) in a patient with PNH. No population exists in the middle of cells with intermediate, weak CD59 expression (PNH type II cells). (Courtesy of J. Davidson and R. Sutherland.)

<table>
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<th>Leukoerythroblastic picture</th>
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<td><strong>Increased demand</strong></td>
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<tr>
<td>• Hemorrhage</td>
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<tr>
<td>• Hemolysis</td>
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<tr>
<td>• Severe infection</td>
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<tr>
<td>• Recovery from bone marrow failure or suppression</td>
</tr>
<tr>
<td>• Sickle cell crisis</td>
</tr>
<tr>
<td>• Thalassemia major</td>
</tr>
<tr>
<td>• Systemic lupus erythematosus</td>
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<tr>
<td><strong>Bone marrow infiltration</strong></td>
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<tr>
<td>• Myelofibrosis</td>
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<tr>
<td>• Other causes of bone marrow fibrosis</td>
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<tr>
<td>• Hematologic malignancies</td>
</tr>
<tr>
<td>• CML, AML, ALL, Hodgkin disease, non-Hodgkin lymphoma, myeloma</td>
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<tr>
<td>• Nonhematologic malignancies</td>
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<tr>
<td>• Metastatic to bone marrow</td>
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<tr>
<td>• Granuloma</td>
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<tr>
<td>• Bone marrow infarction</td>
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<tr>
<td>• Storage disease</td>
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Table 3.11
• Severe megaloblastic anemia
• Severe rickets

CML, chronic myelogenous leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic lymphoma.

Figure 3.64. Leukoerythroblastic anemia, associated with metastasis to the bone marrow or other myelophthisic disease, such as tuberculosis of marrow. Peripheral blood film demonstrating a leukoerythroblastic picture, that is, the presence in the peripheral circulation of nucleated RBCs and immature granulocyte precursors.

Table 3.12
Sickling disorders
• Hb SS
• Hb SC
• Hb Sβ⁰-thalassemia
• Hb Sβ⁺-thalassemia
• Hb SD
• Hb SO-Arab
• Hb S/C-Harlem
• Hb S/S Antilles
• Hb S/Lepore
• Hb C/C-Harlem
• Hb S Antilles (heterozygous)
• Hb S Oman (heterozygous)
Figure 3.65. Sickle cell anemia. **A:** Peripheral blood smear of sickle cell anemia (Hb SS). The numerous curved, elongated erythrocytes with sharp points are classic sickle cells. Sickle cells that appear folded over are called *envelope cells.* Target cells are present, in this case because of hyposplenism from the gradual splenic infarction that occurs in Hb SS patients. Howell–Jolly bodies may be seen as well. **B:** Peripheral blood smear of Hb SS, demonstrating sickle cells with Hb concentrated at one end and absent at the other, called *hemi-lunes* (arrows), a finding seen in Hb SS or Hb SC. **C:** Peripheral blood smear of Hb SS, demonstrating short, stubby, and rhomboid-shaped sickle cells called oat and boat cells (arrows).
Figure 3.66. Sickle cells. A composite figure taken from different sickle cell anemia cases showing various shapes of sickle cells.
Figure 3.67. Sickle cell solubility test. In this test, whole blood is added to a high phosphate buffer with saponin and sodium dithionite, which causes the hemoglobin to become deoxyhemoglobin. Deoxyhemoglobin S is insoluble. The turbidity of the sample on the left indicates the presence of Hb S. The clear sample on the right contains no Hb S. This test is useful for demonstrating the presence of Hb S but is not quantitative and does not distinguish AS from SS. A new variation on this test, using a spot of deoxygenated blood dropped on filter paper, takes advantage of the insolubility of Hb S to distinguish SS from AS by differential spreading of the sample in the paper matrix.
Figure 3.68. Testing for hemoglobinopathy by HPLC. A: High-performance liquid chromatography (HPLC) sample demonstrating hemoglobin S trait (Hb A = 60%, Hb S = 40%). HPLC can separate Hb S from Hb D/G/Lepore, which are seen in the same band on alkaline Hb electrophoresis. B: HPLC sample demonstrating hemoglobin S disease (Hb S = 90%). Note the absence of hemoglobin A.
Figure 3.69. Hemoglobin S/β-thalassemia. Sickle cells and target cells are present in this blood film. The RBCs are microcytic, demonstrated by a diameter smaller than the nucleus of the mature lymphocyte in the bottom central region of this picture. The morphology may appear the same as in a patient with Hb SS/α-thalassemia or Hb SS with iron deficiency. Patients with Hb SS do not have microcytosis.

Figure 3.70. Sickle cell anemia and cholelithiasis. Gallstones extracted from a patient with chronic hemolysis from sickle cell disease. The stones are usually small and multiple and
consist of bilirubin. These stones may provoke cholecystitis. Cholelithiasis also can occur in patients with other hereditary hemolytic disorders, such as hereditary spherocytosis. (Courtesy of Dr. N. F. Olivieri.)

Figure 3.71. Sickle cell retinal changes. A: “Comma” vascular sign: superficial conjunctival vessel that contains densely packed sickle cells (arrow). B: Widened veins and tortuous large retinal vessels. C: Large preretinal hemorrhage of approximately 2 weeks’ duration. D: Old pigmented chorioretinal scar.

Figure 3.72. Sickle cell anemia bony abnormalities. Chest radiographs of the spine reveal central endplate depression with sparing of the anterior and posterior margins of the endplate (arrow). Although not pathognomonic, these radiologic findings are seen most often in sickle cell disease. In addition, the gastric air bubble occupies most of the region under the left hemidiaphragm extending to the left lateral thoracic cage, suggesting the absence of a
Figure 3.73. Sickle cell anemia skin ulcers. The most common site of skin ulcers in sickle cell anemia is the lower limb, often over bony prominences such as the ankles. They often represent vascular occlusion and necrosis of small skin vessels as the initiating event, then fail to heal. The ulcerations often have no antecedent trauma and can progress over time to extend into the dermis and subcutaneous tissue. (Courtesy of Dr. N. F. Olivieri.)

Figure 3.74. Hemoglobin SC disease. Most of the erythrocytes in this blood smear are target cells. Few sickle cells are present, and they tend to be short, stubby, and rhomboid-shaped (oat or boat cells). Irregularly contracted cells also are present. Rarely, hemoglobin C crystals are visible. The diagnosis of hemoglobin SC disease can be difficult using peripheral blood smears alone because few sickle cells are present. It may appear very similar to Hb C disease. Hb SC patients may not demonstrate hyposplenic changes and may have fewer nucleated RBCs than do Hb SS patients.
Figure 3.75. Hemoglobin SC disease. The condensation of Hb crystals in this blood film produces dark, blunt protuberances and other distortions. (From Diggs LW, Bell A. Intraerythrocytic crystals in sickle cell-hemoglobin C disease. Blood. 1965;25:218–223. Copyright American Society of Hematology, with permission.)

Table 3.13

Hemoglobinopathies associated with microcytosis

- β-Thalassemia trait (heterozygous)
- β-Thalassemia major (homozygous)
- α-Thalassemia trait
- Hb H disease
- α-Thalassemia trait and hemoglobin Constant Spring
- Hb C heterozygous and homozygous
- Hb E heterozygous and homozygous
- Hb D disease
- Hb O Arab disease
- Hb Lepore heterozygous and homozygous
- δβ-Thalassemia heterozygous and homozygous
- γδβ-Thalassemia heterozygous and homozygous
- Hereditary persistence of fetal hemoglobin homozygous
- Hereditary persistence of fetal hemoglobin (HPFH); specific types of heterozygous HPFH
Figure 3.76. Hemoglobin C disease. Target cells, irregularly contracted cells, and hemoglobin C crystals are present with microcytosis in this blood smear. Hemoglobin C crystals (arrows) are seen in cells that are otherwise empty of hemoglobin. Hemoglobin C crystals are an uncommon finding. More frequent are target cells, irregularly contracted erythrocytes, and microcytosis. (Courtesy of Dr. A. Chesney.)

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<tbody>
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<td>Target cells</td>
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<td><strong>Microcytic</strong></td>
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<td>• Hemoglobin E heterozygous + homozygous</td>
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<tr>
<td>• β-Thalassemia trait + major</td>
</tr>
<tr>
<td>• α-Thalassemia trait</td>
</tr>
<tr>
<td>• Hb H disease</td>
</tr>
<tr>
<td>• Hb AC + Hb C homozygous</td>
</tr>
<tr>
<td>• Hb Lepore heterozygous + homozygous</td>
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<td>• Hb O Arab disease</td>
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<td>• Hb D disease</td>
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<tr>
<td>• Iron deficiency</td>
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<td>• Hb Lepore trait</td>
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| **Normocytic or macrocytic** |
| • Obstructive jaundice |
| • Liver disease |
| • LCAT deficiency (lecithin-cholesterol acyl transferase deficiency) |
| • Hb SC disease |
| • Hyposplenic state |
| • Hb O Arab disease |
Figure 3.77. Thalassemia trait blood film. Peripheral blood smear in β-thalassemia trait may demonstrate microcytosis and hypochromia. Multiple morphologic changes including target cells, teardrop cells, and rare fragments may occur. These features can appear identical to the morphologic picture of iron deficiency. Basophilic stippling can occur in Mediterranean populations with β-thalassemia trait and is less common in other populations with this disorder. Basophilic stippling may help distinguish β-thalassemia trait from iron deficiency, but is not always present in patients with β-thalassemia trait. RBC indices may help: a normal or slightly decreased hemoglobin with a low MCV/MCH and a low or mildly increased RDW suggests thalassemia. RBC indices may not always distinguish iron deficiency from thalassemia trait, however. Patients also may have combined iron deficiency and β-thalassemia trait and therefore require further testing to exclude the former.

Figure 3.78. Basophilic stippling in thalassemia. Peripheral blood film demonstrating microcytic hypochromic RBCs and basophilic stippling (arrows). Basophilic stippling occurs in thalassemia as well as in other hematologic disorders.
Figure 3.79. Bone marrow in thalassemia. Bone marrow aspirate (A) and biopsy (B) from a case of thalassemia trait. The bone marrow has increased numbers of erythroid precursors (a low myeloid to erythroid ratio) related to the increased peripheral RBC destruction in this disease.
Figure 3.80. Hemoglobin H inclusions. Peripheral blood stained with supravital stain brilliant cresyl blue. The RBC near the top central area (red arrow) demonstrates numerous inclusions, representing precipitated β-globin tetramers, in an evenly diffuse distribution, creating a “golf ball” pattern. Hb H inclusions are seen in α-thalassemia, especially with three α-chain deletions (α−/−−). The difference between the Hb H bodies that appear like dimpled golf balls with diffuse even involvement can be seen from reticulocytes with uneven reticulin deposits (black arrows). Reticulocytes, Heinz bodies, and Howell–Jolly bodies stain positive with brilliant cresyl blue. Reticulocyte inclusions are darker, more net-like, clumped, and uneven in distribution. Heinz bodies are larger and not so numerous. Howell–Jolly bodies are usually single inclusions. These inclusions appear after 10 minutes of incubation at room temperature, whereas Hb H inclusions require incubation at 37°C for 1 to 2 hours. Rare Hb H inclusion bodies may be seen in one or two α-gene deletions in α-thalassemia trait, but in those cases, the absence of identifying these inclusion bodies does not exclude the disorder, which may require molecular studies for definitive diagnosis. In Hb H disease (three α-gene deletion), Hb H bodies are frequent and easily identifiable. (Courtesy of Dr. D. Amato.)
Figure 3.81. Cellulose acetate alkaline (pH 8.4) gel electrophoresis for detection of hemoglobins (Hb). Lane 1 shows the controls for Hb A, Hb F, Hb S, and Hb A_2. Lane 2 shows Hb C trait (AC). Lane 3 shows Hb S trait (AS). Hb A is present in greater amounts than Hb S (usually 60%-40%) Lane 4 shows sickle cell anemia (Hb SS) in a newborn (when Hb F is still the major hemoglobin; no Hb A is present). Hemoglobins that move with Hb S on alkaline gels include D/G/Lepore, and hemoglobins that move with Hb C on alkaline gels include E/O/A_2.

Figure 3.82. High-performance liquid chromatography (HPLC) sample demonstrating increased hemoglobin A_2 (arrow) in a case of β-thalassemia trait. HPLC is an automated way of separating and identifying variant hemoglobins and is more accurate at quantifying hemoglobin A_2 than is Hb electrophoresis. It can separate Hb A_2 from certain other hemoglobins, which is not possible using hemoglobin electrophoresis alone.
Figure 3.83. Capillary electrophoresis sample demonstrating the separation of normal (Hb A, Hb F, Hb A₂) and abnormal (Hb S and Hb C) forms of hemoglobin. Capillary electrophoresis is similar to HPLC in that it provides an automated way to separate and identify variant hemoglobins with high sensitivity.
Figure 3.84. Heinz bodies. Peripheral blood stained with crystal violet supravital stain demonstrating Heinz body inclusions, which are not visible with Romanowsky stains alone. Heinz bodies are purple-blue, large, single, or multiple inclusions attached to the inner surface of the RBC membrane. They represent precipitated normal or unstable hemoglobins. Heinz bodies are more frequently seen postsplenectomy, and in patients with unstable hemoglobins such as Hb H, Hb Kln, and Hb Zurich. Testing should be done within 1 hour after blood is collected. Reticulocytes do not stain positive with crystal violet.

Table 3.15

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<th>Heinz bodies</th>
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<tr>
<td>Oxidative stress</td>
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<td>Glucose-6-phosphate dehydrogenase deficiency, glutathione synthetase deficiency</td>
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<td>Drugs</td>
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<td>Toxins</td>
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<td>Unstable hemoglobins</td>
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Figure 3.85. Hemoglobin H disease. Hb H is β4, a form of α thalassemia, α−/−−, which causes hemolysis due to its instability. This blood film demonstrates microcytosis, hypochromia, and numerous morphologic abnormalities, including target cells, microspherocytes, and fragments. Basophilic stippling may occur. Polychromasia is present. Heinz bodies which are present in these red cells can only be visualized on a supravitaly stained smear.
Figure 3.86. Hydrops fetalis at autopsy in hemoglobin Bart disease. Hepatosplenomegaly in a newborn with hemoglobin Bart disease. Hb Bart is γ4. The loss of all four α-globin genes results in severe anemia, high-output heart failure, splenomegaly, edema, and intrauterine or immediately postpartum death for the affected fetus. Dystocia, eclampsia, and hemorrhage can occur in the mother carrying the affected fetus. (Courtesy of Dr. D. Amato.)
Figure 3.87. α-Thalassemia diagnosis by polymerase chain reaction (PCR) amplification of DNA. Multiplex PCR results for the seven most common deletional mutations of the α-globulin gene cluster. The LIS1 gene at 17p13.3 was included as an amplification control. Using genomic DNA from known genotypes for the following mutant alleles: –α 3.7, –α 4.2, – –FIL, – –MED, and –Sea. Plasmid controls for –THAI and –(α)20.5 were mixed with normal genomic DNA to mimic the heterozygous state. M represents the γ-BstE II molecular weight marker. Lane 1, blank; lane 2, αα/αα; lane 3, –3.7/–4.2; lane 4, αα/– –FIL; lane 5, αα/– –SEA; lane 6, αα/– –MED; lane 7, αα/– –THAI; lane 8, αα/–(α)20.5; lane 9, αα/αα; lane 10, αα/αα; lane 11, – –MED/–α. (Courtesy C. Wei.)
Figure 3.88. β-Thalassemia facial bone abnormalities. These changes include bossing of the skull; hypertrophy of the maxilla, exposing the upper teeth; depression of nasal bridge; and periorbital puffiness. (Courtesy of Dr. N. F. Olivieri.)
**Figure 3.89.** β-Thalassemia major leg ulcer. Leg ulcers can occur in all types of hereditary hemolytic anemias, including sickle cell disease and hereditary spherocytosis. (Courtesy of Dr. N. F. Olivieri.)
Figure 3.90. β-Thalassemia major. Note the pallor, short stature, massive hepatosplenomegaly, and wasted limbs in this undertransfused subject with β-thalassemia major. (Courtesy of Dr. N. F. Olivieri.)
Figure 3.91. β-Thalassemia major blood smear. Unless they have had transfusions, patients with this disease usually have severe anemia. This peripheral blood film demonstrates many nucleated RBCs, microcytosis, and hypochromia with multiple morphologic changes: target cells, teardrop cells, fragments, basophilic stippling, and Pappenheimer bodies. The nucleated RBCs may be dysplastic or show abnormal hemoglobinization. Neutrophilia and thrombocytosis may occur. This patient has undergone splenectomy for hypersplenism and increased transfusion requirements. Howell–Jolly bodies are present.
Figure 3.92. β-Thalassemia bone abnormalities. Note the “hair on end” appearance of the cortical bone caused by expansion of the bone marrow (arrows). The subperiosteal bone grows in radiating striations, which appears as “hairs.” (Courtesy of Dr. N. F. Olivieri.)
Figure 3.93. Kleihauer–Betke test. This peripheral blood from a postpartum woman with fetomaternal hemorrhage demonstrates Hb F–containing fetal cells (dark red) in a background of maternal cells (ghost-like cells). Hb F cells are resistant to acid elution of hemoglobin. Aside from detecting fetal cells in the mother’s blood in a fetomaternal hemorrhage, this test can be used to detect Hb F–containing cells in β-thalassemia, hereditary persistence of hemoglobin F (some types have homogeneous distribution of Hb F in the cells), sickle cell disease, δβ-thalassemia, and myelodysplastic syndrome.
Figure 3.94. Cryoglobulinemia. Precipitated cryoglobulins (arrows in A) may appear as white spots on top of RBCs, usually at the edges of the erythrocyte, and can be easily mistaken for bite cells and blister cells. Purplish clouds of precipitated cryoglobulins overlapping onto the RBCs are shown in both A and B.
HEMOSTASIS AND ITS ROLE IN DISEASE

Hemostasis

Hemostasis is the process by which vascular integrity is maintained. Hemostasis is dependent on multiple components, including vascular endothelium, platelets, von Willebrand factor (vWF), and soluble plasma proteins. The process may be broken down into two main phases termed primary and secondary hemostasis. Primary hemostasis refers to the formation of the platelet plug, and secondary hemostasis refers to the role of the coagulation cascade in forming a fibrin clot, which together with platelets generates thrombus. Briefly, formation of the platelet plug is initiated by platelet adhesion to tissue factor, collagen, von Willebrand factor (vWF), or fibronectin exposed on the subendothelial matrix during vascular injury. This initial contact activates platelets, resulting in increased expression of surface proteins and release of granules that contain factors that enhance coagulation. The activated platelets express on their surface membranes glycoprotein complex IIb–IIIa, which binds fibrinogen, cross-linking the platelets together in a process called aggregation. This process is discussed in more detail in the section on platelets.

Secondary hemostasis refers to the role of the plasma coagulation factors in generating thrombin that cleaves fibrinogen to insoluble fibrin and supports the initial platelet plug. This network of platelets and fibrin is cross-linked by factor XIII to stabilize it and form a chemically stable thrombus in which red cells are enmeshed to provide bulk.

The coagulation cascade of plasma clotting factors is classified into the extrinsic, intrinsic, and common pathways (Fig. 4.1). The extrinsic pathway is initiated when blood is exposed to tissue factor (TF), a transmembrane glycoprotein expressed in vessel walls and endothelial cells. Tissue factor binds factor VII, resulting in its activation to factor VIIa, which activates factor X and generates the prothrombinase complex composed of Xa and Va. The prothrombinase complex cleaves prothrombin (factor II) to thrombin (factor IIa), a key enzyme in the coagulation cascade. Thrombin both cleaves fibrinogen to form fibrin, which is then cross-linked by factor XIII to form a stable clot, and also activates factors V, VIII, and XI, adding to thrombin activation. In contrast, fibrin deposition is limited and controlled by an endogenous anticoagulant system. Antithrombin (AT) is a plasma protein member of the serpin (serine protease inhibitor) family that inhibits the activities of all of the activated coagulation enzymes, particularly factor IIa and Xa. Protein C is a vitamin K–dependent protein that proteolytically inactivates activated factor Va and factor VIIIa, thus blocking their ability to generate more thrombin. Protein C binds to an endothelial cell protein C receptor (EPCR) and is activated by thrombin bound to thrombomodulin, another endothelial cell membrane–based protein, in a reaction that is promoted by a cofactor, protein S (Fig.
Thus, thrombin not only leads to clot formation but also contributes to clot limitation through the activation of protein C. In a parallel fashion, tissue factor pathway inhibitor is a plasma protein that forms a quaternary complex with tissue factor, factor VIIa, and factor Xa that inhibits the extrinsic coagulation pathway.

The extrinsic pathway is thought to be the primary method of activating the coagulation in vivo and is critical for normal hemostasis. This pathway is measured with the prothrombin time (PT).

The intrinsic pathway—composed of factors XII, XI, IX, and VIII—is activated by negatively charged surfaces such as inorganic polyphosphates released from platelet-dense granules upon platelet activation that start a cascade of activation of these components, which again generates the prothrombinase complex of factors Xa and Va and produces fibrin from fibrinogen, as described above. The intrinsic pathway is measured by the activated partial thromboplastin time (aPTT). Maintaining normal hemostasis depends on all these factors working in concert, and alterations in the system, either inherited or acquired, will lead to dysregulation of normal hemostasis with subsequent bleeding or thrombosis (i.e., excessive clotting).

An alternative model of coagulation is the cell-based model proposed by Drs. Hoffman and Monroe in 2001.¹ In this model, coagulation is regulated by properties of cell surfaces in three overlapping stages of initiation, amplification, and propagation, rather than the traditional cascade (Fig. 4.3). This model is appealing because it may more closely approximate what happens in vivo rather than the cascade model, which is based largely on in vitro enzyme activity.

**Fibrinolysis**

As hemostasis refers to the formation of a blood clot to preserve vascular integrity, the fibrinolytic system refers to the removal of blood clots no longer required, via a cascade of serine proteases that degrade fibrin and result in clot dissolution. Activation of the circulating zymogen called plasminogen into its active form, plasmin, is achieved mainly by tissue-type plasminogen activator (t-PA). This catalysis is highly dependent on the presence of fibrin, which by binding both plasminogen and t-PA increases plasmin generation by more than two orders of magnitude. Mechanistically, plasminogen binds to exposed lysine residues formed in fibrin, and these binding sites increase in number during fibrin cleavage, allowing more plasminogen binding to occur, thereby amplifying the process, allowing more plasmin to be generated. Naturally occurring plasmin inhibitors (α₂ antiplasmin, plasminogen activator inhibitor [PAI]-1, and PAI-2) also exist that limit plasmin activity or its generation in the circulation. These include plasma protein forms or inhibitors released by activated platelets. The most potent of these is α₂ antiplasmin. While bound to fibrin, plasmin is largely protected from α₂ antiplasmin, allowing fibrin cleavage to occur. Other key regulatory steps in fibrinolysis occur at the level of the plasminogen activators, as the activities of t-PA and also urokinase-type plasminogen activator (u-PA), the second important endogenous plasminogen activator, are both regulated by PAI-1 and PAI-2.

The most recently described mechanism that limits the fibrinolytic system is via “thrombin activatable fibrinolysis inhibitor.” The plasma protein, thrombin activatable fibrinolysis inhibitor (TAFI), is a carboxypeptidase that specifically cleaves exposed lysine residues from fibrin, thereby removing the ability of plasminogen and t-PA to dock onto lysine-binding sites in fibrin. Because it requires activation by thrombin, TAFI becomes engaged as a direct consequence of coagulation to stabilize and protect clots from premature removal by the
fibrinolytic system.

BLEEDING DISORDERS

Platelet Disorders

Stable clot formation depends on an appropriate platelet number, normal platelet function, and adequate coagulation factors. The role of platelets can be summarized as the three A’s: adhesion, activation, and aggregation. Adhesion refers to the initial binding of a single layer of platelets to sites of vascular injury and is mediated by exposure of tissue factor, collagen, von Willebrand factor (vWF), and fibronectin on the subendothelial matrix during endothelial damage. This initial contact leads to platelet activation, which causes a flipping of the polarity of inner and outer leaflets of the platelet surface membrane. The exposure of anionic phospholipids at the platelet surface serves as platforms for the assemblies of blood coagulation enzyme complexes and release of platelet granules containing factors that further enhance coagulation at the site of vascular injury (Fig. 4.4). Platelet aggregation on the initial layer of adherent platelets at the injury site then occurs when fibrinogen binds to the glycoprotein IIb–IIIa complexes expressed on multiple activated platelets and is converted to fibrin through local activation of the clotting cascade on the platelet surface, forming a platelet plug. Defects at any step in this complex process can lead to bleeding disorders as described in the following sections.

Thrombocytopenia

Thrombocytopenia represents a decrease in the platelet count below the normal range (150,000–450,000/mm³) and is associated with petechial and mucosal bleeding. In evaluating apparently low platelets on the automated complete blood count (CBC), pseudothrombocytopenia must be excluded. Pseudothrombocytopenia is an in vitro phenomenon in which platelets aggregate in the presence of ethylenediamine tetra-acetic acid (EDTA), the anticoagulant in lavender-top specimen tubes used for blood counts. If platelets are found clumped as in Figure 4.5, the count in the CBC is not reliable and must be repeated. Pseudothrombocytopenia can be detected in most cases by redrawing blood for the CBC in a sodium citrate (blue top) tube or by making a peripheral blood smear from a fingerstick blood specimen and counting the platelets manually. Once it is established that the low platelet count is real, the differential diagnosis of thrombocytopenia includes underproduction, excessive consumption or destruction, or sequestration in enlarged spleen or liver. Underproduction may be either hereditary or acquired, as seen in hematologic malignancies or with metastases of solid tumors to the bone marrow. Consumption in vivo can result from active bleeding, from platelet trapping in artificial membranes during cardiopulmonary bypass, or as a manifestation of disseminated intravascular coagulation (DIC). Platelet destruction is seen with idiopathic immune thrombocytopenia (ITP), drug-induced thrombocytopenia, infection-induced thrombocytopenia, and thrombotic microangiopathies (TMAs). Sequestration most often occurs in patients with splenomegaly. Splenomegaly is noted on the physical or radiologic examination of patients with liposomal storage diseases such as Gaucher disease, or with lymphoma, chronic lymphocytic leukemia, autoimmune disorders, thalassemias, portal hypertension, and some infections.

Immune Thrombocytopenic Purpura
One of the most common causes of severe thrombocytopenia (platelet count <50,000/mm\(^3\)) is immune thrombocytopenic purpura (ITP). ITP has a slight female preponderance (1.2–1.7 times more frequent in women than men in different studies) and may have increasing incidence with age. In children, ITP typically arises after a viral infection, has an abrupt onset and a limited course, whereas in adults ITP is more likely to have a gradual onset and to be chronic or relapsing. A patient with severe immune thrombocytopenia usually presents with mucocutaneous bleeding such as epistaxis, gingival bleeding, or a petechial rash; menorrhagia is often the presenting symptom in women of reproductive age (Figs. 4.6 and 4.7). The development of any of these symptoms should prompt an evaluation of the platelet count. Platelet counts below 30,000/mm\(^3\) may lead to petechiae and ecchymosis or bleeding with minor trauma. Platelet counts below 10,000/mm\(^3\) may result in spontaneous bleeding, although clinically significant bleeding is rare in ITP.

ITP is a diagnosis of exclusion and requires isolated thrombocytopenia in the absence of an alternate explanation for low platelets. For example, splenomegaly is rare in ITP. The peripheral blood smear is normal except for low platelet numbers, with large or giant platelets seen on the smear, evidence of platelets being released early from the megakaryocytes in the bone marrow due to high demand. The reticulated platelet count (measured with an acridine orange stain) is elevated in ITP, confirming the increase in “young” platelets. Although tests for antiplatelet antibodies exist, they are not either sensitive or specific enough to warrant regular use in diagnosing ITP. If a bone marrow biopsy is performed, an increased number of morphologically normal megakaryocytes in different stages of differentiation may be observed (Fig. 4.8), in contrast to essential thrombocythemia (ET), in which megakaryocytes are often dysplastic (Fig. 4.9). Bone marrow examination, however, is not required for diagnosis. Treatments for ITP include steroids, intravenous γ-globulin, Rh immunoglobulin (Winrho), splenectomy, rituximab, and thrombopoietin agonists such as eltrombopag and romiplostim (see Fig. 4.8). Splenectomy is the only treatment that is considered curative, but it is being used less frequently because many other therapeutic alternatives are available now.

Drug-Induced Thrombocytopenia

Thrombocytopenia may also be seen as an adverse response to numerous medications. Drug-induced thrombocytopenia may present with severe thrombocytopenia (nadir platelet count <20,000/mm\(^3\)). Drugs cause thrombocytopenia by a variety of mechanisms, including direct antibody interactions that increase the affinity of the antibody for platelet antigens (as for quinine), drug interaction with platelet antigens creating a neoepitope (as for heparin), platelets reacting with a drug metabolite (as for sulfamethoxazole and trimethoprim), or an on-target effect of an therapeutic antiplatelet glycoprotein antibody (as for abciximab). Drug-induced thrombocytopenia resolves after stopping the offending agent and reoccurs after reintroduction of the drug. Heparin-induced thrombocytopenia (HIT) is a special case because it represents both a thrombocytopenic and a hypercoagulable state and will be discussed under the latter topic (Table 4.1).

Infection-Induced Thrombocytopenia

Thrombocytopenia may commonly be seen during severe infections or sepsis, in which it is often a negative prognostic sign associated with DIC. Specific insect-borne infections of red blood cells (RBCs) or white blood cells (WBCs) may also cause thrombocytopenia by other mechanisms such as vascular damage and platelet consumption or DIC. Infections that can cause anemia and thrombocytopenia include viral hepatitis, rickettsial infections, ehrlichiosis, babesia (Fig. 4.10), and Plasmodium falciparum malaria (Fig. 4.11).
Inherited Platelet Disorders

Inherited platelet disorders causing thrombocytopenia are rare. Platelets may be low as part of a bone marrow failure syndrome, or platelet defects that impair platelet production or render platelets less stable in circulation. Such thrombocytopenias may be associated with a syndrome such as Wiskott–Aldrich syndrome (WAS)/X-linked thrombocytopenia (XLT), thrombocytopenia with absent radii (TAR), or MYH9-related thrombocytopenia, or they may be rare primary platelet defects such as in Glanzmann thrombasthenia, Bernard–Soulier syndrome, gray platelet syndrome, or congenital amegakaryocytic thrombocytopenia (CAMT) (Table 4.2). Making the diagnosis of these rare disorders requires advanced testing, the exception being gray platelet syndrome, which can be diagnosed on light microscopy because of the absence of α-granules in these platelets (Fig. 4.12).

Acquired Platelet Disorders

Thrombocytopenia in hematologic malignancies or myelodysplastic syndromes is usually secondary to bone marrow dysfunction or to consumption coagulopathies (DIC) related to malignancy as in acute promyelocytic leukemia [Fig. 4.13] or bone marrow metastasis of solid tumors or trauma, or in severe obstetrical complications. In lymphoproliferative disease or autoimmune diseases where T-cell function is disturbed, ITP is frequently seen either alone or associated with autoimmune hemolytic anemia (as Evans syndrome).

Thrombocytosis

Thrombocytosis represents a marked increase in platelet count to above 500,000/µL and is classified as reactive or primary. In reactive thrombocytosis, the platelets are usually of normal size and have normal reactivity, whereas in primary thrombocytosis platelets are often large or varied in size and have altered functionality. Reactive thrombocytosis occurs in patients with iron-deficiency anemia, hemolysis, connective tissue disorders, inflammatory conditions, tuberculosis, cancer, or after surgery (commonly postsplenectomy). Once reactive thrombocytosis is excluded, primary thrombocytosis or essential thrombocythemia (ET) is likely. ET is characterized by a persistent elevated platelet count and the presence of a JAK2 V617F mutation in 60% of the patients, or an MPL gene substitution or a calreticulin (CALR) mutation; in about 10% of patients with ET, no mutations have been identified. Patients with JAK2 (V617F) mutation are older, have a higher hemoglobin level and WBC count, and a lower platelet count and serum erythropoietin than those with a CALR mutation. Bone marrow megakaryocytes in reactive thrombocytosis show normal maturation, whereas in primary thrombocytosis, megakaryocytes have abnormal morphology. (For further discussion of essential thrombocytosis, see Chapter 8.)

Laboratory Testing of Platelet Function

Platelet functional assays such as the PFA-100 and platelet aggregometry measure qualitative platelet defects and serve as screening tests. In the past, platelet function was assessed by the bleeding time, the time in seconds required for bleeding to stop from a small skin wound made in a standard manner. Bleeding time has since been largely discarded because of variability and the facts that its performance is labor intensive and operator dependent. The PFA-100 test was designed to replace the bleeding time and measures how long a sample of whole blood takes to clot when passed through a special cartridge in which platelets are activated by either collagen + epinephrine or collagen + adenosine diphosphate (ADP) (Fig. 4.14). If the platelets are normally activated by these agonists, the clot forms quickly within the normal time range of the test; if platelet function is deficient, the time to clotting is prolonged or indefinite. This test uses a freshly drawn tube of blood, can be done in the
laboratory rather than at the bedside, does not require the presence of the patient, and the results are less variable than with the classic bleeding time.

The most common cause of platelet dysfunction, by far, is medication taken by the patient, often over-the-counter drugs. Aspirin is a frequently used drug that impairs platelet aggregation and release and does so in very low doses and with a prolonged effect (up to a week). Beyond drug effects on platelets, platelet function may also be intrinsically abnormal (poor) in uremia, von Willebrand disease (vWD), liver disease, autoimmune disease such as lupus, and the hereditary thrombocytopenias, whereas it may be hyperactive in arteriosclerosis and diabetes.

Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) cause a prolongation specifically of the collagen–epinephrine aggregation time in the PFA-100. Other antiplatelet medications such as clopidogrel or ticagrelor, other over-the-counter medications such as guaifenesin, some herbal supplements such as fish oil, or certain antidepressants all can depress platelet function and impair hemostasis. Therefore, a careful medication history is necessary in evaluating hemostasis. In uremia, vWD, and liver disease, both types of PFA-100 results may be abnormal. The PFA-100 has a high negative predictive value; for example, if the PFA-100 gives a normal result, then with a few exceptions (storage pool disease, primary secretion defects, and mild type 1 vWD), primary hemostasis is intact and may eliminate the need for further screening of platelet function. However, if more specific testing is required, complete platelet aggregometry can be performed on either platelet-rich plasma or whole blood. Complete aggregometry includes using a panel of soluble platelet agonists, including ADP, epinephrine, collagen, a thromboxane A2 mimetic, arachidonic acid, thrombin receptor–activating peptide, and ristocetin. By correlating which platelet agonists are able to induce aggregation and which do not, specific defects in platelet function can be determined (Fig. 4.15).11

Coagulation Factor Deficiencies

Inherited Factor Deficiencies

vWD is the most common congenital bleeding disorder, with an estimated prevalence of up to 1% of the population, although the bleeding phenotype is variable. The disease is autosomal dominant in inheritance and represents the most common bleeding disorder in women. Bleeding is typically into skin and mucosae—petechiae and ecchymoses—rather than joint or deep tissue bleeding, and menorrhagia is common in affected women. In vWD, the large multimeric plasma glycoprotein, vWF, necessary for normal platelet adhesion to the subendothelium and also for stabilizing circulating coagulation factor VIII, is decreased or absent. When vWD is inadequate, platelet plug formation is depressed and bleeding occurs in a mucosal and petechial fashion. The severity of vWD ranges from very mild (type I) to severe (type III); in the latter, the vWF level is so low that factor VIII levels are as low as in classic hemophilia, and the clinical phenotype is that of hemophilia A complicated by defective platelet adhesion.

In assessing vWD, several tests are available:

- Antigen level (vWF:Ag)—a quantitative measure of the vWF protein without information about its function
- Activity (vWF:RCo)—a functional assay that measures platelet aggregation after addition of the activator ristocetin to the patient’s plasma
- vWF:RCo/vWF:Ag ratio—the ratio between antigen and activity levels, which can help
distinguish type I from type II vWD

- Factor VIII levels—vWF is required to maintain FVIII in circulation
- Ristocetin-induced platelet aggregation (RIPA and low-dose RIPA)—patients with type IIB vWD demonstrate enhanced platelet aggregation in response to stimulation with low-dose ristocetin
- Multimer assay—vWF functions optimally as a large multimer, and electrophoresis for multimer size helps to distinguish subtypes of vWD

vWD is classified into three main types, as shown in Table 4.3. Type I is a quantitative deficiency of vWF and can be mild, moderate, or severe, depending on the level of the protein deficiency. In von Willebrand assays, this will result in concordantly low von Willebrand antigen and activity, maintaining a preserved vWF:RCo/vWF:Ag ratio. vWD type IC has been described and results from increased VWF clearance from the circulation. Type II vWD represents a qualitative deficiency of vWF activity and is characterized by a decreased ratio of vWF:RCo/vWF:Ag (often <0.7). There are multiple subtypes of type II vWD, including IIA, IIB, IIM, and IIN—each characterized by a different defect. vWD IIA is the most common subtype and results from a deficiency of the high-molecular-weight multimers of vWF, which are required for efficient binding to platelets, that causes the observed bleeding phenotype (Fig. 4.16). vWD type IIB is a gain-of-function mutation that causes increased binding to platelet glycoprotein Ib; however, as this binding occurs inappropriately without hemostatic challenge, it leads to a consumption of platelets and the larger VWF multimers.

As such, treatment of bleeding with deamino-8-D-arginine vasopressin (desmopressin acetate or DDAVP), which releases vWF from endothelial storage sites and thus improves the clinical status in type I vWD, is relatively contraindicated in type IIB owing to the risk of worsening bleeding by increasing the consumption of platelets. VWD IIM is a defect in the ability of vWF to bind platelets. Although both levels of VWF protein and HMW multimers are present, vWF activity is decreased, as measured by platelet aggregation in response to activation with ristocetin. vWD type IIN (Normandy) subtype is distinct in that it can be misdiagnosed as hemophilia A. Type IIN is caused by mutations that decrease the capacity of vWF to bind and stabilize FVIII. These patients will have a prolonged aPTT and low FVIII activity.

Type III vWD, the most severe form, represents an absence of von Willebrand factor and shows no vWF:Ag. Type III vWD is extremely rare and is associated with a severe bleeding phenotype.

In platelet-type or pseudo-vWD, platelet glycoprotein Ib is abnormal and remains in an active form that binds vWF even in the absence of appropriate platelet activation. Pseudo-vWD can be very difficult to distinguish from vWD type IIB in which vWF has increased affinity for platelet gpIb. However, patients with platelet-type vWD will aggregate platelets in the presence of cryoprecipitate in a cryo-challenge test, whereas those with vWD type IIB will not.

Treatment of type I vWD includes avoidance of aspirin and NSAIDs and administration of desmopressin, which releases endogenous vWF from endothelial stores and so raises circulating vWF levels. Specific concentrates of vWF or of factor VIII:vWF are available for severe cases as in type III vWD. More recently recombinant VWF was approved for the on-demand treatment and control of bleeding episodes in adults diagnosed with VWD. Plasma cryoprecipitate, which is rich in vWF, factor XIII, factor XIII, fibrinogen, and fibronectin, can also be used in moderately severe cases, but if used in large volumes may raise fibrinogen and FVIII to potentially hypercoagulable levels.
**Hemophilia A and B**

Hemophilia is an X-linked recessive disorder of hemostasis that causes a lifelong bleeding tendency. Hemophilia A, or factor VIII deficiency, is present in 1 in 5 of 10,000 male births, whereas hemophilia B, a deficiency of factor IX, is seen in approximately 1 in 30,000 male births. Approximately 30% cases of hemophilia result from spontaneous mutations and thus lack a positive family history. The FVIII gene is extremely large, and many different mutations occur that result in hemophilia A. Occasionally, owing to skewed lyonization, female carriers of hemophilia A can have a bleeding tendency. True hemophilia A in females is extremely rare, comprising the offspring of a hemophilic father and hemophilia carrier mother.

Coagulation factors are normally present in the blood at concentrations far in excess of those needed for normal hemostasis. Therefore, hemophilia is classified as clinically mild (>5% of the normal factor level), moderate (<5% but >1%), or severe (<1%). Patients with hemophilia can present with ecchymosis (spontaneous bruising without preceding trauma) and, most commonly, bleeding into joints (hemarthroses) or soft tissues (Fig. 4.17), which over time and many rebleeding episodes can lead to significant inflammation joint deformity and limited range of motion (Figs. 4.18–4.21). Intracranial bleeding is also a dreaded complication. In severe hemophilia, spontaneous bleeding usually manifests in the 1st year of life (classically at circumcision in the newborn), whereas in moderate or mild hemophilia presentation can be variable. As hemophilia is caused by a defect in the intrinsic pathway, patients have a prolonged aPTT, the finding of which should prompt measurement of factor VIII and/or factor IX levels. Usually, only severe hemophiliacs (factor level <1%) require prophylactic factor replacement. However, patients with moderate hemophilia may still have severe bleeding in response to relatively minor trauma and require factor replacement after injuries or before scheduled surgical procedures (Table 4.4). Patients with severe hemophiliacs may also develop antibodies against factor VIII (or factor IX) after extensive treatment with replacement coagulation factors, leading to further exacerbation of bleeding episodes and difficulty with managing their coagulation defect. Special products such as FEIBA (factor VIII inhibitor bypass activity) and recombinant factor VIIa (rVIIa) have been developed to combat such complications, used together with immunosuppressive therapy to suppress the offending autoantibody.

**Hemophilia C: Factor XI Deficiency**

Inherited factor XI deficiency is an autosomal bleeding disorder characterized by reduced levels of factor XI in plasma. Factor XI deficiency is seen primarily in Ashkenazi Jewish populations, where up to 1 in 10 individuals may carry a mutation. In contrast to hemophilia A and B, the level of factor XI in this deficiency does not accurately predict the bleeding phenotype because different mutations may have different phenotypes ranging from asymptomatic to severe. Most bleeding manifestations in patients with severe factor XI deficiency are injury related. When a site of injury with high fibrinolysis is involved, the risk of bleeding is increased in comparison with sites without fibrinolysis. This is because factor XI indirectly increases TAFI and thus leads to reduced fibrinolysis and better-stabilized clot formation, and hence deficiency of factor XI is associated with increased fibrinolysis. The main predictor of bleeding in patients with factor XI deficiency is the clinical history of bleeding (see Table 4.4). Factor XI has a long half-life, and so administration of fresh frozen plasma as replacement usually suffices for treatment or for surgical prophylaxis.
Rare Factor Deficiencies

Deficiencies of other coagulation factors—including II (fibrinogen), V, VII, X, and XIII—are very rare, occurring in 0.5 to 2 of 1,000,000 individuals (Table 4.5). Factor XII deficiency is not associated with a bleeding phenotype, although the aPTT may be significantly prolonged above normal. Indeed, clinically factor XII deficiency has been linked with thrombosis. For deficiencies of factors VII, X, and XIII, replacement factor concentrates are available, but may not be readily available during an acute bleed. In the absence of specific factor concentrate, prothrombin complex concentrate (PCC), cryoprecipitate, or fresh frozen plasma can be used to replace the relevant missing factor. PCC comes in three-factor (II, IX, and X) and four-factor (II, VII, IX, and X) varieties. The 4PCC contains heparin and should be avoided in patients with heparin-induced thrombocytopenia. Cryoprecipitate, as mentioned above, contains fibrinogen, vWF, factor VIII, factor XIII, and fibronectin. Fresh frozen plasma contains all the clotting factors and is readily available in most clinical settings, although it contains much lower concentrations of factor (by definition, 1 U/ml) than in concentrates and a large volume is needed to control bleeding. Lifelong prophylaxis is useful in prevention of bleeding in patients with congenital afibrinogenemia or factor XIII deficiency.

Acquired Factor Deficiencies

There are many acquired coagulation factor deficiencies, ranging from common (e.g., due to medication such as a vitamin K antagonist) to very rare (e.g., acquired hemophilia). Acquired factor deficiencies are often diagnosed when abnormal coagulation studies are noted as part of the workup of another disorder (Table 4.6) or as an effect of a medication. Warfarin is the classic medication that causes abnormal coagulation studies. Warfarin acts as an indirect anticoagulant by limiting access to vitamin K, the essential cofactor in synthesis of coagulation factors II, VII, IX, and X. Because of warfarin’s slow onset of action and wide variability in dose needed from subject to subject, there is increasing clinical use of direct oral anticoagulants (DOACs), which directly block thrombin or factor X, act rapidly, and do not require monitoring of blood levels. As direct factor inhibitors, the DOACs may alter coagulation studies including PT, aPTT, and thrombin time (TT); however, the effects on these tests are less predictable, nonlinear, and vary between individuals (Table 4.7). The DOACs can also cause a false-positive lupus anticoagulant (LA) test. Therefore, patients taking DOACs should not be tested for the LA unless they have discontinued the anticoagulant.19

Acquired hemophilia A is an autoimmune disease caused by spontaneously arising autoantibodies to endogenous factor VIII.20 In contrast to inherited hemophilia, acquired hemophilia is more likely to present with mucocutaneous bleeding or extensive ecchymosis, rather than deep tissue bleeds (Fig. 4.22). Acquired hemophilia is most common in older men, in whom the development of these autoantibodies is idiopathic, but it can also affect women and younger individuals, in whom it may be associated with pregnancy, autoimmune disease, disturbed immunity, or lymphoma. It is diagnosed by a markedly prolonged aPTT that does not correct on mixing patient plasma with normal plasma, plus identification of a factor VIII inhibitor by the Bethesda assay. The diagnosis is often made in the setting of an acute bleed, which should be managed aggressively with both local control of bleeding with bypassing agents such as FEIBA, recombinant factor VIIa (rVIIa), or porcine factor VIII and immunosuppressive therapy to eradicate the autoantibody with agents such as rituximab or cyclophosphamide in idiopathic cases, and treatment of the underlying condition if that can
be identified. An interesting feature of acquired hemophilia A is that once treatment has successfully eliminated the autoantibody, it rarely recurs. Recurrence is more frequent if the autoantibody titer at presentation is very high.

Another mechanism for acquired factor deficiencies is consumption of coagulation factors as in DIC (Table 4.8). The many potential causes of DIC include sepsis, trauma, pregnancy, liver failure, and malignancy. Although DIC results in excessive bleeding that can be difficult to control, it is caused by overactivation of thrombotic pathways and is more correctly a hypercoagulable disorder. DIC is a result of inappropriate activation of thrombotic pathways, by extensive exposure to tissue factor in the case of malignancy or trauma, inflammation in the case of sepsis, or inability to synthesize appropriate natural anticoagulants in the case of liver failure. The common features are bleeding or oozing in very ill patients, which should prompt investigation of coagulation studies where fibrinogen will be low, PT and/or aPTT will be elevated, D-dimers will be elevated, hemoglobin and platelets may be low, and review of a peripheral blood smear will show more than one schistocyte per high-power field (HPF). Treatment is supportive with cryoprecipitate to replace fibrinogen that is being consumed, transfusion of platelets, and treatment of the underlying condition that caused DIC, if possible.

HYPERCOAGULABLE STATES

Venous Thrombosis

Venous thrombosis (VT) is a common problem that affects 900,000 persons annually in the United States. In 1856, Virchow described a triad of risk factors for VT that remain key today—interrupted blood flow (stasis), irritation of the vessel (endothelial injury), and enhanced blood coagulation (hypercoagulability) (Fig. 4.23). Deep venous thrombosis (DVT) is often suspected clinically on the basis of unilateral lower extremity edema or redness (Fig. 4.24) and confirmed on ultrasound (Fig. 4.25).

Many common risk factors for VT, including DVT and pulmonary embolism (PE), are listed in Table 4.9. These risk factors can be divided into inherited and acquired. Some patients with VT may have an underlying thrombophilia, placing them at increased risk for thrombosis. Screening for thrombophilia is indicated in specific clinical scenarios as recommended by published guidelines (Table 4.10).

Hypercoagulable State and Cancer

A diagnosis of unprovoked VT in an adult should, however, prompt age-appropriate cancer screening. Malignancy, especially gastrointestinal (GI) malignancy such as pancreatic cancer, can promote VT through a variety of mechanisms that are not fully understood currently, which, however, may involve the exposure of tissue factor on tumor and/or neoangiogenic blood vessels. Trousseau syndrome consisting of migratory VT in the setting of abdominal cancer is a classic example of this process. VT associated with cancer is currently best treated with low-molecular-weight heparins (LMWHs), although clinical trials evaluating DOACs versus LMWH are underway. VT in the context of cancer is difficult to treat, especially during chemotherapy, and may progress in spite of appropriate anticoagulation. (Figs. 4.26 and 4.27 show DVT and PE; Well’s criteria for PE are outlined in Table 4.11.) Some of the ways that malignancy may affect the various hemostatic pathways are outlined in Table 4.12.
Protein C or S Deficiencies

As can be seen from Figure 4.2, natural anticoagulants have critical roles in regulating and limiting the coagulation cascade and preventing inappropriate thrombosis. Unlike normal coagulation factors, which are normally present in great excess, natural anticoagulants are less abundant so that moderate decreases (e.g., 50% decrease) result in high thrombotic risk. Decreased levels of protein C or S can be inherited or acquired, in the latter case through vitamin K antagonism or depletion (e.g., after surgery). When warfarin administration is initiated, the decrease in protein C occurs before the decrease in vitamin K–dependent coagulation factors because of the very short half-life of protein C, causing a window of hypercoagulability before clinically effective anticoagulation is established. This is the reason that patients need to be “bridged” with a rapidly active anticoagulant such as unfractionated heparin (UFH) or LMWH at the time they are started on a vitamin K antagonist such as warfarin. The rapid decrease in proteins C and S levels is also the mechanism of warfarin-induced skin necrosis, a severe complication of warfarin that can occur in some patients with hereditary protein C and S deficiencies in the absence of proper bridging.

Inherited protein C or S deficiency usually presents as VT and should especially be considered in differential diagnosis of patients with unprovoked VT at a young age, or those with a strong family history of thrombosis, or presenting with an unusual anatomic site of VT such as cerebral VT (Fig. 4.28). While a heterozygote carrier may have variable hypercoagulability, from mild to severe, homozygous deletion of protein C typically presents in utero or shortly after birth with severe purpura fulminans (Fig. 4.29). Homozygous protein C deficiency is fatal unless treated with continuous protein C replacement plus anticoagulation, both of which will be required lifelong.

Antithrombin Deficiency

Antithrombin (AT) is a serine protease synthesized in the liver that inactivates thrombin and factor Xa and, to a lesser extent, factors XIIa, XIa, and IXa, and prekallikrein. Its inhibitory effect is amplified 5,000 to 40,000 fold in the presence of heparin, to which it binds. The half-life of AT is approximately 2.4 days. The gene coding for antithrombin is located at chromosome 1q23–25. Inherited AT deficiency, an autosomal-dominant trait with variable clinical penetrance, is a major risk factor for venous thromboembolism (VTE) and was the first identified inherited thrombophilia.26 Approximately 50% to 90% of untreated heterozygote carriers develop VTE during their lifetime. The estimated prevalence of heterozygote AT deficiency in the general population is between 1 in 500 and 1 in 5,000. Two major types of inherited AT deficiency have been identified. In type I AT deficiency, the AT antigen and activity levels are similarly reduced, whereas in type II, the AT activity is reduced but antigen levels are normal. These two types of AT deficiency in the heterozygous state are associated with approximately 50% of normal AT activity and are estimated to increase by about 20-fold the risk of developing a first episode of VTE. Another variant of AT deficiency contains a defective heparin-binding site; it is about five times as common as type I and type II deficiencies combined, but has approximately one-fifth their risk of VTE.27

Factor V Leiden and Factor II (Prothrombin Gene) G20210A Variants

Factor V Leiden (FVL) is the most common inherited thrombophilia in white populations; it results from a single nucleotide substitution (G1691A, resulting in Arg506Gln) in the factor V molecule, causing resistance to inactivation of factor Va by activated protein C by removing a key cleavage site at which activated protein C cleaves and thereby inactivates
factor Va. As a result, thrombin generation is less well controlled and venous hypercoagulability results (Fig. 4.30). Bertina and colleagues first described this finding in 1994. The prothrombin gene (factor II) G20210A promoter mutation (PGM) is the second most common inherited thrombophilia in the white population and was described in 1996 upon direct sequencing of the prothrombin gene (Fig. 4.31). These two thrombophilias, both inherited as autosomal-dominant traits, represent gain-of-function variants resulting in, respectively, increased activity of factor Va and potentially increased prothrombin levels. The prevalence of these variants in white populations is approximately 7% for FVL and 3% for the PGM. Conversely, this mutation is very rare in Native Americans, Africans, or Asians. However, when evaluating patients presenting with VT, the incidence of FVL and PGM is approximately 20% and 10%, respectively. The relative risk of having VT is 6 and 2.8, respectively, for asymptomatic non-carriers of FVL and PGM compared with. The most common clinical manifestations of carriers of FVL and PGM variants include deep vein thrombosis of the lower extremities and PE. PE appears to be more common in carriers of PGM than in carriers of FVL.

**Arterial Thrombosis**

Hematologic disorders associated with the development of arterial thrombosis include HIT, the antiphospholipid syndrome (APS) including catastrophic APS, and thrombotic microangiopathies (TMA) such as thrombotic thrombocytopenic purpura (TTP), and typical or atypical hemolytic-uremic syndrome (HUS).

**Heparin-Induced Thrombocytopenia**

HIT involves the rapid development of thrombocytopenia in a patient receiving heparin and is often accompanied by or even preceded by arterial or venous thrombosis. Patients are often those who have recently had surgery mostly orthopedic or cardiovascular events. Even a very small amount of heparin (e.g., that used to flush an intravascular line) may suffice to trigger HIT in a patient recently exposed to heparin. HIT is caused by IgG antibodies that develop against the complex of heparin bound to platelet factor 4, a platelet granule glycoprotein that is released from activated platelets and binds to their surface membrane and to the vascular endothelium. These antibody complexes cause further activation of platelets by binding via their Fc portions to FcγIIa (IgG) receptors on platelets or endothelium, causing the activated, antibody-coated platelets to be cleared from the circulation, resulting in thrombocytopenia and simultaneously activating the endothelium to a prothrombotic state.

Such HIT antibodies become detectable in patient serum/plasma at the beginning of the HIT-associated decrease in platelet count, which usually takes a precipitous course. In almost all cases, patients with HIT have experienced a recent immunizing exposure to heparin or certain other PF4-binding polyanions. LMWHs less frequently cause HIT than does unfractionated heparin. The specific diagnosis of HIT rests on heparin exposure, the rapid onset of thrombocytopenia, and the presence of the specific antiheparin/PF4 antibodies. The clinical 4T score was designed for rapid risk stratification of patients having a possible diagnosis of HIT before they underwent more definitive laboratory testing (Table 4.13). The anti-PF4 antibody enzyme immunoassay has become a popular screening test to confirm or rule out HIT when clinical suspicion exists after calculating the 4T score. This test can be performed very quickly (usually within a day) and improves on the specificity of the 4T score. The gold standard test to diagnose HIT is the serotonin release assay, which assesses whether the patient’s plasma activates normal platelets to release serotonin in response to
heparin added to the patient plasma. If HIT antibodies are present, patient plasma plus heparin in certain ratios will cause release of labeled serotonin from normal platelets and an excess of heparin will block the release. Treatment of HIT requires immediate discontinuation of all heparin, avoidance of any heparin coated devices or lines. Substitution of another rapidly acting anticoagulant (since the patient had been originally anticoagulated for a prothrombotic state) until the platelet count rises to the normal range, then continuation of a nonheparin anticoagulant. Until recently, argatroban (IV) as well as fondaparinux had been used as the immediate substitute anticoagulant, but more recently the DOACs are used. HIT is associated with a high risk of morbidity and mortality owing more to venous and arterial thrombosis than to bleeding from the thrombocytopenia (Figs. 4.32 and 4.33).

**Antiphospholipid Syndrome and Catastrophic Antiphospholipid Syndrome**

The antiphospholipid syndrome (APS) is a clinicopathologic diagnosis characterized by the presence of arterial, venous, or microvascular thrombosis and/or pregnancy-related complications in the presence of positive antiphospholipid antibodies (aPL) in the blood (Fig. 4.34). The Sydney criteria are the most recent clinical aid in making the diagnosis (Table 4.14). The aPL include the lupus anticoagulant LA, antiphospholipid antibodies (aCL), and anti-β-2 glycoprotein I (β2GPI) antibodies. The aPL are identified using one of two distinct test processes, namely, solid-phase assays and liquid-phase assays. The former includes anticardiolipin antibodies and anti-β2GPI antibodies, and the latter are centered on clot-based tests that are used to identify the LA (Fig. 4.35). The LA is poorly named, because, although it prolongs certain *in vitro* clotting tests, its activity *in vivo* is to promote thrombosis, that is, to favor hypercoagulability.

