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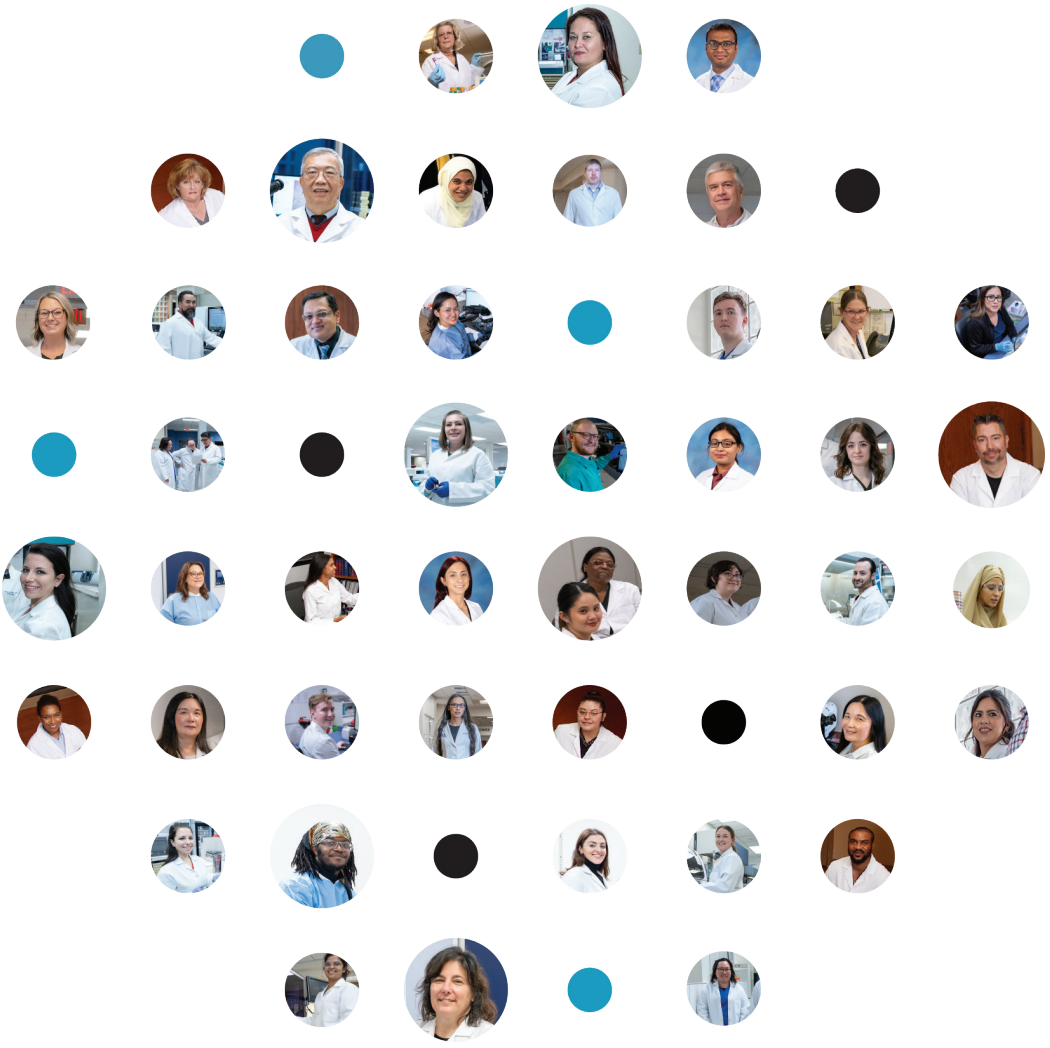
Hematology and Clinical Microscopy Glossary



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1 Blood Cell Identification

Introduction

This glossary corresponds to the master list for hematology, and it will assist survey participants in the proper identification of blood cells in photographs and virtual slides. Descriptions are for cells found in blood smears stained with Wright-Giemsa unless otherwise indicated.

Granulocytes and Monocytes

Basophil, Any Stage

Basophils have a maturation sequence analogous to neutrophils. At the myelocyte stage, when specific granules begin to develop, basophil precursors can be identified. All basophils, from the basophilic myelocyte to the mature segmented basophil, are characterized by the presence of numerous coarse and densely stained granules of varying sizes and shapes. The granules are larger than the granules of neutrophils and most are roughly spherical. The granules are typically blue-black, but some may be purple-red when stained using Wright-Giemsa preparations. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Basophils are comparable in size to neutrophils, ie, 10 to 15 µm in diameter, and the nuclear-to-cytoplasm (N:C) ratio* ranges from 1:2 to 1:3. Basophilia may be seen in several contexts, including in association with myeloproliferative neoplasms, in hypersensitivity reactions, with hypothyroidism, iron deficiency, and renal disease. Basophil granules can be stained with toluidine blue (resulting in a purple color) to differentiate them from the granules of neutrophils.

* For the purpose of this glossary, N:C ratio is defined as the ratio of nuclear volume to cytoplasmic (non-nuclear) cell volume.

Eosinophil, Any Stage

Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter in their mature forms, and 10 to 18 µm in diameter in immature forms. The eosinophil N:C ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is generally evenly filled with numerous coarse, orange-red granules of uniform size. These granules rarely overlie the nucleus and are refractile by light microscopy due to their crystalline structure. This refractile appearance is not apparent in photomicrographs or pictures, however. Due to inherent problems with color rendition on photomicrographs, which is sometimes imperfect, eosinophil granules may appear lighter or darker than on a freshly stained blood film. Discoloration may give the granules a blue, brown, or pink tint. Nonetheless, the uniform, coarse nature of eosinophil granules is characteristic and differs from the smaller, finer granules of neutrophils. Occasionally, eosinophils can become degranulated, with only a few orange-red granules remaining visible within the faint pink cytoplasm.

In the most mature eosinophil form, the nucleus segments into two or more lobes connected by a thin filament. About 80% of segmented eosinophils will have the classic two-lobed appearance. Typically, these lobes are of equal size and round to ovoid or potato shaped with dense, compact chromatin. The remainder of segmented eosinophils will have three lobes and an occasional cell will exhibit four to five lobes.

Eosinophils exhibit the same nuclear characteristics and the same stages of development as neutrophils. Immature eosinophils are rarely seen in the blood, but they are identifiable in bone marrow smears. Immature eosinophils may have fewer granules than more mature forms. The earliest recognizable eosinophil by light microscopy is the eosinophilic myelocyte. Eosinophilic myelocytes often contain a few dark purplish granules (primary granules) in addition to the orange-red secondary granules.

Monocyte

Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. Most monocytes have rounded cytoplasmic borders with smooth edges, but some may have pseudopod-like extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles and/or fine, evenly distributed azurophilic granules. The N:C ratio ranges from 4:1 to 2:1. The nucleus is usually indented, often resembling a three-pointed hat or kidney bean shape, but it can also be folded or band-like. The chromatin is condensed but is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

Monoblast/Promonocyte (Blast Equivalent)

For the purposes of proficiency testing, selection of the response “monoblasts/promonocyte (blast equivalent)” should be reserved for immature-appearing malignant cells in the context of acute myeloid leukemia with monocytic differentiation (eg, acute monoblastic or acute myelomonocytic leukemia, etc), chronic myelomonocytic leukemia, or myelodysplastic neoplasms/syndromes. While small numbers of promonocytes or monoblasts may be identified in normal marrow aspirates, they are generally absent in peripheral blood smears.

A monoblast is a large cell, usually 15 to 25 µm in diameter, with relatively more abundant cytoplasm than a typical myeloblast and a N:C ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has delicate, finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms; in these instances, additional tests (eg, cytochemistry and/or flow cytometry) are required to accurately assign blast lineage.

Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and monocytes. They are generally larger than monocytes, but with similar gray-blue cytoplasm that often contains uniformly distributed fine azurophilic granules. Cytoplasmic vacuolization as seen in mature monocytes is not typical of promonocytes. Promonocyte nuclei show varying degrees of lobulation characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Similar to monoblasts, promonocyte chromatin is open and lace-like, and nucleoli are conspicuous.

Neutrophil, Segmented or Band

Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. Band neutrophils, also known as stabs, constitute 5% to 10% of the nucleated cells in the blood under normal conditions. An increased number of bands may be noted in the blood in a number of physiologic and pathologic states (eg, infectious/inflammatory processes, tissue damage or necrosis, neoplasia, poisoning or intoxication, drug effect, and metabolic abnormalities). The band is round-to-oval and 10 to 18 µm in diameter. The N:C ratio is 1:1.5 to 1:2 and the nuclear chromatin is condensed. The nucleus is indented to more than half the distance to the farthest nuclear margin, but the chromatin is not condensed to a single filament (as is the defining feature of the fully mature neutrophil). The nucleus can assume many shapes: it can

be band-like or sausage-like; S-, C-, or U-shaped; and twisted or folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophils, with specific granules predominating in an otherwise pale cytoplasm.

The segmented neutrophil is the predominant blood leukocyte. It has a similar size to a band neutrophil (ie, 10 to 15 µm in diameter), as well as comparable shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The N:C ratio is 1:3 and the nuclear chromatin is highly condensed. The nucleus is segmented or lobated (with a normal range of three to five lobes). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from the band neutrophil. However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing, it is not required that they be differentiated. For a detailed guideline for the differentiation of segmented and band neutrophils, see Glassy, 2018; for other reading related to the clinical utility of band counts, see Cornbleet, 2002.

Neutrophil, Toxic (To Include Toxic Granulation and/or Döhle Bodies, and/or Toxic Vacuolization)

Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding and either change alone is sufficient to designate a neutrophil as toxic. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Ethylenediaminetetraacetic acid (EDTA) blood collection may produce degenerative vacuolization; in this context, only a few, small, punched out appearing vacuoles may be found. However, as it may be difficult to distinguish toxic from degenerative vacuoles, neutrophil vacuoles should not be labeled as toxic vacuoles unless accompanied by other toxic changes.

Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 µm) and shape (round or elongated or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found at the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum.

Toxic changes result from the action of cytokines released in response to infection, burns, trauma, and granulocyte colony stimulating factor (G-CSF), and they indicate a shortened maturation time and activation of post-mitotic neutrophil precursors.

In the May-Hegglin anomaly, inclusions that resemble Döhle bodies are seen. Unlike Döhle bodies, however, the May-Hegglin inclusion is due to aggregates of non-muscle myosin heavy chain IIA. Also seen in concert with neutrophil abnormalities are thrombocytopenia and giant platelets. The May-Hegglin anomaly is inherited in an autosomal dominant fashion, owing to mutations in *MYH9.*

Neutrophil with Hypersegmented Nucleus

To be considered a neutrophil with a hypersegmented nucleus, the neutrophil should demonstrate six or more lobes. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis. Hypersegmented neutrophils may also be seen in sepsis, renal disease, and myeloproliferative neoplasms. Megaloblastic hematopoiesis occurs when DNA synthesis is impaired. Such conditions include deficiency of cofactors for nucleotide synthesis, such as vitamin B12 and folate, and cases in which patients are receiving a nucleotide analog drug (such as 6-mercaptopurine) or nuclear cofactor blocking agents (such as methotrexate) for the treatment of neoplastic or rheumatologic conditions.

Neutrophil with Pelger-Huët Nucleus (Acquired or Congenital)

Neutrophils with bilobed nuclei in a pince-nez conformation (ie, two round or nearly round lobes connected by a distinct thin filament) are designated as neutrophils with Pelger-Huët nuclei or as Pelger-Huët cells. They may be seen in the context of an inherited autosomal dominant abnormality of nuclear segmentation, referred to as Pelger-Huët anomaly, caused by mutations in the Lamin B Receptor (*LBR*) gene. In patients heterozygous for an *LBR* mutation, virtually all of the neutrophils have bilobed nuclei. Individuals homozygous for an *LBR* mutation, however, typically have single-lobe neutrophils. The nuclear chromatin in Pelger-Huët cells is generally denser than in normal neutrophils. Comparable neutrophil morphology, typically involving only a subset of neutrophils, may be seen in association with a number of conditions including myelodysplastic syndromes and other myeloid malignancies, as a side-effect of certain drugs (eg, sulfonamides, colchicine and mycophenolate mofetil) and in association with certain infections (eg, HIV and *Mycoplasma pneumonia*). To distinguish these cells from neutrophils seen in inherited conditions, these cells are designated as pseudo-Pelger-Huët cells.

Neutrophil with Dysplastic Nucleus and/or Hypogranular Cytoplasm

Dysplastic neutrophils are characteristic of myelodysplastic syndromes. Morphologically, the normal synchronous maturation of nucleus and cytoplasm is lost. As a result, in the cytoplasm, the primary and secondary granules are often decreased or absent, causing the cytoplasm to appear pale and bluish. Dysplastic neutrophils often have cytoplasm so pale that cytoplasmic borders cannot be easily distinguished from the slide background. The nucleus may show abnormal lobation accompanied by a mature chromatin pattern. In some cases, the nucleus has a pince-nez appearance; these cells are known as pseudo Pelger-Huët neutrophils. For proficiency testing purposes, cells with pseudo-Pelger-Huët nuclei are best labelled as Pelger-Huët cells. Dysplastic neutrophils often have abnormal cytochemical reactivity; levels of myeloperoxidase and neutrophil alkaline phosphatase may be low or absent. The dysplastic neutrophils may also exhibit functional defects.

Neutrophil Necrobiosis (Degenerated Neutrophil)

Neutrophil necrobiosis is a common phenomenon that can be seen both in normal individuals and in patients with a variety of medical conditions (including infections, in association with inflammatory disorders, and in malignancies). It is a nondiagnostic and nonspecific finding. Degenerated neutrophils are generally easily identified because they resemble normal segmented neutrophils: they are round to oval cells ranging from 10 to 15 µm in diameter and their N:C ratio is 1:3 or less. The major distinguishing feature is that the nucleus shows karyorrhexis and/or pyknosis. These changes are appreciated when a cell with neutrophilic granules (pale pink cytoplasm with fine lilac granules) contains multiple, unconnected nuclear lobes (karyorrhexis) or a single, dark, round-to-oval nucleus (pyknosis). The chromatin pattern in these karyorrhexic or pyknotic states is also characteristic: dense and homogeneous, without visible parachromatin or nucleoli. The nuclear lobes may fragment into numerous small particles of varying size that can resemble microorganisms such as bacteria or fungi. Also, the nuclear outlines may become indistinct and blurred.

As cellular degeneration continues, the cytoplasm will become hypogranulated, then agranular, and the cytoplasmic borders may become frayed and indistinct. Sometimes, the cells will contain scattered larger azurophilic or dark blue granules (toxic granulation). Vacuolation is frequent. If a cell is too degenerated to be recognized as a neutrophil and lacks recognizable cytoplasm, one should identify it as a basket/smudge cell. On occasion, necrobiotic neutrophils can contain ingested bacteria or fungi. However, the microscopist must be very careful when making this identification since nuclear fragments may appear deceptively similar. Other cells that can resemble degenerated neutrophils include nucleated red blood cells (nRBCs) in the blood and orthochromic normoblasts in the bone marrow. In contrast, however, these erythroid lineage cells have a characteristic pinkish-orange and agranular cytoplasm with a single, often eccentric nucleus with a dense ink-drop appearance.

Neutrophil, Giant Band or Giant Metamyelocyte

Myeloid precursors resulting from megaloblastic hematopoiesis show an increase in size, and they have nuclei that show aberrant maturation, whereby the nucleus appears less mature than the cytoplasm. Although in discussion these changes usually pertain to the neutrophil lineage, they may also be observed in cells in the eosinophil and basophil lineages. Larger-than-normal metamyelocytes and bands with decreased chromatin clumping are seen in the marrow. These cells have diameters 1.5 times those of normal metamyelocytes or bands.

Neutrophil, Metamyelocyte

Metamyelocytes are the first of the postmitotic myeloid precursors. They constitute 15% to 20% of nucleated cells in the bone marrow and may be seen in the blood in pathologic states and in response to stress. They are approximately 10 to 18 µm in diameter. They are round to oval with a N:C ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the maximal nuclear diameter (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic, containing rare azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules.

Neutrophil, Myelocyte

The transition from promyelocyte to myelocyte occurs with the end of production of azurophilic (primary) granules and the beginning of production of lilac or pale orange/pink (specific) granules. Myelocytes are usually confined to the marrow, where they constitute approximately 10% of the nucleated cells. In pathologic states, myelocytes are seen in blood. The myelocyte is smaller than the earlier precursors, usually 10 to 18 µm. The cells are round to oval in shape and have a nuclear-to-cytoplasmic ratio of 2:1 to 1:1. The nucleus is slightly eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping; one side often shows slight flattening. Sometimes a clear space or hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in earlier precursors and is amphophilic. Both azurophilic and specific granules are present in the cytoplasm with specific granules coming to predominate as maturation progresses.

Neutrophil, Promyelocyte

Promyelocytes are round-to-oval cells that are generally slightly larger than myeloblasts, with a diameter of 12 to 24 µm. They are normally confined to bone marrow, where they constitute less than 2% of nucleated cells. However, like myeloblasts, promyelocytes can be seen in the blood in pathologic states. The N:C ratio usually ranges from 5:1 to 3:1. The nucleus is round to oval, has fine chromatin, and contains distinct nucleoli. The cytoplasm is basophilic, more plentiful than in a myeloblast, and contains multiple distinct azurophilic (primary) granules. A paranuclear hof or cleared space is typically present.

Neutrophil, Promyelocyte, Abnormal with/without Auer Rod(s)

The neoplastic cell in acute promyelocytic leukemia is considered to be the neoplastic counterpart of the promyelocyte. However, this leukemic cell differs from the normal promyelocyte in several respects. The nucleus is usually folded, bilobed, or reniform (often with overlapping nuclear lobes). A distinct Golgi zone is typically absent, and cytoplasmic granules, while abundant in the classic hypergranular form of this disease, may differ in appearance, often being coarser or finer than those seen in normal promyelocytes and slightly darker or more reddish in color. In the microgranular variant, very few granules may be visible, and those granules may be very fine. Finally, the abnormal promyelocyte of acute promyelocytic leukemia frequently contains numerous overlapping Auer rods (these types of cells are termed *faggot cells*, derived from the English term for a cord of wood).

Myeloblast, with Auer Rod

Myeloblasts are the most immature cells in the myeloid series. They are normally confined to the bone marrow where they constitute less than 5% of the nucleated cells. They may be present in the blood in leukemic states, myelodysplastic neoplasms/syndromes, myeloproliferative neoplasms, and, very rarely, in leukemoid reactions.

Typical myeloblasts are relatively large, 15 to 20 µm in diameter, round to ovoid cells with high N:C ratios ranging from 7:1 to 5:1, round to oval nuclei with fine chromatin and often distinct nucleoli. The cytoplasm is typically scant, basophilic, and may contain granules with or without Auer rods. Auer rods are pink to red, rod-shaped cytoplasmic inclusions seen in early myeloid cells (eg, myeloblasts and malignant promyelocytes) and represent crystallization of primary granules.

Variable cytomorphology of myeloblasts can make distinguishing one type of abnormal blast cell from another difficult to impossible on Wright-Giemsa stain alone. The only morphologic feature that confirms myeloid lineage is the presence of Auer rods. Additional testing such as cytochemical staining (eg, myeloperoxidase or Sudan black special stains) or immunophenotyping (eg, immunohistochemistry or flow cytometry) may be required to further define the lineage of a blast population.

Erythrocytes

Acanthocyte (Spur Cell)

Acanthocytes are densely stained, spheroidal red blood cells (RBCs) that lack central pallor and have multiple (usually three to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. Spicules may occasionally have branches. Acanthocytes are classically described in association with hereditary abetalipoproteinemia (hereditary acanthocytosis). In addition, these cells are often seen in significant numbers in severe end-stage liver disease, post splenectomy, hepatorenal failure, infantile pyknocytosis, McLeod phenotype, anorexia nervosa, and chronic starvation. (In the latter two disorders, they appear as irregularly shaped erythrocytes with multiple blunt projections imparting an animal cracker-like appearance.) A small number of acanthocytes may be seen in other forms of severe hemolytic anemia, particularly after splenectomy. Acanthocytes are rarely encountered in otherwise normal blood smears (one or two per smear). In such smears, they represent older, senescent RBCs approaching their extremes of life (120 days). It is logical, therefore, that acanthocytes should be more readily found in blood smears in the postsplenectomy state because of diminished splenic activity in removal of such poikilocytes.

Bite Cell (Degmacyte)

Bite cells are RBCs from which precipitated, denatured masses of hemoglobin (Heinz bodies) have been pitted out by the spleen. Precipitation is a function of oxidant injury to hemoglobin by certain drugs or denaturation of unstable hemoglobin variants. Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency may be predisposed to such oxidant injury. The net result of the act of pitting is a variety of peripheral RBC defects, ranging from tiny arc-like nibbles to large bites. Bitten RBCs may show multiple peripheral defects. Symmetrical equatorial defects result in the formation of apple-core-like poikilocytes. Giant single bites may result in the formation of poikilocytes morphologically indistinguishable from the helmet-like cells of microangiopathic hemolytic anemias. As in the fragmentation anemias, spherocytes are also almost invariably present, albeit in small numbers.

Blister Cell/Prekeratocyte

Blister cells are erythrocytes in which the hemoglobin appears to be concentrated on one side of the cell, leaving just a thin membrane on the other side. This produces the appearance of large vacuoles with fuzzy margins. Blister cells are most characteristically seen in sickle cell disease, in which they are considered a sickle cell variant. Similar cells, *eccentrocytes,* may be seen in the setting of oxidant hemolysis. Blister cells may be similar in appearance to prekeratocytes.

Prekeratocytes are RBCs containing one or two sharply defined, usually submembranous, vacuoles. By electron microscopy, these vacuoles are actually *pseudovacuoles,* representing fusion of opposing red-cell membranes with exclusion of intervening hemoglobin. The membrane union is brought about by hemodynamic pressures that have forced opposing RBC membranes to become closely applied to or draped over obstacles, such as nonocclusive thrombi or fibrin strands in small vessels. Dislodgement results in the reappearance of these RBCs in the circulation with stigmata of membrane fusion. By light microscopy, the points of fusion appear as crisply demarcated pseudovacuoles. Rupture of peripheral pseudovacuoles of prekeratocytes results in the formation of keratocytes or *horned cells.* These cells may be morphologically indistinguishable from (or identical to) classic helmet cells. Thus, prekeratocytes and keratocytes are usually found together in the same blood smears and should raise the question of a microangiopathic process. Similar or identical cells are also present in small numbers in iron deficiency anemia.

Echinocyte (Burr Cell, Crenated Cell)

Echinocytes are RBCs with 10 - 30 uniform, short, blunt projections distributed evenly that impart a serrated appearance to the RBC surface. The RBCs retain central pallor and are the same size or slightly smaller than normal RBCs. Their appearance is often the result of an improperly prepared smear (slow drying, thick smears, aged blood and pH alteration of glass slide). Echinocytes that are not artifacts may be indicative of disease, such as uremia or pyruvate kinase deficiency, and are seen post splenectomy, in hepatitis of the newborn, and phosphoglycerate kinase deficiency. Under such circumstances, they should be visible in wet preparations.

Erythrocyte, Normal

An erythrocyte is a mature, non-nucleated biconcave disc-shaped cell of fairly uniform diameter (6.7 to 7.8 µm) with a uniform round area of central pallor. It contains hemoglobin and stains uniformly pink red. The zone of central pallor is due to the biconcavity of the cell and occupies approximately one third (2 to 3 µm) of the cell diameter. Normal erythrocytes circulate in the peripheral blood for approximately 120 days before they undergo catabolism or destruction in the spleen.

Erythrocyte with Overlying Platelet

In preparing a peripheral blood smear, platelets may adhere to or overlap RBCs, suggesting an RBC inclusion or parasite. A correct interpretation depends on carefully examining the morphology of the platelet and comparing the size, staining characteristics, and granularity with known platelets in the same field as well as determining if the platelet is in the same plane of focus as the RBC. Many times the platelet is surrounded by a thin clear zone or halo, which is not a feature of most genuine RBC inclusions.

Fragmented RBC (Schistocyte, Helmet Cell, Keratocyte, Triangular Cell)

Fragmented RBCs are RBCs that have undergone rips and tears when draped over fibrin strands in the microcirculation or have suffered buffeting against unyielding structures in the macrocirculation. Fragments resulting from such trauma reseal by fusion of opposing ends and persist in the circulation, presumably

for a short time. Fragmented RBCs include helmet cells, keratocytes (horn cells), triangulocytes and a more inclusive term, schistocytes. A zone of central pallor is rarely present in fragmented cells. Occasional spherocytes are almost invariably present in association with fragmented cells. These spherocytes are the product of the rounded-up RBC fragments. Fragmented cells are seen in severe burns, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and other microangiopathic hemolytic anemias, in patients with prosthetic cardiac valves or severe valvular stenosis, malignant hypertension, or other mechanical trauma to the cell (eg, march hemoglobinuria, marathon running). When present in large numbers, they may cause the MCV to fall into the microcytic range or interfere with platelet enumeration.

Macrocyte, Oval or Round (Excluding Polychromatophilic RBC)

Macrocytes are abnormally large RBCs (diameter > 8.5 µm). They are best detected by comparing to other RBCs in a smear in the context of the MCV. They may be oval or round. The hemoglobin concentration is normal; cells lack significant polychromasia. (If polychromasia is readily identified, the term polychromatophilic RBC is preferred for proficiency testing purposes). Round macrocytes are associated with reticulocytosis, liver disease, hypothyroidism, and post-splenectomy states. Oval macrocytes are most commonly associated with vitamin B12 or folic acid deficiency. Abnormal RBC maturation (dyserythropoiesis) may also cause oval macrocytosis. Examples include myelodysplastic syndromes and chemotherapy. Oval macrocytes may be mistaken for ovalocytes (elliptocytes). Ovalocytes are often longer than normal RBCs and are significantly narrower. The sides of the cells are nearly parallel, unlike the much more rounded edges of oval macrocytes. The hemoglobin of ovalocytes is often concentrated at the ends, unlike the even peripheral distribution of oval macrocytes. Also, oval macrocytes are much larger than ovalocytes.

Microcyte (with Increased Central Pallor)

Microcytes are smaller than normal RBCs, measuring less than 6 µm in diameter and less than 80 fL in volume. On the blood film, they generally appear smaller than the nucleus of a small lymphocyte. When there is little or no variation in RBC size, morphology is less reliable than instrument-generated MCVs in determining if microcytosis is present. On a peripheral blood film, microcytes retain central pallor, appearing either normochromic or hypochromic. RBCs are considered hypochromic when central pallor exceeds 50% of cell diameter. Although other poikilocytes, such as spherocytes and fragmented RBCs, can be very small in size, these RBCs lack central pallor and should be specifically identified rather than classified as microcytes. Microcytes commonly are seen in iron deficiency anemia, thalassemia, lead poisoning, and some cases of anemia of chronic disease.

