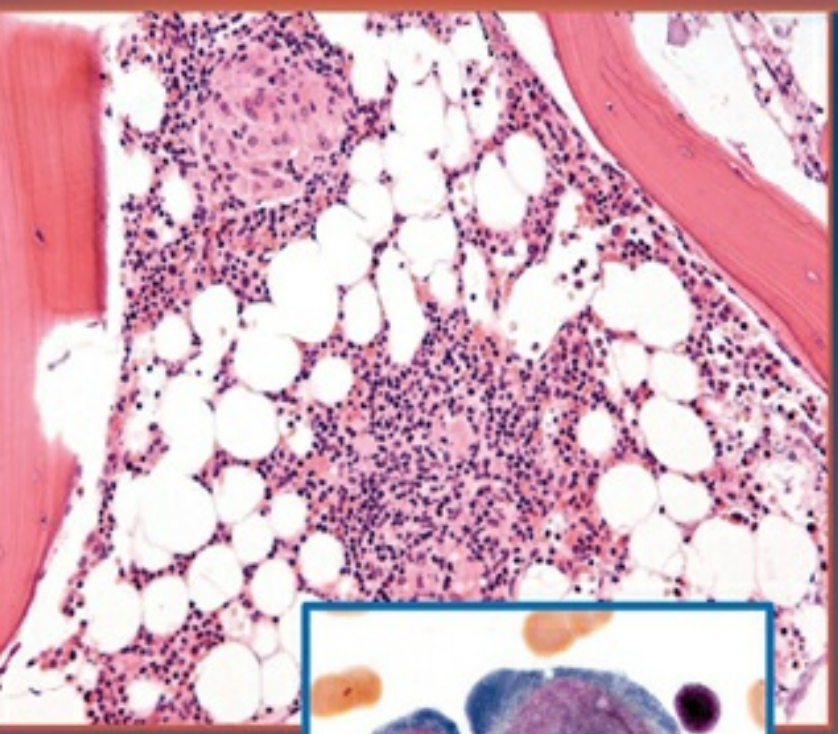
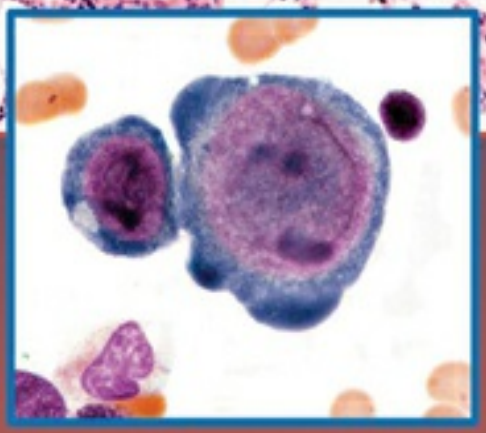
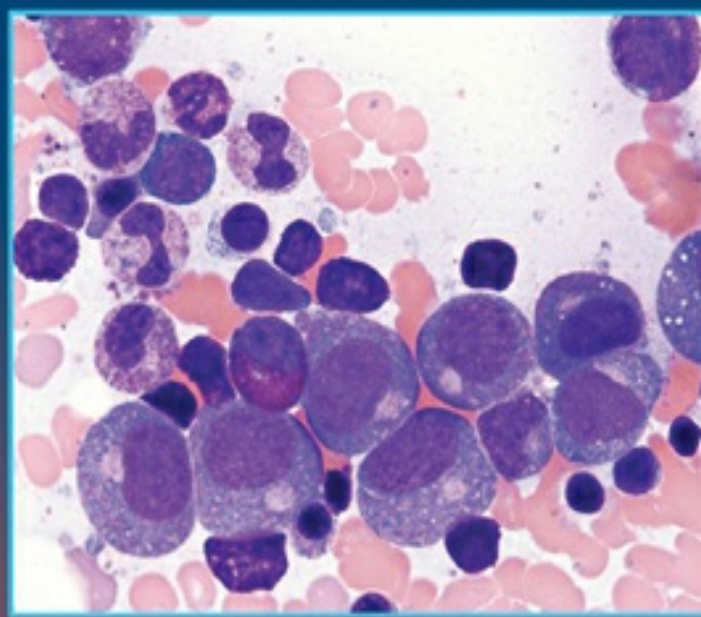
Wintrobe's Atlas of Clinical Hematology

SECOND EDITION







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Wolters Kluwer

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Preface

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

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

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

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

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CHAPTER

1 Approach to the Microscopic Evaluation of Blood and Bone Marrow

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

EXAMINATION OF THE PERIPHERAL BLOOD

Slide Preparation and Staining

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

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

Aniline dyes, containing basic dyes such as methylene blue and acidic dyes such as eosin, are used to stain the smears. The most common methods used today include Wright, Giemsa, or May–Grünwald stains, or a combination of variants, based on the original method developed by Dmitri Romanowsky, a Russian protozoologist, who first used it 1890 to see malaria parasites. Eosin gives a red to orange color to the alkaline components of cells, such as hemoglobin and the granules of eosinophils, that contain an alkaline spermine derivative.

Alkaline methylene blue dye gives a bluish-purple color to the acidic cellular elements, including nucleic acids (DNA, RNA), nucleoproteins, and the granules of basophils, which contain the acid heparin.