Catastrophic antiphospholipid syndrome (CAPS) is a severe complication of APS characterized by extensive thromboses that can be arterial, venous, or microvascular. By definition, CAPS involves multiple organ systems and has a high morbidity and mortality. Diagnosis requires the involvement of at least three organ systems, affected simultaneously by thrombosis in the setting of confirmed aPL. Histopathologic evidence of small vessel occlusion is required to confirm the diagnosis (Table 4.15). Intravenous γ-globulin, plasma exchange, and rituximab and in some case reports eculizumab have been used to treat CAPS. However, the mortality remains about 50%.

**Thrombotic Thrombocytopenic Purpura**

TTP is classically a pentad comprising microangiopathic hemolytic anemia, thrombocytopenia, neurologic changes, renal failure, and fever. However, only a minority of patients (20%–30%) will present with all of these symptoms. TTP is characterized by a marked decrease or absence in activity of ADAMTS13, a plasma metalloprotease that functions to cleave ultra–high-weight vWF (which is strongly procoagulant) to its normal circulating size. Such cleaved vWF supports hemostasis but is not thrombogenic. Therefore, reduced or absent ADAMTS13 activity results in loss of cleavage of high-molecular-weight vWF, leading to inappropriate platelet activation and intravascular platelet-fibrin clot formation (Fig. 4.36). The ADAMTS13 activity is less than 5% in both congenital (rare) and acquired TTP. In the latter, an autoantibody to ADAMTS13 is the usual mechanism for the low levels of the enzyme. Thus, immunosuppression as well as plasmapheresis and plasma replacement is part of the treatment. An ongoing phase 3 randomized control trial comparing plasmapheresis with or without caplacizumab (a single-variable-domain immunoglobulin (Nanobody) directed to the A1 region of von Willebrand factor) is underway.
**Hemolytic Uremic Syndrome**

HUS and atypical HUS may have a clinically similar presentation to TTP, but are caused by inappropriate activation of complement either through shiga toxin produced by bacterial infection in the case of HUS or genetic variants in complement proteins that lower the threshold for complement activation and the platelet count tends to be higher tissue deposition. Renal failure is often more prominent in HUS than TTP, whereas neurologic symptoms may be more prominent in TTP. Although both will cause a thrombotic microangiopathy, HUS may have fewer schistocytes/HPF on blood smears on average than does TTP (Fig. 4.37). Plasmapheresis is not effective in HUS or atypical HUS; in the latter, eculizumab may be useful in combating abnormal complement activation.

**METHODS FOR BETTER DETECTION OF INNATE ABNORMALITIES IN THE HEMOSTATIC SYSTEM**

Approximately 90% of patients with bleeding, thrombotic, and platelet disorders that do not have hemophilia or vWD never obtain a conclusive molecular diagnosis owing to the unavailability of affordable genetic tests. High-throughput sequencing for diagnosing inherited bleeding, thrombotic, and platelet disorders is becoming routine in clinical practice, and the ThromboGenomics platform is an example of this transformation. The aim is for the ThromboGenomics test ([www.thrombogenomics.org.uk](http://www.thrombogenomics.org.uk)) to become the first choice for hemostasis and thrombosis physicians and hematologists requiring a molecular diagnosis for inherited bleeding, thrombotic, and platelet disorders. This platform and the supporting principle of freely accessible expert knowledge about genes, transcripts, and causal variants, and the approach of using human phenotype ontology terms for coding phenotype can be used by reference laboratories to reduce the diagnostic delay in reaching a conclusive molecular diagnosis for these patients.40

**BLEEDING RELATED TO VASCULAR ABNORMALITIES**

Vascular purpuras represent conditions in which bleeding is related to defects in the vasculature rather than in hemostasis or platelet function. These may be inherited, such as hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease) in which mucosal telangiectasias dominant mutations in the endoglin or ALK-1 genes, whose protein products bind TGF-B and govern vascular integrity or acquired. In HHT, fragile telangiectasias form on the skin, mucosae, and in the lungs (Fig. 4.38). Epistaxis and mucosal bleeding are characteristic. The chronic and repeated natures of their bleeding may require parenteral iron administration to treat the resulting iron-deficiency anemias. In Ehlers–Danlos syndrome, genetic defects in collagen synthesis lead to easy bruising, poor wound healing, and bleeding episodes, particularly in type IV Ehlers–Danlos syndrome where the defect is in type III procollagen, the main arterial wall collagen. Such patients are prone to formation of arterial aneurysms and spontaneous arterial dissections, and, in women, to bleeding complications at delivery and in the puerperium.

In addition, vascular purpuras may be acquired, as in scurvy, vitamin C deficiency on a
nutritional basis. Vitamin C is required for proper collagen peptidyl hydroxylation; its lack is associated with perifollicular hemorrhages (Fig. 4.39). Vascular purpuras are characterized by palpable purpura and include leukocytoclastic vasculitis, which may be a drug reaction, and Henoch–Schoenlein purpura, an acute vasculitis with tissue deposition of IgA immune complexes, associated with renal insufficiency. In rickettsial diseases and viral hemorrhagic fevers, purpura is related to vasculitis as well as thrombocytopenia. Effects of age and sun exposure, as well as steroid therapy, may lead to increased cutaneous vascular fragility and result in senile, solar, and steroid-related purpura, consisting mainly of easy bruising and ecchymoses on exposed areas (Figs. 4.40–4.42).

REFERENCES


**Table 4.1**

<table>
<thead>
<tr>
<th>Drugs commonly associated with drug-induced thrombocytopenia</th>
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<tr>
<td>Quinine, quinidine</td>
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<td>Trimethoprim/sulfamethoxazole</td>
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<td>Penicillins</td>
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**Table 4.2**

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<tr>
<th>Genetic platelet disorders</th>
<th>Disorder</th>
<th>Gene/Protein Affected</th>
<th>Mechanism</th>
<th>Phenotype</th>
<th>Diagnosis</th>
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<tr>
<td></td>
<td>Glanzmann thrombasthenia</td>
<td>Glycoprotein IIb–IIa (GP2B, GP3A)</td>
<td>Impaired binding of platelets to fibrinogen</td>
<td>Mild to severe bleeding, petechiae, epistaxis</td>
<td>Aggregometry: impaired with all platelet agonists</td>
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<td>Bernard–Soulier syndrome</td>
<td>Glycoprotein 1b/IX (GPIBA, GPIBB, GP9)</td>
<td>Impaired binding of platelets to vWF</td>
<td>Mild to severe bleeding, may have thrombocytopenia</td>
<td>Aggregometry: impaired agglutination only with ristocetin</td>
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<td></td>
<td>Storage pool disorders</td>
<td>Hermansky–Pudlak (HPS1) Chediack–Hidachi (CHS1/LYST) Wiskott–</td>
<td>Impaired platelet activation due to dense platelet granule defects</td>
<td>Mucocutaneous bleeding, neutropenia, with or without albinism, pulmonary fibrosis</td>
<td>Often on the basis of other abnormalities, absence of dense granules on electron microscopy</td>
</tr>
</tbody>
</table>
Aldrich (WASp)

Gray platelet syndrome[^6] \(\text{NBEAL2, GF11B}\)

- Impaired platelet activation
- Thrombocytopenia, large platelets, no α granules
- Light microscopy: absence of α granules

Congenital amegakaryocytic thrombocytopenia \(\text{C-MPL}[^7]\)

- Insensitivity to thrombopoietin
- Bleeding, severe thrombocytopenia from birth
- Bone marrow aspirate, elevated TPO, genetic testing

MYH9-related \(\text{MYH9}[^8]\)

- Abnormal platelet cytoskeleton causing impaired clot retraction
- Thrombocytopenia, large platelets, often moderate bleeding
- Light microscopy: spindle-shaped inclusion bodies in neutrophils

<table>
<thead>
<tr>
<th>vWD Subtype</th>
<th>Description</th>
<th>aPTT</th>
<th>vWF:Ag</th>
<th>vWF:RCo</th>
<th>VIII</th>
<th>RCo:Ag</th>
<th>RIPA</th>
<th>LD-RIPA</th>
<th>Multi</th>
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<td>I</td>
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<td>Variable loss HMW multimers</td>
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Platelet type

- Normal
- Normal: ↓
- Normal: ↓
- Normal: ↓
- ↑↑: Variable loss HMW multimers

[^6]: Aldrich (WASp)
[^7]: Congenital amegakaryocytic thrombocytopenia
[^8]: MYH9-related

**Table 4.3**

Laboratory characteristics of von Willebrand disease by subtype

<table>
<thead>
<tr>
<th>vWD Subtype</th>
<th>Description</th>
<th>aPTT</th>
<th>vWF:Ag</th>
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<td>I</td>
<td>Reduced vWF protein expression</td>
<td>Normal</td>
<td>↓</td>
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<td>Normal</td>
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<tr>
<td>IIA</td>
<td>Deficiency of HMW multimers</td>
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<td>↓</td>
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<td>IIB</td>
<td>Increased platelet binding and consumption of HMW multimers</td>
<td>Normal</td>
<td>Normal</td>
<td>↓</td>
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<td>↓</td>
<td>None</td>
<td>Normal</td>
</tr>
<tr>
<td>IIN</td>
<td>Defective binding and stabilization of factor VIII</td>
<td>↑</td>
<td>Normal</td>
<td>Normal</td>
<td>↓</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Normal</td>
</tr>
<tr>
<td>III</td>
<td>Absent vWF protein</td>
<td>↑</td>
<td>Absent</td>
<td>Absent</td>
<td>↓</td>
<td>N/A</td>
<td>Absent</td>
<td>None</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Platelet type

- Normal
- Normal: ↓
- Normal: ↓
- Normal: ↓
- ↑↑: Variable loss HMW multimers

vWD, von Willebrand disease; vWF, von Willebrand factor; HMW, high molecular weight.
Type | Hemophilia A (Factor VIII Deficiency) | Hemophilia B (Factor IX Deficiency) | Hemophilia C (Factor XI Deficiency)
---|---|---|---
Inheritance | X-linked | X-linked | Autosomal recessive
Prevalence | 1:5,000 | 1:30,000 | • Common in Ashkenazi Jews
Bleeding type | Hemarthrosis and intramuscular hematomas | Hemarthrosis and intramuscular hematomas | Mucocutaneous bleeding, mainly in areas of extensive fibrinolysis (oral, nasal, and genitourinary areas)
Classification | • Severe (factor VIII <1%) • Moderate (factor VIII ≥1% and <5%) • Mild (factor VIII >5%) | • Severe (factor IX <1%) • Moderate (factor IX ≥1% and <5%) • Mild (factor IX >5%) | • Severe (factor XI <20 IU/dL) • Mild (factor XI ≥20 IU/dL) • Factor XI level does not correlate with bleeding phenotype
Treatment | • Plasma-derived factor VIII concentrates • Recombinant factor VIII concentrates • Gene therapy (clinical trials) | • Plasma-derived factor IX concentrates • Recombinant factor IX concentrates • Gene therapy (clinical trials) | • Plasma • Factor XI plasma-derived concentrate (not available in the United States) • Antifibrinolytics • Recombinant factor VIIa (rVIIa)
Incidence of inhibitor development | • 33% for patients with severe factor VIII deficiency • 25% for patients with mild to moderate deficiency | 3% | 33% of patients with null mutations (Glu117Stop mutation)

---

**Table 4.5**

**Rare coagulation factor deficiencies**

<table>
<thead>
<tr>
<th>Factor Deficiency</th>
<th>Laboratory Abnormality</th>
<th>Treatment for Acute Bleeding</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>PT and aPTT prolonged</td>
<td>PCC or FFP</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>PT and aPTT prolonged</td>
<td>FFP</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>Isolated PT prolonged</td>
<td>FFP; c VIIa or 4-factor PCC</td>
<td>Phenotype may not correlate with factor level</td>
</tr>
<tr>
<td>X</td>
<td>PT and aPTT prolonged</td>
<td>Factor X concentrate or PCC</td>
<td>May be acquired in amyloidosis</td>
</tr>
<tr>
<td>XIII</td>
<td>Normal PT and aPTT</td>
<td>Factor XIII concentrate, cryoprecipitate, or FFP</td>
<td>Routine prophylaxis recommended for severe (&lt;5%) patients</td>
</tr>
</tbody>
</table>
**Afibrinogenemia or dysfibrinogenemia**

PT and aPTT prolonged

Fibrinogen concentrate or cryoprecipitate

Replacement may be required to maintain pregnancy

---

**Table 4.6**

**Acquired coagulation factor deficiencies**

<table>
<thead>
<tr>
<th>Pathologic Process</th>
<th>Specific Factors Affected</th>
<th>Coagulation Study Abnormalities</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin K deficiency</td>
<td>Coagulation factors II, VII, IX, X Anticoagulant proteins C and S</td>
<td>PT/INR prolonged</td>
<td>Administer vitamin K orally, or IV if malabsorption is suspected.</td>
</tr>
<tr>
<td>Liver disease</td>
<td>Factors I, II, V, VII, IX, X, XI Anticoagulant proteins C and S, antithrombin</td>
<td>PT/INR prolonged, fibrinogen decreased or dysfunctional, low platelets</td>
<td>Treat underlying liver disease; patients are at risk of thrombosis as well as bleeding.</td>
</tr>
<tr>
<td>Direct oral anticoagulant use</td>
<td>Thrombin (II)</td>
<td>PT and/or PTT variably prolonged</td>
<td>Four-factor PCC</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation</td>
<td>Fibrinogen (I)</td>
<td>PT and/or PTT prolonged, fibrinogen decreased, low Hb, low platelets</td>
<td>Cryoprecipitate</td>
</tr>
<tr>
<td>Plasma cell dyscrasias and amyloidosis</td>
<td>Consumption of coagulation factor X</td>
<td>PT and PTT prolonged</td>
<td>Treat underlying condition.</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>Loss of LMW proteins</td>
<td>Variable, may have prolonged PTT due to loss of factor IX</td>
<td>Treat underlying condition.</td>
</tr>
<tr>
<td>Acquired hemophilia</td>
<td>Antibody to coagulation factor VIII</td>
<td>PTT prolonged, does not correct in mixing study</td>
<td>FEIBA for acute bleeding; treat underlying condition.</td>
</tr>
<tr>
<td>Acquired von Willebrand disease</td>
<td>vWF, usually shearing of HMW multimers</td>
<td>Loss of HMW multimers on electrophoresis</td>
<td>Treat underlying condition.</td>
</tr>
</tbody>
</table>

LMW, low molecular weight; HMW, high molecular weight; PT, prothrombin time; INR, international normalized ratio; PTT, partial thromboplastin time; PCC, prothrombin complex concentrate; FEIBA, factor VIII inhibitor bypass activity.

---

**Table 4.7**

**Direct oral anticoagulants**

<table>
<thead>
<tr>
<th></th>
<th>Dabigatran (Etexilate)</th>
<th>Rivaroxaban (Xarelto)</th>
<th>Apixaban (Eliquis)</th>
<th>Edoxaban (Savaysa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (INR or seconds)</td>
<td>↑</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>aPTT</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>↑↑↑↑↑</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
### Intrinsic coagulation factors

<table>
<thead>
<tr>
<th>VIII, IX, XI, XII (based on aPTT)</th>
<th>↑</th>
<th>overstimated</th>
<th>overstimated</th>
<th>overstimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogenic anti-Xa assay</td>
<td>—</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Ecarin clotting time</td>
<td>↑</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Major common side effects</td>
<td>Gastrointestinal distress (35%)</td>
<td>Well tolerated</td>
<td>Abnormal hepatic function tests, skin tests</td>
<td>Well tolerated</td>
</tr>
</tbody>
</table>

PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time.

### Table 4.8

**International Society of Thrombosis and Hemostasis (ISTH) DIC scoring system**

1. **Risk assessment:**
   - Does the patient have an underlying disorder known to be associated with overt DIC?
     - If yes: proceed

2. **Order global coagulation tests:**
   - Platelet count
   - Prothrombin time
   - Fibrinogen
   - Soluble fibrin monomers or fibrin degradation products

3. **Score global coagulation test results:**
   - Platelet count:
     - (>100 = 0; <100 = 1; <50 = 2)
   - Soluble fibrin monomers/fibrin degradation products:
     - (No increase = 0; moderate increase = 2; strong increase = 3)
   - Prolonged prothrombin time:
     - (<3 sec = 0; >3 sec but <6 sec = 1; >6 sec = 2)
   - Fibrinogen level:
     - (>1.0 g/L = 0; <1.0 g/L = 1)

4. **Calculate score:**
   - If >5: compatible with overt DIC
   - If <5: suggestive of nonovert DIC

DIC, disseminated intravascular coagulation.

From Toh CH, Hoots WK; SSC on Disseminated Intravascular Coagulation of the ISTH. The scoring system of the Scientific and Standardisation Committee on Disseminated Intravascular Coagulation of the International Society on Thrombosis and Haemostasis: a 5-year overview. *J Thromb Haemost.* 2007;5:604–606.

### Table 4.9

**Inherited and acquired risk factors for thrombosis**

#### Inherited thrombophilias

- Factor V Leiden mutation
- Prothrombin gene mutation
- Protein S deficiency
- Protein C deficiency
- Antithrombin deficiency
- Dysfibrinogenemia

#### Acquired risk factors

- Malignancy
- Surgery or trauma
- Pregnancy/postpartum period
- Exogenous estrogens as in hormonal contraceptives or hormone replacement
Medications such as tamoxifen, raloxifene, thalidomide, lenalidomide
Immobilization
Antiphospholipid syndrome
Myeloproliferative disorders such as polycythemia vera or essential thrombocythemia
Hemolytic anemias such as autoimmune hemolytic anemia or paroxysmal nocturnal hemoglobinuria
Nephrotic syndrome
Protein-losing enteropathies

Table 4.10
Screening guidelines for inherited and acquired thrombophilias

<table>
<thead>
<tr>
<th>Inherited Thrombophilia</th>
<th>Acquired Thrombophilia (e.g., APS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprovoked VTE, or with only minor risk factor</td>
<td>Unprovoked VTE</td>
</tr>
<tr>
<td>VTE (including provoked) in patients &lt;50 years old</td>
<td>Arterial thrombosis in patients &lt;50 years old</td>
</tr>
<tr>
<td>Recurrent VTE</td>
<td>Thrombosis at unusual sites (e.g., splanchnic)</td>
</tr>
<tr>
<td>Skin necrosis associated with VKAs</td>
<td>Late or recurrent pregnancy loss</td>
</tr>
<tr>
<td>Purpura fulminans</td>
<td>Prolonged aPTT</td>
</tr>
<tr>
<td>Strong family history of VTE</td>
<td>Autoimmune disease associated with VTE or pregnancy loss</td>
</tr>
<tr>
<td>Family member with known thrombophilia</td>
<td></td>
</tr>
</tbody>
</table>

VTE, venous thromboembolism; aPTT, activated partial thromboplastin time.

Table 4.11
Wells criteria for pulmonary embolism

<table>
<thead>
<tr>
<th>Variables</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous deep venous thrombosis or pulmonary embolism</td>
<td>1.5</td>
</tr>
<tr>
<td>Heart rate ≥100 bpm</td>
<td>1.5</td>
</tr>
<tr>
<td>Surgery or immobilization within the past 4 wk</td>
<td>1.5</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>1</td>
</tr>
<tr>
<td>Active cancer</td>
<td>1</td>
</tr>
<tr>
<td>Clinical signs of DVT</td>
<td>3</td>
</tr>
<tr>
<td>Alternative diagnosis less likely than PE</td>
<td>3</td>
</tr>
</tbody>
</table>

Scoring:
0–1 point: Low clinical probability
2–6 points: Intermediate clinical probability
≥7 points: High clinical probability for pulmonary embolism

DVT, deep venous thrombosis.

Table 4.12
Hemostatic abnormalities in cancer patients

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>Thrombocytopenia • Marrow infiltration by tumor</td>
</tr>
</tbody>
</table>
| Thrombocytosis | • Chemotherapy effects  
|               | • Biologic response modifiers  
|               | • Monoclonal antibodies and immunotoxins  
|               | • Proteasome inhibitor (bortezomib)  
|               | • DIC  
|               | • Hypersplenism  
|               | • Immune mediated (autoimmune, alloimmune)  
|               | • Thrombotic microangiopathies  
|               | • Reactive  
|               | • Primary myeloproliferative disorders  

| Platelet function abnormalities | • Uremia  
|                                | • Acquired von Willebrand syndrome  
|                                | • Myeloproliferative disorders  

### Abnormalities in coagulation factors and coagulation activation markers

| Hypofibrinogenemia | • Asparaginase  
|                   | • DIC  
| Dysfibrinogenemia | • Hepatocellular carcinoma  
| Factor X (decreased) | • Amyloidosis  
| Decreased coagulation factors | • Impairment in hepatic synthesis  
|                           | • DIC  
|                           | • Vitamin K deficiency  
| Elevated D-dimer and fibrin degradation products | • Inflammation  
|                                               | • Thrombosis  
|                                               | • Fibrinolysis  
|                                               | • DIC  
|                                               | • Renal insufficiency  
|                                               | • Hepatic failure  
| Elevated prothrombin fragment 1 + 2 | • Disseminated malignancies  
|                                      | • DIC  

### Fibrinolysis

| Increased secretion of plasminogen activator | • Acute promyelocytic leukemia  
| Overexpression of annexin II | • Acute promyelocytic leukemia  
| Decreased levels of plasminogen activator inhibitors | • Increased fibrin degradation products and D-dimer  

### Acquired thrombophilias

| Antithrombin deficiency | • Impaired hepatic synthesis of anticoagulant proteins  
|                        | • DIC  
|                        | • L-Asparaginase  
|                        | • Unfractionated heparin  
|                        | • Low-molecular-weight heparin  
| Protein C deficiency | • Impaired hepatic synthesis  
|                       | • DIC  
|                       | • Vitamin K antagonists  
| Protein S deficiency | • Impaired hepatic synthesis  
|                       | • DIC  
|                       | • Vitamin K antagonists  
| Tissue factor pathway inhibitor deficiency | • Impaired hepatic synthesis  
|                                           | • DIC  

DIC, disseminated intravascular coagulation.
Table 4.13

4T Score for heparin-induced thrombocytopenia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>• Fall &gt;50% and nadir ≥20,000/µL</td>
<td>2 points</td>
</tr>
<tr>
<td></td>
<td>• Fall 30% to 50% or nadir 10,000 to 19,000/µL</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>• Fall &lt;30% or nadir &lt;10,000/µL</td>
<td>0 points</td>
</tr>
<tr>
<td><strong>Timing</strong></td>
<td>• Clear onset between days 5 and 10 of heparin exposure, or platelet count fall at ≤1 day if prior heparin exposure within the last 30 days</td>
<td>2 points</td>
</tr>
<tr>
<td></td>
<td>• Consistent with fall in platelet count at 5 to 10 days, but unclear (e.g., missing platelet counts), or onset after day 10, or fall ≤1 day with prior heparin exposure within 30 to 100 days</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>• Platelet count fall at &lt;4 days without recent heparin exposure</td>
<td>0 points</td>
</tr>
<tr>
<td><strong>Thrombosis</strong></td>
<td>• Confirmed new thrombosis, skin necrosis, or acute systemic reaction after intravenous unfractionated heparin bolus</td>
<td>2 points</td>
</tr>
<tr>
<td></td>
<td>• Progressive or recurrent thrombosis, nonnecrotizing (erythematous) skin lesions, or suspected thrombosis that has not been proven.</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>• No thrombosis</td>
<td>0 points</td>
</tr>
<tr>
<td><strong>Other causes</strong></td>
<td>• None apparent</td>
<td>2 points</td>
</tr>
<tr>
<td></td>
<td>• Possible</td>
<td>1 point</td>
</tr>
<tr>
<td></td>
<td>• Definite</td>
<td>0 points</td>
</tr>
</tbody>
</table>

**Sum total points**

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3 points: Low probability</td>
<td></td>
</tr>
<tr>
<td>4–5 points: Intermediate probability</td>
<td></td>
</tr>
<tr>
<td>6–8 points: High probability</td>
<td></td>
</tr>
</tbody>
</table>


Table 4.14

Revised Sydney laboratory criteria for antiphospholipid syndrome

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Test Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupus anticoagulants</td>
<td>Screening, mixing, and confirmation test (ISTH guidelines)</td>
</tr>
<tr>
<td>αCL antibodies</td>
<td>IgG and/or IgM Medium or high titer (&gt;40 units IgG or IgM phospholipid antibody titer or &gt;99th percentile)</td>
</tr>
<tr>
<td>Anti-β2GPI antibodies</td>
<td>IgG and/or IgM Titer &gt;99th percentile</td>
</tr>
</tbody>
</table>

*Note:* Plus positive antiphospholipid results on two or more occasions at least 12 weeks apart.

Table 4.15

Diagnostic criteria for catastrophic antiphospholipid syndrome (CAPS)

**Criteria**

1. Evidence of involvement of ≥3 organs, systems, and/or tissues
2. Development of manifestations simultaneously or in less than a week
3. Confirmation by histopathology of small vessel occlusion in at least one organ tissue
4. Laboratory confirmation of the presence of antiphospholipid antibodies (lupus anticoagulant, α-
antibodies, and/or anti-β_2_\text{GPI} antibodies)

### Classification

#### Definite CAPS

Requires all four criteria

#### Probable CAPS

All four criteria, except for only two organs, systems, and/or sites of tissue involvement or
All four criteria, except for the laboratory confirmation, at least 6 wk apart due to the early death of a patient never tested for antiphospholipid antibodies before CAPS or
Criteria 1, 2, and 4 above or
1, 3, and 4 and the development of a third event in more than 1 wk but less than 1 mo, despite anticoagulation


---

**Figure 4.1.** Coagulation cascade. Factors in the intrinsic (*dashed red box*) and extrinsic (*dashed green box*) pathways are shown converging on the common pathway that generates thrombin. Thrombin cleaves fibrinogen to fibrin. The steps downstream of fibrin activation including cross-linking of the fibrin clot by factor XIII and generation of D-dimers are highlighted in the solid red box.
Figure 4.2. Thrombin is a central regulator of hemostasis and thrombosis. Its complex roles include both procoagulant properties, including activation of fibrinogen to fibrin and activation of factors V and VIII indicated by the blue arrows, plus platelet activation, and anticoagulant properties, including activation of protein C via thrombomodulin and activation of TAFI, indicated by the red arrow. Endothelial effects of thrombin also include contraction of endothelial cells and acquisition of prothrombotic properties, and promotion of cell division.

1. Initiation

2. Amplification

Figure 4.3. Cell-based model of coagulation in which coagulation is regulated by properties of cell surfaces in three overlapping stages of initiation, amplification, and propagation, rather than the traditional cascade. Thrombin is important in each step.
Figure 4.4. Ultrastructure of unstimulated human platelets. Membranous organelles, including the surface-connected canalicular system (SCCS) and dense tubular system (DTS), and cytoplasmic organelles, including mitochondria (M), α-granules (G), dense bodies (DB), coated vesicles (CV), and glycogen (GLY), are visualized at the ultrastructural level. α-Granules contain many coagulation factors, vWF, and antiplasmin; dense bodies contain the platelet agonists calcium, ADP, ATP, and serotonin. Microtubules (MT) are present as cross-sectional and longitudinal profiles at the poles of the discoid platelets. Magnification ×46,000; bar = 0.5 µm. (From Paraskevas F. Lymphocytes and lymphatic organs. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe’s Clinical Hematology. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:227–250.)
Figure 4.5. In pseudothrombocytopenia, the patient actually has normal platelet counts, but the automated cell counter does not count the platelets accurately owing to their clumping (arrow). This is an artifact of drawing blood in a tube with EDTA and can be corrected either by redrawing the specimen in a citrate tube (blue top) or making a bedside peripheral blood smear from a fingerstick. Any low platelet count warrants manual review of the blood smear to rule out pseudothrombocytopenia and confirm platelet count.

Figure 4.6. Patient’s leg showing widespread petechiae in ITP with a platelet count of 11,000/µL. Petechiae do not blanch with pressure because they represent minute
hemorrhages; they may accumulate preferentially in dependent areas such as the lower legs because of hydrostatic pressure. Petechiae may also present initially on the palate and tongue, so examination of the gingiva, oropharynx, and retinas is indicated in a patient with thrombocytopenia because concern for risk of intracranial bleeding is greater if petechiae are present above the level of the neck. Generally, the platelet count will be under 30,000/µL in a patient presenting with petechiae. Platelet counts under 10,000/µL are associated with spontaneous bleeding and warrant immediate intervention.


Figure 4.8. Peripheral blood smear in a patient with ITP postsplenectomy, now on maintenance therapy with eltrombopag (Promacta, oral thrombopoietin agonist). Because of the prior splenectomy, marked poikilocytosis, anisocytosis, Howell–Jolly bodies, and target cells are also noted on the peripheral blood smear. This patient continues to have a low platelet count, and treatment with eltrombopag can cause release of giant platelets such as the one in the middle of the field (arrow).

Figure 4.10. Babesia in a blood smear from a patient with anemia and thrombocytopenia. Organisms can be seen both inside a red blood cell (RBC) and extracellularly.
Figure 4.11. Different forms of malaria in two different patients. **A:** Classic ring form. **B:** Gametocyte form. (Courtesy of Diane Mangraviti.)

Figure 4.12. Two peripheral blood smears from a patient with gray platelet syndrome with NBEAL2 deficiency. The platelets lack visible α granules, and so appear pale and gray. In platelet secretion studies, they fail to secrete α-granule contents. (Courtesy of Drs. Alan Nurden and Paquita Nurden.)

Figure 4.13. Peripheral blood smears from two patients presenting with acute promyelocytic anemia and DIC. Platelets are virtually absent from the smears, and schistocytes are present; a promyelocyte in the center of each field contains Auer rods visible in the cytoplasm. Virtually all patients with acute promyelocytic anemia will have DIC due to hyperfibrinolysis.
Figure 4.14. Schematic representation of the platelet function analyzer (PFA-100) used for whole-blood platelet aggregation. Whole blood is aspirated into a disposable test cartridge through a microscopic aperture cut into a biologically active membrane. These biologically active membranes are coated with platelet agonists, either collagen/epinephrine or collagen/adenosine diphosphate (ADP). Platelets moving through the cartridge contact these agonists, leading to platelet adhesion, activation, and aggregation and resulting in rapid occlusion of the aperture and the cessation of blood flow; this is expressed as a closure time (measured in seconds). If platelet function is not normal, the closure time is prolonged. The usefulness of the PFA-100 is based primarily on its high negative predictive value; a normal PFA-100 generally means that primary hemostasis is intact. Thus, ingestion of aspirin, NSAIDS, other platelet antagonists, storage pool disease, primary storage defects, and mild type I vWD may lead to abnormal (prolonged) closure times. For example, prolonged closure time with collagen/epinephrine and normal closure time with collagen/ADP are typical for aspirin ingestion. Abnormal closure times warrant further screening of platelet function.

<table>
<thead>
<tr>
<th>Coll/EPI</th>
<th>Coll/ADP</th>
<th>Interpre</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ABNORMAL</td>
<td>NORMAL</td>
<td>Aspirin effect</td>
<td></td>
</tr>
<tr>
<td>ABNORMAL</td>
<td>ABNORMAL</td>
<td>Platelet dysfunction or vWD (Type I)</td>
<td></td>
</tr>
<tr>
<td>NORMAL</td>
<td>ABNORMAL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.15. Schematic representation of whole-blood impedance aggregation and light transmission aggregometry. Platelet aggregation studies are difficult, time consuming, and subject to preanalytical variables. A: Impedance aggregation. Citrated whole blood is used to
test platelet functionality. Impedance aggregometry measures the electrical resistance between two electrodes immersed in diluted whole blood stirred in a cuvette at 37°C. Aggregation is stimulated using a number of soluble agonists, resulting in platelet shape change and aggregation. Increased resistance results from increased platelet adhesion and aggregation to the electrodes.\textsuperscript{31} In addition to measuring changes in electrical resistance, platelet impedance aggregometers are also equipped to measure changes in chemiluminescence, also known as lumiaggregometry. Lumiaggregometry measures ATP release from platelet-dense granules upon platelet activation; this method is based on the bioluminescent quantitation of ATP when ATP reacts with luciferin and luciferase (firefly extracts), resulting in light emission captured by a photomultiplier tube. Results are expressed as nmol/10\textsuperscript{8} platelets. B: Light transmission aggregometry. Platelet-rich plasma is prepared by gentle centrifugation and stirred in a cuvette at 37°C placed between a light source and a photocell detector. When soluble agonists are added, platelets undergo shape change, which causes an initial decrease in light transmission, and then begin to aggregate, resulting in less light being scattered and increased light transmission through the cuvette. If aggregation is impaired, different response patterns are recorded depending on the particular platelet function defect and the different agonists used. Lumiaggregometry can also be performed together with light transmission aggregometry.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4_16.png}
\caption{A: Diagram of von Willebrand factor (vWF) domains and their binding sites. B: Cartoon of vWF multimer electrophoresis showing decreased vWF in all multimeric sizes in type I vWD, a selective decrease in high-molecular-weight multimers in type II vWD, and absence of vWF in type III vWD. C: Photograph of electrophoresis of vWF multimers of a normal control in lane 1, patient with type IIB vWD in lane 2, patient with type IIA vWD in lane 3.}
\end{figure}
Figure 4.17. Uvular bleeding in patient with hemophilia A and factor VIII inhibitor.

Figure 4.18. Scan demonstrating retroperitoneal hemorrhage in patient with acquired hemophilia A due to autoantibody to factor VIII. (Courtesy of Dr. Benjamin Weintraub.)
Figure 4.19. Hemarthroses in a patient with severe hemophilia. Recurrent hemarthroses in these patients can lead to severe arthritis and disfigurement of joints.

Figure 4.20. Elbow with limited range of motion in a patient with hemophilia A. He has only about 5 degrees of motion in his elbow.

Figure 4.21. Elbow and knee joints in a patient with hemophilia show thickening of
synovium with deposition of calcium (A and B); increased intercondylar notch (C); increased density and decreased interarticular space (A–D); and lipping along the borders of the joint surfaces (D). (From Powell JS, Rodgers GM. Inherited coagulation disorders. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe’s Clinical Hematology. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:1143–1185.)

**Figure 4.22.** Ecchymosis (spontaneous bruising) on forearm of patient with acquired hemophilia. Spontaneous ecchymoses and hematomas as well as ecchymoses with trauma with sudden onset are features of acquired hemophilia, caused by autoantibodies to factor VIII.

**Figure 4.23.** Virchow’s triad depicting the clinical features that promote thrombosis.
Figure 4.24. Two patients with lower extremity DVT. A: Leg edema that is greater in the right than in the left leg and prominent superficial veins resulting from congested blood behind the DVT. B: Marked bilateral leg edema due to bilateral DVTs as well as redness greater in the left leg than the right. Measuring the circumference of the affected (and unaffected) limbs is a more sensitive way of emphasizing the edema than direct observation.
Figure 4.25. Venous sonogram of a patient showing thrombosis in common femoral vein.

Figure 4.26. Ultrasonography of DVT in femoral vein. Note how thrombus (arrow in A) is echogenic relative to blood in vasculature and is not compressible (arrow in B).
Figure 4.27. CT images of saddle emboli in bilateral pulmonary arteries in a thrombophilic patient. Hypercoagulability can be inherited or acquired. Although not every VTE warrants a hypercoagulable workup, VTE does warrant age-appropriate cancer screening. Arrows point to filling defect, indicating the presence of a pulmonary embolism.
Figure 4.28. Cerebral venous angiogram is a young woman with cerebral venous thrombosis. The patient presented with headache and was diagnosed with cerebral venous thrombosis seen as an absence of filled vessels on the left side of the brain compared with the right side. A thrombophilia workup revealed heterozygous factor C deficiency.
**Figure 4.29.** Purpura fulminans in a foot. Homozygous protein C deficiency untreated is not compatible with life. Lifelong treatment with protein C replacement starting in utero is possible. (From Graessle WR. Nonblanching rashes. In: Chung EK, ed. Visual Diagnosis and Treatment in Pediatrics. 3rd ed. Philadelphia, PA: Wolters Kluwer; 2015:564–572.)

**Figure 4.30.** A schematic showing the factor V gene and the mutations associated with factor V Leiden. The G1691A mutation eliminates an important activated-protein C-dependent cleavage site in factor V that is key for the inactivation of factor Va, thus producing the APC “resistance,” impairing thrombin regulation of hemostasis, and permitting venous hypercoagulability.

**Figure 4.31.** A schematic showing the most common prothrombin gene mutation G20210A. Note that the mutation is in the 3’ untranslated promoter region of the molecule and acts to permit greater than normal activity of the prothrombin gene, potentiating thrombin generation.
Figure 4.32. Necrotic fingers in a patient with heparin-induced thrombocytopenia with thrombosis. The swollen hand and fingers show early digital gangrene as well as cyanosis. Pink marks on wrist and palm indicate locations of arterial pulses for monitoring by Doppler ultrasound. Heparin-induced thrombocytopenia produces both venous and arterial thrombosis.

Figure 4.33. Skin infarction in a patient with heparin-induced thrombocytopenia with thrombosis.
Figure 4.34. Diagnostic criteria of antiphospholipid syndrome include complications of either pregnancy or vascular thrombosis as well as the presence of an antiphospholipid antibody. LA, lupus anticoagulant; β2-GPI, β2-glycoprotein I; aCL, anticardiolipin.
Figure 4.35. A: Interpretation of mixing results in aPTT testing. It is first determined that the patient’s aPTT is prolonged above the normal range. Patient’s plasma is then mixed 1:1 with normal pooled plasma, and the aPTT is measured either immediately upon mixing or after incubation at 37°C for a specified time (usually 60 minutes). Prolongation of either immediate or incubated aPTT using this mixture of patient and normal plasma is consistent with a lupus anticoagulant (LA), that is, by a circulating coagulation inhibitor. In contrast, correction of a prolonged aPTT to normal by 1:1 mixing with normal plasma is consistent with a coagulation factor deficiency in the patient’s plasma. B: The dilute Russell’s viper toxemia test (DRVVT) and the silicate contact test (SCT) can be used to confirm the presence of LA. If the DRVVT is positive, the DRVVT screen/DRVVT confirm > 1.2 indicates the presence of LA. Similarly, if the SCT is positive, the SCT screen/SCT confirm > 1.2 indicates the presence of LA. False positives for the DRVVT can include heparin and anti-Xa NOACs. False positives for the SCT can also include heparin and anti-Xa NOACs.
venom time (dRVVT) evaluates the causes of an unexplained prolonged aPTT by adding dilute Russell’s viper venom (RVV), a direct activator of FV and FX, to the test plasma. The dRVVT is poor in phospholipids, making it sensitive to LA, which is an antiphospholipid antibody. An initial dRVVT screen is performed on both the patient plasma and control normal pooled plasma (NPP). In the presence of calcium, RVV directly activates factor X to Xa in plasma, resulting in a clot. Therefore, the dRVVT unaffected by the contact factor abnormalities, factor VII, VIII, and IX deficiencies or inhibitors or by heparin (up to 1 U/mL). The ratio of the patient’s clotting time to that of the NPP clotting time is the dRVVT screen ratio. Next, plasma samples are similarly tested but with the addition of excess phospholipid (PL), which neutralizes the LA, resulting in a shorter clotting time. The ratio of the patient’s clotting time to the NPP clotting time in this second test is referred to as the dRVVT confirm ratio. If the dRVVT screen/dRVVT confirm is >1.2, an LA is considered to be present. However, false-positive results occur in the presence of high-dose heparin (>1 U/mL), the new oral anticoagulants (anti-Xa inhibitors), and warfarin, so an accurate drug history is essential. Many laboratories further check a negative dRVVT in a clinically suspicious setting with a more sensitive test, the silica clotting time (SCT). The SCT reagents contain a low dose of phospholipid, which makes the test more sensitive to LA as a screening test for the latter. As in the dRVVT, confirmatory SCT for LA uses a high PL concentration to neutralize the LA, thus demonstrating its PL-dependence. Both dRVVT and SCT are expressed as a ratio of ratios. If the SCT screen/SCT confirm is greater than 1.2, this is consistent with the presence of an LA. Similarly, false-positive results can be seen in the presence of heparin, the new oral anticoagulants (anti-Xa inhibitors), and warfarin. A similar type of testing can be run for prothrombin times to detect the presence of an LA that is directed against prothrombin.
Figure 4.36. Histopathology and histochemistry of thrombotic thrombocytopenic purpura (TTP) and other microangiopathic disorders. A: TTP (heart); B: TTP (brain); C: Shiga toxin–associated hemolytic-uremic syndrome (kidney); D–F: atypical HUS (kidney); G: disseminated intravascular coagulation (kidney); H: tumor cell invasion of microvasculature in metastatic neoplasm (soft tissue); and I: proliferative vasculitis of lupus, causing microvascular stenosis (soft tissue). The endothelial cells and the vessel wall are intact in TTP and disseminated intravascular coagulation. A, D, E, H, and I used hematoxylin and eosin stain; B used immunohistochemical stain for vWF (brown); C and G used Carstair stain for fibrin; and F used Jones silver stain. (From Tsai H-T. Thrombotic thrombocytopenia purpura, hemolytic-uremic syndrome, and related disorders. In: Greer JP, Arber DA, Glader B, et al, eds. Wintrobe’s Clinical Hematology. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:1077–1096.)
Figure 4.37. Peripheral blood smear from two patients with thrombotic thrombocytopenic purpura with microangiopathic hemolytic anemia characterized by absence of platelets, multiple schistocytes, and polychromasia indicative of increased erythrocyte production in the setting of hemolysis. One nucleated red blood cell (RBC) is shown in each smear. ADAMTS13 activity was undetectable (<5%) in both patients.

Figure 4.39. Scurvy. Extensive ecchymosis of both legs was seen in this psychiatric patient, who had normal platelets and coagulation parameters. The patient admitted to not consuming any citrus fruits or juice for an extended period of time. The ecchymoses resolved after treatment with high doses of vitamin C. Ascorbic acid is required to promote peptidyl hydroxylation of procollagen. In severe deficiency, abnormal collagen results, which
weakens the support of vascular structures, leading to capillary fragility. Petechiae form initially around follicles, but can coalesce to form extensive skin bleeding as seen in this photograph as well as gingival and muscular bleeding. Mentally ill patients, alcoholics, and pediatric and elderly patients eating very limited diets are at risk for this otherwise rare disease now.

Figure 4.40. Scan of retroperitoneal hemorrhage from patient taking excessive amount of warfarin.
Figure 4.41. Extensive hematomas in a patient with cirrhosis and increased fibrinolysis.
Figure 4.42. Drug-induced purpura without thrombocytopenia. Multiple ecchymoses due to idiosyncratic response to venlafaxine (Effexor), which can impair platelet function. (Courtesy of Dr. Anita Aggarwal.)
Benign diseases of white blood cells (WBC) can result in either quantitative or qualitative changes in the WBC. The qualitative changes may be functional and/or morphologic. Both leukocytosis and leukopenia are commonly encountered laboratory findings and can be seen in benign and neoplastic disorders. The clinical findings are extremely helpful in identifying the underlying etiology. One of the first steps is to review the peripheral blood (PB) smear to confirm the leukocyte differential and to evaluate the morphology of each cell type. Further testing is guided by the morphologic findings on the PB smear and on the lineage that is increased or decreased.

The following sections provide a brief overview of benign leukocyte disorders based on the cell lineage that is increased or decreased.

### NONNEOPLASTIC DISORDERS OF NEUTROPHILS

The normal reference ranges for WBC counts including relative percentages and absolute cell counts vary by subject age and hospital or outpatient population. Each hospital laboratory must validate reference ranges in its patient population. Total WBC counts are higher in infants, with newborns having the highest WBC count and absolute neutrophil count (ANC) of any age. By the first few weeks of age through early adolescence, lymphocytes become the predominant WBC. This gradually shifts and neutrophils are the predominant WBC in teenagers and adults.\(^1\) The neutrophil count also differs by race with individuals of black African descent having lower ANCs.\(^2\) Granulopoiesis occurs in the bone marrow and is a closely regulated system of cell proliferation and maturation. The time required for granulopoiesis in the marrow from blasts to neutrophils varies from 1 to 3 weeks. Once released into the peripheral circulation, neutrophils circulate for only a few hours before egressing to tissues. In the PB, there is equilibrium between the circulating pool and the marginated pool of neutrophils adherent to vascular endothelium.

### Neutrophilia

Neutrophilia is defined as an ANC that exceeds age-related normal range. The normal reference interval (established for each laboratory separately) is approximately 1.8 to 7.0 × 10⁹/L in adults and 1.0 to 8.5 × 10⁹/L in young children.\(^3\) The peripheral neutrophil count only monitors neutrophils in the circulating pool. The primary mechanisms for reactive peripheral neutrophilia are listed in Table 5.1.

Causes of reactive neutrophilia include a broad spectrum of disorders (Table 5.2).\(^4\) The most common cause is bacterial infection. Occasional viral infections such as severe acute respiratory distress syndrome or Hantavirus pulmonary syndrome can result in neutrophilia.
Another common cause is administration of granulocyte-colony stimulating factor (G-CSF). Other medications including steroids or acute stressful events such as burns or trauma are also not infrequently encountered causes of reactive neutrophilia.

Peripheral smears of reactive neutrophilia, as seen in infections, usually demonstrate toxic granulation, Döhle bodies, and vacuolization (Fig. 5.1A–D). Döhle bodies are pale cytoplasmic inclusions that are parallel stacks of rough endoplasmic reticulum with bound ribosomes. Toxic granulation represents either retained primary granules or altered uptake of stain by secondary granules. Prominent cytoplasmic vacuoles in neutrophils are usually associated with sepsis. Variable degree of left shift in the granulocytic lineage can be seen. In adults with infections, the left shift is usually mild (bands and metamyelocytes) with absent or rare blasts. With peripheral reactive neutrophilia, the bone marrow demonstrates granulocytic hyperplasia with a full spectrum of maturation (Fig. 5.1E). However, the left shift can be prominent in infections in pediatric patients and in patients receiving G-CSF (Fig. 5.2A). In these patients, binucleate neutrophils or very large neutrophils can sometimes be present and should not be interpreted as dysplasia (Fig. 5.2B, C). The hypergranulation induced by G-CSF therapy shows a high density of granules, which stain redder than toxic granulation and often obscure the nucleus (Fig. 5.2D). Left shifted granulocytic maturation can be seen in the marrow with G-CSF administration (Fig. 5.2E).

Neutrophilia with left shift can be seen in various hematopoietic neoplasms, especially chronic myeloid leukemia (CML). CML usually has very high WBC (>50 × 10^9/L) with prominent left shift with a “myelocyte bulge” and associated basophilia (Fig. 5.3). Toxic granulation is usually absent. Blasts can be increased. Splenomegaly can be seen in CML. In cases with findings suggestive of CML, molecular or cytogenetic testing for BCR/ABL1 gene rearrangement is warranted. In reactive neutrophilia, WBC count is usually less than 30 × 10^9/L, except in pediatric patients with infection or patients receiving G-CSF or having tumors that are secreting G-CSF or infections in post-splenectomy states. Basophils are usually not increased. Blasts are very rare, and are usually seen only in patients receiving G-CSF or in pediatric patients. Reactive neutrophilia typically does not cause splenomegaly. Chronic neutrophilic leukemia (CNL) is a myeloproliferative neoplasm that presents with marked leukocytosis (>30 × 10^9/L) with absolute neutrophilia. In CNL, the peripheral smear does not demonstrate significant left shift (immature granulocytes are <10% of WBC), patients often have splenomegaly, and mutational analysis is positive for CSF3R mutations in over 80% of cases.

In a patient with absolute neutrophilia, the distinction between reactive and neoplastic etiology (especially CML) requires integration of clinical, hematologic, morphologic, and other laboratory parameters. It is extremely important to carefully review the clinical history for possible reactive etiologies of neutrophilia.

**Neutropenia**

Neutropenia is defined as ANC in blood below age-related normal range (≤2.5 × 10^9/L in infants and ≤1.5 × 10^9/L in all other patient age groups). Approximately 25% of healthy children and adults of African ancestry have ANC of 1.0 to 1.5 × 10^9/L that is considered a normal race-related variant. The degree of neutropenia is classified based on ANC; mild neutropenia is defined as ANC of 1.0 to 1.5 × 10^9/L, moderate neutropenia as ANC of 0.5 to 1.0 × 10^9/L, and severe neutropenia as ANC below 0.5 × 10^9/L. Patients with severe neutropenia are at high risk for infections.

Neutropenia can be isolated or associated with abnormalities in other lineages. Most
causes of isolated neutropenia are either constitutional or acquired nonneoplastic disorders. In neoplastic processes, neutropenia is often associated with abnormalities in other lineages.

Neutropenia can be transient or chronic. Chronic neutropenia is usually defined as an ANC below $1.5 \times 10^9/L$ lasting for more than 3 months. Transient isolated neutropenia is usually secondary to infections or certain drugs. These infections include cytomegalovirus, Epstein–Barr virus (EBV), human immunodeficiency virus (HIV), influenza, parvovirus B19, Brucella, paratyphoid, tuberculosis, tularemia, typhoid, Anaplasma phagocytophilum (Fig. 5.4) and other rickettsias, Plasmodium vivax and Plasmodium falciparum. The drugs commonly associated with neutropenia are anticonvulsants (carbamazepine, valproate), antimicrobials (sulfonamides, penicillins, trimethoprim/sulfamethoxazole), antipsychotic (clozapine, olanzapine, phenothiazines), antiheumatics (gold, levamisole, penicillamine), antithyroid (methimazole, propylthiouracil), and other drugs such as aminopyrine, deferiprone, rituximab, and levamisole-adulterated cocaine. The mechanisms underlying peripheral neutropenia include proliferation defect, maturation defect, an abnormal distribution, and peripheral destruction (Table 5.3). In practice, more than one of these mechanisms may play a role in a given patient.

The common causes of neutropenia based on age of the patient are listed in Table 5.4. The most common cause of neutropenia in neonates/infants is infection that can be acquired or vertically transmitted such as HIV. Infants can also develop neutropenia due to maternal factors such as hypertension, medications given to the mother and maternal antibodies that cross the placenta and attack fetal neutrophils. Autoimmune neutropenia of infancy usually develops between 1 and 3 years of age. It is not associated with other immune aberrations and usually resolves spontaneously. Congenital neutropenia syndromes are usually recognized during childhood because of associated infections. The clinical, molecular, PB, and bone marrow features of congenital neutropenic disorders are listed in Table 5.5. In older children, infection, often viral, is the most common cause of neutropenia.

Cyclic neutropenia is a rare, autosomal-dominant disorder due to mutations in the gene for neutrophil elastase (ELANE or ELA-2) usually confined to exons 4 and 5 in 80% of affected patients. These patients have regular oscillations of severe neutropenia lasting for 4 to 6 days that occur with a 21-day periodicity. During periods of profound neutropenia, patients are predisposed to developing painful mouth ulcers, fever, and bacterial infections. The diagnosis of cyclic neutropenia can be established by serial WBC counts at least three times per week for a minimum of 6 weeks to observe at least two neutrophil nadirs. The PB also usually shows relative monocytosis at the nadir of neutropenia. A bone marrow aspiration and biopsy is not required for diagnosis; however, when performed, it demonstrates maturation arrest in myeloid lineage preceding peripheral neutropenia. Management includes the administration of very low amounts of G-CSF shortly before the neutropenic phase of each cycle to counteract the maturation arrest, reduce the nadir, and diminish the cycling. This approach alleviates the symptoms. Cyclic neutropenia is not associated with an increased risk for leukemia or myelodysplasia.

In adults, medication is the most common cause of acquired neutropenia in an outpatient setting and it is important to query this history. In adults, autoimmune neutropenia is often associated with an immune underlying disorder such as systemic lupus erythematosus (SLE), thyroid disease, or rheumatoid arthritis. The neutropenia is usually self-limited with spontaneous recovery after 2 or 3 years. There may be an increased number of large granular lymphocytes (LGL) in the blood and/or bone marrow. The constellation of neutropenia, splenomegaly, and rheumatoid arthritis is recognized as Felty syndrome. In some of these patients, the LGL proliferation is neoplastic.
Neutropenia can also be seen in myelodysplastic syndromes (MDS). In this setting, neutropenia is often accompanied by abnormalities in other lineages. On the peripheral smear, the neutrophils may show dysplastic features including hypogranularity and nuclear segmentation abnormalities.

Age at onset and duration of neutropenia are critical factors in determining the etiology of neutropenia. Transient neutropenia is common in children and likely secondary to infection. Family history and clinical findings can provide clues to the diagnosis of constitutional neutropenia. In adults, history of infections or medication use, morphologic evaluation of neutrophil morphology, percentage of LGL or other lineage abnormalities on the peripheral smear are helpful in evaluating the etiology of neutropenia.

**Neutrophilic Disorders with Abnormal Morphology**

Table 5.6 summarizes a list of nonneoplastic disorders presenting with abnormal neutrophil morphology. Although these morphologic changes are not specific for a particular etiology, they can provide essential information to guide further evaluation. A detailed clinical history including complete medication list is necessary for evaluation of the underlying etiology.

**Functional Defects of Granulocytes**

Patients with constitutional neutrophil functional defects are extremely rare but are susceptible to recurrent bacterial and fungal infections. Most constitutional neutrophil function defects are manifested in infancy or early childhood and are listed in Table 5.7. Acquired neutrophil function defects are much more common but usually not severe. Conditions associated with acquired neutrophil function defects include acute myeloid leukemia (AML), MDS, chronic renal failure, poorly controlled diabetes mellitus, numerous medications (corticosteroid, epinephrine, aspirin, colchicine), autoimmune disorders, chronic infection (e.g., HIV), trauma, surgery, thermal injury, and so forth.

**NONNEOPLASTIC DISORDERS OF LYMPHOCYTES**

**Lymphocytosis**

Absolute lymphocytosis is typically defined as a lymphocyte count greater than $4.0 \times 10^9/L$ in adults and greater than $8.8 \times 10^9/L$ in children. These values provide general ranges; specific upper and lower limits of normal may vary in different laboratories. Lymphocytosis can be either a reactive polyclonal proliferation or a clonal expansion. The distinction between reactive and clonal lymphocytosis is important as it has implications for monitoring and therapy. Clonal lymphocytosis is usually seen as a monotonous population of lymphocytes on the peripheral smear. It will be discussed in a separate chapter.

The causes of benign lymphocytosis are summarized in Table 5.8. The most common cause is viral infections, particularly infectious mononucleosis caused by EBV. The characteristic morphologic finding is the presence of an increased number of reactive lymphocytes of variable morphology (Fig. 5.14). Correlation with heterophile antibody test is useful in establishing a diagnosis. Many other viruses can cause similar clinical and morphologic features that are referred to as infectious mononucleosis-like syndromes.

Large granular lymphocytosis can be transient or chronic and can be neoplastic or reactive. Reactive etiologies of large granular lymphocytosis are listed in Table 5.9. T-cell large granular lymphocytic (T-LGL) leukemia is a neoplasm that is often associated with
neutropenia and by definition is a clonal T-cell process with persistence of the clone for at least 6 months.\textsuperscript{19} On the PB smear, large granular lymphocytes (LGL) have a similar morphology in both reactive and neoplastic processes (Fig. 5.16).

Reactive lymphocytosis in most cases is due to an increase in T cells. However, rare etiologies can result in an increased number of polytypic B cells. Persistent polyclonal B lymphocytosis is a syndrome observed in young to middle-aged women with a strong association with cigarette smoking. These patients present with a polyclonal increase in B cells in the PB and binucleate lymphocytes identified on the PB smear. They also show an increase in serum immunoglobulin M (IgM) with or without lymphadenopathy and splenomegaly. Most patients have a stable clinical course on long-term follow-up even though they demonstrate frequent cytogenetic and molecular abnormalities including an extra isochromosome for the long arm of chromosome 3 and \textit{BCL2/IGH} gene arrangements.\textsuperscript{20,21}

Reactive lymphocytosis commonly demonstrates a spectrum of lymphocyte morphologies including variably sized lymphocytes. In children, lymphocytosis is usually benign with a heterogeneous morphology. If the lymphocytosis is composed of a monotonous population of lymphocytes, flow cytometric immunophenotyping is a useful tool to exclude a neoplastic process.

**Lymphopenia**

Lymphopenia is defined as an absolute lymphocyte count less than $1.0 \times 10^9$/L in adults or less than $2.0 \times 10^9$/L in children.\textsuperscript{3} Causes of lymphopenia are listed in Table 5.9. Among them, HIV infection is the most common cause. The lymphocytes are decreased in number but show normal morphology.

**NONNEOPLASTIC DISORDERS OF MONOCYTES**

**Monocytosis**

Absolute monocytosis is defined as a monocyte count greater than $1.0 \times 10^9$/L.\textsuperscript{3} Monocytosis can represent a reactive phenomenon or a neoplastic disorder involving monocytes and their precursors. A variety of conditions can raise the monocyte count, including autoimmune disorders (e.g., rheumatoid arthritis, SLE, and inflammatory bowel disease), chronic infections (e.g., mycobacteria, listeria or ehrlichiosis; see Fig. 5.4), underlying solid or hematopoietic tumors, environmental exposure such as alcoholic liver disease, chemotherapy, stem cell transplant, GM-CSF or glucocorticoid administration, hemolytic anemia, cyclic neutropenia, and chronic neutropenia (Fig. 5.17). In neutropenic patients, the monocytosis represents a compensatory phenomenon. The most common cause of reactive monocytosis is chronic infection.