Nucleated Red Blood Cell, Normal or Abnormal Morphology

The term nRBC is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nRBC is at the orthochromic stage of differentiation. Both megaloblastic and dysplastic changes can be seen in these circulating RBCs, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nRBC as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroid precursors present in the marrow.

For the purposes of proficiency testing, it is adequate to identify a cell as an nRBC when it is present in the peripheral blood, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes).

Ovalocyte (Elliptocyte)

The terms elliptocytes and ovalocytes are used to describe RBCs appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. Hemoglobin is often concentrated at the ends, producing a dumbbell appearance. A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte skeletal membrane proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells are prominent. Some ovalocytes may superficially resemble oval macrocytes but are not as large as macrocytes and tend to be less oval with sides that are nearly parallel. The ends of ovalocytes are always blunt and never sharp, unlike those of sickle cells.

Polychromatophilic (Non-nucleated) Red Blood Cell

A polychromatophilic RBC is a non-nucleated, round or ovoid RBC that represents the final stage of RBC maturation after exiting the bone marrow. It is larger than a mature erythrocyte and usually lacks central pallor. It primarily contains hemoglobin with a small amount of RNA, and thereby stains pale purple to pink-gray with Romanowsky or Wright-Giemsa stain. These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. With supravital staining, reticulocytes reveal deep blue granular and/or filamentous structures. This reticulin network is called the *substantia reticulofilamentosa.* The amount of precipitated RNA and intensity of polychromasia varies inversely with the age of the reticulocyte. The intensity of the polychromasia will vary with the amount of RNA and the age of the cell, with younger cells (ie, earlier polychromatophilic red cells) appearing more purple or blue and relatively more mature cells (ie, later polychromatophilic red cells) appearing more pink-gray. Automated technologies for assessing reticulocytes improve the accuracy and precision of determining reticulocyte numbers.

Red Blood Cell Agglutinates

RBC agglutination occurs when RBCs cluster or clump together in an irregular mass in the thin area of the blood film. Usually, the length and width of these clumps are similar (14 by 14 µm or greater). One must distinguish this abnormality from rouleaux formation. Individual RBCs often appear to be spherocytes due to overlapping of cells in RBC agglutinates. This misperception is due to obscuring of the normal central pallor of the RBCs in the clump. Autoagglutination is due to cold agglutinins, most commonly an IgM antibody. Cold agglutinins can arise in a variety of disease states and are clinically divided into cases occurring after viral or Mycoplasma infections, cases associated with underlying lymphoproliferative disorders or plasma cell dyscrasias (cold agglutinin disease), and chronic idiopathic cases that are more frequently seen in elderly women. RBC agglutinates can also be found in cases of paroxysmal cold hemoglobinuria that exhibit a similar clinical pattern and can occur after viral infections. This disorder is caused by an IgG antibody that binds to the RBCs at low temperature (eg, in the extremities during cold weather) and then causes hemolysis when the blood returns to the warmer central circulation.

Rouleaux

Rouleaux formation is a common artifact that can be observed in the thick area of virtually any blood film. This term describes the appearance of four or more RBCs organized in a linear arrangement that simulates a stack of coins. The length of this arrangement (18 µm or more) will exceed its width (7 to 8 µm), which is the diameter of a single red cell. The central pallor of the RBCs is generally apparent, but it may be obscured due to overlapping of the cells' cytoplasm. When noted in only the thick area of a blood film, rouleaux formation is a normal finding and not associated with any disease process. True rouleaux formation is present when seen in the thin area of a blood film. It is often associated with a proteinaceous, blue-staining background. True

rouleaux formation is due to increased amounts of plasma proteins, primarily fibrinogen, and globulins. It is seen in a variety of infectious and inflammatory disorders associated with polyclonal increases in globulins and/or increased levels of fibrinogen. Rouleaux formation associated with monoclonal gammopathies can be seen in multiple myeloma and in malignant lymphomas such as Waldenstrom macroglobulinemia.

Sickle Cell (Drepanocyte)

RBCs appearing in the shape of a thin crescent with two pointed ends are called sickle cells. The polymerization of deoxygenated hemoglobin S may cause RBCs to appear in one or more of the following forms: crescent-shaped, boat-shaped, filament-shaped, holly-leaf form, or envelope cells. These cells usually lack central pallor. Sickle cells may be seen particularly in the absence of splenic function or after splenectomy in patients with the various forms of sickle cell anemia including hemoglobin SS disease, SC disease, SD disease, and S-beta-thalassemia.

Spherocyte

Spherocytes are identified as densely staining, spherical, or globular RBCs with normal or slightly reduced volume (ie, normal or low MCV) and increased thickness (more than 3 µm), but with decreased diameter (usually less than 6.5 µm) and usually without central pallor. These cells appear denser than normal RBCs and are commonly found in hereditary spherocytosis and immune hemolytic anemias. Microspherocytes (spherocytes measuring 4 µm or less in diameter) are frequently seen in severe burns or microangiopathies and represent rounded-up fragments of RBCs.

Stomatocyte

Stomatocytes are RBCs in which the central pallor is not round but straight or appears as a curved rod-shaped slit, giving the RBCs the appearance of a smiling face or a fish mouth. Stomatocytes have a low surface-to-volume ratio induced by either loss of surface or, more commonly, a gain in cell volume due to altered permeability and increased water uptake (hydrocytosis). These cells have a decreased MCHC. They appear as uniconcave cup-like cells in solution or by electron microscopy and as stomatocytes on air-dried smears. Stomatocytes account for less than 3% of RBCs in normal adult individuals. Newborns may have more stomatocytes than adults. They are also a common artifact arising from slow drying of smears. Stomatocytes not due to this artifact are commonly seen in hereditary stomatocytosis, in association with Rh-null disease, or in patients with liver disease.

Target Cell (Codocyte)

Target cells are thin RBCs with an increased surface membrane-to-volume ratio. They are often flattened out on the smears and may appear macrocytic. Target cells are believed to arise from disturbances in RBC membrane cholesterol and lecithin content or decreased cytoplasmic hemoglobin content. Target cells are characterized by a central hemoglobinized area within the surrounding area of pallor, which in turn is surrounded by a peripheral hemoglobinized zone giving target cells the appearance of a sombrero or a bull's-eye. Target cells associated with hemoglobin C may have a slightly reduced or normal MCV, whereas those associated with hemoglobin E disorders or hemoglobin H disease exhibit microcytosis of varying degree. Target cells are usually seen in thalassemias, iron deficiency anemia, following splenectomy or in patients who are jaundiced or who have chronic liver disease. In the latter two conditions, the MCV may be normal or increased. Target cells may also appear as artifacts from slow drying the slides in a humid environment or from specimens anticoagulated with excessive EDTA. The drying artifact results in the presence of numerous target cells in some fields, but none or few in other fields.

Teardrop Cell (Dacrocyte)

RBCs appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end are called teardrop cells. These are commonly seen in patients with bone marrow fibrosis, but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias, and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and nonhematologic malignancies may also be accompanied by dacrocytosis. Teardrop cells may be seen as an artifact of slide preparation. Such dacrocytes are usually easily recognized due to the fact that their tails all point in the same direction.

Erythrocyte Inclusions

Basophilic Stippling (Coarse)

Basophilic stippling may be either fine or coarse. Fine stippling is seen in reticulocytes. It is barely discernible in the RBC and is not of any clinical consequence. Coarse stippling, on the other hand, is clinically significant and suggests impaired hemoglobin synthesis. It is readily visible and made up of relatively evenly distributed blue-gray granules in Wright-Giemsa-stained RBCs. Coarse stippling results from abnormal aggregates of ribosomes and polyribosomes in reticulocytes. Iron-containing mitochondria in the aggregates may further accentuate the stippling. Heavy metal poisoning, including lead and arsenic poisoning, hemoglobinopathies, thalassemia, sideroblastic anemias, 5’ nucleotidase deficiency, and myelodysplastic syndromes are disorders commonly associated with coarse basophilic stippling.

Hemoglobin C Crystal

Hemoglobin C crystals within RBCs are dense structures with rhomboidal, tetragonal, or rod shapes. They often distort the cell and project beyond its rim. The classic shape resembles the Washington monument. The crystals are often surrounded partly by a clear area or blister devoid of hemoglobin. Hemoglobin C crystals are readily seen after splenectomy in patients with hemoglobin C disease or SC disease. Crystals in hemoglobin SC disease are more pleomorphic and may be multiple parallel or nonparallel, irregular, finger-like projections of uneven length with blunt or pyramidal-shaped ends.

Howell-Jolly Body

Howell-Jolly bodies are small, round, dark purple homogeneous masses that measure about 1 µm in diameter. They are larger, more rounded, and darker staining than Pappenheimer bodies and are composed of DNA. They are formed in the process of RBC nuclear karyorrhexis or when an aberrant chromosome becomes separated from the mitotic spindle and remains behind after the rest of the nucleus is extruded. Normally, the spleen is very efficient in removing Howell-Jolly bodies from RBCs, but if the spleen is missing or hypofunctional, they may be readily found in the peripheral blood. Howell-Jolly bodies are usually present singly in a given RBC. Multiple Howell-Jolly bodies within a single RBC are less common and are typically seen in megaloblastic anemia.

Pappenheimer Bodies (Iron or Wright Stain)

Pappenheimer bodies are small, angular, irregularly distributed, dark inclusions appearing either singly or in small groups near the cell periphery. They are less than 1 µm in diameter and thus are smaller than Howell-Jolly bodies. Unlike Heinz bodies, they are visible on Wright-Giemsa-stained smears. Their preferential location beneath the cell membrane aids in distinguishing them from more diffusely distributed

basophilic stippling. Pappenheimer bodies stain positively with iron stains, such as Prussian blue, indicative of the presence of iron (siderocytes). Wright-Giemsa stain does not stain the iron, but rather the protein matrix that contains the iron. Pappenheimer bodies are formed as the RBC discharges its abnormal iron-containing mitochondria. An autophagosome is created that digests the offending organelles. If the autophagosome is not discharged out of the cytoplasm or removed by the pitting action of the spleen, the inclusions will be visible on Wright-Giemsa-stained blood films. Their true nature and unequivocal distinction from basophilic stippling or Howell-Jolly bodies is confirmed by iron staining. Pappenheimer bodies are seen in iron overloaded states, hemolytic anemias, thalassemia, sideroblastic anemias, postsplenectomy, and hyposplenism.

Lymphocytes and Plasma Cells

Lymphoblast

Lymphoblasts are the most immature cells of the lymphoid series. They are most commonly seen in acute lymphoblastic leukemia (ALL) and lymphoid blast crisis of chronic myelogenous leukemia (CML). These round-to-oval cells range in size from 10 to 20 µm. The N:C ratio varies from 7:1 to 4:1. Morphologically, lymphoblasts are variable in appearance, at times within a single case. At one end of the spectrum are small lymphoblasts (previously called L1 subtype) with dense but not clumped chromatin, inconspicuous or absent nucleoli, and extremely scanty cytoplasm. At the other end are large lymphoblasts (previously called L2 subtype) with finely dispersed chromatin, variable numbers of distinct nucleoli, and moderate amounts of cytoplasm, closely resembling myeloblasts. The nuclear contours of lymphoblasts range from round to convoluted. The cytoplasm is typically slightly to moderately basophilic and is usually agranular. Auer rods are absent. Because lymphoblasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone and use of other techniques (such as immunophenotypic analysis) is required. Lymphoblasts can be indistinguishable from other types of blasts and lymphoma cells. For purposes of proficiency testing, one should identify individual cells exhibiting this immature type of morphology as blast cells.

Lymphocyte

While most normal lymphocytes are fairly homogeneous, they do exhibit a range of normal morphology. Lymphocytes are small, round-to-ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round-to-oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

Lymphocyte, Large Granular

Large granular lymphocytes are medium-to-large cells with round nuclei, dense chromatin, and no visible nucleoli. The cytoplasm is moderate to abundant, clear and lightly basophilic, and contains several variable coarse, unevenly distributed, small azurophilic granules. These cells are found in small numbers in blood smears from normal individuals, but they may be increased in association with reactive lymphocytes. Cell surface marker studies show that these cells are natural killer cells or suppressor/cytotoxic T-lymphocytes.

Lymphocyte, Reactive (Includes Plasmacytoid and Immunoblastic Forms)

The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. The classic example is infectious mononucleosis (acute Epstein-Barr virus infection). Reactive lymphocytes can also be found in a variety of other viral infections (including cytomegalovirus, adenovirus, or acute HIV infection) protozoal infections (such as toxoplasmosis), some drug reactions, connective tissue diseases, and after major stress to the body's immune system. A variety of reactive lymphocyte forms have been described and they are often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2.

The most common type of reactive lymphocyte resembles a larger lymphocyte and corresponds to a Downey type II cell. These cells have round-to-oval nuclei, moderately condensed chromatin (giving it a smeared appearance), and absent or indistinct nucleoli. They contain abundant, pale gray-blue cytoplasm. Granules, if present, are usually small and few in number. Frequently, these reactive lymphocytes have an amoeboid cytoplasm that partially surrounds adjacent red cells and has a darker-staining, furled margin. Basophilia radiating out from the nucleus may also be present.

Immunoblasts and immunoblastic-like reactive lymphocytes are large cells (15 to 20 µm) with round-to-oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli. These may resemble lymphoma cells or blasts. Their cytoplasm is moderately abundant and stains deeply basophilic. The N:C ratio is high (3:1 to 2:1). These reactive lymphocytes correspond to Downey type III cells.

Another type of reactive lymphocyte is referred to as a Downey I cell. These cells are rare. These cells possess scant to moderate amounts of basophilic cytoplasm. The nuclei often appear indented, folded, or lobulated. The chromatin is condensed. A few small vacuoles may be present. Granules may also be apparent.

Plasmacytoid lymphocytes resemble plasma cells and are intermediate in size (10 to 20 µm) and round to oblong in shape. They have round nuclei that are centrally placed or slightly eccentric. The chromatin is slightly to moderately coarse and forms small dense masses or a meshwork of strands resembling that of plasma cells. Nucleoli are generally not visible, but some cells may have one or two small irregular nucleoli. The cytoplasm is moderately abundant, homogeneous, and light blue to deep slate-blue, and it may show a perinuclear clear zone, or hof.

Malignant Lymphoid Cell (Other Than Blast)

Lymphoma cells can exhibit a variety of appearances depending on the lymphoma subtype, and definitive diagnosis can be difficult. These cells can exhibit a variety of sizes, shapes, and nuclear and cytoplasmic characteristics. Cell size ranges from 8 to 30 µm and the N:C ratio varies from 7:1 to 3:1. It is critical to obtain an accurate clinical history, since knowledge of a previous diagnosis of lymphoma greatly aids in the identification of these cells. Supplemental studies, such as immunophenotyping, are often necessary to arrive at a diagnosis. In blood smears, it may be difficult to distinguish reactive lymphocytes from lymphoma cells. The most important distinction between these cells is the difference in their N:C ratios. The N:C ratio tends to be low in reactive lymphocytes, while it is high in lymphoma cells. In addition, reactive lymphocytes are characterized by their wide range of morphologic appearances within the same blood smear. In contrast, while lymphoma cells can exhibit a wide range of morphologic appearances, any individual case tends to show a more monotonous population of the abnormal cells.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): CLL/SLL cells may be the same size as normal lymphocytes but are often slightly larger. The nucleus is typically round, although a small nuclear indentation may be present. The cells have clumped chromatin and a scant amount of pale blue cytoplasm. Nucleoli are inconspicuous. Occasional prolymphocytes are often seen. Individual CLL/SLL cells may be difficult to distinguish from normal lymphocytes. Clues to a diagnosis of CLL/SLL include a high white blood cell (WBC) count with absolute lymphocytosis, and nuclear chromatin that often appears cracked, resulting in a nucleus that resembles a soccer ball. The presence of occasional prolymphocytes is an additional clue to the diagnosis as these are not seen in normal or reactive blood smears. For proficiency testing purposes, lymphocytes in a CLL/SLL smear may be identified as either “malignant lymphoid cell (other than blast)” or “lymphocyte,” and both are considered acceptable.

Prolymphocytes (CLL/SLL or prolymphocytic leukemia): Prolymphocytes are larger lymphoid cells that are seen in cases of CLL, where they usually comprise less than 10% of lymphoid cells. They can also be found in prolymphocytic transformation of CLL, and B and T-cell prolymphocytic leukemia (PLL). These round-to-ovoid cells range from 10 to 18 µm, and the N:C ratio varies from 5:1 to 3:1. They are larger than normal lymphocytes and typical CLL cells and are similar in size to lymphoblasts. A centrally placed, oval-to-round nucleus and a moderate amount of homogeneously staining, blue cytoplasm is typical. The cytoplasm is more abundant than in normal lymphocytes or blasts and may contain a few azurophilic granules. The nucleus shows somewhat condensed chromatin (coarser than in lymphoblasts and more open than in mature lymphocytes) with indistinct parachromatin and, typically, a single, prominent nucleolus. Occasionally, these cells may exhibit more than one nucleolus.

Follicular lymphoma (low grade): Low-grade follicular lymphoma cells are slightly larger than normal lymphocytes. The majority of nuclei are clefted, indented, folded, convoluted, or even lobulated. The chromatin is moderately coarse, and one or more nucleoli may be present. The cytoplasm is scant to moderate and often basophilic.

Hairy cell leukemia: Hairy cells, typical of hairy cell leukemia, are round-to-ovoid lymphoid cells that measure 12 to 20 µm (larger than normal, mature lymphocytes). Their N:C ratio ranges from 4:1 to 2:1, and they contain moderate-to-abundant, pale blue to gray-blue cytoplasm. The cell borders are often indistinct, secondary to the presence of characteristic elongated, fine (hairy), cytoplasmic projections. These projections are frequently irregular and may be thick, blunted, smudged, serrated, or short. The cytoplasm typically is agranular, although occasional fine azurophilic granules may be seen. Small vacuoles can be present and often give a mottled appearance to the cytoplasm. The nuclei of hairy cells are usually oval to indented, but may be folded, bean-shaped, angulated, or dumbbell-shaped and are either centrally or eccentrically located. The chromatin is usually homogeneous, finer than in normal lymphocytes or chronic lymphocytic leukemia cells, and evenly distributed with scant intervening parachromatin. Nucleoli, if present, are generally small and single. Occasional cells may have multiple small nucleoli or a single large nucleolus.

Burkitt lymphoma: Burkitt lymphoma cells are medium-to-large cells (10 to 25 µm) with a round-to-oval nucleus and moderately coarse chromatin with one or more prominent nucleoli. The cytoplasm is moderately abundant, deeply basophilic, and often contains numerous small and uniformly round vacuoles.

Mycosis fungoides/Sézary syndrome: Sézary cells are classically found in patients with leukemic manifestations of mycosis fungoides, a form of primary cutaneous T-cell lymphoma. These cells are usually round to oval, but they can be irregular. They range in size from 8 to 20 µm, and their N:C ratio varies from 7:1 to 3:1. Smaller Sézary cells are slightly bigger than normal lymphocytes and have folded, grooved, or convoluted nuclear membranes, which may give them a cerebriform appearance. The chromatin is dark and hyperchromatic without visible nucleoli. Larger Sézary cells can be more than twice the size of normal lymphocytes. The nucleus is also convoluted and cerebriform appearing with hyperchromatic chromatin.

Often, the nuclear membrane is so folded that the nucleus may appear lobulated or even similar to a cluster of berries. Some cells may exhibit a small nucleolus, although this is not a prominent feature. Both large and small Sézary cells have scant, pale blue to gray agranular cytoplasm, and they may contain one or several small vacuoles that lie adjacent to the nucleus. While the appearance of Sézary cells is distinctive, other T-cell lymphomas and some cases of B-cell lymphoma can mimic Sézary cells. Small populations of Sézary-like cells have been reported in normal, healthy individuals, comprising up to 6% of lymphocytes.

Large cell lymphoma: These cells may exhibit some of the most abnormal morphologic appearances. They are large (20 to 30 µm) and have scant to moderate amounts of basophilic cytoplasm. The nuclei are generally round to oval, but they may be angulated, folded, indented, or convoluted. Nucleoli are prominent, and they may be single or multiple. Vacuoles can occasionally be seen in the cytoplasm. These cells can be easily confused with blasts, and additional studies such as immunophenotyping are often necessary to make the correct diagnosis.

Plasma Cell, Morphologically Mature/Abnormal/Containing Inclusion (eg, Dutcher Body, Russell Body)

Plasma cells represent terminally differentiated B-lymphocytes and are a normal constituent of the bone marrow, where they usually comprise less than 5% of the cellularity. They are rarely seen in normal peripheral blood. They range in size from 10 to 20 µm, and they are often oval shaped with relatively abundant cytoplasm and eccentrically located nuclei. The N:C ratio is 1:2. Their nuclei are usually round to ovoid with prominently coarse and clumped chromatin that is often arranged in a cartwheel-like or clock-face pattern. Occasional benign plasma cells are binucleated. Nucleoli are absent. The cytoplasm stains gray blue to deeply basophilic. A prominent hof, or perinuclear zone, of pale or lighter staining cytoplasm is typically seen adjacent to one side of the nucleus. This area corresponds to the Golgi zone, which is prominent in cells that produce large amounts of protein, such as immunoglobulin in the case of plasma cells. Cytoplasmic granules are absent, and scattered vacuoles of varying size may be seen. In IgA-type myelomas, plasma cells may have pink-red cytoplasm (so-called *flame cells*).

Immature or atypical plasma cells in the bone marrow or, less commonly, in the blood are associated with a variety of plasma cell dyscrasias, including multiple myeloma (plasma cell myeloma), lymphoplasmacytic lymphoma (Waldenström macroglobulinemia), and amyloidosis. Malignant plasma cells show a wide spectrum of morphologic features and may include some or all forms of plasmablasts, immature plasma cells, and mature plasma cells. The cells range from those that are easily recognized as plasma cells to those that are difficult to classify without ancillary studies or clinical data. Binucleated and multinucleated forms may be frequent and, when present, often display immature nuclear characteristics. Atypical mitotic figures may also be found. Malignant plasma cells in the peripheral blood may be numerous in cases of plasma cell leukemia.

Plasmablasts represent the most immature form in the maturation sequence of plasma cells. They are larger than mature plasma cells, measuring 25 to 40 µm in diameter. The cell border is often ragged with cytoplasmic bleb and bud formation. Nuclei are round to oval and may be eccentric or centrally placed. The N:C ratio is typically 2:1 to 1:1, which is higher than is seen in mature plasma cells. The nuclear chromatin is dispersed and fine with one or more prominent nucleoli. The cytoplasm is pale to deep blue. A perinuclear hof is usually discernible, but it is less prominent than in mature plasma cells. Although plasmablasts are a normal constituent of the bone marrow, they are present in very low numbers and are very rarely identified except in malignant conditions (eg, plasma cell myeloma and other plasma cell dyscrasias). Thus, identification of a plasmablast is considered abnormal.

Plasma cells normally produce and secrete immunoglobulins. This protein product may appear in different forms within the cytoplasm. When production within a particular plasma cell is increased or when there is a blockage in secretion, accumulation of immunoglobulin occurs and may appear as inclusions called Dutcher bodies or Russell bodies. Dutcher bodies are typically grainy or textured and are seen within or beneath the nucleus. They represent cytoplasmic invaginations rather than true intranuclear inclusions. In contrast, Russell bodies are homogeneous with punched-out edges and can be found throughout the cell. If they overlie the nucleus they can be confused with Dutcher bodies. When Russell bodies are numerous and fill the cytoplasm, the cell is called a Mott cell. These findings may occur in mature, immature, or malignant plasma cells. Dutcher bodies are more closely associated with malignancies such as Waldenström macroglobulinemia, while Russell bodies may be seen in normal or neoplastic plasma cells. Occasionally, immunoglobulin accumulations in plasma cells may form crystalline cytoplasmic structures.

Megakaryocytes and Platelets

Erythrocyte with Overlying Platelet

In preparing a peripheral blood smear, platelets may adhere to or overlap RBCs, suggesting an RBC inclusion or parasite. A correct interpretation depends on carefully examining the morphology of the platelet and comparing the size, staining characteristics, and granularity with known platelets in the same field as well as determining if the platelet is in the same plane of focus as the RBC. Many times, the platelet is surrounded by a thin clear zone or halo, which is not a feature of most genuine RBC inclusions.

Megakaryocyte (normal, abnormal, or *nuclear fragment*)

Megakaryocyte nuclei or *nuclear fragments* are uncommonly observed in the peripheral blood. After subtotally discharging their cytoplasm to form platelets, mature megakaryocytes may enter the bloodstream and sometimes be observed in peripheral smears. Circulating megakaryocytes are not necessarily indicative of neoplasia, as they can be seen in neonates. On the other hand, they are more commonly detected in leukoerythroblastic reactions and myeloproliferative neoplasms (such as in chronic myeloid leukemia and primary myelofibrosis). The cell nucleus in the peripheral blood smear is single-lobed or, less commonly, multilobated. The chromatin is smudged or puddled and is surrounded by a scant amount of cytoplasm. At times, the cytoplasm is barely perceptible on light microscopy, giving the impression of a bare or naked megakaryocyte nucleus. However, upon closer inspection, a small amount of wispy, frilly, or fragmented cytoplasm can still be seen. There may be a few localized areas of cytoplasmic blebs or adherent platelets. If the nuclear characteristics are not appreciated, megakaryocyte nuclei may be mistakenly identified as lymphocytes. Finding megakaryocyte cytoplasmic fragments and giant platelets in the blood film are helpful clues to the origin of the bare nucleus. It is important to remember that these cells are not degenerating cells, and, therefore, the chromatin pattern does not have the characteristics of basket cells. In bone marrow aspirates, a megakaryocytic nucleus that has been artifactually stripped of cytoplasm by smear preparation may sometimes be seen.

Rarely, circulating mature or precursor megakaryocytes appear in peripheral blood smears with ample amounts of pink-purple to wine-red, flocculent cytoplasm, as would be observed in bone marrow aspirate smears of normal individuals.

For CAP proficiency testing purposes in peripheral blood smears, it is sufficient to identify any mature megakaryocyte – whether it is normal, abnormal, a nuclear fragment, or a circulating micromegakaryocyte – as a megakaryocyte. On the other hand, blasts of megakaryocytic origin (ie, megakaryoblasts in cases of acute megakaryoblastic leukemia, as an example) are best classified for proficiency testing purposes as blasts, rather than as megakaryocytes.

Megakaryocyte morphology (whether normal or abnormal) is further discussed within the bone marrow section.