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

Slide Examination

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

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

The final reporting also includes an assessment of red cell morphology and an estimate of the platelet count. Evaluation of red cell morphology includes reporting of cell size, estimation of the hemoglobin content, shape, inclusions, or structural abnormalities. In the blood of a healthy individual, the red cells appear as uniform discs that range 6 to 8 μm in diameter with a slightly pale central area. Red blood cells (RBCs) smaller than 6 μm in diameter are microcytic, and those that are larger than 9 μm are macrocytic. Abnormal variation in cell size, or anisocytosis, is commonly seen in anemias and is reflected in a wider RDW (RBC distribution width) or coefficient of variation of RBC volume in automated

blood counts. The color of the red cells reflects the hemoglobin content, and increased area of central pallor is seen in hypochromic cells, whereas a denser central coloration is observed in hyperchromic cells or in spherocytes. Abnormal (increased) variation in RBC shape, or poikilocytosis, is reported by identifying the presence of specific atypical forms including elliptocytes, spherocytes, target cells, sickle cells, tear drop cells (dacrocytes), schistocytes, acanthocytes, and echinocytes, among others. Reporting of inclusions or granules in the red cells (such as Howell–Jolly bodies, basophilic stippling, or Pappenheimer bodies) or intracellular parasites (e.g., *Plasmodium* sp. or *Babesia* sp.) requires evaluation at higher magnification (×40 to ×100).

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

BONE MARROW EXAMINATION

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

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

EXAMINATION OF THE BONE MARROW ASPIRATE

SMEAR AND TOUCH PREPARATION

Slide Preparation and Staining

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

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

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

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

Slide Examination

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

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

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

EXAMINATION OF THE BONE MARROW BIOPSY AND CLOT SECTION

Processing for Histologic Sections

The trephine biopsy specimen should be taken at right angle to the cortex and be at least 1.5 cm in length. A long core biopsy increases the likelihood of finding focal lesions (i.e., lymphoma, granuloma, and metastatic disease). After the touch preparations are made, the

trephine core specimen is placed in a container with appropriate fixative. Methods for fixation vary and can significantly affect morphologic detail and immunohistology. Commonly used fixatives include B5, Bouin's, zinc formaldehyde, isotonic buffered saline, or formaldehyde and glutaraldehyde. Typically, neutral buffered formalin is used with a 6-hour fixation period, but the fixation times can also range from 1 hour to a maximum of over 24 hours, depending on the method. Decalcification is a necessary step to soften the bone, allowing cutting of thin sections without tearing them. However, decalcification chelates the storage iron, affects the morphologic detail and immunohistochemistry, and interferes with molecular assays.

A bone marrow clot preparation is processed in a similar manner as the trephine core biopsy with the exception of the decalcification step. A clot section can provide additional information, especially if the trephine core biopsy is inadequate. It is generated at the time of the bone marrow aspiration, when a portion of the aspirate containing marrow particles is allowed to form a clot. Various agents may be used to facilitate clotting. The clot is then placed into an appropriate fixative without further decalcification. An important advantage of the clot preparation is that it may be used for FISH analysis or extraction of nucleic acid material suitable for molecular-based testing. Furthermore, lack of decalcification produces more reliable immunoreactivity, although interpreting these stains may be more challenging than on the trephine biopsy section.

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

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

Slide Examination

Histologic sections represent thin slices through the specimen, which preserves the overall architecture of the marrow and connective tissue elements. Compared to aspirate smears, histologic sections allow better evaluation of the marrow cellularity and number of megakaryocytes. In the clot section, however, the cellular marrow is somewhat contracted and lacks the full architecture, thus the trephine biopsy section is ideal for assessment of

cellularity. Similar to the estimate performed on bone marrow aspirate smears, cellularity is evaluated by comparing the volume of the hematopoietic cells to the adipose cells and stromal elements that make up the total marrow space. Cellularity is assessed with reference to the patient's age, because bone marrow cellularity varies with age. The subcortical intertrabecular spaces are frequently hypocellular and therefore these areas should be excluded from the cellularity assessment.

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

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

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

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

Table 1.1

Hematology reference values in normal adults

Test	Men		Women	
	Conventional Units	**SI**	**Conventional Units**	**SI**
Hemoglobin	14.0–17.4 g/dL	140–175 g/L	12.3–15.3 g/dL	123–153 g/L
Hematocrit (volume of packed red cells)	41.5–50.4	0.415–0.504	36–45	0.36–0.45
Red cell count	4.5–5.9 × 10^6/	4.5–5.9 ×	4.5–5.1 × 10^6/	4.5–5.1 ×

	μL	10^{12}/L	μL	10^{12}/L
White cell count	4.4–11.3 × 10^3/μL	4.4–11.3 × 10^9/L	4.4–11.3 × 10^6/μL	4.4–11.3 × 10^9/L
Mean corpuscular volume (fL)	80–96	80–96	80–96	80–96
Mean corpuscular hemoglobin (pg)	27.5–33.2	27.5–33.2	27.5–33.2	27.5–33.2
Mean corpuscular hemoglobin concentration	33.4–35.5 g/dL	334–355 g/L	33.4–35.5 g/dL	334–355 g/L
Platelet count	150–450 × 10^3/μL	150–450 × 10^9/L	150–450 × 10^3/μL	150–450 × 10^9/L
Reticulocyte percentage	0.5%–2.5%	0.005–0.025	0.5%–2.5%	0.005–0.025
Reticulocyte count	22,500–147,500/mm^3	22.5–147.5 × 10^9/L	22,500–147,500/mm^3	22.5–147.5 × 10^9/L
Sedimentation rate (Westergren) <50 yr of age (mm/h)	0–15	0–15	0–20	0–20

SI, Systéme International d'Unites.