Monocytosis can be transient in settings including post chemotherapy or stem cell transplant, recovery phase of cyclic neutropenia, drug reaction, acute infection, or tissue injury. Chronic monocytosis can be seen in chronic inflammatory disorders, chronic infections, or neoplasms.

In neoplastic disorders, the monocytes are part of the neoplastic clone. Immature monocytes and dysplastic abnormal monocytes can also be present (Fig. 5.18). If monocytosis is persistent (>3 months) and a reactive etiology is not identified, further workup for a neoplastic process such as chronic myelomonocytic leukemia in adults or juvenile myelomonocytic leukemia in children is warranted.
Monocytopenia
Monocytopenia is a decrease in circulating monocytes below the lower reference value of 0.2 \( \times 10^9/L \). Although monocytopenia may occur in any nonneoplastic or neoplastic diseases associated with pancytopenia (e.g., acute infections, stress, autoimmune disorders, treatment with glucocorticoids, aplastic anemia, AML, treatment with myelotoxic drugs), a decrease in monocytes is a constant and important feature of hairy cell leukemia. Deficiency or absence of monocytes can also occur in patients with mutations of the hematopoietic transcription factor gene, GATA2. Affected patients sometimes present with nontuberculous mycobacterial infection, especially at cutaneous sites (i.e., MonoMAC syndrome), genital human papillomavirus infection, or lymphedema. These patients are at risk of progression to MDS/AML.

PANCYTOPENIA
Pancytopenia refers to hemoglobin, WBC count, and platelet count below age/sex-related normal ranges (anemia, leukopenia, and thrombocytopenia). The etiologies/mechanisms of pancytopenia are listed in Table 5.10. Constitutional pancytopenia is rare and usually manifests in early infancy; Fanconi anemia is the most common type. Acquired pancytopenia is more common in clinical practice and is often nonneoplastic. Clinical history of infection, nutritional deficiencies, exposure to toxins, autoimmune disorders, and chemotherapy is important for evaluation of pancytopenia. Assessment of morphologic abnormalities of various lineages such as macrocytic anemia or hypersegmentation of neutrophils provides clues to nutritional deficiency (Fig. 5.23). Evaluation of all hematopoietic lineages for dysplasia and presence or absence of circulating neoplastic cells such as blasts, hairy cells, or lymphoma cells is necessary to exclude neoplastic etiologies. If a reactive etiology for pancytopenia is not identified, further workup, including a bone marrow biopsy is helpful to determine the underlying etiology.

NONNEOPLASTIC DISORDERS OF EOSINOPHILS AND BASOPHILS
Eosinophilia
Eosinophilia is defined as an absolute eosinophil count (AEC) more than 0.5 \( \times 10^9/L \) independent of age. PB eosinophilia has been divided into mild (0.5–1.5 \( \times 10^9/L \)), marked (>1.5 \( \times 10^9/L \)), and massive (>5.0 \( \times 10^9/L \)) eosinophilia. Eosinophilia can be transient, episodic, or persistent (chronic). Persistent eosinophilia is defined as PB eosinophilia recorded on at least two occasions with a minimum time interval of 4 weeks. Eosinophilia may be neoplastic or reactive. The common causes of reactive eosinophilia are listed in Table 5.11. Common causes of eosinophilia are allergic diseases (Fig. 5.24), parasitic infections (Fig. 5.25), and secondary reactions to drugs. Allergic disorders are the most common cause in industrialized nations; parasitic infections are the most common cause of eosinophilia with a history of travel to parasite-endemic areas, and medications/drugs are most common cause in hospitalized patients. Paraneoplastic eosinophilia may be seen in neoplastic diseases including Hodgkin lymphoma, T-cell
lymphoma, B-lymphoblastic leukemia, mastocytosis, and carcinomas.

Hypereosinophilia (HE) in the PB is defined as an absolute eosinophil count $>1.5 \times 10^9/L$ and is usually persistent. A hypereosinophilic syndrome (HES) is defined by the presence of HE (as defined above), with eosinophil-mediated organ damage and/or dysfunction. It may be neoplastic where eosinophilic expansion is clonal and occurs in the setting of an underlying stem cell, myeloid, or eosinophilic neoplasm; secondary where eosinophilic expansion is driven by overproduction of cytokines by other cell types and is polyclonal; or idiopathic where the underlying cause of HE remains unknown despite extensive etiologic workup.

Evaluation for the underlying etiology of eosinophilia must include a thorough history that queries symptoms of organ involvement, medical conditions, exposure to medications, occupational and recreational exposures, travel, and prior eosinophil counts.

**Basophilia**

Basophilia is defined as an absolute basophil count exceeding $0.2 \times 10^9/L$. Reactive basophilia is very rare. Causes of reactive basophilia include allergic/hypersensitivity disorders, chronic inflammatory conditions, endocrinopathy, renal disease, infections, irradiation, and carcinomas. Basophilia is more commonly seen in association with clonal disorders. The most common cause of basophilia is chronic myelogenous leukemia (Fig. 5.26). Evaluation of granulocytes including the presence of left shift or leukocytosis is important to differentiate neoplastic from reactive etiologies. Basophils frequently degranulate on blood smear that can make their identification difficult.

**NONNEOPLASTIC DISEASES OF THE SPLEEN**

Nonneoplastic diseases of the spleen include both infectious and noninfectious disorders and can be classified based on the pattern of splenic involvement (Table 5.12). The following section will discuss the salient features of noninfectious and cystic disorders of the spleen.

Reactive follicular hyperplasia can be seen at any age; however, it is more common in children and younger adults. It can be caused by both acute and chronic immunologic stimuli (e.g., bacterial infections, autoimmune diseases including hemolytic processes). It may present with mild to moderate splenomegaly. Morphologically, variably prominent germinal centers with well-defined marginal and mantle zones are present (Fig. 5.27). The germinal centers are positive for CD20, CD10, bcl-6, and negative for bcl-2 immunohistochemical stains. Increased number of plasma cells and small plasma cell aggregates can be identified in the red pulp.

Reactive lymphoid hyperplasia without germinal center hyperplasia is the most common pattern encountered in viral infections (infectious mononucleosis, herpes simplex virus), transplant recipients, and immunosuppressed individuals (e.g., steroid-treated immune thrombocytopenic purpura, patients with rheumatoid arthritis on methotrexate) and in functional immunodeficiencies encountered in infants and elderly individuals. These patients can present with modest splenomegaly.

Patients with multicentric Castleman disease present with mild to moderate splenomegaly, constitutional symptoms such as fever, and frequently show a host of hematologic and immunologic abnormalities (anemia and hypergammaglobulinemia). Histologic evaluation demonstrates hyperplastic or regressively transformed follicles with significant red pulp plasmacytosis.
Common variable immunodeficiency (CVID) can present in childhood or in adults. Patients often present with recurrent infections. The spleen can be normal to enlarged in size. The histologic features vary dependent on the primary pathogenetic deficiency in lymphoid stimulatory molecules. They can show follicular hyperplasia or follicular atrophy. Granulomas may be present. In some cases, immunoblastic proliferation and atypical cells resembling Hodgkin or Reed–Sternberg cells may be present. Careful clinical history of immunodeficiency must be obtained to avoid misinterpretation of significant immunoblastic proliferation associated with CVID as malignant lymphoma. Immunohistochemical stains show a mixed lymphoid population with variable proportions of B and T cells.

Autoimmune lymphoproliferative syndrome (ALPS) is a rare heritable lymphoproliferative syndrome due to mutations in \textit{Fas} (CD95), \textit{Fas ligand}, \textit{caspase 8}, or \textit{caspase 10} genes. It usually presents in patients younger than 2 years of age as generalized lymphadenopathy, splenomegaly, and autoimmunity. It is frequently associated with immune cytopenias. These patients are at an increased risk for development of non-Hodgkin and Hodgkin lymphoma. Histologic evaluation of the spleen demonstrates prominent white pulp with follicular hyperplasia and expansion of marginal zones. There is marked expansion of periarteriolar lymphoid sheath and red pulp due to the infiltration by a mixed population of small T cells, T-cell immunoblasts, and polyclonal plasma cells. Double-negative T cells (CD4- and CD8-negative) are the hallmark of the disease and can be predominantly found in the red pulp.

Gaucher disease is the most common storage disorder affecting the splenic red pulp. It is an inherited disorder caused by mutations in the \textit{GBA} gene that greatly reduce or eliminate the activity of \(\beta\)-glucocerebrosidase resulting in accumulation of glucocerebroside and related substances within cells. It has two major forms: neuronopathic and nonneuronopathic. The neuronopathic form presents as hepatosplenomegaly and mental deterioration in the first year of life with death in infancy or early childhood. The nonneuronopathic form is the form most commonly encountered in splenectomy specimens. The disease is mostly seen in Ashkenazi Jews with an insidious onset in late childhood or adulthood with no mental retardation. Patients present with pancytopenia, hepatomegaly, splenomegaly, and adrenal involvement. Bone lesions including pathologic fractures, lytic lesions, and avascular necrosis of the femoral head may be present. On histologic evaluation, there is expansion of the red pulp with distention of splenic cords by large pale-stained macrophages (Fig. 5.28). Gaucher cells have a characteristic “wrinkled-silk” cytoplasm that often appears brownish in H&E-stained sections and is distinguishable from that of Niemann–Pick cells, which is foamy or bubbly owing to the presence of numerous small clear vacuoles (Fig. 5.29). Rarely, macrophages engorged with \textit{Mycobacterium avium} may resemble Gaucher cells; however, Gaucher cells are negative for acid-fast stains. Gaucher-like cells can also be seen in CML, probably as a result of rapid marrow cell turnover. In contrast, Niemann–Pick disease is a lysosomal storage disorder caused due to lack of sphingomyelinase resulting in accumulation of sphingomyelin within cells.

Certain hematologic disorders may result in characteristic findings in the spleen. Extramedullary hematopoiesis refers to the accumulation of hematopoietic precursor cells in the spleen (Fig. 5.30). It is a normal finding in the fetus and premature infants. It can be seen as a reactive process in the spleen in patients with severe anemia (e.g., thalassemia major), post bone marrow transplant and immunodeficiency disorders.

Epidermoid cyst (true cyst) in the spleen usually presents in childhood to young adults as a single cyst with a trabeculated appearance covered by a shiny lining. Histologic sections demonstrate a thin, fibrous wall with epithelial lining that can be squamous (more often),
transitional, or columnar. Pseudocyst in the spleen shows a similar clinical presentation as true cyst but is more common than true cyst. It is believed to result from degradation of a splenic hematoma of posttraumatic origin or to be a consequence of cystic degeneration of a splenic infarct or infarcted hemangioma. Histologic sections show a fibrous wall without epithelial lining. Parasitic (Echinococcal) cyst occurs in residents of areas of the world where the parasite is endemic (e.g., Greece); in the United States, the disease is rare but has been reported in California, Arizona, New Mexico, and Utah. Adults are most commonly affected and they usually have significant exposure to animal vectors such as cattle, sheep, pigs, or deer or exposure to the feces of dogs, wolves, or coyotes. These cysts are often multilocular with a granular wall with small granules in cyst contents. Histologic sections demonstrate a fibrous wall with daughter cysts or brood capsules containing parasites with scolices (Fig. 5.31).

IMMUNODEFICIENCY DISORDERS

Primary Immunodeficiency Disorders

Primary immunodeficiencies include predominantly humoral defects, predominantly T-cell defects, combined immunodeficiencies, and immunodeficiencies associated with other major defects such as phagocytic function and complement deficiencies.

Primary humoral immunodeficiency refers to diseases resulting from impaired antibody production either due to a molecular defect intrinsic to B cells or a failure of interactions between B and T cells. Cellular immunity is intact. Clinically, these patients demonstrate recurrent, often severe upper and lower respiratory tract infections with encapsulated bacteria (e.g., *Streptococcus pneumoniae*, *Haemophilus influenzae*). Chronic diarrhea is seen in both children and adults. Workup for patients with suspected antibody deficiency includes measurement of total serum IgG, IgA, and IgM, as well as specific antibody titers to protein and polysaccharide antigens. The salient features of primary humoral immunodeficiency disorders are listed in Table 5.13.32,33

Combined immunodeficiency syndromes are a heterogeneous group of disorders due to a disturbance in the development and function of both T and B cells. Severe combined immunodeficiency disorders (SCID) are a spectrum of disorders with severe combined defects of humoral and cellular immunity that often lead to early death from overwhelming infection. The classic symptoms of SCID are recurrent severe infections, chronic diarrhea, and failure to thrive. Thymic defects are common. The typical laboratory abnormalities observed in SCID include low to absent T cell numbers and function. Hypogammaglobulinemia is often present.

Other inherited immunodeficiency disorders include Griscelli syndrome, Chédiak–Higashi syndrome, chronic granulomatous disease, Wiskott–Aldrich syndrome (WAS), ataxia-telangiectasia and autoimmune lymphoproliferative disorder. Only the latter three entities will be briefly described here.

Autoimmune lymphoproliferative syndrome is a genetic defect that includes mutations in genes relevant for the Fas pathway of apoptosis including Fas, Fas ligand, and caspase 10. The inheritance pattern is autosomal dominant in 90% of cases related to Fas mutations.34 Most patients are children who present with generalized lymphadenopathy, autoimmune phenomena, with or without splenomegaly. Typically, these patients do not develop opportunistic infections but are at an increased risk of developing lymphoma. PB
demonstrates increased T cells that are double negative (CD4-negative, CD8-negative). Lymph nodes demonstrate marked paracortical expansion by lymphocytes, polytypic plasma cells, and immunoblasts (Fig. 5.32). The spleen shows expanded red pulp due to increased T cells.

Ataxia-telangiectasia is a rare autosomal recessive disorder. It typically presents in infancy or early childhood with neurologic deficits, oculocutaneous telangiectasia, and both humoral and cellular immunodeficiency. The genetic defect involves abnormalities in the ATM gene resulting in defective DNA repair and chromosomal instability. These patients develop sinopulmonary infections and also have increased incidence of malignancy, radiation sensitivity, and diabetes mellitus due to insulin resistance. Laboratory findings demonstrate elevated serum α-fetoprotein levels in the absence of liver disease, and lymphocytopenia due to decreased CD4-positive T cells. Ataxia-telangiectasia is a rare autosomal recessive disorder. It typically presents in infancy or early childhood with neurologic deficits, oculocutaneous telangiectasia, and both humoral and cellular immunodeficiency. The genetic defect involves abnormalities in the ATM gene resulting in defective DNA repair and chromosomal instability. These patients develop sinopulmonary infections and also have increased incidence of malignancy, radiation sensitivity, and diabetes mellitus due to insulin resistance. Laboratory findings demonstrate elevated serum α-fetoprotein levels in the absence of liver disease, and lymphocytopenia due to decreased CD4-positive T cells.

Wiskott-Aldrich syndrome is a rare autosomal recessive disease caused by mutations in the WAS gene located at Xp11.22-23 that encodes the WAS protein. There is a wide spectrum of disease severity due to WAS gene mutations, ranging from classic WAS phenotype to a milder form characterized by isolated thrombocytopenia, called X-linked thrombocytopenia, to X-linked neutropenia. The classic WAS phenotype is characterized by bleeding secondary to significant thrombocytopenia, recurrent infections (bacterial and opportunistic), and eczema. Lymphadenopathy and hepatosplenomegaly are commonly present. These patients also have autoimmune disorders and an increased risk of lymphoproliferative disorders, particularly at extranodal sites. The laboratory findings in WAS include significant thrombocytopenia (below 50 × 10⁹/L) with small platelets on the PB smear, decreased number and function of T cells, low to normal IgG and IgM and high IgA and IgE, defective antibody responses to some vaccine antigens, normal to increased natural killer cell numbers, but reduced cytotoxicity, decreased function of regulatory T cells and normal number of neutrophils but decreased chemotaxis of phagocytic cells. Lymph nodes and spleen show varying degrees of T-cell zone depletion.

Secondary Immunodeficiency

Secondary immunodeficiency can result from a wide array of disease processes including infections (bacterial, viral, mycobacterial, and parasitic), immunosuppressive therapy, malignancies, autoimmune disorders, burns, environmental exposure (toxic chemicals and radiation), disorders of biochemical homeostasis (diabetes mellitus, uremia, malnutrition, and cirrhosis), aging, and pregnancy. The clinical features, laboratory findings, and sequelae vary depending on the underlying etiology.

Hypergammaglobulinemia

An increase in serum immunoglobulins may be clonal or polyclonal. Polyclonal gammopathy is often diagnosed when an elevated total serum protein value triggers serum protein electrophoresis analysis. The presence of a broad-based peak or band, usually of γ mobility, suggests a polyclonal increase in immunoglobulins. Polyclonal gammopathy represents diffuse activation of B cells and should prompt evaluation for an underlying condition. Common etiologies are infectious, inflammatory, or reactive processes. Polyclonal gammopathy may on occasion be present without evidence of an underlying process. The most common disorders associated with polyclonal hypergammaglobulinemia are liver disease (autoimmune hepatitis, viral hepatitis, primary biliary cirrhosis, ethanol-related liver disease), connective tissue disease (Sjögren syndrome, rheumatoid arthritis, systemic lupus erythematosus), chronic infections, hematologic disorders, and non-hematologic...
malignancies. Polyclonal gammopathy, along with bone marrow plasmacytosis, is a common finding in HIV-infected patients.

REFERENCES

Table 5.1
Mechanisms for reactive neutrophilia

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Causes and Findings</th>
</tr>
</thead>
</table>

303
Demargination
- Neutrophils attached to endothelium are released back into circulation
- Seen in stressful conditions accompanied by endogenous adrenaline release
- ANC doubled within a few minutes
- Not associated with a left shift

Decreased transit to solid tissues
- Neutrophils remain in the blood for about 12 hours before leaving the circulation and entering solid tissues
- Administration of corticosteroids can delay the transit from blood to solid tissues resulting in a mild to moderate neutrophilia
- Not associated with a left shift

Mobilization from the maturation–storage pool of the bone marrow
- A large reserve compartment of neutrophils, bands, and metamyelocytes in the bone marrow is released into the circulation
- Infections and other inflammatory processes are the most common causes
- May cause a mild left shift

Increased production
- G-CSF mediated
- Increased granulopoiesis in the bone marrow
- Seen with administration of G-CSF or in chemokine-producing neoplasms and chronic inflammation
- Requires days to several weeks

Table 5.2
Causes of reactive neutrophilia

<table>
<thead>
<tr>
<th>Cause</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>• Bacterial infections</td>
</tr>
<tr>
<td></td>
<td>• Occasional viral infections (HPS, SARS)</td>
</tr>
<tr>
<td>Medication, hormones, endogenous overproduction</td>
<td>• G-CSF</td>
</tr>
<tr>
<td></td>
<td>• CSF-producing tumor</td>
</tr>
<tr>
<td></td>
<td>• Corticosteroids (therapeutic or endogenous production)</td>
</tr>
<tr>
<td></td>
<td>• Epinephrine (therapeutic or endogenous production)</td>
</tr>
<tr>
<td></td>
<td>• Lithium</td>
</tr>
<tr>
<td>Inflammatory and metabolic disorders</td>
<td>• Tissue necrosis (burns, trauma, infarct, acute gout)</td>
</tr>
<tr>
<td></td>
<td>• Inflammatory disorders (collagen vascular disorder)</td>
</tr>
<tr>
<td></td>
<td>• Metabolic disorders (ketoacidosis, uremia, eclampsia)</td>
</tr>
<tr>
<td>Other</td>
<td>• Stress/severe exercise</td>
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<tr>
<td></td>
<td>• Pregnancy</td>
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<tr>
<td></td>
<td>• Smoking</td>
</tr>
<tr>
<td></td>
<td>• Toxins (venoms)</td>
</tr>
<tr>
<td>Constitutional (rare)</td>
<td>• Leukocyte adhesion deficiency</td>
</tr>
<tr>
<td></td>
<td>• Hereditary neutrophilia</td>
</tr>
<tr>
<td></td>
<td>• Familial cold urticaria</td>
</tr>
</tbody>
</table>

ANC, absolute neutrophil count; G-CSF, granulocyte-colony stimulating factor.

HPS, Hantavirus pulmonary syndrome; SARS, severe acute respiratory distress syndrome; G-CSF, granulocyte-colony stimulating factor.
Figure 5.1. Peripheral blood smear from an adult patient with bacterial infection. **A:** Peripheral neutrophilia (40×). **B:** Toxic granulation in neutrophils (100×). **C:** Döhle bodies seen as pale blue cytoplasmic inclusions (100×). **D:** Cytoplasmic vacuoles in neutrophil (100×). **E:** Marrow core biopsy with granulocytic hyperplasia with a full spectrum of maturation (20×).
Figure 5.2. Peripheral blood smear from an adult patient receiving G-CSF. **A:** Immature granulocytes on the peripheral smear (100×). **B:** Binucleate neutrophils with pseudo Pelger–Huët morphology (40×). **C:** Large neutrophil (100×). **D:** Neutrophil with toxic granulation that has high density of red granules (100×). **E:** Bone marrow smear with granulocytic hyperplasia with markedly left shifted granulocytic maturation (100×).
**Figure 5.3.** Peripheral smear in a patient with chronic myeloid leukemia. **A:** Marked granulocytosis with left shift and basophilia (40×). **B:** Left shift with predominantly myelocytes and basophilia. Neutrophils do not show toxic granulation (100×).

**Figure 5.4.** Morulae detected in a granulocytic band on a peripheral blood smear in a patient infected with ehrlichiosis (100×). (Courtesy of Dr. Kristie White.)

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Causes of Neutropenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation defect</td>
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<tr>
<td>Stem cell defect</td>
<td>Congenital neutropenia</td>
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<td></td>
<td>Immune disorders</td>
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<td></td>
<td>Following myeloablative therapies</td>
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<td></td>
<td>Medication-associated agranulocytosis</td>
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<tr>
<td>Bone marrow replacement</td>
<td>Myelophthisis</td>
</tr>
<tr>
<td></td>
<td>Myelofibrosis</td>
</tr>
<tr>
<td>Maturation defect</td>
<td>Megaloblastic anemia</td>
</tr>
<tr>
<td></td>
<td>Myelokathexis</td>
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<tr>
<td>Abnormal distribution</td>
<td>Hypersplenism</td>
</tr>
<tr>
<td></td>
<td>Defective release of bone marrow neutrophils (rare)</td>
</tr>
<tr>
<td>Peripheral destruction</td>
<td>Infections</td>
</tr>
</tbody>
</table>
### Table 5.4

**Age-related causes of neutropenia**

<table>
<thead>
<tr>
<th>Age</th>
<th>ANC</th>
<th>Causes of Neutropenia</th>
</tr>
</thead>
</table>
| Infant| ≤2.5 × 10⁹/L | • Infection (most common cause)  
• Maternal hypertension/medications/alloantibodies  
• Autoimmune neutropenia of infancy  
• Metabolic disorders  
• Constitutional neutropenia                                                                 |
| Child | ≤1.5 × 10⁹/L | • Infection (most common cause)  
• Autoimmune neutropenia  
• Immunodeficiency  
• Bone marrow replacement by neoplasms  
• Myeloablative therapy  
• Megaloblastic anemia  
• Aplastic anemia  
• Medications  
• Constitutional neutropenia  
• Idiopathic                                                                 |
| Adult | ≤1.5 × 10⁹/L | • Medications (most common cause)  
• Infection  
• Chronic illness  
• T-cell large granular lymphocytic leukemia  
• Hypersplenism  
• Immunodeficiency  
• Bone marrow replacement  
• Myeloablative therapy  
• Megaloblastic anemia  
• Copper deficiency  
• Immune disorders including white cell aplasia  
• Aplastic anemia  
• Idiopathic                                                                 |

ANC, absolute neutrophil count.

### Table 5.5

**Clinical, molecular, and hematologic features of congenital neutropenias**

<table>
<thead>
<tr>
<th>Neutropenia Type</th>
<th>Clinical Features</th>
<th>Molecular Features</th>
<th>Peripheral Blood Findings</th>
<th>Bone Marrow Findings</th>
</tr>
</thead>
</table>
| Cyclic neutropenia | Inherited mutation resulting in neutropenia (<0.2 × 10⁹/L), without congenital physical abnormalities  
21 d cycle of neutropenia with fever and aphthous ulcers followed by recovery of ANC | AD or S: ELANE mutation | Neutropenia, relative monocytosis at the nadir of ANC | Maturation arrest in myeloid lineage preceding peripheral neutropenia |
| Severe congenital | Inherited mutation resulting in | AD: ELANE mutation | Neutropenia and relative | Maturation arrest in myeloid lineage |
| Neutropenia (including Kostmann syndrome) | Neutropenia due to increased apoptosis of myeloid cells; severe pyogenic infections; no characteristic dysmorphic features | AR: HAX1 mutation  
X-linked: WAS mutation  
Dominant negative: GF11 mutation | Monocytosis | Promyelocyte/myelocyte stage, with decreased or absent neutrophil, with monocytosis, and thrombocytosis |
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<tr>
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</thead>
<tbody>
<tr>
<td>Shwachman–Diamond syndrome</td>
<td>Predominant neutropenia, pancreatic insufficiency, and skeletal abnormalities</td>
<td>AR: SBDS mutation</td>
<td>Initial neutropenia, may develop other cytopenias</td>
<td>Variable cellularity, cases are hypocellular and transient trilineage dysplasia, increased propensity to develop AA</td>
</tr>
<tr>
<td>Chédiak–Higashi syndrome</td>
<td>Oculocutaneous albinism, recurrent pyogenic infections, mild bleeding tendency, accelerated phase with HLH</td>
<td>AR: CHS1/LYST mutations and abnormalities of lysosome-related organelles</td>
<td>Giant cytoplasmic inclusions in granulocytes, neutropenia (Fig. 5.6)</td>
<td>Complete myeloid maturation is pre-giant cytoplasmic inclusions in leukocyte precursor cells</td>
</tr>
<tr>
<td>Myelokathexis (may be a part of WHIM syndrome)</td>
<td>Profound neutropenia, recurrent infections, warts</td>
<td>AD, truncating mutations in CXCR4</td>
<td>Profound neutropenia, neutrophils show aberrant segmentation with long, thin, filamentous strands connecting pyknotic nuclear lobes (Fig. 5.7)</td>
<td>Hypercellular marrow due to impaired release of neutrophils from bone marrow</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Bone marrow failure, congenital abnormalities including skin hyperpigmentation, microphthalmia, radial ray anomalies, genitourinary abnormalities, and short stature</td>
<td>Autosomal or X-linked recessive disorder, caused by 13 separate genes comprising the “Fanconi anemia pathway”; increased spontaneous chromosomal breakages and hypersensitivity to DNA cross-linking agents</td>
<td>Thrombocytopenia and/or anemia in early stages with eventual progression to pancytopenia</td>
<td>Hypocellular marrow (Fig. 5.8)</td>
</tr>
</tbody>
</table>
| Dyskeratosis congenita | Bone marrow failure, variable mucocutaneous abnormalities including nail dystrophy | AD: mutations in TERT, TERC, or TINF2 gene  
AR: mutations in TERT, NOP10, NHP2, or WRAP53; X-linked recessive mutations in | Thrombocytopenia or anemia followed by pancytopenia | Gradual development of aplastic anemia |
<table>
<thead>
<tr>
<th>DKC1 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mutations in these genes result in very short telomeres)</td>
</tr>
</tbody>
</table>

AD, autosomal dominant; AR, autosomal recessive; S, sporadic; ANC, absolute neutrophil count; AA, aplastic anemia; HLH, hemophagocytic lymphohistiocytosis; WHIM, warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis.

**Figure 5.5.** Marrow aspirate smear (A) and core biopsy (B) with granulocytic maturation arrest at the myelocyte stage (40×) in a patient with severe congenital neutropenia.
Figure 5.6. Large cytoplasmic granules in a neutrophil from a patient with Chédiak–Higashi syndrome (100×). (Courtesy of Dr. Anne Deucher.)

Figure 5.7. Hypersegmented neutrophil with thin filamentous strands connecting pyknotic nuclear lobes in a patient with myelokathexis (100×).
**Figure 5.8.** Markedly hypocellular bone marrow with markedly decreased megakaryocytes in a 2-year-old child with Fanconi anemia (20×).

<table>
<thead>
<tr>
<th>Morphologic Abnormality</th>
<th>Features</th>
<th>Disorders</th>
<th>Other Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelger–Huët change</td>
<td>Bilobed or non-segmented neutrophil nuclei (<a href="#">Fig. 5.9</a>)</td>
<td>Pseudo Pelger–Huët changes: Secondary to medications (mycophenolate mofetil, tacrolimus, ganciclovir, sulfonamides, valproic acid and colchicine, chemotherapy)</td>
<td>Changes are reversible upon discontinuation of medications; neutrophils function normally</td>
</tr>
<tr>
<td>Hypersegmented neutrophils</td>
<td>≥5% neutrophils with 5 nuclear lobes or 1 neutrophil with ≥6 nuclear lobes (<a href="#">Fig. 5.10</a>)</td>
<td>Vitamin B₁₂, folate or iron deficiency; Medication exposure (sulfonamide, chemotherapy)</td>
<td>Macrocytic anemia in vitamin B₁₂ or folate deficiency</td>
</tr>
<tr>
<td>Nuclear shape abnormalities</td>
<td>Nuclear segmentation abnormalities, pyknotic nuclei</td>
<td>Myelokathexis (AD)</td>
<td>Neutropenia, bone marrow abnormalities, skeletal abnormalities, growth retardation</td>
</tr>
<tr>
<td>Cytoplasmic inclusions</td>
<td>Large blue cytoplasmic</td>
<td>May–Hegglin anomaly (AD)</td>
<td>Thrombocytopenia, enlarged platelets,</td>
</tr>
<tr>
<td>within neutrophils</td>
<td>inclusions resembling giant Döhle bodies (Fig. 5.11)</td>
<td>variable neutropenia, inclusions also in eosinophils, basophils, and monocytes</td>
<td></td>
</tr>
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<td>---------------------</td>
<td>-------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Intense azurophilic granulation (Fig. 5.12)</td>
<td>Alder–Reilly anomaly (AR)</td>
<td>Associated with different types of genetic mucopolysaccharide disorders</td>
<td></td>
</tr>
<tr>
<td>Giant cytoplasmic granules (Fig. 5.6)</td>
<td>Chédiak–Higashi syndrome (AR)</td>
<td>Neutropenia, thrombocytopenia, partial oculocutaneous albinism, frequent pyogenic infections and peripheral neuropathy</td>
<td></td>
</tr>
<tr>
<td>Döhle bodies and toxic granulation</td>
<td>Infection or G-CSF treatment</td>
<td>Left shifted granulocytic cells</td>
<td></td>
</tr>
<tr>
<td>Pseudo Howell–Jolly bodies (Fig. 5.13)</td>
<td>HIV infection, immunosuppressive therapy, chemotherapeutic agents</td>
<td>Cytopenia in HIV patients</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic vacuoles</td>
<td>Copper deficiency, sepsis, ethanol toxicity</td>
<td>Cytopenia in copper deficiency</td>
<td></td>
</tr>
<tr>
<td>Giant neutrophils</td>
<td>HIV infection, G-CSF therapy</td>
<td>Cytopenia in HIV patients</td>
<td></td>
</tr>
</tbody>
</table>

AD, autosomal dominant; AR, autosomal recessive; G-CSF, granulocyte-colony stimulating factor; HIV, human immunodeficiency virus.

**Figure 5.9.** Neutrophils with pseudo Pelger–Huët nuclei in a patient on tacrolimus post heart transplant. The neutrophils show bilobed nuclei (arrows) with a pince-nez appearance (40×).
Figure 5.10. Hypersegmented neutrophil demonstrating six nuclear lobes in a patient with vitamin $\text{B}_{12}$ deficiency (100×).

Figure 5.11. Giant Döhle body-like cytoplasmic inclusion in a neutrophil adjacent to a large platelet in a patient with May–Hegglin anomaly (100×). (Courtesy of Dr. Benjamin Rosen.)

Figure 5.13. Howell–Jolly body-like cytoplasmic inclusions in a neutrophil from a patient with HIV (100×). (Courtesy of Dr. Anne Deucher.)

<table>
<thead>
<tr>
<th>Table 5.7</th>
<th>Constitutional functional defects of granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of Defect</strong></td>
<td><strong>Disease</strong></td>
</tr>
<tr>
<td>Disorders of Chédiak–Higashi</td>
<td>AR; disorder of CHS1 gene resulting in abnormal lysosomal</td>
</tr>
</tbody>
</table>
granules Higashi syndrome trafficking and defective cytoplasmic degranulation

<table>
<thead>
<tr>
<th>Disorders of oxidative metabolism</th>
<th>Chronic granulomatous disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorder</td>
<td>Predominantly X-linked, rarely AR; mutations in genes encoding subunits of NADPH oxidase, defective phagocytosis; recurrent infections, lymphadenopathy, hepatosplenomegaly and anemia of chronic disease</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disorders of adhesion and motility</th>
<th>Leukocyte adhesion deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorder</td>
<td>AR disorders where neutrophils fail to adhere to and migrate through the endothelium into tissues: <em>(Type I (common): deficiency of and/or defects in CD18)</em> <em>(Type II (rare): defective rolling of hematopoietic cells due to absence of fucosylated carbohydrate ligands)</em> <em>(Type III (rare): defective activation of β integrins)</em></td>
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<table>
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<tr>
<th>Table 5.8 Causes of benign lymphocytosis</th>
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</thead>
<tbody>
<tr>
<td><strong>Cause</strong></td>
</tr>
<tr>
<td>Infection</td>
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<td></td>
</tr>
<tr>
<td>Stress</td>
</tr>
<tr>
<td>Drug reaction</td>
</tr>
<tr>
<td>Large granular lymphocytosis</td>
</tr>
<tr>
<td>Persistent polyclonal B-cell lymphocytosis</td>
</tr>
</tbody>
</table>

AR, autosomal recessive; NADPH, nicotinamide adenine dinucleotide phosphate.

EBV, Epstein–Barr virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus.
Figure 5.14. Peripheral blood smear from a patient with infectious mononucleosis. A: Large lymphocyte with abundant pale blue cytoplasm, slightly dispersed chromatin, and indistinct nucleoli as well as large granular lymphocyte with abundant cytoplasm and azurophilic granules (100×). B: Immunoblast with moderate amount of basophilic cytoplasm, oval nucleus, and coarse chromatin (100×).
Figure 5.15. Lymphocyte in a 6-month-old child with pertussis showing cleaved nucleus (100×).
Figure 5.16. Peripheral smear from a patient post bone marrow transplant. A: Increased large granular lymphocytes (40×). B: Large granular lymphocyte with moderate to abundant cytoplasm and prominent azurophilic granules (100×).

Table 5.9

<table>
<thead>
<tr>
<th>Cause</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutional</td>
<td>Severe combined immunodeficiency disorders</td>
</tr>
<tr>
<td></td>
<td>DiGeorge syndrome</td>
</tr>
<tr>
<td></td>
<td>Wiskott–Aldrich syndrome</td>
</tr>
<tr>
<td></td>
<td>Idiopathic CD4 T-lymphocytopenia</td>
</tr>
<tr>
<td>Acquired</td>
<td>HIV infection</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Other viral infection (SARS, influenza, hepatitis)</td>
</tr>
<tr>
<td></td>
<td>Bacterial infection (tuberculosis, typhoid fever, brucellosis)</td>
</tr>
<tr>
<td></td>
<td>Autoimmune disorders (rheumatoid arthritis, SLE, myasthenia gravis)</td>
</tr>
<tr>
<td></td>
<td>Systemic disease (sarcoidosis, renal insufficiency)</td>
</tr>
<tr>
<td></td>
<td>Therapy (radiation, chemotherapy, immunosuppressive agents)</td>
</tr>
<tr>
<td></td>
<td>Others (alcohol abuse, zinc deficiency, malnutrition, stress, exercise, trauma, abnormalities of thoracic duct, protein-losing enteropathy)</td>
</tr>
</tbody>
</table>

HIV, human immunodeficiency virus; SARS, severe acute respiratory distress syndrome; SLE, systemic lupus erythematosus.

**Figure 5.17.** Reactive monocytosis in a patient post chemotherapy (40×). The monocytes have indented nucleus with mature chromatin.
Figure 5.18. Peripheral smear from a patient with chronic myelomonocytic leukemia. A: Peripheral monocytosis with rare blast and hypogranular neutrophil (40×). B: Monocyte with slightly immature chromatin but more mature than a blast equivalent (100×).

Table 5.10
Nonneoplastic causes of pancytopenia

<table>
<thead>
<tr>
<th>Cause</th>
<th>Mechanisms</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased production</td>
<td>Constitutional</td>
<td>Fanconi anemia</td>
</tr>
<tr>
<td>(ineffective hematopoiesis)</td>
<td>BM failure</td>
<td>Shwachman–Diamond syndrome</td>
</tr>
<tr>
<td></td>
<td>syndrome</td>
<td>Dyskeratosis congenita</td>
</tr>
</tbody>
</table>
| Acquired BM failure | Aplastic anemia  
|                   | PNH  
|                   | Infections (Fig. 5.19), toxins, autoimmune disorders or HLH (Fig. 5.20)  
|                   | Chemotherapy  
| Nutritional deficiency | Vitamin B\textsubscript{12}/folate deficiency, cachectic states secondary to severe malnutrition (Fig. 5.21) or copper deficiency (Fig. 5.22)  
| Peripheral destruction or sequestration | Hypersplenism  
|                   | Splenomegaly secondary to various etiologies  

BM, bone marrow; PNH, paroxysmal nocturnal hemoglobinuria; HLH, hemophagocytic lymphohistiocytosis.

**Figure 5.19.** Patient with disseminated histoplasmosis and peripheral pancytopenia. **A:** Yeast forms of histoplasma within a macrophage in the cerebrospinal fluid (100×). **B:** Marrow biopsy from the patient with nonnecrotizing granuloma (10×).

**Figure 5.20.** Marrow aspirate smear demonstrating macrophage with ingested red cells and leukocytes in a patient with hemophagocytic lymphohistiocytosis secondary to Epstein–Barr virus infection (40×).
Figure 5.21. Marrow biopsy from a patient with anorexia nervosa demonstrating gelatinous transformation of fat (also called serous fat atrophy) and virtually acellular marrow (10×). There is atrophy of fat cells with deposition of extracellular gelatinous pink-purple substance.

Figure 5.22. Marrow aspirate smear from a patient with copper deficiency due to increased zinc supplementation. Erythroid and granulocytic precursors demonstrate the presence of cytoplasmic vacuoles (100×).
Figure 5.23. Patient with megaloblastic anemia. A: Peripheral smear with hypersegmented neutrophil (100×). B: Marrow aspirate smear with marked nuclear-cytoplasmic asynchrony in the erythroid and granulocytic precursors; terminal dyserythropoiesis with unequal nuclear budding (100×).

<table>
<thead>
<tr>
<th>Cause</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergy</td>
<td>Asthma, hay fever, allergic rhinitis, sinusitis</td>
</tr>
<tr>
<td>Drugs</td>
<td>Recombinant interleukin, many medications</td>
</tr>
<tr>
<td>Infection</td>
<td>Helminthic infection, fungal, protozoal, bacteria, viral</td>
</tr>
<tr>
<td>Autoimmune disorders</td>
<td>Churg–Strauss syndrome, Kimura disease, polyarteritis nodosa, sarcoidosis</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Pulmonary disorders</td>
<td>Loeffler syndrome, hypersensitivity pneumonia, bronchiectasis</td>
</tr>
<tr>
<td>Skin lesions</td>
<td>Pemphigus, bullous pemphigoid, angiolympoid hyperplasia, Wells syndrome, eosinophilic panniculitis</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>Inflammatory bowel disease, eosinophilic gastroenteritis</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Classical Hodgkin lymphoma, T-cell lymphoproliferative disorders, Langerhans cell histiocytosis, carcinoma</td>
</tr>
</tbody>
</table>

**Figure 5.24.** Peripheral eosinophilia in a patient secondary to phenytoin therapy (40×).

**Figure 5.25.** Microfilaria in the peripheral blood from a patient with lymphatic filariasis.
Figure 5.26. Basophilia in a patient with chronic myelogenous leukemia (100×). Basophils with coarse dark purple granules almost obscuring the nucleus. The bottom of the image demonstrates a partially degranulated basophil.

Table 5.12  
Nonneoplastic disorders involving the spleen

<table>
<thead>
<tr>
<th>Pattern of Involvement</th>
<th>Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>White pulp involvement</td>
<td>• Reactive follicular hyperplasia</td>
</tr>
<tr>
<td></td>
<td>• Reactive lymphoid hyperplasia without germinal center formation</td>
</tr>
<tr>
<td></td>
<td>• Castleman disease</td>
</tr>
<tr>
<td></td>
<td>• Common variable immunodeficiency</td>
</tr>
<tr>
<td></td>
<td>• Autoimmune lymphoproliferative syndrome</td>
</tr>
<tr>
<td>Red pulp involvement</td>
<td>• Storage disorders</td>
</tr>
<tr>
<td></td>
<td>• Extramedullary hematopoiesis</td>
</tr>
<tr>
<td>Cysts</td>
<td>• Epidermoid cyst (true cyst)</td>
</tr>
<tr>
<td></td>
<td>• Pseudocyst</td>
</tr>
<tr>
<td></td>
<td>• Parasitic (echinococcal) cyst</td>
</tr>
<tr>
<td>Granulomatous diseases</td>
<td>• Sarcoidosis</td>
</tr>
<tr>
<td></td>
<td>• Miliary tuberculosis</td>
</tr>
<tr>
<td></td>
<td>• Histoplasmosis</td>
</tr>
<tr>
<td></td>
<td>• Coccidioidomycosis</td>
</tr>
<tr>
<td></td>
<td>• Lipogranulomas</td>
</tr>
<tr>
<td>Non-granulomatous</td>
<td>• Infectious mononucleosis</td>
</tr>
<tr>
<td>diseases</td>
<td>• Cytomegalovirus infection</td>
</tr>
<tr>
<td></td>
<td>• Mycobacterium avium-intracellularare</td>
</tr>
<tr>
<td></td>
<td>• Malaria</td>
</tr>
<tr>
<td></td>
<td>• Pyogenic bacterial infections (abscess)</td>
</tr>
</tbody>
</table>
Figure 5.27. Spleen with hyperplastic follicle with marginal zone hyperplasia in a patient with autoimmune hemolytic anemia (20×). (Courtesy of Dr. Attilio Orazi and Dr. Dennis O’Malley.)
Figure 5.28. Numerous macrophages with “wrinkled silk” cytoplasm (A; 10×) within the red pulp of spleen and (B; 40×) in bone marrow in a patient with Gaucher disease. (Courtesy of Dr. Attilio Orazi.)
Figure 5.29. Numerous macrophages with vacuolated/bubbly cytoplasm within the red pulp of spleen in a patient with Niemann–Pick disease (20×). (Courtesy of Dr. Attilio Orazi.)

Figure 5.30. Extramedullary hematopoiesis demonstrating erythroid precursors (black arrows) and megakaryocyte (blue arrow) within sinusoids in splenic red pulp from a patient with thalassemia (20×).
**Figure 5.31.** Eosinophilic laminated lining of a splenic echinococcal cyst (20×).
Figure 5.32. Lymph node from a patient with autoimmune lymphoproliferative syndrome. **A:** Lymph node with markedly expanded paracortex (4×). **B:** A mixed population of lymphocytes, plasma cells (black arrows), and immunoblasts (blue arrows) within the paracortex (20×). (Courtesy of Dr. Yi Xie.)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Underlying Defects</th>
<th>Clinical Features</th>
<th>Laboratory and Morphologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common variable immunodeficiency</td>
<td>Failure of B-cell differentiation</td>
<td>Variable age at presentation, recurrent infections</td>
<td>Decreased serum IgG and IgA levels, normal number of B cells in PB;</td>
</tr>
<tr>
<td></td>
<td>with impaired</td>
<td>(upper and</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 5.13
Primary humoral immunodeficiency disorders

331
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
<th>Symptoms</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selective IgA deficiency</td>
<td>Failure of terminal differentiation of B cells into IgA-producing cells; mutation in <em>IGAD1</em> and <em>IGAD2</em>; familial clustering with no distinct Mendelian inheritance pattern</td>
<td>Respiratory and/or gastrointestinal infections in 50% of patients, autoimmune disorders in a third of patients, allergies in 10%–15% of patients, increased risk of life-threatening anaphylactic reaction when administered blood products or γ-globulins</td>
<td>No serum IgA with normal IgG and IgM; normal number of B cells in PB and normal T-cell function</td>
</tr>
<tr>
<td>Hyper IgM syndrome</td>
<td>Defective immunoglobulin isotype switching; most common inheritance is X-linked; most common genetic defect is mutations in the gene for CD40 ligand, other defects in genes involving CD40, activation-induced cytidine deaminase, uracil DNA glycosylate and NF-κB essential modulator</td>
<td>Usually presents in the first or second year of life with recurrent pyogenic bacterial infections, severe opportunistic infections, chronic neutropenia, autoimmune diseases, and increased risk for hepatic carcinoma</td>
<td>Markedly decreased levels of IgG, IgA, and IgE with elevated or normal levels of IgM, normal or increased circulating B cells, normal number of T cells; lymph nodes show only primary follicles with absent or abortive germinal centers and markedly decreased to absent follicular dendritic cells</td>
</tr>
<tr>
<td>Hyper IgE-syndrome (Job syndrome)</td>
<td>AD forms: mutations in <em>STAT3</em>; AR forms: mutations in <em>TYK2</em> and <em>DOCK8</em>; defects in neutrophil chemotaxis in 80% of patients</td>
<td>Often presents in childhood with skin infections (<em>Staphylococcus</em> abscesses), pruritus, sinopulmonary infections, coarse facial features, recurrent fractures and hyperextensible joints</td>
<td>Elevated serum IgE level and peripheral eosinophilia</td>
</tr>
<tr>
<td>X-linked agammaglobulinemia (Bruton)</td>
<td>Gene defect at Xq21.3-q22 encoding <em>BTK</em></td>
<td>Increased infections with encapsulated bacteria and blood-borne</td>
<td>Hypogammaglobulinemia or agammaglobulinemia, deficient antibody</td>
</tr>
<tr>
<td>Disorder</td>
<td>Gene</td>
<td>Inheritance</td>
<td>Associated Conditions</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>-------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Agammaglobulinemia</td>
<td>Gene; X-linked</td>
<td>Inheritance</td>
<td>Viruses (enteroviruses), absence or near absence of tonsils and adenoids, increased risk of lymphomas</td>
</tr>
<tr>
<td>X-linked lymphoproliferative disorder (Duncan syndrome)</td>
<td>Mutations in SAP/SH2D1A, X-linked recessive, ineffective T and natural killer cell interaction with B cells resulting in lack of immune surveillance for EBV</td>
<td>Average age at presentation is 2–3 yr; most common clinical presentations are fulminant infectious mononucleosis often complicated by HLH, dysgammaglobulinemia and B-cell lymphoma</td>
<td>Polyclonally activated CD8-positive T cells during EBV infection, following EBV infection: increased IgM and decreased IgG</td>
</tr>
</tbody>
</table>

AD, autosomal dominant; AR, autosomal recessive; PB, peripheral blood; HLH, hemophagocytic lymphohistiocytosis; NF-κB, nuclear factor kappa B; EBV, Epstein–Barr virus.
Acute leukemias are hematologic malignancies characterized by increased numbers of myeloid or lymphoid blasts. The term “acute,” historically referring to a rapid onset and promptly fatal outcome, now indicates the relatively undifferentiated nature of the leukemic cells. The World Health Organization (WHO) Classification of hematopoietic and lymphoid tissue tumors is the most widely accepted and universally applied classification of acute leukemias with diagnostic criteria of disease entities based on the integrated combination of clinical, morphologic, immunophenotypic, and genetic characteristics. The WHO Classification divides acute leukemias into myeloid, lymphoid, and ambiguous lineage (mixed) phenotypes, depending on the origin of the blast cell.

The overall annual incidence of these disorders in the general population is about 4 per 100,000, with approximately 70% of them being acute myeloid leukemia (AML). AML accounts for about 15% of childhood leukemias and for approximately 80% to 90% of acute leukemias in adults, with the median age at diagnosis being about 70 years. Acute lymphoblastic leukemia (ALL) is primarily a childhood disease, with the peak incidence between the ages of 2 and 3 years. It diminishes in frequency until it reaches a nadir from about the ages of 25 to 50, after which it increases to achieve a second, but minor, peak at ages older than 80.

The etiology of most cases is unknown, but a few patients have had previous exposure to ionizing radiation, cytotoxic chemotherapeutic agents, or chemicals such as benzene. Several congenital diseases, such as Down syndrome, Bloom syndrome, and Turner syndrome, have an increased incidence of AML, as do certain types of bone marrow failure, such as Fanconi anemia, the Blackfan–Diamond syndrome, and rare individuals with familial RUNX1 and ETV6 mutations. Very rare familial forms of acute leukemia (e.g., RUNX1, ETV6, ANKRD26 and GATA2 gene mutations) have also been recognized. Patients with myelodysplastic and myeloproliferative disorders have varying, but elevated risks of developing AML. Heavy cigarette smoking also increases the incidence.

At the time of diagnosis, most patients with acute leukemia have nonspecific symptoms, such as fatigue, lethargy, and weight loss. Some complaints, such as dyspnea, angina, and dizziness, arise from anemia. Fever from the disease itself or from an infection related to neutropenia can be the presenting manifestation. Bleeding, such as epistaxis or cutaneous ecchymoses, may occur from thrombocytopenia or from disseminated intravascular coagulation (DIC) in patients with acute promyelocytic leukemia (APL). Bone pain and tenderness can develop from bone marrow expansion or direct periosteal involvement. Gums may swell from leukemic infiltration, especially in the monocytic types of AML.

On physical examination, lymph node enlargement and hepatosplenomegaly may be detectable, more commonly in ALL than in AML. Pallor from anemia and petechiae or ecchymoses from thrombocytopenia or DIC may be apparent. Approximately 5% to 20% of patients with AML and ALL have skin infiltration with leukemic cells (leukemia cutis).
sometime during the course of their disease. In about 5% to 10% of these patients, the cutaneous lesions precede the diagnosis, in about 35% to 45% they are simultaneous, and in about 55% they appear afterward, usually months later. They are typically erythematous or violaceous papules or nodules, but plaques, macules, palpable purpura, or ulcers also can occur. Patients with AML also may develop Sweet syndrome, characterized by an acute onset of fever and tender violaceous plaques that are often extensive, may affect the mucous membranes, and sometimes develop into blisters. Biopsies demonstrate mature neutrophils in the dermis. The fever and skin lesions disappear with systemic corticosteroid therapy, but recurrences are common with AML. Occasionally, prior to, or concurrent with, the diagnosis of AML, patients develop myeloid sarcomas, which are tumors of leukemic cells outside the bone marrow. These tumors can involve lymph nodes, skin, periosteum, extramedullary bone, and soft tissues. They often affect the subperiosteal bony structures of the skull, sternum, ribs, vertebrae, and pelvis.

At the time of presentation, the blood smear typically reveals decreased red cells and platelets, with the white count varying from leukopenia to marked leukocytosis. A decrease in the number of mature neutrophils is common. Circulating blasts are usually detectable. *A leukemic leukemia*, in which blasts are not apparent in the peripheral blood, is slightly more common in AML than in ALL. With DIC, which occurs primarily with APL, microangiopathic features may be present, including red cell fragments, marked polychromatophilia, microcytes, and profound thrombocytopenia. In patients with preceding myelodysplastic syndromes (MDS), features of MDS are typically apparent, such as hypolobulated and hypogranular neutrophils, giant and agranular platelets, and erythrocytic macrocytosis and poikilocytosis.

All patients with suspected leukemia should undergo bone marrow aspirate and biopsy, with cytogenetic analysis, molecular testing, cytochemical analysis, and immunophenotyping done on the cells to delineate the correct classification of the leukemia. Usually, the presence of leukemia is obvious on bone marrow examination of Wright–Giemsa-stained aspirates: typically hypercellular, with sheets of blasts replacing the normally maturing cells in the erythroid, myeloid, and megakaryocytic lines. The distinction between a myeloid or lymphoid origin of blasts is crucial to classifying acute leukemia, as different types of acute leukemia require different treatment strategies. The presence in the cytoplasm of Auer rods—red, needle-like structures thought to be coalescences of primary granules—indicates a myeloblast. Otherwise, the distinction requires cytochemical or immunophenotypic studies. The first step is to use stains specific for myeloperoxidase (MPO) activity or for myeloid granules, and those that detect cells of monocytic lineage, such as nonspecific esterase. Concurrently, flow cytology is used to distinguish between minimally differentiated AML and ALL, to detect monocytic, erythroid or megakaryoblastic leukemia, to discriminate between B- and T-cell forms of ALL, and to establish the tumor-specific immunophenotypic signature that will be used in subsequent marrow samples in order to rule out minimal residual disease (MRD).

The criterion for the diagnosis of AML in most cases is that myeloblasts constitute at least 20% of the nucleated cells in the blood (based on counting 200 cells) or the bone marrow (counting 500 cells). The abnormal promyelocytes in APL and the promonocytes in AML with monocytic differentiation are considered blast equivalents. The diagnosis of AML can also be established in cases with evidence of t(8;21)(q22;q22) translocation, inv(16) (p13.1;q22) or t(15;17)(q22;q12) translocation regardless of the number of blasts or blast equivalents.
ACUTE MYELOID LEUKEMIAS

These disorders are defined as clonal expansions of myeloid blasts, most commonly in the blood or bone marrow, but occasionally presenting as tumor masses (myeloid sarcoma) in other tissues, such as skin and lymph nodes. The WHO classification (Table 6.1) separates AML into seven general groups: (1) AML with recurrent genetic abnormalities; (2) AML with myelodysplasia-related changes; (3) therapy-related AML and MDS; (4) AML not otherwise categorized; (5) myeloid sarcoma; (6) myeloid proliferations related to Down syndrome; and (7) blastic plasmacytoid dendritic cell neoplasm. The last two entities are exceedingly rare in routine daily practice. The fourth group—AML not otherwise categorized—is a revision of the previously used FAB classification and divides cases of AML based on morphology and immunophenotype.

AML with Recurrent Genetic Abnormalities

These disorders have cytogenetic abnormalities, most commonly breaks in chromosomes in which the fragments join other chromosomes (translocations). These rearrangements create fusion genes that regulate the production of abnormal proteins.

**AML with t(8;21)(q22;q22) (RUNX1-RUNX1T1, previously called AML1-ETO)** constitutes about 5% to 10% of AML cases, predominantly in younger patients. The t(8;21)(q22;q22) translocation results in a fusion protein, RUNX1-RUNX1T1 (also called AML1-ETO) a transcription factor. The blasts are typically large, with abundant basophilic cytoplasm, often with Auer rods and numerous, sometimes very large, azurophilic granules. Dysplasia in the form of abnormal nuclear segmentation and cytoplasmic staining may be present in promyelocytes, myelocytes, and mature neutrophils.

**AML with inv(16)(p13q22) or t(16;16)(p13;q22)** is found in about 10% of cases of AML, primarily in younger patients. The translocation results in a fusion protein, CBFB-MYH-11, a transcription factor. The bone marrow usually has elements of both granulocytic (including myeloblasts) and monocytic differentiation (including monoblasts, promonocytes, and monocytes), combined with abnormal eosinophils. Eosinophil precursors contain abnormally large, purple granules that can be sufficiently numerous to obscure the nuclei. In addition to detection by FISH, the RUNX1-RUNX1T1 and CBFB-MYH11 transcript levels can be measured using quantitative RT-PCR (Figure 6.24). The transcript levels can be used as a way to measure MRD, and serial monitoring of the transcript levels can be utilized to risk stratify and identify patients at high risk of relapse post-chemotherapy.

**AML with t(15;17)(q22;q12) or APL** constitutes about 5% of AML. Abnormal promyelocytes are present, either hypergranular or hypogranular (microgranular). The t(15;17)(q22;q12) translocation results in a fusion protein, PML-RARa, which leads to transcriptional repression and arrest of promyelocyte differentiation. In the hypergranular form, the cytoplasm is packed with pink, red, or purple granules that are usually large, but may be fine. Bundles of Auer rods are present in most cases. The nuclei, which may be bilobed, are irregular in size and variable in shape, and may be reniform (kidney-shaped). APL is often associated with DIC, and has a high rate of early mortality due to hemorrhage if not treated promptly. Treatment should be started immediately if morphology is suggestive, without waiting for confirmation by flow cytometry or cytogenetics.

**AML with 11q23 abnormalities**, which constitutes about 5% of AML, occurs at any age, but is more common in children. Some cases develop after treatment with topoisomerase II inhibitors. Monocytic differentiation, with monoblasts and promonocytes predominating, is the most common morphologic pattern. Patients may have gum infiltration, leukemia cutis,
and DIC. Monoblasts are large cells with round nuclei that usually contain lacy chromatin and large prominent nucleoli. The abundant basophilic and sometimes vacuolated cytoplasm may form pseudopods and contain scattered, fine azurophilic granules.