Platelet, Giant (Macrothrombocyte)

Giant platelets are larger than 7 µm, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, the term giant platelet is used when the platelet is larger than the size of the average RBC in the field, assuming a normal MCV. The periphery of the giant platelet may be round, scalloped, or stellate. The cytoplasm may contain a normal complement of fine azurophilic granules, or the granules may fuse into giant forms. Giant platelets are a rare finding in normal peripheral blood, but may be seen in many different reactive, neoplastic, and inherited conditions. Reactive causes include conditions in which platelet turnover is markedly increased, such as immune thrombocytopenia or severe leukemoid reactions. Giant platelets are most often seen in myeloproliferative neoplasms and myelodysplastic syndromes. The inherited conditions associated with giant platelets are rare and have associated thrombocytopenia. This group of disorders is termed congenital macrothrombocytopenias and includes May-Hegglin anomaly and Bernard-Soulier syndrome.

Platelet, Hypogranular

Hypogranular platelets either lack granules entirely or have a substantially reduced number of the granules found in normal platelets. The cells may be normal in size, shape, and configuration, or they may be enlarged and misshapen. The cytoplasm stains pale blue or blue gray. If no granules are present, the presence of zoning is needed to confidently identify the structure as a megakaryocyte fragment or platelet. Zoning refers to the normal alternation of lighter and darker areas within the cytoplasm of a platelet. Cytoplasmic fragments from cells other than megakaryocytes generally do not show zoning. Hypogranular and other dysplastic platelet forms are typically seen in myeloproliferative neoplasms and myelodysplastic syndromes. Hypogranular platelets are also seen in the very rare inherited condition of alpha granule deficiency, termed *gray platelet syndrome.* When platelets are entirely agranular, they may be easy to miss on peripheral blood film review without careful scrutiny. Difficult venipuncture may sometimes cause degranulation of some platelets. Rarely, prominent platelet degranulation resulting in platelet hypogranularity may be seen as an EDTA-induced artifact.

Platelet, Normal

Platelets, also known as thrombocytes, are small, blue-gray fragments of megakaryocytic cytoplasm. Most measure 1.5 to 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 to 7 µm in diameter, may also be seen in normal blood films. Fine, purple-red granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. These granules are platelet alpha granules. Platelet delta granules (or dense granules) are not visible on light microscopy. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins. They are typically single but may form aggregates, particularly in fresh (fingerstick) preparations.

Platelet Satellitism

Platelet satellitism, also known as *platelet rosettes,* is a rare peripheral blood finding that is due to the clumping and adherence of four or more platelets to a neutrophil, or very rarely to a monocyte. Neutrophil phagocytosis of platelets may occasionally be seen when satellitism is present. Platelet satellitism is an in vitro phenomenon that results from the interaction of EDTA and immunoglobulin, which nonspecifically binds to platelets. The antibody-coated platelets then bind to the surface of neutrophils or monocytes. The platelets and neutrophils are normal in morphology and function. This phenomenon has no clinical significance, but platelet satellitism causes spurious thrombocytopenia with automated cell counters because the cellular aggregates are counted as leukocytes rather than platelets.

Microorganisms

Babesia sp.

Babesia microti and related organisms are intracellular parasites that are often confused with malaria. The organisms range in size from 1 to 5 µm, mimicking the ring forms of malaria. They may be round, oval, elongated, ameboid, or pyriform. Pyriform organisms form a Maltese cross after division into four organisms, as *Babesia* will form teardrop-shaped organisms that occur in pairs at right angles to one another. In addition, Schüffner's granules are absent, as are the schizont and gametocyte forms of malaria. Organisms are smaller and more commonly extracellular with *Babesia* than with *Plasmodium* species. The tetrad arrangement of the merozoites and the lack of other findings on the peripheral blood smear are most helpful in distinguishing these organisms from malaria. Other potential look-alikes include platelets or stain precipitate overlying erythrocytes. Thick blood films are preferred for diagnosis, where one will see tiny chromatin dots and wispy cytoplasm.

Bacteria (Cocci or Rod), Extracellular

Although bacteremia is relatively common, it is quite unusual to identify bacteria on a random blood film. In most cases, this finding represents an overwhelming infection. When present, individual organisms are typically 1 µm in size, although there is considerable variation in size and shape. Organisms can range from cocci to bacilli and can occur singly, in clusters, or in chains. A Gram stain can be useful in confirming the presence of bacteria and in separating organisms into Gram-positive and Gram-negative groups. The most likely error in interpretation is to misidentify stain precipitate as microorganisms. Avoid this error by remembering that bacteria tend to be relatively uniform in size and shape, while stain precipitate is often irregular in shape and individual grains vary considerably in size. In addition, extracellular bacteria may represent a stain contaminant. Conduct a careful search for intracellular organisms, as this finding indicates a true bacteremia.

Bacteria (Spirochete), Extracellular

Pathogenic spirochetes include members of the genera *Leptospira, Borrelia,* and *Treponema,* but only *Borrelia* is encountered on peripheral blood films. These bacteria are 5 to 25 µm long and 0.2 to 0.5 µm wide, with 4 to 30 helical coils. The organisms can be seen in fresh wet-mount preparations, on thin Giemsa-stained blood films, or on thick Giemsa-stained blood preparations. A concentration technique can be used to detect rare organisms in patients with low-level bacteremia. Fiber, thread, or hair contamination may mimic spirochetes, but these lack uniform coiling.

Fungi, Extracellular

Extracellular fungi are most commonly seen in the bone marrow but can rarely be identified in peripheral blood films. When visualized, they indicate a serious infection. The most frequently seen fungus in the blood and bone marrow is *Histoplasma capsulatum,* but the organisms are nearly always present within macrophages as 1 to 2 µm budding yeast forms. They are only rarely seen in an extracellular location, usually when the cell membranes of the macrophages have ruptured. The other organisms, such as *Coccidioides, Cryptococcus, Candida,* and *Aspergillus,* occur less frequently but are more commonly extracellular. They are rarely seen in blood. The appearance of the fungal form is dependent upon the specific organism. *Coccidioides* typically shows mature spherules ranging between 20 to 60 µm in diameter which often contain endospores ranging from 2 to 4 µm. *Cryptococcus* is a round to oval yeast-like fungus ranging from 3.5 to 8 µm or more in diameter, usually with a thick mucopolysaccharide capsule, and demonstrating a narrow neck when budding. *Candida* can appear in blood and bone marrow as either yeast-like organisms with budding or as pseudohyphae. *Aspergillus* is typically identified by its septate 4 µm wide hyphae with characteristic 45° branching. Most organisms will stain with a periodic acid-Schiff (PAS) stain, but they are best accentuated by Gomori's methenamine silver (GMS) staining.

Leukocyte with Intracellular *Anaplasma/Ehrlichia*

Recognized as an arthropod-borne infectious agent in humans, members of the genus *Anaplasma* (previously *Ehrlichia*) are small, Gram-negative, obligate intracellular organisms currently classified as rickettsiae. On Wright-stained preparations, Anaplasma species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either neutrophils (*A. phagocytophilium)* or monocytes and macrophages (*A. chafeensis*). The morulae are microcolonies of organisms.

Leukocyte with Intracellular Bacteria

As noted under "Bacteria (cocci or rod), extracellular," it is very unusual to see bacteria on a routine blood film. This finding usually represents an overwhelming infection. When present, the bacteria may be ingested by neutrophils or monocytes and can be seen within the cytoplasm of these cells. Although leukocytes with phagocytized bacteria are rare in the blood film, they are commonly seen in infected body fluids. When present within neutrophils, bacteria can be difficult to distinguish from toxic granulation. However, toxic granulation tends to involve nearly all of the cytoplasm of the neutrophil, whereas engulfed bacteria are usually few in number. In addition, bacteria are typically larger than toxic granules, measuring around 1 µm in size, and are more defined in shape, ranging from cocci to bacilli and arranged singly, as diplococci, in clusters or in chains. They can be accentuated and confirmed with a Gram stain.

Leukocyte with Intracellular Fungi

Fungi are only rarely visualized in peripheral blood. When present, the fungi are usually seen within the cytoplasm of monocytes, macrophages, or neutrophils. Phagocytized fungi are usually localized within a vacuole that forms a clear halo around the organism. Usually the number of organisms present is scant. Clinical history and blood cultures are also very important in making the appropriate identification. *Histoplasma capsulatum* is most frequently seen. *Candida albicans* can be seen, but it is exceptionally rare. Although other fungi can be grown from blood cultures and therefore are present in the circulation, the level of fungemia is so low that they are virtually never visualized on a blood film. Intracellular fungi can be confused with precipitated stain overlying a leukocyte, large toxic granules, Döhle bodies, or large bacterial cocci.

Microfilaria

There are eight main species of filariae that infect humans. The microfilariae of five of the species circulate in the blood, some on a regular periodicity and others sporadically. The other three species do not circulate and are identified from small biopsies of skin and subcutaneous tissue. All microfilariae have elongated, cylindrical bodies with one tapered end, one rounded end, and smooth contours. Nuclei are arranged in a chain, filling most of the body. Some species are covered with a thin transparent sheath. They vary from 160 to 315 µm in length and 3 to 10 µm in width on a stained blood film. Microfilariae circulate in low numbers and can be difficult to detect on a thin blood film. To increase sensitivity, thick smears (such as those used in diagnosing malaria), concentration methods, or membrane filtration are used. Once the organisms are identified in the blood, speciation is usually possible using various morphologic parameters, including size, shape, presence or absence of an investing sheath, and the disposition of nuclei in the tail. The patient's travel history is also helpful, as various species occur in different parts of the world. These morphologic and geographic features have been reviewed in many texts. Microfilariae should not be confused with trypanosomes, chains of bacteria or fungi, nor with artifacts such as fibers or threads.

Plasmodium sp. (Malaria)

There are five species of *Plasmodium* that cause the clinical disease known as malaria: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi.* Species may be distinguished by different shapes and appearance of the various stages of development. The ring forms of all five types of malaria are usually less than 2 µm in diameter. Trophozoites range from 3 to 8 µm, depending on the species. Schizonts and gametocytes range from approximately 5 to 11 µm. Erythrocytes infected by *P. ovale* and *P. vivax* are enlarged. Schüffner stippling (a golden brown to black pigment in the cytoplasm of the infected erythrocyte) is most conspicuous in infections with *P. ovale* and *P. vivax.* Multiple stages of organism development are seen in the peripheral blood with all species except P*. falciparum,* where the peripheral blood usually contains only ring forms and gametocytes (unless infection is very severe). Multiple ring forms within one erythrocyte are also most common with *P. falciparum* and are not seen with *P. malariae.* Mixed infections may occur. Potential look-alikes include platelets overlying RBCs, clumps of bacteria or platelets that may be confused with schizonts, masses of fused platelets that may be confused with a gametocyte, precipitated stain, *Babesia* infection, and contaminating microorganisms (bacteria, fungi, etc). Often infected cells are present in low numbers and difficult to identify in thin blood films. Use of a thick smear or concentration methods increases the ability to identify malarial parasites in the blood.

Protozoa (Non-malarial)

The trypanosomes are protozoan hemoflagellates, along with *Leishmania,* and are characterized by the presence of a kinetoplast. The trypomastigote stage is seen in the peripheral blood and shows a long, slender body with a kinetoplast at the posterior end, an undulating membrane and axoneme extending the entire length, and a flagellum at the anterior end, representing an extension of the axoneme. Trypomastigotes of the *Trypanosoma brucei* group are up to 30 µm long with graceful curves and a small kinetoplast. Trypomastigotes of *T. cruzi* are shorter (20 µm), with S and C shapes and a larger kinetoplast. Trypanosomes should not be confused with artifacts, such as fibers, threads, or microfilarial organisms.

Miscellaneous

Blast Cell

A blast is a large, round-to-oval cell, 10 to 20 µm in diameter. In the blood film, the cell may appear flattened or compressed by adjacent RBCs. The nuclear-to-cytoplasmic ratio is high, varying from 7:1 to 5:1. The blast often has a round-to-oval nucleus, but sometimes it is indented or folded. The blast cell has fine, lacy or reticular chromatin. One or more prominent nucleoli may be seen. The cytoplasm is variably basophilic and typically agranular. The morphologic features of a blast cell do not permit determination of the cell lineage, ie, myeloblast versus lymphoblast. The one exception is the presence of Auer rods, which are diagnostic of myeloid lineage (ie, myeloblast). Other cells may have the appearance of a blast, including some lymphoma cells. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections, or, less commonly, cytochemical staining (eg, peroxidase or Sudan black) is required to determine the lineage of a given blast cell.

As blasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Blasts may rarely be morphologically indistinguishable from lymphoma cells. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable.

Cryoglobulin

Cryoglobulins are immunoglobulins that precipitate when cooled. They may cause a clinical syndrome that can include joint pain, Raynaud's phenomenon, skin lesions, and renal abnormalities. Rarely, cryoglobulins may be observed in routine peripheral blood smears. Typically, these immunoglobulin precipitates take the form of cloud-like, extracellular masses of blue, amorphous material. The intensity of staining of these aggregates varies from case-to-case, such that they range from very pale, barely visible deposits to obvious, dense masses. Rarely, cryoglobulins may be diffusely distributed in a blood smear as fine droplets. Phagocytosis of cryoglobulin by neutrophils or monocytes may also be rarely seen, producing pale blue to clear cytoplasmic inclusions that may mimic vacuoles.

Leukocyte Containing Alder-Reilly Anomaly Inclusion(s)

Alder-Reilly anomaly inclusions are large, purple, or purplish black, coarse, azurophilic granules resembling the primary granules of promyelocytes. They are seen in the cytoplasm of virtually all mature leukocytes and, occasionally, in their precursors. At times, clear zones or halos surround the granules. The prominent granulation in lymphocytes and monocytes distinguishes these inclusions from toxic granulation, which only occurs in neutrophils. Alder-Reilly anomaly inclusions are seen in association with the mucopolysaccharidoses, a group of inherited disorders caused by a deficiency of lysosomal enzymes needed to degrade mucopolysaccharides (or glycosaminoglycans).

Leukocyte Containing Chediak-Higashi Inclusion(s)

Giant, often round, red, blue, or greenish gray granules of variable size are seen in the cytoplasm of otherwise typical leukocytes (granulocytes, lymphocytes, and monocytes) and sometimes erythrocyte precursors normoblasts or megakaryocytes in patients with Chediak-Higashi syndrome. In the blood the disease is manifested by the presence of medium to large peroxidase positive inclusions in the leukocytes. These may be single or in aggregates. These arise due to a poorly understood lysosomal trafficking abnormality that

results in fusion of primary (azurophilic) and, to a lesser extent, secondary (specific) lysosomal granules, resulting in poor function in killing phagocytized bacteria.

Mast cell, abnormal

Circulating mast cells (MCs) are abnormal and should prompt investigation for mast cell leukemia (MCL). MCs in MCL exhibit a range of morphologies, from immature to mature forms. The immature forms include: (1) Promastocytes, which are atypical MCs with bi- or multilobated nuclei; (2) Metachromatic blasts, which are metachromatic granulated blast-like cells; and (3) Multinucleated or highly pleomorphic MCs. The atypical mature MCs include: (1) Spindle-shaped MCs with an oval nucleus and elongated, often hypogranulated cytoplasm with focal granule accumulation; and (2) Well-differentiated MCs, which are round-shaped, enlarged MCs with a round, central to slightly eccentric nucleus that is usually obscured by a heavily granulated cytoplasm.

MCL can occur de novo (primary MCL) or as a progression from antecedent systemic mastocytosis (secondary MCL). Diagnosis of MCL requires the presence of ≥ 20% atypical MCs in bone marrow aspirate and meeting the diagnostic criteria for systemic mastocytosis. The overall prognosis for MCL is poor, with a median survival time ranging from 2 to 31 months.

Mitotic Figure

A cell containing a mitotic figure is variable in size. The cytoplasm has color and granulation characteristic of the resting cell. When a cell undergoes mitosis, typical nuclear features are no longer present. Instead, the nucleus appears as a dark, irregular mass, often with a clear central zone. It may take various shapes, including a daisy-like form or a mass with irregular projections. In metaphase, the individual chromosomes become visible. Arranged equatorially, the chromosomes begin to separate and move toward opposite poles. Rarely, the anaphase or telophase of mitosis may be seen, characterized by two separating masses of chromosomes forming two daughter cells. A mitotic cell can be distinguished from a degenerating cell by a relatively compact nucleus (or nuclei). A degenerating cell often displays a pyknotic nucleus that has been fragmented into numerous purple, roundish inclusions.

Squamous Epithelial Cell/Endothelial Cell

Squamous epithelial cells are large (30 to 50 µm), round-to-polyhedral-shaped cells with a low nuclear-to-cytoplasmic ratio (1:1 to 1:5). The nucleus is round to slightly irregularly shaped, with dense, pyknotic chromatin, and no visible nucleoli. The abundant cytoplasm is lightly basophilic and may show keratinization or a few blue kerato-hyaline granules. Epithelial cells from deeper layers of the epidermis have larger nuclei with a high nuclear-to-cytoplasmic ratio. In contrast to squamous carcinoma, contaminant squamous epithelial cells lack nuclear atypia. Skin epithelial cells rarely may contaminate peripheral blood, particularly when smears are obtained from finger or heel punctures. Endothelial cells have an elongated or spindle shape, approximately 5 µm wide by 20 to 30 µm long, with a moderate nuclear-to-cytoplasmic ratio (2:1 to 1:1). The oval or elliptical nucleus occasionally is folded and has dense-to-fine, reticular chromatin. One or more nucleoli may be visible. The frayed cytoplasm tapers out from both ends of the nucleus and may contain a few azurophilic granules. Endothelial cells (lining blood vessels) rarely may contaminate peripheral blood, particularly when smears are obtained from finger or heel punctures. When present as a contaminant in blood smears, endothelial cells may occur in clusters.

Artifacts

Basket Cell/Smudge Cell

A basket cell or smudge cell is most commonly associated with cells that are fragile and easily damaged in the process of making a peripheral blood smear. The nucleus may either be a nondescript chromatin mass or the chromatin strands may spread out from a condensed nuclear remnant, giving the appearance of a basket. Cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, but there is no recognizable cytoplasm to give a clue to the origin of the cell. They are seen most commonly in disorders characterized by lymphocyte fragility, such as infectious mononucleosis and chronic lymphocytic leukemia. Basket cells should not be confused with necrobiotic neutrophils, which have enough cytoplasm to allow the cell to be classified.

Stain Precipitate

Stain precipitate on a Wright-Giemsa smear is usually due to unclean slides or improper drying of the stain on the smear. Oxidized stain appears as metachromatic red, pink, or purple granular deposits on and between cells. The stain may adhere to RBCs and be mistaken for inclusions, parasites, or infected cells. The size of the stain deposits is variable, and this can be helpful in discerning their origin. Yeast and bacteria have a more uniform morphology than precipitated stain. Organisms are usually rare and dispersed throughout the slide. They do not circulate in large aggregates. Stain deposits, on the other hand, may be very focal and intense.

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2 Bone Marrow Cell Identification

Introduction

This glossary corresponds to the master list for hematology, and it will assist survey participants in the proper identification of blood cells in photographs and virtual slides. Descriptions are for cells found in aspirated bone marrow particle slides stained with Wright-Giemsa and cytochemical stains, such as iron stain and others.

Granulocytes and Monocytes

Basophil, Any Stage

Basophils have a maturation sequence analogous to neutrophils. At the myelocyte stage, when specific granules begin to develop, basophil precursors can be identified. All basophils, from the basophilic myelocyte to the mature segmented basophil, are characterized by the presence of numerous coarse and densely stained granules of varying sizes and shapes. The granules are larger than the granules of neutrophils and most are roughly spherical. The granules are typically blue-black, but some may be purple-red when stained using Wright-Giemsa preparations. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Basophils are comparable in size to neutrophils, ie, 10 to 15 µm in diameter, and the nucleus-to-cytoplasm (N:C) ratio* ranges from 1:2 to 1:3. Basophilia may be seen in several contexts, including in association with myeloproliferative neoplasms, in hypersensitivity reactions, with hypothyroidism, iron deficiency, and renal disease.

* For the purpose of this glossary, N:C ratio is defined as the ratio of nuclear volume to cytoplasmic (non-nuclear) cell volume.

Eosinophil, Any Stage

Eosinophils are round-to-oval leukocytes that are present in the blood, bone marrow, and tissues of normal individuals. They are generally easily recognized due to their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter in their mature forms, and 10 to 18 µm in diameter in their immature forms. The eosinophil N:C ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is generally evenly filled with numerous coarse, orange-red granules of uniform size. These granules rarely overlie the nucleus and are refractile by light microscopy due to their crystalline structure. This refractile appearance is not apparent in photomicrographs or pictures, however. Due to inherent problems with color rendition on photomicrographs, which is sometimes imperfect, eosinophil granules may appear lighter or darker than on a freshly stained blood film. Discoloration may give the granules a blue, brown, or pink tint. Nonetheless, the uniform, coarse nature of eosinophil granules is characteristic and differs from the smaller, finer granules of neutrophils. Occasionally, eosinophils can become degranulated, with only a few orange-red granules remaining visible within the faint pink cytoplasm.

In the most mature eosinophilic form, the nucleus is segmented into two or more lobes connected by a thin filament. About 80% of segmented eosinophils will have the classic two-lobed appearance. Typically, these lobes are of equal size and round to ovoid or potato shaped with dense, compact chromatin. The remainder of segmented eosinophils will have three lobes and an occasional cell will exhibit four to five lobes.

Eosinophils exhibit the same nuclear characteristics and the same stages of development as neutrophils. Immature eosinophils are rarely seen in the blood, but they are found in bone marrow smears. Immature eosinophils may have fewer granules than more mature forms.

The earliest recognizable eosinophilic form by light microscopy is the eosinophilic myelocyte. Eosinophilic myelocytes often contain a few dark purplish granules in addition to the orange-red secondary granules.

Eosinophil, Any Stage with Atypical/Basophilic Granulation

Eosinophils with atypical/basophilic granules are typically the same size as their normal counterparts. Any stage of eosinophilic maturation may be affected. This finding is more commonly seen in the myelocyte stage. The abnormal granules resemble basophilic granules. The granules are purple violet in color and usually larger than normal eosinophilic granules at the immature stages. These atypical granules are usually admixed with normal eosinophilic granules in the cytoplasm. Although the atypical granules resemble basophilic granules, they differ from normal basophilic granules by lacking myeloperoxidase and toluidine blue reactivity.

Eosinophils with atypical/basophilic granules (also referred to as harlequin cells) are associated with clonal myeloid disorders and are most often seen in acute myeloid leukemia with the recurrent cytogenetic abnormality involving *CBFB-MYH11,* inv(16)(p13.1q22) or t(16;16)(q13.1;q22).

Mast Cell

The MC is a large (ie, 15 to 30 µm), round or elliptical cell with a small, round nucleus and abundant cytoplasm packed with black, bluish black, or reddish-purple metachromatic granules. Normal MCs are differentiated from basophils by the fact that they are larger (often twice the size of blood basophils), have more abundant cytoplasm, and have round rather than segmented nuclei. The cytoplasmic granules are smaller, more numerous, more uniform in appearance, and less water-extractable than basophil cytoplasmic granules. Although both MCs and basophils are primarily involved in allergic and anaphylactic reactions via release of bioactive substances through degranulation, the content of their granules is not identical. Both MC and basophil granules can be differentiated from neutrophilic granules by positive staining with toluidine blue resulting in a purple color in the former.

Mast Cell, Atypical, Spindled

Atypical MCs may exhibit a variety of morphologic and architectural features that are not typically seen in normal/reactive MCs in bone marrow specimens. Atypical MC morphology includes elongation and spindled cytoplasm, cytoplasmic hypogranularity, nuclei with immature blast-like chromatin, and bilobated or multilobated nuclei.

The number of atypical MCs seen in an aspirate smear may be less than that in the biopsy due to associated fibrosis. However, increased numbers of atypical MCs seen singly as well as in clusters and sheets may be appreciated in the aspirate smear. Architectural features are thus typically appreciated in the bone marrow biopsy and include perivascular and/or paratrabecular aggregates of MCs.

These atypical morphological and architectural findings are seen in a clonal neoplastic MC disease known as mastocytosis. Mastocytosis may be either cutaneous or systemic. Systemic disease is usually identified in the

bone marrow. Further subclassification is defined by the distribution of the neoplastic MCs and the associated clinical, laboratory, and molecular genetic findings.

Monocyte

Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. Most monocytes have rounded cytoplasmic borders with smooth edges, but some may have pseudopod-like extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles and/or fine, evenly distributed azurophilic granules. The N:C ratio ranges from 4:1 to 2:1. The nucleus is usually indented, often resembling a three-pointed hat or kidney bean shaped, but it can also be folded or band-like. The chromatin is condensed but is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

Monoblast/Promonocyte (Blast Equivalent)

For the purposes of proficiency testing, selection of the response “monoblasts/promonocyte (blast equivalent)” should be reserved for immature-appearing malignant cells in the context of acute myeloid leukemia with monocytic differentiation (eg, acute monoblastic or acute myelomonocytic leukemia, etc), chronic myelomonocytic leukemia, or myelodysplastic neoplasms/syndromes. While small numbers of promonocytes or monoblasts may be identified in normal marrow aspirates, they are generally absent in peripheral blood smears.

A monoblast is a large cell, usually 15 to 25 µm in diameter, with relatively more abundant cytoplasm than a typical myeloblast and a N:C ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has delicate, finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms; in these instances, additional tests (eg, cytochemistry and/or flow cytometry) are required to accurately assign blast lineage.

Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and monocytes. They are generally larger than monocytes, but with similar gray-blue cytoplasm that often contains uniformly distributed fine azurophilic granules. Cytoplasmic vacuolization as seen in mature monocytes is not typical of promonocytes. Promonocyte nuclei show varying degrees of lobulation characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Similar to monoblasts, promonocyte chromatin is open and lace-like, and nucleoli are conspicuous.

Neutrophil, Segmented or Band

Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. The band is round to oval and 10 to 18 µm in diameter. The nuclear-to-cytoplasmic ratio is 1:1.5 to 1:2 and the nuclear chromatin is condensed. The nucleus is indented to more than half the distance to the farthest nuclear margin, but the chromatin is not condensed to a single filament (as is the defining feature of the fully mature neutrophil). The nucleus can assume many shapes: it can be band-like or sausage-like; S-, C-, or U-shaped; and twisted or folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophilic cells, with specific granules predominating in the pale cytoplasm.

The segmented neutrophil, the mature cell of the myeloid series and the predominant white cell in blood, mimics its immediate precursors in size (10 to 15 µm), shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The N:C ratio is 1:3, the most mature of any cell in the neutrophilic series, and the nuclear chromatin is highly condensed. The nucleus is segmented or lobated

(three to five lobes normally). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from its precursor, the band neutrophil. However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing, it is not required that they be differentiated (for a detailed guideline for the differentiation of segmented and band neutrophils, see Glassy, 2018. For other reading related to the clinical utility of band counts, see Cornbleet, 2002).