Adapted from Perkins SL. Normal blood and bone marrow values in humans. In: Greer JP, Foerster J, Lukens JN, et al, eds. *Wintrobe's Clinical Hematology*. 11th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2004:2697.

Table 1.2

Red blood cell characteristics at various ages

Age	Lowest Normal Hb (g/dL)	Normal Red Blood Cell Size Mean Corpuscular Volume (fL)	Fetal Hb (%)
Birth	14.0	100–130	55–90
1 mo	12.0	90–110	50–80
2 mo	10.5	80–100	30–55
3–6 mo	10.5	75–90	5–25
6 mo–1 yr	11.0	70–85	<5
1–4 yr	11.0	70–85	<2
4 yr–puberty	11.5	75–90	<2
Adult female	12.0	80–95	<2
Adult male	14.0	80–95	<2

Hb, hemoglobin.

From Means RT Jr, Glader B. Anemia: general considerations. In: Greer JP, Arber DA, Glader B, et al, eds. *Wintrobe's Clinical Hematology*. 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:588.

Table 1.3

Differential counts from bone marrow aspirates from 12 healthy men

	Mean (%)	Observed Range (%)	95% Confidence Limits (%)
Neutrophilic series (total)	53.6	49.2–65.0	33.6–73.6
Myeloblasts	0.9	0.2–1.5	0.1–1.7
Promyelocytes	3.3	2.1–4.1	1.9–4.7
Myelocytes	12.7	8.2–15.7	8.5–16.9
Metamyelocytes	15.9	9.6–24.6	7.1–24.7
Band	12.4	9.5–15.3	9.4–15.4
Segmented	7.4	6.0–12.0	3.8–11.0

Eosinophilic series (total)	3.1	1.2–5.3	1.1–5.2
Myelocytes	0.8	0.2–1.3	0.2–1.4
Metamyelocytes	1.2	0.4–2.2	0.2–2.2
Band	0.9	0.2–2.4	0–2.7
Segmented	0.5	0–1.3	0–1.1
Basophilic and mast cells	0.1	0–0.2	—
Erythrocytic series (total)	25.6	18.4–33.8	15.0–36.2
Pronormoblasts	0.6	0.2–1.3	0.1–1.1
Basophilic	1.4	0.5–2.4	0.4–2.4
Polychromatophilic	21.6	17.9–29.2	13.1–30.1
Orthochromatic	2.0	0.4–4.6	0.3–3.7
Lymphocytes	16.2	11.1–23.2	8.6–23.8
Plasma cells	1.3	0.4–3.9	0–3.5
Monocytes	0.3	0–0.8	0–0.6
Megakaryocytes	0.1	0–0.4	—
Reticulum cells	0.3	0–0.9	0–0.8
Myeloid to erythrocyte (M:E) ratio	2.3	1.5–3.3	1.1–3.5

From Smock KJ, Perkins SL. Examination of the blood and bone marrow. In: Greer JP, Arber DA, Glader B, et al, eds. *Wintrobe's Clinical Hematology.* 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:13.

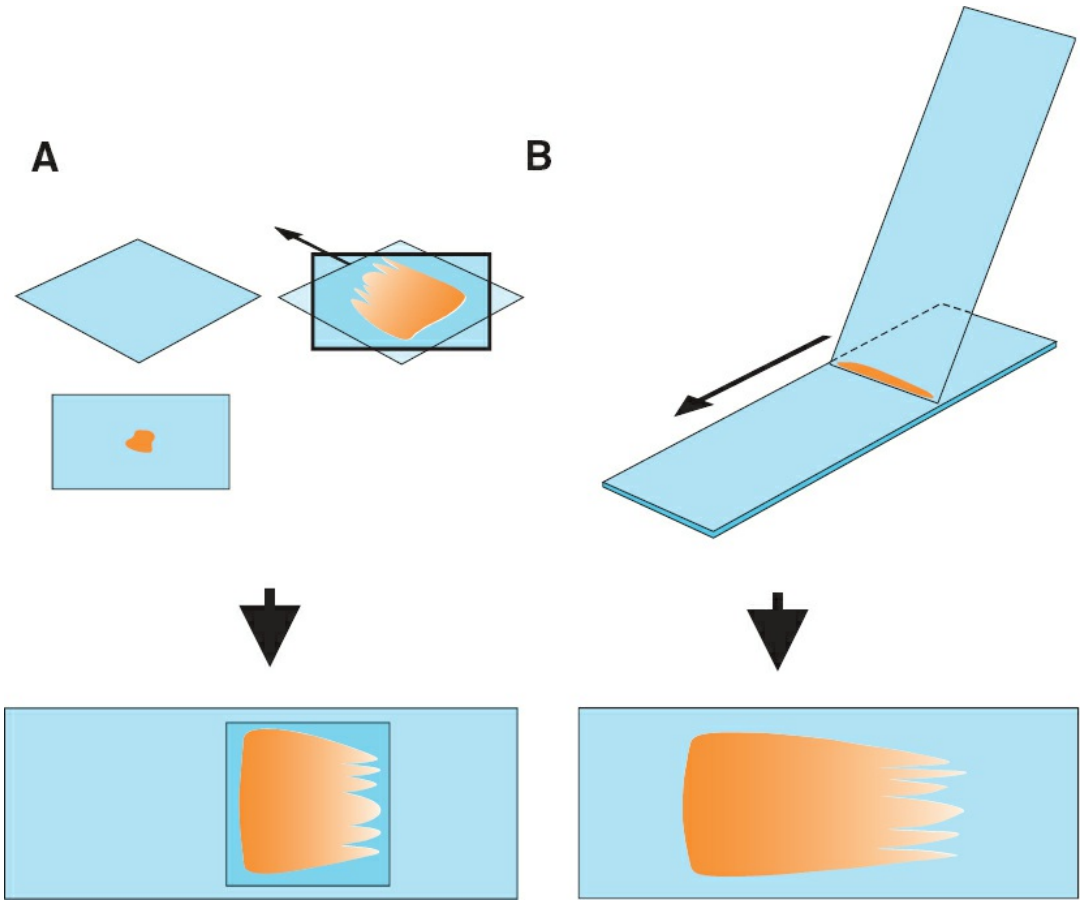

Figure 1.1. Preparation of blood smears. Blood smears may be prepared by the coverslip or slide wedge method. **A:** Coverslip smears are prepared by placing a drop of blood in the