**AML with Myelodysplasia-Related Changes**

This type of AML occurring primarily in older adults has dysplasia in at least 50% of cells in two or more cell lines, including megakaryocytes. It is also diagnosed in patients with a previous diagnosis of MDS or MDS/MPN or in AML cases with an MDS-related cytogenetic abnormality. Abnormalities in granulopoiesis include hypogranular cytoplasm and hypolobulated or bizarrely segmented nuclei. Abnormal erythropoiesis is characterized by ring sideroblasts, vacuolated cytoplasm, and nuclei that are multiple, fragmented, or megaloblastic. Abnormal megakaryocytes are small or have single-lobed or multiple, discrete nuclei.

**Therapy-Related AML**

These occur as a consequence of cytotoxic drugs, radiation therapy, or both. One type follows alkylation agents or radiation therapy, most commonly about 5 years later. Myelodysplasia (MDS) usually occurs first, with evidence of bone marrow failure, to which many succumb without developing AML. About two-thirds of patients have MDS with multilineage dysplasia, and about 25% have refractory anemia with excess blasts. Whether during the MDS phase or after development into overt AML, these patients usually have dysplasia in all hematopoietic cell lines.

A second type of treatment-associated AML occurs after therapy with topoisomerase II inhibitors, such as etoposide and doxorubicin. The average interval between the cytotoxic treatment and the occurrence of AML is about 33 months, usually without an intervening MDS phase. Most cases are acute myelomonocytic or monoblastic leukemias.

**AML Not Otherwise Categorized**

The classification depends on the morphologic and cytochemical characteristics of the blasts and their degree of differentiation and maturation.

**AML, minimally differentiated**, constitutes about 5% of AML and occurs mainly in adults. By morphology and light microscopic cytochemistry, the blasts show no myeloid differentiation. They are medium-sized, have an agranular basophilic cytoplasm, round or slightly indented nuclei with one or two nucleoli, and dispersed chromatin. On cytochemical studies fewer than 3% of the blasts react to Sudan black, α-naphthyl acetate, or stains that detect MPO.

**AML, without maturation** is responsible for about 10% of cases of AML, usually in adults. Azurophilic granules and Auer rods in the cytoplasm of the blasts may suggest their myeloid nature; in other cases, the blasts resemble lymphoblasts, from which they are differentiated by positivity to MPO stains or Sudan black in at least 3% of blast cells.

**AML with maturation** constitutes about 30% to 45% of cases of AML and may occur in all ages. Blasts may show azurophilic granules and Auer rods, and evidence of maturation is present with >10% of the marrow cells being promyelocytes, myelocytes, and mature neutrophils and <20% being monocytes. The neutrophils may show abnormally increased or decreased segmentation and lobulation. Basophils, eosinophils, and mast cells may be increased.

**Acute myelomonocytic leukemia** accounts for about 15% to 25% of AML, usually in the elderly and sometimes in patients who have had preceding chronic myelomonocytic
leukemia. Both neutrophilic and monocytic cells and their precursors are present, each constituting at least 20% of the marrow cells. Circulating monocytes may be numerous (≥5 × 10⁹/L). Monoblasts are large cells with round nuclei containing one or more prominent nucleoli and abundant basophilic cytoplasm, sometimes with fine azurophilic granules, vacuoles, and pseudopod formation. Promonocytes have a less basophilic and more granulated cytoplasm, containing occasional vacuoles and azurophilic granules. The nuclei are irregular and indented.

**Acute monoblastic and acute monocytic leukemia** each account for about 5% of AML, the former more common in children, the latter in adults. In both, at least 80% of the leukemic cells are in the monocytic line. In acute monoblastic leukemia, at least 80% of the monocytic cells are monoblasts; in acute monocytic leukemia, most of them are promonocytes.

**Acute erythroid leukemias** include two subtypes, erythroleukemia (erythroid/myeloid) and pure erythroid leukemia. The former constitutes about 5% of AML; the latter is very rare. In erythroleukemia, at least 50% of the nucleated cells in the bone marrow are erythroid and at least 20% of the nonerythroid cells are myeloblasts. The erythroid cells are dysplastic, containing multiple and megaloblastoid nuclei, the cytoplasm often possessing poorly delineated, coalescing vacuoles. The myeloblasts are similar to those in AML with and without maturation. Some cases of erythroleukemia evolve from a MDS. In *pure erythroid leukemia*, >80% of the marrow cells are erythroid. The erythroblasts have deeply basophilic, often agranular, cytoplasm that may contain poorly delineated vacuoles. The round nuclei have fine chromatin and one or more nucleoli.

**Acute megakaryoblastic leukemia** affecting all ages accounts for about 5% of AML. At least 50% of the blasts are from the megakaryocyte lineage. The megakaryoblasts are often pleomorphic and have a basophilic, often agranular, cytoplasm that may demonstrate pseudopod and bleb formation, indicating budding platelets. The nuclei have fine chromatin and one to three nucleoli. Dysplastic platelets may be visible in the blood, as may be circulating micromegakaryocytes and megakaryocyte fragments.

**Myeloid Sarcoma**
AML can also manifest in extramedullary tissues, with or without bone marrow involvement. Myeloid sarcoma can occur as an isolated case, or more often occur in patients with history of leukemia or other myeloid malignancies. Myeloid sarcoma can occur in the soft tissue, lymph nodes, or the periosteum. Similarly, leukemia cutis can occur in the skin, most commonly extremities, back trunk, and face. Extramedullary leukemia is diagnosed by obtaining biopsy of the affected site, and immunohistochemistry and flow cytometry are used to establish the diagnosis. Similar cytogenetic abnormalities associated with AML have been reported in these cases, and treatment is similar to that of AML, with systemic chemotherapy.

**Acute Leukemias of Ambiguous Lineage**
In less than 4% of cases of leukemia, diagnostic tests currently available have several drawbacks: (1) they cannot determine whether the blasts have a myeloid or lymphoid origin (*acute undifferentiated leukemia*); (2) they indicate two populations of cells, each having a distinct lineage from myeloid or T or B lymphocytes (*acute bilineal leukemia*); or (3) they indicate that the blasts individually have markers of two or three lines of myeloid, T lymphocytes, and B lymphocytes (*acute biphenotypic leukemia*). In acute undifferentiated leukemia, the blasts lack any distinguishing characteristics, whereas in the bilineal and biphenotypic forms, the leukemic cells may resemble lymphoblasts, myeloblasts, or monoblasts.
ACUTE LYMPHOBLASTIC LEUKEMIAS

The WHO classification divides these leukemias into two forms, depending on whether the precursor cell is a T or B lymphocyte. The distinction between leukemia and lymphoma in these cases depends on whether the disease presents with abnormal cells in the blood and bone marrow or whether the abnormal cells appear primarily in lymph nodes or extranodal sites outside the bone marrow. Arbitrarily, if the patient has a mass lesion and fewer than 25% lymphoblasts in the bone marrow, the designation is lymphoma.

B-Cell Lymphoblastic Leukemia/Lymphoma

About 75% of cases of this disease occur in children below the age of six. About 85% of ALL are B-cell lymphoblastic leukemias. Enlarged lymph nodes, liver, and spleen are common. The leukocyte count varies widely. Lymphoblasts are pleomorphic and vary from small to large, with nuclei containing either prominent or inconspicuous nucleoli, compact or dispersed chromatin. The blue or blue-gray cytoplasm is usually scant, but may be abundant. Coarse azurophilic granules may be present. Diagnosis is made using immunohistochemistry and flow cytometry. B-lymphoblasts are characterized by expression of B-cell antigens (CD19, CD79a, and occasionally CD20), in addition to markers of immaturity such as CD10, TdT, and CD34. A number of cytogenetic abnormalities can occur in B-ALL, including hyperdiploidy, hypodiploidy, t(9;22), and mixed-lineage leukemia-1 (MLL-1) gene rearrangement. Among the cytogenetic abnormalities seen in B-ALL, t(9;22)(q34;q11), also known as the Philadelphia chromosome, is particularly important to distinguish, as tyrosine kinase inhibitors that inhibit the resulting fusion protein BCR-ABL are an important component of treatment for Ph+ B-ALL. Similar to chronic myeloid leukemia, BCR-ABL transcript levels can be monitored by using quantitative RT-PCR in Ph+ B-ALL, and can be used to detect MRD after systemic treatment. In Ph- B-ALL cases, flow cytometry can be used to assess MRD in evaluating response to treatment stratify patients for risk of recurrence of leukemia after induction therapy.

T-Cell Lymphoblastic Leukemia/Lymphoma

This disorder accounts for about 15% of childhood ALL and about 25% of adult ALL. The leukocyte count is often markedly elevated, and a mediastinal mass is often present. In T-cell lymphoblastic lymphoma, there is minimal or no bone marrow involvement by the T lymphoblasts. The lymphoblast morphology resembles that of precursor B-cell ALL, with a wide variation in morphology. Similar to B-ALL, diagnosis is made using immunohistochemistry and flow cytometry. T lymphoblasts are usually TdT-positive, cytoplasmic CD3-positive, and variably express CD2, CD5, CD7, CD1a, CD99, and CD34. Neoplastic cells may be double-negative or double-positive for CD4 and CD8, or, less commonly, be either CD4- or CD8-positive. Similar to B-ALL, a variety of cytogenetic abnormalities and translocations involving transcription factors are found in T-ALL. In particular, an activating mutation in NOTCH1, a transmembrane receptor, is found in about 60% of T-ALL cases.

REFERENCES


Table 6.1

<table>
<thead>
<tr>
<th>WHO classification of acute myeloid leukemia</th>
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<tbody>
<tr>
<td><strong>Acute myeloid leukemia with recurrent genetic abnormalities</strong></td>
</tr>
<tr>
<td>• AML with t(8;21)(q22;q22)</td>
</tr>
<tr>
<td>• AML with abnormal bone marrow eosinophils inv(16)(p13q22) or t(16;16)(p13;q22)</td>
</tr>
<tr>
<td>• Acute promyelocytic leukemia—AML with t(15;17)(q22;q12)</td>
</tr>
<tr>
<td>• AML with 11q23 abnormalities</td>
</tr>
<tr>
<td><strong>Acute myeloid leukemia with multilineage dysplasia</strong></td>
</tr>
<tr>
<td>• Following a myelodysplastic syndrome or myelodysplastic/myeloproliferative disorder</td>
</tr>
<tr>
<td>• Without antecedent myelodysplastic syndrome</td>
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</table>
Acute myeloid leukemia and myelodysplastic syndromes, therapy-related

- Alkylating agent–related
- Topoisomerase type II inhibitor–related (some may be lymphoid)
- Other types

Acute myeloid leukemia, not otherwise categorized

- AML minimally differentiated
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocytic leukemia
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Table 6.2

Cytogenetic risk groups in adult AML

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Cytogenetic Abnormality</th>
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<tbody>
<tr>
<td>Favorable</td>
<td>t(8;21)(q22;q22); RUNX1-RUNX1T1; inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11</td>
</tr>
<tr>
<td></td>
<td>Mutated NPM1 without FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td>Intermediate I</td>
<td>Mutated NPM1 and FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td></td>
<td>Wild-type NPM1 and FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td></td>
<td>Wild-type NPM1 without FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td>Intermediate II</td>
<td>t(9;11)(p22;q23); MLLT3-MLL</td>
</tr>
<tr>
<td></td>
<td>Cytogenetic abnormalities not classified as favorable or adverse</td>
</tr>
<tr>
<td>Adverse</td>
<td>inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1</td>
</tr>
<tr>
<td></td>
<td>t(6;9)(p23;q34); DEK-NUP214</td>
</tr>
<tr>
<td></td>
<td>t(v;11)(v;q23); MLL rearranged –5 or del(5q); –7; abnl(17p); complex karyotype</td>
</tr>
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</table>


Table 6.3

Acute promyelocytic leukemia: chromosomal translocations and fusion products

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Frequency (%)</th>
<th>Molecular Fusion Product (X-RARα)</th>
<th>Function “X” Gene</th>
<th>Retinoid Sensitive</th>
<th>Chemotherapy Sensitive</th>
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<tbody>
<tr>
<td>(15;17)</td>
<td>95</td>
<td>PML-RARα</td>
<td>Transcriptional</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(11;17)</td>
<td>&lt;5</td>
<td>PLZF-RARα</td>
<td>Developmental/differentiation control</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(5;17)</td>
<td>&lt;1</td>
<td>NPM-RARα</td>
<td>Ribonucleoprotein maturation and transport</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(11;17)</td>
<td>&lt;1</td>
<td>NuMA-RARα</td>
<td>Structural role in mitosis, apoptosis, and interphase nuclear matrix</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>(17;17)</td>
<td>&lt;1</td>
<td>STAT 5b-RARα</td>
<td>Signal transduction, transcriptional factor</td>
<td>–</td>
<td>?</td>
</tr>
</tbody>
</table>

*, sensitive; –, not sensitive; ±, may be sensitive; NPM, nucleophosmin; NuMA, nuclear mitotic apparatus; PLZF, promyelocytic leukemia zinc finger; PML, promyelocytic leukemia; RARα, retinoic acid receptor-α; STAT 5b, signal transducer and activator of transcription 5b; “X,” RARα partner gene.
Figure 6.1. Clinical presentation of acute leukemia. Bone marrow replacement by leukemic blasts in acute leukemia can lead to decreased production of red blood cells, platelets, and leukocytes resulting in anemia, thrombocytopenia, and leukopenia. **A** and **B**: Conjunctiva and skin pallor in patients with leukemia who have severe anemia. (Courtesy of Dr. I. Quirt.) **C**: This patient presented with submandibular adenopathy from acute myelomonocytic leukemia.
Figure 6.2. Sweet syndrome (acute febrile neutrophilic dermatosis) and AML. Sweet syndrome is most often idiopathic, but 10% to 20% of cases are associated with various malignancies, including AML, myelodysplastic syndrome, lymphoproliferative disorders, and multiple myeloma. A–C: The lesions are usually tender, swollen red papules, or plaques that are most commonly distributed on the extremities, face, and neck. D: Patients with malignancy who have Sweet syndrome frequently have bullous and mucosal membrane lesions, whereas those with idiopathic Sweet syndrome do not. In addition to the skin findings, patients with Sweet syndrome usually also have fever and high erythrocyte sedimentation rates. (Courtesy of the Crookston Collection.)
Figure 6.3. Conditions that may precede and signal AML. A: Pyoderma gangrenosum. Approximately 10% of cases of pyoderma gangrenosum are associated with hematologic disorders, most commonly with AML, but also with myeloproliferative syndromes and multiple myeloma. Pyoderma gangrenosum can precede the diagnosis of leukemia or be part of the initial presentation. The lesions are painful, coalescing papules or pustules that can evolve to large ulcers. (Courtesy of Dr. I. Quirt.) B: Clubbing of fingers can herald the onset of various malignancies, including AML.
Figure 6.4. Leukemia cutis. Skin infiltration with leukemic blast cells, or leukemia cutis, most commonly occurs in monocytic forms of AML. Leukemia cutis can also develop in any acute or chronic leukemia, including the leukemic phase of lymphomas and hairy cell leukemia. As shown in these photographs from different cases of AML with leukemia cutis, this condition is highly variable in appearance and can include widespread papules, nodules, macules, palpable purpura, plaques, or ulcers. Pain, tenderness, and pruritus are uncommon. (Courtesy of the Crookston Collection.)
Figure 6.5. Extramedullary involvement by AML as gingival hyperplasia, seen in these three cases of untreated AML at presentation (C and D are two views of the same case), is most common in acute monocytic leukemias and usually resolves with effective leukemia chemotherapy. (Courtesy Drs. Galbraith and Quirt.)
Figure 6.6. Thrombocytopenic purpura in AML. Purpura can be classified as petechiae, ecchymoses, or hematomas depending on the size, shape, and depth of blood extravasation. Petechiae are superficial, pinpoint (<3 mm), red or purple, nonblanching macules that mostly occur in dependent areas (A) and if severe can become confluent (B). C: Ecchymoses (bruises) are larger, flat, extravasating lesions. When ecchymoses become palpable, they are termed hematomas. Hematomas contain larger amounts of extravasated blood.

Figure 6.7. AML and disseminated intravascular coagulation (DIC). Extensive purpura is present on the soles of a patient with acute promyelocytic leukemia and DIC. Fibrinolysis is now considered an important component of the bleeding tendency in acute promyelocytic leukemia. (Courtesy of Dr. I. Quirt.)
Figure 6.8. AML and DIC. Brain sections from a case of AML complicated by DIC shows multiple scattered tiny hemorrhages in white matter with some forming small hematomas. (Courtesy of Dr. J. Bilbao.)

Figure 6.9. Hyphema in AML. Thrombocytopenia has caused bleeding into the anterior chamber of the eye (hyphema).
Figure 6.10. Ocular manifestations of thrombocytopenia in AML. A: Subconjunctival hemorrhages in the right eye. B: A fundus photograph shows intraretinal hemorrhages in the left eye. C: A fundus image demonstrates a hyperemic disc with peripapillary intraretinal hemorrhages and pseudo-Roth spots. D: Fundus of right eye has a subhyaloid hemorrhage with fluid level. (Courtesy of Dr. F. Altomare.)

Figure 6.11. Pulmonary hemorrhage from AML-induced thrombocytopenia. An autopsy specimen from a case of diffuse pulmonary hemorrhage caused by thrombocytopenia secondary to AML shows a congested lung filled with blood.
Figure 6.12. AML with neutropenia. Extensive fungal infection of tongue (A) and perirectal abscess (B) in a case of AML with neutropenia. (Courtesy of Drs. A. Lutynski and I. Quirt.)
Figure 6.13. Fatal pulmonary infection in AML with neutropenia. **A:** Lung fixed in formalin from an autopsied case of AML presenting with neutropenia and extensive lobar pneumonia from aspergillosis. **B:** The liver from a different case of AML. Cut surface discloses multiple abscesses from aspergillosis. (Courtesy of Dr. I. Wanless.)
Figure 6.14. Demonstration of extreme leukocytosis in AML. A: A tube of centrifuged whole blood from a patient with leukocytosis (WBC = 310,000) due to acute leukemia is shown on the right, compared with normal (WBC = 5,000) on the left. The prominent buffy coat from the patient consists entirely of leukemic blasts (arrow). B: An autopsy liver specimen shows tiny nodules (arrows in inset) of leukemic blasts filling the hepatic vasculature in a case of AML with a high blast count.
**Figure 6.15.** Blast morphology in AML. The upper panel shows a pleomorphic population of cells that are aligned in the middle panel according to size and chromatin patterns. The bottom panel illustrates the distinguishing features of the two blasts most commonly seen in aspirate smears, the myeloblast and erythroblast.
Figure 6.16. Chromatin patterns. A: An aspirate smear in AML shows diverse chromatin patterns in different marrow cell populations. The blasts are characterized by “open” (uncondensed or lacy or immature) chromatin with prominent nucleoli. B: This schematic diagram categorizes cells from A, with, from left to right, “open” (lacy chromatin or immature) to increasingly “closed” (condensed or mature) patterns of chromatin.

Figure 6.17. Myeloperoxidase staining of blasts in AML. A: AML, minimally differentiated. By definition, this type of AML shows MPO staining in less than 3% of the total blast population. A mature granulocyte serves as positive control. B: MPO staining in AML, without maturation. Weak to moderate punctate staining is present in the cytoplasm of more
than 3% of the blasts (a myelocyte serves as a positive control). C: MPO staining and AML with maturation. Strong cytoplasmic staining is present in blasts and abnormal maturing granulocytes. An Auer rod staining strongly with MPO is present.

**AML CLASSIFICATION, DEFINITION OF THE “BLAST”**

**Morphology: histochemistry and CD45 vs side scatter**

Figure 6.18. Patterns of cytoplasmic granulation in various types of AML. Cytoplasmic granulation as seen in standard Wright–Giemsa staining (left column), MPO cytochemistry (middle column), and side scatter by flow cytometry (right column) correlates with the degree of blast differentiation in various types of AML. AML, minimally differentiated, is shown with the typical immature, agranular, MPO-negative blasts with low side scatter.
compared with the more mature cells of acute promyelocytic leukemia with the classic hypergranular cytoplasm (with multiple Auer rods here), strong MPO staining, and the characteristic "comet-shaped" diffusely spread pattern characteristic of blasts with high side scatter.

**AML CLASSIFICATION, DEFINITION OF THE “BLAST”**

*Cytoplasmic features: histochemistry and CD45 vs side scatter*

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**ACUTE MYELOMONOCYCTIC LEUKEMIA (M4)**

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**ACUTE MONOCYTIC LEUKEMIA (M5)**

---

**ACUTE ERYTHROID LEUKEMIA (M6)**

---

**ACUTE MEGAKARYOCYTIC LEUKEMIA (M7)**

---

**Figure 6.19.** Morphology of cytoplasm in various types of AML. Cytoplasmic features are shown with standard Wright–Giemsa staining (left column), cytochemistry (middle column), and CD45 versus side scatter by flow cytometry (right column) in various types of AML. Demonstrated here are acute myelomonocytic leukemia with two distinct blast populations, acute monocytic leukemia with ample esterase-positive cytoplasm, acute
erythroid leukemia (pure erythroleukemia type shown here) with coarse PAS-positive cytoplasmic granules, CD45 weak/negative blasts, and acute megakaryocytic leukemia with CD61+ blasts that are weakly CD45-positive. (Histograms courtesy J. Davidson and T. Anderson.)

**AML CLASSIFICATION, DEFINITION OF THE “BLAST”**

Cytoplasmic features: histochemistry and CD45 vs side scatter

**ACUTE MYELOMONOCYTIC LEUKEMIA (M4)**

**ACUTE MONOCYTIC LEUKEMIA (M5)**

**ACUTE ERYTHROID LEUKEMIA (M6)**

**ACUTE MEGAKARYOCYTIC LEUKEMIA (M7)**

*Figure 6.20.* Morphology and histochemical staining characteristics used to differentiate lymphoid, myeloid, and monocytoid leukemic blasts. MPO, myeloperoxidase; SBB, Sudan black B; PAS, periodic acid–Schiff; ANAE, α-naphthyl acetate esterase; ANAE/NaF, ANAE with fluoride inhibition (butyrate substrates now more commonly in use); CAE,
Figure 6.21. AML with maturation with t(8;21) translocation. Aspirates show a spectrum of differentiated myeloid precursors ranging from blasts to mature segmented bands and neutrophils.
Figure 6.22. AML with t(8;21) translocation. A: Aberrant expression of the B-cell marker CD19 by myeloid blasts is commonly seen in AML with t(8;21). B: Aspirate smear shows granulated blasts mixed with abnormal mature myeloid forms. A long slender, tapered Auer rod is present in the cytoplasm of a partially degenerated blast in the upper right area of the slide. C: Auer rod morphology is shown here from several cases of AML with t(8;21). The blasts in this type of AML often appear more differentiated and may contain long, tapering Auer rods.
Figure 6.23. A: The t(8;21)(q22;q22) gene rearrangement. The ideograms of chromosomes 8, 21 and the respective derivative chromosomes are depicted below in color, and the corresponding G-banded chromosome pairs are shown above. The arrows indicate the breakpoints on the respective chromosomes. Green, red, and yellow colors indicate expected FISH signals using the LSI AML1 and LSI ETO probe as shown in the lower panel. B: FISH of the AML1/ETO fusion gene. The AML1 gene at 8q22 (green) and the ETO gene at 21q22 (red) co-localize to generate yellow fusion signals (arrows), indicating a reciprocal translocation involving these loci.
**Figure 6.24.** Minimal residual disease assessment in AML with t(8;21). Quantitative PCR for AML-ETO mRNA is shown here for a period of 3 years following diagnosis. (Courtesy of Dr. S. Kamel-Reid.)
Figure 6.25. AML with t(8;21) translocation and pseudo–Chédiak–Higashi inclusions. A: Aspirate smear shows numerous granulated blasts and differentiated myeloid precursors. Many of the blasts contain pseudo–Chédiak–Higashi inclusions (arrows). B: The t(8;21) leukemia cells include two blasts, each containing long thin Auer rods and, in the lower right corner, a myelocyte. C: Three blasts are shown here, one with an Auer rod. D: Myeloperoxidase stains show heavy block-like staining of the Auer rods (arrows) in this unusual case of AML with t(8;21). Pseudo–Chédiak–Higashi inclusions also occur in other AML subtypes.
Figure 6.26. AML with inv(16)  

A: Aspirate smear with a dual population of small and large blasts with prominent eosinophilia.  

B: Abnormal eosinophils with large basophilic granules are typically associated with inv(16).  

C: Biopsy shows hypercellular marrow with monocytic blasts and eosinophilia. Note the large monocytic blasts with folded nuclear contours.
Figure 6.27. The inv(16)(p13.1q22) rearrangement. A: The ideograms of the normal and inverted chromosome 16 are to the left in color and the corresponding G-banded pair is to the right. The arrows on the normal chromosome 16 ideograms indicate the breakpoints on the short and long arms of chromosome 16. B: Metaphase FISH using a dual color CBFB (16q22) probe set. The CBFB gene is labeled with a red fluorochrome at the 5’ end and with a green fluorochrome at the 3’ end. When an inv(16)(p13.1q22) is present, the red and green signals split apart such that the red signal is now on the other arm. A yellow signal indicates the normal chromosome 16. C: Acute myelomonocytic leukemia with eosinophilia and inv16. An aspirate smear reveals a dimorphic blast population made up of large monocytoid and small myeloblast cells with eosinophilia.

Figure 6.28. AML with inv(16). A: Aspirate smear shows granular monocytoid blasts with dysplastic eosinophilic/basophilic precursors. B: Bone marrow biopsy displays a hypercellular marrow composed of sheets of uniformly spaced monocytoid blasts with eosinophilia. C: High-power view of the biopsy reveals uniformly spaced monocytoid blasts with characteristic folded nuclear contours associated with eosinophilia.
Figure 6.29. **A–C:** AML with inv(16). Aspirate smears from three cases of AML with inv(16) showing monocytic blasts mixed with dysplastic eosinophilic/basophilic precursors (“eosinobasophils”). The latter show the characteristic dual-staining cytoplasmic granules commonly seen in AML with inv(16). **D:** Examples of the dysplastic eosinophilic and basophilic precursors seen in several different cases of AML with inv(16).
Figure 6.30. AML with inv(16). A: A pleomorphic blast population composed of large cells with lower nuclear:cytoplasmic (N:C) ratios, compared to smaller blasts with high N:C ratios is present in this aspirate smear. Abnormal eosinophil precursors exhibiting the classical dual-staining cytoplasmic granules are prominent. B: Butyrate esterase stains show that approximately one-half of the blast population, particularly the larger blasts, display positive staining. C: Myeloperoxidase stains demonstrate strong “block-like” cytoplasmic staining in approximately one-half of the blast population, including the abnormal eosinophils.
AML with t(15;17)(q22;q12) or acute promyelocytic leukemia (APL)

Figure 6.31. Acute promyelocytic leukemia. A: CD45 versus side scatter plot shows the classic comet-shaped pattern from granulated blasts. B: Blood smear displays pancytopenia, low platelets, and one abnormal bilobed promyelocyte. A single Auer rod appears in the two o’clock position of this APL cell. C: Aspirate smear shows numerous abnormal promyelocytes, many containing fused primary granules in the form of either classical Auer rods (long arrow) or other bizarre forms (short arrow). D and E: Fluorescent in situ hybridization (FISH) of the PML/RARA fusion gene. The PML gene at 15q22 (red) and RARA gene at 17q12~21 (green) co-localize to generate yellow signals, indicating a reciprocal translocation involving these loci. F: A biopsy demonstrates replacement of the marrow by uniformly spaced, large, immature cells with folded nuclei and ample cytoplasm.
Figure 6.32. Minimal residual disease assessment in AML with t(15;17) translocation. Quantitative PCR for PML-RAR-α and mRNA is shown here for a period of 15 months following diagnosis. (Courtesy of Dr. S. Kamel-Reid.)

Figure 6.33. Minimal residual disease and response to treatment and in AML with t(15;17) translocation. Quantitative PCR for PML-RAR-α mRNA is shown here for a period of 21 months following diagnosis and shows cycles of treatment response and relapse. (Courtesy of Dr. S. Kamel-Reid.)
Figure 6.34. Acute promyelocytic leukemia, microgranular variant. **A:** Peripheral smear shows leukocytosis and severe thrombocytopenia with increased numbers of early myeloid cells displaying the characteristic bilobed nuclei of microgranular APL cells. **B:** Closer inspection of the APL cells demonstrates the characteristic bilobed or “pinched” nuclei and very fine, dust-like cytoplasmic granules. **C:** High-power view of a bone marrow biopsy reveals a hypercellular bone marrow with uniformly spaced, large immature hematopoietic cells with abundant eosinophilic-staining cytoplasm and bilobed nuclei.
Figure 6.35. Acute promyelocytic leukemia with disseminated intravascular coagulation. A: Peripheral blood smear from a case of APL shows a leukocytosis with thrombocytopenia and schistocytes. B: Aspirate smears demonstrate increased numbers of abnormal promyelocytes with bizarre cytoplasmic granulation including typical Auer rods (shown on the left), large coarse granules (shown in the right central area), and fine, dust-like eosinophilic granules.
Figure 6.36. Auer rod morphology in acute promyelocytic leukemia. Picture shows variations on the appearance of promyelocytes in several different cases of APL.
Acute promyelocytic leukemia, microgranular variant

Figure 6.37. Acute promyelocytic leukemia, microgranular variant. A: Peripheral blood smear from a case of microgranular variant APL demonstrates leukocytosis and thrombocytopenia. The characteristic abnormal bilobed promyelocytes are present. B: Close examination of the abnormal promyelocytes in this case reveals fine, dust-like eosinophilic granules within the cytoplasm. Auer rods are difficult to find in many of these cases. C: A bone marrow biopsy shows replacement of bone marrow by monotonous sheets of uniformly spaced bilobed immature hematopoietic precursors.
Figure 6.38. Acute promyelocytic leukemia, microgranular variant. Peripheral blood smear from a case of the microgranular variant of APL discloses leukocytosis and thrombocytopenia with increased numbers of abnormal seemingly agranular, bilobed promyelocytes.
Figure 6.39. Acute promyelocytic leukemia, microgranular variant. A: Blood smear shows increased numbers of abnormal bilobed promyelocytes with hypogranular cytoplasm and thrombocytopenia. These large immature hematopoietic cells with folded nuclear contours can be confused with monocytic blasts. B: Bone marrow biopsy demonstrates a monotonous proliferation of uniformly spaced large cells with characteristic bilobed (buttock-like) nuclei and ample cytoplasm.

Figure 6.40. Acute promyelocytic leukemia in response to therapy. A: Core biopsy at
diagnosis of acute promyelocytic leukemia with sheets of promyelocytes and absence of myeloid maturation. Normal trilineage hematopoiesis is markedly decreased. B: Core biopsy 20 days after the diagnosis following treatment with all-trans retinoic acid (ATRA). Numerous mature neutrophils are seen. Occasional erythroid precursors and megakaryocytes are also present. Neutrophils show persistence of PML-RARA gene rearrangement, consistent with tumor cell differentiation.
Acute promyelocytic leukemia

A

B

C

MPO
Figure 6.41. Acute promyelocytic leukemia with bone marrow fibrosis. A: Hypocellular aspirate smear demonstrates rare, abnormal, heavily granulated promyelocytes. B: Bone marrow biopsy shows architectural distortion suggesting significant marrow fibrosis and replacement by a monotonous immature hematopoietic cell population. Higher magnification of the biopsy (inset) discloses monotonous foci composed of uniformly spaced immature, bilobed hematopoietic precursors. C: Anti-MPO immunostaining reveals the infiltration of the bone marrow space is caused by MPO-positive immature hematopoietic cells with strong granular staining (inset).

Figure 6.42. AML with 11q23 abnormalities. A and B: FISH of a MLL rearrangement. The MLL gene at 11q23 is labeled with a green fluorochrome at the telomeric (tel) end and with a red fluorochrome at the centromeric (cen) end. A: A yellow fusion signal indicates an intact MLL gene. B: Translocations involving the MLL (HRX) gene split the red and green signals apart. C: The t(9;11)(p22;q23) rearrangement. The ideograms of chromosomes 9 and 11 and the respective derivative chromosomes are to the left in color, and the corresponding G-banded chromosome pairs are to the right. The arrows indicate the breakpoints on the respective chromosomes. D: Acute monocytic leukemia with t(9;11) translocation. Well-differentiated monocytic blasts predominate in this aspirate smear.
Figure 6.43. AML with cup-like nuclear morphology. In acute myeloid leukemia, this distinctive nuclear morphology, also referred to as “thumbprinting,” has been associated with FLT3 ITD mutation, NPM1 mutation, or both. Testing for these mutations is recommended in cases of AML with cup-like morphology.
AML with multilineage dysplasia

A

B

C
Figure 6.44. AML with multilineage dysplasia. A: Aspirate smear with increased blasts and a dysplastic mature erythroid precursor displays irregular nuclear contours (arrow). B: Aspirate smear shows several giant hypogranular bands (arrows) and a dysplastic erythroid precursor with asymmetric binucleation is situated just below the centrally located hypogranular band. C: Aspirate smear reveals increased blasts and two dysplastic micromegakaryocytes (arrows).

Figure 6.45. AML, minimally differentiated. A: Flow cytometric scattergram shows blasts with low side scatter. B: Low-power magnification of aspirate smear demonstrates hypercellular marrow fragments consisting exclusively of monotonous sheets of primitive
mononuclear cells. **C:** High-power view of aspirate smear discloses nucleolated undifferentiated blasts. **D:** Less than 3% of the blasts stain positively for MPO. A late myeloid precursor serves as a positive internal control (arrow).
AML, without maturation (FAB: M1)
Figure 6.46. AML, without maturation. A: Flow cytometry scattergram shows a discrete population of CD45+ blasts displaying weak side scatter signal consistent with low cytoplasmic granularity. B: Aspirate smear demonstrates immature myeloid precursors, some of which show the presence of numerous primary granules in the cytoplasm. C: MPO stains of aspirate smear show a minor subpopulation of positive-staining blasts with punctate cytoplasmic staining (arrows). Most of the blasts are very pale as they were not counterstained and are MPO negative.

AML with maturation (FAB: M2)

Figure 6.47. AML, with maturation. A: Blood smear displays a spectrum mainly immature and rare mature myeloid precursors. B: MPO stains reveal strong granular cytoplasmic staining in many leukemic blasts. Two MPO-positive Auer rods are present (arrow).
Figure 6.48. AML with maturation. **A:** Aspirate smear shows a leukemic blast population composed mostly of promyelocytes that could be confused with a case of acute promyelocytic leukemia. However, the latter often shows bilobed nuclei and a more homogeneous blast population compared with cases of AML with differentiation. **B:** Myeloperoxidase stains show strong diffuse staining that obscures nuclear morphology. Molecular testing for t(15:17) is the key to the correct diagnosis. **C:** A hypercellular clot section consists almost exclusively of large blasts with “open” chromatin pattern (vesicular nuclei), two to three small nucleoli, and indented nuclear contours.
Figure 6.49. Acute myelomonocytic leukemia. A: Flow cytometric scattergram shows dual population of blasts with the monocytic blasts displaying more side scatter and stronger CD45 staining than do the myeloblasts. B: An aspirate smear demonstrates the same dimorphic blast population composed of monocytes and myeloblasts (open and closed arrows, respectively).
Figure 6.50. Acute myelomonocytic leukemia. A: Blood smear shows a distinctive dimorphic blast population consisting of immature nucleolated myeloblasts and mature monocytes. Fragments of cytoplasm also are present. B: Aspirate smear stained with butyrate esterase displays positive staining in only the larger sized monocytic blast population. C: The immunostained bone marrow biopsy shows clusters of CD34-positive myeloid blasts interspersed among negative-staining monocytic blasts. Note that the endothelial cells lining the vessel on the left side of the slide act as CD34-positive internal controls. D: Immunohistochemistry for monocytic marker CD68 shows positive staining in the monocytic blast subpopulation, corresponding to approximately 50% of all leukemia cells.
Acute myelomonocytic leukemia, (FAB: M4)

**Figure 6.51.** Acute myelomonocytic leukemia. **A:** Blood smear shows a dual population of small and large blasts, myeloblasts and monoblasts, respectively. On the left side are three mature monocytic blasts characterized by large size, low nuclear-to-cytoplasmic (N:C) ratios with somewhat condensed chromatin, absent nucleoli, irregularly folded nuclear contours, and vacuolated, finely granular, neutral-staining cytoplasm. The right upper corner of the slide contains three myeloblasts characterized by medium-size, higher N:C ratios, open chromatin, and scant cytoplasm. A small mature lymphocyte with closed chromatin is present in the right lower corner. **B:** Myeloperoxidase staining demonstrates strong granular cytoplasmic staining in a subpopulation of blasts. **C:** Butyrate esterase stains display strong positive staining in one blast. A neutrophil near the left upper corner serves as a built-in negative control.
Figure 6.52. Blast morphology in acute monocytic leukemia. This composite photograph including 16 different cases of acute monoblastic and monocytic leukemias illustrates the wide variation in blast morphology seen in this type of AML. Undifferentiated monoblasts with high N:C ratios, open chromatin, nucleoli, and basophilic-staining cytoplasm are present in the upper panels, promonocytes in the middle panels, and differentiated monocytic blasts with closed chromatin pattern and lobulated nuclei are shown in the lower panels.
Figure 6.53. Acute monoblastic/monocytic leukemia. A: A flow cytometric scattergram illustrates the moderate CD45 expression and intermediate side scatter properties typical for monocytic blasts. B: Bone marrow aspirate features promonocytes characterized by large size with low nuclear-to-cytoplasmic (N:C) ratios, nuclei with moderately open chromatin, folded nuclear contours (some kidney bean-shaped), absent nucleoli, and agranular, slightly basophilic-staining cytoplasm. For comparison, an arrow points to a small mature lymphocyte with higher N:C ratio and condensed chromatin. (Courtesy of T. Anderson.)
Figure 6.54. Acute monoblastic leukemia (esterase-negative). A: This peripheral smear shows leukocytosis consisting of large undifferentiated blasts with low to moderate N:C ratios and basophilic-staining cytoplasm. B: An aspirate smear shows clusters of these immature cells. By flow cytometric evaluation, these blasts displayed CD45 versus side scatter properties characteristic of monocytes and were CD11C+/CD4+. C: Butyrate esterase stains are negative in this particular case of acute monoblastic leukemia. A benign histiocyte located near the bottom of the slide serves as internal positive control.
Figure 6.55. Acute monoblastic leukemia. A: A bone marrow aspirate smear from acute monoblastic leukemia displays cytoplasmic granularity that is focally localized to the Golgi area (arrows). B: Butyrate esterase stains show localized positivity restricted to the perinuclear granules, confirming the monocytic origin for this case of acute monoblastic leukemia with heavily granulated blasts.
Figure 6.56. Acute monoblastic leukemia with marrow fibrosis. A and B: Bone marrow biopsy at low and high power, respectively. A: Increased cellularity and architectural distortion, such as swirling and lining up of individual marrow cells (so-called Indian filing), suggest the presence of significant bone marrow fibrosis. B: Large, monotonous-appearing, wide-spaced, immature hematopoietic cells that replace the entire marrow cavity. C and D: In this case, CD68 stains only the benign reactive histiocytes interspersed among the negative-staining leukemic monoblasts. E and F: Immunohistochemistry for lysozyme stains with equal intensity in both the benign histiocytes and leukemic monoblasts.
Acute monocytic leukemia, (FAB: M5a)

**Figure 6.57.** A: Acute monoblastic leukemia. Nucleolated monoblasts are present in this peripheral blood smear. B: A bone marrow aspirate smear shows monotonous sheets of monoblasts displaying open chromatin with multiple prominent nucleoli and vacuolated, basophilic-staining cytoplasm. (Compare with Burkitt lymphoma in Chapter 9.) C: Strong, diffuse, cytoplasmic staining by butyrate esterase confirms the monocytic origin of these leukemic blasts. An erythroid cluster on the left side of the slide serves as a negative control.
Figure 6.58. Acute monoblastic leukemia. A: Monoblasts surround a large, benign histiocyte with long tentacles of cytoplasmic projections packed with dark-staining pigment (likely iron) in this case of acute monoblastic leukemia. B: In the same case, the two lower cells are monocytic leukemic blasts and the larger cell in the upper area is a benign histiocyte. Histiocytes, usually “innocent” benign bystanders in marrow diseases, are characterized by oval-shaped nuclei, smooth nuclear contours, “bland” chromatin patterns, and ample neutral-staining cytoplasm. Often, histiocytes are stuffed with iron pigment and/or digested cellular debris. C: Positive butyrate esterase staining is seen in the numerous leukemic monoblasts (on the right) and a benign histiocyte (long arrow). An erythroid precursor serves as a negative control (short arrow).
Figure 6.59. A: Acute monoblastic leukemia, esterase negative. A peripheral blood smear displays two circulating promonocytes and a small mature lymphocyte. The two promonocytes are characterized by large size, low N:C ratios, distinctly lobulated nuclear contours, and ample “agranular” neutral-staining cytoplasm. B: A high-power view of a bone marrow aspirate smear shows monoblasts with bilobed nuclei reminiscent of the microgranular variant of acute promyelocytic leukemia. C: Butyrate esterase histochemistry demonstrates negative staining in the promonocytes (a benign histiocyte serves as positive control).

Rare types of AML: Acute monocytic leukemia with t(8;16)
Figure 6.60. Acute monoblastic leukemia with t(8;16) translocation. An aspirate smear shows a pleomorphic population of immature monocytic precursors. Although this translocation has been associated with prominent hemophagocytosis, in this particular case only a rare blast was seen engulfing RBCs as shown on the right.

Figure 6.61. Acute monocytic leukemia. A: High-magnification view of a blood smear shows four large mature monocytes that have low N:C ratios, lobulated nuclei with moderately closed chromatin pattern and neutral staining, and finely granular, vacuolated cytoplasm. B: Aspirate smear shows clusters of monocytic blasts that have a more immature appearance than do the circulating blasts.
Figure 6.62. Acute monocytic leukemia. A and B: Blood smears show peripheral monocytosis consisting of mature monocytes that have a closed chromatin pattern, lobulated nuclei, and abundant granular, vacuolated cytoplasm. C: Bone marrow biopsy demonstrates hypercellular marrow consisting almost exclusively of monocytoid precursors.
Figure 6.63. Acute erythroid leukemia, pure erythroid type. A: Bone marrow aspirate smear consists entirely of a heterogeneous mixture of dysplastic erythroid blasts, many displaying cytoplasmic vacuolation. B: PAS stains show the characteristic coarse granular red staining in immature leukemic erythroid precursors, compared with a negative-staining mature erythroid precursor shown in the left upper corner.
Acute erythroid leukemia (erythroid/myeloid type): the "non-erythroid blast count"

**Figure 6.64.** Acute erythroid leukemia, erythroid/myeloid type. **A:** Aspirate smear shows a mixture of dysplastic erythroid precursors and immature myeloid cells, including numerous blasts. **B:** All the erythroid cells from the field in **A** were digitally removed, clearly demonstrating a nonerythroid blast count exceeding 20% (blasts shown by arrows).
Figure 6.65. Acute erythroid leukemia, erythroid/myeloid type. A: Aspirate smear shows erythroid hyperplasia, increased numbers of blasts, and hemophagocytosis. In the center of the figure, a benign-appearing histiocyte engulfs cellular debris. Hemophagocytosis can occur in both benign and malignant bone marrow disorders, including acute erythroid leukemia. B: When all the erythroid cells are digitally removed from A, it is apparent that the nonerythroid blast count exceeds 20%.
Figure 6.66. Acute erythroid leukemia, pure erythroid type. **A:** Blood smear shows a leukoerythroblastic picture with marked poikilocytosis and a nucleated RBC. **B:** Bone marrow aspirate smear reveals only scattered megaloblastic erythroid precursors. **C:** Biopsy at medium magnification shows a hypercellular marrow comprising a heterogeneous mononuclear cell population. **D:** Biopsy at higher magnification shows replacement of the bone marrow cavity by a mixed erythroid population. **E:** Immunohistochemistry for the erythroid lineage marker glycophorin C demonstrates positive staining in the leukemic cells, and negative-staining megakaryocytes serve as controls.
Figure 6.67. Acute erythroid leukemia (pure erythroleukemia type). A: Bone marrow aspirate smear shows erythroid hyperplasia and numerous dysplastic erythroid blasts with vacuolated cytoplasm. B: Biopsy demonstrates increased numbers of erythroid precursors, which, especially in biopsies, can be confused morphologically with plasma cells (a plasma cell is shown by an arrow in the right upper corner). C: PAS stains of aspirate smear demonstrate granular and diffuse staining in immature and mature erythroid precursors, respectively.
Figure 6.68. A and B: Acute erythroid leukemia versus reactive erythroid hyperplasia. Bone marrow aspirate smears show increased numbers of erythroid blasts associated with dysplastic mature erythroid forms displaying irregular nuclear contours. Teardrop RBCs related to the bone marrow fibrosis also are visible. C: Biopsy shows erythroid hyperplasia with nests consisting exclusively of monotonous immature erythroid forms. D: PAS-stained aspirate shows the typical coarse granular pattern of staining in immature erythroid precursors (long arrows) compared with the diffuse staining seen in the more mature forms (short arrows).
Figure 6.69. Acute megakaryoblastic leukemia. A: Aspirate shows a blast that is positive for the megakaryocytic marker CD61 (on the left) with a negative-staining lymphocyte (on the right). B–D: Biopsy shows fibrotic, hypercellular marrow composed entirely of atypical megakaryocytes. In C, CD61 is positive only in the larger, more differentiated blasts. Fibrosis often precludes flow cytometry, and often the diagnosis of this disease rests solely on immunohistochemically stained biopsies.
Figure 6.70. Acute megakaryoblastic leukemia. **A:** Aspirate smear shows four blasts with multiple, broad-based cytoplasmic projections reminiscent of platelet production by megakaryocytes. **B:** Cohesive clustering of malignant megakaryocyte precursor cells in bone marrow aspirate. **C** and **D:** Bone marrow biopsies show hypercellularity with many giant cells present. **E:** Hematopoietic cells are replaced by large immature cells, some displaying megakaryocytic features.
Figure 6.71. Acute megakaryoblastic leukemia. A: Aspirate smear shows blasts with cytoplasmic blebbing reminiscent of platelet production by megakaryocytes. B: Bone marrow biopsy demonstrates increased blasts, some of which display megakaryocytic differentiation. C: Reticulin stains of a biopsy at low magnification display a diffuse increase in the number of fibers throughout the bone marrow space. D: Higher magnification of the bone marrow biopsy stained for reticulin shows thickened fibers that encircle individual bone marrow cells.
Figure 6.72. Acute basophilic leukemia. A: Blood smear shows circulating immature and dysplastic basophilic precursors. B: Bone marrow aspirate smear demonstrates cohesive clumps of cells consisting exclusively of abnormal basophil precursors. C: Marrow biopsy reveals a hypercellular bone marrow replaced by monotonous-appearing small cells with abundant “agranular” cytoplasm (representing basophils degranulated during tissue processing).
Figure 6.73. Acute myeloid leukemia; flow cytometry detection of minimal residual disease. 
A: A minute population of neoplastic myeloid blasts (in black; arrow) is seen in the background of normal regenerating myeloid blasts (in green). Tumor cells represent 0.03% of all cells and express bright CD34, dim CD117, and aberrant CD7. B: One month later, the neoplastic population is more prominent at 1% of analyzed cells (arrow).

Figure 6.74. Acute myeloid leukemia; flow cytometry detection of minimal residual disease. A small subset of the CD34-positive, CD117-positive myeloid blasts shows aberrant expression of TdT (0.15% of analyzed cells; arrow).
Chloromas (granulocytic sarcomas): CD45 negative acute monocytic leukemia

Figure 6.75. Myeloid sarcoma. A: Low magnification of a myeloid sarcoma arising in the chest wall of a patient suffering from acute monocytic leukemia. Tumor cells infiltrate the adjacent fat. B: The malignant behavior of this lesion is shown by the invasive growth pattern consisting of finger-like projections of tumor surrounding fat globules. C: Immunohistochemistry for CD45 shows negative staining in this tumor derived from leukemic monocytes. Two lymphocytes displaying strong CD45 staining are present on the left side of the slide and serve as positive controls. D: Anti-CD68 shows strong cytoplasmic staining in the monocytic leukemia cells.
Figure 6.76. Acute myeloid leukemia in cerebrospinal fluid (CSF). Hematopoietic blasts are seen in this CSF specimen contaminated with blood. The blasts have the typical open chromatin pattern seen in immature cells, unlike the closed pattern of small mature lymphocytes (arrows).
Figure 6.77. Leukemia cutis. A: Nests of large leukemic cells infiltrate the dermis in this skin biopsy from a patient with acute myeloid leukemia. B: Leukemic infiltration extends into the subcutaneous fat. C: Anti-MPO immunostains show positive-staining leukemia cells invading subcutaneous fat and a blood vessel.
Figure 6.78. Myeloid sarcoma in appendix. AML presenting as acute appendicitis with neutropenia and systemic aspergillosis. **A:** Low-power view of the appendix shows transmural involvement by leukemic cells. **B:** High-power view of the appendix wall reveals infiltration by large leukemic cells with some residual small mature lymphocytes shown on the upper left side. **C** and **D:** Low- and high-power views, respectively, illustrating CD117-positive leukemic cells infiltrating the entire intestinal wall. In **D,** negative-staining residual lymphocytes are present in the upper left side of the slide. **E** and **F:** PAS stains show angioinvasive aspergillus.
Figure 6.79. Myeloid sarcomas from multiple patients involving lymph nodes. A: Resected lymph node reveals almost total effacement by a diffuse proliferation of large pale-staining cells. Residual nodules of lymphocytes are present (arrows). B: Immunostaining for CD45 is positive in residual lymphoid aggregates (arrows), whereas most of the infiltrating leukemic cells are negative. C: Immunostaining for the myeloid marker CD15 shows positive-staining leukemic cells surrounding a negative-staining nodule of residual lymphocytes (arrow). D and E: Low- and medium-magnification views, respectively, of a surgically resected lymph node show replacement of nodal tissue by pale-staining leukemic cells. Residual lymphocytes are present on the right side of both figures. F: Immunostaining for CD117 demonstrates large positive-staining leukemic cells and negative-staining residual small lymphocytes. G: Anti-MPO stains show positive staining in the large leukemic cells, with negative-staining lymphocytes on the right.

Chloromas (granulocytic sarcomas)

Figure 6.80. Myeloid sarcoma with monocytic differentiation involving lymph node. A and B: Low- and high-power photographs of a fine-needle biopsy of a lymph node reveal
effacement of architecture by sheets of large, uniformly spaced, monotonous, leukemic cells. 

C: Residual small, mature lymphocytes are present near the right lower corner. Immunostaining for CD117 (C and D) and lysozyme (E and F) shows positive leukemic cells surrounding negative-staining residual lymphocytes.

**Figure 6.81.** Small cell carcinoma of lung. A: Medium-power view shows a cohesive nest of carcinoma cells with scalloped borders and nuclear molding present in the aspirate. B: High-power view of the aspirate shows very large pleomorphic malignant cells. An arrow delineates a paranuclear blue inclusion. C: Necrotic metastatic tumor composed of “ghost” cells appears in the biopsy specimen.
Acute leukemia mimics: Neuroblastoma
Figure 6.82. Neuroblastoma in bone marrow. A: An aspirate smear demonstrates an esthesioneuroblastoma in the bone marrow with blast-like cells possessing fine cytoplasmic projections. B: Loose aggregates of malignant primitive cells. C: A clump of malignant cells displays nuclear molding. D and E: Low- and high-power views, respectively, of biopsy show bone marrow replacement by small, round, blue cells separated by fibrovascular bundles.

Figure 6.83. Rhabdomyosarcoma. A: Hypocellular aspirate smear from a case of undifferentiated rhabdomyosarcoma presenting in the bone marrow discloses blast-like cells. B: Pretreatment biopsy demonstrates marrow replacement by an undifferentiated small round blue cell tumor. C: Immunostaining of the pretreatment biopsy for the skeletal muscle marker sarcomeric actin shows strong staining in many of the tumor cells. D and E: Posttreatment biopsy reveals differentiation into sarcomeric actin positive tube-like structures.
Figure 6.84. Ewing sarcoma of the bone marrow. **A:** A touch prep shows marrow replacement by undifferentiated, blast-like primitive cells. **B** and **C:** Biopsy displays infiltration of marrow by loose nests of malignant cells separated by fibrovascular bundles. **D:** Immunostaining for CD99 reveals strong cytoplasmic staining.
Figure 6.85. Blast morphology in ALL. In the left column of this composite figure are three examples from different blood smears illustrating the three morphologic variants of blasts: L1, L2, and L3 as seen in acute lymphoid leukemias. On the far right side are the corresponding cartoons. The L1 blast is common and can be confused, especially in infants, with the normal small mature lymphocytes that are shown in many of the blood smears for comparison.
Figure 6.86. ALL blasts. ALL blasts, in particular the L2 morphologic variants, can look like typical AML blasts (large cells with moderate N:C ratios and very prominent nucleoli). Morphology can be misleading when trying to determine lineage of blast. Immunohistochemistry and immunophenotyping are necessary to distinguish ALL from AML.
Figure 6.87. B-cell lymphoblastic leukemia/lymphoma (B-cell ALL). A: Lymphoid leukemic blasts with characteristic low CD45 expression and low side-scatter properties are shown (arrow). B–G: Blood smears from six different cases of B-cell ALL showing variations of morphology compared with small mature lymphocytes. Arrows delineate blasts with lower N:C ratios and nucleoli.
Acute leukemia, in particular B-cell ALL, but also some AMLs, especially those coexpressing B-cell markers, harbors the t(9;22) translocation. This fact has important implications for initial treatment decisions, and RT-PCR for BCR-ABL should be considered for all adult B-cell ALLs and those AMLs with B-cell marker expression profiles. RT-PCR primer sets should include those that can detect the p190 BCR-ABL transcript (more common in the Ph-positive acute leukemia), as well as the p210 transcript (more common in chronic myeloid leukemia). (Courtesy of Dr. S. Kamel-Reid.)

Figure 6.88. Philadelphia chromosome (Ph)–positive acute leukemia. Acute leukemia, in particular B-cell ALL, but also some AMLs, especially those coexpressing B-cell markers, harbors the t(9;22) translocation. This fact has important implications for initial treatment decisions, and RT-PCR for BCR-ABL should be considered for all adult B-cell ALLs and those AMLs with B-cell marker expression profiles. RT-PCR primer sets should include those that can detect the p190 BCR-ABL transcript (more common in the Ph-positive acute leukemia), as well as the p210 transcript (more common in chronic myeloid leukemia). (Courtesy of Dr. S. Kamel-Reid.)

Figure 6.89. ALL with fibrosis. A and B: A case of B-cell ALL with architectural distortion on hematoxylin and eosin (H&E)–stained biopsy sections includes lining up of individual
blasts (so-called Indian filing) and swirling patterns that suggest significant marrow fibrosis. 

C: The latter is confirmed with reticulin stains showing increased numbers of thickened fibers. Marrow fibrosis often precludes flow cytometric evaluation of leukemic cells from dry aspirate smears. In these cases, efforts should be made either to disaggregate biopsy specimens for flow cytometry or, alternatively, to acquire biopsies for immunohistochemistry.

**Figure 6.90.** Flow cytometry detection of minimal residual disease in B-ALL. There is a small population of CD19 dim–positive, CD20 dim–positive, CD10-positive, CD34-positive, TdT-positive, light chain–negative neoplastic B-lymphoblasts (0.1% of analyzed cells; black dots). Neoplastic blasts are present in the background of normal maturing B cells (hematogones; green dots) and polytypic mature B cells (red dots).
Figure 6.91. T-cell lymphoblastic leukemia/lymphoma (T-ALL). T-ALL often presents with a high blast count and a mass in the mediastinum or other tissues. **A:** Chest of a young man with a mediastinal mass eroding through the sternum. **B:** Axial CT scan with intravenous contrast at the level of the upper thorax discloses an anterior mediastinal soft tissue mass consistent with leukemia/lymphoma. (Courtesy of Dr. I. Quirt.)
**Figure 6.92.** T-cell lymphoblastic leukemia/lymphoma (T-ALL). A: Flow cytometry scattergram shows blasts positive for cytoplasmic CD3 expression, a T-cell–specific marker that is usually positive in T-ALL. B and C: Aspirate smears disclose sheets of L2 type blasts with high mitotic rate and strong block-like cytoplasmic PAS positivity, respectively.
Figure 6.93. Flow cytometry detection of minimal residual disease in T-ALL. There is a small population of surface CD3–negative, cytoplasmic CD3–positive, CD2-positive, CD7 bright–positive, CD5 dim–positive, CD34-positive, CD4-negative, and CD8-negative T lymphoblasts (in red and black; arrows). Neoplastic cells are present in the background of reactive mature T and NK cells (in green).