Neutrophil, Toxic (To Include Toxic Granulation and or Döhle Bodies, and/or Toxic Vacuolization)

Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding. Either change alone is sufficient to designate a neutrophil as toxic. Toxic granulation is the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia.

Ethylenediaminetetraacetic acid (EDTA) blood collection may produce degenerative vacuolization. In this context, only a few, small, punched-out-appearing vacuoles may be found. However, as it may be difficult to distinguish toxic from degenerative vacuoles, neutrophil vacuoles should not be labeled as toxic vacuoles unless accompanied by other toxic changes.

Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 µm) and shape (round, elongated, or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found in the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum. Toxic changes result from the action of cytokines released in response to infection, burns, trauma, and granulocyte colony stimulating factor (G-CSF) therapy. They indicate a shortened maturation time and activation of post-mitotic neutrophil precursors.

In the May-Hegglin anomaly, inclusions that resemble Döhle bodies are seen. Unlike Döhle bodies, however, the May-Hegglin inclusion is due to aggregates of nonmuscle myosin heavy chain IIA. The May-Hegglin anomaly is inherited in an autosomal dominant fashion, owing to mutations in *MYH9* gene.

Neutrophil with Hypersegmented Nucleus

To be considered a neutrophil with hypersegmented nucleus, the neutrophil should demonstrate six or more lobes separated by thin filaments. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis. Hypersegmented neutrophils may also be seen in sepsis, renal disease, and myeloproliferative neoplasms.

Megaloblastic hematopoiesis occurs when DNA synthesis is impaired. Such conditions include deficiency of cofactors for nucleotide synthesis, such as vitamin B12 and folate, and cases when patients are receiving a nucleotide analog (such as 6-mercaptopurine) or nuclear cofactor blocking agents (such as methotrexate) for neoplastic or rheumatologic conditions.

Neutrophil with Pelger-Huët Nucleus (Acquired or Congenital)

Neutrophils with bilobed nuclei in the pince-nez conformation (ie, two round or nearly round lobes connected by a distinct thin filament) are designated as neutrophils with Pelger-Huët nuclei or as Pelger-Huët cells. They may be seen in the context of an inherited autosomal dominant abnormality of nuclear segmentation,

referred to as Pelger-Huët anomaly, which is caused by mutations in the Lamin B Receptor (*LBR*) gene. In patients heterozygous for an *LBR* gene mutation, virtually all of the neutrophils have bilobed nuclei. Individuals homozygous for an *LBR* gene mutation, however, typically have single-lobe neutrophils. The nuclear chromatin in Pelger-Huët cells is generally denser than in normal neutrophils. Comparable neutrophil morphology, typically involving only a subset of neutrophils, may be seen in association with a number of conditions including myelodysplastic syndromes and other myeloid malignancies, as a side-effect of certain drugs (eg, sulfonamides, colchicine, and mycophenolate mofetil) and in association with certain infections (eg, HIV and *Mycoplasma pneumonia*). To distinguish these cells from neutrophils seen in inherited conditions, these cells are designated as pseudo-Pelger-Huët cells.

Neutrophil with Dysplastic Nucleus and/or Hypogranular Cytoplasm

Dysplastic neutrophils are characteristic of myelodysplastic syndromes. Morphologically, the normal synchronous maturation of nucleus and cytoplasm is lost. As a result, in the cytoplasm, the primary and secondary granules are often decreased or absent, causing the cytoplasm to appear pale and bluish. The nucleus shows abnormal lobation accompanied by a mature chromatin pattern. In some cases, the nucleus has a pince-nez appearance. These cells are known as pseudo-Pelger Huët neutrophils. For proficiency testing purposes, cells with pseudo-Pelger Huët nuclei are best defined as Pelger Huët cells. Dysplastic neutrophils often have abnormal cytochemical reactivity; levels of myeloperoxidase and leukocyte alkaline phosphatase may be low or absent. The dysplastic neutrophils may also exhibit functional defects. In addition, dysplastic cytoplasmic and nuclear changes may be seen in maturing granulocytic cells in the bone marrow, frequently appearing as dyssynchrony between the cytoplasmic and nuclear maturation and/or cytoplasmic hypogranularity.

Neutrophil Necrobiosis (Degenerated Neutrophil)

Neutrophil necrobiosis is a common phenomenon that can be seen both in normal individuals and in patients with a variety of medical conditions (including infections, in association with inflammatory disorders, and in malignancies). It is a nondiagnostic and nonspecific finding. Degenerated neutrophils are generally easily identified because they resemble normal segmented neutrophils. They are round-to-oval cells ranging from 10 to 15 µm in diameter and their N:C ratio is 1:3 or less. The major distinguishing feature is that the nucleus shows karyorrhexis and/or pyknosis. These changes are appreciated when a cell with neutrophilic granules (pale pink cytoplasm with fine lilac granules) contains multiple, unconnected nuclear lobes (karyorrhexis) or a single, dark, round-to-oval nucleus (pyknosis). The chromatin pattern in these karyorrhexic or pyknotic states is also characteristic: dense and homogeneous, without visible parachromatin or nucleoli. The nuclear lobes may fragment into numerous small particles of varying size that can resemble microorganisms such as bacteria or fungi. Also, the nuclear outlines may become indistinct and blurred.

As the cellular degeneration continues, the cytoplasm will become hypogranulated, then agranular, and the cytoplasmic borders may become frayed and indistinct. Sometimes, the cells will contain scattered larger azurophilic or dark blue granules (toxic granulation). Vacuolation is frequent. If a cell is too degenerated to be recognized as a neutrophil and lacks recognizable cytoplasm, one should identify it as a basket/smudge cell. On occasion, necrobiotic neutrophils can contain ingested bacteria or fungi. However, the microscopist must be very careful when making this identification since nuclear fragments may appear deceptively similar. Other cells that may resemble degenerated neutrophils are orthochromic normoblasts in the bone marrow. In contrast, however, these erythroid lineage cells have a characteristic pinkish-orange and agranular cytoplasm with a single, often eccentric nucleus with a dense ink-drop appearance.

Neutrophil, Giant Band or Giant Metamyelocyte

Myeloid precursors resulting from megaloblastic hematopoiesis show an increase in size, and they have nuclei that show aberrant maturation where the nuclear features appear less mature than the cytoplasmic features. Although these changes are usually discussed in terms of the neutrophil series, they may also be observed in cells in the eosinophil and basophil cell lines. Larger than normal metamyelocytes and bands with decreased chromatin clumping are seen in the marrow. These cells have diameters 1.5 times those of normal metamyelocytes or bands.

Neutrophil, Metamyelocyte

Metamyelocytes are the first of the postmitotic myeloid precursors. They constitute 15% to 20% of nucleated cells in the bone marrow and may be seen in the blood in pathologic states and in response to stress. They are approximately 10 to 18 µm in diameter. They are round to oval with a nuclear-to-cytoplasmic ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the maximal nuclear diameter (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic, containing rare azurophilic (primary) granules and many fine lilac or pale orange/pink specific granules.

Neutrophil, Myelocyte

The transition from promyelocyte to myelocyte occurs with the end of production of azurophilic (primary) granules and the beginning of production of lilac or pale orange/pink (specific) granules. Myelocytes are usually confined to the marrow, where they constitute approximately 10% of the nucleated cells. In pathologic states, myelocytes are seen in blood. The myelocyte is smaller than the earlier precursors, usually 10 to 18 µm. The cells are round to oval in shape and have a nuclear-to-cytoplasmic ratio of 2:1 to 1:1. The nucleus is slightly eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping. One side often shows slight flattening. Sometimes a clear space or hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in earlier precursors and is amphophilic. Both azurophilic and specific granules are present in the cytoplasm with specific granules coming to predominate as maturation progresses.

Neutrophil, Promyelocyte

Promyelocytes are round-to-oval cells that are generally slightly larger than myeloblasts, with a diameter of 12 to 24 µm. They are normally confined to bone marrow, where they constitute less than 2% of nucleated cells. However, like the myeloblast, promyelocytes can be seen in the blood in pathologic states. The nuclear-to-cytoplasmic ratio is high (5:1 to 3:1). The nucleus is round to oval, has fine chromatin, and contains distinct nucleoli. The cytoplasm is basophilic, more plentiful than in a myeloblast, and contains multiple distinct azurophilic (primary) granules. A paranuclear hof or cleared space is typically present.

Neutrophil, Promyelocyte, Abnormal with/without Auer Rod(s)

The neoplastic cell in acute promyelocytic leukemia is considered to be the neoplastic counterpart of the promyelocyte. However, this leukemic cell differs from the normal promyelocyte in several respects. The nucleus is usually folded, bilobed, or reniform (often with overlapping nuclear lobes). A distinct Golgi zone is typically absent. Cytoplasmic granules, while abundant in the classic hypergranular form of this disease, may differ in appearance, often being coarser or finer than those seen in normal promyelocytes and slightly darker or more reddish in color. In the microgranular variant, few granules may be visible, and those granules may be very fine. Finally, the abnormal promyelocyte of acute promyelocytic leukemia frequently contains numerous

overlapping Auer rods (these types of cells are termed *faggot cells*, derived from the English term for a cord of wood).

Myeloblast, with Auer Rod

Myeloblasts are the most immature cells in the myeloid series. They are normally confined to the bone marrow, where they constitute less than 3% of the nucleated cells. They may be present in the blood in leukemic states, myelodysplastic syndromes, myeloproliferative neoplasms, and, very rarely, leukemoid reactions. The myeloblast is usually a large cell, 15 to 20 µm in diameter, with a high nuclear-to-cytoplasmic (N:C) ratio, usually 7:1 to 5:1 with typically basophilic cytoplasm. Myeloblasts may occasionally be smaller, similar to the size of a mature myeloid cell. The cell and nucleus are usually rounded, although irregularly shaped or folded nuclei may be present. The myeloblast nucleus has finely reticulated chromatin pattern with distinct nucleoli present. Leukemic myeloblasts may also exhibit a few delicate granules and/or Auer rods. Distinguishing one type of abnormal blast cell from another is not always possible using Wright-Giemsa stains alone. Additional testing such as cytochemical staining (eg, using myeloperoxidase or Sudan black), or immunophenotyping by flow cytometry may be required to further define the lineage of a given blast population.

Auer rods are pink or red, rod-shaped cytoplasmic inclusions seen in neoplastic early myeloid forms and occasionally in early monocytic forms in patients with myeloid lineage leukemia. These inclusions represent a crystallization of azurophilic (primary) granules. A cell containing multiple Auer rods clumped together is termed a faggot cell. These cells are most commonly seen in acute promyelocytic leukemia.

Erythrocytes

Erythrocyte

An erythrocyte is a mature, nonnucleated RBC of fairly uniform size (6.7 to 7.8 µm in diameter) and shape (round or slightly ovoid biconcave disc). Erythrocytes contain hemoglobin and stain pink red. A central zone of pallor is seen due to the biconcavity of the cell and occupies approximately one-third of the cell diameter. Normal erythrocytes circulate in the peripheral blood for approximately 120 days before they undergo catabolism or destruction in the spleen.

Erythrocyte Precursor, Normal (Includes Pronormoblast, Basophilic Normoblast, Polychromatophilic Normoblast, and Orthochromic Normoblast)

Mature erythrocytes are derived from erythrocyte precursors in the bone marrow. The earliest recognizable erythroid precursor is the pronormoblast (proerythroblast, erythroblast). From this stage, the maturation sequence progresses through the basophilic, polychromatophilic, and orthochromic normoblast stages until the nucleus is extruded and an anucleate cell exits the bone marrow and enters the peripheral blood. The pronormoblast, basophilic normoblast, and polychromatophilic normoblast are all capable of cell division. In the bone marrow, erythroid maturation requires approximately seven days to reach the polychromatophilic normoblast stage. Another three days is required for the cell to reach the orthochromic normoblast stage, extrude the nucleus, and enter the peripheral blood.

Pronormoblast (Proerythroblast): Pronormoblasts, morphologically the most immature cells of the erythrocytic series, are large, round-or-ovoid cells measuring 17 to 24 µm in diameter. The nucleus is round or slightly oval and contains one or more prominent nucleoli. The chromatin is finely reticulated or

lacy and blast-like without clumping. A perinuclear halo is usually evident. The cytoplasm stains darker blue (more basophilic) than that of a myeloblast and lighter blue than basophilic normoblasts. The N:C ratio is approximately 8:1.

Normoblast, Basophilic: Basophilic normoblasts are slightly smaller 10 to 17 µm in diameter, slightly smaller than pronormoblasts, but similar in cellular and nuclear shape. The chromatin is coarse-trabecular and beady in appearance. The chromatin should not show any significant condensation or clumping. The nuclei of large or early basophilic normoblasts may reveal single nucleoli, but those of small or late basophilic normoblasts lack nucleoli. A perinuclear halo is often visible. The cytoplasm is more abundant than pronormoblasts, lacks any reddish coloration, and is intensely basophilic, imparting a royal blue color. The N:C ratio is approximately 6:1.

Normoblast, Polychromatophilic: Polychromatophilic normoblasts are round or ovoid cells, but are slightly smaller (10 to 15 µm in diameter) than earlier erythroid precursors. The nucleus is round and may have a cartwheel or checkerboard appearance due to prominent chromatin condensation and clumping. It lacks nucleoli and may be placed centrally or eccentrically. A perinuclear halo is visible. The cytoplasm is abundant and stains as admixtures of blue-gray and pink gray, depending upon the relative proportions of RNA and hemoglobin. The N:C ratio is approximately 4:1.

Normoblast, Orthochromic: Orthochromic normoblasts are round or ovoid cells and are even smaller than the polychromatophilic normoblasts (8 to 12 µm in diameter). The nucleus is also very small, often pyknotic, and sometimes appears as a homogeneous mass of dense chromatin. It is often eccentrically placed and at times may be extruding or fragmented. The cytoplasm usually stains uniformly pinkish orange with little or no basophilia and lacks the variegated appearance of polychromatophilic normoblasts. The N:C ratio is approximately 1:2.

Erythrocyte Precursor, Abnormal/Dysplastic Nuclear Features (Includes Pronormoblast, Basophilic Normoblast, Polychromatophilic Normoblast, and Orthochromic Normoblast)

Dysplastic nRBCs are similar size to their normal counterparts in the erythrocytic series but characteristically exhibit strikingly abnormal nuclear features. Compared to the round nucleus of normal erythroid precursors, dysplastic erythrocytes often have a misshapen nucleus due to nuclear budding (lobation or rosette formation) or fragmentation. Multinucleation is also common, and internuclear bridging by thin strands of chromatin may be present. Megaloblastic changes may also be present as manifested by dyssynchrony of nuclear and cytoplasmic maturation where the nuclear features appear less mature than those seen in the cytoplasm (see below). The cytoplasm shows normal hemoglobinization. Some dysplastic RBCs may be vacuolated, contain multiple Howell-Jolly bodies, or exhibit coarse basophilic stippling. Erythroid dysplasia may be seen in a variety of benign disorders (eg, vitamin B12, folate, or copper deficiency) or malignant conditions (eg, myelodysplastic syndromes, acute myeloid leukemias).

Erythrocyte Precursor with Parvovirus Inclusion

The virus preferentially infects erythroid precursors resulting in very large pronormoblasts with visible eosinophilic nuclear inclusions. Often the markedly enlarged pronormoblasts will be rarely seen and widely scattered but may appear in small clusters. Other erythroid precursor stages are typically absent (erythroid aplasia), although in immunosuppressed patients, erythroid hyperplasia may be seen with viral inclusions present in all stages of erythroid differentiation. The enlarged pronormoblast nuclear membranes may appear to be dissolving. The viral nuclear inclusions appear as glassy, poorly defined lucencies in Wright-Giemsa-stained bone marrow smears but are more sharply defined in fixed tissue sections.

Erythrocyte Precursor with Megaloblastic Changes/Maturation

Megaloblastic changes are the result of defective DNA synthesis and occur in a variety of disorders. Vitamin B12 deficiency and folate deficiency are the classic examples of megaloblastic maturation, but stem cell abnormalities associated with myelodysplasia, toxins, drugs, or any number of other extrinsic factors may also alter DNA production. Megaloblastic erythroid precursors are larger than the corresponding normal cells of the erythrocytic series and are characterized by nuclear and cytoplasmic maturation dyssynchrony. This is manifest by delayed nuclear maturation relative to the degree of cytoplasmic maturation (ie, cells have an immature chromatin pattern compared to the degree of cytoplasmic hemoglobinization). Coexisting features of dyserythropoiesis, such as multinucleation, abnormal nuclear shapes, and cytoplasmic Howell-Jolly bodies, are often also seen. RBCs with megaloblastic changes are classified into similar stages of development as their normal counterpart cells. The assumption is that cytoplasmic maturation is appropriate, and thus cell identification is based on cytoplasmic characteristics. Megaloblastic change is often difficult to appreciate in early erythroid precursors and is more easily recognized in polychromatophilic and orthochromic normoblasts.

Erythrocyte Precursor with Vacuolated Cytoplasm

Normal erythrocyte precursors do not contain cytoplasmic vacuoles. When present, vacuoles appear as variably sized, round cytoplasmic holes in the cytoplasm. Periodic Acid-Schiff (PAS) will stain the vacuoles red pink. Cytoplasmic vacuoles may be seen in a variety of conditions, including ethanol abuse, chloramphenicol therapy, copper deficiency, riboflavin deficiency, phenylalanine deficiency, hyperosmolar coma, and Pearson syndrome. In addition, erythroblasts in cases of acute erythroid leukemia also typically demonstrate deeply basophilic cytoplasm with prominent vacuolization.

Sideroblast (Iron Stain)

Sideroblasts are nucleated erythroid precursors that contain cytoplasmic inclusions called siderosomes, which stain blue with Prussian Blue (Perls stain). Siderosomes are randomly distributed in the cytoplasm and are not concentrated around the nucleus as seen in ring sideroblasts. Siderosomes consist of ferritin (an iron storage protein) wrapped in a lysosomal membrane. In normal bone marrow, approximately 30% to 50% of erythrocyte precursors are sideroblasts, with up to five siderosomes per cell. Under normal physiologic conditions the number of siderosomes decreases as the normoblast matures. Bone marrow sideroblasts are usually at the polychromatophilic or orthochromic stage of maturation. Nonnucleated RBCs that contain siderosomes are referred to as siderocytes. Siderosomes visible in mature RBCs on Wright-Giemsa-stained peripheral smears are termed Pappenheimer bodies. Siderocytes and sideroblasts are not normally found in peripheral blood.

Sideroblast, Ring (Iron Stain)

Sideroblasts are nucleated erythroid precursors that contain cytoplasmic inclusions called siderosomes, which stain blue with Prussian Blue (Perls stain). Siderosomes consist of ferritin (an iron storage protein) wrapped in a lysosomal membrane. In contrast to normal sideroblasts in which siderosomes are scattered randomly throughout the cytoplasm, ring sideroblasts are characterized by siderosomes concentrated adjacent to the nucleus where they form a partial or complete perinuclear ring. By definition, a ring sideroblast must contain five or more siderosomes encircling at least one-third of the nucleus. The perinuclear location occurs due to iron accumulation within mitochondria, which are normally concentrated adjacent to the nucleus. Iron accumulation in mitochondria is usually associated with defects in heme or globin synthesis. Ring sideroblasts are not present in normal blood or bone marrow and are seen in sideroblastic anemias, myelodysplastic syndromes, in association with some toxins, and in other dyserythropoietic conditions.

Lymphocytes and Plasma Cells

Hematogone

Hematogones are benign B-lymphocyte precursor cells that are a normal cellular constituent of the bone marrow. The cells are typically small, but show some variability in size, ranging from 10 to 20 µm. Nuclei are round or oval, sometimes with a shallow nuclear indentation. Nucleoli are absent or indistinct. The chromatin is characteristically condensed and homogeneous. The cytoplasm is very scant and often not discernible. Hematogones are most frequently encountered in the bone marrow of infants and young children, particularly following a viral infection, during recovery from chemotherapy, or in association with bone marrow transplant. A small number of hematogones may be seen in the bone marrow of adults. The morphologic appearance of individual hematogones is often indistinguishable from lymphoblasts as seen in acute lymphoblastic leukemia. Thus, distinguishing small groups of hematogones from residual acute lymphoblastic leukemia often requires ancillary studies such as immunophenotyping. Unlike lymphoblasts, which are commonly seen in blood smears of patients with acute lymphoblastic leukemia, hematogones are not identifiable in the peripheral blood.

Lymphoblast

Lymphoblasts are the most immature cells of the lymphoid series. They are most commonly seen in acute lymphoblastic leukemia (ALL) and lymphoid blast crisis of chronic myeloid leukemia (CML). These round-to-oval cells range in size from 10 to 20 µm. The N:C ratio varies from 7:1 to 4:1. Morphologically, lymphoblasts are variable in appearance, even at times within a single case. At one end of the spectrum, are small lymphoblasts with dense, but not clumped chromatin, inconspicuous or absent nucleoli, and extremely scanty cytoplasm. At the other end are large lymphoblasts with finely dispersed chromatin, variable numbers of distinct nucleoli, and moderate amounts of cytoplasm, closely resembling myeloblasts. The nuclear contours of lymphoblasts range from round to convoluted. The cytoplasm is typically slightly to moderately basophilic and is usually agranular. Auer rods are absent. As lymphoblasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Lymphoblasts can be indistinguishable from other types of blasts and lymphoma cells. For purposes of proficiency testing, one should identify individual cells exhibiting this immature type of morphology as blast cells.

Lymphocyte

While most normal lymphocytes are fairly homogeneous, they do exhibit a range of normal morphology. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round-to-oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

Lymphocyte, Large Granular

Large granular lymphocytes are medium-to-large cells, 15 to 25 µm, with round nuclei, dense chromatin, and no visible nucleoli. The cytoplasm is moderate to abundant, clear or lightly basophilic, and contains several variable coarse, unevenly distributed small azurophilic granules. These cells are found in small numbers in blood smears from normal individuals, but they may be increased in association with certain infections,

autoimmune disease, port-transplant setting, among other etiologies. Cell surface marker studies show that these cells are either natural killer cells or suppressor/ cytotoxic T-lymphocytes.

Malignant Lymphoid Cell (Other Than Blast)

Lymphoma cells can exhibit a variety of appearances, depending on the lymphoma subtype, and definitive diagnosis can be difficult. These cells can exhibit a variety of sizes, shapes, and nuclear and cytoplasmic characteristics. Cell size ranges from 8 to 30 µm, and the N:C ratio varies from 7:1 to 3:1. Some mature lymphocyte malignancies typically present as a leukemia (ie, lymphoma cells circulating in the blood) such as hairy cell leukemia, while others may occur in a tissue site and secondarily involve the blood (eg, follicular lymphoma). Chronic lymphocytic leukemia/small lymphocytic lymphoma is somewhat unique in that it may present in the blood as a leukemia, in the tissue as a lymphoma, or in both sites at the same time. It is critical to obtain an accurate clinical history, since knowledge of a previous diagnosis of lymphoma greatly aids in the identification of these cells. Supplemental studies, such as immunophenotyping, are often necessary to arrive at a diagnosis. In blood smears or bone marrow aspirates, it may be difficult to distinguish reactive lymphocytes from lymphoma cells. The most important distinction between reactive lymphocytes and lymphoma cells is the difference in their N:C ratios. The N:C ratio tends to be low in reactive lymphocytes, while it is high in lymphoma cells. In addition, reactive lymphocytes are characterized by their wide range of morphologic appearances within the same blood smear or bone marrow aspirate. In contrast, while lymphoma cells can exhibit a wide range of morphologic appearances, any individual case tends to show a more monotonous population of the abnormal cells. Examples of lymphoma cells that may be seen in the blood or bone marrow are described below:

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): CLL/SLL cells may be the same size as normal lymphocytes but are often slightly larger. The nucleus is typically round, although a small nuclear indentation may be present. The cells have scant cytoplasm. Nucleoli are inconspicuous. Occasional prolymphocytes are often seen, usually representing less than 10% of lymphoid cells. Prolymphocytes are larger cells, 10 to 18 mm in diameter, with a round, centrally located nucleus, clumped chromatin, and a characteristic single prominent nucleolus. The cytoplasm is homogeneously blue, may contain a few azurophilic granules, and is more abundant than in normal lymphocytes or blasts. Individual CLL/SLL cells may be difficult to distinguish from normal lymphocytes. Clues to a diagnosis of CLL/SLL include a high WBC count with absolute lymphocytosis, and nuclear chromatin that often appears cracked, resulting in a nucleus that resembles a soccer ball. The presence of occasional prolymphocytes is an additional clue to the diagnosis. For proficiency testing purposes, lymphocytes in a CLL/SLL smear may be identified as either “malignant lymphoid cell (other than blast)” or “lymphocyte” and both are considered acceptable.

B-cell prolymphocytic leukemia: B-PLL is a neoplasm of B prolymphocytes that requires immunophenotyping (eg, flow cytometry) for diagnosis. Morphologically, the diagnosis requires more than 55% of lymphoid cells in the blood to be prolymphocytes. In most cases of B-PLL, prolymphocytes represent greater than 90% of lymphoid cells, and the WBC count is markedly elevated with extensive infiltration of prolymphocytes in the bone marrow. See “chronic lymphocytic leukemia/small lymphocytic lymphoma” above for a morphologic description of prolymphocytes.

Follicular lymphoma (low grade): Low-grade follicular lymphoma cells are slightly larger than normal lymphocytes. The majority of nuclei are clefted, indented, folded, convoluted, or even lobulated. The chromatin is moderately coarse, and one or more nucleoli may be present. The cytoplasm is scant to moderate and often basophilic.

Hairy cell leukemia: Hairy cells, typical of hairy cell leukemia, are round-to-ovoid lymphoid cells that measure 12 to 20 µm (larger than normal, mature lymphocytes). Their N:C ratio ranges from 4:1 to 2:1, and they contain moderate-to-abundant pale blue to gray-blue cytoplasm. The cell borders are often indistinct secondary to the presence of characteristic elongated, fine (hairy), cytoplasmic projections. These projections are frequently irregular and may be thick, blunted, smudged, serrated, or short. The cytoplasm typically is agranular, although occasional fine azurophilic granules may be seen. Small vacuoles can be present and often give a mottled appearance to the cytoplasm. The nuclei of hairy cells are usually oval to indented, but may be folded, bean-shaped, angulated, or dumbbell-shaped. The nuclei are either centrally or eccentrically located. The chromatin is usually homogeneous, finer than in normal lymphocytes or chronic lymphocytic leukemia cells, and evenly distributed with scant intervening parachromatin. Nucleoli, if present, are generally small and single. Occasional cells may have multiple small nucleoli or a single large nucleolus.

Burkitt lymphoma: Burkitt lymphoma cells are medium-to-large cells (10 to 25 um) with a round-to-oval nucleus and moderately coarse chromatin with one or more prominent nucleoli. The cytoplasm is moderately abundant, deeply basophilic, and it often contains numerous small and uniformly round vacuoles.