center of a coverslip and spreading the blood by rotating a second coverslip over it. **B:** Wedge smears are prepared by placing a drop of blood on a slide and using a second slide to push the blood out along the length of the slide. (Adapted from Bauer J. *Clinical Laboratory Methods.* 9th ed. St Louis, MO: CV Mosby; 1982. From Smock KJ, Perkins SL. Examination of the blood and bone marrow. In: Greer JP, Arber DA, Glader B, et al, eds. *Wintrobe's Clinical Hematology.* 13th ed. Philadelphia, PA: Wolters Kluwer; 2014:9.)

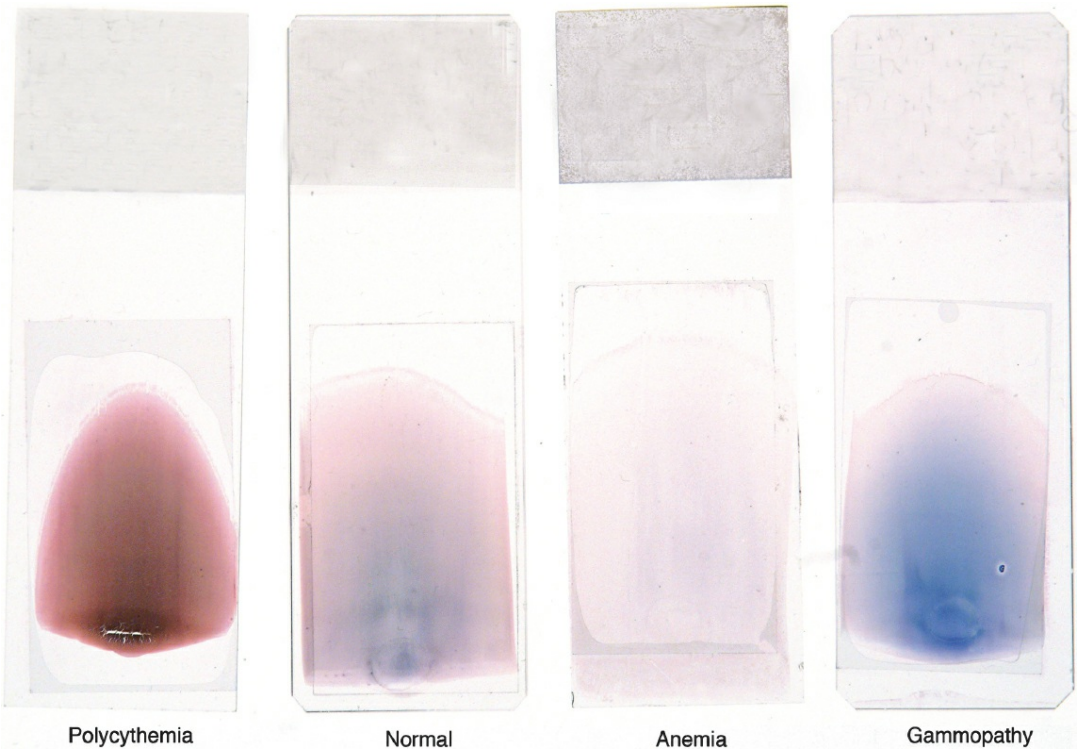

Figure 1.2. Macroscopic appearance of peripheral blood smears. Abnormalities in the patient's red cell concentration and the presence of marked gammopathy can be reflected in the gross appearance of the blood smears. The smear on the left, from a patient with polycythemia vera and a hemoglobin of 20 g/dL, appears noticeably redder and darker compared to the normal (**center left**; hemoglobin = 14 g/dL) or to the pale smear from an anemic patient (**center right**; hemoglobin = 7 g/dL). The blood smear on the right, from a patient with plasma cell myeloma, is visibly bluish because high levels of circulating monoclonal immunoglobulins take up the basophilic stains.