Figure 6.95. Overall survival of patients up to 60 years treated in UK Medical Research Council AML 10 and AML 12 trials according to cytogenetics. (With permission from Grimwade D. Impact of cytogenetics on clinical outcome in AML. In: Karp J, ed. Acute Myelogenous Leukemia. Totawa, NJ: Humana Press, 2007:181.)

Figure 6.96. AML diagnosis requires a multiparameter approach: morphology, flow cytometry, cytogenetics, and molecular analysis.
Two types of mutations can lead to constitutive activation of FLT3: internal tandem duplications in the juxtamembrane domain and point mutations in the activation loop domain. Figure shows the clinical outcome in 550 AML patients with a normal karyotype according to FLT 3/ITD and NPM 1 mutant status. Both markers were prognostically significant predictors of survival (p < 0.0001), and together identified three prognostic groups: good (FLT3/ITD– NPM 1+), intermediate (FLT 3/ITD– NPM1− or FLT 3/ITD+ NPM1+), and poor (FLT 3/ITD + NPM1−). (With permission from Gale RE, Green C, Allen C, et al. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008;111:2776–2784.)
CHAPTER 7
Myelodysplastic Syndromes

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The myelodysplastic syndromes (MDSs) are a heterogeneous group of disorders caused by a clonal expansion of hematopoietic stem cells in which maturation is abnormal and cell production is ineffective, resulting in various degrees of cytopenia with anemia being the most common. MDS usually occurs in older adults with the median age at diagnosis of 65 years. Although the risk of developing MDS increases with age, therapy-related MDS can develop at any age and MDS can rarely affect children. MDS has been associated with exposure to environmental factors (including chemotherapy and radiation), certain inherited genetic abnormalities, and preceding acquired hematologic conditions (Table 7.1).

Many patients are asymptomatic and present with cytopenia(s) on routine blood count analysis. Some patients may present with symptoms and/or complications from a previously unrecognized cytopenia, such as infection, bleeding, easy bruising, and general fatigue. Complete blood count analysis typically shows normocytic or macrocytic anemia that is usually associated with inadequately low reticulocyte production and increased red blood cell distribution width reflecting anisocytosis. Anemia can be isolated or associated with leukopenia and/or thrombocytopenia; MDS only rarely presents with leukopenia and/or thrombocytopenia in the absence of anemia. The bone marrow is typically hypercellular for age despite peripheral blood cytopenias. In addition to the quantitative abnormalities described above, the cells in peripheral blood and bone marrow demonstrate dysplasia that involves one or more hematopoietic lineages (Table 7.2). Erythrocytes may show macrocytosis, anisocytosis, basophilic stippling, and Pappenheimer bodies. Poikilocytosis is usual and includes target cells, acanthocytes, elliptocytes, stomatocytes, red cell fragments, and teardrop cells (Fig. 7.1). Erythroid precursors in the bone marrow may show nuclear and/or cytoplasmic abnormalities. The former consist of budding, internuclear bridging, the presence of more than one nucleus per cell, karyorrhexis (fragmentation), abnormal chromatin (either fine or dense), and megaloblastic changes, in which the nucleus is enlarged and less mature than would be expected based on the degree of cytoplasmic hemoglobinization (Fig. 7.2). Cytoplasmic abnormalities include vacuolization and ring sideroblasts, which are erythroblasts that, on iron stain, contain at least five ferritin granules encircling at least one-third of the nucleus (Figs. 7.3 and 7.4).

Evidence of abnormal granulopoiesis on the peripheral smear includes nuclear hypersegmentation or hyposegmentation, Döhle bodies, decreased or absent cytoplasmic granules, and the presence of immature cells, including blasts (which should not exceed 20% of the leukocytes) (Fig. 7.5). The nuclei may exhibit dense chromatin clumping, and many are hypolobulated, with a single lobe or two joined by a thin band of chromatin, resembling the congenital Pelger–Huët anomaly (Fig. 7.6). Such pseudo Pelger–Huët cells, if frequent on a blood smear, strongly suggest an underlying MDS. Eosinophils and basophils may have diminished granules and/or decreased nuclear lobulation (Fig. 7.1). In the bone marrow biopsy, granulopoiesis may be architecturally disturbed whereby blasts and immature

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myeloid precursors occur centrally in the marrow space rather than in their typical paratrabecular location, a finding designated as abnormal localization of immature precursors (ALIP) (Fig. 7.7). Abnormal thrombopoiesis on the peripheral smear is manifested by giant platelets and forms with decreased or absent granules. In the bone marrow, dysplastic megakaryocytes are small (micromegakaryocytes), and possess abnormal nuclei that are multiple and widely separated or have decreased or absent lobulation (Figs. 7.8 and 7.9). In all hematopoietic lineages, the dysplasia should affect at least 10% of the cells within the lineage to be considered significant according to the WHO Classification criteria.

Morphologic dysplasia can be complemented by evaluation of “immunophenotypic dysplasia” by multiparameter flow cytometric (FCM) analysis, especially in patients with borderline/minimal dysplasia and low blast counts. The International/European LeukemiaNet Working Group for Flow Cytometry in MDS has developed an FCM MDS score based on a simple approach using the following widely applicable parameters/antigens: side scatter cell properties, CD10, CD34, and CD45 (Table 7.3; Fig. 7.10). A FCM score of at least 2 has high specificity for an MDS diagnosis and may be adopted as a basic protocol for the diagnostic workup of MDS patients. These parameters may be supplemented by the analysis of altered expression of additional antigens on myeloblasts, maturing myeloid cells, and monocytes (Table 7.4; Fig. 7.11). However, until uniform FCM diagnostic criteria for MDS are developed, reference ranges for each parameter should be validated based on individual laboratory practices.

Patients with MDS should always undergo genetic evaluation that not only helps establish disease clonality and classify MDS subtypes but also determines prognostic risk group and helps guide therapy. The presence of certain recurrent cytogenetic abnormalities, as detected by routine karyotype, in patients with cytopenia is considered MDS-defining even in the absence of significant dysplasia (Table 7.5). Recent technological advances in high-throughput genomic sequencing have revealed a panoply of MDS-specific somatic mutations. Up to 90% of MDS patients demonstrate at least one acquired somatic mutation compared to a much lower (50% to 60%) rate of abnormalities detected by conventional karyotype. Mutations in more than 40 genes have been identified in MDS that fall into several general categories, including transcription factors, epigenetic regulators and chromatin-remodeling factors, pre-mRNA splicing factors, and signaling molecules (particularly in the RAS pathway) (Table 7.6). Detection of MDS-specific somatic mutations has drastically changed the current understanding of MDS biology and has the potential to improve diagnostic criteria, prognostication, and monitoring response to treatment. For example, the presence of SF3B1 mutation has been included as a diagnostic criterion for MDS with ring sideroblasts in the revised 4th edition WHO Classification.

Many mutations have been found to be associated with patient outcome and some of the mutations (e.g., EXH2, ASXL1, TP53, RUNX1, and ETXV6), have been shown to independently predict clinical behavior independent of other established prognostic variables. The International Prognostic Scoring System (IPSS), recently revised in 2012 (IPPS-R), has been in use for the past two decades in newly diagnosed MDS patients in order to assess their prognosis and to help in the timing of therapy and to decide among treatment options. These scoring systems are based on the degree of cytopenia, bone marrow blast counts and cytogenetic findings. The IPSS-R is considerably more complex than the IPSS, using a larger number of blast strata, five instead of three karyotype categories, and more detailed assessments of cytopenias in each lineage (Table 7.7). The combined data from the above parameters are used to calculate five risk groups (Table 7.8). The IPSS-R has been widely
adopted in clinical practice as a basis for treatment decisions and appears to predict outcome both at diagnosis and dynamically in patients being followed at later timepoints. Although MDS-associated mutations have not been incorporated into the IPSS-R, future prognostic risk systems will likely incorporate the mutational profile in patient risk grouping. Treatment options for patients with MDS typically fall into one of three broad categories: supportive care (antibiotics and red cell and platelet transfusions for symptomatic anemia and thrombocytopenia), low-intensity therapies (hypomethylating agents, immunosuppressants, and lenalidomide) and high-intensity therapy (multiagent chemotherapy and allogeneic transplant). Although low-intensity therapies are typically used in patients with very low-risk and low-risk IPSS-R scores, high-intensity therapies are usually reserved for high-risk and very high–risk IPSS-R score patients. This lessens treatment-related morbidity and mortality in patients with a relatively good prognosis, and allows for the aggressive treatment of disease in those with a poor prognosis. Patients with intermediate IPSS-R scores may benefit from either of these approaches. Lower risk patients with anemia (especially in the context of MDS with ring sideroblasts) and serum erythropoietin (EPO) levels below 500 U/L may benefit from erythropoiesis-stimulating agents used with or without a granulocyte colony-stimulating factor (G-CSF); patients with higher serum EPO levels rarely respond to erythropoiesis-stimulating agents. Similarly, G-CSF and thrombopoietin receptor agonists may be used in MDS patients with neutropenia and thrombocytopenia, respectively, but none of these agents improve survival. The therapeutic approach to MDS will likely increasingly be governed by somatic mutations that can help define responsiveness to particular treatment regimens. For example, certain mutations, such as DMNT3A and TET2, appear to be associated with a superior response to hypomethylating agents. Lenalidomide, a thalidomide analogue, is most effective in MDS with del(5q) lacking TP53 mutation, with a 67% response rate and a median duration of response of more than 2 years. Allogeneic transplant, although the only curative modality, is currently used in less than 10% MDS patients, due to advanced age, the presence of comorbidities, and lack of suitable donors. It remains a treatment of choice for children and patients under 40 years of age and is also used in some older patients with higher risk disease. However, the presence of TP53 mutation is associated with poor outcome in MDS treated with allogeneic transplant.

Although establishing an MDS diagnosis relies on the presence of cytopenia(s) and morphologic dysplasia, these features are also present in a variety of neoplastic as well as non-neoplastic conditions. Some patients have prolonged unexplained cytopenia(s), yet do not meet diagnostic criteria for MDS (due to lack of sufficient dysplasia, increased blasts, or a defining cytogenetic abnormality), a condition designated as idiopathic cytopenia of undetermined significance (ICUS). Moreover, MDS-associated mutations may occur in cytopenic or non-cytopenic older individuals who do not have MDS, termed clonal cytopenia of undetermined significance (CCUS) and clonal hematopoiesis of indeterminate potential (CHIP). Mutation frequency increases with age and individuals with clonal mutations are at a higher risk of development of a hematologic malignancy. However, mutations by themselves do not currently establish an MDS diagnosis and in the absence of diagnostic criteria for a myeloid neoplasm, a diagnosis of CCUS/CHIP should be considered. Reflecting the recent pivotal developments in the molecular biology of MDS, the terminology and diagnostic criteria of MDS have been updated in the 2016 revision of WHO MDS Classification. The current classification of MDS includes six distinct entities and one provisional entity and is based primarily on bone marrow findings. Despite the presence of genetic abnormalities in greater than 90% of cases, most of the MDS subtypes are still mainly defined by the degree of dysplasia and blast count, reflecting our incomplete understanding of the disease.
understanding of MDS molecular pathogenesis and challenges in distinguishing MDS from ICUS and CHIP. Importantly, certain features exclude a diagnosis of MDS, even in the context of cytopenia and significant morphologic dysplasia (Table 7.11).

MDS WITH SINGLE LINEAGE DYSPLASIA

MDS with single lineage dysplasia (MDS-SLD) is an indolent MDS subtype that accounts for 5% to 10% of MDS cases, with a median survival of about 5 years and acute leukemia development in less than 5% of patients. Dysplasia affects over 10% of cells in only one hematopoietic lineage, ring sideroblasts are rare (<15% of erythroid cells) or absent, blasts are less than 5% of cells in the marrow and less than 1% of cells in the blood, and there is single or bi-cytopenia. The most common presentation is with anemia and dysplasia isolated to the erythroid lineage, previously termed refractory anemia in older MDS classifications. On the peripheral blood smear the red cells are normocytic or macrocytic. Cytogenetic abnormalities occur in 25% to 50% of cases.

MDS WITH RING SIDEROBLASTS

The subtype MDS with ring sideroblasts (MDS-RS) is characterized by ring sideroblasts affecting at least 15% of bone marrow erythroid precursors on an iron-stained bone marrow aspirate smear, or at least 5% ring sideroblasts in the presence of an SF3B1 mutation; blasts must constitute less than 5% of the bone marrow cells and less than 1% of the peripheral blood leukocytes (Fig. 7.13). It is further divided into subtypes with single lineage dysplasia (MDS-RS-SLD), which by definition must be limited to the erythroid lineage and be accompanied by anemia with or without one other cytopenia, and multilineage dysplasia (MDS-RS-MLD). The SF3B1 mutation, which affects RNA splicing, is strongly associated with the presence of ring sideroblasts and correlates with a favorable prognosis in MDS. Cytogenetic abnormalities occur in less than 10% of cases with single lineage dysplasia, but are more common in cases with multilineage dysplasia. Prognosis in MDS-RS is variable and appears to be poorer in cases with multilineage dysplasia, those that lack SF3B1 mutation, and those that have additional non-SF3B1 mutations such as TP53 and ASXL1.

MDS WITH MULTILINEAGE DYSPLASIA

MDS with multilineage dysplasia (MDS-MLD) is one of the more common MDS subtypes and the diagnosis requires cytopenia(s) and dysplastic changes (≥10% of cells) in at least two hematopoietic lineages; blasts must comprise less than 5% of the bone marrow cells and less than 1% of the peripheral blood leukocytes. There may be a single cytopenia, two cytopenias, or pancytopenia. The median survival is 2.5 to 3 years, and the risk of developing leukemia is approximately 10%. Cytogenetic abnormalities occur in about 50% of cases.

MDS WITH EXCESS BLASTS

MDS with excess blasts (MDS-EB) is an aggressive MDS subtype in which blasts are
increased in the bone marrow and/or peripheral blood, but not sufficiently to meet the criteria for acute leukemia, which is at least 20% of nucleated cells in the blood or bone marrow. Two subtypes exist: MDS-EB1 has 5% to 9% blasts in the bone marrow or 2 to 5% blasts in the blood, and no Auer rods, whereas MDS-EB2 has 10% to 19% blasts in the bone marrow, or 5% to 19% blasts in the blood, or Auer rods (Figs. 7.9, 7.12, and 7.13). MDS-EB accounts for approximately 40% of cases of MDS. About 25% of MDS-EB1 and 33% of MDS-EB2 progress to acute leukemia, and their respective median survivals are 18 and 10 months. 35% to 50% of MDS-EB patients have cytogenetic abnormalities, which are often complex or high risk (Fig. 7.14).

**MYELODYSPLASTIC SYNDROME WITH ISOLATED DEL(5Q) (5Q– SYNDROME)**

Unlike the other MDS subtypes, this entity is primarily defined by a cytogenetic abnormality, a deletion in the long arm of chromosome 5, with variability in the actual break points and the size of the deletion (Fig. 7.14). In the revised 2016 WHO Classification, the del(5q) abnormality may be isolated or occur with one other cytogenetic abnormality, with the exception of monosomy 7 or del(7q). Most patients are middle-aged to older women whose major problem is refractory macrocytic anemia; the platelet count is often normal and some patients have thrombocytosis. The bone marrow is usually hypercellular, with dysplasia most prominent in the megakaryocytes, which are increased and have hypolobulated nuclei; erythroid dysplasia is also often present, but dysgranulopoiesis is rare (Fig. 7.15 and Fig. 7.16). Blasts are less than 5% of cells in the bone marrow and less than 1% of cells in the blood. Patients typically have a favorable prognosis and often have an excellent response to lenalidomide. Progression to acute leukemia occurs in less than 25% of cases.

**MYELODYSPLASTIC SYNDROME, UNCLASSIFIABLE**

Patients can be assigned to the myelodysplastic syndrome, unclassifiable (MDS-U) category for one of three reasons:

- Patients manifest features of one of the lower-grade MDS subtypes (any subtype except for MDS-EB), but have 1% blasts in the peripheral blood measured on at least two separate occasions.
- Patients with MDS that would otherwise be classified as MDS-SLD, MDS-RS-SLD, or MDS with isolated del(5q), but with pancytopenia (hemoglobin <10 g/dL, platelets <100 × 10^9/L, absolute neutrophil count <1.8 × 10^9/L)
- Patients with persistent unexplained cytopenia and absence of sufficient dysplasia in any hematopoietic lineage, but who have acquired cytogenetic abnormalities that are considered MDS-defining (Table 7.5)

MDS-U is rare and its median survival and risk of developing leukemia are unknown. The MDS-U subtype with 1% peripheral blood blasts appears to have a similar prognosis to MDS-EB1.
Refractory cytopenia of childhood (RCC) is a rare MDS affecting children and is considered as a provisional entity in the revised 2016 WHO Classification. Unlike MDS affecting adults, most cases of RCC manifest with a hypocellular marrow and may be difficult to distinguish from aplastic anemia. RCC is otherwise morphologically similar to MDS-MLD, usually with trilineage dysplasia and frequent small, hypolobated megakaryocytes. By definition, blasts are less than 5% of the bone marrow cells. MDS in children with increased blasts is classified as MDS-EB, similar to that observed in adults.

REFERENCES

13. Wong KF, Chan JK. Are ‘dysplastic’ and hypogranular megakaryocytes specific markers

<table>
<thead>
<tr>
<th>Table 7.1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Predisposing factors and epidemiologic associations of myelodysplastic syndromes</strong></td>
</tr>
<tr>
<td><strong>Heritable predisposition</strong></td>
</tr>
<tr>
<td>Constitutional genetic disorders</td>
</tr>
<tr>
<td>Down syndrome (trisomy 21)</td>
</tr>
<tr>
<td>Trisomy 8 mosaicism</td>
</tr>
<tr>
<td>Familial monosomy 7</td>
</tr>
<tr>
<td>Neurofibromatosis 1</td>
</tr>
<tr>
<td>Germ cell tumors (embryonal dysgenesis)</td>
</tr>
<tr>
<td>Congenital neutropenia (Kostmann syndrome or Shwachman–Diamond syndrome)</td>
</tr>
<tr>
<td>Diamond–Blackfan anemia</td>
</tr>
<tr>
<td>DNA repair deficiencies and telomere biology disorders</td>
</tr>
<tr>
<td>Fanconi anemia</td>
</tr>
<tr>
<td>Ataxia telangiectasia</td>
</tr>
<tr>
<td>Bloom syndrome</td>
</tr>
<tr>
<td>Xeroderma pigmentosum</td>
</tr>
<tr>
<td>Dyskeratosis congenita</td>
</tr>
<tr>
<td>Germline mutations</td>
</tr>
<tr>
<td>Germline predisposing mutation without a preexisting condition or organ dysfunction (CEBPA or DDX41 mutation)</td>
</tr>
<tr>
<td>Germline predisposing mutation with preexisting platelet disorder (RUNX1, ANKRD26, or ETV6 mutation)</td>
</tr>
<tr>
<td>GATA2 mutation</td>
</tr>
<tr>
<td><strong>Acquired</strong></td>
</tr>
<tr>
<td>Mutagen exposure</td>
</tr>
<tr>
<td>Genotoxic therapy</td>
</tr>
<tr>
<td>Alkylators</td>
</tr>
<tr>
<td>Topoisomerase II interactive agents</td>
</tr>
<tr>
<td>β-Emitters (phosphorus-32)</td>
</tr>
</tbody>
</table>
Autologous bone marrow transplantation
Radiation exposure to hematopoietic bone marrow
Environmental or occupational exposure (e.g., benzene)
Tobacco
Aplastic anemia
Paroxysmal nocturnal hemoglobinuria


<p>| Table 7.2 | Morphologic abnormalities in myelodysplastic syndromes |</p>
<table>
<thead>
<tr>
<th>Lineage</th>
<th>Peripheral Blood</th>
<th>Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid</td>
<td>Ovalomacrocytes</td>
<td>Megaloblastoid erythropoiesis</td>
</tr>
<tr>
<td></td>
<td>Elliptocytes</td>
<td>Nuclear budding</td>
</tr>
<tr>
<td></td>
<td>Acanthocytes</td>
<td>Ring sideroblasts</td>
</tr>
<tr>
<td></td>
<td>Stomatocytes</td>
<td>Internuclear bridging</td>
</tr>
<tr>
<td></td>
<td>Teardrops</td>
<td>Karyorrhexis</td>
</tr>
<tr>
<td></td>
<td>Nucleated erythrocytes</td>
<td>Nuclear fragments</td>
</tr>
<tr>
<td></td>
<td>Basophilic stippling</td>
<td>Cytoplasmic vacuolization</td>
</tr>
<tr>
<td></td>
<td>Howell–Jolly bodies</td>
<td>Multinucleation</td>
</tr>
<tr>
<td>Myeloid</td>
<td>Pseudo Pelger–Huët anomaly</td>
<td>Defective granulation</td>
</tr>
<tr>
<td></td>
<td>Auer rods</td>
<td>Maturation arrest at myelocyte stage</td>
</tr>
<tr>
<td></td>
<td>Hypogranulation</td>
<td>Increase in monocytoid forms</td>
</tr>
<tr>
<td></td>
<td>Nuclear sticks</td>
<td>Abnormal localization of immature precursors</td>
</tr>
<tr>
<td></td>
<td>Hypersegmentation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ring-shaped nuclei</td>
<td></td>
</tr>
</tbody>
</table>


<p>| Table 7.3 | Flow cytometric MDS score assignment “Ogata score” based on the four principal parameters |</p>
<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Measurement(s)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in CD34+ myeloblasts</td>
<td>Percentage of all nucleated cells</td>
<td>1</td>
</tr>
<tr>
<td>Decrease in CD34+ B-cell progenitors</td>
<td>Percentage of all CD34+ cells</td>
<td>1</td>
</tr>
<tr>
<td>Significant increase or decrease in lymphocyte to myeloblast CD45 ratio</td>
<td>CD45 mean fluorescence intensity on lymphocytes divided by CD45 mean fluorescence intensity on myeloblasts</td>
<td>1</td>
</tr>
<tr>
<td>Decrease in granulocyte to lymphocyte side scatter (SSC) peak channel ratio</td>
<td>SSC channel number with maximum CD10+ granulocyte divided by SSC channel number with maximum lymphocytes</td>
<td>1</td>
</tr>
</tbody>
</table>

*Reference ranges for each parameter should be validated based on individual laboratory practice; the flow cytometric score of ≥2 shows specificity of >90%.

<table>
<thead>
<tr>
<th>Table 7.4</th>
<th>Aberrant flow cytometric features on blasts, myeloid, and monocytic cells in myelodysplastic syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Population</td>
<td>Abnormalities Seen in Myelodysplastic Syndrome</td>
</tr>
</tbody>
</table>

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### CD34+ blasts
- Increased numbers (variably defined as >2% or ≥3%)
- Altered CD11b, CD13, CD15, CD33, CD45, CD64, or CD65 expression
- Decreased CD38 or HLA-DR expression
- Aberrant CD2, CD5, CD7, or CD56 expression

### Myeloid cells
- Increased expression of CD36, CD117, or HLA-DR
- Altered CD11b, CD13, CD15, CD16, or CD45 expression
- Decreased CD10 expression or side scatter on granulocytes
- Aberrant CD34, CD19, CD5, CD56, or CD7 expression

### Monocytes
- Decreased CD45 or side scatter
- Altered HLA-DR, CD11b, CD14, or CD64 expression
- Aberrant CD2, CD5, CD7, CD16, CD34, CD36, or CD56 expression

---

### Table 7.5
Recurring chromosomal abnormalities in MDS

<table>
<thead>
<tr>
<th>Gain or Loss of Chromosomal Material (Relatively Common)</th>
<th>Other Translocations and Inversions (Relatively Uncommon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>–7 or del(7q)</td>
<td>t(3;3)(q21;q26), inv(3)(q21q26), t(3;21)(q26;q22)</td>
</tr>
<tr>
<td>–5 or del(5q)</td>
<td>Other 3q21 and 3q26 translocations</td>
</tr>
<tr>
<td>+8*</td>
<td>t(1;7)(p11;p11)</td>
</tr>
<tr>
<td>+21, –21</td>
<td>t(2;11)(p21;q23)</td>
</tr>
<tr>
<td>–17 and unbalanced translocations at 17p</td>
<td>t(11;16)(q23;p13)</td>
</tr>
<tr>
<td>–20 or del(20q)*</td>
<td>t(6;9)(p23;q34)</td>
</tr>
<tr>
<td>del(11q)</td>
<td>t(2;11)(p21;q23)</td>
</tr>
<tr>
<td>–Y*</td>
<td>i(17q)</td>
</tr>
<tr>
<td>del(9q)</td>
<td></td>
</tr>
<tr>
<td>+6</td>
<td></td>
</tr>
<tr>
<td>del(12p) and unbalanced translocations at 12p</td>
<td></td>
</tr>
<tr>
<td>–13 or del(13q)</td>
<td></td>
</tr>
</tbody>
</table>

*Del(20q), +8, and –Y abnormalities are not considered MDS-defining and cannot in isolation be used to make a diagnosis of MDS.

### Table 7.6
Frequency of the most common MDS-associated somatic mutation and their effect on overall survival

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Frequency Range</th>
<th>Effect on Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF3B1</td>
<td>15%–32%</td>
<td>Good</td>
</tr>
<tr>
<td>SRSF2</td>
<td>10%–17%</td>
<td>Poor</td>
</tr>
<tr>
<td>U2AF1</td>
<td>7%–12%</td>
<td>Poor</td>
</tr>
<tr>
<td>ZRSR2</td>
<td>3%–11%</td>
<td>Neutral/poor</td>
</tr>
<tr>
<td>PRPF8</td>
<td>1%–4%</td>
<td></td>
</tr>
<tr>
<td><strong>Epigenetic regulators</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TET2</td>
<td>20%–32%</td>
<td>Neutral</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>5%–13%</td>
<td>Poor</td>
</tr>
<tr>
<td>IDH1/IDH2</td>
<td>4%–12%</td>
<td>Poor</td>
</tr>
<tr>
<td>ASXL1</td>
<td>11%–23%</td>
<td>Poor</td>
</tr>
<tr>
<td>EZH2</td>
<td>5%–12%</td>
<td>Poor</td>
</tr>
<tr>
<td>UTX</td>
<td>1%</td>
<td></td>
</tr>
</tbody>
</table>
### Transcription factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Frequency</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUNX1</td>
<td>8%–15%</td>
<td>Poor</td>
</tr>
<tr>
<td>TP53</td>
<td>4%–14%</td>
<td>Poor</td>
</tr>
<tr>
<td>ETV6</td>
<td>2%–5%</td>
<td>Poor</td>
</tr>
<tr>
<td>WT1</td>
<td>&lt;1%</td>
<td></td>
</tr>
</tbody>
</table>

### Signaling molecules

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Frequency</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS/KRAS</td>
<td>5%–10%</td>
<td>Poor</td>
</tr>
<tr>
<td>CCSNK1A1</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>CBL</td>
<td>2%–5%</td>
<td></td>
</tr>
<tr>
<td>JAK2</td>
<td>2%–5%</td>
<td></td>
</tr>
<tr>
<td>FLT3</td>
<td>1%–4%</td>
<td>Poor</td>
</tr>
<tr>
<td>GNAS</td>
<td>&lt;1%</td>
<td></td>
</tr>
</tbody>
</table>

MDS, myelodysplastic syndrome.

### Table 7.7

Revised International Prognostic Scoring System (IPSS-R): prognostic variables in myelodysplastic syndrome

<table>
<thead>
<tr>
<th>Prognostic Variable</th>
<th>Points Scored for Each Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Cytogenetics*</td>
<td></td>
</tr>
<tr>
<td>Bone marrow blasts (%)</td>
<td>≤2</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>≥10</td>
</tr>
<tr>
<td>Platelets (×10^9/L)</td>
<td>≥100</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>≥0.8</td>
</tr>
</tbody>
</table>

*Cyto genetic definitions: Very good: —Y, del(11q); Good: normal, del(5q), del(12p), del(20q), double including del(5q); Intermediate: del(7q), +8, +19, 1q17q, any other single or double independent clones; Poor: —7, inv(3)/t(3q)/del(3q), double including —7/del(7q), complex: 3 abnormalities; Very poor: complex: >3 abnormalities.

### Table 7.8

The Revised International Prognostic Scoring System (IPSS-R): risk group and score assignment and prognosis

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>IPSS-R Group</th>
<th>Median Overall Survival (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low</td>
<td>≤1.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Low</td>
<td>&gt;1.5–3.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&gt;3–4.5</td>
<td>3.0</td>
</tr>
<tr>
<td>High</td>
<td>&gt;4.5–6.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Very high</td>
<td>&gt;6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### Table 7.9

Differential diagnosis of the myelodysplastic syndromes

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cytopenia</th>
<th>Dysplasia</th>
<th>Defining Cytogenetic</th>
<th>Somatic</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal cytopenia of unknown significance (CCUS)</td>
<td>Yes</td>
</tr>
<tr>
<td>(&gt;6 mo)</td>
<td>No</td>
</tr>
<tr>
<td>Clonal hematopoiesis of indeterminate potential</td>
<td>Yes or no</td>
</tr>
<tr>
<td>(CHIP)</td>
<td>No</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>Usually</td>
</tr>
<tr>
<td>Yes or no</td>
<td>t(8;21)(q22;q22)</td>
</tr>
<tr>
<td>通常</td>
<td>No</td>
</tr>
<tr>
<td>t(16;16)(p13.1q22)</td>
<td>Yes or no</td>
</tr>
<tr>
<td>t(15;17)(q24.1;q21.1)</td>
<td>No</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia</td>
<td>Yes (anemia), and monocytosis</td>
</tr>
<tr>
<td>Usually</td>
<td>No</td>
</tr>
<tr>
<td>Yes (90%)</td>
<td>JAK2, MPL, or CALR and SF3B1</td>
</tr>
<tr>
<td>MDS with ring sideroblasts and thrombocytosis</td>
<td>Yes, and thrombocytosis</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
<td>Yes or No</td>
</tr>
<tr>
<td>No or minimal</td>
<td>No</td>
</tr>
<tr>
<td>JAK2, MPL, or CALR</td>
<td>May be present</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>Yes</td>
</tr>
<tr>
<td>No or minimal</td>
<td>No</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Yes</td>
</tr>
<tr>
<td>No or minimal</td>
<td>No</td>
</tr>
<tr>
<td>PIGA or others</td>
<td>No</td>
</tr>
<tr>
<td>HIV infection</td>
<td>Yes or No</td>
</tr>
<tr>
<td>Often</td>
<td>No</td>
</tr>
<tr>
<td>Nutritional deficiency (vitamin B₁₂, folate, copper)</td>
<td>Yes</td>
</tr>
<tr>
<td>Often</td>
<td>No</td>
</tr>
<tr>
<td>Medications</td>
<td>Yes</td>
</tr>
<tr>
<td>Often</td>
<td>No</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 7.10

WHO Classification of myelodysplastic syndromes (MDS)¹⁵

<table>
<thead>
<tr>
<th>Revised 2016 Classification of MDS</th>
<th>2008 Classification of MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS syndrome with single lineage dysplasia (MDS-SLD)</td>
<td>Refractory cytopenia with unilineage dysplasia (RCUD)</td>
</tr>
<tr>
<td>MDS syndrome with ring sideroblasts (MDS-RS)</td>
<td>MDS-RS and single lineage dysplasia (MDS-RS-SLD)</td>
</tr>
<tr>
<td>MDS-RS and multilineage dysplasia (MDS-RS-MLD)</td>
<td>Refractory anemia with ring sideroblasts (RARS)</td>
</tr>
<tr>
<td>MDS with multilineage dysplasia (MDS-MLD)</td>
<td>Refractory anemia with mutililineage dysplasia and ring sideroblasts (RCMD-RS)</td>
</tr>
<tr>
<td>MDS with excess blasts (MDS-EB)</td>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>Same</td>
</tr>
<tr>
<td>MDS, unclassifiable</td>
<td>Same</td>
</tr>
<tr>
<td>Refractory cytopenia of childhood (provisional)</td>
<td>Same</td>
</tr>
</tbody>
</table>

Table 7.11
Features excluding a diagnosis of MDS, even in the context of cytopenia and significant morphologic dysplasia

- ≥1 × 10⁹/L peripheral blood monocytes; such cases are classified as chronic myelomonocytic leukemia (CMML).
- The presence of significant and persistent thrombocytosis (≥450 × 10⁹/L) and/or leukocytosis (white blood cell count ≥13 × 10⁹/L), which warrants classification as a MDS/MPN overlap disease; however, MDS with t(3;3) or inv(3) cytogenetic abnormalities or fulfilling features of MDS with isolated del(5q) are allowed to have thrombocytosis.
- Cases with ≥20% peripheral blood or bone marrow myeloblasts are classified as AML.
- Cases with the AML-defining cytogenetic abnormalities inv(16), t(16;16), t(8;21), or t(15;17) are classified as AML irrespective of the blast count.

MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; AML, acute myeloid leukemia.

**Figure 7.1.** Dysplastic nuclear features in circulating cells. Composite image taken from several cases of myelodysplastic syndrome showing dysplastic nuclear features seen in circulating granulocytes (nuclear hypolobation or hyperlobation with or without cytoplasmic hypogranulation; pseudo-Pelger-Huet nuclei), and RBCs (numerous Pappenheimer bodies).
Figure 7.2. Normocytic, megaloblastic, and dysplastic features of the erythroid lineage. Composite figure showing several examples of normal (normocytic) versus megaloblastic and dysplastic erythroid precursors at different stages of maturation. Megaloblastic precursors are typically larger in size than normal precursors and exhibit asynchronous nuclear and cytoplasmic maturation. The latter features are best appreciated in more mature forms, where cytoplasmic maturation proceeds in the form of hemoglobin production while nuclear maturation is delayed. Dyserythropoiesis is manifested primarily by nuclear contour abnormalities such as budding, irregularity (flower-like lobulation), and asymmetric multinucleation.

Figure 7.4. Dysplastic findings in megakaryocytes (A–E), erythroids (F–J), and granulocytic precursors (K–O) in bone marrow aspirate smears. A: Primitive megakaryoblasts, including

Figure 7.5. Dysplastic features in circulating neutrophils. Hypolobulation, “stringy” chromatin, and pseudo-Barr bodies are included in the spectrum of the abnormal nuclear features seen in MDS. The cytoplasm is often hypogranular or agranular.

Figure 7.6. Comparison of dysplastic hypogranular neutrophils to normal forms. Several examples of dysplastic, hypogranular neutrophils are shown here on the left side of each panel (indicated by the arrows), whereas normal neutrophils are shown on the right side; in MDS, dysplastic features are seen usually only in a subpopulation of the affected lineage, whereas staining artifacts usually cause all granulocytes to appear hypogranular in the smear.
Figure 7.7. Bone marrow biopsy in MDS showing a markedly hypercellular marrow with abnormally located immature precursors (“ALIP”). Normal maturing granulopoiesis usually appears as indiscrete, poorly circumscribed foci, located primarily, but not exclusively, in paratrabecular and perivascular locations. These normal foci of granulopoiesis are made up predominantly of mature, segmented neutrophils. In MDS, immature granulocytic precursors can occur in small clusters found away from bony trabeculae and vascular structures (arrows).
Figure 7.8. Dysplastic megakaryocytes seen in the bone marrow biopsy and bone marrow aspirate. The dysplastic forms are characterized by small size, rounded nuclear contours, and often widely separated multiple nuclei. Micromegakaryocytes in the bone marrow biopsy may be difficult to detect on routine histology and can be highlighted by immunohistochemistry using megakaryocytic markers.
Figure 7.9. Immunohistochemistry in MDS. A: CD34 staining of MDS-EB1, showing scattered and occasionally clustered blasts. B: CD34 staining of MDS-EB2, showing frequent clustered blasts. C: CD61 immunostain of MDS with multilineage dysplasia highlights small, dysplastic megakaryocytes with simplified, rounded nuclei. D: p53 Immunostain in therapy-related MDS, showing many strongly positive cells, typically correlating with the presence of TP53 mutation, complex karyotype, and poor prognosis.

Figure 7.10. Flow cytometry MDS Ogata score based on side scatter cell properties, CD10, CD34, and CD45. A and D: Different marrow cell populations are shown based on CD45 and side scatter properties: erythroblasts in brown, granulocytes in green, monocytes in dark blue, lymphocytes in light blue, and blasts in red. In comparison with the normal bone marrow, the MDS bone marrow shows abnormal location of myeloblasts (shown in red) and lower side scatter characteristics of granulocytic cells (shown in green). B and E: Increase in CD34+ blasts is shown. C and F: There is an absence of hematogones (lymphoid precursors) in the MDS marrow. (Courtesy of Dr. Sa A. Wang, MD Anderson Cancer Center, Houston, TX.)
Figure 7.11. Expanded flow cytometry MDS panel using additional antibodies. In comparison with the normal control, the MDS bone marrow shows altered CD13 and CD16 antigen expression on myeloid cells (A and C) and aberrant CD56 expression on blasts (B and D). (Courtesy of Dr. Sa A. Wang, MD Anderson Cancer Center, Houston, TX.)
Figure 7.12. Myelodysplastic syndrome with excess blast 2 (MDS-EB2). Aspirate smear shows dysplastic small megakaryocytes (micromegakaryocytes), a dysplastic hypogranular/hypolobulated neutrophil, and increased numbers of blasts (arrows).
Figure 7.13. Hypoplastic MDS with excess blasts. A: The biopsy is hypocellular with a predominance of erythroid elements. B and C: Increased blasts and small megakaryocyte are difficult to appreciate on routine histology, but are revealed by a CD34 (B) and CD61 immunostain (C), respectively.

Figure 7.14. Complex karyotype of a patient with MDS-EB2. This karyotype includes, among other aberrations, arrows indicate del(5q) and monosomy 7.

Figure 7.15. del(5q) cytogenetic abnormality. A: Ideogram of the normal chromosome 5 with breakpoints shown by arrows and the del(5q). B: The corresponding G-banded chromosomes. C and D: Corresponding FISH results. The EGR-1 gene at 5q31, labeled with a red fluor, is usually deleted (arrow). The loci at 5p15.2, including D5S5721 and D5S523, are...
labeled with a green fluor and serve as intrachromosomal controls.

**Figure 7.16.** Myelodysplastic syndrome with isolated del(5q) chromosome abnormality (5q–syndrome). Aspirate smear show increased numbers of the characteristic small hypolobated megakaryocytes with rounded nuclei.
Myeloproliferative neoplasms (MPNs) are a group of clonal disorders of the hematopoietic stem cells that share similar features, such as hypercellular marrow and evolution to acute leukemia (Table 8.1). In addition, these disorders are associated with bone marrow fibrosis either at presentation or upon disease progression. Unlike myelodysplastic syndromes (MDS), MPNs demonstrate absence of bone marrow dysplasia with normal maturation and effective cell production causing elevated blood levels of one or more cell lines (i.e., erythrocytosis, leukocytosis, and thrombocytosis) (Table 8.2). Patients tend to have hepatosplenomegaly (an unusual finding in MDSs) because of sequestered blood cells, extramedullary hematopoiesis, or infiltration by neoplastic cells (Figs. 8.1 and 8.2). However, similar to MDS, MPNs may evolve into acute leukemia, albeit at lower rates. These diseases differ from acute leukemias in the slower onset and more protracted course, measured in months to years for MPNs, compared to weeks for untreated acute leukemias.

MPNs usually occur in middle-aged adults. The clinical and hematologic features of the various MPNs significantly overlap and an accurate diagnosis of a specific MPN subtype requires careful integration of morphologic, cytogenetic/molecular findings and strict adhesion to the WHO criteria for diagnosing of MPNs. Advances in gene expression analysis and next-generation sequencing have led to identification of unique somatic mutations allowing to significantly improve the diagnostic and prognostic criteria for MPNs, which have been reflected in the revised 2016 WHO Classification of Tumors of Hematopoietic and Lymphoid Neoplasms1–4 (Table 8.3). Most MPNs develop through acquired clonal abnormalities in genes encoding cytoplasmic or receptor protein tyrosine kinases that result in constitutively active tyrosine kinase promoting growth and replication through various signal transduction pathways in a cytokine-independent manner.5,6 BCR-ABL1 fusion protein, the result of a reciprocal translocation that gives rise to an abnormal Philadelphia chromosome, was the first tyrosine kinase to define a specific MPN subtype7 and traditionally MPN subtypes have been divided into the Ph chromosome–positive group, comprising chronic myeloid leukemia (CML) as a sole entity, and the Ph chromosome–negative group that includes the remaining MPN subtypes. The classic Ph chromosome–negative MPNs are primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET) —three distinct clinical entities which share specific mutually exclusive somatic mutations (JAK2, MPL, and CALR) that result in dysregulation of the JAK/STAT signaling pathway leading to abnormal hematopoiesis, cytokine overproduction, and development of bone marrow fibrosis.3,8–11 Chronic neutrophilic leukemia (CNL), another rare Ph chromosome–negative MPN, has recently been connected to an activating somatic mutation in the receptor for colony stimulating factor 3 gene (CSF3R), which has been shown to induce constitutive phosphorylation of JAK2, STAT3, AKT, and ERK.12,13

In addition to the “driver” mutations that largely define the MPN phenotype, a constantly increasing number of coexisting mutations in epigenetic modifiers, such as TET2, DMNT3A,
ASXL1, EZH2, and the genes involved in hematopoietic signaling, such as NRAS, CBL, have been described in Ph chromosome–negative MPNs that may precede the “driver” mutations or occur at a later disease stage. Some of these mutations, such as TP53, TET2, SH2B3, and IDH1, are more frequently seen in leukemic transformation phase of MPNs, which, in addition to the acquisition of cytogenetic abnormalities, suggests their role in disease progression.

Another sign of MPN progression is the development of bone marrow fibrosis, which has been associated with increased cytogenetic aberrations and higher number of specific mutations. Chronic inflammation has been suggested as a driver of clonal evolution in MPNs and a potential trigger of bone marrow fibrosis, which is likely a part of polyclonal stromal reaction to the proliferation of neoplastic cells. Aberrant production of fibrogenic factors, such as transforming growth factor-β, fibroblast growth factor, and lysyl oxidase, by the neoplastic cells (particularly megakaryocytes) leads to the stimulation of marrow cells including fibroblasts, osteoblasts, and endothelial cells with consequent development of fibrosis and osteosclerosis (Figs. 8.3 and 8.4).

This chapter reviews seven MPNs and summarizes the revised diagnostic and prognostic criteria based on the new clinical, laboratory, morphologic, and molecular genetic data that have emerged in the last decade.

**CHRONIC MYELOID LEUKEMIA, BCR-ABL1-POSITIVE**

CML, BCR-ABL1–positive, is the most common MPN and accounts for approximately 15% to 20% of all adult leukemias with an annual incidence of 1 to 2 cases per 100,000 with a slight male predominance. It typically occurs at ages 40 to 60, with about 20% to 50% of patients asymptomatic, the diagnosis having been suggested by hepatosplenomegaly on physical examination (Fig. 8.5) or abnormal results—leukocytosis, anemia, or thrombocytosis—on routine hematologic testing. When symptoms occur (Table 8.4), they usually relate to splenomegaly (left upper quadrant discomfort or early satiety), problems from an increased white cell production (bone pain, mild fever, night sweats, weight loss), or anemia (dyspnea, fatigue, pallor).

CML is defined by the presence of the Philadelphia chromosome, a derivative chromosome 22 that harbors the BCR-ABL1 fusion gene, the result of reciprocal translocation t(9;22)(q34;q11) (Fig. 8.6). The BCR-ABL1 fusion gene results in the formation of the BCR-ABL1 fusion protein with constitutive tyrosine kinase activity of ABL1. Although most cases of CML can be diagnosed based on peripheral blood findings combined with detection of BCR-ABL1 by molecular genetic techniques (karyotype, fluorescence in situ hybridization [FISH] or reverse transcription polymerase chain reaction [RT-PCR]), a bone marrow biopsy and aspirate are essential for morphologic evaluation to confirm disease phase.

The disease goes through three stages: chronic phase (CML-CP), accelerated phase (CML-AP), and blast phase (CML-BP). In the chronic phase, which is present at the time of the diagnosis in approximately 85% of patients, the blood studies typically show mild anemia and leukocytosis that usually exceeds 25 × 10⁹/L (median white count of about 170 × 10⁹/L), primarily comprising neutrophils in various stages of maturation, especially myelocytes and mature neutrophils (Table 8.5). Basophils are universally increased, and eosinophilia is common (Fig. 8.7). The platelet count is normal or elevated, and may exceed 1,000 ×
10^9/L, but resulting thrombosis is unusual. \textsuperscript{21,22} Serum lactate dehydrogenase and uric acid are commonly increased, reflecting the underlying excessive cell proliferation. The bone marrow shows hypercellularity due to marked granulocytic hyperplasia, blasts constitute less than 5% of the cells, and megakaryocytes are small and hypolobular (Figs. 8.8 and 8.9). Eosinophils may be prominent (Fig. 8.10). In about 50% of patients, the megakaryocytes are increased in number, and, especially in this group, but also in others, reticulin fibrosis of the bone marrow may be apparent. Because of the excessive hematopoiesis, the number of cells that eventually die increases, and macrophages containing the lipids from the dead cells may be visible as sea-blue histiocytes or pseudo-Gaucher cells (Fig. 8.11).

In the era of therapy with tyrosine kinase inhibitors (TKI) the accelerated phase is becoming less common and currently there are no universally accepted criteria for its definition. The existing diagnostic WHO criteria for CML that include hematologic, morphologic, and cytogenetic and molecular parameters\textsuperscript{25} have been revised to include the “provisional response-to-TKI” criteria (Table 8.6). The accelerated phase should be diagnosed if any one or more of the criteria is present. Morphologic signs of the accelerated phase include peripheral blood basophilia of at least 20% and 10% to 19% of blasts in peripheral blood or bone marrow (Fig. 8.12). The finding of even less than 10% of lymphoblasts in peripheral blood or bone marrow warrants a complete clinical and molecular genetic workup as leukemic transformation may be imminent. Bone marrow findings suggestive of, but not definitive for, CML-AP are the presence of large clusters or sheets of abnormal small megakaryocytes associated with marked reticulin or collagen fibrosis; these morphologic findings are usually present in conjunction with other criteria of the accelerated phase.

The blast phase is defined by one or more of these features: (1) blasts accounting for at least 20% of peripheral white cells or nucleated bone marrow cells; (2) blasts proliferating in extramedullary sites, such as the skin, lymph nodes, and spleen; and (3) large aggregates of blasts occurring in the bone marrow. The blasts are usually myeloid but in about 20% to 30% of cases, they are lymphoid, usually B lymphoblasts (Figs. 8.13–8.16).

Development of small molecule TKI targeting the constitutively active tyrosine kinase has been one of the most successful stories in modern medicine improving the 10-year survival rate from 20% to 90%.\textsuperscript{6} Current guidelines recommend the three commercially available TKI (imatinib, dasatinib, and nilotinib) as the first-line treatment for chronic phase CML, with most patients demonstrating an excellent response within the first year of treatment.\textsuperscript{26} The TKI therapy is continued indefinitely as long as it is tolerated and the treatment milestones are met. Treatment response in CML is defined as hematologic, cytogenetic, and molecular\textsuperscript{27,28} (Table 8.7). Patients should continuously undergo molecular and/or cytogenetic monitoring. Chromosome banding analysis of at least 20 marrow cell metaphases is necessary to determine the degree of cytogenetic response or to identify secondary abnormalities associated with disease progression (Fig. 8.17). Quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) is generally performed on peripheral blood (Fig. 8.18). Whenever possible, both cytogenetic and molecular tests are recommended until complete cytogenetic response (CCyR) or major molecular response (MMR) are achieved\textsuperscript{28} (Fig. 8.19; Table 8.8). Raising levels of BCR-ABL1 transcripts may be associated with disease progression or development of drug resistance. Conventional Sanger sequencing is recommended to detect mutations affecting TKI binding either by directly altering an amino acid on the drug-binding site (e.g., T315I, F317J, F359C/V) or indirectly by altering protein conformation (e.g., G250E, Q252H, E255K/V).\textsuperscript{29} An alternative TKI or allogeneic stem cell transplant therapies are used in patients who become intolerant to a first-line TKI,
show excessive toxicity, treatment failure, or suboptimal response. The prognosis of CML in accelerated or blast phases is dismal, particularly for patients with prior TKI therapy, as many of them develop resistance (Fig. 8.20). There is a significant relapse rate even after successful treatment with TKI and these patients should be considered for transplantation.

CHRONIC NEUTROPHILIC LEUKEMIA

CNL is a very rare MPN in which marrow granulocytic hypercellularity results in persistent blood neutrophilia. Mature neutrophils and bands constitute most of the neutrophils, with few being less immature forms. The neutrophils may appear normal, but sometimes contain coarse granules. The bone marrow shows normal numbers of blasts or promyelocytes, but myelocytes and mature neutrophils are increased (Fig. 8.21). Splenomegaly and hepatomegaly usually are present. Until recently, CNL was a diagnosis of exclusion requiring an extensive workup for other reactive or neoplastic conditions presenting with neutrophilia. However, the discovery of mutations in the CSF3R gene affecting the receptor for colony stimulating factor in a large subset of CNL patients has changed the approach to CNL and the WHO diagnostic algorithm has been revised to include the presence of CSF3R T618I or other activating CSF3R mutation as a diagnostic criterion of CNL (Table 8.9). Mutational frequency of CSF3R in CNL has been reported to be between 89% and 100%. CSF3R mutations, driver mutations in CNL, are often accompanied by recurrent mutations, such as SETBP1 and ASXL1, present in other myeloid neoplasms. The presence of these mutations in nearly all CNL cases greatly simplifies its distinction from reactive neutrophilia. Two types of CSF3R mutations have been described: membrane proximal, the main mutations that include T618I (exon 14 C-to-T substitution at nucleotide 1853) and T615A, and truncating mutations often seen in association with T618I/T615A. Although both mutational types demonstrate growth factor–independent proliferation, JAK-STAT activation and resulting sensitivity for a JAK1/2 inhibitor ruxolitinib is only seen in the presence of the membrane proximal type. The truncating mutations are sensitive to the multikinase inhibitor dasatinib. The presence of CSF3R mutations is uncommon in other myeloid neoplasms and has been described in rare cases of de novo acute myeloid leukemia (AML; <1%) and atypical CML (3%), the main differential diagnosis for CNL. Both, CNL and atypical CML present with persistent peripheral blood leukocytosis; however, in contrast to CNL where immature myeloid cells are rare (<10% of the blood WBC), in atypical CML, promyelocytes, myelocytes, and metamyelocytes account for at least 10% of blood WBC. Unlike CNL, atypical CML displays dysgranulopoiesis (e.g., nuclear hypolobularity, abnormal chromatin clumping) with or without dyserythropoiesis or dysmegakaryopoiesis (Fig. 8.22; Table 8.10).

Allogeneic stem cell transplant is the preferred therapy option for CNL patients with hydroxyurea as the first-line drug therapy. Investigational therapy includes the use of ruxolitinib, a JAK1/2 inhibitor, in patients who failed hydroxyurea; this treatment may provide symptom palliation without necessarily affecting disease outcome.

POLYCYTHEMIA VERA

PV is one of the classic Philadelphia-negative MPNs characterized by increased red cell production independent of the mechanisms that normally regulate erythropoiesis associated
with the somatic gain-of-function mutations in the cytoplasmic tyrosine kinase JAK2 that lead to hypersensitivity to cytokines and trilineage proliferation. The incidence of PV is estimated at 1.9/100,000 per year with a slight male predominance and the average age of 60 at diagnosis. Many patients are asymptomatic, and the diagnosis may be suspected by the findings of plethora and splenomegaly on examination or abnormalities on a routine blood count. The symptoms typically arise from the erythrocytosis, which causes hyperviscosity and a tendency for venous and arterial thromboses, such as myocardial infarctions, strokes, and venous thromboses of the legs (Table 8.11). In some patients, concomitant platelet abnormalities abet this propensity to clot. Venous occlusion of the intraabdominal vessels—such as the portal, hepatic (Budd–Chiari syndrome), and mesenteric veins—is especially suggestive of PV. Other features presumably related to the erythrocytosis include dizziness, tinnitus, headache, paresthesias, visual disturbances, and painful feet, sometimes associated with digital ischemia and ulcerations despite palpable pulses. A finding probably originating from platelet abnormalities is erythromelalgia—a combination of a burning sensation, erythema, and warmth in the hands and feet that is worsened by exercise, dependency of the extremity, and heat and that is reduced by elevation, cooling, and aspirin. Hemostatic problems, such as epistaxis, gingival hemorrhage, and gastrointestinal bleeding, are common and probably relate to abnormal platelet function and, in about one-third of patients, primarily those with marked thrombocytosis, to the development of acquired von Willebrand disease. The reduction in von Willebrand factor apparently occurs from absorption of large von Willebrand multimers onto platelets, resulting in their removal from the circulation and subsequent destruction. Another symptom, present in about one-half of patients, is itching on exposure to hot water (aquagenic pruritus), probably caused in part by histamine release from basophils. Excessive cell production can lead to weight loss and sweating, presumably from hypermetabolism, and to hyperuricemia, which often leads to attacks of gout. Findings on examination include hypertension and ruddy cyanosis, apparent on the nose, cheeks, lips, ears, neck, and digits. The eyes can appear bloodshot because of conjunctival plethora, and fundus examination may reveal distended, tortuous, and unusually violaceous vessels. Approximately 70% of patients have palpable splenomegaly and almost half have hepatomegaly.

The diagnosis of PV requires fulfillment of the WHO criteria that are based on hemoglobin and/or hematocrit levels, bone marrow biopsy morphologic findings, presence of JAK2 V617F or JAK2 exon 12 mutation and subnormal serum erythropoietin levels, the only minor criterion that is not required if the first three criteria are met (Table 8.12). One of the major criteria, the JAK2 V617F mutation, is present in greater than 95% of PV patients, which although establishes disease clonality, is not specific, as the same mutation is present in a large proportion of ET and PMF cases, although mutant allele burden and evidence of JAK2 V617F homozygosity is significantly higher in PV. On the other hand, JAK2 exon 12 mutation, a rare mutation seen in only 3% of patients, is specific to PV (Table 8.14).

PV progresses through three stages that define clinical presentation and morphologic findings: pre-polycythemic/early phase, polycythemic phase, and post-polycythemic myelofibrosis (post-PV MF) phase. Most PV patients present in the polycythemic phase that is associated with a significantly increased red cell mass leading to markedly elevated hematocrit values, frequently above 60%, leukocytosis of greater than 10,000/μL, and thrombocytosis of greater than 450,000/μL. Because excessive red cell production depletes iron stores, erythrocytes may be microcytic and hypochromic (Fig. 8.23). Other findings sometimes seen on a peripheral blood smear include polychromatophilia, basophilic stippling, nucleated red cells, and markedly enlarged and misshapen platelets.
Patients presenting in early-phase PV, also known as masked PV, may have hemoglobin and hematocrit values that are just slightly elevated; if a red cell mass evaluation is not performed, such patients may be mistakenly diagnosed as ET, especially, those presenting with very high platelet counts. However, discrimination between masked PV and ET is straightforward on morphologic evaluation of a bone marrow biopsy that shows findings similar to those seen in the polycythemic stage of PV. To better capture the early-phase PV patients, the WHO has lowered the hemoglobin/hematocrit levels and included bone marrow biopsy findings as one of the required major criteria to reflect the importance of morphologic evaluation.

Bone marrow biopsy findings in PV (both early and overt polycythemic stages) include age-adjusted hypercellularity and trilineage proliferation, which includes pleomorphic megakaryocytes often occurring in loose clusters. Some patients, especially those with more pronounced megakaryocytic proliferation, may display MF-1 bone marrow fibrosis. Iron stores are nearly always absent.

After a variable time, which is often of many years, approximately 12% to 21% of PV patients progress to a “spent” phase of the disease that is associated with the development of post-PV MF. This phase is characterized by anemia and other cytopenias caused by ineffective erythropoiesis, marrow fibrosis, hypersplenism, and extramedullary production of cells with shortened life spans. The spent phase occurs in up to 50% of patients and, on average, appears about 10 years after the diagnosis. The blood smear shows leukoerythroblastosis (the presence in the peripheral blood of nucleated red cells and cells in the granulocyte series that are more immature than bands [e.g., metamyelocytes, myelocytes]), poikilocytosis, and teardrop cells. The bone marrow demonstrates reticulin and collagen fibrosis (at least MF-2), areas of trilineage hypocellularity, intrasinusoidal hematopoiesis, and osteosclerosis. Atypical megakaryocytes may be present. Less than 10% of PV will develop accelerated or blast phases which may resemble AML or MDS.

To date, no drug has been shown to improve survival, delay in development of marrow fibrosis, or lower risk of leukemic transformation in PV. However, current available treatment modalities extend patient survival to over 13 years. The overall treatment goals in PV patients are to alleviate clinical symptoms and prevent complications associated with thrombotic events and progression to myelofibrosis and/or AML/MDS. Patients without active thrombosis or at low risk of thrombotic complications are treated with therapeutic phlebotomy. In high-risk patients, phlebotomy is supplemented by cytoreductive agents, such as hydroxyurea and interferon-α. Therapeutic considerations for patients with progression to post-PV MF include allogeneic stem cell transplant, JAK1/JAK2 inhibitors, or other investigational disease-modifying agents (these therapies are discussed in more detail in the next section).