Mycosis fungoides/Sézary syndrome: Sézary cells are classically found in patients with leukemic manifestations of mycosis fungoides, a form of primary cutaneous T-cell lymphoma. These cells are usually round to oval but can be irregular. They range in size from 8 to 20 µm, and their N:C ratio varies from 7:1 to 3:1. Smaller Sézary cells are slightly bigger than normal lymphocytes and have folded, grooved, or convoluted nuclear membranes that may give them a cerebriform appearance. The chromatin is dark and hyperchromatic without visible nucleoli. Larger Sézary cells can be more than twice the size of normal lymphocytes. The nucleus is also convoluted and cerebriform, appearing with hyperchromatic chromatin. Often, the nuclear membrane is so folded that the nucleus may appear lobulated or even like a cluster of berries. Some cells may exhibit a small nucleolus, although this is not a prominent feature. Both large and small Sézary cells have scant, pale blue to gray agranular cytoplasm and may contain one or several small vacuoles that lie adjacent to the nucleus. While the appearance of Sézary cells is distinctive, other T-cell lymphomas and some cases of B-cell lymphoma can mimic Sézary cells.

Large cell lymphoma: These cells may exhibit some of the most abnormal morphologic appearances. They are large (20 to 30 µm) and have scant to moderate amounts of basophilic cytoplasm. The nuclei are generally round to oval, but they may be angulated, folded, indented, or convoluted. Nucleoli are prominent and may be single or multiple. Vacuoles can occasionally be seen in the cytoplasm. These cells can be easily confused with blasts, and additional studies such as immunophenotyping are often necessary to make the correct diagnosis.

Plasma Cell, Morphologically Mature/Abnormal/Containing Inclusion (eg, Dutcher body, Russell body)

Plasma cells represent terminally differentiated B-lymphocytes and are a normal constituent of the bone marrow, where they usually comprise less than 5% of the cellularity. They are rarely seen in normal peripheral blood. They range in size from 10 to 20 µm and are often oval shaped with relatively abundant cytoplasm and eccentrically located nuclei. The N:C ratio is 1:2. Their nuclei are usually round to ovoid with prominently coarse and clumped chromatin that is often arranged in a cartwheel-like or clock-face pattern. Occasional benign plasma cells are binucleated. Nucleoli are absent. The cytoplasm stains gray blue to deeply basophilic. A prominent hof, or perinuclear zone, of pale or lighter staining cytoplasm is typically seen adjacent to one side of the nucleus. This area corresponds to the Golgi zone, which is prominent in cells that produce large amounts of protein, such as immunoglobulin in the case of plasma cells. Cytoplasmic granules are absent, and scattered vacuoles of varying size may be seen. In IgA type myelomas, plasma cells may have pink-red cytoplasm (so-called *flame cells*).

Immature or atypical plasma cells in the bone marrow or, less commonly, in the blood are associated with a variety of plasma cell dyscrasias, including multiple myeloma (plasma cell myeloma), lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia), and amyloidosis. Malignant plasma cells show a wide spectrum of morphologic features and may include some or all forms of plasmablasts, immature plasma cells, and mature plasma cells. The cells range from those that are easily recognized as plasma cells to those that are difficult to classify without ancillary studies or clinical data. Binucleated and multinucleated forms may be frequent and, when present, often display immature nuclear characteristics. Atypical mitotic figures may also be found. Malignant plasma cells may also be seen in the peripheral blood and may be numerous in cases of plasma cell leukemia.

Plasmablasts represent the most immature form in the maturation sequence of plasma cells. They are larger than mature plasma cells, measuring 25 to 40 µm in diameter. The cell border is often ragged with cytoplasmic bleb and bud formation. Nuclei are round to oval and may be eccentric or centrally placed. The N:C ratio is typically 2:1 to 1:1, which is higher than is seen in mature plasma cells. The nuclear chromatin is dispersed and fine with one or more prominent nucleoli. The cytoplasm is pale to deep blue. A perinuclear hof is usually discernible but is less prominent than in mature plasma cells. Although plasmablasts are a normal constituent of the bone marrow, they are present in very low numbers and are very rarely identified except in malignant conditions (eg, plasma cell myeloma and other plasma cell dyscrasias). Thus, identification of a plasmablast is considered abnormal.

Plasma cells normally produce and secrete immunoglobulins. This protein product may appear in different forms within the cytoplasm. When production within a particular plasma cell is increased or when there is a blockage in secretion, accumulation of immunoglobulin occurs. This finding can occur in mature, immature, or malignant plasma cells. These plasma cells range from 10 to 25 µm, and the N:C ratio varies from 1:2 to 1:3. Accumulations of immunoglobulin sometimes appear as intranuclear inclusions called Dutcher bodies. While Dutcher bodies appear to be within the nucleus, they are actually pseudoinclusions that occur when a cytoplasmic globule invaginates through the nucleus or is surrounded by the nucleus. The immunoglobulin globules may also appear as large cytoplasmic eosinophilic globules called Russell bodies. When multiple Russell bodies are present, the cell is called a Mott cell. Occasionally, immunoglobulin inclusions in plasma cells may form crystalline structures in the cytoplasm.

Megakaryocytes

Megakaryocyte or Precursor, Normal

Megakaryocytes are the largest bone marrow hematopoietic cells. They are derived from bone marrow stem cells and are responsible for platelet production. During development, the cell does not divide. Instead, the nucleus undergoes nuclear replication without cell division (endomitosis or endoreduplication), giving rise to a hyperdiploid nucleus with several lobes and each lobe roughly contains a normal complement of chromosomes. The cytoplasm of a precursor megakaryocyte is initially scant, basophilic and agranular, then progressively becomes more voluminous. It starts to display a few fine azurophilic granules in paranuclear regions, then becomes more uniformly dispersed throughout the cytoplasm in the later stages of maturation.

The normal mature, platelet-producing megakaryocyte typically measures between 50 to 100 µm in diameter, although its size can vary from as small as 25 µm to as large as 160 µm. Nuclear lobes are of various sizes, connected by large bands or fine chromatin threads. The chromatin is coarse and clumped to pyknotic. The abundant cytoplasm stains purple-pink or wine-red and contains fine azurophilic granules that may be clustered, producing a checkered pattern.

The normal end-stage megakaryocyte undergoes pyknosis through apoptosis (programmed cell death), where the cell has been liberated of much of its cytoplasm as it fragments into platelets. This end-stage megakaryocyte can appear as a pyknotic nucleus, which is left behind to be phagocytized by marrow histiocytes. In bone marrow aspirates, some megakaryocyte nuclei can also be artifactually stripped of cytoplasm by smear preparation.

For proficiency testing purposes, the term “megakaryocyte or precursor, normal” almost always refers to a mature cell rather than one of the maturation stages. A single megakaryoblast in an otherwise normal bone marrow aspirate is a relatively rare occurrence and morphologic features alone are insufficient to correctly identify an isolated megakaryoblast. On the other hand, megakaryoblasts (such as those seen in leukemic megakaryoblastic transformation of a myeloid neoplasm or acute megakaryoblastic leukemia) are best classified as blasts for proficiency testing purposes, not as abnormal megakaryocytes.

Megakaryocyte or Precursor, Abnormal

Megakaryocytic abnormalities manifest in a wide variety of aberrations in cell size, nuclear shape (with separation of nuclear lobes), and cell location. Rare dysmorphic megakaryocytes can be seen in otherwise normal bone marrow aspirate smears. Therefore, the presence of increased numbers of abnormal forms in a single case provides greater support for neoplasia. For a diagnosis of myeloid neoplasia or dysmegakaryocytopoiesis, the revised 4th edition of the WHO classification advises a threshold of at least 10% abnormal forms of 30 or more total megakaryocytes. Other investigators have suggested an even higher threshold of 30% to 40% abnormal megakaryocytes for a confident diagnosis of myeloid neoplasia.

Micromegakaryocytes are abnormally small megakaryocytes that usually measure less than 30 µm in diameter, about the size of a promyelocyte or smaller. The nuclear-to-cytoplasmic ratio is 1:1 or 1:2. The nucleus may be unilobed or can display widely separated bilobed nuclei. The cytoplasm can vary from agranular and pale blue to being flocculent (resembling tufts of wool) with numerous purple-pink granules Micromegakaryocytes may be found in the marrow or circulating in the peripheral blood. When found in the bone marrow, micromegakaryocytes are considered characteristic for myeloid neoplasia, in the appropriate context.

Increased numbers of **nonlobulated and hypolobulated mature megakaryocytes of any size** are useful findings for myeloid neoplasia. Small nonlobulated and hypolobulated nuclei or so-called *dwarf megakaryocytes,* usually associated with chronic myeloid leukemia (CML), are an example. They are larger than promyelocytes, yet smaller than the typical 50 - 100 µm size of normal mature megakaryocytes. Though historically equated to micromegakaryocytes, dwarf megakaryocytes are described as distinct from true micromegakaryocytes in cases of MDS by the 2017 revised 4th edition of the WHO classification. Significantly increased numbers of hypolobulated megakaryocytes of normal or slightly increased size are considered typical for MDS with isolated del(5q).

Normal megakaryocyte nuclei are connected in series, either by broad bands or thin threads of chromatin, and are mononuclear. In contrast, **multinucleated megakaryocytes** or **megakaryocytes with widely separated nuclei** with round and similarly sized nuclear lobes are considered useful findings in dysplasia and/or myeloid neoplasia. Though rare, widely spaced trilobed pawn ball megakaryocytes are characteristic.

Very large abnormal megakaryocytes are highly variable in morphology. Some show **increased nuclear lobation,** while others are hypolobated. While late-stage pyknotic megakaryocytes are a normal component of bone marrows, **significantly increased numbers of pyknotic megakaryocytes** are abnormal. The naked or bare nuclei are composed of dark masses of chromatin and are undergoing apoptosis (programmed cell death).

On biopsy specimens, abnormal megakaryocytes may cluster together, sometimes close to bony trabeculae. Normal megakaryocytes are usually well separated from each other and located away from the trabeculae.

Historically, cytoplasmic features (like vacuolation, agranular or hypogranular cytoplasm, and persistent basophilia with nucleo-cytoplasmic dyssynchrony) were thought to be important in the assessment of dysplasia. However, assessment of cytoplasmic abnormalities show only a weak correlation to hematologic neoplasia. As a result, cytoplasmic features do not factor into current criteria for megakaryocytic dysplasia.

For proficiency testing purposes, the term “megakaryocyte or precursor, abnormal” always refers to a mature cell and not a megakaryoblast. Similarly, megakaryoblasts (like those identified in acute megakaryoblastic leukemia) are best classified as blasts, not as abnormal megakaryocytes.

Microorganisms

The bone marrow of infected patients can demonstrate nonspecific reactive changes, such as granulocytic hyperplasia, reduced erythropoiesis, or megakaryocytic hyperplasia. Macrophages, occasionally with hemophagocytosis, may be observed. The morphologic detection of organisms within cells is uncommon in the marrow. Blood and/or bone marrow culture testing is indicated in evaluating a patient for infection.

Fungi, Extracellular

Probably the most frequently seen fungus in the bone marrow is *Histoplasma capsulatum.* The organisms are nearly exclusively present within macrophages as 1 to 2 µm budding yeast forms but can be seen in neutrophils. They are only rarely seen in an extracellular location, usually when the cell membranes of the macrophages have ruptured. Other organisms, such as *Coccidioides, Cryptococcus, Candida,* and *Aspergillus,* occur less frequently but are more commonly extracellular. The appearance is dependent upon the specific organism. *Coccidioides* typically shows mature spherules ranging between 20 to 60 µm in diameter, which often contain endospores ranging from 2 to 4 µm. *Cryptococcus* is a round to oval, yeast-like fungus ranging from 3.5 to 8 µm or more in diameter, usually with a thick mucopolysaccharide capsule, demonstrating a narrow neck when budding. *Candida* can appear in bone marrow as either yeast-like organisms with budding or as pseudohyphae. *Aspergillus* is typically identified by its septate 4-µm-wide hyphae with characteristic 45° branching. Most organisms will stain with a Periodic Acid-Schiff (PAS) stain (and the Gomori’s methenamine silver stain for fungi).

Erythrocyte Precursor with Parvovirus Inclusion

The virus preferentially infects erythroid precursors and very large pronormoblasts with visible eosinophilic nuclear inclusions. Often the markedly enlarged pronormoblasts will be rarely seen and widely scattered but may appear in small clusters. Other erythroid precursor stages are typically absent (erythroid aplasia), although in immunosuppressed patients, erythroid hyperplasia may be seen with viral inclusions present in all stages of erythroid differentiation. The enlarged pronormoblast nuclear membranes may appear to be dissolving. The viral nuclear inclusions appear as glassy, poorly defined lucencies in Wright-Giemsa-stained bone marrow smears but are more sharply defined in fixed tissue sections.

Macrophage Containing Fungi, *Leishmania* or *Toxoplasma*

Histoplasma capsulatum is a 1 to 2 µm budding yeast that typically is present in large numbers within the cytoplasm of macrophages within the bone marrow. The organisms may manifest a crescent or ring shape, and they also may be surrounded by a small halo. Both are artifacts. The amastigote form of *Leishmania* has

a similar size and appearance to *H. capsulatum* within bone marrow macrophages, but it is recognized by the additional presence of a dot-like kinetoplast associated with each organism. The unicellular tachyzoites of *Toxoplasma gondii* also imitate *Histoplasma* morphologically but do not stain positively with the Gomori's methenamine silver (GMS) stain. The biggest diagnostic problem with this group of organisms is their ability to imitate each other, but they can also be confused with other budding yeast organisms, large bacterial cocci, or phagocytized material, particularly cells. If the macrophage has ruptured, extracellular organisms may be mistaken for platelets.

Macrophage Containing Mycobacteria

The mycobacteria are responsible for a variety of clinical infections, including tuberculosis and leprosy. At least 25 species of mycobacteria are causative agents of human disease and several species can infect the bone marrow. The two species that most commonly involve the bone marrow are *Mycobacterium tuberculosis* and *Mycobacterium avium* complex.

M. tuberculosis elicits a granulomatous response with or without caseous necrosis typically identified in the biopsy, while *M. avium-intracellulare* is usually seen in large numbers within bone marrow macrophages with or without a granulomatous response. When a granulomatous response is present, organisms may be rare and difficult to find. The mycobacteria are straight to slightly curved bacilli varying from 0.2 to 0.6 µm in width and 1 to 10 µm in length. They are acid fast (due to the high lipid content in the cell wall) and may appear beaded on acid-fast stain. The organisms appear as nonrefractile negative images or as clear or red refractile beaded rods on Romanowsky-stained preparations. The incidence of disseminated *M. avium-intracellulare* infection has increased as the population of patients infected with HIV has expanded. Because this organism often does not elicit a granulomatous response, some authors have advocated routine use of the acid-fast stain (and the Gomori's methenamine silver stain for fungi) on marrow biopsies in all patients with HIV.

Miscellaneous

Blast Cell

A blast is a large, round to oval cell, 10 to 20 µm in diameter. The nuclear-to-cytoplasmic ratio is high, approximately 7:1 to 5:1. The blast often has a round-to-oval nucleus, but sometimes it is indented or folded. The blast cell has fine, lacy, reticular chromatin. One or more prominent nucleoli may be seen. The cytoplasm is basophilic and typically agranular.

The morphologic features of a blast cell do not permit determination of the cell lineage, (ie, myeloblasts versus lymphoblast (see lymphoblast entry). The one exception is the presence of Auer rods, which are diagnostic of myeloid lineage (ie, myeloblast). Other cells that may have the appearance of a blast include some lymphoma cells. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections, or, less commonly, cytochemical staining (eg, peroxidase or Sudan black) is required to determine the lineage of a given blast cell.

As lymphoblasts and myeloblasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone and additional studies, such as immunophenotypic analysis, must be performed for lineage assignment. Blasts may rarely be morphologically indistinguishable from lymphoma cells. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable.

Gaucher Cell, Pseudo-Gaucher Cell

A Gaucher cell is a form of histiocyte (macrophage) that is ovoid and measures 20 to 90 µm in diameter with a low nuclear-to-cytoplasmic ratio (less than 1:3). It contains a small, round, or oval nucleus with indistinct nucleoli. The chromatin is coarse. The cytoplasm is abundant, lipid-laden (containing glucosylcerebroside), and stains gray to pale blue. Fibrillar, reticular, crumpled-cellophane, or wrinkled-tissue-paper appearance of the cytoplasm is characteristic. This distinctive linear striation results from lamellar bodies stacked within secondary phagolysosomes. A morphologic variant shows less striking linear striation and contains a small number of fine, blue cytoplasmic granules. The cells stain for PAS and lysosomal enzymes, such as acid phosphatase (tartrate-resistant) and nonspecific esterase. Gaucher disease is an inherited deficiency of beta-glucocerebrosidase, leading to accumulation of glucosylcerebroside in a variety of tissues, including bone, liver, lung, and brain.

Pseudo-Gaucher cells are indistinguishable from true Gaucher cells on light microscopy, although they differ ultrastructurally. They are phagocytic cells engaged in catabolism of glycoside from the membranes of dead cells. These macrophages have normal amounts of beta-glucocerebrosidase enzyme and are postulated to arise from excessive cell breakdown with an overload of glucoceramide.

Histiocyte, Sea-Blue

These bone marrow cells are macrophages (histiocytes) that have abundant cytoplasm filled with variably sized bluish or bluish green globules or granules of insoluble lipid pigment called ceroid. Ceroid, which is Latin for wax-like, is a pigment of uncertain identity thought to represent partially digested globosides derived from cell membranes. They are distinguished from hemosiderin-laden macrophages (siderophages) by a negative Prussian blue stain. Small numbers of sea blue histiocytes may be seen in normal marrow and should not be considered a pathologic finding. Large numbers occur in marrow, spleen, and liver in an inherited disorder of unknown cause called the sea blue histiocyte syndrome. Occasional to moderate numbers of sea blue histiocytes can be seen in other lipid storage diseases, hyperlipidemias, chronic myeloid leukemia, patients on hyperalimentation, and any disorder with massively increased intramedullary cell destruction.

Leukocyte Containing Chediak-Higashi Inclusion(s)

Giant, often round, red, blue, or greenish gray granules of variable size are seen in the cytoplasm of otherwise typical leukocytes (granulocytes, lymphocytes, and monocytes) and sometimes normoblasts or megakaryocytes in patients with Chediak-Higashi syndrome. In the blood the disease is manifested by the presence of medium to large peroxidase positive inclusions in the leukocytes, and this is the basis of a clinical diagnostic test for this disorder. These may be single or in aggregates. A poorly understood lysomal trafficking abnormality results in fusion of primary (azurophilic) and, to a lesser extent, secondary (specific) lysosomal granules, resulting in poor function in killing phagocytized bacteria.

Lipocyte (Adipocyte, Fat Cell)

The lipocyte, a normal constituent of yellow or fatty bone marrow, is a large (25 to 75 µm in diameter) cell with a very small, densely staining, eccentric nucleus. The fat-laden cytoplasm is abundant and often consists of a single, colorless fat vacuole, giving the cell a signet-ring appearance. Alternately, it may appear to contain numerous large fat vacuoles, separated by delicate, light blue or pink cytoplasm. Eosinophilic fibrils may be present, both within the cytoplasm and extending outward from the cell margins. The lipocyte, a fat-producing cell, should be distinguished from a macrophage with phagocytized fat (or lipophage). The lipid-laden macrophage contains small, uniform lipid particles, giving the cytoplasm a foamy or bubbly appearance.

Macrophage (Histiocyte)

A macrophage is a large (15 to 80 µm in diameter) phagocytic cell. It is irregular in shape, frequently with shaggy margins and bleb-like or filiform pseudopodia. The nucleus usually is round or oval, but occasionally it may be indented. The nuclear membrane is distinct, and the nuclear chromatin is fine with a spongy, reticular pattern. One or more small nucleoli may be seen. The frayed, streaming cytoplasm is abundant, pale gray blue, and often granulated (coarse, azurophilic granules).

Phagocytized material (white cells, RBCs, platelets, nuclei or their remnants, and microorganisms) may be present in native or degraded form within the cytoplasm. Cytoplasmic vacuoles may be abundant and may contain phagocytized material or appear empty. Iron is stored in bone marrow macrophages as ferritin or hemosiderin (demonstrated with Prussian blue stain). The stored iron arises almost exclusively from phagocytosis and degradation of senescent or defective erythrocytes.

Less phagocytic macrophages sometimes are referred to as histiocytes. They have fewer lysosomal granules and may play a role in antigenic presentation to lymphocytes, cell-cell interactions in the immune system, and production of mediators important in inflammatory and immune responses. Histiocytes may cluster together, forming an epithelioid agglomeration, or fuse to form multinucleated giant cells. These aggregated epithelioid histiocytes often are prominent components of marrow granulomas, a finding best appreciated in the bone marrow biopsy.

Macrophage Containing Abundant Small Uniform Lipid Vacuole(s)/Droplet(s) (Lipophage)

The lipophage is a macrophage containing uniform, small lipid vacuoles that completely fill the cytoplasm. These fat-filled inclusions may originate from extracellular fatty material or from the membranes of ingested cells.

Macrophage Containing Hemosiderin (Siderophage)

The siderophage is a macrophage containing the coarsely granular iron-protein complex known as hemosiderin. They are granules that are dark blue with the Wright stain, arising from iron byproduct. The Prussian blue stain can confirm the identity of intracytoplasmic iron and stains hemosiderin a vivid lighter blue. Hemosiderin pigment should be differentiated from melanin and anthracotic pigment.

Macrophage Containing Cell (Hemophagocytosis)

The cytoplasm of macrophages may contain one or more intact erythroid cells as well as degraded erythroid forms within vacuoles. With further digestion, dark blue hemosiderin granules may be evident. Phagocytosis of erythrocytes often occurs concomitantly with macrophage ingestion of lymphocytes, neutrophils, and/or platelets (hemophagocytosis).

Metastatic Tumor Cell or Tumor Cell Clump

Metastatic tumor cells are larger than most bone marrow cells, except megakaryocytes, varying from approximately 15 µm to 100 µm in diameter, with a highly variable nuclear-to-cytoplasmic ratio (7:1 to 1:5). They frequently adhere in tight clusters, forming syncytial sheets or mulberry-like aggregates (morulae), best detected at the periphery of the aspirate smear. Within a given sample, the tumor cells may be polymorphous, varying in cell size and shape. Likewise, nuclei are round, spindle-shaped, or pleomorphic; and multiple nuclei of unequal size and shape may be present. The nuclear chromatin usually is finely reticulated, often with prominent parachromatin spaces. One or more large nucleoli may be seen. Rapidly

proliferating tumors can show many mitotic forms and many small apoptotic cells with nuclear pyknosis or karyorrhexis. The amount of cytoplasm is variable and is scant in small cell tumors (eg, small cell carcinoma, neuroblastoma, retinoblastoma, rhabdomyosarcoma, and Ewing sarcoma), and plentiful in others, particularly adenocarcinoma. The cytoplasm may be intensely basophilic and may contain granules, fine to large vacuoles, or bluish cytoplasmic debris. The cytoplasm often appears frayed on the aspirate smear due to pulling apart of cohesive tumor cells. Keratin formation may be apparent in squamous carcinoma.

Nonhematopoietic malignant cells are frequently not aspirable (dry tap) due to associated marrow fibrosis. Thus, tumor cells may not be detected in marrow smears. Biopsy sections are preferred for the detection of metastatic tumors. However, tumor cells may be identified in touch imprints of the biopsy. Immunohistochemistry are useful in distinguishing metastatic neoplasia from hematopoietic malignancy and in determining tumor origin. The presence of a leukoerythroblastic reaction (ie, immature granulocytes plus nRBCs) in the blood is associated with involvement of bone marrow by metastatic tumor.

Mitotic Figure

A cell containing a mitotic figure is variable in size; it may or may not be larger than the surrounding cells. The cytoplasm has color and granulation characteristic of the resting cell. When a cell undergoes mitosis, typical nuclear features are no longer present. Instead, the nucleus appears as a dark, irregular mass, often with a clear central zone. It may take various shapes, including a daisy-like form or a mass with irregular projections. In metaphase, the individual chromosomes become visible. Arranged equatorially, they begin to separate and to move toward opposite poles. Rarely, the anaphase or telophase of mitosis may be seen, with two separating masses of chromosomes forming two daughter cells. A mitotic cell can be distinguished from a degenerating cell by a relatively compact nucleus (or nuclei). A degenerating cell often displays a pyknotic nucleus that has been fragmented into numerous purple, roundish inclusions. Although the bone marrow is normally a rapidly dividing tissue, only small numbers of mitoses are found in normal marrow aspirates.

Niemann-Pick Cell, Foamy Macrophage

Niemann-Pick disease is an inherited deficiency of the lysosomal enzyme sphingomyelinase, leading to extensive accumulation of sphingomyelin in a variety of tissues, including the bone marrow. The Niemann-Pick cell is a sphingomyelin-laden histiocyte of variable size (20 to 90 µm in diameter) with abundant cytoplasm (N:C ratio less than 1:10). The cell has one or more small, round nuclei with coarse chromatin. The cytoplasm is vacuolated and foamy with a mulberry-like appearance. Some variants of Niemann-Pick disease have mixtures of foamy macrophages and sea-blue histiocytes, representing breakdown of the stored sphingomyelin to ceroid. Blood lymphocytes and monocytes also may display cytoplasmic vacuoles containing sphingomyelin. Although foamy macrophages characterize Niemann-Pick disease, they may be seen in other conditions. These include inherited deficiencies in the metabolism of lipid materials (eg, gangliosidoses, Fabry disease, and lactosyl ceramidosis) or excess accumulation of lipid material in bone marrow macrophages (eg, hyperlipidemias, thalassemias, rheumatoid arthritis, sickle cell anemia, thrombocytopenic purpura, infectious mononucleosis, chemotherapy-induced marrow aplasia, hepatitis, and chronic renal failure). The foamy macrophages in these disorders differ slightly from Niemann-Pick cells in that their vacuoles may be larger and more irregular.

Osteoblast

The osteoblast is a bone-forming cell, producing bone matrix (osteoid), that when mineralized becomes lamellar bone. It is large (25 to 30 µm in diameter) and often elliptical. It contains a round or ovoid nucleus with one or more nucleoli. The nucleus may be partially extruded from the cell. The cytoplasm is abundant, stains blue gray, and may have an indistinct, streaming border. A prominent clear zone (hof) is usually

evident a small distance away from the nucleus. Although osteoblasts resemble plasma cells, they may be distinguished by their larger size (at least twice as large as plasma cells), elliptical shape, lightly basophilic cytoplasm, prominent clear zone away from (rather than next to) the nucleus, fine reticular chromatin, and one or more nucleoli. Osteoblasts often occur singly or in clusters in the marrow aspirates of growing children and small numbers may be seen in adult specimens.