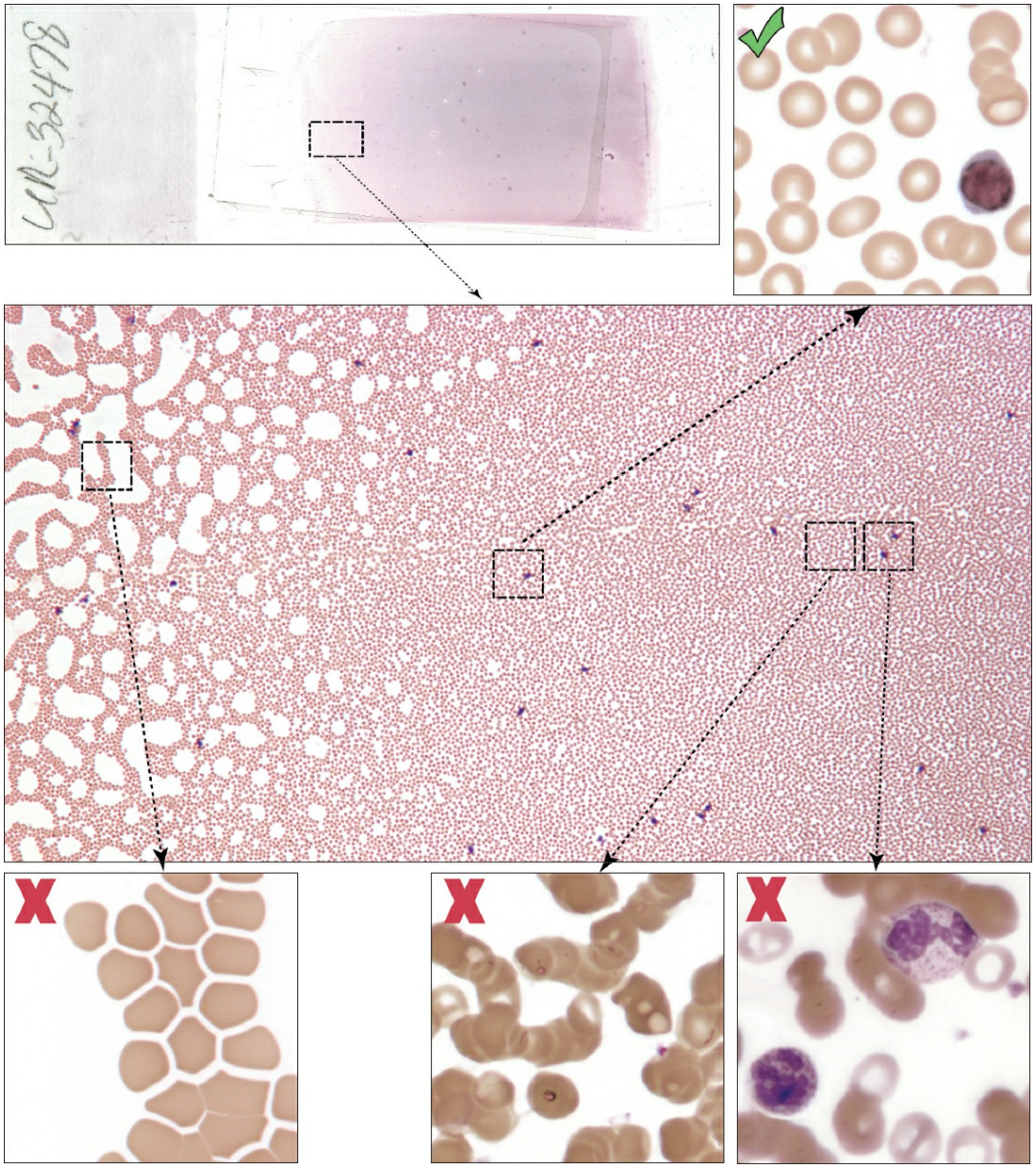

Figure 1.3. Microscopic approach to peripheral blood smears for red cell morphology. Red cells are best evaluated in areas where they are well spaced in a single layer without touching each other and exhibit central pallor **(right upper panel)**. Conversely, when red cells are examined too close to the feathered edge of the slide, they appear misshapen, flattened, and falsely hyperchromic **(left lower panel)**, whereas red cells in densely packed areas often appear shrunken and clumped **(right lower panels)**. Therefore, examining cells in an optimal area is required for accurate diagnosis.