**PRIMARY MYELOFIBROSIS**

PMF is the least common Ph-negative MPN that is characterized by a clonal proliferation of megakaryocytes and granulocytic precursors in the bone marrow through dysregulation of the JAK/STAT signaling pathway that is accompanied by various degrees of marrow fibrosis and extramedullary hematopoiesis. In contrast with PV and ET, two other classic subtypes of Ph-negative MPNs, PMF demonstrates a significantly reduced median overall survival estimated at 6.5 years and an increased risk of leukemic transformation. A number of prognostic
scoring systems have been developed to stratify patents into four risk categories (low, intermediate-1, intermediate-2, and high) associated with significantly different overall survival and leukemic transformation rate.\textsuperscript{56,57} Such patient stratification is based on clinical (age \textgreater 65 years, presence of constitutional symptoms and transfusion requirement), hematologic (leukocyte count \textgreater 25,000/\(\mu\)L, hemoglobin \textless 10 g/dL, platelets \textless 100,000/\(\mu\)L, and circulating blasts \textgreater 1%), and cytogenetic (complex karyotype or sole or two abnormalities that include inv(3), −5/5q−, −7/7q−, +8, 11q23 rearrangements, 12p− or i(17q)) parameters.

Although accurate, this scoring system does not reflect the complex genetic landscape of PMF. In contrast to the unfavorable karyotype that is present only in 7\% of PMF patients, mutations in JAK2 V617F, CALR, and MPL are found in approximately 60\%, 20\%, and 5\%, respectively (Table 8.13). Approximately 10\% to 15\% of PMF patients lack any of these mutations and are designated as “triple-negative.”\textsuperscript{3} These mutational groups are associated with different median survival, and the mutational analysis of JAK2, CALR, and MPL should be performed for diagnostic and prognostic purposes. The most recently described CALR mutation is associated with the longest median survival (15.9 years), followed by mutated MPL (9.9 years) and mutated JAK2 V617F (5.9 years); the “triple-negative” group is associated with the shortest median survival of 2.3 years.\textsuperscript{51} In addition, the presence of ASXL1, SRSF2, and EZH2 mutations has been shown to be independently associated with inferior outcome, such as premature death and leukemic transformation identifying “high molecular risk” patients.\textsuperscript{58}

PMF occurs mainly in middle-aged and elderly patients, and the median age at presentation is 67 years.\textsuperscript{59} About 30\% to 40\% of patients are asymptomatic at diagnosis, which may be suggested by splenomegaly, the hallmark of PMF, or abnormal blood tests. Symptoms typically occur from anemia, hypermetabolism from high cell turnover, splenomegaly, or thrombocytopenia (Table 8.14).\textsuperscript{60} Hypermetabolism may cause weight loss, fever, sweats, or problems associated with hyperuricemia, including attacks of gout or renal stones. Anemia can lead to complaints of fatigue, dyspnea, weakness, and palpitations. Splenomegaly can produce left upper quadrant discomfort, a sensation of early satiety from compression of the stomach, or diarrhea from pressure on the bowels. On examination, splenomegaly is nearly universal and often enormous, and hepatomegaly is present in about one-half of the patients.\textsuperscript{61} Thrombocytopenia or altered platelet function may cause cutaneous hemorrhage, and, occasionally, extramedullary hematopoiesis in the skin produces erythematous or purplish nodules, papules, and plaques. Extramedullary hematopoiesis is most common in the spleen and liver, but can also affect many other sites, such as the dura mater, lymph nodes, lung, and pleura (Fig. 8.28).\textsuperscript{62}

PMF represents a stepwise evolution from an initial early (prefibrotic or minimally fibrotic) phase to an overt fibrotic phase. Clinical, laboratory, and morphologic findings depend on the disease phase. Approximately one-third of the patients are diagnosed during the prefibrotic phase, in which the characteristic findings are borderline anemia, thrombocytosis, and mild leukocytosis clinically mimicking ET.\textsuperscript{63} The blood smear may be unremarkable or only show occasional teardrop-shaped erythrocytes (dacrocytes) and large platelets. Discrimination between early/prefibrotic PMF and ET is clinically relevant, as patients presenting in the prefibrotic PMF phase demonstrate worse overall and event-free survival, higher rate of progression to fibrotic stage, and transformation to acute leukemia.\textsuperscript{64} Because of a significant overlap in clinical, laboratory, and molecular findings, the distinction between prefibrotic PMF and ET remains based predominantly on bone marrow biopsy morphologic assessment that is reflected in the new WHO criteria for early (prefibrotic or minimally fibrotic) PMF (Table 8.15).\textsuperscript{4} Unlike ET, the marrow cellularity in prefibrotic PMF
is increased because of granulocytic and megakaryocytic proliferation. Megakaryocytes are seen in loose and tight clusters, and demonstrate an atypical appearance because of the presence of an increased nuclear:cytoplasmic ratio, abnormal chromatin clumping and hyperchromatic changes producing the so-called “bulbous” or “cloud-like” nuclei; presence of bare megakaryocytic nuclei is another commonly observed finding (Table 8.16; Figs. 8.29 and 8.30). Fibrosis at this stage is absent or minimal (MF-0 or MF-1). The prominent hematologic findings of the overtly fibrotic phase (MF-2 or MF-3) are anemia and a leukoerythroblastic blood smear (nucleated erythrocytes, immature granulocytes) with numerous dacrocytes. White cell and platelet counts vary widely, but a few myeloblasts are common, and platelet abnormalities include large size, bizarre shape, circulating megakaryocyte nuclei, and micromegakaryocytes (Fig. 8.31). Bone marrow aspiration is usually unsuccessful, but the bone marrow biopsy demonstrates variable cellularity, with increased deposition of reticulin and/or collagen fibrosis and numerous atypical megakaryocytes in large aggregates. Osteosclerosis (new bone formation) may be visible (Figs. 8.32 and 8.33). Overt PMF, especially the advanced stages, may be difficult to distinguish from fibrotic stages of ET and PV. The diagnosis of overt PMF requires meeting three major and at least one minor WHO criteria that are based on clinical, morphologic, laboratory, and molecular findings (Table 8.17).

One of the important diagnostic and prognostic morphologic findings in PMF is the presence of myelofibrosis. Absent or minimal myelofibrosis (MF-0 and MF-1) define early/prefibrotic PMF, whereas advanced myelofibrosis grades (MF-2 and MF-3) define overt PMF, as well as progression of ET and PV to the post-ET myelofibrosis (post-ET MF) and post-PV MF stages. Progressive bone marrow fibrosis is associated with increasing density of reticulin fibers and collagen deposition, and is graded on a semiquantitative scale that utilizes reticulin and trichrome stains (Figs. 8.34 and 8.35; Table 8.18). Strict adherence to the WHO myelofibrosis grading criteria significantly improves accuracy and interobserver reproducibility. In addition, in the era of the novel disease-modifying agents (see below) that have been shown to diminish or resolve bone marrow fibrosis, dynamic myelofibrosis grade is utilized for treatment response assessment and prognosis. It is important to mention that myelofibrosis is not specific to Ph-negative MPNs and may accompany a large number of other neoplastic and nonneoplastic conditions, which should be excluded, especially in the absence of any clonal mutations (Table 8.19).

To date, allogeneic stem cell transplant remains the only potential curative modality for patients with PMF, post-PV MF, and post-ET MF, and should be considered for all eligible patients with estimated survival rates of less than 3 years. However, most of these patients are not candidates for stem cell transplant and their care is directed toward alleviation of debilitating symptoms associated with progressive marrow fibrosis, such as anemia, extramedullary hematopoiesis, and massive splenomegaly. Traditional treatments do not generally delay or reverse bone marrow fibrosis, which prompted development of novel disease-modifying agents that have demonstrated antifibrotic activity in preclinical and clinical studies. Ruxolitinib, a JAK1/JAK2 inhibitor, has been approved for patients with advanced myelofibrosis, and has been shown to reduce splenomegaly, improve quality of life, and prolong overall survival. In addition, long-term therapy with ruxolitinib has resulted in resolution or improvement in bone marrow fibrosis and cellularity, suggesting that long-term treatment may delay progression. Currently, a number of ongoing clinical trials are investigating the role of other agents, such as the histone deacetylase inhibitor panobinostat, the telomerase inhibitor imetelstat, and recombinant pentraxin-2 (PRM-151), in preventing disease progression.
ET is a clonal MPN that predominantly affects megakaryocytes resulting in sustained excessive thrombocytosis. The average age at diagnosis is about 50 to 60 years; however, up to 20% of patients are below the age of 40 years at presentation. Most patients are asymptomatic presenting with an incidental finding of thrombocytosis on routine blood tests. When symptoms occur, they usually are related to vessel thrombosis or abnormal vascular reactivity, such as dizziness, headaches, visual disturbances, transient ischemic attacks, digital ischemia, and paresthesias. One form of vascular abnormality is erythromelalgia, a combination of erythema, burning pain, and warmth of the hands and feet that is exacerbated by dependency, heat, or exercise and is relieved by elevation, cooling, and aspirin. It is typically asymmetric. Thrombosis usually involves arteries, but venous occlusion also can occur. Hemorrhage is less frequent and is usually associated with extremely high platelet counts above 1,000/µL. Sometimes bleeding occurs from acquired von Willebrand disease associated with marked thrombocytosis, in which the large von Willebrand multimers adhere to circulating platelets and are removed prematurely from the circulation. Splenomegaly, usually moderate and nonprogressive, is present in about 50% of patients, and is associated with platelet sequestration rather than with extramedullary hematopoiesis.

The platelet count commonly exceeds 1,000 × 10^9/L, and the most prominent finding on blood smear is the large number of platelets, which vary considerably in size, ranging from small to giant (diameter larger than an erythrocyte) (Fig. 8.36). White cells are typically normal in number and appearance, as are the erythrocytes, unless hemorrhage has led to iron deficiency anemia.

A variety of reactive and neoplastic conditions can present with an increased platelet count mimicking ET (Table 8.20), and an accurate diagnosis of ET relies on a composite assessment of clinical, morphologic, and molecular findings, and fulfillment of the WHO criteria (Table 8.21). Detection of JAK2 V617F, CALR, and MPL mutations in approximately 90% of ET cases confirms the presence of a clonal MPN-associated thrombocytosis allowing distinction from reactive condition-associated thrombocytosis. However, these mutations are not present in approximately 10% of ET, so-called “triple-negative” ET (Table 8.13). In these patients, the ET diagnosis relies on the detection of other somatic mutations, exclusion of reactive and certain neoplastic conditions, and meeting the morphologic criteria for ET.

The bone marrow is normocellular for age with markedly increased numbers of megakaryocytes often of large size with hyperlobulated “stag horn” nuclei, which commonly form loose aggregates. In contrast to PMF, the megakaryocytes seen in ET show normal maturation and lack atypia (Figs. 8.37 and 8.38). Minimal reticulin fibrosis (MF-1) may occur, especially in association with loose megakaryocytic clusters, but a higher fibrosis grade and presence of collagen fibrosis suggests another diagnosis. As discussed in the Primary Myelofibrosis section, ET should be distinguished from an early/prefibrotic stage PMF; that distinction could be efficiently made on morphologic evaluation of bone marrow biopsy (Table 8.16; Fig. 8.39). Another potential mimicker of ET is myelodysplastic/MPN with ring sideroblasts and thrombocytosis (MDS/MPN with RS-T) that, similar to ET, presents with persistent thrombocytosis at least 450 × 10^9/L that may be associated with JAK2 V617F, CALR, or MPL mutations. However, unlike ET, patients with MDS/MPN with RS-T demonstrate various degree of anemia. Bone marrow evaluation shows erythroid proliferation with unilineage or multilineage dysplasia and presence of at least 15% ring sideroblasts (Fig. 8.40). Detection of SF3B1 mutation with or without JAK2 V617F, CALR, or
MPL mutations strongly supports a diagnosis of MDS/MPN with RS-T (Table 8.22).4

The life expectancy of patients with strictly WHO-defined ET is nearly normal with a 15-year survival of approximately 80% and the 10-year risk of progression to AML or myelofibrosis of less than 1%.87 Risk factors for survival in ET include advanced age, leukocytosis, and thrombocytosis, and the current risk stratification in ET, similar to PV, is designed to estimate the likelihood of thrombotic complications rather than the risk of fibrotic or leukemic transformation.89 Predictors of arterial thrombosis include age older than 60 years, prior thrombosis, leukocytosis of greater than $11 \times 10^9/L$, cardiovascular risk factors, and presence of JAK2 V617 mutation.83 In contrast, only male gender is associated with venous thrombosis. CALR mutation is associated with high platelet count at presentation and a lower risk of thrombosis when compared to the JAK2 mutation with no difference in the rate of myelofibrosis transformation.9 Therapeutic goals in ET are prevention of thrombotic and bleeding complications and alleviation of vasomotor symptoms. Low-risk patients are either observed or treated with low-dose aspirin; treatment of high-risk patients includes aspirin, hydroxyurea, and interferon-α.87

**CHRONIC EOSINOPHILIC LEUKEMIA, NOT OTHERWISE SPECIFIED**

Chronic eosinophilic leukemia, not otherwise specified (CEL NOS), is a clonal MPN defined by an increased number of eosinophils in peripheral blood and bone marrow that excludes all genetically defined myeloid neoplasms associated with eosinophilia (Table 8.23). Idiopathic hypereosinophilic syndrome (HES) is defined by persistent unexplained eosinophilia without underlying causes with associated organ damage and absence of eosinophil clonality. Idiopathic HES is a diagnosis of exclusion and may include some unrecognized cases of CEL NOS in which clonality cannot be proven, or cases of cytokine-driven eosinophilia.90

Both CEL NOS and idiopathic HES are extremely rare; however, true incidence and prevalence are unknown.91 The disease has male predominance. Patients present with peripheral blood eosinophilia of at least $1.5 \times 10^9/L$ and various combinations of symptoms related to tissue infiltration by eosinophils or the toxic materials that eosinophils produce. Most patients with these disorders are symptomatic with constitutional complaints, such as pruritus, fever, and fatigue, or with problems related to a specifically affected area, such as cough, peripheral neuropathy, or rashes.92 Some patients present with life-threatening conditions because of the rapid evolution of cardiovascular and neurologic complications. Heart involvement includes a restrictive cardiomyopathy caused by endomyocardial fibrosis and scarring of the mitral or tricuspid valves, leading to valvular regurgitation.90

The blood smear shows markedly increased eosinophils, with mainly mature forms, but with variation in size, granulation, and nuclear segmentation. Basophilia, neutrophilia, or monocytosis may be present. The bone marrow is hypercellular and demonstrates eosinophilic proliferation, usually with normal maturation and no significant dysplasia. Charcot–Leyden crystals are often plentiful (Fig. 8.41). In the absence of clonal genetic abnormalities, the morphologic findings of increased number of myeloblasts ($\geq 2\%$ in peripheral blood and 5%–19% in bone marrow) or lineage dysplasia support the diagnosis of CEL, NOS (Table 8.24).93,94 A diagnosis of idiopathic HES can be made in patients with persistent peripheral blood eosinophilia of at least $1.5 \times 10^9/L$, tissue damage due to eosinophilic infiltration, and absence of reactive or clonal eosinophilia.
The prognosis of WHO-defined CEL NOS is poor with the median survival of less than 2 years with a high risk of leukemic transformation.  

CHRONIC MYELOPROLIFERATIVE NEOPLASM, UNCLASSIFIABLE

Chronic myeloproliferative disease, unclassifiable, refers to disorders in which MPN is definitely present, based on clinical and laboratory findings, but the features fail to conform to the diagnostic entities delineated above. The disease may (1) be early in its course and not yet fully developed into a clear diagnostic category; (2) be late in the course and end-stage findings, such as advanced myelofibrosis or osteosclerosis, have replaced earlier diagnostic features; or (3) possess characteristics of two or more myeloproliferative disorders. Presence of BCR-ABL1 or PCM1-JAK2 translocations, rearrangements of PDGFRα, PDGFRβ, or FGFR1, or other disease-defining abnormalities exclude this entity.

REFERENCES

11. Mesa RA, Verstovsek S, Cervantes F, et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): consensus on terminology by the International Working Group for Myelofibrosis Research and Treatment (IWG-


### Table 8.1

<table>
<thead>
<tr>
<th>The revised 2016 WHO classification of myeloproliferative neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic myeloid leukemia (CML), BCR-ABL1–positive</strong></td>
</tr>
<tr>
<td><strong>Chronic neutrophilic leukemia (CNL)</strong></td>
</tr>
<tr>
<td><strong>Polycythemia vera (PV)</strong></td>
</tr>
<tr>
<td><strong>Primary myelofibrosis (PMF)</strong></td>
</tr>
<tr>
<td>- Primary myelofibrosis, prefibrotic/early stage</td>
</tr>
<tr>
<td>- Primary myelofibrosis, overt fibrotic stage</td>
</tr>
<tr>
<td><strong>Essential thrombocytemia (ET)</strong></td>
</tr>
<tr>
<td><strong>Chronic eosinophilic leukemia, not otherwise specified (CEL NOS)</strong></td>
</tr>
<tr>
<td><strong>Myeloproliferative neoplasm (MPN), unclassifiable</strong></td>
</tr>
</tbody>
</table>


### Table 8.2

<table>
<thead>
<tr>
<th>Comparison of clinical, morphologic, and molecular features between myeloproliferative neoplasms and myelodysplastic syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Features</strong></td>
</tr>
<tr>
<td>----------------------------------------------------------------</td>
</tr>
<tr>
<td>Bone marrow cellularity</td>
</tr>
<tr>
<td>Maturation</td>
</tr>
</tbody>
</table>
### Hematopoiesis

<table>
<thead>
<tr>
<th>Peripherally Cell counts</th>
<th>Effective</th>
<th>Ineffective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood cell counts</td>
<td>Increased (erythrocytosis, neutrophilia, thrombocytosis)</td>
<td>Decreased (anemia, leukopenia, thrombocytopenia)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell morphology</th>
<th>Mostly normal*</th>
<th>Defined by presence of lineage(s) dysplasia</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Organomegaly</th>
<th>Present (splenomegaly, hepatomegaly)</th>
<th>Absent</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Evolution to acute leukemia</th>
<th>Present (different but low rates)</th>
<th>Present (high rates)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Molecular or cytogenetic features</th>
<th>Defined by mutations or rearrangements of tyrosine kinase genes†</th>
<th>Mostly shared with acute myeloid leukemia</th>
</tr>
</thead>
</table>

*Overt cell dysplasia is usually absent at initial MPN stages; however, dysplasia may develop in later stages or upon disease progression.

†In addition to disease-defining tyrosine kinase mutations/rearrangements, additional mutations in myeloid-associated genes may be present at presentation or upon disease progression.

### Table 8.3

**Molecular genetic findings in myeloproliferative neoplasms (MPNs)**

<table>
<thead>
<tr>
<th>Unique “Driver” Molecular Abnormalities</th>
<th>Common Most Frequent Molecular Mutations*</th>
<th>Cytogenetic Aberrations†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myeloid leukemia</td>
<td>BCR-ABL1</td>
<td>TP53, RB1, MYC, RAS, AML1, EVI1, CDKN2A</td>
</tr>
<tr>
<td>Chronic neutrophilic leukemia</td>
<td>CSF3R T618I or other CSF3R activating mutations</td>
<td>Chromatin modifiers: ASXL1, EZH2, Spliceosome: SRSF2, SF3B1, DNA methylation: TET2, DNMT3A, IDH1, IDH2, TP53</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>JAK2 V617F</td>
<td>Activated signaling: RAS, CBL</td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
<td>JAK2 V617F, MPL exon 10, CALR exon 9</td>
<td></td>
</tr>
<tr>
<td>Essential thrombocythemia</td>
<td>JAK2 V617F, MPL exon 10, CALR exon 9</td>
<td></td>
</tr>
<tr>
<td>Chronic eosinophilic leukemia, NOS</td>
<td>No unique somatic mutations</td>
<td></td>
</tr>
<tr>
<td>MPN, unclassifiable</td>
<td>No unique somatic mutations</td>
<td></td>
</tr>
</tbody>
</table>

*Some molecular abnormalities can precede the “driver” mutation or acquired during disease progression.

†Some cytogenetic abnormalities are associated with accelerated phase and/or leukemic transformation.

### Table 8.4

**Symptoms and signs of chronic phase chronic myeloid leukemia (CML) at presentation**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Patients Experiencing Sign or Symptom (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>83</td>
</tr>
<tr>
<td>Weight loss</td>
<td>61</td>
</tr>
<tr>
<td>Abdominal fullness and anorexia</td>
<td>38</td>
</tr>
<tr>
<td>Easy bruising or bleeding</td>
<td>35</td>
</tr>
</tbody>
</table>
Table 8.5

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Patients Exhibiting Abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleated erythrocytes</td>
<td>98</td>
</tr>
<tr>
<td>Dyserythropoietic (binucleate)</td>
<td>12</td>
</tr>
<tr>
<td>Target cells</td>
<td>2</td>
</tr>
<tr>
<td>Giant platelets</td>
<td>2</td>
</tr>
<tr>
<td>Megakaryocytic nucleoli</td>
<td>24</td>
</tr>
<tr>
<td>Binucleate of lobular leukocyte nuclei</td>
<td></td>
</tr>
<tr>
<td>Blasts</td>
<td>2</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>2</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>6</td>
</tr>
<tr>
<td>Hypogranular leukocytes</td>
<td></td>
</tr>
<tr>
<td>Myelocytes</td>
<td>8</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
<td>2</td>
</tr>
<tr>
<td>Basophils</td>
<td>12</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>4</td>
</tr>
<tr>
<td>Cells in mitosis</td>
<td>14</td>
</tr>
<tr>
<td>Giant metamyelocytes</td>
<td>24</td>
</tr>
<tr>
<td>Pelger–Huët cells</td>
<td>4</td>
</tr>
<tr>
<td>Hypersegmented neutrophils</td>
<td>12</td>
</tr>
<tr>
<td>Leukocytes with mixed basophil-eosinophil granules</td>
<td>8</td>
</tr>
</tbody>
</table>

**Note:** As shown in the figures in this chapter, a marked left-shifted leukocytosis with an accompanying basophilia is among the most important peripheral smear findings in CML. Numerous morphologic abnormalities, as described in this table, also are observed. The white blood cell counts in CML may range from 20,000 to over 500,000/μL; the higher white blood cell count helps distinguish CML from reactive leukocytoses, atypical CML, and chronic myelomonocytic leukemia.


Table 8.6

**WHO diagnostic criteria for chronic myeloid leukemia, BCR-ABL1–positive, accelerated phase**

**Clinical**
- Persistent or increasing WBC (>10 × 10⁹/L), unresponsive to therapy
- Persistent or increasing splenomegaly, unresponsive to

**Cytogenetic**
- Additional Ph+, trisomy 8, isochromosome 17q, trisomy 19, complex karyotype, or abnormalities of 3q26.2
- Any new clonal chromosomal abnormality during therapy


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therapy
• Persistent thrombocytosis (>1,000 × 10^9/L), unresponsive to therapy
• Persistent thrombocytopenia (<100 × 10^9/L) unrelated to therapy

Morphologic
• 20% or more basophils is peripheral blood
• 10%–19% blasts in the peripheral blood and/or bone marrow

“Provisional” response-to-TKI criteria
• Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response to the first TKI) or
• Any hematologic, cytogenetic, or molecular indications of resistance to two sequential TKIs or
• Occurrence of two or more mutations in BCR-ABL1 during TKI therapy

Table 8.7
Definitions of hematologic, cytogenetic, and molecular response in chronic myeloid leukemia, BCR-ABL1–positive

<table>
<thead>
<tr>
<th>Response</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic</strong></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>WBC &lt;10 × 10^9/L</td>
</tr>
<tr>
<td></td>
<td>Basophils &lt;5%</td>
</tr>
<tr>
<td></td>
<td>Absence of myelocytes, promyelocytes, and blasts on peripheral blood smear</td>
</tr>
<tr>
<td></td>
<td>Platelet count &lt;450 × 10^9/L</td>
</tr>
<tr>
<td></td>
<td>Nonpalpable spleen</td>
</tr>
<tr>
<td><strong>Cytogenetic</strong></td>
<td></td>
</tr>
<tr>
<td>Major</td>
<td>Complete: no Ph+ metaphases or &lt;1% BCR-ABL1+ nuclei of ≥200 nuclei by FISH</td>
</tr>
<tr>
<td></td>
<td>Partial: 1%–35% Ph+ metaphases</td>
</tr>
<tr>
<td>Minor</td>
<td>36%–65% Ph+ metaphases</td>
</tr>
<tr>
<td>Minimal</td>
<td>66%–95% Ph+ metaphases</td>
</tr>
<tr>
<td>None</td>
<td>&gt;95% Ph+ metaphases</td>
</tr>
<tr>
<td><strong>Molecular</strong></td>
<td></td>
</tr>
<tr>
<td>MR&lt;sup&gt;4,5&lt;/sup&gt;</td>
<td>Ratio of BCR-ABL1 to ABL1 (or other housekeeping gene) ≤0.0032% (≥4.4 log reduction) on international scale</td>
</tr>
<tr>
<td>MR&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Ratio of BCR-ABL1 to ABL1 (or other housekeeping gene) ≤0.01% (≥4 log reduction) on international scale</td>
</tr>
<tr>
<td>MR&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Ratio of BCR-ABL1 to ABL1 (or other housekeeping gene) ≤0.1% (≥43 log reduction) on international scale (also termed “major molecular response”)</td>
</tr>
<tr>
<td>MR&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Ratio of BCR-ABL1 to ABL1 (or other housekeeping gene) ≤1% (≥2 log reduction) on international scale (corresponds to complete cytogenetic response)</td>
</tr>
</tbody>
</table>

Table 8.8
Cytogenetic and molecular monitoring for patients with chronic myeloid leukemia, BCR-ABL1–positive

<table>
<thead>
<tr>
<th>At diagnosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Karyotype of marrow cell metaphases</td>
<td>FISH to identify variant, cryptic translocations</td>
</tr>
<tr>
<td>Qualitative PCR to identify transcript type</td>
<td></td>
</tr>
<tr>
<td>During treatment</td>
<td>QR-PCR to determine BCR-ABL1 transcript levels, every 3 mo until MMR and then every 6 mo</td>
</tr>
</tbody>
</table>

and/or
Karyotype of marrow cell metaphases at 3, 6, and 12 mo until CCyR, then every 12 mo

At progression QR-PCR, mutational analysis, karyotype of marrow cell metaphases; blasts immunophenotype

Table 8.9
WHO diagnostic criteria for chronic neutrophilic leukemia (CNL)

1. Peripheral blood leukocytosis ≥25 × 10⁹/L
   - Segmented neutrophils and bands constitute ≥80% of the white blood cells
   - Promyelocytes, myelocytes, and metamyelocytes constitute <10% of WBC
   - Rare myeloblasts
   - Monocyte count <1 × 10⁹/L
   - Absence of dysgranulopoiesis
2. Hypercellular bone marrow
   - Increased neutrophils in percentage and number
   - Normal neutrophil maturation
   - Myeloblasts <5% of nucleated cells
3. Not meeting WHO criteria for CML, BCR-ABL1–positive, PV, ET, or PMF
4. Absence of PDGFRα, PDGFRβ, or FGFR1, or PCM1-JAK2
5. Presence of CSF3R T618I or other activating CSF3R mutation
   - In the absence of a CSF3R mutation, persistent neutrophilia (≥3 mo), splenomegaly, and no identifiable cause of reactive neutrophilia, including absence of a plasma cell neoplasm or, if present, demonstration of clonally myeloid cells by cytogenetic or molecular studies


Table 8.10
WHO diagnostic criteria for atypical chronic myeloid leukemia, BCR-ABL1–negative

- Peripheral blood leukocytosis due to increased numbers of neutrophils and their precursors (promyelocytes, myelocytes, metamyelocytes) composing ≥10% of leukocytes
- Dysgranulopoiesis, which may include abnormal chromatin clumping
- No or minimal absolute basophilia; basophils usually <2% of leukocytes
- No or minimal absolute monocytosis; monocytes <10% of leukocytes
- Hypercellular BM with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages
- Less than 20% blasts in the blood and bone marrow
- No evidence of PDGFRA, PDGFRB, or FGFR1 rearrangement, or PCM1-JAK2
- Not meeting WHO criteria for BCR-ABL1–positive chronic myeloid leukemia, primary myelofibrosis, polycythemia vera, or essential thrombocythemia


Table 8.11
Physical findings and symptoms in polycythemia vera

<table>
<thead>
<tr>
<th>Physical Findings</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenomegaly</td>
<td>70</td>
</tr>
<tr>
<td>Skin plethora</td>
<td>67</td>
</tr>
<tr>
<td>Conjunctival plethora</td>
<td>59</td>
</tr>
<tr>
<td>Engorged retinal vessels</td>
<td>46</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>40</td>
</tr>
</tbody>
</table>

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Systolic blood pressure >140 mm Hg  72
Diastolic blood pressure >90 mm Hg  32

**Symptoms**

- Headache  48
- Weakness  47
- Pruritus  43
- Dizziness  43
- Diaphoresis  33
- Visual disturbances  31
- Weight loss  29
- Paresthesias  29
- Dyspnea  26
- Joint symptoms  26
- Epigastric discomfort  24


**Table 8.12**

**WHO diagnostic criteria for polycythemia vera (PV)**

Diagnosis of PV requires meeting either all three major criteria, or the first two major criteria and the minor criterion.

**Major criteria:**

1. Hemoglobin >16.5 g/dL in men and >16.0 g/dL in women
   or
   Hematocrit >49% in men and >48% in women
   or
   Increased red cell mass (>25% above mean normal predicted value)
2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (different in size)
3. Presence of JAK2 V617F or JAK2 exon 12 mutation

**Minor criterion:**

Subnormal serum erythropoietin levels


**Table 8.13**

**Shared driver mutations in Philadelphia-negative myeloproliferative neoplasms**

<table>
<thead>
<tr>
<th></th>
<th>Polycythemia Vera (%)</th>
<th>Primary Myelofibrosis (%)</th>
<th>Essential Thrombocythemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2 V617F</td>
<td>&gt;95</td>
<td>60–65</td>
<td>60–65</td>
</tr>
<tr>
<td>JAK2 exon 12</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CALR</td>
<td>—</td>
<td>20–25</td>
<td>20–25</td>
</tr>
<tr>
<td>MPL</td>
<td>—</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>“Triple-negative”</td>
<td>—</td>
<td>10–15</td>
<td>5–10</td>
</tr>
</tbody>
</table>

**Table 8.14**

**Clinical findings at diagnosis among patients with primary myelofibrosis**
Very common findings (>50% of cases)

- Splenomegaly
- Hepatomegaly
- Fatigue
- Anemia
- Leukocytosis
- Thrombocytosis

Common findings (10%–50% of cases)

- Asymptomatic
- Weight loss
- Night sweats
- Bleeding
- Splenic pain
- Leukocytopenia
- Thrombocytosis
- Thrombocytopenia

Uncommon findings (<10% of cases)

- Peripheral edema
- Portal hypertension
- Lymphadenopathy
- Jaundice
- Gout

Table 8.15
WHO diagnostic criteria for prefibrotic/early primary myelofibrosis

Requires all three major criteria and at least one minor criterion.

**Major criteria:**
1. Megakaryocytic proliferation with atypia, without reticulin fibrosis >1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for BCR-ABL1–positive CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker or absence of minor reactive reticulin fibrosis

**Minor criteria:**
Presence at least one of the following, confirmed in two consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 × 10^9/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range

LDH, lactate dehydrogenase.


Table 8.16
Comparison of bone marrow morphologic findings in prefibrotic primary myelofibrosis (PMF) and essential thrombocythemia (ET)

<table>
<thead>
<tr>
<th>Early/Prefibrotic PMF</th>
<th>ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity (age-matched)</td>
<td>Increased</td>
</tr>
</tbody>
</table>
### WHO diagnostic criteria for overt primary myelofibrosis (PMF)

Requires all three major criteria and at least one minor criterion.

**Major criteria:**
1. Presence of megakaryocytic proliferation and atypia accompanied by either reticulin and/or collagen fibrosis grades 2 or 3
2. Not meeting the WHO criteria for *BCR-ABL1*–positive CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker or absence of minor reactive reticulin fibrosis

**Minor criteria:**
Presence at least one of the following, confirmed in two consecutive determinations:
- Anemia not attributed to a comorbid condition
- Leukocytosis $\geq 11 \times 10^9/L$
- Palpable splenomegaly
- LDH increased to above upper normal limit of institutional reference range
- Leukoerythroblastosis

LDH, lactate dehydrogenase.


### WHO grading of myelofibrosis

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF-0</td>
<td>Scattered linear reticulin with no intersections (crossovers) corresponding to normal bone marrow</td>
</tr>
<tr>
<td>MF-1</td>
<td>Loose network of reticulin with many intersections, especially in perivascular areas</td>
</tr>
<tr>
<td>MF-2</td>
<td>Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen† and/or focal osteosclerosis</td>
</tr>
<tr>
<td>MF-3</td>
<td>Diffuse and dense reticulin with extensive intersections and coarse bundles of collagen,† often associated with osteosclerosis</td>
</tr>
</tbody>
</table>

*Fiber density should be assessed in hematopoietic (cellular) areas.
†In grades MF-2 and MF-3, an additional trichrome stain is recommended.


### Conditions in which myelofibrosis may occur

#### Neoplastic conditions
- Chronic myeloproliferative neoplasms
- Primary myelofibrosis
- Polycythemia vera
| Chronic myeloid leukemia, \textit{BCR-ABL1}–positive |
| Acute megakaryoblastic leukemia (M7) |
| Myelodysplasia with fibrosis |
| “Transitional” agnogenic myeloid metaplasia–myelodysplastic myeloproliferative syndrome |
| Other acute myeloid leukemias |
| Acute lymphoid leukemia |
| Hairy-cell leukemia |
| Myeloma |
| Carcinoma |
| Systemic mastocytosis |

**Nonneoplastic conditions**

- Granulomatous disease
- Paget disease
- Hypoparathyroidism
- Hyperparathyroidism
- Osteoporosis
- Renal osteodystrophy
- Vitamin D deficiency
- Gray platelet syndrome
- Systemic lupus erythematosus
- Systemic sclerosis

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Table 8.20

| Reactive and neoplastic conditions that can present with thrombocytosis |

**Nonneoplastic conditions**

- Nonneoplastic hematologic conditions
  - Acute blood loss
  - Acute hemolytic anemia
  - Iron deficiency anemia
  - Treatment of vitamin B\textsubscript{12} deficiency
  - Rebound effect after treatment for ITP or ethanol-induced thrombocytopenia
- Inflammatory conditions
  - Rheumatoid disorders
  - Vasculitides
  - IBD
  - Celiac disease
  - POEMS syndrome
  - Tissue damage
    - Trauma, myocardial infarction, thermal burns
  - Infections
  - Exercise
  - Allergic/medication reactions
  - Asplenia

**Neoplastic conditions**

- Other myeloproliferative neoplasms
  - Polycythemia vera, early stage
  - Primary myelofibrosis, early/prefibrotic stage
  - Chronic myeloid leukemia, \textit{BCR-ABL1}–positive
  - Myelodysplastic syndrome
  - MDS with isolated del(5q)
  - Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis

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Table 8.21

WHO diagnostic criteria for essential thrombocythemia

Diagnosis of ET requires meeting all four major criteria or the first three major and the minor criteria.

**Major criteria:**
- Platelet count \( \geq 450 \times 10^9/L \)
- Bone marrow biopsy showing megakaryocytic hyperplasia with enlarged, mature megakaryocytes with hyperlobulated nuclei, no significant increase or left-shift in myelopoiesis or erythropoiesis and minor reticulin fibrosis (MF-1)
- Not meeting criteria for \( BCR-ABL1 \)–positive CML, PV, PMF, MDS or other myeloid neoplasms
- Presence of \( JAK2 \), \( CALR \) or \( MPL \) mutations

**Minor criterion:**
- Presence of a clonal marker or no evidence of reactive thrombocytosis


Table 8.22

Comparison of bone marrow morphologic findings in myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN with RS-T) and essential thrombocythemia

<table>
<thead>
<tr>
<th></th>
<th>MDS/MPN with RS-T</th>
<th>Essential Thrombocythemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity (age-matched)</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Myeloid hyperplasia</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Megakaryocytic proliferation</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Megakaryocyte morphology</td>
<td>Hypolobate</td>
<td>Stag horn hyperlobated nuclei</td>
</tr>
<tr>
<td>Megakaryocyte size</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Megakaryocyte clusters</td>
<td>Absent</td>
<td>Loose</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>MF-0 or MF-1</td>
<td>MF-0</td>
</tr>
<tr>
<td>Ring sideroblasts on iron stain</td>
<td>Present ( \geq 15% )</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Table 8.23

Primary and secondary causes of eosinophilia

**Primary (clonal) eosinophilia**
- Myeloid neoplasms
  - Chronic myeloid leukemia, \( BCR-ABL1 \)–positive
  - Acute myeloid leukemia with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)
  - Acute myeloid leukemia with t(8;21)(q22;q22.1)
  - Chronic myelomonocytic leukemia
- Myeloid and lymphoid neoplasms associated with \( PDGFR A, PDGFR B, \) or \( FGFR1 \) rearrangements or \( PCM1-JAK2, ETV6-JAK2, \) or \( BCR-JAK2 \) fusion genes
- Mastocytosis
- Lymphoid neoplasms
  - T-cell lymphoma
  - Hodgkin lymphoma
  - Acute lymphoblastic leukemia
  - Lymphocyte-variant hyper eosinophilic

**Secondary (nonclonal) eosinophilia**
- Infections
- Parasites
- Specific fungal infections
- Allergy
- Drug reactions
- Collagen vascular disease

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• Churg–Strauss syndrome
• Wegener granulomatosis
• Systemic lupus erythematosus
• Pulmonary diseases
  • Idiopathic acute or chronic eosinophilic pneumonia
  • Tropical pulmonary eosinophilia
  • Allergic bronchopulmonary aspergillosis
• Gastroenteritis
• Adrenal insufficiency
• Immunologic conditions

Table 8.24
Diagnostic criteria for chronic eosinophilic leukemia, not otherwise specified

1. Presence of eosinophilia (eosinophil count ≥1.5 × 10^9/L)
2. Not meeting WHO criteria for BCR-ABL1 positive chronic myeloid leukemia, PV, ET, PMF, CNL, CMML or aCML
3. No rearrangement of PDGFRA, PDGFRB or FGFR1; no PCM1-JAK2, ETV6-JAK2 or BCR-JAK2 fusion gene
4. The blast cell count in the peripheral blood and bone marrow is less than 20% and inv(16)(p13.1q22), t(16;16)(p13.1;q22), t(8;21)(q22;q22.1) and other diagnostic features of AML are absent
5. There is a clonal cytogenetic or molecular genetic abnormality or blast cells are ≥2% in the peripheral blood or ≥5% in the bone marrow


Figure 8.1. Splenomegaly in myeloproliferative neoplasms. A: Excised spleen from a case of chronic myeloid leukemia, BCR-ABL1–positive, showing a markedly enlarged spleen
weighing more than 1,000 g and measuring approximately 30 cm in greatest dimension (label represents 5 cm). **B:** Diffusely expanded red pulp made up of extramedullary hematopoiesis.

**Figure 8.2.** Hepatosplenomegaly and osteosclerosis in myeloproliferative neoplasms. **A:** Axial computed tomography (CT) scan of the upper abdomen showing homogenously enlarged spleen and liver in a patient with primary myelofibrosis. **B:** Coronal CT scan with bone windows in a patient with primary myelofibrosis showing a mildly enlarged spleen (normal is <14 cm). Increased density of the visualized bones is present.
Figure 8.3. Osteosclerosis in primary myelofibrosis. Chest radiographs, PA (A) and lateral (B) views, show diffuse increased density throughout bony skeleton in a patient with advanced primary myelofibrosis with osteosclerosis. C: The density of ribs is prominent on CT scan (computer tomography scan).

Figure 8.4. Osteosclerosis in primary myelofibrosis. A and B: Normal adult bone marrow consists of cellular and fat elements interspersed among thin bony trabeculae that appear, on
gross examination, as red and white tissue, respectively. C and D: In primary myelofibrosis, by contrast, cellular marrow is replaced by deposition of collagen and appears uniformly white.

Figure 8.5. Cachexia and hepatosplenomegaly in myeloproliferative neoplasms. Massive hepatosplenomegaly in a woman with chronic myeloid leukemia, BCR-ABL1–positive.
Figure 8.6. In chronic myeloid leukemia (CML), chromosomal translocation results in formation of BCR-ABL1 fusion protein. A: FISH for BCR-ABL1. CML is defined by the presence of the Philadelphia chromosome, the derivative chromosome 22 containing BCR-ABL1 that results from the reciprocal translocation of the breakpoint cluster region gene (BCR) from chromosome 22 with the Abelson gene (ABL1) from chromosome 9, or molecular genetic evidence of the BCR-ABL1 fusion product. FISH analysis may be performed on interphase cells or metaphase spreads, blood, or marrow. A probe directed at the ABL1 gene is labeled with a “red” fluorophore, whereas a probe directed at the BCR gene is labeled with a “green” fluorophore. After denaturation of DNA, both probes are added to the patient’s cells and allowed to hybridize. Detection of two yellow signals resulting from the presence of the BCR-ABL1 fusion gene and reciprocal translocation indicates the presence of BCR-ABL1, the t(9;22) (q34;q11.2) translocation. If two red and two green signals are detected, no translocation has occurred at ABL or BCR, and there is no evidence of CML. (Courtesy of Cytogenetics Laboratory, Stanford Medical Center, Stanford, CA.) B: Chromosomal translocation between chromosomes 9 and 22 leads to juxtaposition of sequences from BCR and ABL1 (detected by karyotype or FISH) to form a chimeric 210-kDa mRNA (p210 transcript detected and measured by RT-PCR) that is transcribed into the
oncogenic fusion protein \textit{BCR-ABL1}. In contrast to the t(9;22) of acute leukemias, which usually show the p190 \textit{BCR/ABL1} fusion protein, CML almost exclusively shows the p210 fusion product of this translocation. This reflects the breakpoint within the major breakpoint cluster region of the \textit{BCR} gene, typically between exons b2 and b3, or, as shown here, between exons b3 and b4.
**Figure 8.7.** Chronic myeloid leukemia, BCR-ABL1–positive, chronic phase. **A** and **B:** Peripheral blood smear shows a marked leukocytosis with left-shifted myelopoiesis and basophilia. **C:** A high-power examination of a basophil at left contrasted with a lymphocyte is shown. To the left of the basophil, a purple haze of granule contents is present.

**Figure 8.8.** Chronic myeloid leukemia, BCR-ABL1–positive, chronic phase. **A:** Low-magnification view of an aspirate smear with the numerous small, mononuclear megakaryocytes and hyperplastic, left-shifted granulocytes. **B:** High-power view with small megakaryocytes and increased numbers of immature myeloid precursors that include eosinophils and basophils. The bone marrow aspirate in the chronic phase of CML usually contains fewer than 5% blasts, but they may range up to 9%.
Figure 8.9. Chronic myeloid leukemia, BCR-ABL1–positive, chronic phase. A bone marrow biopsy shows a hypercellular marrow with granulocytic hyperplasia and the typical small “dwarf” megakaryocytes.
Figure 8.10. Chronic myeloid leukemia, BCR-ABL1–positive, chronic phase. A: Aspirate smear at low magnification showing the characteristic small “dwarf” megakaryocytes (arrows) and granulocytic hyperplasia. B and C: Basophilia and eosinophilia are characteristic of CML. Basophils, both granulated (solid black arrows) and degranulated forms (open black arrows) along with eosinophil precursors (red arrows) are part of the myeloid hyperplasia in CML. Hybrid cells with mixed eosinophil-basophil granulation (eobasophils) also can be present (arrows and inset).
Figure 8.11. Chronic myeloid leukemia, BCR-ABL1–positive, chronic phase. Histiocytic response in CML. High cell turnover in rapidly growing neoplasms such as CML is often accompanied by increased phagocytic activity by reactive histiocytes. A: “Sea blue” histiocytes in a CML aspirate smear. B: “Starry sky” pattern from scattered tangible body macrophages. C: Increased numbers of small megakaryocytes are seen in the upper middle portion, with five pseudo-Gaucher–type histiocytes on the right side.

Figure 8.12. Chronic myeloid leukemia, BCR-ABL1–positive, accelerated phase. A: Peripheral blood smear shows a leukoerythroblastic picture with two blasts (arrows) and basophilia. B and C: Hypercellular biopsies with granulocytic hyperplasia and scattered Gaucher-type histiocytes.
Figure 8.13. Chronic myeloid leukemia, BCR-ABL1-positive, blast crisis. A: Blood smear shows neutrophilia with left-shift and increased numbers of blasts (arrows). B: Aspirate smear shows increased numbers of small megakaryocytes and clusters of cells made up almost entirely of blasts (arrows).
Figure 8.14. Chronic myeloid leukemia, BCR-ABL1–positive, blast crisis. A: A hypercellular biopsy with increased numbers of blasts. B: By immunohistochemistry, approximately 25% to 30% of the bone marrow cells are CD34\(^+\) blasts. Blast phase or blast crisis in CML is defined by 20% or more blasts in the peripheral blood or bone marrow. Large numbers of blasts on the bone marrow biopsy, as shown here, also are sufficient for a diagnosis of blast phase. Similarly, the development of nonsplenic, extramedullary myeloid tumors is usually sufficient for the blast phase according to WHO criteria. C: Bone marrow biopsy of CML in the chronic phase with less than 10% CD34\(^+\) marrow blasts is shown for comparison.

Figure 8.15. Chronic myeloid leukemia, BCR-ABL1–positive, myeloid blast phase. A high-
power field from an aspirate smear illustrating increased numbers of large blasts (solid arrows) with abundant granular cytoplasm, “open” chromatin pattern, and inconspicuous nucleoli. Dysplastic mature granulocytes accompany the leukemic blasts (open arrows).

Figure 8.16. A–C: Chronic myeloid leukemia, BCR-ABL1–positive, myeloid blast phase. A: Flow cytometric scattergram displaying leukemic myeloid blasts coexpressing both myeloid (CD33) and B-cell (CD19) markers from a case of acute myeloid leukemia evolved from CML. B and C: Blood smears showing blasts and increased platelets and myeloperoxidase (MPO) stains (C) highlight an Auer rod in one of the myeloid blasts. D and E: Chronic myeloid leukemia, BCR-ABL1–positive, lymphoid blast phase. D: Left-shifted myelopoiesis, eosinophilia, basophilia, and increased blasts are shown in this blood smear from CML in lymphoid blast crisis. MPO stain demonstrates the lack of expression of MPO in the two lymphoid blasts in the upper left corner. E: The three darkly stained neutrophils near the bottom of the slide serve as an internal positive control. Lymphoid blast crisis has traditionally been defined as a blast cell proliferation that is TdT-positive, but more detailed
immunophenotyping shows expression of other precursor B-cell markers such as CD19 and CD10. Weak coexpression of myeloid-associated antigens such as CD13 and CD33 also is common.

Figure 8.17. Chronic myeloid leukemia, karyotype. This karyotype from a patient with CML demonstrates clonal evolution, which may be present at the time of transformation to either the accelerated phase or the blast phase. Clonal evolution in this patient shows trisomy 8 and isochromosome 17q, in addition to the presence of the Philadelphia chromosome, t(9;22) (q34;q11). An extra Philadelphia chromosome also may be seen in clonal evolution in CML. (Courtesy of Cytogenetics Laboratory, Stanford Medical Center, Stanford, CA.)

Figure 8.18. Quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) for measuring BCR-ABL1 transcripts. Precise quantitation of transcript levels is done by real-time/reverse transcriptase PCR of peripheral blood or bone marrow samples, commonly using a TaqMan-based assay. In the illustration, known standard transcripts are run in parallel with the patient sample. The $C_t$ (threshold cycle) at which the amplified products are detectable depends on the number of transcripts present in the specimen and is calculated from the inflection points. The $C_t$ of different transcript levels is used to plot a standard curve. The BCR-ABL1 transcript level of the patient sample is back-calculated from the standard curve. QRT-PCR is used for monitoring residual disease. (Courtesy of Molecular Pathology Laboratory, Stanford Medical Center, Stanford, CA.)
Figure 8.19. Minimal residual disease detection in the follow-up of a chronic myeloid leukemia patient treated with imatinib mesylate therapy. BCR-ABL1 transcripts are serially measured over time using reverse transcriptase polymerase chain reaction to assess response to therapy. As shown here, transcript levels are expected to fall with treatment. A major molecular response occurs when the transcript levels decrease to greater than 3 log levels within 1 year, as seen in this patient. (Courtesy of Molecular Pathology Laboratory, Stanford Medical Center, Stanford, CA.)

Figure 8.20. Chronic myeloid leukemia biopsy in a patient resistant to imatinib mesylate therapy. The bone marrow biopsy shows the typical features of CML, including an atypical megakaryocytic hyperplasia with dwarf forms possessing hypolobated nuclei and megakaryocytes with separate nuclear lobes. A myeloid hyperplasia also is present, with increased numbers of blasts containing vesicular nuclei.
Figure 8.21. Chronic neutrophilic leukemia (CNL). A: Peripheral blood findings of CNL include a neutrophilia, usually with less than 5% immature granulocytes. Neutrophils may display toxic granulation or appear unremarkable. B: A bone marrow biopsy discloses a myeloid hyperplasia with no increase in blasts.
Figure 8.22. Atypical chronic myeloid leukemia, BCR-ABL1–negative. A: Peripheral blood smear shows neutrophilia. The neutrophils are dysplastic demonstrating hypogranular cytoplasm, abnormal nuclear lobation, including pseudo Pelger–Huët cells, and abnormal chromatin clumping. B: Bone marrow aspirate shows myeloid cells with left-shifted maturation, as evidenced by the increased number of myelocytes, and dysgranulopoiesis similar to blood smear. C: Bone marrow biopsy shows hypercellular marrow with marked myeloid hyperplasia and left-shifted myelopoiesis.

Figure 8.23. Polycythemia vera (PV). This blood smear demonstrates hypochromic microcytes (arrows), a feature of the iron deficiency commonly associated with PV. Absent stainable iron on marrow examination is typical. The red blood cell mass may be decreased in PV patients with concurrent iron deficiency; laboratory studies may need to be repeated following iron therapy. This 41-year-old man had splenomegaly with a white blood cell count of 29,000/μL, hemoglobin 19 g/dL, MCV 77 fL, and a platelet count of 804,000/μL. The proliferative or erythrocytotic phase typically has elevations of red blood cells, white blood cells, and platelets. White cells may be left-shifted and show a slight basophilia, but basophils are not typically as numerous as in CML. Platelet counts can exceed 600,000/μL, which may cause confusion with essential thrombocytemia.
Figure 8.24. Polycythemia vera, polycythemic phase. The bone marrow biopsy is hypercellular, showing panmyeloisis (trilineage proliferation) and loose clusters of pleomorphic megakaryocytes. (Boxed area in A is shown at higher magnification in B.) Marrow fibrosis is typically minimal in this stage of the disease. Pre-polycythemic/early-stage PV demonstrates morphologic findings similar to the polycythemic phase, so bone marrow biopsy is essential for the correct diagnosis.
Figure 8.25. Polycythemia vera, post-polycythemic phase. The post-polycythemic phase of PV is associated with marrow fibrosis and shows peripheral blood and bone marrow changes similar to or identical with changes seen in primary myelofibrosis, including a leukoerythroblastic smear with frequent dacrocyes and giant and bizarre platelets. This blood smear shows a leukoerythroblastic picture with dacrocyes and giant platelets. The spent phase will typically follow 10 to 15 years of the proliferative phase of the disease. Once in the post-polycythemic phase of the disease, median survival is a few years. Cases of myelodysplastic transformation are reported in the literature, which are favored to be therapy-related.
Figure 8.26. Polycythemia vera, post-polycythemic phase. The marked marrow fibrosis seen in this bone marrow biopsy from a patient with spent-phase PV manifests itself as a lining up of cells with a “streaming” quality on hematoxylin and eosin (H&E) stain (A). Reticulin (B) and trichrome (C) stains confirm the presence of fibrosis grade MF-3. Bone marrow aspirates at this stage of disease are typically “dry taps” yielding only blood. In this biopsy, a very large dilated sinus occupies the middle of the field, whereas a focus of bony remodeling appears in the top left corner. Scattered foci of pleomorphic megakaryocytes in clusters are present on higher power. Morphologic differentiation between post-polycythemic myelofibrosis and primary myelofibrosis may not be possible without a history of PV.

Figure 8.27. Polycythemia vera, transformation to AML. A: Blood smear from a patient with a long history of PV that shows increased blasts and numerous bizarre, giant platelets. Two large blasts dominate the field, with cytoplasmic blebbing reminiscent of platelet production by megakaryocytes. Positive expression of CD41 and CD61 confirmed megakaryocytic lineage of these blasts. B and C: Marrow replacement by a pleomorphic population of blasts associated with dense sclerosis is seen in the biopsy. Approximately 10% to 20% of PV patients will eventually transform to acute myeloid leukemia within 15 years of diagnosis.
Figure 8.28. Extramedullary hematopoiesis in primary myelofibrosis (PMF). The liver and spleen are the most common sites for extramedullary hematopoiesis. **A:** Discrete tumor masses such as this retroperitoneal mass can occur virtually in any site, however. **B:** As shown on cut section, these “tumors” are usually made up of bloody, friable tissue ("red currant jelly–like") pieces.
Figure 8.29. Primary myelofibrosis, prefibrotic phase. A: The bone marrow biopsy shows trilineage proliferation as shown here, particularly early in the disease when marrow fibrosis is less prominent (the prefibrotic phase of PMF). B: Megakaryocytes are seen in loose and tight clusters and demonstrate characteristic appearance of abnormal patterns of chromatin clumping with “bulbous” or “cloud-like” nuclei; presence of bare megakaryocytic nuclei is a typical finding. C: Reticulin stain demonstrates minimal fibrosis (grade MF-1).

Figure 8.30. Primary myelofibrosis, prefibrotic phase. Myeloid and megakaryocytic proliferation with dilated sinusoids and intrasinusoidal hematopoiesis is described in PMF and shown here. (Boxed area in A is shown at higher magnification in B.) Distinguishing the prefibrotic phase of PMF from other MPNs, especially essential thrombocythemia, can be quite difficult. The diagnosis of prefibrotic PMF is favored by the presence of marrow that is hypercellular for the patient’s age due to granulocytic and megakaryocytic hyperplasia, with loose or tight clusters of megakaryocytes displaying “bulbous” or “cloud-like” nuclei. Serial bone marrow biopsies will show an increase in fibrosis in patients with CIM.
Figure 8.31. Primary myelofibrosis. This leukoerythroblastic smear, displaying frequent dacrocytes and thrombocytosis with giant and bizarre platelets, is characteristic of the fibrotic phase of PMF, the phase of disease in which most patients are diagnosed. In this disease, the platelet count is variable, with giant and bizarre forms and sometimes bare megakaryocytes seen. The anemia typically is accompanied by a mild reticulocytosis, frequent teardrop-shaped red blood cells, and circulating nucleated red blood cells. Typically, a left-shifted leukocytosis of 15,000 to 30,000/μL occurs. Basophilia or eosinophilia is found in 10% to 30% of cases.
Figure 8.32. Primary myelofibrosis, fibrotic phase. Bone marrow biopsies during the fibrotic phase of PMF show a replacement of marrow cellularity by fibrosis. Clonal studies demonstrate that the trilineage hematopoiesis is monoclonal in PMF patients and the fibrosis is reactive. A and B: Atypical megakaryocyte clustering is prominent, with collections of medium-sized to giant megakaryocytes, often adjacent to sinuses (as in B) and bony trabeculae. Features of architectural distortion, such as the lining up of individual marrow cells, are common. C: Megakaryocytes often contain hyperchromatic nuclei with coarse lobulations.
Figure 8.33. Primary myelofibrosis, osteosclerosis, and transformation to AML. A: Blood film shows blasts, neutrophilia, and teardrop RBCs. B and C: Biopsies reveal sclerosis of bone trabeculae and blasts. Osteosclerosis occurs in late-stage PMF with broad, irregular trabeculae replacing the marrow space. Although stem cell transplant may reverse the marrow fibrosis in patients with PMF, the osteosclerosis remains. This case of long-standing PMF with osteosclerosis is complicated by AML.
Figure 8.34. Grading of marrow fibrosis on reticulin stain. A: Myelofibrosis grade 0 shows delicate reticulin fibrosis around blood vessels. B: MF grade 1 shows a loose network of reticulin fibers especially in perivascular areas. C: MF grade 2 shows diffuse increase in reticulin fibers with numerous intersections. Trichrome stain reveals a mild increase in collagen fibrosis (inset). D: MF grade 3 shows diffuse and dense increased in thick reticulin fibers with encasing bone marrow cells. Trichrome stain reveals dense collagen fibrosis (inset).
**Figure 8.35.** Collagen fibrosis deposition in primary myelofibrosis, trichrome stain. **A:** A mild increase in collagen fibers in the interstitial areas usually accompanies MF grade 2. **B:** Dense collagen bundles are associated with MF grade 3 fibrosis.
Figure 8.36. Essential thrombocytemia (ET). Peripheral blood findings show thrombocytosis with large and giant platelets and normal erythrocytes (as shown here). A mild leukocytosis, usually less than 30,000/μL, also may be present, as can circulating megakaryocyte nuclear fragments or even micromegakaryocytes.
Figure 8.37. Essential thrombocythemia. A: Bone marrow aspirate at low power shows numerous large megakaryocytes with multilobulated nuclei and abundant cytoplasm (inset). B: Clumps of platelets at low power, a common finding in ET.
Figure 8.38. Essential thrombocythemia. The bone marrow biopsy is normocellular (as adjusted for age) with a marked increase in the numbers of megakaryocytes arranged in loose clusters throughout the marrow. The megakaryocytes of ET are larger than those in reactive conditions or CML and contain hyperlobulated “stag horn–like” nuclei.