Osteoclast

Osteoclasts are involved in bone resorption and are frequently located along the bone trabeculae. They are very large cells, approximately 100 µm in diameter. Although osteoclasts resemble megakaryocytes, they are distinguished by the presence of an even number of multiple round-to-ovoid, uniformly shaped, and widely separated nuclei. The chromatin may be dense or reticular, and each nucleus usually contains one or more small, prominent nucleoli. The cytoplasm is abundant, with frayed margins, stains blue or purple to pale pink, and contains many fine reddish-purple granules. Osteoclasts are most frequently seen in marrow aspirate samples from children, patients with Paget disease, or in the clinical setting of hyperparathyroidism.

Squamous Epithelial Cell/Endothelial Cell

Squamous epithelial cells and endothelial cells are nonhematopoietic cells that can be found in a bone marrow aspirate. Squamous epithelial cells are large (30 to 50 µm), round-to-polyhedral-shaped cells with a low N:C ratio (1:1 to 1:5). The nucleus is round to slightly irregular with dense, pyknotic chromatin and no visible nucleoli. The abundant cytoplasm is lightly basophilic and may show keratinization or a few blue kerato-hyaline granules. Epithelial cells from deeper layers of the epidermis have larger nuclei with a high N:C ratio. In contrast to squamous carcinoma, contaminant squamous epithelial cells lack nuclear atypia. Squamous epithelial cells are usually derived from the skin and are removed during the bone marrow biopsy procedure. When present, these should be distinguished from metastatic tumor cells, macrophages, or fibroblasts.

Endothelial cells are a normal component of the bone marrow, lining capillaries, and sinuses. They have an elongated or spindle shape, approximately 5 µm wide by 20 to 30 µm long, with a moderate nuclear-to-cytoplasmic ratio (2:1 to 1:1). The oval or elliptical nucleus occasionally is folded and has dense-to-fine, reticular chromatin. One or more nucleoli may be visible. The frayed cytoplasm tapers out from both ends of the nucleus and may contain a few azurophilic granules. Endothelial cells have a similar, if not identical, appearance to fibroblast-like cells that make up the skeletal framework of the bone marrow.

Stromal Cell

Stromal cells of the bone marrow are elongated cells, with poorly demarcated (wispy) cell borders, and may have bipolar or multipolar cytoplasmic processes, agranular gray-blue cytoplasm. The nucleus is small, round to elongated with inconspicuous nucleoli. Stromal cells often occur in cohesive clusters on aspirate smears, forming the scaffolding of the marrow particle along with fat cells, capillaries and macrophages. These cells make extracellular matrix proteins and are progenitors for bone, cartilage, and adipocytes. Stromal cells are generally inconspicuous in normal marrow aspirate smears and are most readily seen in hypocellular marrow specimens.

While atypical MCs may mimic stromal cells, atypical MCs are distinguished by blast-like chromatin, associated granular/hypogranular MCs, and characteristic peritrabecular or perivascular localization in bone marrow core biopsies.

Artifacts

A variety of artifacts can be identified in a bone marrow aspirate. They may be present due to fixation, biopsy technique, or specimen processing.

EDTA: If a specimen is anticoagulated with EDTA and there is a delay in the preparation of smears, artifacts can appear in the cells. These can include the appearance of dyserythropoiesis with nuclear lobulation and fragmentation as well as cytoplasmic vacuoles.

Specimen processing/suboptimal staining: If the slides are fixed before adequately drying, cellular outlines can appear indistinct, and the nucleus can appear to be leaking into the cytoplasm. Uptake of water in methanol when used in fixation can cause RBCs to appear refractile with sharp round inclusions. Overstaining or understaining of aspirate smears can result in erroneous cell identification. Stain precipitate on the slides may be due to unclean slides or improper drying of the stained smears. Contaminated stain components may result in the presence of bacterial or fungal organisms in the smear, but the organisms are typically extracellular.

Technique: If the aspirate specimen is partially clotted before smears are made, small clots can be mistaken for spicules and may lead to inaccurate assessment of cellularity or erroneous determination of absent iron stores with iron stains. Extensive platelet clumping can also mimic spicules and hinder cell distribution and staining. Thick smears may result in poor staining of cells and poor cytologic detail.

Basket Cell/Smudge Cell

A basket cell or smudge cell is most commonly associated with cells that are fragile and easily damaged in the process of making a peripheral blood smear. The nucleus may either be a nondescript chromatin mass, or the chromatin strands may spread out from a condensed nuclear remnant, giving the appearance of a basket. Cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, but there is no recognizable cytoplasm to give a clue to the origin of the cell. They are seen most commonly in disorders characterized by lymphocyte fragility, such as infectious mononucleosis and chronic lymphocytic leukemia. Basket cells should not be confused with necrobiotic neutrophils, which have enough cytoplasm to allow the cell to be classified.

Stain Precipitate

Stain precipitate on a Wright-Giemsa smear is usually due to unclean slides or improper drying of the stain on the smear. Oxidized stain appears as metachromatic red, pink, or purple granular deposits on and between cells. The stain may adhere to RBCs and be mistaken for inclusions, parasites, or infected cells. The size of the stain deposits is variable, and this can be helpful in discerning their origin. Yeast and bacteria have a more uniform morphology than precipitated stain. Organisms are usually rare and dispersed throughout the slide; they do not circulate in large aggregates. Stain deposits, on the other hand, may be very focal and intense.

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3 Urine Sediment Cell Identification

Introduction

The following descriptions of objects found in urine are intended only as a guide to photograph-based proficiency testing. More complete descriptions of the subject are available in standard atlases and textbooks, some of which are listed in the references to this section. In clinical practice, the decision to perform microscopic examination should be based on a variety of factors, including the patient information, appearance of the urine, and results of the chemical analysis. Individual laboratories should have defined criteria and protocols which determine the performance of microscopy on the urinary sediment. This microscopic examination is the most time consuming and requires well-trained, knowledgeable personnel. Typically, the urine sediment is initially examined as an unstained wet preparation. However, other microscopic techniques including brightfield, phase-contrast, and polarizing microscopy aid in the visualization of formed elements in the urine. The use of polarized microscopy is useful in distinguishing many birefringent crystals, oval fat bodies, and fibers from nonbirefringent casts and other structures. The photographs presented in proficiency testing surveys are typically unstained wet preparations, but split-screen photographs may be used to demonstrate other techniques.

Urinary Cells

Erythrocyte

Under high power, unstained RBCs in wet preparations appear as pale, often biconcave, yellow-orange discs. They vary in size but are usually about 7 to 8 µm in diameter. With dissolution of hemoglobin in old or hypotonic specimens, cells may appear as faint, colorless circles or ghosts. These ghost membranes are more defined with phase-contrast microscopy.

RBCs may become crenated in hypertonic urine and appear as small, shrunken cells with irregular edges and surfaces. The surface crenations may resemble granules, and these cells may be confused with small WBCs, though crenated RBCs are much smaller than granulocytes. RBCs may be confused with fat droplets, starch granules, or yeast cells. Fat droplets (mineral oil or vaginal creams) show great variation in size and are usually highly refractile. Yeast cells are oval to round, generally smaller than erythrocytes, nearly colorless, and often show budding. Starch granules are larger and have a central indented or folded area.

Small numbers of erythrocytes may be found in the urine sediment of normal patients. Hematuria, or the presence of increased numbers of RBCs in the urine, suggests possible disease somewhere in the kidney or urinary tract. Generalized bleeding disorders, trauma, and the use of anticoagulants also may produce hematuria. Contamination of the urine by menstrual blood frequently causes falsely positive test results. nRBCs and sickle cells are only rarely seen in the urine of patients with sickle cell disease. Macrophages containing ingested RBCs may be seen in the urine of patients with chronic hematuria.

Erythrocyte, Dysmorphic

Dysmorphic RBCs are suggestive of glomerular bleeding, typically glomerulonephritis. They may also be seen in pyelonephritis and rhabdomyolysis and are a sign of a serious renal disease. When examined by phase-contrast microscopy, these RBCs demonstrate loss of the limiting membrane or the presence of cytoplasmic blebs (Mickey Mouse ears). Dysmorphic RBCs should not be confused with yeast as they contain hemoglobin and lack a refractile capsule.

Leukocytes (Eosinophil, Lymphocyte, Neutrophil, and Monocyte)

Eosinophil, Unstained

Eosinophils in urine are difficult to recognize without a stain. In unstained wet preparations, eosinophils appear slightly larger than neutrophils and may be oval or elongated. Cytoplasmic granules are less prominent. In fresh specimens, two or three large nuclear segments are apparent.

Eosinophil, Stained

Eosinophils are recognized by their characteristic bright orange-red spherical granules. These granules are larger than primary or secondary granules in neutrophils. The nucleus typically has two or three lobes separated by a thin filament. Urinary eosinophils, unlike those found in blood smears, may not stain with the Wright-Giemsa stain, but Hansel's stain may enhance their visibility. In general, eosinophils are not normally seen in the urine; more than one percent is considered significant. Increased numbers (greater than one percent) are found in patients with interstitial nephritis.

Lymphocyte, Unstained

Rare lymphocytes are normally present in urine but are difficult to recognize. Only slightly larger than erythrocytes, they have round nuclei and a small amount of smooth, nongranulated cytoplasm. Increased numbers of small lymphocytes may occur in the urine during the first few weeks after renal transplant rejection.

Lymphocyte, Stained

Normal lymphocytes are small cells with dense chromatin. Their round-to-ovoid nuclei may be notched or slightly indented. The scant to moderately abundant light blue cytoplasm may contain a few fine azurophilic granules. Urine lymphocytes prepared by cytocentrifugation may differ morphologically from those in blood films. The mature or quiescent lymphocyte appears slightly larger and often contains more abundant cytoplasm than is found in blood smears. Sometimes a small nucleolus may also be seen in cytocentrifuge preparations. Plasma cells and atypical lymphocytes are rare in urine and should be reported.

Monocyte/Macrophage, Stained

Monocytes and macrophages are phagocytic cells of variable size. The continuum of monocyte/macrophage morphology can range from the typical blood monocyte to the vacuolated, activated stage of a macrophage. The cells are usually large (14 to 30 µm), with abundant blue-gray cytoplasm containing sparse azurophilic granules. The nucleus may be round or oval, indented, lobulated, band-like, or folded. The chromatin is fine and lacy and may contain small nucleoli. Binucleated forms may be seen. Sometimes there is evidence

of active phagocytosis, such as ingested material, post ingestion vacuoles, or remnants of digested products. Occasionally, a single large cytoplasmic vacuole displaces the nucleus, suggesting the signet ring appearance of some tumor cells. Macrophages containing lipid globules may form oval fat bodies identical to those formed by renal tubular cells. Monocytes/macrophages may be seen with chronic inflammation and radiation therapy.

Neutrophil, Unstained

In unstained wet preparations, neutrophils appear as colorless granular cells, typically 10 to 12 µm or nearly twice the size of a red cell. The size of the neutrophils can vary with the condition of the urine. In freshly voided urine, nuclear detail is well-defined. With cellular degeneration, nuclear segments fuse into a single, round nucleus, and cytoplasmic granules may be lost, making distinction from renal tubular cells difficult or impossible. Neutrophils are smaller than renal tubular cells. In dilute or hypotonic urine, neutrophils swell. There may be small intracytoplasmic vacuoles and loss of nuclear segmentation. Neutrophils containing these refractile granules are moving due to Brownian motion and are called *glitter cells.* Neutrophils are actively phagocytic and can often be seen extending pseudopods. Increased numbers of leukocytes in the urine, principally neutrophils, are seen in most urinary tract disorders, particularly acute infections. Small numbers of neutrophils, usually less than five per high power field (hpf), may be found in normal urine specimens.

Neutrophil, Stained

The neutrophil nucleus is segmented into two to five lobes that are connected by a thin filament of chromatin. The abundant, pale pink cytoplasm contains many fine, lilac-colored granules. The nuclear lobes may appear eccentric, and the cytoplasm may be vacuolated. Nuclear pyknosis and fragmentation in degenerating neutrophils can make recognition difficult. Cytocentrifuge (cytospin) preparation may impart artifacts, cellular distortion, and cellular degeneration.

Other Mononuclear Cells, Unstained

Epithelial Cell, Unstained

Squamous, transitional, and renal tubular epithelial (RTE) cells may be found in cytocentrifuge urine preparations. Squamous cells are the most common epithelial cells in the urine. All have a low, nuclear-to-cytoplasmic ratio. Binucleated cells are occasionally seen. Squamous and transitional cells have a small, round nucleus with dense nuclear chromatin and abundant cytoplasm. Small keratohyaline granules may be found in squamous cells. Transitional cells tend to be more rounded and may appear in clusters. Cuboidal and columnar epithelial cells have eccentric, round-to-oval nuclei, moderately coarse chromatin, and abundant cytoplasm that may contain vacuoles.

Neutrophil/Macrophage with Intracellular Bacteria, Stained

Bacteria within a neutrophil or macrophage usually appear dark blue to black on Wright-Giemsa stain, but it may be better defined using a Gram stain. They are uniform in appearance, round or rod-shaped, single, diploid, or formed in small chains, depending upon the particular organism. It is important to distinguish bacteria from the normal cytoplasmic granules present within a neutrophil or macrophage. Bacteria of similar appearance may also be present extracellularly. Phagocytosed bacteria are a significant indicator of infection and should be characterized as completely as possible.

Parabasal Cell, Basal Cell (Vaginal Fluid only)

Parabasal cells and basal cells are located in the deeper layers of the squamous epithelium in the vaginal tract. These cells are increased in numbers when the upper layers of the squamous epithelium have been damaged or lost due to injury, trauma, or an inflammatory process. Parabasal cells are also increased in numbers in direct cervical smears and are derived from areas of squamous metaplasia of the endocervical epithelium.

Parabasal cells vary in size from 12 to 30 µm in diameter, about a quarter to half the size of superficial squamous cells. They tend to have a round-to-oval shape with smooth borders and occasional small vacuoles in the cytoplasm. They can appear in clusters and may be angulated and have irregular polygonal shapes. Their nuclei are round to oval, and the nuclear-to-cytoplasmic ratio is higher than seen in superficial squamous cells.

Basal cells are rarely seen in vaginal smears unless a pathologic process has damaged the squamous epithelium. These cells are smaller than parabasal cells and are round to oval in shape. They resemble very small parabasal cells. They have scant cytoplasm, and their nuclei are about the same size as those of parabasal cells. However, due to their smaller size, basal cells have a higher nuclear-to-cytoplasmic ratio than parabasal cells.

Renal Tubular Epithelial Cell

RTE cells are derived from the epithelium lining the nephron. They vary in size from approximately two to five times the size of RBCs, up to three times as large as a neutrophil (15 to 35 µm). Typically, they range from polyhedral in shape, to oval or elongated with granular cytoplasm. The single nucleus is round and sometimes eccentric. Renal tubular cells originating from the proximal tubule may show a microvillous border on the apical side with an eccentric nucleus displaced toward the basal side, which may be visible with brightfield microscopy. Disintegrating RTE cells become swollen and frayed, and the cytoplasm is often indistinct. In wet preparations, RTE cells may be difficult to distinguish from degenerating neutrophils, mononuclear leukocytes, or transitional epithelial cells. RTE cells generally display more cytoplasmic granularity and are less rounded and swollen appearing than transitional epithelial cells. RTE cells are the most clinically important epithelial cells found in the urine. Increased numbers are found in many diseases affecting the kidney, especially in cases of acute tubular necrosis, viral infections involving the kidney, and renal transplant rejection.

In viral infections, such as rubella and herpes, RTE cells may contain inclusion bodies. Especially large intranuclear inclusions are seen in cytomegalovirus infection.

Cytoplasmic inclusions may be found in cases of lead poisoning. These inclusions are most obvious in Papanicolaou-stained preparations.

Columnar or polyhedral cuboidal epithelial cells, with or without cilia, are occasionally found in urine and are difficult to distinguish from RTE cells. They originate in the prostate gland, seminal vesicles, or periurethral glands. Columnar epithelial cells from gut mucosa can also be found in urine containing fecal material as a result of fistula formation and in fluid from ileal bladders.

The glomerular filtrate of patients with nephrosis or lipiduria contains large amounts of lipids, such as cholesterol and/ or triglycerides, which are partially reabsorbed by the renal tubular cells. These lipids are toxic and accumulate in the cytoplasm of degenerating tubular epithelial cells. Enlarged, lipid-laden RTE cells are called oval fat bodies. Spherical intracytoplasmic lipid droplets, rich in cholesterol esters, form a Maltese cross when viewed with the polarizing microscope. Triglyceride-rich fat droplets stain positively with Oil Red O or Sudan dyes. Several days after an episode of hemoglobinuria, RTE cells containing orange-yellow to

colorless intracytoplasmic hemosiderin granules may appear in the urine. The hemosiderin granules stain positively with Prussian blue.

Spermatozoa

Spermatozoa may be found in the urine of males who have undergone prostatectomy and have retrograde ejaculation or in voided specimens obtained from males shortly after ejaculation. Sperm are also seen in normal women's urine following intercourse. Spermaturia should be noted and discussed with patients' providers, in males and females under the age10 years, as well as institutionalized patients, such as nursing homes. In wet preparations, the sperm head is about 4 to 6 µm long, usually tapering anteriorly. It is smaller and narrower than an RBC. The slender tails are about 40 to 60 µm long. The head may be separated from the tail, making identification more difficult. Tailless forms should not be confused with RBCs, being smaller, ovoid and lacking hemoglobin.

Squamous Epithelial Cell

These large (30 to 50 µm), flat cells are derived from the lining of the female urethra, the distal male urethra, or from external skin, or vaginal mucosa. Increased numbers of epithelial cells in urine suggest perineal, vaginal, or foreskin contamination. They may also be seen in males with prostatic disease, or after administration of estrogen. In wet preparations, squamous cells are about five to seven times as large as an RBC and larger than most transitional epithelial cells. A single small, condensed, round, polygonal, or oval central nucleus about the size of a small lymphocyte (10 to 12 µm) is seen in flat, round, or rectangular cells. Binucleation occurs, although less frequently than in transitional epithelial cells, and is often associated with reactive or inflammatory changes. The cell membrane is usually well defined, with occasional curled or folded edges, and there may be fine cytoplasmic granulation. Degenerating squamous cells have granular swollen cytoplasm with a frayed cell border and a pyknotic nucleus. Sheets of squamous epithelial cells, accompanied by many rod-shaped bacteria and/or yeast, occur with contamination of the urine by vaginal secretion or exudates.

Clue Cell (Squamous Epithelial Cell with Bacteria)

In vaginal specimens, squamous epithelial cells which have a stippled or granular, very refractile cytoplasm with shaggy borders due to the presence of numerous coccobacillary bacteria are known as clue cells. Clue cells are one diagnostic finding seen in bacterial vaginosis. Bacterial vaginosis is a clinical syndrome resulting from replacement of the normal *Lactobacillus* species in the vagina with high concentrations of anaerobic bacteria, *Gardnerella vaginalis,* and/or *Mycoplasma hominis.*

Transitional Epithelial Cell (Urothelial Cell)

Urothelial cells line the urinary tract from the renal pelvis to the distal part of the urethra in the male and to the base of the bladder in the female. They vary in size (20 to 40 µm), usually averaging about four to six times the size of an RBC. They are usually round or pear-shaped and smaller than a squamous cell. The nucleus is well defined, oval or round, and usually central. Binucleate cells may occur. Transitional epithelial cells can occur singly, in pairs, or in small groups (syncytia). In wet preparations, they appear smaller and plumper than squamous epithelial cells and have a well-defined cell border. They may be spherical, ovoid, or polyhedral. The smaller cells resemble RTE cells. Some, called *tadpole cells,* have elongated cytoplasmic processes, indicating a direct attachment to the basement membrane. Small vacuoles and/or cytoplasmic inclusions may be present in degenerating cells.

Small numbers of transitional epithelial cells are normally present in the urine. Increased numbers, usually accompanied by neutrophils, are seen with infection. Clusters or sheets of transitional cells are found after urethral catheterization or with urinary tract lesions.

Urinary Casts

Urinary casts are cylindrical objects that form in the distal tubules and collecting ducts as a result of solidification of protein within the tubule lumen. Any material present within the tubules is trapped in the matrix of the cast. Casts are subclassified based on their appearance and composition (eg, WBCs, RBCs, granules, bacteria). Casts must be distinguished from mucous threads and rolled up squamous epithelial cells. Filtered polarized light microscopy is helpful in distinguishing highly birefringent synthetic fibers from the true casts which are usually nonbirefringent.

Broad casts are defined as being wider than twice the length of an RTE cell. While this is a nonspecific term, as RTE cells are not often found in the same field as the cast in question, it is a helpful reference standard to have in mind when evaluating casts. Broad casts are important as they are considered to originate in dilated, atrophic tubules, and the term renal failure casts is often applied to them. It is possible to recognize broad granular casts, broad waxy casts, etc. They are important to identify and report as their presence suggests chronic renal disease.

Bacterial Cast

Bacterial casts often are misclassified as granular or cellular casts. However, bacterial forms can be seen on close inspection using phase or differential interference contrast (Nomarski) microscopy. Gram staining of the sediment is also helpful. Most of these casts contain segmented neutrophils. Urine containing large numbers of WBCs and granular or WBC casts is pathognomonic for acute pyelonephritis and should be carefully examined for the presence of bacterial casts. Yeast forms may be seen in casts from patients with fungal pyelonephritis.

Cellular Cast, Neutrophil

These cellular casts are most prevalent in pyelonephritis. The cast may be crowded with cells or have only a few clearly defined cells present in the matrix, often at one end. They contain predominantly intact, segmented neutrophils, with cell membranes and nuclei clearly visible in most of the cells. The nucleus of the segmented neutrophil may be degenerated and rounded, precluding categorization of the cell.

Cellular Cast, Renal Tubular Epithelial

These casts contain RTE cells within their matrix that are usually intact and irregularly dispersed over the surface. However, in some RTE casts, the cells may be lined up in columns or rows, indicating sloughing of the epithelium of an entire tubule. Often the specific cell type of a cellular cast cannot be determined, and the casts are reported as “Cellular Casts” without specifying the cell type. The concomitant finding of cellular casts and RTE cells in a urine sediment is diagnostically helpful. As RTE cells degenerate, their nuclei become pyknotic and dense. The cast matrix may contain granules thought to arise from degenerated RTE cells. While the cast matrix may be scant or difficult to visualize due to overlying RTE cells, it must be present in order to diagnose a cast. RTE casts are found in a wide variety of kidney diseases but are most prominent in diseases that cause damage to the kidney tubules.

Fatty Cast

Fatty casts contain large numbers of spherical, highly refractile fat droplets of varying size in the cast matrix or within oval fat bodies in the cast. Fat may be stained with Sudan stain or examined with polarized light to demonstrate the birefringent Maltese-cross pattern of cholesterol esters. Fatty casts often are associated with marked proteinuria and the nephrotic syndrome.

Granular Cast

Granular casts may contain many fine or coarse granules that are most often evenly dispersed over the cast, but they may be confined to one area or loosely scattered. They may also include degenerated cell remnants. Distinction between coarsely and finely granular casts has no clinical relevance. Granular casts are found in normal urine as well as in urine from individuals with renal disease.

Hyaline Cast (Includes Nonhemoglobin Pigmented Cast)

Hyaline casts are colorless, homogeneous, and translucent, and they have a low refractive index. They have a smooth or finely wrinkled surface and may appear tortuous or coiled. Inclusion granules may occasionally be seen in the cast matrix. These casts are usually present in small numbers in normal urine, but they may be more prevalent after strenuous physical exercise or physiological stress.

Large quantities of pigmented material may be absorbed into the cast matrix, transforming urobilinogen to a yellow color. This type of cast is called a pigmented cast (nonhemoglobin pigmented).

Red Blood Cell Cast

This type of case is rare but always clinically significant. The predominant cells are intact erythrocytes, densely or loosely covering the hyaline or granular matrix. The RBCs may be shrunken or crenated when compared with those in the surrounding urine. A yellow or red-brown color is seen when a large number of RBCs fill the cast. RBCs are of uniform size within the cast, as opposed to fat globules, which vary in size. Numerous causes of acute nephritis, particularly with glomerular injury, may produce blood casts or RBC casts. The casts are usually multiple and tend to vary somewhat in length. They are narrower than waxy casts. They are seen in 70% to 80% of cases of acute tubular necrosis and are a significant clinical finding.

Waxy Cast

Waxy casts are usually broad and stubby, with blunt ends that may appear broken off. They have well-defined parallel margins that may be serrated or notched. The colorless or waxy yellow interior is dense and homogeneous. They are thought to arise from the degeneration of cellular casts, and they are frequently associated with severe or progressive renal disease.

Urinary Crystals

At Acid pH

Ampicillin crystals appear in the urine following large intravenous doses of the antibiotic ampicillin. They are long, slender, colorless crystals that aggregate into irregular sheaves after refrigeration.

Cystine crystals are clear, colorless, and hexagonal. There may be a wide variation in crystal size. They demonstrate weak birefringence when viewed with polarized light. The reduction of cysteine to cystine in the cyanide-nitroprusside test produces a cherry-red color, supporting the crystal morphology. However, the

nitroprusside test is also positive with cysteine and homocystine, and in urine with large amounts of ketones. These crystals are present in large numbers in patients with cystinosis, a congenital autosomal recessive condition. It is the most common cause of aminoaciduria and causes renal stones. Definitive diagnosis is dependent upon chromatography and quantitative amino acid analysis. One or two percent of all renal calculi are composed of radiopaque cystine, which may produce obstruction and infection at any level of the urinary tract. Cystine crystals are distinguished from the hexagonal variant of uric acid using the cyanide nitroprusside test.

Sulfonamide crystals may form renal calculi, especially in a dehydrated patient, but with the use of water-soluble sulfonamides, this is infrequently seen today. They are colorless to yellow-brown or green-brown and precipitate at a low acid pH. Small, brown acid urate crystals found in slightly acid pH may be confused with sulfonamide crystals.

Sulfadiazine crystals appear as bundles of long needles with eccentric binding that resemble stacked wheat sheaves, fan shapes, or spherical clumps with radiating spikes. Sulfamethoxazole crystals are dark brown, divided or fractured spheres.

Uric acid crystals occur at low acid pH. They are usually yellow to brown in color and birefringent. Uric acid is the most polymorphic crystal seen in urine. Common forms are four-sided, flat, and whetstone. They vary in size and shape, including six-sided plates, needles, lemon-shaped forms, spears or clubs, wedge shapes, and stars. Large numbers may indicate hyperuricemia and are associated with nephrolithiasis.

Amorphous urate crystals are tiny granules composed of sodium, potassium, calcium, or magnesium salts of uric acid and are often referred to as *brick dust.* These colorless or red-brown aggregates of granular material occur in cooled standing urine, and they must be distinguished from bacteria. Large quantities may impart a pink or red color to urine sediment. This may give the appearance of hematuria.