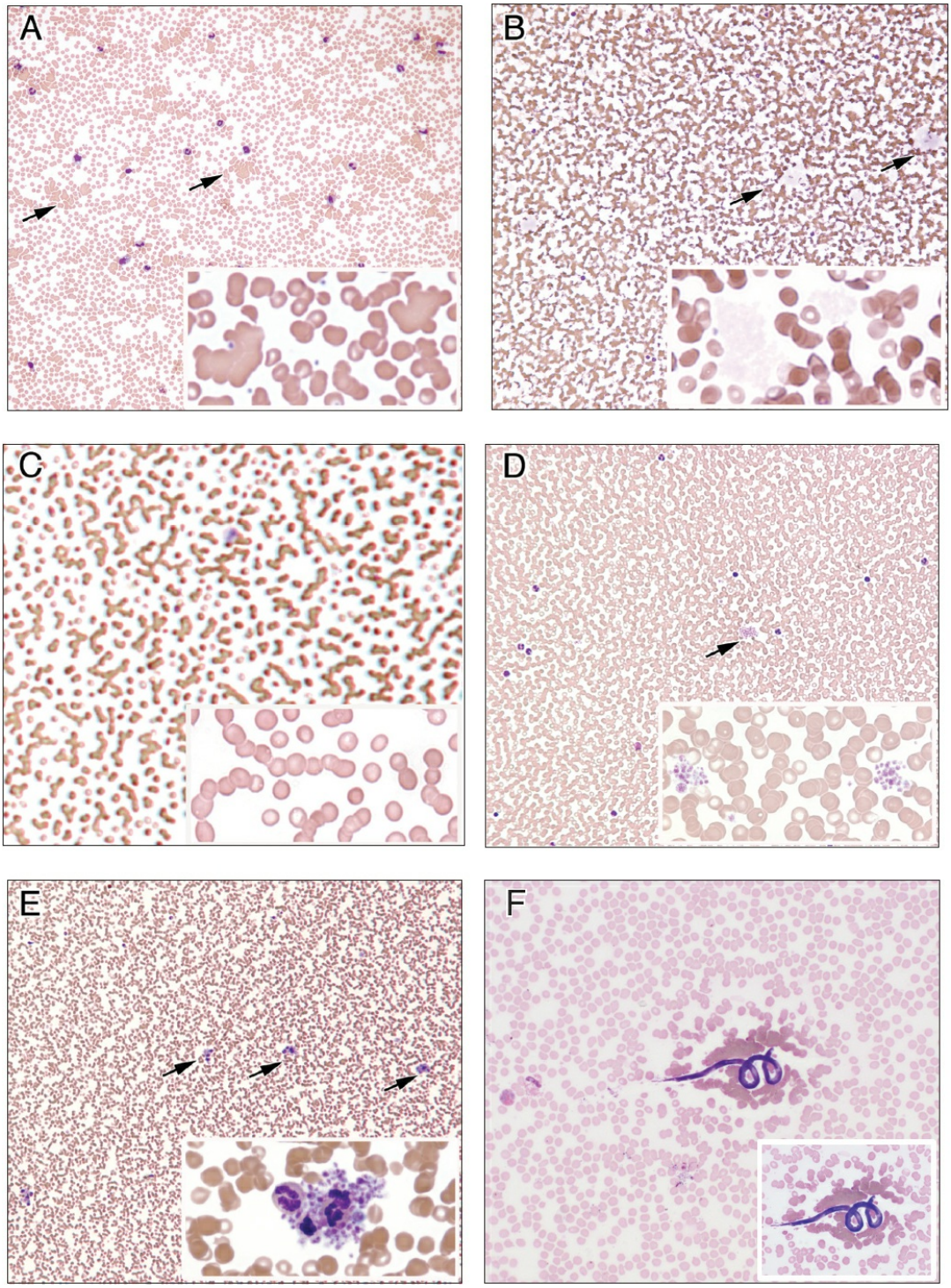

Figure 1.4. Examination of peripheral blood smears at low magnification. Abnormal aggregates, precipitated proteins, and parasites are best seen by scanning large areas of the smear at low-power magnification. The low-power views show suspicious areas to investigate, which are confirmed by the higher power insets. **A:** Red cell aggregation caused by a cold agglutin. **B:** Cryoglobulin precipitates causing red cell aggregation. **C:** Rouleaux (stacking of red cells) caused by the presence of a monoclonal immunoglobulin. **D:** Marked platelet aggregation caused by the anticoagulant EDTA, which can result in pseudothrombocytopenia. **E:** Aggregation of leukocytes and platelets caused by EDTA. **F:** Circulating microfilaria (×20; *inset*, ×50).