Figure 8.39. Morphologic overlap between ET and early/prefibrotic PMF. This patient was originally diagnosed as ET (A), but subsequent bone marrow biopsy showed marked fibrosis and atypical hyperchromatic megakaryocytes characteristic of the fibrotic phase of PMF (B).
Figure 8.40. Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis. A: Biopsy shows a hypercellular marrow with erythroid and megakaryocytic hyperplasia. B: Dysmegakaryopoiesis in the form of hypolobate megakaryocytes and megakaryocytes with separated nuclear lobes is present. C: Aspirate smear demonstrates dysplastic megakaryocytes (separate nuclear lobes) and erythroid elements (megaloblastoid maturation and nuclear irregularities). D: Numerous ring sideroblasts are evident on Prussian blue stain. Molecular analysis demonstrated the presence of SF3B1 mutation. (Courtesy of Dr. R. Hasserjian, Massachusetts General Hospital, Boston, MA.)
Figure 8.41. Chronic eosinophilic leukemia and idiopathic hypereosinophilic syndrome (HES). A: Peripheral blood smear shows numerous normal or slightly atypical-appearing eosinophils. B: Aspirate smear reveals increased eosinophils. C: The biopsy shows a markedly hypercellular marrow with sheets of eosinophils. This case, as often seen in hypereosinophilic syndromes, was difficult to classify because no evidence existed for parasitic, allergic, or other known causes of eosinophilia and marrow cytogenetics were negative. CEL is usually distinguished from HES by the presence of increased blood (≥2%) or marrow (5%–19%) blasts, or the presence of a clonal cytogenetic abnormality.
The diagnosis and classification of lymphoid tumors is a multistep process involving integration of clinical, morphologic, phenotypic, and molecular data. The first step in the evaluation of pathologic specimens for lymphoma is distinguishing benign from malignant lymphoproliferations. Generally, lymphomas are characterized by distortion of the normal nodal or tissue architecture, a monotonous-appearing cellular proliferation, and “atypical” features, like necrosis or a high mitotic rate. However, some indolent lymphoproliferations can be quite subtle to identify morphologically; in these cases, integration of clinical and morphologic data with flow cytometry and other molecular testing may help establish a diagnosis. Once the diagnosis of a lymphoma is made, the World Health Organization (WHO) classification provides a framework for subtyping lymphoid neoplasms into three major divisions: mature B-cell lymphoma, mature T/natural killer (NK)-cell lymphoma, and Hodgkin lymphoma. About 85% of lymphoid neoplasms are of B-cell origin; nearly all the rest derive from T cells. Tumors arising from NK cells are rare. A complete discussion of this classification is beyond the scope of this text; this chapter will briefly review features typical of the most common lymphoproliferative disorders.

MATURE B-CELL LYMPHOMA

B-Cell Ontogeny and the Mature B-Cell Neoplasms

The entire B-cell line originates from precursor B lymphoblasts in the bone marrow that differentiate into mature B cells expressing surface immunoglobulin. The lymphoblasts are the putative cells of origin of precursor B acute lymphoblastic leukemia and lymphoma. The naïve marrow B cells are the cells of origin of some chronic lymphocytic leukemias (CLLs)/small lymphocytic lymphomas (SLLs). Immature B cells exit the bone marrow and undergo variable (V), diversity (D), and joining (J) gene segment (VDJ) recombination of immunoglobulin genes, differentiating into naïve B cells. The naïve B cells leave the marrow to circulate in the blood and travel to the cortex of lymph nodes, where they occupy primary follicles (those without germinal centers) and secondary follicles (those with germinal centers) in the mantle zone, which surrounds the germinal centers. These cells are the source of mantle cell lymphoma (MCL). When naïve B cells encounter antigen, they transform into blasts and travel to the center of primary follicles, forming the germinal center, where the cells are called centroblasts. These large cells, whose vesicular nuclei contain nucleoli, are thought to be the source of most large B-cell lymphomas and Burkitt lymphoma. The centroblasts mature to centrocytes, which are medium-sized, cleaved cells with inconspicuous nucleoli from which follicular lymphomas are thought to arise. The centrocytes eventually leave the germinal center and undergo plasmacytic differentiation. It is these cells that give rise to the activated B-cell subtype of diffuse large B-cell lymphoma. Post-germinal center B
cells differentiate further into: (1) antibody-secreting plasma cells, from which plasma cell myeloma and Waldenström macroglobulinemia originate, and (2) memory B cells, which are found in the areas around follicles (marginal zone) and are the origin of marginal zone lymphomas of the spleen, lymph nodes, and mucosa-associated lymphoid tissue (MALT). The origin of cells in mature B-cell neoplasms ranges in differentiation from naïve B cells—resting lymphoid cells that have surface immunoglobulin but have not yet encountered antigens—to mature plasma cells, which produce immunoglobulins in response to previous antigen exposure. The classification of these disorders, which account for >80% of lymphoid neoplasms, generally depends on morphologic characteristics, immunophenotyping of the cells, and anatomic involvement (i.e., whether the process is primarily disseminated [often leukemic], extranodal, or nodal). In addition, molecular diagnostics (e.g., karyotype, fluorescent in situ hybridization [FISH] studies, and specific point mutations) play a role in lymphoma diagnosis. In this chapter, the mature B-cell neoplasms have been organized by cell of origin to reflect the role of B-cell ontogeny in lymphomagenesis.

**Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma**

CLL/SLL is a clinically heterogeneous disease consisting of monoclonal small B-lymphocytes expressing CD19, CD5, and CD23. The presentation can be predominantly leukemic with bone marrow involvement (i.e., CLL) or may present in lymph nodes without a significant leukemic component (i.e., SLL). Most patients are asymptomatic at the time of diagnosis, their median age is about 65, and the male:female ratio is approximately 2:1. When symptoms occur, they commonly include fatigue related to anemia from bone marrow replacement, splenomegaly, or immune-related hemolysis caused by a warm-reactive polyclonal IgG; the latter occurs in about 10% to 25% of patients during the course of disease.

CLL is characterized by a monoclonal B-cell lymphocytosis in peripheral blood of at least $5 \times 10^9/L$ with a specific immunophenotype: CD5 and CD23 positivity with low levels of surface immunoglobulin and CD20. The blood smear shows an increased number of mature small lymphocytes with little cytoplasm and dense, clumped chromatin. Nucleoli are not usually visible, and many cells, being more fragile than normal lymphocytes, disrupt during the preparation of the smear, producing “smudge cells,” in which the cytoplasm is lost and the nucleus spreads out. Some cells may be prolymphocytes, which are larger than mature lymphocytes, possess nucleoli, and have more cytoplasm. The presence of a small circulating CD5-positive monoclonal B-cell population (i.e., $<5 \times 10^9/L$) in the absence of lymphadenopathy is termed *monoclonal B-cell lymphocytosis* (MBL), which precedes the development of virtually all cases of CLL. MBL is seen in 12% of healthy individuals and is divided into “high count” and “low count” at a threshold of $0.5 \times 10^9/L$. Individuals with “low count” MBL have little risk of disease; “high count” MBL should be followed for progression to CLL. Disease prognosis varies by immunophenotype, cytogenetic findings, and mutational status. Analysis of the immunoglobulin heavy chain gene for somatic hypermutation has demonstrated that CLL consists of two subsets: an unmutated form and a mutated form. Unmutated CLL arises from naïve B cells and mutated CLL arises from memory B cells. In the immunochemotherapy era, the presence of somatic hypermutation in the IgH gene was associated with good prognosis, whereas expression of CD38, ZAP-70, and CD49d were markers of an aggressive course. Deletion of 13q is a favorable cytogenetic finding, whereas del(17p), del(11q), and del(6q) tend to indicate poor prognosis. The molecular landscape of CLL/SLL is heterogeneous, with mutations in *TP53, NOTCH1, SF3B1,* and *BIRC3* frequently seen and associated with a worse prognosis. As therapeutic
options have evolved to include B-cell receptor antagonists (e.g., inhibitors of Bruton tyrosine kinase or phosphoinositol triphosphate kinase), the predictive value of these markers is also in flux and is the subject of active study.

About 5% of patients with CLL/SLL develop Richter syndrome. The CLL/SLL transforms into a non-Hodgkin lymphoma, usually diffuse large B-cell type. Characteristic features of Richter syndrome include fever, weight loss, rapid increase in lymph node size, and elevated serum lactic dehydrogenase (LDH). Patients with transformed CLL should be reevaluated for signs of progression, including genetic or karyotypic evolution.

**B-Cell Prolymphocytic Leukemia**
This rare disorder predominantly affects older adults (median age, 70), with a male:female ratio of 1.6:1. Most patients have marked splenomegaly and lymphocytosis without enlarged peripheral lymph nodes. By definition, prolymphocytes constitute over 55% of the circulating lymphoid cells, but in most cases they exceed 90%. About half of the patients have anemia and thrombocytopenia. The prolymphocytes are twice the size of small lymphocytes and possess a small amount of pale blue cytoplasm and round nuclei, which contain moderately condensed chromatin and a conspicuous central nucleolus. The bone marrow shows diffuse infiltration with these abnormal cells.

**Mantle Cell Lymphoma**
This lymphoma, which constitutes about 4% to 5% of non-Hodgkin lymphoma, occurs primarily in adults, with a median age at diagnosis of about 60 years and a male predominance of at least 2:1. It is thought to arise from naive B cells normally present in the mantle zone and develops along two distinct pathways. *Classical MCL* involves lymph nodes and other extranodal sites, has unmutated immunoglobulin heavy chain variable genes, *IGHV*, and may be aggressive. In contrast, *leukemic non-nodal MCL* is limited to the bone marrow, peripheral blood, and spleen, has mutated *IGHV*, and is generally indolent. The neoplastic cell closely resembles centrocytes, appearing as small- to medium-sized lymphocytes with sparse cytoplasm and irregular or cleaved nuclei containing moderately dispersed chromatin and inconspicuous nucleoli. In the *blastoid variant* of classical MCL, the neoplastic cells appear enlarged with a fine chromatin pattern. Bone marrow involvement in MCL includes interstitial, focal, paratrabecular, and diffuse patterns. The prognosis of MCL is slightly worse than the other small mature B-cell lymphomas; individuals with the blastoid variant fare worst, with a median overall survival of slightly more than a year.

Most cases of MCL are driven by a translocation of cyclin D1 and the immunoglobulin heavy chain gene, which can be detected by immunohistochemical testing for cyclin D1 or by FISH studies. A recently described subset of MCLs is negative for cyclin D1; these cases generally express SOX-11.

**Follicular Lymphoma**
Follicular lymphoma constitutes about 20% of all lymphoma in the United States, primarily affecting adults, with a median age of about 60 years and equal gender distribution. Most patients have widespread disease at diagnosis, with diffuse lymph node enlargement, bone marrow involvement in about 40% of patients, and circulating neoplastic cells in about 10%. In 25% to 35% of cases, at a rate of 1% to 3% per year, the disease transforms into a large B-cell lymphoma, usually diffuse.

Follicular lymphoma arises from germinal center B lymphocytes; these cells recapitulate the constituency of normal lymphoid follicles: centrocytes and centroblasts. The centrocytes
are small with cleaved or otherwise irregular nuclear contours. Centroblasts are larger with oval nuclei with open chromatin and prominent nucleoli. Histologic examination of the lymph node generally shows back-to-back follicles, which are composed entirely of neoplastic cells. The proportion of centroblasts then determines the grade: grade 1 or 2 (low grade) has 0 to 15 centroblasts per high-power field (hpf); grade 3a has more than 15 centroblasts/hpf with centrocytes also present; grade 3b has more than 15 centroblasts/hpf without centrocytes. Thus, grade 3b can often be difficult to distinguish from diffuse large B-cell lymphoma. In peripheral blood smears, the neoplastic cells are commonly smaller than normal lymphocytes, have very sparse cytoplasm, and possess cleft nuclei. Bone marrow involvement is generally paratrabecular and is composed primarily of centrocytes. Follicular lymphoma is characterized by a rearrangement of \[BCL2\] with the immunoglobulin heavy chain gene in 80% to 90% of cases, resulting in a translocation of chromosomes 14 and 18 and expression of BCL2 by immunohistochemistry (IHC). Markers of germinal center cell origin, such as CD10, BCL6, LMO2, and HGAL, are also positive in follicular lymphoma.

**Diffuse Large B-Cell Lymphoma**

The large B-cell lymphomas are defined by lymphoid cells with nuclei that are twice the size of a small lymphocyte. There are numerous distinct variants of large B-cell lymphoma, many of which are vanishingly rare. Diffuse large B-cell lymphoma accounts for 25% of non-Hodgkin lymphoma and is generally a disease of the elderly. Patients commonly present with a rapidly enlarging mass, which can be in virtually any site. Bone marrow involvement, however, is rare. The proliferation is generally diffuse, with obliteration of normal follicular dendritic meshworks.

Diffuse large B-cell lymphoma is a heterogeneous group and can arise from B cells at different stages of maturation. Gene expression profiling studies of diffuse large B-cell lymphoma have identified two distinct subgroups with an expression profile characteristic of either normal germinal center cells (GCB-like) or activated blood memory B cells (ABC-like) and have demonstrated that patients with the ABC subtype have a worse prognosis when treated with immunochemotherapy. IHC, including the Hans algorithm, which relies on expression of CD10, BCL6, and MUM1, can be used to identify GCB and non-GC subgroups with reasonable accuracy, although the prognostic value of IHC-defined cell of origin remains unclear. Expression of MYC and BCL2 are commonly evaluated by IHC, and positivity for both may be associated with poor outcomes. Cytogenetic analysis for rearrangements of \[MYC\], \[BCL2\], and \[BCL6\] is commonly performed (see below). A subset of DLBCL is Epstein–Barr virus (EBV) driven (\[EBV^+ DLBCL, NOS\]) and may be associated with some degree of immunosuppression.

**High-Grade B-Cell Lymphoma**

This category encompasses large B-cell lymphomas with either morphologic or molecular features that are thought to indicate poor prognosis. There are two subgroups that compose this category: (1) Large B-cell lymphomas with blastoid or Burkitt-like features may be classified as high-grade B-cell lymphoma, not otherwise specified, although at present, there is little consensus about what morphologic features indicate more aggressive disease; or (2) large B-cell lymphomas with \[MYC\] and either \[BCL2\] or \[BCL6\] rearrangements, known historically as “double-hit lymphoma,” also fall into this category as high-grade B-cell lymphoma, with rearrangements of \[MYC\] and \[BCL2\] and/or \[BCL6\].

**Mediastinal Large B-Cell Lymphoma**
This subtype of diffuse large B-cell lymphoma commonly affects young women and presents with low stage, bulky mediastinal disease, causing airway obstruction. Histologically, the B cells are large and embedded in a background of prominent sclerosis. *MYC*, *BCL2*, and *BCL6* gene rearrangements are uncommon.

**Primary Effusion Lymphoma**
This subtype of large B-cell lymphoma was originally identified in the early days of the human immunodeficiency virus (HIV) epidemic. Most cases arise in immunocompromised hosts (HIV most commonly, but also organ transplant recipients and elderly individuals) and, while they tend to involve body cavities (e.g., pleural, pericardial, or peritoneal), rare extracavitary “solid” cases of primary effusion lymphoma have been reported in various nodal and extranodal sites. It is universally associated with HHV8 infection and a subset of cases shows coinfection with EBV. The neoplastic cells are large and plasmablastic with eccentric nuclei, a perinuclear clear zone, and prominent nucleoli. The cytoplasm is basophilic and may contain vacuoles. These cells express CD45 and plasma cell markers (CD138, CD38, CD30). They typically do not express B- or T-cell antigens or surface immunoglobulin. Immunostaining for LANA, an HHV8-associated antigen, can be useful in diagnosis.

**Burkitt Lymphoma**
Burkitt lymphoma is the most common form of non-Hodgkin lymphoma in children; however, the majority of cases arise in adults. It occurs in three distinct epidemiologic settings. The *endemic* form is EBV-driven and affects young children from equatorial Africa and Papua New Guinea. Common sites of involvement include the bones of the face, gonads, and kidneys. The *sporadic* form occurs worldwide, predominantly arising in extranodal sites in children and young adults. The minority of cases are associated with EBV. The *immunodeficiency-associated* form of Burkitt lymphoma can be seen not only in HIV-positive individuals, but also in patients with iatrogenic immunosuppression. In immunodeficient hosts, Burkitt lymphoma more frequently involves the bone marrow and lymph nodes.

Burkitt lymphoma is classically described as having a “starry sky” appearance, with sheets of dark neoplastic lymphocytes punctuated by scattered benign histiocytes with clear cytoplasm. The neoplastic, uniform-appearing cells have vacuolated cytoplasm, clumped chromatin, and often multiple nucleoli. The classic immunophenotype is positive for CD10 and BCL6 with no expression of BCL2 or TdT. The Ki-67 proliferation index should be almost 100%. *MYC* rearrangements are seen in all cases, most commonly with the Ig heavy chain locus on chromosome 14, or rarely with the κ or λ light chain loci on chromosomes 2 or 22, respectively. The diagnosis of Burkitt lymphoma can be difficult in cases with atypical morphology or an aberrant immunophenotype.

**Marginal Zone Lymphoma**
Post-germinal center memory B cells give rise to marginal zone lymphoma, which is further subclassified based on the sites of involvement into splenic, nodal, and extranodal (MALT) lymphoma. Marginal zone lymphomas generally lack CD5, CD10, and CD23 expression; at present, sensitive and specific markers are an area of active research. The diagnosis tends to be one of exclusion. Thus, although splenic, nodal, and extranodal marginal zone lymphoma share a common cell of origin, they have distinct epidemiologic, pathologic, and molecular features.
Splenic Marginal Zone Lymphoma

This rare disorder, previously called splenic lymphoma with villous lymphocytes, is a B-cell neoplasm involving the splenic white pulp and bone marrow with a small component of circulating neoplastic cells. The splenic white pulp architecture, which normally consists of reactive germinal centers, is altered by a proliferation of larger “monocytoid” B cells in the marginal zone. The germinal centers are colonized by small centrocyte-like cells with some extension into the red pulp. Most patients are over 50 years old, and both genders are equally affected. Hepatitis C infection has been associated with splenic marginal zone lymphoma (SMZL), and hepatitis C virus-related SMZLs may respond to antiviral therapy. The major clinical feature is splenomegaly and hypersplenism. About one-third of patients have a monoclonal gammopathy, which may complicate distinction from lymphoplasmacytic lymphoma (LPL). In the peripheral blood, the lymphocytes may have a “villous” morphology with an eccentric nucleus and thin projections from the opposing pole of the cell. In other cases, the lymphocytes may be more “plasmacytoid.” In the bone marrow, sinusoidal infiltration is characteristically seen, with the neoplastic lymphocytes forming linear proliferations inside the marrow sinusoids. However, nodular involvement can also be seen. There are no specific immunohistochemical or molecular markers of marginal zone lymphoma; the diagnosis is made based on morphology and after exclusion of other low-grade B-cell lymphomas. NOTCH2 and KLF2 mutations may be seen in a minority of cases, resulting in disruption of the NF-κB signaling pathway.

Extranodal Marginal Zone B-Cell Lymphoma of Mucosa-Associated Lymphoid Tissue (MALT Lymphoma)

This neoplasm, usually an adult disease with a median age of about 60 years, constitutes about 8% of non-Hodgkin lymphomas. It arises in association with chronic antigenic stimulation or autoimmune disorders, such as Sjögren disease and Hashimoto thyroiditis. The prototypical MALT lymphoma arises in the stomach in association with Helicobacter pylori gastritis and in many cases can be cured with antibiotics. Other common sites are the lungs, salivary glands, eye, skin, and thyroid. Bone marrow involvement is uncommon but secondary spread to lymph nodes may be seen. In the involved tissue, in contrast to other low-grade B-cell lymphomas, the neoplastic infiltrate is heterogeneous, including small lymphocytes, plasma cells, and monocytoid B cells. The latter are small cells with generous, pale cytoplasm. Larger cells may be present as well; a predominance of large cells would be worrisome for transformation to a high-grade process. The lymphocytes may invade the epithelium and destroy it, forming a “lymphoepithelial lesion”; however, this finding is neither required nor specific for a diagnosis of MALT lymphoma.

There are no immunophenotypic findings specific to marginal zone lymphoma; in general, they tend to be negative for CD5, CD10, and Cyclin D1. Translocations involving the MALT1 gene on chromosome 18 and the BCL10 gene on chromosome 1 have been reported. The presence of genetic alterations portends a worse prognosis and a poor response to noncytotoxic forms of treatment.

Nodal Marginal Zone Lymphoma

This rare small B-cell lymphoma most commonly presents in the sixth and seventh decade. Because of the highly variable, nonspecific presentation and pathologic features, the diagnosis requires careful correlation of clinical and pathologic data. Most patients present with lymphadenopathy and a minority will also have cytopenias, elevated LDH, elevated M-
protein or bone marrow involvement. Marginal zone lymphoma has variable morphology, but the most common histologic pattern is a diffuse nodal proliferation of a heterogeneous population of cells (lymphocytes, monocytoid cells, plasmacytoid cells, and plasma cells). IRTA1 has recently been shown to be a specific immunohistochemical marker for nodal marginal zone lymphoma. The translocations associated with extranodal marginal zone lymphoma are not seen in the nodal subtype, which tends to have simple chromosomal gains and losses.

**Waldenström Macroglobulinemia (Lymphoplasmacytic Lymphoma)**

This clonal disorder is characterized by the production of monoclonal IgM in association with bone marrow infiltration by a proliferation of cells exhibiting a spectrum of differentiation, from small lymphocytes to plasma cells. The median age is about 65, and males slightly outnumber females. Some patients are asymptomatic, but most develop problems related to tissue infiltration of bone marrow, lymph nodes, and spleen by the malignant cells; circulating IgM; or amyloid.

The bone marrow may show any pattern of involvement, but most commonly it is a subtle interstitial proliferation. There are no specific immunohistochemical markers for LPL. Most LPLs are CD20+, CD5−, CD10−, which can make them challenging to distinguish from marginal zone lymphoma. The L265P mutation in MYD88 is present in 90% of cases. Rarely, MYD88 mutations other than L265P may be present. In addition, up to 35% of LPLs may have a nonsense or frameshift mutation in CXCR4 similar to those found in the warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome.

Circulating IgM forms aggregates and binds water, sometimes resulting in hyperviscosity. The level of IgM does not necessarily correlate well with clinical manifestations. Ophthalmologic features include visual blurring and decreased acuity, associated with retinal hemorrhages, exudates, and dilated, tortuous veins (“sausage links” or “boxcars”). The increase in plasma volume can cause congestive heart failure. The impaired blood transit through the microvasculature can cause neurologic symptoms of dizziness, headache, deafness, confusion, nystagmus, vertigo, and ataxia. The combination of damage to vessel walls from the diminished blood flow and the interaction of monoclonal IgM with clotting factors and platelets can lead to bleeding, such as epistaxis, oral mucosal hemorrhage, and cutaneous ecchymoses. The IgM can precipitate on cooling, creating a type I cryoglobulinemia in up to 20% of patients, but less than 5% have symptoms related to it, such as cutaneous vasculitis, Raynaud phenomenon, or cold urticaria.

The monoclonal IgM also can behave as an autoantibody, and up to 20% of patients have a peripheral neuropathy from antibodies against glycoproteins in the nerves. It is usually a distal, symmetrical, chronic demyelinating process. The macroglobulins also may interact with red cell antigens at temperatures below 37°C, causing a chronic hemolytic anemia called cold-agglutinin disease. In cold agglutinin disease, the monoclonal IgM has anti-I activity and fixes complement in the cold (<37°C); the IgM then dissociates from red cells at core body temperature, leaving the red blood cells (RBCs) coated only with complement. The coated cells are rapidly cleared from the circulation. Red cell agglutination in this syndrome characteristically is as grape-like clusters of RBCs rather than as rouleaux.

Deposition of IgM in the kidneys can lead to proteinuria and renal insufficiency. Firm, flesh-colored papules and nodules can form from IgM in the dermis, and occasional patients can develop gastrointestinal problems of diarrhea, malabsorption, and bleeding from monoclonal protein present in the intestinal wall. Monoclonal light chains can form amyloid, which is clinically evident in about 2% of patients with Waldenström macroglobulinemia.
A normocytic, normochromic anemia is common and can arise from bone marrow infiltration, splenomegaly, hemolysis, hepcidin-related iron deficiency, dilution by increased plasma volume, and hemorrhage. On peripheral blood smear, rouleaux formation is common, and sometimes red cell agglutination leads to clumping of erythrocytes. Increased numbers of small lymphocytes, some resembling plasma cells (“plasmacytoid lymphocytes”) with abundant basophilic cytoplasm, are common. Plasma cells also may be visible.

**Hairy Cell Leukemia**

The median age of patients with this disease is approximately 55 years, and the male:female ratio is about 4:1. In most patients, the neoplastic B-lymphoid cells affect primarily the bone marrow and spleen, causing splenomegaly, monocytopenia, and neutropenia in most patients. About 50% have pancytopenia at the time of diagnosis. The disease, which accounts for about 2% of adult leukemias, predisposes to bacterial infections because of neutropenia, but also causes diminished cell-mediated immunity. The result is an increased susceptibility to nontuberculous mycobacteria, fungi, *Listeria monocytogenes*, *Toxoplasma gondii*, *Pneumocystis pneumonia*, and various viruses. Some patients have other rheumatologic disorders, including systemic sclerosis, polymyositis, and polyarteritis nodosa. Nearly all patients with this disease have palpable splenomegaly, often to gargantuan size, and about 40% have hepatomegaly.

In addition to cytopenia in one or more cell lines, peripheral smears in about 85% of cases reveal hairy cells—small- to medium-sized lymphoid cells that possess a round, kidney-shaped, oval or bilobed, and commonly eccentric, nucleus with ground-glass chromatin, but absent or inconspicuous nucleoli, and abundant, pale blue cytoplasm with numerous irregular, thin, surface projections resembling hairs. Because of associated fibrosis, bone marrow aspiration often results in a dry tap. Bone marrow biopsies are usually hypercellular and classically demonstrate interstitial infiltration with mononuclear cells possessing abundant cytoplasm and prominent cell borders, creating a “fried-egg” appearance. The fibrosis produces a net-like pattern affecting areas of hairy cell infiltration. Hairy cells have acid phosphatase activity that is resistant to tartrate and, in >95% of cases, a tartrate-resistant acid phosphatase (TRAP) stain is positive. Almost all cases of hairy cell leukemia (HCL) harbor the *BRAF* V600E mutation, which is not seen in other small B-cell lymphomas or hairy cell variant. In the rare variant form of hairy cell leukemia (HCL-v), however, the TRAP stain is usually negative, the white cell count is elevated, neutropenia and monocytopenia are absent, and the hairy cells have prominent nucleoli, similar to the cells of prolymphocytic leukemia. HCL-v is characterized by *MAP2K1* mutations in ~50% of cases, resulting in dysregulation of the mitogen-activated protein kinase signaling pathway.

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**MATURE T-CELL AND NK-CELL NEOPLASMS**

The classification of these neoplasms depends on combining histologic, immunophenotypic, genetic, and especially, clinical features because morphologic and immunophenotypic abnormalities may be diverse within a specific disorder and yet similar among different diseases. As a group, these disorders have specific geographic foci (especially Asia), have infrequent lymph node involvement, demonstrate cell death (apoptosis) and necrosis, are accompanied by an increased incidence of the hemophagocytic syndrome, and often are associated with viral infections, especially with the EBV. In contrast to the B-cell lymphomas, classification of mature T-cell lymphomas according to cell of origin is difficult.
because of our limited understanding of T-cell ontogeny. We will discuss the most common T- and NK-cell neoplasms below.

**T-Cell Prolymphocytic Leukemia**

This disorder accounts for about 2% of adult small lymphocytic leukemias and is primarily a disease of people more than 50 years old, with most patients having impressive splenomegaly. Generalized lymph node enlargement and hepatomegaly are common. Skin nodules or diffuse papular rashes occur in about 25% of cases; pleural effusions or ascites occur in about the same number. The major laboratory finding is a very high white cell count, exceeding \(200 \times 10^9/L\) in about two-thirds of patients. Anemia and thrombocytopenia are also common. The diagnosis is established on peripheral blood films, where most leukocytes are prolymphocytes, with a well-defined central nucleolus and a deeply basophilic cytoplasm that often demonstrates blebs or protrusions. These cells are larger than normal small lymphocytes, but in 20% of cases of T-cell prolymphocytic leukemia (the small-cell variant), the cells are small, with more condensed nuclear chromatin and no apparent nucleolus on routine staining, but nucleoli are detectable, however, on electron microscopy.

Bone marrow aspirates show the same cells that are present on blood smears. Bone marrow biopsies show heavy infiltration that can be interstitial, nodular, diffuse, or mixed. The number of reticulin fibers is increased.

**T-Cell Large Granular Lymphocytic Leukemia/Chronic NK Lymphoproliferative Disorder**

The median age of patients with this disorder is about 60 years, and many have clinical and serologic evidence of rheumatoid arthritis. About 40% are asymptomatic at the time of diagnosis. The most prominent clinical feature is splenomegaly, present in about 50%; lymph node enlargement is rare. The white cell count is elevated because of an increased number of large granular lymphocytes, which usually appear normal and have round or oval, eccentric nuclei with condensed chromatin and abundant basophilic cytoplasm containing small or large purplish granules. To fulfill diagnostic criteria, these cells should exceed \(2 \times 10^9/L\) for at least 6 months without an alternative explanation, although occasionally patients with fewer cells may meet criteria for this diagnosis if they show other features of T-LGL. T-LGL must be differentiated from chronic NK cell lymphoproliferative disorder (CLPD-NK). Peripheral blood flow cytometry is helpful in identifying an increased CD8+, CD57+ T-cell population with a monotypic pattern of killer immunoglobulin-like receptors CD158a, CD158b, and CD158e. Polymerase chain reaction (PCR) for the T-cell receptor will show a monoclonal gene rearrangement in T-LGL but not in CLPD-NK. This lymphoproliferation of CLPD-NK is generally indolent, but the course may be complicated by severe neutropenia and/or thrombocytopenia, sometimes associated with bacterial infections or pure red cell aplasia. Bone marrow involvement is variable and often minimal, the typical pattern being intrasinusoidal. Patients with neutropenia usually have normal immature granulocytes but decreased neutrophils (maturation arrest), and patients with thrombocytopenia characteristically demonstrate adequate or increased megakaryocytes. Anemia may be accompanied by red cell aplasia or hypoplasia. Mutations in \(STAT3\) and \(STAT5B\) are frequently seen.

**Aggressive NK-Cell Leukemia**

This disorder typically occurs in adolescents or young adults and is more common in Asians.
than in whites. Patients present acutely with fever, cytopenias, lymphadenopathy, and hepatosplenomegaly. Peripheral blood smear shows large atypical lymphocytes with fine cytoplasmic granules and irregular, hyperchromatic nuclei. The bone marrow involvement is variable and may be associated with hemophagocytosis. Most cases are associated with EBV infection.

**Adult T-Cell Leukemia/Lymphoma**

This disorder, caused by infection with the retrovirus human T-cell leukemia virus type 1 (HTLV-1), is endemic in Japan, the Caribbean, and parts of central Africa. Most patients have acquired the infection at an early age through breast milk or exposure to blood, and the lifetime cumulative risk of later developing leukemia or lymphoma, which occurs at a median age of about 55 years, is approximately 2%. The typical clinical features include generalized lymph node enlargement, hypercalcemia, hepatosplenomegaly, and skin lesions, which can be nodules, papules, or a diffuse scaly rash. Four clinical patterns occur. Most common is the *acute variant*, which includes constitutional symptoms, diffuse lymph node enlargement, skin rash, hepatosplenomegaly, hypercalcemia with or without lytic bone lesions, high numbers of circulating leukemic cells, and an elevated serum LDH. Some patients have opportunistic infections from decreased cell-mediated immunity. The *lymphomatous variant* consists of generalized lymph node enlargement without circulating leukemic cells. Hypercalcemia and elevated LDH may be present. The *chronic variant* has prominent skin lesions, mostly an exfoliative process, but no hypercalcemia. The white cell count is elevated, with over 10% being leukemic cells. Serum LDH is slightly increased. The *smoldering variant* has a normal white cell count with less than 3% neoplastic cells, normal serum calcium and LDH, and no enlargement of lymph nodes, spleen, or liver. Pulmonary involvement and skin rashes may be present.

Peripheral blood smears classically show pleomorphic lymphocytes, with clumped chromatin, prominent nucleoli, and lobated nuclei (i.e., “flower cells”). A subset may appear more blast-like with fine chromatin. Bone marrow involvement is common and in most cases the cells are quite pleomorphic with prominent nucleoli. The bony structures may appear abnormal because of increased osteoclastic activity. The neoplastic lymphocytes secrete chemokines that drive bone resorption, resulting in hypercalcemia and osteolytic lesions. Adult T-cell leukemia/lymphoma is a neoplastic process arising from T-regulatory cells, which function to suppress the immune system. They express CD4 and CD25 and are generally negative for CD7. They also frequently express CCR4, which may be targeted therapeutically. This proliferation of T-regulatory cells and the consequent immunosuppression correlates with the frequent opportunistic infections seen in these patients.

**Extranodal NK-/T-Cell Lymphoma, Nasal Type**

This disorder, virtually always associated with EBV when it involves the nose, is more common in males than in females and is more frequent in Asia and Latin America than elsewhere. The disease typically occurs in adults and classically presents as a nasal mass. From these areas, it can disseminate to distant sites, such as the gastrointestinal tract, cervical lymph nodes, and the skin, where nodules and ulcers may form. When the disease originates outside the nasal cavity, systemic symptoms can occur.

The lymphoma cells commonly are intermingled with numerous benign cells, such as small lymphocytes, histiocytes, eosinophils, and plasma cells, making the disease often appear inflammatory rather than neoplastic. The process is destructive, typically causing
ulceration and necrosis. It tends to occur around vessels, which it destroys. The tumor cells are diverse in size and appearance. They may have irregular and elongated nuclei, often undergoing mitosis, with granular chromatin. The cytoplasm is commonly pale. The peripheral blood film rarely discloses neoplastic cells. Similarly, the bone marrow rarely is involved with neoplastic cells. The cells are usually NK cell in origin and are usually positive for CD2, CD56, and cytotoxic granule proteins TIA-1 and granzyme B. A subset arises from cytotoxic T cells and would have a clonal T-cell receptor gene rearrangement detectable by PCR. Karyotype studies may show deletion of 6q or 8p.

**Hepatosplenic T-Cell Lymphoma**

Hepatosplenic T-cell lymphoma is a disorder of the innate immune system, arising from cytotoxic γ/δ T-cells. It classically arises in adolescents and young men, but a subset is associated with immunosuppression, especially patients exposed to azathioprine. The main clinical feature is marked hepatosplenomegaly without lymphadenopathy, usually accompanied by thrombocytopenia. The peripheral blood film rarely shows neoplastic cells, but they are usually present on the bone marrow aspirate as medium-sized lymphocytes with dispersed chromatin and mildly basophilic cytoplasm. In the liver and spleen, the neoplastic cells are medium sized and percolate through the hepatic and splenic sinuses. The marrow biopsy shows interstitial or intrasinusoidal infiltration with medium- to large-sized lymphoid cells with a rim of pale cytoplasm. Their immunophenotypic profile is variable, but classically the cells express CD56 and TIA1. Isochromosome 7q and trisomy 8 are common cytogenetic findings. Recent studies have shown mutations in STAT5B in about 40% of cases.

**Mycosis Fungoides and Sézary Syndrome**

This disorder occurs most commonly in adults, with a male: female ratio of about 2:1. It is a cutaneous T-cell lymphoma that begins as flat areas of skin scaling and erythema that may be asymptomatic or pruritic. At varying intervals, but typically after several years, it may progress to cause dusky red to violaceous plaques—sharply demarcated lesions that are elevated above the surrounding normal skin. Sometimes, lymph nodes are enlarged, but biopsies commonly show a reactive pattern, rather than neoplastic infiltrates. If the disease continues to advance, the next stage is the formation of cutaneous tumors. Only then does the lymphoma tend to spread to extracutaneous sites, typically, lymph nodes, spleen, liver, and lungs.

Confident pathologic diagnosis of mycosis fungoides can be very difficult, especially in the early stages, and numerous skin biopsies, sometimes taken over intervals of months to years, may be necessary before the characteristic findings are clearly present. The diagnostic abnormality consists of dermal and epidermal infiltration of small- to medium-sized T cells with irregular nuclei whose convolutions resemble the brain (cerebriform nuclei). In the dermis, infiltrates typically are present at the epidermal border and consist of small lymphocytes, eosinophils, and the neoplastic cells. Single or small numbers of the neoplastic cells may be present in the epidermis, but sometimes many aggregate there to form Pautrier abscesses. One variant of mycosis fungoides is pagetoid reticulosis, in which a chronic solitary plaque is present, and the neoplastic cells are located only in the epidermis. Another is follicular mucinosis, in which the lymphoma cells are present only in hair follicles, where they cause mucinous degeneration. Clinically, the lesions are indurated papules or plaques, which in hairy areas can cause alopecia.

Sézary syndrome is a systemic variant of mycosis fungoides characterized by erythroderma, generalized lymph node enlargement, and the presence of circulating
neoplastic cells. As in mycosis fungoides, the skin shows dermal and epidermal infiltration with cerebriform lymphocytes. The peripheral blood film has numerous small or large neoplastic cells with cerebriform nuclei containing condensed chromatin. Because these cells may appear in small numbers in both benign skin diseases and early stages of mycosis fungoides, most criteria require that their level exceed 1,000/mm$^3$.

**Peripheral T-Cell Lymphoma, Unspecified**

This category includes about 50% of the peripheral T-cell lymphomas in Western countries. Most cases occur in adults, and the disease usually presents with nodal involvement, but disseminated disease is common, often with circulating neoplastic cells and affected extranodal sites, especially the skin. Constitutional symptoms, such as weight loss, fever, and fatigue, are frequent.

These lymphomas cause diffuse infiltration of lymph nodes with neoplastic cells that are variable, but most commonly medium- to large-sized cells with irregular, pleomorphic nuclei and prominent nucleoli. Vascular proliferation in the lymph node is common, and often a mixed inflammatory reaction is prominent, including eosinophils, plasma cells, small lymphocytes, and epithelioid histiocytes. Multinucleated cells resembling Reed–Sternberg cells and “clear cells” with very pale cytoplasm may be present. Two rare subtypes are the T-zone and lymphoepithelioid cell variants. The former has small- to medium-sized neoplastic cells in intact follicles. The latter has small cells and numerous clusters of epithelioid histiocytes.

**Nodal Peripheral T-Cell Lymphoma with T-Follicular Helper Phenotype**

Patients with this disorder tend to present with constitutional symptoms, rashes, polyclonal hypergammaglobulinemia, immunosuppression, and generalized lymphadenopathy. It generally occurs in older adults and has an aggressive course because of the associated immunodeficiency. Peripheral blood involvement is rare. Lymph nodes show prominent arborizing vasculature with expansion of the interfollicular areas by atypical T cells with clear cytoplasm (so-called “clear-cell immunoblasts”). Lymph node follicles may appear atrophic or may be absent. The cell of origin is the T-follicular helper cell (TFH), which classically expresses CD4 and CD10. The neoplastic T cells need to express two or three TFH-related antigens, such as PD1, CD10, BCL6, CXCL13, ICOS, SAP, and CCR5. These cells induce proliferation of follicular dendritic cells and recruit B cells to the lymph node. An IgH gene rearrangement can be seen in 30% of cases, in addition to T-cell receptor gene rearrangement, which is present in over 90%. Sequencing studies have identified frequent mutations in RHOA, IDH2, DNMT3A, and TET2 in these cases.

**Anaplastic Large-Cell Lymphoma, ALK-Positive**

This disorder causes about 3% of adult non-Hodgkin lymphoma and about 10% to 20% of childhood lymphomas. The usual clinical features are generalized lymph node enlargement and constitutional symptoms, including fever. The disease is commonly widespread at the time of diagnosis, typically involving such extranodal sites as skin, bone, lung, and liver. The neoplastic cells are pleomorphic, but despite this lymphoma having three variants—common, lymphohistiocytic, and small-cell—all cases include some characteristic cells called “hallmark cells” because they are present in all types. These have eccentric nuclei shaped like horseshoes or kidneys, sometimes with a perinuclear eosinophilic area. They are usually, but not always, large. In the **common variant**, which accounts for about 70% of cases, large hallmark cells typically predominate, and they possess abundant cytoplasm that is clear,
basophilic, or eosinophilic. Multiple nuclei may occur, the chromatin usually is dispersed, and nucleoli are prominent. Sometimes, the hallmark cells are less numerous than the large neoplastic cells with rounded nuclei. The neoplastic cells are positive for CD30 and ALK. ALK is a tyrosine kinase receptor that is normally not expressed in lymphocytes. Various genetic alterations cause a fusion of the ALK gene with other partners, leading to overexpression of the ALK protein. Although molecular methods can be used to detect ALK translocations, because of the wide variety of potential partners, immunohistochemical stains are the most sensitive method of detecting ALK alterations. ALK-positive anaplastic large-cell lymphoma (ALCL) has a better prognosis than its ALK-negative counterpart (discussed below), with a 5-year survival rate of 80%. Neoplastic cells are rarely visible in the peripheral blood film, where they are large and pleomorphic. On bone marrow aspirates, they are also usually sparse. Bone marrow biopsies may show the large, pleomorphic, and sometimes multinucleated lymphoma cells in an interstitial, focal, or diffuse pattern.

**Anaplastic Large-Cell Lymphoma, ALK-Negative**

ALK-negative ALCL is morphologically similar to ALK-positive cases but has a different epidemiology, prognosis, and molecular background. ALK-negative ALCL tends to affect individuals in their 40s to 60s and there is a mild male predominance. In comparison with ALK-positive cases, the prognosis is poor with a 5-year overall survival of 49%. Approximately 30% of ALK-negative cases harbor a DUSP22-IRF4 rearrangement, and these cases have a prognosis comparable with ALK-positive ALCL. A smaller subset of patients (<10%) have translocations involving TP63, and these cases have extremely poor outcomes (17% 5-year overall survival). These molecular alterations can be detected by FISH or next-generation sequencing platforms.

**HODGKIN LYMPHOMA**

Hodgkin lymphomas constitute about 30% of all lymphomas. Hodgkin lymphomas are defined by the presence of diagnostic tumor cells, known as Reed–Sternberg cells, in the appropriate cellular environment. The WHO classification divides this disorder into two major categories: nodular lymphocyte predominant Hodgkin lymphoma and classical Hodgkin lymphoma, which has four subtypes—nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted Hodgkin lymphoma. In classical Hodgkin lymphoma, the Reed–Sternberg cells are single or multinucleated cells with prominent central nucleoli. They are derived from a pre-apoptotic germinal center B cell in which there is epigenetic silencing of B-cell genes and downregulation of B-cell transcription factors Oct2 and BOB1. These cells coexpress surface antigens CD15 and CD30 with weak nuclear expression of PAX-5, a B-cell marker. They are negative for CD45. In contrast, the neoplastic cell population in nodular lymphocyte predominant Hodgkin lymphoma is the “LP” or “popcorn” cell, which is derived from a germinal center B cell with somatic hypermutation. These cells are further along the B-cell development pathway and consequently express B-cell markers like CD20, Oct2, and BOB1. They are negative for CD15 and CD30.

The neoplastic cells rarely appear in the peripheral blood smear. Even with marrow involvement, they are also uncommon in the bone marrow aspirate, where they appear as large cells with two nuclei and prominent nucleoli. On bone marrow biopsy, neoplastic cells are present in about 10% of cases and usually exist in a mixture of small lymphocytes,
eosinophils, and macrophages. The pattern can be either focal or diffuse. Variations include the presence of numerous Reed–Sternberg cells with few other cells, a fibrotic marrow with few neoplastic cells, and a hypocellular marrow with scattered foci of neoplastic and reactive cells.

**Nodular Lymphocyte Predominant Hodgkin Lymphoma**
This group accounts for 5% of Hodgkin lymphoma and typically occurs in the fourth to sixth decade of life. It usually causes localized lymph node enlargement in cervical, axillary, and inguinal lymph nodes. The histology is characterized by a nodular infiltrate of small lymphocytes and histiocytes with scattered large atypical cells called L&H cells (“lymphocytic and/or histiocytic Reed–Sternberg cells”). In contrast to classical Hodgkin lymphoma, neutrophils and eosinophils are rare. There may be morphologic overlap between cases of nodular lymphocyte–predominant Hodgkin lymphoma and T-cell/histiocyte-rich forms of large B-cell lymphoma; a broad panel of immunohistochemical stains is necessary to resolve this differential. The disease has a fairly indolent course with a good prognosis despite frequent relapses. The overall survival is 80% at 10 years.

**Classical Hodgkin Lymphoma**
This group accounts for 95% of cases of Hodgkin lymphoma and has a bimodal age distribution with a peak between 20 and 30 years and a smaller peak in the elderly. Most patients present with localized lymph node enlargement affecting cervical, mediastinal, axillary, or para-aortic regions. Primary extranodal involvement is rare, as is bone marrow infiltration, except in advanced disease or severely immunodeficient individuals. All subtypes of classical Hodgkin lymphoma will have variably numerous Reed–Sternberg cells with the identical immunophenotype (described above). The epidemiology, association with immunosuppression and EBV, and the background reactive infiltrate will vary by subtype.

**Nodular Sclerosis Hodgkin Lymphoma**
This type accounts for about 70% of classical Hodgkin lymphoma, with a median age of 28 and equal gender distribution. Most patients present with stage II disease, and about 40% have B symptoms. The most common site of involvement is the mediastinum. Lymph nodes have a thickened capsule with broad bands of fibrosis dividing the lymphoid tissue into nodules composed of a polymorphous cell population, with small lymphocytes, plasma cells, eosinophils, and macrophages admixed with less numerous Reed–Sternberg cells. Because of formalin fixation, the cytoplasm retracts around these large cells, leaving a white halo (or “lacuna”) around them, earning them the title “lacunar cells.” These neoplasms are EBV-associated in 10% to 40% of cases.

**Mixed Cellularity Hodgkin Lymphoma**
This type accounts for about 25% of cases of classical Hodgkin lymphoma, is more frequent in male patients, as well as in patients with HIV or those from the developing world, and shows evidence of EBV infection in at least 70% of cases. The average age is about 35 to 40 years. Patients commonly have advanced disease and B symptoms, with widespread enlargement of peripheral lymph nodes. The normal nodal architecture is effaced by an expansion of the interfollicular areas by an infiltrate of eosinophils, histiocytes, and plasma cells admixed with numerous Reed–Sternberg cells. Fibrosis is noticeably absent.
**Lymphocyte-Rich Classical Hodgkin Lymphoma**

In this disease, which constitutes about 5% of classical Hodgkin disease, peripheral lymph node enlargement is the main feature, and patients usually have stage I or II disease without B symptoms. About 70% of patients are male. The lymph nodes can show a diffuse or nodular pattern with numerous small lymphocytes, no neutrophils or eosinophils, and scattered Reed–Sternberg and lacunar cells. About 40% have evidence of infection with EBV.

**Lymphocyte-Depleted Classical Hodgkin Lymphoma**

This type, which accounts for less than 1% of classical Hodgkin lymphoma, often is associated with HIV infection. The median age is 37 years, and about 75% of patients are male. Peripheral lymph nodes are less commonly involved than are retroperitoneal lymph nodes, abdominal organs, and bone marrow. Most patients have advanced disease with B symptoms. The lymph nodes have a relative paucity of lymphocytes and sheets of neoplastic cells. Evidence of Epstein–Barr infection is common.

**ACKNOWLEDGMENTS**

The authors thank Dr. Amy Chadburn for her assistance in preparing this chapter.

**BIBLIOGRAPHY**


### Table 9.1
Pathologic features in the differential diagnosis of small B-cell lymphomas

<table>
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<tr>
<th>Lymphoma Type</th>
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<th>Immunophenotype</th>
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<td>Follicular lymphoma</td>
<td>Nodular (follicular)</td>
<td>Lymphocytes with irregular cleaved nuclei (centrocytes) and admixed large cells (centroblasts)</td>
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<td>Diffuse with proliferation centers</td>
<td>Small lymphocytes with round nuclei and scant cytoplasm</td>
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<td>Lymphoplasmacytic lymphoma</td>
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<td>Diffuse or vaguely nodular</td>
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<td>Nodal marginal zone B-cell lymphoma</td>
<td>Interfollicular and perisinusoidal</td>
<td>Small lymphocytes with round, folded nuclei and abundant cytoplasm ± plasma cells</td>
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medium-sized lymphocytes with round nuclei and abundant clear cytoplasm ± plasma cells ± plasma cells

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Ig, immunoglobulin; +, positive; −, negative.

Figure 9.1. Lymph node with follicular hyperplasia. This low-power image shows a normal lymph node with a thin fibrous capsule (Cp) surrounding the paracortex (PCx), medulla (Md), and hilum (H). Within the paracortex, numerous reactive follicles (F) in the cortex are surrounded by distinct darker staining mantle zones (M). (From Mirza MK, Sheehan AM. Hematopoietic system. In: Husain AN, ed. Biopsy Interpretation of Pediatric Lesions. Philadelphia, PA: Wolters Kluwer; 2014:212–253.)
Figure 9.2. Mantle cell lymphoma; nodular type and mantle zone pattern. **A** and **B**: Neoplastic nodules of medium-sized lymphoid cells with slightly irregular nuclear contours efface the lymph node architecture. Some of the nodules retain a distinctly mantle zone pattern, in which expanded collars of mantle zones surround germinal centers (arrows). **C**: The high-power view shows cytologic detail of the mantle lymphoma cells with a histiocyte nucleus (H) for size comparison.
Figure 9.3. Mantle cell lymphoma; blastoid variant. This peripheral blood smear shows medium to large atypical lymphoid cells with fine chromatin. The blastoid variant of mantle cell lymphoma is associated with a poor prognosis and p53 mutation. (From Farhi DC, Chai CC, Edelman AS, et al. B-cell neoplasms. In: Pathology of Bone Marrow and Blood Cells. Philadelphia, PA: Lippincott Williams & Wilkins; 2004:293–314.)

Figure 9.5. Mantle cell lymphoma; multiple lymphomatous polyposis. Sections from the jejunum show widespread polyoid lesions composed of submucosal nodules of small lymphoid cells that were cyclin D1+/CD5+/CD20+ B cells by immunohistochemistry.
Figure 9.6. Mantle cell lymphoma; nodular type. A: This lymph node is effaced by a nodular expansion of medium-sized lymphoid cells with a closed chromatin pattern and fairly regular nuclear contours. B: Low-power view of a reticulin-stained lymph node section highlights the nodular pattern of involvement. C: Admixed with the mantle lymphoma cells are occasional follicular dendritic cells, one delineated by an arrow showing classic “kissing” nuclei.
Figure 9.7. Mantle cell lymphoma; marrow involvement. Low- (A and C) and high- (B and D) power views of bone marrow biopsies from two cases of mantle cell lymphoma. The pattern of marrow involvement and the cytologic appearance of mantle cell lymphoma can be quite variable. Two different cases are demonstrated here, with one revealing a nodular pattern of infiltration (A and B) and the other displaying a diffuse pattern (C and D). High-power views show small- to medium-sized (endothelial nuclei for comparison are delineated by arrows) lymphoma nuclei with condensed chromatin pattern and slightly irregular nuclear contours.
Figure 9.8. Mantle cell lymphoma; t(11;14)(q13;q32) rearrangement. The diagnostic t(11;14) of mantle cell lymphoma is depicted here. The ideograms of chromosomes 11, 14, and the respective derivative chromosomes are illustrated on the left in color, and the corresponding G-banded chromosome pairs are on the right. Arrows indicate the breakpoints on the respective chromosomes. The t(11;14) is detectable in almost all cases of mantle cell lymphomas. This translocation juxtaposes the immunoglobulin heavy chain (IGH) and the cyclin D1 gene (CCND1), resulting in overexpression of cyclin D1.

Figure 9.9. Mantle cell lymphoma; FISH of the IGH/CCND1 fusion gene. A standard FISH assay that is used to detect the t(11;14) in either formalin-fixed/paraffin-embedded or fresh tissues is illustrated here. The CCND1 gene at 11q13 (red) and the IGH gene at 14q32 (green) co-localize to generate yellow signals, indicating a reciprocal translocation involving
these loci. The t(11;14)(q13;q32) occurs in 95% of cases by FISH, although it may not be readily detectable by standard cytogenetics.

Figure 9.10. Follicular lymphoma in spleen. This gross splenectomy specimen shows tiny macroscopically visible neoplastic nodules of follicular lymphoma diffusely involving the entire spleen.
Figure 9.11. Reactive follicular hyperplasia. An enlarged submandibular lymph node shows numerous follicles of varying size that do not appear crowded. Focally (blue arrow), the lymphoid tissue extends outside the capsule into adjacent adipose tissue. This extracapsular tissue, however, contains reactive follicles similar to the follicles inside the capsule. The follicles contain prominent, well-defined germinal centers that have a reactive appearance, with frequent tingible body macrophages (black arrows) and mitotic lymphocytes (red arrow). Undisrupted, well-demarcated, broad mantle zones surround the follicles. Immunohistochemistry was negative for BCL2 protein in the follicles.
Figure 9.12. Follicular lymphoma, grade 1. Fibroadipose tissue is involved by crowded, diffusely distributed, uniformly sized aggregates composed almost exclusively of well-spaced centrocytes with cleaved nuclear contours. For comparison, the lower right corner of the bottom right smear shows benign centrocytes with regularly contoured nuclei and condensed chromatin (arrows).
Figure 9.13. Follicular lymphoma, grade 2. The lymph node architecture is completely effaced by uniform, crowded follicles, some of which merge into each other. Focal invasion into the capsule, without extension into perinodal tissue, is present. The neoplastic follicles consist of small cleaved (centrocytes) and large noncleaved (centroblasts; red arrows) cells. An average of nine large noncleaved cells was present in 10 high-power fields. A rare follicular dendritic cell (fdc) is also visible among the lymphoma cells. Immunophenotype (by immunohistochemistry and flow cytometry) of the lymphoma cells revealed CD20⁺/CD10⁺/CD23⁺/CD5⁻/CD11c⁻/BCL2⁺/BCL6⁺. MIB-1 staining showed positivity in approximately 25% of the follicular lymphoma cells.
Figure 9.14. Follicular lymphoma, grade 3. This lymph node shows numerous uniformly spaced and sized nodules composed almost exclusively of large nucleolated centroblasts. Squeezed between the malignant nodules of centroblasts are linear arrays of centrocytes.
Figure 9.15. Follicular lymphoma, grade 3 (3b subtype). A retroperitoneal lymph node is replaced by numerous, back-to-back lymphoid follicles that often merge together. The follicles consist of monotonous, intermediate to large cells with irregular nuclei, vesicular chromatin, small distinct nucleoli, and scanty, basophilic cytoplasm. The proliferation extends through the lymph node capsule into surrounding adipose tissue (arrow). Immunophenotyping of the lymphoma cells reveals CD20+/CD10+/CD23+/CD5−/BCL6+.

Figure 9.16. Follicular lymphoma; cells in bone marrow aspirate smear. Medium-sized atypical lymphoid cells with cleaved nuclei are present in this aspirate from a marrow
replaced by follicular lymphoma.