Microscopically, amorphous urates and phosphates appear identical. Amorphous urates form in acid urine and are less commonly seen than amorphous phosphates. Amorphous urates have no clinical significance, but are seen more frequently in concentrated urine, as in cases of fever or dehydration. They collect in groups and appear shapeless or amorphous.

Amorphous urates are technically considered crystals, but their very small size does not allow identification as specific types of crystals.

At Neutral or Acid pH

Bilirubin crystals are occasionally seen in urine containing large amounts of bilirubin and usually accompany bile-stained cells. Small brown needles cluster in clumps or spheres or on cells or hyaline casts. Bilirubin crystals indicate severe liver disease.

Calcium oxalate crystals vary in size and may be much smaller than RBCs. The dihydrate form appears as small colorless octahedrons that resemble stars or envelopes. They are sometimes described as two pyramids joined at the base. Oval, elliptical, or dumbbell monohydrate forms are less commonly seen. All calcium oxalate crystals are birefringent. Patients who consume foods rich in oxalic acid, such as tomatoes, apples, asparagus, oranges, or carbonated beverages, may have large numbers of calcium oxalate crystals in their urine. Calcium oxalate crystals are seen in large numbers in ethylene glycol poisoning. Although oxalate crystals are usually not an abnormal finding, they may suggest the cause of renal calculi.

Cholesterol crystals are rarely seen. They are large, flat, clear, colorless rectangular plates or rhomboids that often have one notched corner. They are frequently accompanied by fatty casts and oval fat bodies. Cholesterol crystals polarize brightly, producing a mixture of many brilliant hues within each crystal. They may be confused with radiographic contrast media, but cholesterol crystals are not associated with a high urinary specific gravity.

Hippuric acid crystals are a rare component of acid urine. They are typically found in persons who eat a diet rich in benzoic acid, such as one rich in certain vegetables, but they may also be seen in patients with acute febrile illnesses or liver disease. Hippuric acid crystals are colorless to pale yellow and, unlike uric acid, may occur as hexagonal prisms, needles, or rhombic plates. They are birefringent when examined with polarized light but lack the interference colors usually seen with uric acid. While both types of crystals are soluble in NaOH, only hippuric acid is soluble in alcohol. Hippuric acid crystals are also soluble in hot water, alkali, and ether.

Leucine crystals are rare and may be found in the urine in hereditary disorders of amino acid metabolism and in severe liver disease. These highly refractile brown, spherical crystals have a central nidus and spoke-like striations extending to the periphery. Leucine spherules are birefringent, demonstrating a pseudo-Maltese cross appearance with polarized light. Leucine crystals dissolve in acid and alkali and can be distinguished from sulfa crystals by a negative diazo reaction.

Tyrosine crystals may be seen in hereditary tyrosinosis or with hepatic failure. They appear as silky, fine, colorless-to-black needles, depending on focusing. Clumps or sheaves form after refrigeration. They should not be confused with ampicillin or acyclovir crystals. A drug history and a positive nitronapthol test will confirm the presence of tyrosine.

At Neutral to Alkaline pH

Ammonium biurate crystals may be associated with phosphate crystals generally in aged/stored alkaline urine. Biurates appear as crystalline yellow-brown smooth spheres with radial or concentric striations. The thorn apple variety has projecting horns. These crystals should not be confused with sulfonamide crystals.

Amorphous phosphate crystals form colorless or brown granular aggregates. They are similar in appearance to amorphous urates but occur in alkaline, rather than acid, urine. Amorphous phosphates are technically considered crystals, but their very small size does not allow identification as specific types of crystals. They collect in groups and appear shapeless or amorphous.

Ammonium magnesium (triple) phosphate crystals are typically colorless, often large clear prisms with a coffin-lid appearance. Triple phosphate crystals assume a characteristic four-armed, feathery appearance as they dissolve. They are birefringent and are often accompanied by amorphous phosphates and bacteria.

Organisms

Bacteria

Rod-shaped bacteria (bacilli), most commonly Gram-negative enteric organisms, are identified in wet mounts as rod-shaped organisms of medium size. Large, longer bacilli seen in urine are likely to be Gram-positive lactobacilli from vaginal or fecal contamination. Cocci are more difficult to identify in wet mounts, and they must be distinguished from amorphous phosphates and amorphous urates.

Abnormal, elongated bacillary forms, about the size of yeast cells with swollen centers, are occasionally seen in urine. Their appearance is due to bacterial cell wall damage induced by antibiotics, of the penicillin group, in patients being treated for urinary tract infections.

Stained bacteria may be round or spherical (cocci) or rod-shaped (bacilli). They can appear singly or in groups, clusters, pairs, or chains of variable length and may be seen in both intracellular and extracellular locations. They stain deeply basophilic with Wright-Giemsa. Gram stain may be helpful for further classification. If found within a cell, the more specific diagnosis of “neutrophil/macrophage with phagocytized bacteria, stained” should be used. The fact that bacteria are regular and uniform in appearance is helpful in distinguishing them from cellular constituents, especially granules and phagocytized debris, and from crystals such as amorphous urates.

Helminth

Schistosoma haematobium is a trematode that inhabits the veins of the bladder, prostate, vagina, and uterus. It is most often present in the urine of patients from Africa and the Middle East who have schistosomiasis. Large oval eggs, about 150 µm long, with a distinct terminal spine, accumulate in the bladder wall. Eggs containing embryos eventually pass into the urinary bladder, usually accompanied by neutrophils and many RBCs.

Protozoa

Trichomonas vaginalis primarily causes vaginal infections, but it is also capable of infecting the urethra, periurethral glands, bladder, and prostate. The normal habitat of *T. vaginalis* is the vagina and the prostate. This protozoan flagellate has only a trophozoite stage. It is pyriform, or pear-shaped, with a length of 7 to 23 µm. There is a single nucleus and a stout central axostyle protruding from the posterior end of the body. Additional morphologic features include four anterior flagella and an undulating membrane in the anterior half, from which projects a single posterior flagellum. In wet mounts, it demonstrates a jerky, rotating, nondirectional leaf-like motion. This is a required diagnostic feature that obviously cannot be illustrated in the photomicrographs used for proficiency surveys. Rippling of the undulating membrane can be seen for several hours after cessation of motility. Degenerating forms resemble large oval cells, without visible flagella, and they may be easily confused with neutrophils or other leukocytes.

Yeast/Fungi

Candida albicans is characteristically a colorless, ovoid form with a single bud. The 5 to 7 µm, thick-walled cells stain poorly with aqueous stains in wet preparations but are strongly positive with Gram staining. They may be branched and/or have terminal budding forms. Stained yeast and fungi may assume a variety of forms. They are regular in contour and usually basophilic on Wright-Giemsa stain. They may be within or outside of cells and may have a clear capsule surrounding them. The most commonly encountered yeast is *C. albicans.* The spores may form pseudohyphae, up to 50 µm in length, that branch and may have terminal budding. The ovoid shape, budding, and pseudohyphae distinguish yeast from RBCs and fat droplets.

Miscellaneous/Exogenous

Fat Droplet

Free, highly refractile fat droplets in urine or stool are seen as dark spherules under low power, and clear spheres of varying size under high power. Fat droplets may represent endogenous triglycerides, neutral fats,

cholesterol esters, or combinations of all three. In urine, they may be observed in association with fat-laden cells or casts, and they are usually seen in patients with the nephrotic syndrome. Fat droplets should not be confused with uniform, nonrefractile RBCs containing hemoglobin or starch granules with a central indentation or fold.

Fecal Contamination of Urine

Fecal material in the urine may be due to a fistula between the colon and urinary tract or caused by contamination of the urine with feces during collection. Skeletal muscle fibers, yellow brown in color, often are seen as remnants of undigested meat in stool specimens. Columnar epithelial cells from gut mucosa and squamous epithelial cells from anal mucosa are rarely seen. Columnar cells have a distinct cell border, round nucleus, and smooth cytoplasm and may be vacuolated.

Ileal urinary bladders are formed from a segment of ileum to which the ureters are attached. Ileal bladder urine usually contains large numbers of degenerating columnar cells, neutrophils, macrophages, and bacteria. Cells are not stained yellow brown, in contrast to urine contaminated with fecal material.

Fiber

Hair and synthetic/natural fibers from clothing, cotton balls, dressings, and disposable diapers can be found in urine or stool specimens. Most fibers are large, long, and sometimes twisted. Short cellulose fibers from disposable diapers resemble large, broad, waxy casts, but unlike waxy casts they are birefringent. Fibers are well-defined, flat, refractile, and colorless and often contain fissures, pits, or cross-striations.

Mucus

Mucus strands or threads arising from glands in the lower urinary and vaginal tracts are frequently found in urinary sediments. Translucent delicate strands may form long, wavy, intertwined aggregates. They constitute the background material in the field and are more obvious with phase microscopy. Mucous threads should not be confused with fibers or hyaline cases as they are thinner and nonbirefringent.

Pollen Grain

Pollen grains contaminate urine and urine containers, often on a seasonal basis. They are usually large, about 20 µm or greater in diameter, tend to be rounded or regularly shaped, and have a well-defined thick cell wall. They may have short, regular, thorny projections. Some are yellow tan. They may resemble worm ova.

Stain Precipitate

Crystal violet-safranin and similar stains, such as Sternheimer-Malbin, which are used for wet urinary sediments, crystallize, especially at alkaline pH. They form brown-to-purple, needle-shaped crystals that sometimes aggregate in star-shaped clusters. Wright-Giemsa stain precipitate appears as metachromatic granular deposits on and between cells and may be confused with bacteria, yeast, or other parasites. The size of stain precipitate varies, unlike bacteria and yeast, which have a more uniform morphology.

Starch Granule

Starch granules from surgical gloves or other sources are a frequent contaminant of body fluids. Granule size varies from that of an RBC to four to six times larger. The usual form is colorless and irregularly rounded with a central slit or indentation, often described as looking like a beach ball with crossed polarizing filters. The granules form white Maltese crosses against a black background.

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4 Cerebrospinal Fluid (CSF) and Body Fluid Cell Identification

Introduction

The value of routine evaluation of body fluids has been amply documented. Concentration by cytocentrifugation allows for the evaluation of fluids with low cell counts, as well as adequate preservation of cytologic detail. The following descriptions are based primarily on fluids that are prepared by cytocentrifugation, air-dried, and stained with Wright-Giemsa. Most of the material used for preparation of CAP survey cell identification images has been processed in a similar manner.

Erythrocytes

Erythrocyte

These are typical erythrocytes without nuclei and similar to those present in the peripheral blood. They are not typically found in normal body fluid samples and reflect hemorrhage or traumatic contamination. They may also be seen in association with many disease states such as malignancy or pancreatitis. Erythrocytes may appear crenated in certain fluids, but that finding is not clinically significant.

Erythrocyte, Nucleated

These cells are found uncommonly in body fluids and are usually derived from peripheral blood contamination in which circulating nRBCs are present. Occasionally, they may arise from accidental aspiration of the bone marrow in an infant or an adult with osteoporosis. When the nRBCs are a result of accidental marrow contamination, they are at earlier stages (polychromatophilic and basophilic normoblast) and may also be associated with immature myeloid cells. The cytoplasm should be carefully evaluated to distinguish these cells from necrobiotic cells. nRBCs due to peripheral blood contamination tend to be at a later stage of development (orthochromatophilic normoblast).

Lymphocytes and Plasma Cells

Lymphocyte

The cytologic features of lymphocytes from body fluids prepared by cytocentrifugation may differ from those in peripheral blood smears. Changes induced by cytocentrifugation may include cytoplasmic spreading, nuclear convolutions, and nucleolar prominence. The mature, or quiescent, lymphocyte appears slightly larger than its counterpart on blood smears, often with more abundant cytoplasm but usually smaller than neutrophils and monocytes. Because of the high speed used in cytocentrifugation, a small nucleolus may be seen, and this should not be interpreted as indicative of lymphoma or immaturity. A few azurophilic granules may be noted in the lymphocytes on slides prepared by cytocentrifugation and do not by themselves denote an abnormality.

For proficiency testing purposes, it is not important to distinguish between normal and reactive lymphocytes, as this distinction is often difficult and subjective. However, it is imperative to distinguish lymphocytes, including reactive, from malignant cells (such as lymphoma cells and blasts). In contrast to malignant lymphoid cells, which demonstrate a monotonous population of neoplastic cells, there is usually a spectrum of lymphocyte morphology present in reactive conditions. In many instances, identification of malignant lymphoid cells requires the use of ancillary techniques, including flow cytometry and molecular analysis.

Lymphoma Cell

The morphology of lymphoma cells is dependent upon the specific nature of the lymphoproliferative process, but the cell population is typically relatively monotonous. Large cell lymphomas are distinguished from reactive lymphocytes by their high nuclear-to-cytoplasmic ratio, vesicular chromatin pattern, irregular nuclear contours, prominent, often large nucleoli, and lack of a clear perinuclear hof/Golgi region.

Follicular lymphoma cells are slightly larger than normal lymphocytes and have high nuclear-to-cytoplasmic ratios. The chromatin pattern may appear dense or hyperchromatic, and some of the nuclei may show deep clefts or irregular contours.

Lymphoblastic lymphoma cells appear similar to the blasts described in the Miscellaneous Cells section and sometimes contain a more folded or convoluted nuclear pattern.

Chronic lymphocytic leukemia/small lymphocytic lymphoma demonstrates a uniform population of small lymphocytes that often cannot be distinguished morphologically from normal resting lymphocytes. Sometimes, however, they are slightly enlarged with prominent parachromatin clearing, and occasional prolymphocytes may be present. Prolymphocytes are large cells with clumped chromatin, abundant basophilic cytoplasm, and a characteristically prominent central nucleolus.

Lymphoid cells (including lymphoma cells) in body fluids characteristically demonstrate a dyscohesive, or dispersed, cell pattern. However, cytocentrifugation artifact may result in small cellular aggregates. Large clumps of tightly cohesive cells with continuous outer borders are more characteristic of malignant nonhematopoietic cells.

Immunocytochemical studies and flow cytometric immunophenotypic studies are very useful to distinguish malignant from reactive lymphocytes and lymphoma from nonhematopoietic neoplasms.

Plasma Cell (Normal/Abnormal)

Normal Plasma cell: Plasma cells are terminally differentiated forms of B lymphocytes. Plasma cells can be seen in body fluids but are not normally present. They may be seen in infectious or inflammatory processes. They have round-to-oval, eccentrically placed nuclei with condensed, clumped chromatin. The cytoplasm is deeply basophilic, often with a paranuclear clear zone or Golgi region. Occasionally, the cytoplasm may contain immunoglobulin-filled vacuoles that appear clear. Binucleated plasma cells occasionally can be seen. Mesothelial cells may resemble plasma cells, but they are usually larger in size, have more centrally placed nuclei with smooth rather than ropey chromatin, and usually lack the perinuclear clear zone.

Abnormal Plasma cell: Malignant plasma cells may resemble normal plasma cells, but they may also have prominent nucleoli, irregularly shaped nuclei, more open chromatin, absent perinuclear halo, and high nuclear-to-cytoplasmic ratios. Plasma cells may be binucleated or multinucleated. In some rare situations, the nuclear-to-cytoplasmic ratio may be so altered and the cytologic features so atypical, that it is difficult to recognize the cells as of plasma cell origin. Special studies such as immunophenotyping may be necessary to confirm the plasma cell lineage and monoclonality indicating malignancy.

Granulocytes

Basophil, Mast Cell, Normal or Abnormal

Basophils and MCs are derived from separate progenitor cells in the bone marrow. Basophils and MCs are not normally found in body fluids, but when present, they are most commonly associated with inflammatory conditions, foreign body reactions, and/or parasitic infections.

Basophils and MCs are recognized by characteristic granules that stain dark blue to black with Wright-Giemsa, and that may overlay or obscure the nucleus. The nucleus of the basophil is segmented, and the chromatin is condensed or smudged. The granules of a basophil are larger than the azurophilic granules of a neutrophilic promyelocyte and are often irregular in shape. MCs are usually larger than basophils, with a low nuclear-to-cytoplasmic ratio, and a round or oval nucleus that is usually obscured by abundant granules. These granules are smaller, more numerous, rounder, and more regular than basophil granules.

Circulating MCs are abnormal and should prompt investigation for mast cell leukemia (MCL). MCs in MCL exhibit a range of morphologies, from immature to mature forms. The immature forms include (1) promastocytes, which are atypical MCs with bi- or multilobated nuclei; (2) metachromatic blasts, which are metachromatic granulated blast-like cells; and (3) multinucleated or highly pleomorphic MCs. The atypical mature MCs include (1) spindle-shaped MCs with an oval nucleus and elongated, often hypogranulated cytoplasm with focal granule accumulation; and (2) well-differentiated MCs, which are round-shaped, enlarged MCs with a round, central to slightly eccentric nucleus that is usually obscured by a heavily granulated cytoplasm.

MCL can occur de novo (primary MCL) or as a progression from antecedent systemic mastocytosis (secondary MCL). Diagnosis of MCL requires the presence of ≥ 20% atypical MCs in bone marrow aspirate and meeting the diagnostic criteria for systemic mastocytosis. The overall prognosis for MCL is poor, with a median survival time ranging from 2 to 31 months.

Eosinophil, Any Stage

The eosinophil is recognized by its characteristic round, orange-pink to orange-red granules. These are larger than the primary or secondary granules seen in neutrophils. Particularly large numbers of eosinophils may be seen in foreign body reactions, parasitic infection, and when air is inadvertently introduced into a body cavity such as during thoracentesis.

Neutrophil, Immature, includes Metamyelocyte, Myelocyte, Promyelocyte

Immature stages of the myeloid series are infrequently found in body fluids, unless there is an accompanying increase in those same cells in the peripheral blood. Patients with chronic myeloid leukemia or chronic myelomonocytic leukemia may have soft tissue involvement, and increased numbers of immature myeloid cells may be seen in fluids from these patients. Immature granulocytic (and erythroid) cells can be found when there is marrow contamination of fluid, most commonly in CSF.

Neutrophil, Segmented or Band

The segmented or band neutrophil is usually easily recognized. The nuclear lobes often appear eccentric in cytocentrifuge preparations. The cytoplasm may contain toxic granules or vacuoles with inflammatory states. Intracellular bacteria, crystals, or debris may be seen in pathologic conditions. If intracellular foreign material is present, the more specific identifications such as “neutrophil/macrophage with phagocytized bacteria” or “neutrophil/macrophage containing crystal” should be used for purposes of identification in CAP Surveys.

Neutrophils in body fluids can show morphologic change due to autolysis, including nuclear pyknosis and fragmentation, making recognition of cell type difficult. These autolytic neutrophils can be mistakenly identified as nRBCs. However, persistence of a few azurophilic granules in the cytoplasm provides a clue to the neutrophilic origin. Neutrophils in samples from the stomach, intestine, or stool often show striking degenerative changes. For the purpose of proficiency testing, the identification “degenerative cell, NOS” should be chosen if the cell of origin can no longer be recognized.

Mononuclear Phagocytic Cells

Macrophage/Monocyte, including macrophage containing abundant uniform small lipid vacuoles/droplets, ie, lipophage

Monocytes are derived from a common myeloid progenitor cell in the bone marrow where they mature and then enter the circulation. Macrophages evolve from monocytes after their migration into tissues and body fluids. Monocyte/macrophage morphology in fluids is quite variable, ranging from the typical monocyte of the peripheral blood to a vacuolated, activated stage with the morphology of a typical macrophage. Monocytes are usually large (12 to 20 µm) with abundant blue-gray cytoplasm and often containing sparse azurophilic granules. The nucleus is round to oval and may show indentation, giving it a kidney bean or horseshoe shape. The chromatin is lacy and small nucleoli may be apparent.

Macrophages are larger cells (15 to 80 µm) with abundant cytoplasm showing evidence of active phagocytosis. Ingested material may include other cells, infectious organisms (eg, fungal elements or bacteria), hemosiderin (hemosiderophage), lipid (lipophage), and acellular debris. Macrophages have light blue cytoplasm and variable numbers of small, delicate cytoplasmic azurophilic granules. One or more round-to-oval, typically eccentric nuclei are present and occasionally prominent nucleoli may be seen. Alveolar macrophages are usually the predominant cells in bronchoalveolar lavage (BAL) fluid, which is obtained by instilling sterile saline into the alveolar spaces and then removing it through a fiberoptic bronchoscope. They appear similar to macrophages found in other body fluids; however, they may contain anthracotic (black) pigment in their cytoplasm, particularly in specimens from smokers or city dwellers. Macrophages can at times be difficult to differentiate from mesothelial cells. Mesothelial cells are usually larger than macrophages/monocytes and usually show a biphasic staining cytoplasm and surface microvilli (see “Mesothelial Cell” section below).

The lipophage is a macrophage containing abundant small, uniform, lipid vacuoles that completely fill the cytoplasm. These fat-filled inclusions may originate from extracellular fatty material or from the membranes of ingested cells. Lipophages may be present in CSF following cerebral infarcts, injections of intrathecal chemotherapy, or post-irradiation. They may be present in pleural fluid associated with chylothorax or with extensive cell membrane destruction.

Macrophage Containing Erythrocyte(s), ie, Erythrophage

The erythrophage is a macrophage that has ingested RBCs, usually due to hemorrhage from trauma or a bleeding disorder. As phagocytic activity may persist following acquisition of the specimen, the presence of erythrophagocyto-sis does not always imply in vivo erythrophagocytosis. However, it can be an important clue to prior hemorrhage. Erythrophagocytosis is also seen in hemophagocytic syndromes in which it is usually accompanied by leukophagocytosis.

Macrophage Containing Hemosiderin, ie, Siderophage

The siderophage is a macrophage containing the coarsely granular iron-protein complex known as hemosiderin. Hemosiderin is an iron byproduct from digested RBCs that appears as coarse, dark blue granules within macrophages on Wright stain. Siderophages may be seen in conditions that cause bleeding in any body cavity such as with alveolar or intracranial hemorrhage, and may be detected in body fluids months after the initial event. Hemosiderin pigment should be differentiated from melanin and anthracotic pigment. The Prussian blue stain can confirm the identity of intracytoplasmic iron and stains hemosiderin a vivid lighter blue.

Macrophage Containing Neutrophil(s), ie, Neutrophage

The neutrophage is a macrophage containing one or more phagocytosed neutrophils. In the early stages of ingestion, the characteristic segmented nucleus of the neutrophil will be evident and is surrounded by a large, clear zone of cytoplasm. As digestion of the neutrophil proceeds, the nucleus becomes round and pyknotic. Finally, remnants of digested nuclei of neutrophils appear as smaller, dark purple to black, homogeneous inclusions. However, these inclusions are larger than the small azurophilic lysosomal granules characteristic of macrophages. The remnant nuclei should be distinguished from bacteria and yeast, which are usually much smaller and have a more uniform appearance. Bacteria display either a coccal or bacillary morphology, whereas yeast often display budding forms. Special histochemical stains can help identify infectious organisms. Darkly staining blue-black hemosiderin granules (from breakdown of RBCs) should also be distinguished from digested leukocyte debris.

For purposes of identification in CAP Surveys, a macrophage should be termed a “macrophage containing neutrophil(s) (neutrophage)” when the phagocytized nuclear inclusion is clearly identifiable as originating from a segmented neutrophil. If a macrophage contains microorganisms, the identifications of “neutrophil/macrophage with phagocytized bacteria” or “neutrophil/macrophage with phagocytized fungi” should be used.

Neutrophil/Macrophage Containing Crystal

Crystals may be present within the cytoplasm of a neutrophil/macrophage and are most frequently seen in synovial fluids. Crystals can be seen in conditions such as gout, pseudogout, or hemorrhage (hematoidin crystals). They may vary in shape, size and color, and may not be readily apparent on Wright-Giemsa stain, thus further evaluation with polarized light microscopy is required if the presence of crystals is suspected. For proficiency testing in CAP Surveys, when crystals are present within a neutrophil or macrophage, this more specific identification of “Neutrophil/Macrophage Containing Crystal” should be chosen.

Lining Cells

Bronchial Lining Cell

Bronchial lining cells may be obtained as a contaminant in BAL fluid, indicating sampling from the bronchial tree. These cells have a unique appearance imparting a columnar shape with a row of cilia at one end and a basally oriented oval-to-round nucleus at the other end. The nucleus contains coarsely stippled chromatin and inconspicuous nucleolus. Bronchial lining cells are seen as single cells or in small clusters.

Endothelial Cell/Capillary

Endothelial cells line blood vessels. They are a normal component of tissue and are rarely found in body fluids though occasionally an intact capillary may be present as a contaminant. Capillary segments in body fluids are arranged in a longitudinal overlapping pattern in two rows, sometimes with a visible lumen, similar to their appearance in intact tissue. Endothelial cells have an elongated or spindled shape, measure approximately 5 µm wide by 20 to 30 µm long and have a moderate nuclear-to-cytoplasmic ratio (2:1 to 1:1). The frayed cytoplasm tapers out from both ends of the nucleus and may contain a few azurophilic granules. The oval or elliptical nucleus occasionally is folded and has dense to fine, reticular chromatin. One or more small nucleoli may be visible.

Mesothelial Cell

The mesothelial cell (20 to 50 µm) normally lines pleural, pericardial, and peritoneal surfaces. These cells can be shed individually or in clusters. When found in pairs or clusters, mesothelial cells have articulated or coupled cell borders with a discontinuous outer border (clear spaces or windows) between many of the cells. The nuclear-to-cytoplasmic ratio of mesothelial cells is low (less than 1:1). The nucleus is round to oval in shape with a well-defined nuclear membrane and regular contour. Nuclei are usually single and centrally located. Binucleation and multinucleation may occur, sometimes with overlap, however, the nuclei will maintain relatively similar physical characteristics. The chromatin varies from dense to fine, but it is evenly distributed. One or more nucleoli may be present. The cytoplasm is light to dark blue and may have a grainy texture or a crystalline/ground glass appearance particularly in the perinuclear area. With some staining techniques, the peripheral and perinuclear cytoplasmic regions may demonstrate relatively pale staining , imparting a two-tone quality to the cytoplasm. While this variability in cytoplasmic staining is associated with mesothelial cells, it is not specific to mesothelial cells and can be seen in other cells including malignant cells. Cytoplasmic vacuoles, blebs, and/or fragmentation may occur with cellular degeneration. Reactive mesothelial cells, seen in chronic effusions or inflammatory processes, may show variable enlargement of overall size, less condensed chromatin, and prominent nucleoli. However, the nucleus still retains a definitive, smooth nuclear membrane. Mitotic figures occasionally are seen within mesothelial cells. Mesothelial cells can be phagocytic and resemble macrophages, resulting in forms that have a morphology intermediate between mesothelial cells and macrophages.