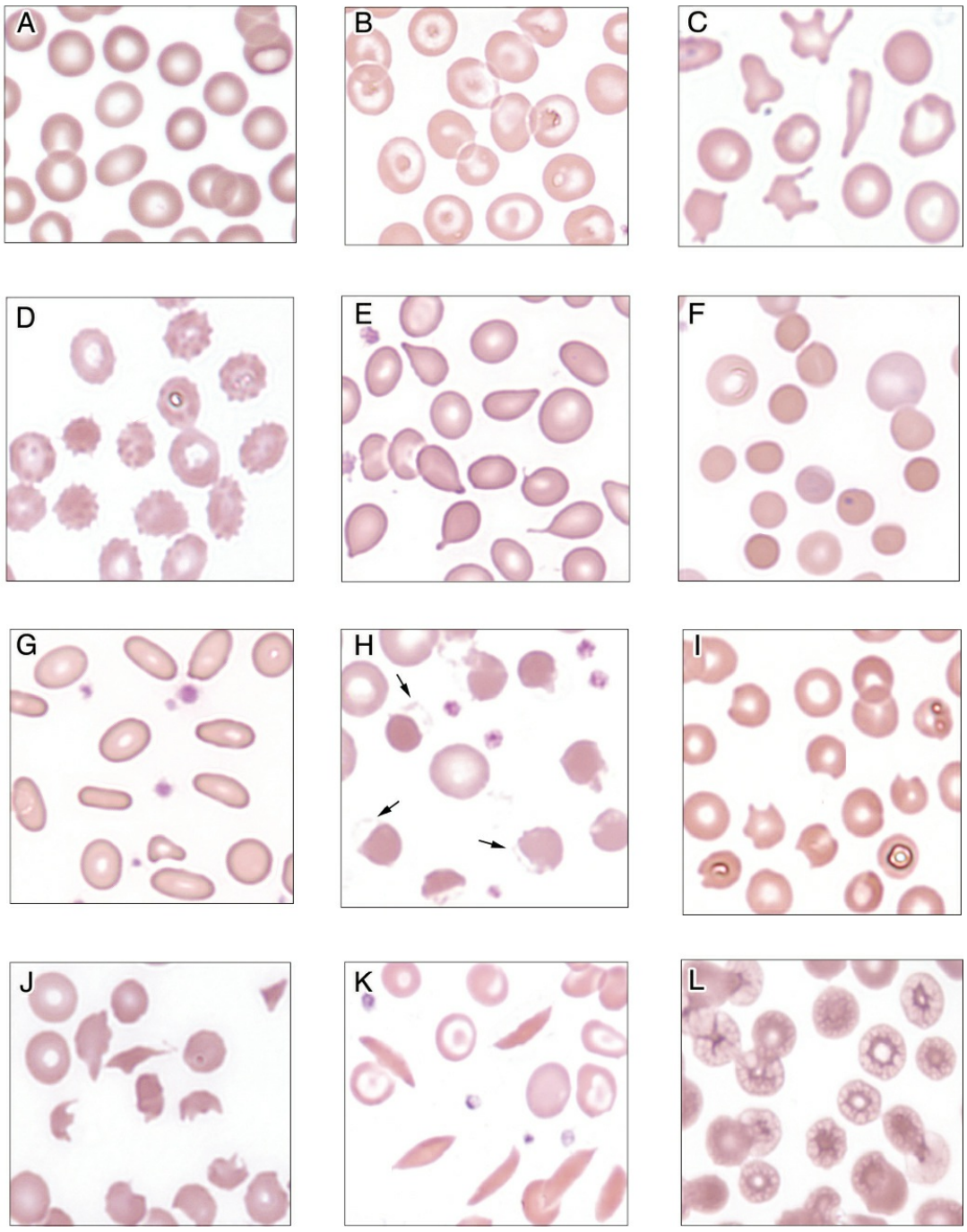

Figure 1.5. Oil immersion views of normal RBCs and various poikilocytes (i.e., abnormally shaped RBCs), which occur in different diseases. **A:** Normal red blood cells, which are quite uniform in shape and size. **B:** Target cells as seen in hemoglobin C. **C:** Acanthocytes (spur cells) seen in liver disease. **D:** Echinocytes or burr cells. **E:** Dacrocytes or teardrop RBCs (characteristic of myelofibrosis). **F:** Spherocytes seen in immune hemolysis or spherocytosis. **G:** Ovalocytes and elliptocytes. **H:** Blister cells (*arrows*). **I:** Bite cells. **J:** Schistocytes as in disseminated intravascular coagulation or thrombotic thrombocytopenic purpura. **K:** Sickled RBCs and target cells as in sickle cell anemia and sickle-β thalassemia. **L:** Dehydration artifact: this last effect, stemming from faulty smear preparation, should not be confused with an abnormal finding.

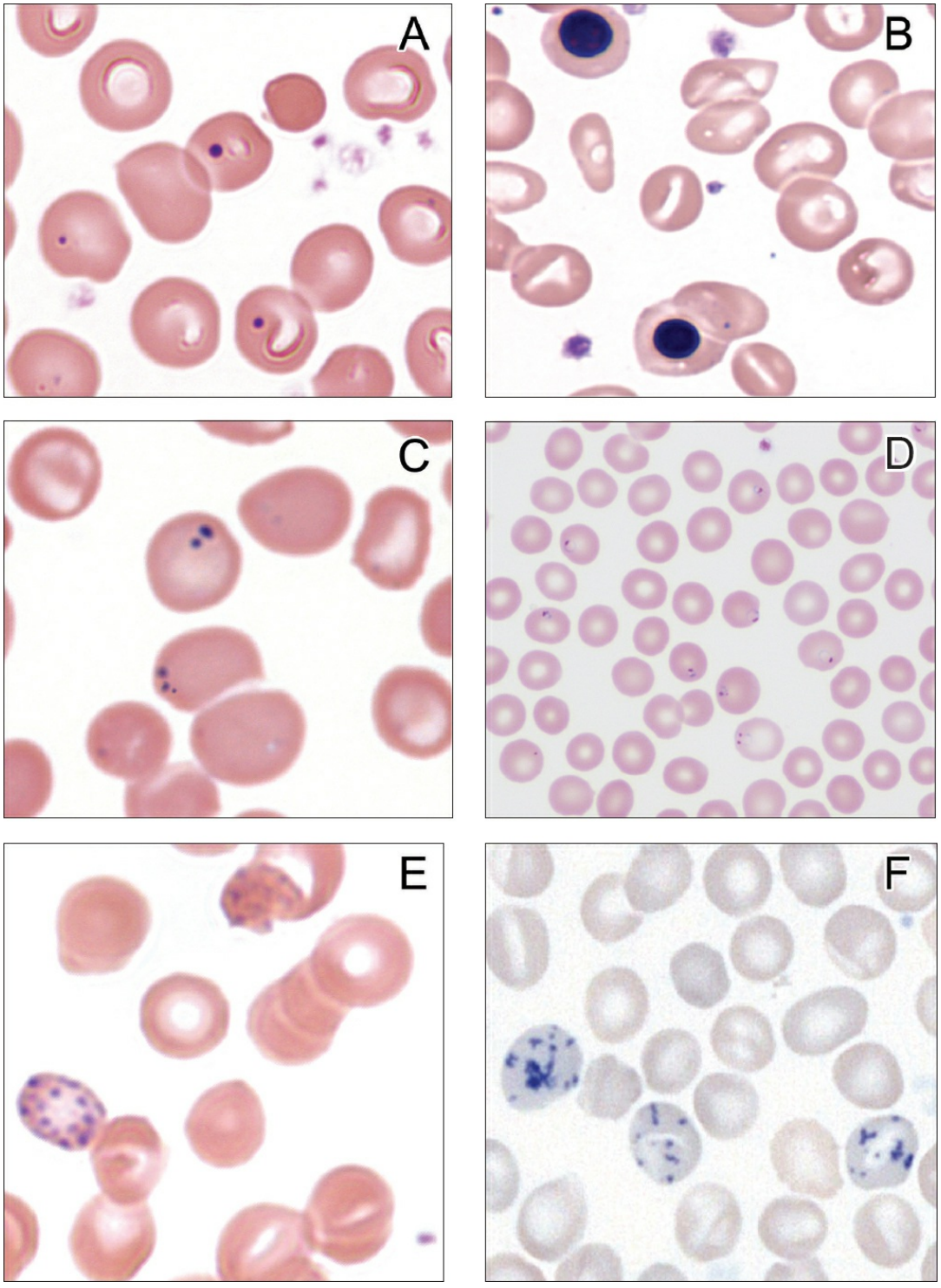

Figure 1.6. Abnormal inclusions in red blood cells. **A:** Howell–Jolly bodies. **B:** Nucleated RBCs (orthochromic normoblasts) seen in the peripheral blood with acute anemia, severe hemolysis, and myelophthisis. **C:** Pappenheimer bodies. **D:** Trophozoites of *Plasmodium falciparum*. **E:** Coarse basophilic stippling in a case of lead poisoning. **F:** Heinz body inclusions in a patient who has undergone a splenectomy. Heinz bodies can only be demonstrated by using the crystal violet supravital stain. Additional illustrations of these RBC inclusions can be found in Chapter 3 on Anemia.