**Figure 9.17.** Follicular lymphoma; marrow involvement. **A and B:** This bone marrow biopsy demonstrates extensive involvement by follicular lymphoma, with the multiple paratrabeicular lymphoid aggregates closely hugging bony trabeculae. **C:** Closer inspection reveals that the lymphoid infiltrate consists primarily of small lymphocytes with coarse chromatin pattern and irregular nuclear contours.
Figure 9.18. Follicular lymphoma; marrow involvement. **A:** Medium-power view of biopsy from a case of follicular lymphoma show the characteristic paratrabecular aggregates. **B–D:** Paratrabecular collections of CD20+/CD10+/BCL2+ small lymphoid cells are typical when this disease affects the bone marrow. **D:** Note the nuclear staining pattern for BCL2 (inset).
CHRONIC LYMPHOCYTIC LYMPHOMA

FOLLICULAR LYMPHOMA

LARGE B-CELL LYMPHOMA
Figure 9.19. Nuclear morphology of chronic lymphocytic leukemia (A), follicular lymphoma (B), and large B-cell lymphoma (C) lymphocytes in biopsy. Although lymphomas should not be classified based on their appearance in bone marrow, this composite figure highlights the differences in nuclear morphology among these three different B-cell lymphoproliferative disorders.

Figure 9.20. The t(14;18)(q32;q21) rearrangement. The ideograms of chromosomes 14 and 18 and of the respective derivative chromosomes are to the left in color. The corresponding G-banded chromosome pairs are to the right. Arrows indicate the breakpoints on the respective chromosomes. Most cases of follicular lymphoma have a t(14;18) reciprocal chromosomal translocation. This results in overexpression of the BCL2 gene and, ultimately, increased production of the antiapoptotic protein BCL2. This protein is not detected in high amounts in benign, reactive germinal center cells.

Figure 9.21. FISH of the IGH/BCL2 fusion gene. Diagrams in the left panels show the chromosomal localization of breakpoints in the two component genes. FISH of the translocated genes (right panels) demonstrates that the BCL2 gene at 18q21 (red) and the
IGH gene at 14q32 (green) co-localize to generate yellow signals, indicating a reciprocal translocation involving these loci.

**Figure 9.22.** IGH gene rearrangement analysis of lymphoid proliferations. Primers complementary to the framework 3 (FR3) region of the V genes and JH genes in the IGH locus are used in PCR amplification, which provides a sensitive assay to detect clonal B-cell populations. Fluorescently labeled PCR amplicons are separated and sized by capillary electrophoresis. Malignant B-cell proliferations are clonal, a feature that helps distinguish them from reactive polyclonal proliferations. **A:** A polyclonal pattern with multiple peaks in a Gaussian distribution. **B:** A monoclonal pattern with a prominent peak. (Courtesy of Dr. Wayne Tam.)
Figure 9.23. Diffuse large B-cell lymphoma involving lymph node. This lymph node shows the homogenous, fish-flesh appearance of lymphoma totally replacing the entire lymph node.

Figure 9.24. Diffuse large B-cell lymphoma involving spleen. Multiple large discrete tumors are visible in this gross splenectomy specimen.
Figure 9.25. Diffuse large B-cell lymphoma involving cerebrospinal fluid (CSF). This CSF sample stained with Wright–Giemsa demonstrates numerous large, pleomorphic cells with multilobulated nuclei and a high mitotic rate.

Figure 9.26. Diffuse large B-cell lymphoma involving bone marrow. As with many of the mature B-cell lymphomas, the appearance of DLBL in the marrow is highly variable, and classifying these diseases based solely on the morphologic appearance in the marrow is discouraged. Shown here are mid-trabecular aggregates composed almost exclusively of atypical, large lymphoid cells with the characteristic vesicular (or “glassy”) chromatin pattern and one to two prominent nucleoli of DLBCL. An aspirate smear from the same case shows a pleomorphic population of highly atypical, large lymphoid cells (right lower panel).
Figure 9.27. Marrow involvement by T-cell/histiocyte-rich large B-cell lymphoma. Low- (top), medium- (middle), and high- (bottom) power views demonstrate numerous large, poorly circumscribed, mid-trabecular aggregates made up of a heterogeneous population of large, atypical lymphoid cells with vesicular nuclei admixed with small mature lymphocytes. CD20-immunostained biopsy sections accentuate the nodular pattern of marrow involvement by the minor subpopulation of large malignant-appearing B cells that are admixed with benign reactive small T cells. This case could be mistaken for marrow involvement by Hodgkin lymphoma.
Figure 9.28. Diffuse large B-cell lymphoma (centroblastic, with multilobated cells). This lymph node shows marked sclerotic thickening of its capsule, with complete effacement of the architecture by extensive, noncohesive sheets of monotonous, large cells. A high mitotic rate with tripolar mitotic figure (white triangle) and multilobed nuclei (small black triangle) is seen, as well as numerous apoptotic bodies (small arrows in bottom right smear). Immunophenotype of the lymphoma cells by flow cytometry and immunohistochemistry (data not shown) revealed a CD20+/CD10+/BCL6+/κ+ immunophenotype.
Figure 9.29. Primary mediastinal large B-cell lymphoma. An axial CT scan through upper thorax at the level of the aortic arch shows a large mass (arrow) mostly involving the anterior mediastinum.

Figure 9.30. Primary mediastinal large B-cell lymphoma. This autopsy specimen with transversely cut lungs and mediastinum at the level of the aortic arch demonstrates a large, fibrotic, infiltrative mass encasing the entire aortic arch and extending into the adjacent lung.
Figure 9.31. High-grade B-cell lymphoma, not otherwise specified. A biopsy from the base of the tongue shows necrosis and extensive infiltration of tissue with atypical large lymphoid cells composed of immunoblasts with large, single, central nucleoli (white arrows) and centroblasts with multiple smaller nucleoli situated near the nuclear membrane (black arrows). Rearrangements of MYC, BCL2, and BCL6 were not identified by FISH.
Figure 9.32. A and B: High-grade B-cell lymphoma with \textit{MYC} and \textit{BCL2} rearrangement (“double-hit lymphoma”). These blood smears show circulating, large nucleolated lymphoid cells, some with clefted nuclear contours. For comparison, mature small lymphocytes are present near the lower left corners of both figures. C: The bone marrow biopsy is totally replaced by sheets of poorly preserved, malignant cells that display extensive crush artifact. FISH for \textit{BCL2} and \textit{MYC} rearrangement was positive in this case. These “double-hit” lymphomas, as well as diffuse large B-cell lymphoma, when confined to blood and marrow at the time of diagnosis, are easily misdiagnosed as precursor B-cell lymphoblastic leukemia/lymphoma.
Figure 9.33. High-grade B-cell lymphoma with MYC and BCL6 rearrangement. This cervical lymph node specimen showed total effacement by a proliferation of large atypical lymphoid cells with prominent nucleoli and in association with tangible body macrophages. The morphology resembles Burkitt lymphoma, but in light of the karyotype, which revealed translocations involving MYC and BCL6, this was classified as a high-grade B-cell lymphoma. (From Kluin KH, Rosati S. Burkitt lymphoma and related disorders. In: Orazi A, Foucar K, Knowles DM, et al, eds. Knowles’ Neoplastic Hematopathology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2014:566–583.)

Figure 9.34. Intravascular large B-cell lymphoma. This gastric biopsy from a 45-year-old
man shows normal overall architecture, but higher magnification reveals a vessel filled with highly atypical large CD20+ B-cell lymphoma cells.

Figure 9.35. Angiocentric diffuse large B-cell lymphoma (lymphomatoid granulomatosis). An autopsy lung from a patient with lymphomatoid granulomatosis reveals extensive involvement with multiple necrotizing lesions.
Figure 9.36. Lymphomatoid granulomatosis, grade 3/3. **A:** Low-power view showing effacement of normal lung architecture. **B:** Lung biopsy showing large lymphoid cells restricted to blood vessels and displaying a vasculitic pattern of involvement, with fibrinoid necrosis and angioinvasion. Commonly EBV-positive, this large B-cell lymphoma variant often is accompanied with a predominant T-cell background and usually manifests as pulmonary or paranasal sinus involvement.
Figure 9.37. Intravascular large B-cell lymphoma. Sections of cerebrum from autopsied brain reveal widespread involvement of small vessels by large atypical lymphoid cells that stain positively as CD20+ B-cells. The lymphoma cells extend through the walls of the vessels but do not invade brain parenchyma.
Figure 9.38. Diffuse large B-cell lymphoma, ALK-positive. A and B: A supraclavicular lymph node biopsy shows dense fibrous tissue and extensive coagulative tumor necrosis intimately associated with sheets of large, malignant lymphoid cells. C: The malignant cells have giant, eosinophilic staining nucleoli (black arrow) and mitotic figures (white arrow). D: Immunostaining for ALK-1 shows strong granular staining with focal enhancement in the Golgi region. Additional immunostaining revealed the lymphoma cells were CD138+/EMA+ and negative for B-cell and T-cell markers. In this case, approximately 50% of the lymphoma cells were positive for the proliferation marker MIB1.
Figure 9.40. Burkitt lymphoma. **A:** The biopsy of an abdominal mass shows, at low power, the classic “starry sky” appearance of rapidly proliferating malignant cells intermixed with larger histiocytes actively engulfing tumor debris. **B:** Higher power shows intermediate-sized cells that are uniform in size and shape, with multiple small basophilic nucleoli. FISH for MYC translocation was positive in this case.
Figure 9.41. Burkitt lymphoma involving bone marrow. This bone marrow biopsy demonstrates total replacement by rapidly growing tumor cells, some of which exhibit apoptosis admixed with “starry-sky” histiocytes engulfing tumor debris.
Figure 9.42. Burkitt leukemia. This blood smear reveals L3-type lymphoblasts with multiple nucleoli and basophilic staining, vacuolated cytoplasm.

Figure 9.43. Burkitt lymphoma/leukemia showing c-MYC translocations. Translocations of c-MYC including t(8;14), t(2;8), and t(8;22) have been reported in nearly all cases of Burkitt lymphoma. The t(8;14)(q24.1;q32) rearrangement is the most common and is found in 60% to 70% of cases. The ideograms of chromosomes 8, 14, and the respective derivative chromosomes are to the left in color; the corresponding G-banded chromosome pairs are to the right. Arrows indicate the breakpoints on the respective chromosomes.
Figure 9.44. FISH for c-MYC translocations in Burkitt lymphoma/leukemia. The region centromeric to the c-MYC gene is labeled with a red fluor and the region approximately 1 Mb telomeric to the c-MYC gene is labeled with a green fluor. When a translocation occurs involving the c-MYC gene, the red and green signals split apart. A yellow fusion signal indicates an intact c-MYC gene. This approach can detect the t(8;14), t(2;8), and t(8;22) involving the c-MYC locus at 8q24.
Figure 9.45. Plasmablastic lymphoma. This figure shows fragments of a solitary nasal tumor from an HIV-negative patient with no evidence of paraproteinemias. The tumor contains sheets of atypical plasmacytoid cells with a high mitotic rate. Plasmablastic lymphoma commonly presents at extranodal sites and is EBV-positive in 75% of cases.

Figure 9.46. Enlarged lymph nodes. Gross lymph node specimens have been cut to show characteristic homogenous pattern (“fish flesh” appearance) of a lymph node diffusely involved by chronic lymphocytic leukemia/small lymphocytic lymphoma (A), as compared with the nodular, heterogeneous appearance of a lymph node involved with metastatic cancer (B).
Figure 9.47. Mature B-cell neoplasms in peripheral blood. **A:** Chronic lymphocytic leukemia has lymphocytosis and “soccer ball” nuclei. Frequent smudged or “basket” cells, which are nuclear remnants of damaged cells, can be seen. **B:** Mantle cell lymphoma has small lymphocytes, condensed chromatin, and scant cytoplasm. **C:** Hairy cell leukemia has lymphopenia, ample cytoplasm with a central nucleus, and cytoplasmic projections. **D:** Splenic marginal zone lymphoma has lymphocytosis and irregular, eccentric nuclei. (A, B, and D: from Orazi A, Foucar K, Knowles DM, et al, eds. Knowles’ Neoplastic Hematopathology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2014. C: from Anderson SC, Poulsen KB. Anderson’s Atlas of Hematology. Philadelphia, PA: Lippincott Williams & Wilkins; 2003.)
Figure 9.48. Chronic lymphocytic leukemia (CLL); blood smear. **A:** Low-power view of a blood smear shows lymphocytosis with smudge cells. **B:** Higher power view demonstrates the typical appearance of the CLL cell: a small mature lymphocyte with condensed, clumpy chromatin pattern ("soccer ball" pattern) and regular nuclear contours. CLL cells can be difficult to distinguish from normal circulating lymphocytes.

Figure 9.49. Chronic lymphocytic leukemia/small lymphocytic lymphoma. An internal mammary node is diffusely effaced by a proliferation of small, mature lymphocytes with high N:C ratios and condensed chromatin. Pale staining proliferation centers are composed of prolymphocytes and immunoblasts—larger cells with vesicular nuclei and nucleoli. Arrows delineate the boundary of the proliferation centers.
Figure 9.50. Chronic lymphocytic leukemia/small lymphocytic lymphoma. The lymph node is diffusely replaced by small mature lymphocytes with high N:C ratios and condensed chromatin. Coalescent proliferation centers consisting of prolymphocytes and immunoblasts surround the smaller, mature-appearing CLL cells.
Figure 9.51. Chronic lymphocytic leukemia/small lymphocytic lymphoma; diffuse marrow involvement. A blood smear demonstrates mature lymphocytosis (A), and various magnified views of a marrow biopsy are shown (B–D). The biopsy shows a diffuse pattern of involvement of marrow by CLL, with total effacement of the architecture by small, mature lymphoid cells possessing regular nuclear contours.
Figure 9.52. Chronic lymphocytic leukemia; patterns of marrow involvement. Low- and high-power views of bone marrow biopsies are shown on the left and right, respectively, demonstrating the three patterns commonly seen in marrow involvement by CLL: nodular, interstitial, and diffuse. Nodular infiltration indicates the presence of foci of lymphoma cells separated by unaffected bone marrow and is typically distributed in a mid-paratrabecular pattern in CLL (top). Interstitial infiltration is marked by diffuse, patchy bone marrow invasion by individual CLL cells interspersed between hematopoietic and fat cells. Diffuse infiltration involves complete effacement of bone marrow architecture by sheets of lymphoma cells.
Historically, cytogenetic abnormalities such as del(17)(p13.1) and del(11)(q22) have been associated with rapid disease progression and poor survival, whereas del(13)(q14.3) as the sole abnormality suggested good prognosis in CLL. The significance of trisomy 12 is undetermined. This slide shows results from an interphase FISH panel performed on CLL cells. A: Deletion of the ATM gene (red) at 11q22.3 compared with a centromere 11 control (green). B: Trisomy 12 as indicated by three centromere 12 signals. C: Deletion of the 13q14.3 region (red) as compared to the LAMP1 gene (green) at 13q34. D: Deletion of the p53 gene (red) at 17p13 as compared with a centromere 17 control (green). Loss of p53 is associated with more aggressive disease.
Figure 9.54. Chronic lymphocytic leukemia; spleen. CLL involvement of organs is often diffusely infiltrative, but with retention of the underlying architecture. In this gross specimen, the spleen is homogeneously enlarged by diffuse infiltration of the red pulp.

Figure 9.55. Prolymphocytic leukemia (PLL). A blood smear demonstrates large, nucleolated lymphoid cells in a case of PLL transformed from CLL. For comparison, a normal small lymphocyte is present in the upper left corner of the figure.

Figure 9.56. Richter transformation. This lymph node is diffusely effaced by a dual population of small CLL/SLL and large atypical B cells. Different areas of the lymph node are composed exclusively of either the smaller CLL/SLL or larger B-cell lymphoma cells, as
shown in the bottom left and right smears, respectively.

**Figure 9.57.** Splenic marginal zone lymphoma (SMZL); gross appearance of a splenectomy specimen. Multiple small (1- to 3-mm) discrete nodules of expanded white are diffusely distributed throughout the splenic parenchyma.
Figure 9.58. Splenic marginal zone lymphoma; hilar lymph node involvement. The spleen is diffusely infiltrated by macroscopically visible nodular expansions of white pulp. Perivascular nodules made up of monotonous-appearing, well-spaced, medium-sized lymphoid cells are shown.
**Figure 9.59.** Splenic marginal zone lymphoma; blood smear. The typical cells in this disease are mature-appearing, medium-sized lymphocytes with ample cytoplasm that forms villous projections. A normal small lymphocyte is seen in the right upper corner.
Figure 9.60. Splenic marginal zone lymphoma; marrow involvement. A: Marrow biopsy shows a mostly sinusoidal pattern of marrow involvement by lymphoma. B and C: High-power views show small-sized lymphoma nuclei (compared with the larger endothelial nucleus; arrow in B), which possess a condensed chromatin pattern and regular nuclear contours. Low- (D) and high- (E) power views of CD20-immunostained biopsy sections accentuate the sinusoidal pattern of marrow involvement characteristic of this lymphoma.
Figure 9.61. Mucosa-associated lymphoid tissue (MALT) lymphoma of sclera. Clinical photograph of eye showing fleshy “salmon-color patch” of the superficial ocular surface. Ocular MALT lymphoma can be associated with *Chlamydia psittaci*.
Figure 9.62. Mucosa-associated lymphoid tissue lymphoma. This gastrectomy specimen shows a thickened stomach wall (arrow in A) from MALT lymphoma with a well-demarcated ulcer (B).
Figure 9.63. Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue lymphoma. This gastric biopsy demonstrates extensive involvement by well-spaced, medium-sized lymphoid cells that invade and destroy glands to form lymphoepithelial lesions (*arrow*).
Figure 9.64. Mucosa-associated lymphoid tissue lymphoma; small bowel. This MALT lymphoma involves a very well-defined segment of small bowel. Note the circumferential and transmural involvement of the bowel wall. MALT lymphoma of the small bowel can be linked to Campylobacter jejuni infection.
Figure 9.65. Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue lymphoma. This partial gastrectomy specimen demonstrates a MALT lymphoma ulcerating through the muscularis mucosae. Uniformly, well-spaced, medium-sized lymphoid cells invade and destroy glands to form lymphoepithelial lesions (bottom right image).
Figure 9.66. Mucosa-associated lymphoid tissue lymphoma; right parotid gland. An axial CT scan through the lower jaw reveals an enhancing soft tissue mass in the region of the right parotid gland.
Figure 9.67. Extranodal mucosa-associated lymphoid tissue lymphoma; parotid gland. A lymph node and subjacent parotid gland demonstrate a vaguely nodular proliferation (A and B) of well-spaced monocytoid lymphocytes invading germinal centers (C) and glandular structures to form lymphoepithelial lesions (D). Nodules of MALT lymphoma are seen filling sinuses (E), and these exhibit a characteristic uniformly well-spaced pattern reminiscent of the “fried egg” appearance of hairy cell leukemia involving the bone marrow (F).
Figure 9.68. Extranodal mucosa-associated lymphoid tissue lymphoma; axillary lymphadenopathy.
Figure 9.69. Mucosa-associated lymphoid tissue lymphoma; lymph node involvement by extranodal marginal zone B-cell lymphoma. A cervical lymph node from a case of MALT lymphoma of the parotid gland shows diffuse involvement by uniformly well-spaced, medium-sized lymphoid cells. Islands of MALT lymphoma are visible in the medium-power photomicrograph (arrows in upper right image).
Figure 9.70. Waldenström macroglobulinemia; lymphoplasmacytic lymphoma. Right flank mass in a patient suffering from recurrent Waldenström macroglobulinemia.
Figure 9.71. Waldenström macroglobulinemia. This lymph node is completely effaced by diffuse sheets of monotonous-appearing plasma cells, some showing intranuclear inclusions of monoclonal immunoglobulin in the form of Dutcher bodies (arrows).
Figure 9.72. Waldenström macroglobulinemia. A–C: Bone marrow biopsy sections show diffuse replacement of the marrow by a small lymphocytic/plasma cell population that exhibits restricted IgM expression by immunohistochemistry. D: Mixed population of lymphocytic/plasmacytoid cells is seen in a marrow aspirate smear.
Figure 9.73. Hairy cell leukemia (HCL); blood smears and bone marrow biopsies. This is a composite figure from four cases of HCL. These examples illustrate the variability in the appearance of HCL cells in blood smear (top panels). HCL cells are small to medium in size with mature chromatin and display ample, neutral-staining cytoplasm that may form hair-like villous projections. The distinctive “fried egg” appearance of HCL in biopsy specimens is shown in the bottom panels. HCL usually is associated with extensive fibrosis that results in failed attempts to obtain cellular aspirate smears (dry taps).
Figure 9.74. Hairy cell leukemia; electron micrographs. Transmission electron micrographs of cells from a case of hairy cell leukemia show numerous cytoplasmic villous projections at low magnification (A), and at higher power (B), the characteristic ribosomal–lamella complexes composed of concentrically arranged sheets of membranes alternating with rows of ribosomes are seen.
Figure 9.75. T-cell lymphoproliferative disorders; blood smear. Morphologic appearance of four T-cell lymphoma/leukemia disorders in blood: precursor T-cell lymphoblastic leukemia/lymphoma (T-ALL); T-cell prolymphocytic leukemia (T-PLL); Sézary syndrome/mycosis fungoides; and adult T-cell leukemia/lymphoma (ATLL).
Figure 9.76. Adult T-cell leukemia/lymphoma (ATLL). This blood smear from a case of ATLL shows a pleomorphic population of large cells with polylobated nuclei (“flower cells”). By flow cytometry, these cells were CD3+/CD4+/CD2+/CD7−/CD25+. Serology for HTLV-1 was positive.
Figure 9.77. Adult T-cell leukemia/lymphoma. Large atypical lymphoid cells, “flower cells” with convoluted nuclei, are seen circulating in blood (A) and in involved lymph nodes (B–E). ATLL cells are typically CD4+/CD25+ (D and E) and TdT-negative.
Figure 9.78. Extranodal NK-/T-cell lymphoma, nasal type. Ethmoid sinus tissue fragments from a 38-year-old woman show extensive necrosis and an angiocentric, pleomorphic population of large atypical lymphoid cells mixed with some histiocytes, small lymphocytes, and rare plasma cells. The lymphoma cells were positive for T-cell markers, CD56, and EBV.
Figure 9.79. Hepatosplenic T-cell lymphoma. **A:** An axial CT scan of the upper abdomen reveals a homogeneously enlarged liver. The spleen is absent. **B:** Blood smear showing medium-sized lymphoid cells with pale rims of cytoplasm and slightly irregular nuclear contours. **C:** The lymphoma cells typically express the γ/δ T-cell receptor, as shown in the flow cytometric scattergram (arrow).

Figure 9.80. Hepatosplenic T-cell lymphoma. **A:** Liver biopsy shows a sinusoidal infiltrate of small lymphocytes. **B** and **C:** The lymphocytes are positive for CD3 and negative for CD4, CD5, CD8, and TCR-β chain (βF1), consistent with a T-cell neoplasm of γ/δ T-cell origin. **D:** Traditionally in HSTCL, the lymphocytes express TIA1 but not granzyme B. (A–D from Feldman AL, Dogan A. Peripheral T-cell lymphomas. In: Orazi A, Foucar K, Knowles DM, et al, eds. Knowles’ Neoplastic Hematopathology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2014:59–697.)
Figure 9.81. Mycosis fungoides. Generalized erythrodermic type.
Figure 9.82. Mycosis fungoides involving the lower extremities with hyperkeratosis and fissuring of soles.
Figure 9.84. Mycosis fungoides. Epidermal involvement with single-cell exocytosis and individual large, atypical lymphoid cells with convoluted nuclear contours (arrow) infiltrating the epidermis (epidermotropism). The lymphoma cells in this particular case by immunohistochemistry displayed an abnormal T-cell phenotype: CD4$^+$ T-cells with aberrant loss of CD7.
Figure 9.85. Nodal peripheral T-cell lymphoma (PTCL) with T-follicular helper cell phenotype. A: The normal lymph node architecture is effaced by a mixed-cell infiltrate consisting of small lymphocytes, scattered plasma cells, eosinophils, and transformed lymphocytes. B: The infiltrate is accompanied by a marked increase in arborizing thin-walled blood vessels lined by plump endothelial cells. C: Clusters of atypical, transformed lymphocytes with abundant clear cytoplasm, so-called clear cell immunoblasts, are present. There is a background increase in CD3+ small T cells (D) with large atypical clear lymphoid cells strongly CD10+ (E). The atypical clear lymphoid cells by flow cytometry and immunohistochemistry were CD10+/CD3+ T cells with loss of CD7. PCR studies were negative for B-cell clonality, Epstein–Barr virus, and human herpes virus 8 in this case.
Figure 9.86. Enteropathy-type T-cell lymphoma. This figure shows sections near a small-bowel fistula from a 53-year-old man with a history of celiac disease. A large ulcerating lesion with transmural extension into the surrounding serosa is associated with necrosis and an extensive multifocal infiltrate composed mostly of atypical large lymphoid cells with vesicular nuclei that were positive for T-cell markers CD3, CD2, and CD5, with aberrant loss of CD7 by immunohistochemistry (data not shown).
Figure 9.87. Peripheral T-cell lymphoma. The lymph node architecture is disrupted by a diffuse interfollicular proliferation of clusters of “clear” lymphoid cells admixed with centrocytes. The morphologic spectrum for this disease entity is broad, with this particular case composed primarily of large cells.
Figure 9.88. Subcutaneous panniculitis-like T-cell lymphoma (large-cell variant). A subcutaneous neck nodule shows adipose tissue infiltrated by a heterogeneous population of fibroblasts, histiocytes, and atypical lymphoid cells. The lymphocytes characteristically form a rim around the subcutaneous fat. The large atypical lymphoid cells stained positively for T-cell markers such as CD8 (inset at bottom).
Figure 9.89. Anaplastic CD30+ large-cell lymphoma involving spleen. Nodules of lymphoma are seen near the capsule (arrows).
Figure 9.90. Primary cutaneous anaplastic CD30⁺ large-cell lymphoma. This skin biopsy from the upper arm of a 54-year-old woman reveals deep infiltration into subcutaneous tissue by poorly defined nodules of large, anaplastic cells with multilobulated nuclei. Many of the cells are “hallmark cells,” with lobulated nuclei forming horseshoe or wreath-like configurations. Immunostains were positive for T-cell markers and CD30.
Figure 9.91. Anaplastic CD30$^+$ large-cell lymphoma, ALK-1–positive. This inguinal lymph node from a 19-year-old man displays a sinusoidal pattern of involvement by large pleomorphic CD30$^+$/ALK-1$^+$ T cells. The diffuse pattern of sinusoidal spread, similar to metastatic carcinoma, preserves nodal architecture.
Figure 9.92. Anaplastic CD30+ large-cell lymphoma, ALK-1–positive. A and B: Nests of large malignant CD30+/ALK1+ cells fill sinuses throughout this lymph node. C and D: Lymphoma cell with an atypical multilobated or horseshoe-shaped (wreath-like) nucleus (arrow). Numerous mitotic figures are present. E: The t(2;5) translocation associated with anaplastic large-cell lymphoma results in the expression of a ALK-1 fusion protein that can be detected either by immunohistochemistry (as here) or FISH. F: Immunohistochemistry for CD30 is also positive.
Figure 9.93. Translocation of t(2;5)(p23;q35) detected by cytogenetics and FISH in diffuse anaplastic large-cell lymphoma. Approximately one-third of anaplastic CD30+ large-cell lymphomas harbor the t(2;5) translocation, which is associated with a favorable outcome in this disease. A: Ideograms of chromosomes 2 and 5, with the respective derivative chromosomes to the left in color. The corresponding G-banded chromosome pairs are to the right. Arrows indicate the breakpoints on the respective chromosomes. B: FISH of an ALK rearrangement. C: The region telomeric to the ALK gene is labeled with a red fluor, and the region centromeric to the ALK gene is labeled with a green fluor. When a translocation occurs involving the ALK gene, the red and green signals split apart. A yellow fusion signal indicates an intact ALK gene.
PCR is a sensitive assay used to detect clonal T-cell populations in which primers are used to amplify the V and J regions of the TCRG gene. Fluorescently labeled DNA is separated by capillary electrophoresis to identify a monoclonal peak. However, finding a monoclonal T-cell population by PCR is not specific for a diagnosis of lymphoma; in many reactive T-cell lymphoproliferations (e.g., infection, immunodeficiency, atopic disorders), a clonal population may be detected. **A:** A polyclonal pattern with multiple peaks in a Gaussian distribution is shown. **B:** A monoclonal T-cell population is shown with a single prominent peak. (Courtesy of Dr. Wayne Tam.)

**Figure 9.94.** Capillary electrophoresis of the T-cell receptor γ locus in T-cell lymphoma.

**Figure 9.95.** Gross appearance of a group of resected lymph nodes matted together by nodular sclerosis Hodgkin lymphoma. A vague nodular appearance can be discerned.
Figure 9.96. Hodgkin lymphoma of spleen. Multiple small nodules of Hodgkin lymphoma are distributed throughout the splenic parenchyma.

Figure 9.97. Nodular lymphocyte–predominant Hodgkin lymphoma. This lymph node is effaced by a small lymphocyte/histiocyte infiltrate and occasional “L&H” (popcorn) cells that are large cells with multilobulated nuclei and very fine neutral-staining nucleoli (bottom). In this disease, distinct from classical Hodgkin disease, these cells are typically CD45+/CD20+/BCL6+/CD30−/CD15−.
Figure 9.98. Nodular sclerosis Hodgkin lymphoma. A spinal accessory node from a 31-year-old woman shows a thickened capsule with the architecture distorted by broad bands of collagenous fibrosis interspersed with nodular aggregates composed primarily of small lymphocytes, scattered histiocytes, occasional eosinophils, plasma cells, and classic Reed–Sternberg cells. Classic Reed–Sternberg cells are large and binucleated with prominent central nucleoli (bottom).
Figure 9.99. Nodular sclerosis Hodgkin lymphoma; syncytial variant. A lymph node from a 32-year-old woman is distorted by wide bands of collagen sclerosis and nodules consisting mostly of highly atypical, large, multinucleated cells that stain positively for CD30 (bottom right) and CD15.
Figure 9.100. Mixed-cellularity Hodgkin lymphoma. This lymph node from a 37-year-old woman displays diffuse proliferation of small lymphocytes, eosinophils (Eo), plasma cells (Pc), and histiocytes (H) admixed with occasional classic Reed–Sternberg (RS) cells, which are characterized by a bilobed nucleus, vesicular chromatin, and prominent, dual, eosinophilic nucleoli. In addition, mononuclear RS variants (mononuclear RS) actually are often easier to find in this and other subtypes of Hodgkin lymphoma.
Figure 9.101. Lymphocyte-rich classical Hodgkin lymphoma. An enlarged lymph node is replaced by a vaguely nodular proliferation of small lymphocytes interspersed with classical RS cells (A and B), L&H cells (C), clusters of histiocytes (D), and immunohistochemistry showing the typical RS phenotype in classical Hodgkin lymphoma: positive for CD30 (E) and CD15 (F) and negative for CD45 (G).
Figure 9.102. Lymphocyte-depleted Hodgkin lymphoma. This axillary lymph node is totally replaced by a primarily large-cell infiltrate accompanied by some small mature lymphocytes. Numerous classic Reed–Sternberg cells (CD30+/CD15+/CD45−) were present in addition to bizarre forms with multilobulated nuclei mimicking wreath cells of anaplastic large-cell lymphoma.

Plasma cells are terminally differentiated B cells responsible for immunoglobulin production. Plasma cell disorders represent a group of malignancies in which clonal plasma cells give rise to a spectrum of pathology, depending on the degree of plasmacytosis in the bone marrow, the amount and type of immunoglobulin secretion, and the tropism of the immunoglobulin deposition in the body. The appearance of the plasma cells in the bone marrow may vary widely in these neoplasms, ranging from similar to normal mature plasma cells to very immature plasmablastic cells with high nuclear:cytoplasmic ratios. Abnormal plasma cells may produce immunoglobulin to such a degree that vacuoles full of immunoglobulin can either aggregate in the cytoplasm, in which case they are called Russell bodies, or penetrate the nucleus, then termed Dutcher bodies. A subset of immunoglobulins can also produce a highly eosinophilic appearance to the plasma cell cytoplasm, especially in the case of monoclonal IgA isotype antibodies, thereby giving rise to the term “flame cell.”

Plasma cell disorders are characterized by secretion of a monoclonal immunoglobulin (either whole or light chain only; very rarely heavy chain only) which can be quantified by serum protein electrophoresis (SPEP). The monoclonal immunoglobulin is referred to as an M-spike (because of the characteristic peaked appearance of the γ region on the densitometry scan of an electrophoresis gel), a monoclonal paraprotein, or an M-protein. The M-spike isotype is determined via immunofixation, in which parallel SPEPs in a gel are each impregnated with a specific antibody to the heavy chain of immunoglobulin IgG, IgA, or IgM and the light chains, κ or λ. The presence of an M-protein will produce a distinct pattern on the immunofixation gel correlating with the isotype.

Clonal plasma cells often produce excess free κ- or λ-light chains which are not bound to heavy chains, in addition to the intact M-protein. These circulate in the blood and are excreted via the kidneys. Free light chains are normally reabsorbed in the proximal tubule of the nephron; however, when in great excess, they can be found in the urine and are then called Bence Jones proteins. Similar to serum testing, a Bence Jones protein can be identified by urine protein electrophoresis and immunofixation. Free light chains in the serum can also be measured by an immunologic assay using antibodies that will detect only free, and not bound, light chains.

The presence of an M-spike that has been confirmed to have a single heavy and/or light chain by immunofixation is referred to as a monoclonal gammopathy. Although monoclonal gammopathy is most often linked to plasma cell neoplasms, there are many hematologic and immunologic disorders in which a monoclonal gammopathy can also be present. These conditions range in clinical impact from benign diseases (such as monoclonal gammopathy of undetermined significance [MGUS], rheumatologic diseases, skin disorders, chronic viral infections) to malignancies of B cells, including multiple myeloma (MM), Waldenström macroglobulinemia (WM), and B-cell lymphomas (Table 10.1). In the case of connective tissue disorders, the circulating autoantibody associated with the disease, such as rheumatoid
factor, can manifest as an M-spike on SPEP testing thus producing an associated monoclonal gammopathy.\textsuperscript{7}

Monoclonal gammopathies may present as type I cryoglobulinemia. Type I cryoglobulinemia involves a monoclonal immunoglobulin, usually of the IgG or IgM subtype, that precipitates from serum and plasma at temperatures below 37°C. The most common sources of these monoclonal immunoglobulin are WM, monoclonal plasma cell disorders, or other lymphoproliferative disease.\textsuperscript{8,9} Type I cryoglobulinemia may be asymptomatic or present with a constellation of signs and symptoms such as acrocyanosis, gangrene of distal extremities, hyperviscosity syndrome, skin purpura or ulceration, peripheral neuropathy, arthralgias, and membranoproliferative glomerulonephritis. Type II cryoglobulinemia results from a combination of a monoclonal protein with a polyclonal IgG and is associated with immune complex-mediated vasculitis often in conjunction with hepatitis C infection.\textsuperscript{10}

There are two rare severe skin diseases that are highly associated with monoclonal gammopathy, although the pathophysiology is obscure and not usually associated with an underlying plasma cell disorder. Schnitzler’s disease, a neutrophilic urticarial dermatosis, is associated with monoclonal IgM-κ in over 75% of cases.\textsuperscript{11} This debilitating condition is characterized by the development of chronic nonpruritic urticarial lesions with signs of systemic inflammation, including periodic fevers and leukocytosis. Schnitzler’s disease has recently been discovered to respond to anakinra, an interleukin-1 receptor antagonist.\textsuperscript{12} Scleromyxedema is a form of papular mucinosis that is associated with IgG-λ monoclonal gammopathy in 70% to 90% of the cases.\textsuperscript{13} In this condition, papules filled with mucin form and coalesce on the skin, leading to induration and restriction of movement in a scleroderma-like process. Scleromyxedema responds to high-dose intravenous immunoglobulin with occasional responses to immunomodulating novel agents used to treat MM.

**MONOClonAL Gammopathy of UNDetermined Significance**

MGUS is the most common plasma cell disorder. MGUS arises from a limited clonal proliferation of plasma cells in the bone marrow which secrete M-protein but otherwise has no clinical complications. The World Health Organization (WHO) definition of MGUS is a serum M-spike of less than 3 g/dL associated with less than 10% monoclonal plasma cells in the marrow, the absence of end organ damage, and no evidence of a lymphoma, as many lymphomas can be associated with a monoclonal gammopathy incidentally. MGUS is common in the elderly, and its incidence increases with aging; approximately 3% of persons over the age of 50 and 5% of persons over the age of 75 in the United States have MGUS.\textsuperscript{14} There is an increased risk of persons with MGUS to progress to symptomatic MM of approximately 1% per year. The risk of progression is higher when there is a greater amount of M-protein present, a non-IgG isotype of M-protein, and an abnormal free κ:λ light chain ratio.\textsuperscript{15} Studies of sera collected prior to a new diagnosis of MM suggest that all cases of MM have a preceding MGUS condition.\textsuperscript{16,17}

Monoclonal gammopathy of renal significance (MGRS) describes conditions where the M-protein, or portion thereof, harms the kidney.\textsuperscript{18} In these patients, low-level M-proteins have the capacity to damage glomeruli by a variety of mechanisms that lead to glomerulonephropathy and renal failure. In monoclonal immunoglobulin deposition disease,
free light or heavy chains or both form extracellular non-organized deposits predominantly in
the glomeruli and, in some patients in renal tubules as well, causing nephron damage
manifested by Bence Jones proteinuria, heavy albuminuria, and renal insufficiency.\textsuperscript{19} The
causative protein can often be readily determined by examination of the urine by
immunoelectrophoresis and immunofixation, although renal biopsy with immunofluorescence
is the gold standard for diagnosis. A bone marrow biopsy should also reveal a clonal
population of plasma cells sharing the same isotype of monoclonal protein involved in the
MGRS. Free light chains that are misfolded may also form organized aggregates in the renal
parenchyma in the form of amyloid deposition, leading to nephrotic range albuminuria, in
contrast to the Bence Jones proteinuria seen in light chain deposition disease. Renal failure is
the most common manifestation of primary amyloidosis (discussed below); however, other
organ damage caused by amyloid deposition is commonly seen. Rarely, monoclonal protein
deposition has also been detected in renal biopsies from patients with proliferative
membranous glomerulonephritis. Free light chains may also deposit on nerves and lead to a
peripheral neuropathy in plasma cells disorders, including MGUS, MM, and POEMS
(polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin
changes) syndrome.\textsuperscript{20} A specific IgM antibody called myelin-associated glycoprotein (MAG)
can cause demyelinating peripheral nerve damage.\textsuperscript{21}

In free light chain deposition disease, extrarenal symptoms are rare; however, free heavy
chain deposition disease often has other systemic morbidities depending on the heavy chain
isotype. In the heavy chain diseases, mutated and truncated free heavy chains are unable to
form disulfide bonds with corresponding light chains to create an intact immunoglobulin.
Antiserum will react with the corresponding heavy chain involved, but not with light chains. In
\(\alpha\)-chain disease, (also called Seligmann’s disease), the free \(\alpha\)-chains have a predilection for
deposition in the aerodigestive tract, where IgA is normally present in high amounts. Patients
with \(\alpha\)-chain disease are usually of Middle Eastern or Northern African descent and can
present with symptoms of malabsorption or dyspnea, depending on whether the deposition
involves primarily the gastrointestinal or respiratory tract. \(\gamma\)-Chain disease (also called
Franklin disease) is less common than \(\alpha\)-chain disease. \(\gamma\)-Chain disease occurs most
frequently in conjunction with a history of autoimmune disease, such as rheumatoid arthritis,
and more commonly affects women. \(\gamma\)-Chain disease can be diagnosed concurrently with a
lymphoma in the majority of new cases and often involves Waldeyer ring. Fevers,
lymphadenopathy, palatal edema, and impaired immunity are the most common symptoms.

\section*{MULTIPLE MYELOMA}

MM is the second most common hematologic malignancy, representing approximately 10%
of all blood cancers.\textsuperscript{22} MM is a chronic and incurable malignancy characterized by responses
to treatment followed by relapses requiring retreatment.\textsuperscript{23} MM has classically been defined
by the presence of all the following criteria: (1) at least 10% bone marrow clonal
plasmacytosis or more than one plasmacytoma elsewhere in the body; (2) detectable M-
protein in the blood or urine; and (3) end organ damage resulting from the plasmacytosis.\textsuperscript{24}
The end organ damage is often summarized by the acronym CRAB which stands for
hypercalcemia, renal dysfunction, anemia, and bone lytic lesions. A recent update has been
made to the definition of MM by the International Myeloma Working Group (IMWG) to
define MM as the presence of clonal plasmacytosis in the marrow or a plasmacytoma plus
one or more of the following biomarkers of malignancy: at least 60% bone marrow
plasmacytosis, free light chain (κ:λ or vice versa) ratio of at least 100, or more than one focal lesion on a MRI study. The presence or lack of M-protein further defines the MM into secretory and nonsecretory types. Thus, a person may have a biomarker of malignancy without symptoms and still have the diagnosis of MM. The diagnosis of smoldering myeloma (SMM) is made when a person has a clonal marrow plasmacytosis and/or M-protein present yet does not otherwise fulfill other criteria for either MGUS or MM. Patients with SMM have an elevated risk of transformation to active MM at approximately 10% per year for the first 5 years from diagnosis, then 3% per year for the next 5 years, followed thereafter at a rate of 1% per year. Most patients with SMM eventually do develop MM.

Like MGUS, MM is more common in older individuals, with a median age of 70. Twice as many men are affected as women. There is a racial disposition as well, with approximately twice the incidence in persons of African descent than that found in Caucasians. Exposure to pesticides imparts a slight risk in developing MM, as well as does chronic antigen stimulation from infection or autoimmune disease; however, most cases are idiopathic. Familial myeloma and monoclonal gammopathy have also been observed but occur relatively infrequently, at only two to four times the usual risk of developing MM, which is currently estimated at 6.5 per 100,000 persons per year.

Clinical features of MM relate to the CRAB end organ damage and immunodeficiency. The most common presenting symptom of MM is a complication of a lytic bone lesion, present in approximately 60% of patients at diagnosis, often leading to pathologic compression fractures of the vertebral bodies. These bony lesions can be seen on x-ray, although the more sensitive techniques of MRI and PET-CT modalities are used with increasing frequency for imaging. Malignant plasma cells carve out niches in the marrow through manipulation of cytokines that limit osteoblast activity and stimulate increased osteoclastogenesis. This activity can lead to the development of lytic bone lesions which have a “punched out” appearance on x-ray. Osteoporosis is also a consequence of this heightened osteoclastic activity and contributes to compression fractures and bone pain which are among the most common presenting features of MM. The cytokine activity of the malignant plasma cell can lead to systemic hypercalcemia with symptoms of constipation, confusion, and ultimately renal failure.

Anemia is the most common presenting laboratory feature in MM and results in fatigue and reduced exercise tolerance. MM can lead to anemia through multiple mechanisms, including occupation of space in the marrow, renal complications, and systemic inflammation with high interleukin-6 levels, leading to increased hepcidin production characteristic of the anemia of chronic disease. The replacement of normal marrow elements with MM cells can occasionally lead to leukoerythroblastosis on a blood smear. More commonly, the M-protein interacts with sialic acid on the erythrocytes and promotes red blood cell stacking, giving rise to rouleaux. The presence of rouleaux will also increase the erythrocyte sedimentation rate. Renal failure occurs at presentation in about 20% of newly diagnosed patients usually due to cast nephropathy. Cast nephropathy occurs when excess light chains overwhelm the reabsorptive capacity of the proximal renal tubules, which leads to intratubular aggregation of light chains, tubular obstruction, and local inflammation with acute kidney injury. Hypercalcemia—with symptoms of constipation, abdominal pain, and confusion—is present at diagnosis less commonly in about 15% of patients and contributes to worsening of renal function.

The dysregulated immunoglobulin production in MM leads to a relative humoral immunodeficiency from immunoparesis (reciprocal reduction in uninvolved immunoglobulins). Twenty percent of patients with MM present with infection, most
commonly from encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Deposition of M-protein or free light chains on nerve endings can lead to peripheral neuropathy, present in about 15% of persons at diagnosis.\(^{33,34}\) Approximately 20% of patients are asymptomatic at diagnosis and are identified through screening laboratory testing alone, most commonly identified by an elevated serum total protein or increased globulin gap (serum total protein minus serum albumin).

MM is often defined by the isotype of M-protein secreted. IgG subtype is most common at 60% of cases, followed by IgA (20%), free light chain only (10%–15%), and IgD, IgE, IgM, and nonsecretory at approximately 1% each. Rarely, unchecked or undiagnosed MM can lead to the production of so much M-protein that hyperviscosity of the blood develops, leading to symptoms of blurry vision, headache, dyspnea, and bleeding. Hyperviscosity occurs most often in IgM lymphoplasmacytic lymphoma (WM) and occasionally in IgA myeloma, given that IgA and IgM are larger proteins (dimer and pentamer, respectively) and thus meet a lower threshold for interference with blood flow.\(^9\) Rarely it may be seen in patients with very high IgG levels and the rare myeloma patient with an IgM isotype. Hyperviscosity is treated initially with plasmapheresis; however, this is a temporary measure only and treatment must be directed at the underlying MM to prevent recurrence. Particular M-protein isotypes (often IgM) can also lead to type I cryoglobulinemia.

As MM undergoes treatment and subsequent multiple relapses, the disease may decrease production of an intact immunoglobulin M-protein and secrete free light chains instead, termed a free light chain escape.\(^{35}\) Late stage MM may also stop producing M-protein or free light chains altogether, manifesting as plasmacytomas only, often extramedullary in nature involving virtually any part of the body accompanied by high serum lactate dehydrogenase levels.\(^{36}\) Late stage MM (and rarely at presentation) may also present as plasma cell leukemia, where a population of clonal plasma cells loses its restriction to the bone marrow and circulates in the bloodstream. By definition, plasma cell leukemia is defined as a circulating plasmacytosis of more than 2,000 cells/μL or when over 20% of all circulating white blood cells are plasma cells.\(^{37}\) Plasma cell leukemia usually carries a poor prognosis compared to MM.

Myeloma is a clinically and molecularly heterogeneous disease, and prognosis varies depending on disease stage, underlying cytogenetic abnormalities, and patient factors such as age and overall health.\(^{38}\) The most commonly used staging system is the International Staging System (ISS), which is defined by serum albumin and β-2 microglobulin alone.\(^{39}\) A recent update indicates that the revised ISS (R-ISS) takes into account elevated serum lactate dehydrogenase levels (a marker for extramedullary disease) and the presence of adverse cytogenetic abnormalities (Table 10.2).\(^{40}\) Depending on disease risk, median survival can vary widely from 43 months in patients at the highest stage to greater than a decade in those patients with stage I disease. Longer survival is seen for R-ISS stage III than ISS stage III due to advances made in treatment of MM between the development of the two staging systems including the use of immunomodulating agents and the proteasome inhibitors and the increased use of high-dose melphalan with the support of autologous transplants.

**AL (PRIMARY) AMYLOIDOSIS AND POEMS**

Amyloidosis is a disease driven by deposition of protein as self-propagating β-pleated sheets in organ tissue. Amyloid can be seen histologically by Congo red staining and apple green birefringence when viewed under polarized light. AL or primary amyloidosis refers to
amyloid consisting of monoclonal free light chains secreted by an underlying plasma cell neoplasm.\textsuperscript{41} AL amyloidosis is present in 10\% of cases of MM or may occur as a separate feature of a clonal plasma cell disorder, primary amyloidosis, that does not yet meet criteria for MM. As MGUS occurs relatively frequently, a diagnosis of AL requires screening for familial amyloidosis by history and, if not accompanied by overt MM, testing of the amyloid fibrils by immunocytochemistry or mass spectroscopy to confirm the diagnosis of a light chain–related amyloidosis rather than one caused by an inherited amyloidogenic protein.\textsuperscript{42} The median age at diagnosis for AL amyloidosis is 63, which is significantly younger than MM. Two-thirds of AL amyloidosis cases are λ-light chain isotype, whereas the remainder are κ. The light chain deposition in organs leads to clinical symptoms sooner than that expected from the degree of associated plasmacytosis; only 20\% of primary amyloidosis patients progress in plasma cell growth to develop overt MM.

The clinical consequences of AL amyloidosis are dependent on the tropism of the light chain for target organs. Most commonly, light chain deposition occurs around capillaries, leading to fragility and easy bruising which may manifest as periorbital ecchymoses. Deposition in the heart leads to arrhythmia through disruption of the cardiac conduction system, making sudden cardiac dysfunction the leading cause of death from AL amyloid. Because of these features, the staging system of cardiac AL amyloidosis is based on serum levels of N-terminal pro-brain natriuretic peptide and cardiac troponin.\textsuperscript{43} Infiltration of the renal parenchyma and glomerulopathy in renal AL amyloidosis can often produce nephrotic range albuminuria. Gastroparesis or malabsorption may occur with gastrointestinal tract involvement with AL amyloid. Other clinical manifestations may include restrictive lung disease from pleural involvement and macroglossia from tongue infiltration. Other soft tissue involvement may cause carpal tunnel syndrome or hoarseness. AL amyloidosis has a median survival of 2 years, far less than would be expected for MM alone.

A syndrome of light chain deposition called POEMS syndrome can also be seen as a consequence of light chain deposition, usually of λ isotype.\textsuperscript{44} Multiple criteria must be fulfilled to confirm a diagnosis of POEMS, three major and at least one minor. The polyneuropathy seen is often sensory and demyelinating in nature and with monoclonal gammopathy serve as the two required major criteria for the diagnosis. The other major criteria include Castleman disease (angiofollicular lymph node disease), sclerotic bone lesions, and elevated vascular endothelial growth factor levels. The minor criteria are organomegaly, endocrinopathy, skin changes, extravascular volume overload, thrombocytosis, and erythrocytosis. Organomegaly refers to enlargement of the liver and spleen from light chain infiltration. Endocrinopathy refers to hypogonadism, hypothyroidism, adrenal dysfunction, or diabetes from light chain involvement of endocrine tissues. The skin changes can be hypertrichosis, hyperpigmentation, or skin thickening. Like AL amyloidosis, POEMS is often a low plasma cell burden disease in which the light chain deposition leads to pathologic consequences before the plasmacytosis develops to symptomatic MM.

**SOLITARY PLASMACYTOMA**

A plasmacytoma is a collection of monoclonal plasma cells that form a tumor. Plasmacytomas arise most often in concert with a diagnosis of systemic MM; however, approximately 5\% of patients may present with a solitary plasmacytoma without evidence of a systemic plasma cell disorder.\textsuperscript{45} A solitary bone plasmacytoma is associated with an M-spike in approximately two-thirds of cases and a 60\% chance of progression to MM.\textsuperscript{46}
Extramedullary plasmacytomas arise outside of bone and may involve any part of the body; however, there is a predilection for the aerodigestive tract. By definition, solitary plasmacytomas present without CRAB features, and bone marrow evaluation is negative for monoclonal plasmacytosis. Solitary plasmacytomas may be cured with radiotherapy in approximately 30% of patients, with better outcomes seen for smaller size and extramedullary location. The majority of patients who do not achieve local control, especially with solitary plasmacytoma of bone, eventually develop MM.

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Non-Hodgkin lymphoma  
Chronic lymphocytic leukemia  
Castleman disease  
Post-transplant monoclonal gammopathy

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Psoriatic arthritis  
Immune complex vasculitis/type II cryoglobulinemia  
Chronic inflammatory demyelinating polyneuropathy

**Chronic viral infections**

Human immunodeficiency virus  
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**Skin disorders**

Scleromyxedema  
Schnitzler’s syndrome  
Pyoderma gangrenosum

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$\beta_2M$, serum $\beta$-2 microglobulin; LDH, lactate dehydrogenase; high-risk CA, the following cytogenetic abnormalities by interphase fluorescence in situ hybridization (FISH): del 17p, t(4;14), or t(14;16).

Figure 10.1. A: Normal blood smear. B: Blood smear from a patient with multiple myeloma. Immunoglobulins are acidic which leads to higher avidity for the blood smear stain and a bluish appearance. C: The feathered edge of a blood smear in a patient with multiple myeloma. Red blood cells should be separated in this location; however, interaction of sialic acid on the RBC membrane with excess immunoglobulin leads to stacking or rouleaux formation.
Figure 10.2. Serum protein electrophoresis. The point of application on the gel is marked by the arrow. Serum proteins separate based on charge and weight into a distinct banding pattern.

Figure 10.3. Densitometry view of serum electrophoresis. Albumin is the largest peak and closest to the positive electrode and the next peaks are globulins: α-1, α-2, β, and γ. The γ-peak is closest to the negative electrode. The presence of a monoclonal protein is characterized by a sharp, well-defined “M-spike” with a single heavy chain and a similar band with a κ- or λ-light chain. The absolute amount of monoclonal immunoglobulin, involved κ- or λ-light chain, and κ/λ ratio is followed to determine myeloma response to therapy and progression of disease. A broad diffuse band with one or more heavy chains and κ- and λ-light chains characterizes a polyclonal protein.
Figure 10.4. Serum protein electrophoresis and immunofixation. ELP, protein electrophoresis lane. 

A: Sample from healthy patient. No monoclonal banding is present. A dark albumin band (arrow) is seen at the top of the gel as it is the most abundant protein in normal serum samples.

B: Sample from a patient with multiple myeloma. Distinct bands are seen in the immunofixation lanes for heavy chain G and light chain λ. This patient has an IgG-λ type myeloma.

C: Urine protein electrophoresis and immunofixation. This patient has κ-light chain Bence Jones proteinuria quantified at 4.37 g/day. Note the faint albumin band (arrowhead). Albumin is blocked from urine excretion by the glomerulus in functioning kidneys. In this patient with myeloma, there is slight glomerular damage.

Figure 10.5. Familial myeloma and monoclonal gammopathy in two African American families. 

A: In this previously reported family, the propositus (arrow) had dialysis-dependent renal failure due to multiple myeloma at diagnosis and reported his sister died of multiple myeloma. Two of his three brothers had biclonal gammopathy on screening protein electrophoreses. (Adapted from Jain M, Ascensao J, Schechter GP. Familial monoclonal gammopathy and myeloma: a report of eight African American families. Am J Hematol. 2009;84:34–38.)

B: In the case of this family, at diagnosis the propositus had congestive heart failure due to cardiac amyloidosis documented by biopsy. Bone marrow biopsy showed a predominant λ-positive plasmacytosis associated with hyperdiploidy. His sister was previously diagnosed and under treatment for multiple myeloma, and two of his four children had monoclonal gammopathy on screening.
Figure 10.6. Sixteen examples of differences in plasma cell morphology seen in multiple myeloma. Multiple nuclei, plasmablastic morphology with increased nucleus:cytoplasm ratio and prominent nucleoli, and intracellular aggregates of immunoglobulin are seen.
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Figure 10.8. Plasma cell leukemia. **A:** Low-power view of peripheral blood smear demonstrating rouleaux and leukocytosis. **B:** Circulating plasma cells. **C:** Atypical lymphocyte with an asymmetric nucleus and vacuolization which could be mistaken for a circulating plasma cell.
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Figure 10.11. A: Osteoclast activation in myeloma. Clavicle biopsy from a myeloma patient showing multiple osteoclasts in resorption lacunae (arrow) surrounding a group of myeloma cells (arrowhead). Factors produced by myeloma cells or by their interaction with the bone marrow stroma include receptor-activated nuclear factor κB ligand (RANKL), macrophage inflammatory factor 1 α (MIP1-α), and interleukin-6 activate osteoclast resorption. Bone loss is further exaggerated by osteoblastic inhibitory factors produced by the myeloma microenvironment including dickkopf-1 (DKK-1) and others. (From Mundy GR, Raisz LG, Cooper RA, et al. Evidence for secretion of an osteoclast stimulating factor in myeloma. N Engl J Med. 1974;291:1041–1045. Copyright © 1974 Massachusetts Medical Society. Reprinted with permission.) B: Osteonecrosis of the jaw as a complication of bisphosphonate therapy. This patient has erosion of the mandible with delayed healing. Bisphosphonates inhibit osteoclast activity and thus help prevent worsening of myeloma-induced lytic lesions. However, bone remodeling cannot proceed normally and osteonecrosis of the jaw may result, especially after surgical manipulation, such as a dental extraction.

Figure 10.12. Solitary plasmacytoma. A: A chest x-ray shows a large lytic lesion in the right scapula in a patient with no other bone lesions and a small monoclonal protein. B: A year after radiation treatment of the scapula lesion the x-ray shows a small decrease in the size of the lesion with minor evidence of sclerosis. The monoclonal M protein was no longer detected. There was no evidence of recurrence after a decade of follow-up of this patient.
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translocations involving the IgH locus: t(4;14), t(14;16); del(17p) [which are best detected by interphase fluorescent in situ hybridization (iFISH)], del(1p), amp(1q), and del(13), when detected by conventional cytogenetics. A: A multiple myeloma karyotype with del(13) (q14;q22) (first arrow) and a derivative 14 chromosome (second arrow) from the translocation t(11;14)(q13;q32) along with trisomies of chromosomes 4, 11, 20. B: Another myeloma case with the translocation t(11;14)(q13;q32) (first arrow pointing to the loss in 11q and the second arrow pointing to the gain in 14q) along with other structural aberrations
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