Synoviocyte/Synovial Lining Cell

Synovial lining cells cover the nonarticular surface of the joint cavity. Synoviocytes are large (20 to 40 µm) cells with a round-to-oval shape and a round-to-oval nucleus with a distinct membrane and regular contour. Occasional multinucleated forms occur, but nuclei typically are similar in size and shape. The chromatin varies from dense to finely granular and one or more nucleoli may be present. Cytoplasm is abundant, basophilic, and agranular with an often uneven or grainy texture. Degenerative changes may occur, including multiple small vacuoles or cytoplasmic blebs. Overall, the appearance of synovial lining cells is similar to that of mesothelial cells in serous fluids. Their presence in synovial fluid is expected and has no diagnostic significance.

Ventricular Lining Cell/Ependymal or Choroid Cell

Cerebrospinal fluid samples may contain lining cells from the cerebral ventricles (ependymal cells) or choroid plexus (choroidal cells or choroid plexus cells), particularly in neonates or in the presence of a ventricular shunt or reservoir. Choroidal and ependymal cells are not diagnostically significant in CSF samples but must be distinguished from malignant cells. These cells are large (20 to 40 µm), and may occur singly or in clumps. Clumps may be loose aggregates or tight clusters with indistinct cell borders. The cytoplasm is typically

amphophilic and grainy, but occasionally it is more basophilic (a feature often associated with ependymal cells), and microvilli may be present (a feature of choroidal cells). Nuclei are eccentrically placed and are round to oval with a well-defined, smooth nuclear membrane and regular nuclear contours. Occasionally, the nucleus may appear pyknotic. Chromatin is distributed evenly and is reticulated or dense. Nucleoli are inconspicuous. Extensive degeneration of ventricular lining cells may occur so that only naked nuclei remain.

Miscellaneous Cells

Blast Cell

Blasts may be present in body fluids of patients with acute leukemia. They are typically present as dispersed, isolated cells though centrifugation artifact may produce spurious groups. Blasts are large, 10 to 20 µm in diameter, round-to-oval cells, with a high nuclear-to-cytoplasmic ratio, and round-to-oval nucleus, that is occasionally indented or folded, a feature that may be accentuated by cytocentrifugation. The cytoplasm is basophilic and varies from scant to abundant depending on the type of leukemia, and may contain azurophilic granules or Auer rods. The nuclear chromatin is typically fine or lacey, and one or more nucleoli may be present. Nucleoli are more prominent in cytocentrifuge slides. it is not possible to further characterize a given blast cell as myeloid or lymphoid in the absence of lineage-associated findings, such as Auer rods, or cytochemical or immunophenotypic data. This is particularly true for body fluid samples, where cytospin preparation artifact may alter or obscure morphologic details. Degenerative changes also may occur if the fluid specimen is not processed promptly.

Chondrocyte/Cartilage Cell

Chondrocytes are typically seen in the synovial fluid of patients with osteoarthritis, as well as after joint trauma or surgery. Rarely, chondrocytes are seen on cytospin preparations of CSF samples, likely obtained as the needle nicks the vertebral cartilage during lumbar puncture. This is a more common occurrence in infants or adults with a narrow intervertebral space. Chondrocytes have round or oval, dark nuclei that are typically centrally placed. The cytoplasm is dense and homogenous often with a perinuclear clear zone.

Degenerating Cell, Not Otherwise Specified (NOS)

Degenerating cells with pyknotic (highly condensed) nuclei or nuclear karyorrhexis (fragmentation) may occasionally be seen in body fluids.

Autodigestion or autolysis of neutrophils may occur as they attempt to remove foreign material. As the nucleus becomes pyknotic, it fragments, and with further autolysis, it may appear as one or more indistinct, light purple inclusion(s). The nuclear lobes may fragment into numerous small particles of varying sizes that resemble microorganisms. Cytoplasmic granules may become less prominent or may fuse (particularly with toxic granulation). The cytoplasmic borders may become frayed and indistinct. Cytoplasmic vacuole formation is common.

Autolytic neutrophils with eccentric, dense, round nuclei, and pale cytoplasm may resemble nRBCs. In contrast, autolytic neutrophils tend to maintain some degree of cytoplasmic granulation, while nucleated red cells do not contain granules.

Actively dividing cells, such as malignant cells, reactive lymphocytes, and mesothelial cells may more readily undergo degenerative changes in body fluids. The cytoplasm may show a swollen, vacuolated, or frayed appearance. The nuclear chromatin may show coarse condensations separated by enlarged parachromatin spaces.

Ventricular lining cells often will not appear intact when shed into CSF or ventricular fluid. Only bare nuclei with pieces of frayed cytoplasm will be seen.

All cell types may undergo degenerative changes in fluids with prolonged storage or after infusion of sclerosing agents into a body fluid cavity.

Germinal Matrix Cell

Germinal matrix cells, also known as undifferentiated leptomeningeal cells, are small blast-like cells that typically occur in clusters. They have a high nuclear-to-cytoplasmic ratio and delicate nuclear chromatin, and they may have a single small nucleolus. Nuclear molding may occur. Immunophenotypically, these cells are of neural origin. Germinal matrix cells originate from the subependymal cell layer in the lateral ventricles. These cells are pluripotent and can give rise to mature neuronal and glial cells. Significant amounts of vascular germinal matrix persist until about 32 weeks of gestation. As migration of neuronal and glial precursors proceeds into the cerebral cortex, the germinal matrix layer progressively thins and breaks into small islands, which may persist through the first postnatal year of life. The germinal matrix has a thin, fragile microvasculature, often prone to hemorrhage in premature infants. Germinal matrix cells may be found in neonatal CSF in association with hydrocephalus, after intraventricular hemorrhage, following ventriculostomy, or after placement of a ventricular-peritoneal shunt.

Lupus Erythematosus (LE) Cell

The LE cell is characteristically associated with systemic lupus erythematosus (SLE), however, they have been described in effusions of patients with other autoimmune disorders (eg, rheumatoid arthritis), multiple myeloma, Hodgkin lymphoma and in the setting of certain medications. Around 25-30% of pleural and pericardial (and less often peritoneal or synovial) effusions from SLE patients harbor LE cells. LE cells may form in vitro, and serous fluids standing at room temperature for a prolonged period of time may have more LE cells. LE cells are intact neutrophils or macrophages that contain a large, homogeneous, glassy cytoplasmic inclusion (so-called hematoxylin body) that distends the cytoplasm and displaces the nucleus. The inclusion is antibody coated generated nuclear material with a characteristic magenta color on Wright stains. Although assessing effusions for LE cells is no longer considered a sensitive or specific test for the diagnosis of SLE or other autoimmune diseases, identifying an LE cell in a patient with an unknown diagnosis is useful in guiding further laboratory evaluation.

Tart cells should be distinguished from LE cells. Tart cells are macrophages that have phagocytized the nucleus of another cell, but in contrast to the true LE cell, the ingested nucleus remains intact and nonhomogenized. Tart cells are found more frequently in serous fluids, and they are not associated with SLE or other autoimmune disorders.

Malignant Cell, Nonhematopoietic

A variety of neoplastic cells may be found in body fluids, although their presence in synovial fluid is rare. Their morpholo-gy is dependent on that of the primary underlying malignancy. Malignant cells may be numerous and clustered or appear as rare single cells. Cytologic features of malignant cells on cytocentrifuge preparations include high nuclear-to-cytoplasmic ratio, increased cell and nuclear size, irregularly shaped nuclei, atypical nuclear chromatin patterns, large nucleoli, and a tendency to form large clusters, frequently with nuclear molding. Occasionally, a cell cluster may recapitulate an organoid structure, such as pseudo-gland formation with adenocarcinoma.

Megakaryocyte

Occasionally, bone marrow hematopoietic elements can be introduced into the CSF during the lumbar puncture procedure. Megakaryocytes appear as large cells with a multilobated nucleus and distinctive finely granular cytoplasm.

Neural Tissue/Neuron

Neural tissue consists of capillary fragments, neurons (ganglion cells), glial cells, or fragments of these cells within fibrillar cerebral cortex tissue. They can be seen in the CSF of patients who have experienced intracranial hemorrhage, signifi-cant head trauma, or recent neurosurgery. Neural tissue and/or neurons are also more frequently encountered when CSF is collected from the ventricles through a shunt or reservoir device. The neural tissue fragments in Wright-Giemsa-stained preparations appear as basophilic or amphiphilic, fibrillar, finely granular matrix containing glial cells with small bland nuclei with indistinct cytoplasm. The neural tissue matrix may also contain neurons and capillaries, or be acellular. Intact neurons are evidenced by their pyramidal/angular-shape with round-to-oval nuclei, reticulated nuclear chromatin, a single prominent nucleolus, and basophilic cytoplasm that may contain lipofuscin pigment. Isolated glial cells resemble monocytes and hence are more difficult to accurately identify. Inflammatory cells may be seen within degenerating neural tissue. If necessary, immunocytochemistry can be used to confirm the suspected nature of such elements.

Squamous Epithelial Cell

Squamous cells derived from skin may be found in fluids as contaminants. Squamous epithelial cells are large (30 to 50 µm), round-to-polyhedral-shaped cells with a low nuclear-to-cytoplasmic ratio (1:1 to 1:5). The nucleus is round to slightly irregular with a dense, pyknotic chromatin pattern, and no visible nucleoli. The abundant cytoplasm is lightly basophilic and may show evidence of keratinization or contain a few blue keratohyaline granules. Epithelial cells from deeper layers of the epidermis have larger nuclei with a high nuclear-to-cytoplasmic ratio. In contrast to squamous cell carcinoma, contaminant squamous epithelial cells lack nuclear atypia.

Crystals

Calcium Pyrophosphate Dihydrate (CPPD) Crystal

These crystals are found in synovial fluid of patients with arthritis, pseudogout, or metabolic diseases such as hypothyroidism. CPPD crystals are intracellular, usually 1 to 20 µm long, and rod-shaped, rhomboid, diamond, or square. They can be differentiated from monosodium urate (MSU) crystals by polarizing microscopy with a first-order red compensator. The CPPD crystals are blue when the long axis of the crystal is parallel to the slow ray of light from the color compensator (positive birefringence); MSU crystals are yellow (negative birefringence).

Monosodium Urate (MSU)/Uric Acid Crystal

Pathognomonic of gout, MSU crystals are found in synovial fluid. They are found either intracellu-larly or extracellularly and are described classically as needle-like. They are 2 to 20 µm in length and 0.2 to 1 µm thick. Intracellular crystals are present in acute attacks of gout. The main differential of MSU crystals are CPPD crystals. However, these are reliably distinguished by use of a polarizing microscope and a first-order

red compensator. The MSU crystal is yellow when the long axis of the crystal is parallel to the slow ray of light from the color compensator (negative birefringence); the CPPD crystal is blue (positive birefringence).

Cholesterol Crystal

These crystals are extracellular and are one of the larger crystals found in fluids. The most common form is flat, plate-like with a notch in one corner. Occasionally they may be needle-like. They are transparent and appear as a negative impression on Wright stained slides. Cholesterol crystals are strongly birefringent when viewed with polarizing filters and are found in chronic effusions, especially in rheumatoid arthritis patients.

Crystal, Not Otherwise Specified (NOS)

Steroid crystals may occasionally be seen, especially in synovial fluids. For example, betamethasone acetate occurs as blunt-ended rods, 10 to 20 µm long. Steroid crystals may be either positively or negatively birefringent and interfere with the diagnosis of crystal-associated arthritis. Other structures that can be confused with crystals include fragments of degenerated cartilage and foreign material from prosthetic devices.

Hematin/Hematoidin Crystal

Hematin and hematoidin crystals both result from the breakdown of hemoglobin in tissue. Hematin is a porphyrin compound. Hematoidin is similar to bilirubin. The crystals may be found anywhere in the body approximately two weeks after bleeding/hemorrhage. The crystal may be either intracellular or extracellular, are bright yellow on Wright stain and have a rhomboid shape. Unlike hemosiderin granules, hematin and hematoidin do not stain with iron stains.

Microorganisms

Intracellular and extracellular organisms such as bacteria and yeast may be found in body fluids, particularly during the acute stage of infection. The organisms are uniform in structure and staining characteristics. Bacteria must be differentiated from stain precipitate and nonspecific phagocytic debris. A wide variety of parasites may be found in body fluids. Organisms usually have characteristic features that allow identification.

Anaplasma/Ehrlichia

Members of the genus *Anaplasma* (previously *Ehrlichia*) are small, Gram-negative obligate intracellular organisms currently classified as rickettsiae. On Wright-stained preparations, *Anaplasma* species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either neutrophils (*A. phagocytophilia*) or monocytes and macro-phages (*A. chafeensis*). The morulae are microcolonies of elementary bodies.

Bacteria, Extracellular

A wide variety of bacteria can be seen in body fluids, including bacilli, cocci, and filamentous bacteria. All are best seen under oil immersion magnification, and they may be seen in an intracellular or extracellular location. However, when they are intracellular, the more specific identification of “neutrophil/macrophage with intracellular bacteria” should be used for proficiency testing purposes. Bacilli are rod-shaped bacteria, while cocci are spherical. Filamentous bacteria are bacilli that grow in a branching pattern. They can be mistaken for fungal hyphae but are typically smaller and narrower.

Most bacteria have a basophilic hue on Wright-Giemsa stain. A Gram stain can be useful in separating these microorganisms into Gram-positive (blue/purple) and Gram-negative (pink) groups. An acid-fast stain is also useful in identifying certain filamentous bacteria. The most likely error in interpretation is to misidentify stain precipitate as microorganisms. In contrast to bacteria that tend to be relatively uniform in size and shape, stain precipitate is irregular in shape and individual grains vary considerably in size.

Neutrophil/Macrophage Containing Bacteria

Bacteria within a neutrophil or macrophage are notable for their uniform appearance, and may be round (cocci) or rod-shaped (bacilli), single, diploid, or in small chains depending upon the species present. They usually appear dark blue/purple on Wright-Giemsa stain. Gram stain may be helpful for further characterization. Bacteria of similar appearance may also be present extracellularly. It is important to distinguish bacteria from the normal cytoplasmic granules or debris present within a neutrophil or macrophage. For proficiency testing, when bacteria are present within a neutrophil or macrophage, this more specific identification should be chosen.

Neutrophil/Macrophage Containing Fungi

Fungi or yeast may be seen within a neutrophil or macrophage. Their shape is distinctive and regular, occasionally showing budding, or a clear capsule may be present around them. They appear basophilic when stained with Wright-Giemsa stain. As with intracellular bacteria, fungi should be distinguished from normal or degenerating intracellular granules and other constituents. For proficiency testing, when fungi/yeast are present within a neutrophil or macrophage, this more specific identification should be selected.

Parasite

A wide variety of parasites may be found in body fluids. The organisms usually have characteristic features that allow identification. Both unicellular (eg, amoeba, Giardia) and multicellular (eg, tapeworms, roundworms) parasites can be encountered.

Yeast/Fungi, Extracellular

Yeast and fungi may assume a variety of forms and may be intracellular or extracellular. If located intracellularly, the more specific identification of “neutrophil/macrophage with intracellular fungi” should be used for proficiency testing purposes. They are regular in contour and usually basophilic on Wright-Giemsa stain. The most commonly encountered yeast is *Candida albicans*. It is ovoid and measures 5 to 7 µm, and it has a thick wall. The spores may form pseudohyphae that branch and/or have terminal budding forms. These pseudohyphae may be up to 50 µm in length. These micro-organisms can be highlighted by GMS (Gomori methenamine silver) staining.

In the CSF, *Cryptococcus* is the most commonly encountered fungus. This microorganism is a round-to-oval, yeast-like fungus which stains lightly basophilic on Wright-Giemsa. It ranges from 3.5 to 8 µm or more in diameter and usually has a thick mucopolysaccharide capsule that is accentuated by mucicarmine stain. Budding forms display a narrow neck.

Miscellaneous Findings

Fat Droplet

Fat droplets are found free in body fluids as translucent or nearly translucent spheres of varying size. They are refractile and anucleate. Fat droplets may be endogenous or exogenous in origin.

Mitotic Figure

When a cell undergoes mitosis, the regular features of a nucleus are no longer present. Instead, the nucleus appears as a dark, irregular mass. It may take various shapes, including a daisy-like form or a mass with irregular projections. On rare occasions, the telophase of mitosis may be seen as two separating masses of irregularly shaped nuclear material (chromosomes). A cell containing a mitotic figure may or may not be larger than surrounding cells. A mitotic figure may occasionally be difficult to distinguish from a degenerating cell. In a degenerating cell, the nucleus is often fragmented into a single or multiple dark blue-purple, round, homogeneous cytoplasmic object(s), without discernable chromosomal structures.

Stain Precipitate

Wright-Giemsa stain precipitate appears as metachromatic granular deposits on and between cells. It may be confused with bacteria, yeast, or other microorganisms. The size of stain precipitate granules, however, varies in contrast to microorganisms, which have a more uniform appearance.

Starch Granule

Starch granules are highly refractile contaminants (classically from medical glove powder) whose size varies from the diameter of an RBC to four to six times larger. With Wright-Giemsa stain, they are blue to blue-purple, hexagonal or rounded with a central irregular slit or indentation.

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5 Clinical Microscopy Miscellaneous Cell Identification

Introduction to Vaginal Wet Preparations

Wet preparations of vaginal secretions are often examined to diagnose causes of vaginal discharge. The consistency, color, pH, odor of the discharge, and the presence or absence of microorganisms in wet preparations are paramount to the evaluation process. Vaginal secretions from the posterior vaginal pool are collected on a cotton or Dacron (polyethylene terephthalate)-tipped swab and mixed with a few drops of nonbacteriostatic saline either directly on a slide for microscopic evaluation or mixed in a tube with normal saline. If secretions are first mixed in a tube, the tip of the collection swab is subsequently pressed against a glass slide to express the fluid. The slide, regardless of preparation method, is evaluated by brightfield or phase microscopy. Some authors suggest that a drop of vaginal fluid plus a drop of 10% potassium hydroxide (KOH) solution, covering it with a cover slip, and examining it with brightfield or phase microscopy enhances the detection of fungal organisms. The postcoital test is another type of vaginal wet preparation performed in the preovulatory period on cervical mucus, 2 to 12 hours following intercourse, to assess the interaction between the sperm and cervical mucus as well as the number of sperm and sperm motility.

Vaginal Cells

Fern Test

The fern test, in conjunction with the nitrazine pH test, is highly sensitive and specific for the detection of ruptured placental membranes. The fern test requires a vaginal pool fluid sample that is allowed to air dry on a microscope slide for five to seven minutes. The slide is then examined microscopically at low power. A positive test reveals an elaborate arborized crystallization pattern (ferning) and indicates the presence of amniotic fluid in the vaginal vault. The arborization pattern is best visualized when the substage condenser is lowered to accentuate the diffraction pattern. The fern test is able to detect amniotic fluid leak as early as 12 weeks of gestation. Inadvertent contamination by cervical mucus may produce a similar arborization pattern (and is thus a potential cause for false positive results), though the pattern is less elaborate and is typically not seen after the first trimester due to high levels of progesterone.

Squamous Epithelial Cell

The vagina is lined by squamous epithelium which matures under the influence of estrogen. The most superficial (mature) squamous cells are large (30 to 50 µm), polygonal cells with a small, hyperchromatic nucleus. In wet preparations, squamous cells are about five to seven times larger than an RBC and have abundant transparent cytoplasm that may be curled or folded. The squamous cell membrane is usually well-defined on brightfield and phase microscopy. In response to chronic injury or irritation (eg, uterine prolapse), changes of cellular degeneration may be seen including cytoplasmic granularity and fraying, pyknotic nuclei, or anucleate squamous cells.

Parabasal Cell, Basal Cell

Parabasal cells and basal cells are immature squamous cells that comprise the deeper layers of the vaginal epithelium. Their presence in wet preparations is the hallmark of epithelial atrophy. Both cell types may also be encountered in cases of squamous metaplasia.

Parabasal cells vary in size from 12 to 30 µm in diameter, are round to oval, and typically have dense cytoplasm with increased nuclear-to-cytoplasmic ratios compared to mature squamous cells. They can form loosely aggregated clusters or broad sheets which may cause them to appear more angulated and polygonal.

Basal cells are rarely seen in vaginal smears unless a pathologic process has damaged the squamous epithelium. Compared to parabasal cells, basal cells have similar nuclear features but less cytoplasm and are therefore smaller in overall size.

Clue Cell (Squamous Epithelial Cell with Adherent Bacteria)

Clue cells are squamous epithelial cells covered with coccobacilli and associated with bacterial vaginosis (BV), a nonsexually transmitted disorder characterized by thin, milky discharge and a distinctive odor. BV is traditionally attributed to *Gardnerella vaginalis,* though other bacteria may be culpable. Clue cells have a heavy stippled or granular, refractile cytoplasm with shaggy or bearded cell borders due to the heavy coating of bacteria. The majority of the cell surface should be covered by bacteria to identify it as a clue cell. Occasional irregular keratohyalin granules in the cytoplasm of squamous cells should not be confused with adherent bacteria.

Organisms

Trichomonas

Trichomonas vaginalis is the etiologic agent of trichomoniasis, a sexually transmitted disease that primarily infects the vagina but can also infect the urethra, periurethral glands, bladder, and prostate. *T. vaginalis* is a pyriform, or pear-shaped, protozoan flagellate that ranges 7 to 23 µm in length. There is a single, small eccentric nucleus, and the cytoplasm often contains delicate red granules. The flagella are often inconspicuous. In wet mounts, it demonstrates a jerky, rotating, nondirectional, leaf-like motion. Rippling of the undulating membrane can be seen for several hours after cessation of organism motility.

Spermatozoa

The sperm head is smaller than RBCs at about 4 to 6 µm long, usually tapering anteriorly Spermatozoa tails are thin and about 40 to 60 µm long. The head may be separated from the tail, making identification more difficult.

Yeast/Fungi

Candida albicans is a colorless, ovoid, 5 to 7 µm, thick-walled yeast cell often accompanied by filamentous pseudohyphae up to 50 µm long, with occasional budding. The cells stain poorly with aqueous stains in wet preparations, but they are strongly Gram positive and are easily identified on Wright-stained preparations.

References

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Introduction to Nasal Smears and Stained Stool for Eosinophils

Stool smears for detection of leukocytes are prepared from fecal material obtained by a wooden applicator and placed on a glass slide. The smear is allowed to air dry for several minutes and is then stained with a Wright-Giemsa stain.

Nasal smears are prepared by having the patient blow their nose into a nonabsorbent material (eg, waxed paper, plastic wrap). A swab is then used to transfer the mucus to a glass slide. A thin translucent smear is essential as cytologic detail is lost if the smear is too thick. The smear is then allowed to air dry and is stained. In nasal smears, usual Wright-Giemsa blood stains may yield bluish rather than red granules in eosinophils. Some laboratories use the Hansel stain, as eosinophils stain bright red, whereas neutrophils and mucus debris have a blue color.

Since the characteristics of eosinophils and neutrophils are the most important features in nasal and stool smears, these are described below.

Eosinophil, Stained

Eosinophils are recognized by their characteristic bright orange-red, spherical granules. The granules are large in comparison to neutrophil granules. Eosinophils typically have a bilobed nucleus separated by a thin filament. Occasionally, more than two lobes may be seen.

Neutrophil, Stained

The neutrophil nucleus is segmented or lobulated (two to five lobes) and is connected by a thin filament of chromatin. The abundant cytoplasm contains many fine, lilac granules.

In smears, artifacts, cellular distortion, and cellular degeneration are common. The nuclear lobes may appear eccentric, and the cytoplasm may contain toxic granules or be vacuolated.

Neutrophils may show morphologic changes due to autolysis, including nuclear pyknosis and fragmentation, making recognition of the cell type difficult.

References

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KOH Preparations for Fungi

The KOH preparation is the simplest method to microscopically identify fungi or yeasts from epidermal skin scrapings, hair roots, or nail clippings. KOH digests proteinaceous debris and dissolves the chitinous cell walls of skin cells and hair. The result is a cleared background in which hyphal elements, yeast cells, and arthrospores (structures resulting from hyphae fragmenting into individual cells) can be detected.

To make a KOH smear, a drop of 10% KOH solution is placed in the center of a clean glass slide. The specimen to be examined (hair, skin flake, piece of nail, etc) is placed in the KOH. A coverslip is then placed over the material, and the slide is gently heated for 5 to 10 minutes. The coverslip is then compressed to spread the material and is examined with a brightfield microscope with the condenser lowered to increase contrast. In laboratories where it is available, phase microscopy or interference microscopy can be used to increase detection. If fluorescent microscopy is available, calcofluor white can be added to enhance detection. Care must be taken when reading this type of preparation since tissue fragments, fibers, and cholesterol crystals may be mistaken for hyphae by the inexperienced observer.

Several species of fungi cause infection of the skin. *Tinea versicolor* consists of areas of depigmented-to-brown-red areas of skin on the trunk. It is due to growth of *Malassezia* in the cells of the stratum corneum. In the KOH prep, one sees many short, stubby hyphal segments (3 to 5 µm in diameter) admixed with budding, spheroidal yeast cells (4 to 6 µm in diameter). *Microsporum, Epidermophyton,* and *Trichophyton* species can cause several types of infection depending on the structures involved. *Tinea corporis* (ringworm) shows circular patches with a red, vesiculated border and central scaling that result from infection of sites other than the feet, groin, face, or hand. *Tinea pedis* (athlete's foot) consists of red, scaling areas in the interdigital spaces of the feet due to infection of these areas. *Tinea capitis* consists of scaling, bald patches on the scalp due to infection of the hair by fungal elements that either invade (endothrix) or surround (ectothrix) the hair shaft. In all of these conditions, if a preparation is made from the active border of advancing infection, one would see slender hyphal forms (3 to 5 µm in diameter), often breaking into arthrospore-like segments.

References

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Pinworm Preparations

Helminth (Includes Pinworm)

Enterobius sp. (pinworm) have characteristic ova. The eggs are elongate or ovoid, with a thick, colorless shell, 50 to 60 µm long and 20 to 32 µm wide. Typically, they are conspicuously flattened on one side, which helps distinguish them from hookworm eggs, which also have thinner shells. The egg of the whipworm (*Trichuris trichiura*), another human colonic nematode, is about the same size as a pinworm egg, but it is barrel-shaped with a transparent plug at each end.

Specimen collection is by the paddle test, which involves pressing a plastic paddle (coated with an adhesive surface) or piece of cellophane tape against the perianal region in the morning. The tape is then applied to a glass slide on which a small amount of toluidine has been placed to partially clear the tape and eliminate distracting air bubbles. Testing multiple samples over several days may be necessary to establish the diagnosis.

Strongyloides stercoralis (rhabditiform larva) is an intestinal nematode. The mature form and eggs are rarely seen. However, the rhabditiform larvae can be found in the duodenal contents and stool, which comprise the diagnostic form. The larva is slender and measures about 16 by 225 µm. The head has a short buccal cavity, distinguishing it from hookworm larva, which has long buccal cavities. The tail is notched, in contrast to the pointed tail of hookworm larvae.

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