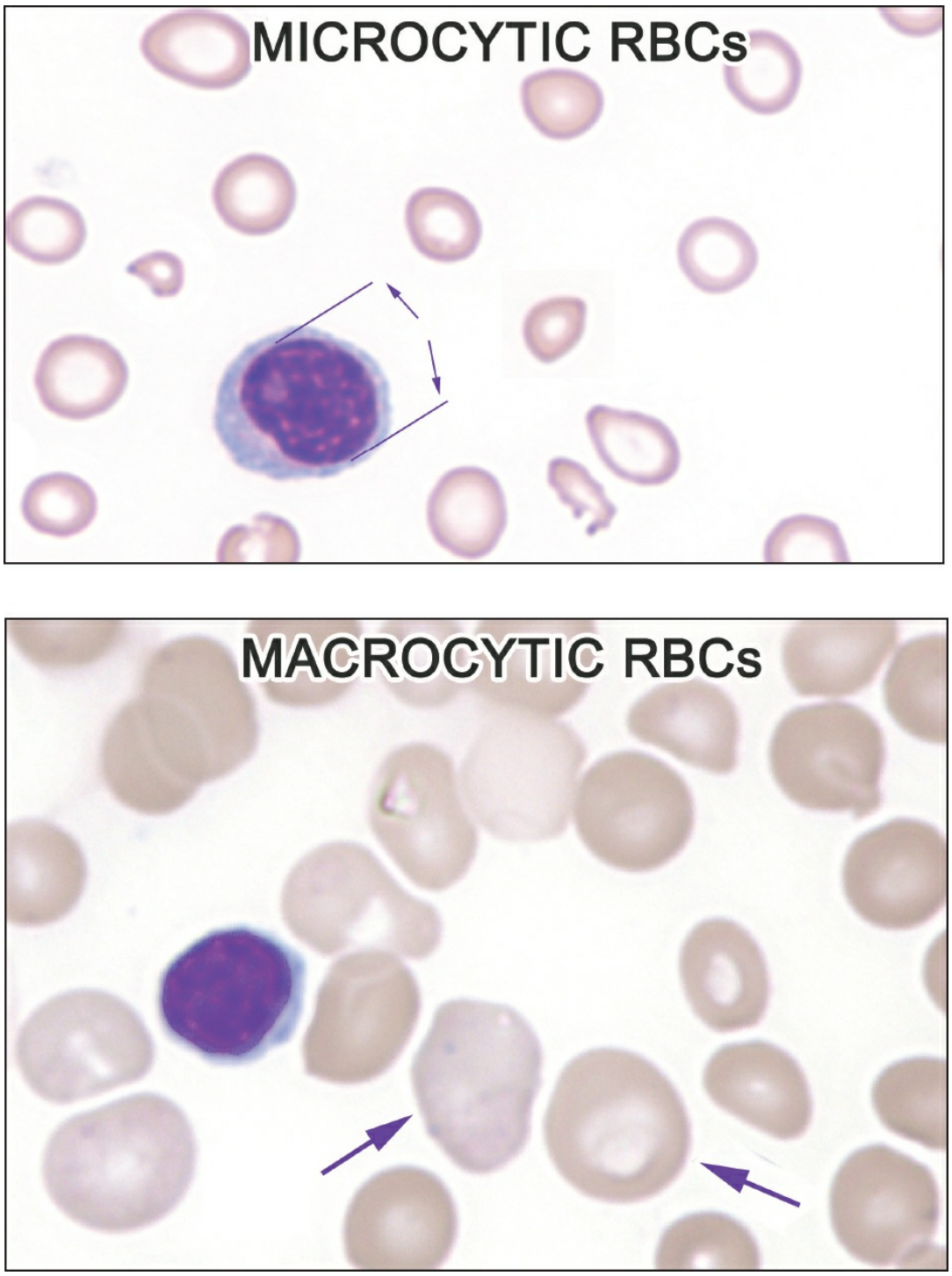

Figure 1.7. Red blood cell morphology: size variation or anisocytosis. RBC size is best measured by automated hematology analyzers, but marked size differences can be estimated by comparing the diameters of red cells to those of lymphocyte nuclei. Normal erythrocytes are approximately the same size as the nuclei of small lymphocytes, about 8 μm in diameter. The top panel shows numerous microcytic red cells that are significantly smaller than the diameter of the lymphocyte nucleus. The microcytic cells are also hypochromic, with a large region of central pallor and just a thin peripheral rim that is hemoglobinized. The bottom panel demonstrates two macrocytic red cells (*arrows*), the one on the left is a polychromatophilic red cell, likely a reticulocyte. In macrocytic anemias, RBCs are often of varying size with only a small population of macroovalocytes. RDW is increased both in macrocytic and in microcytic anemias.

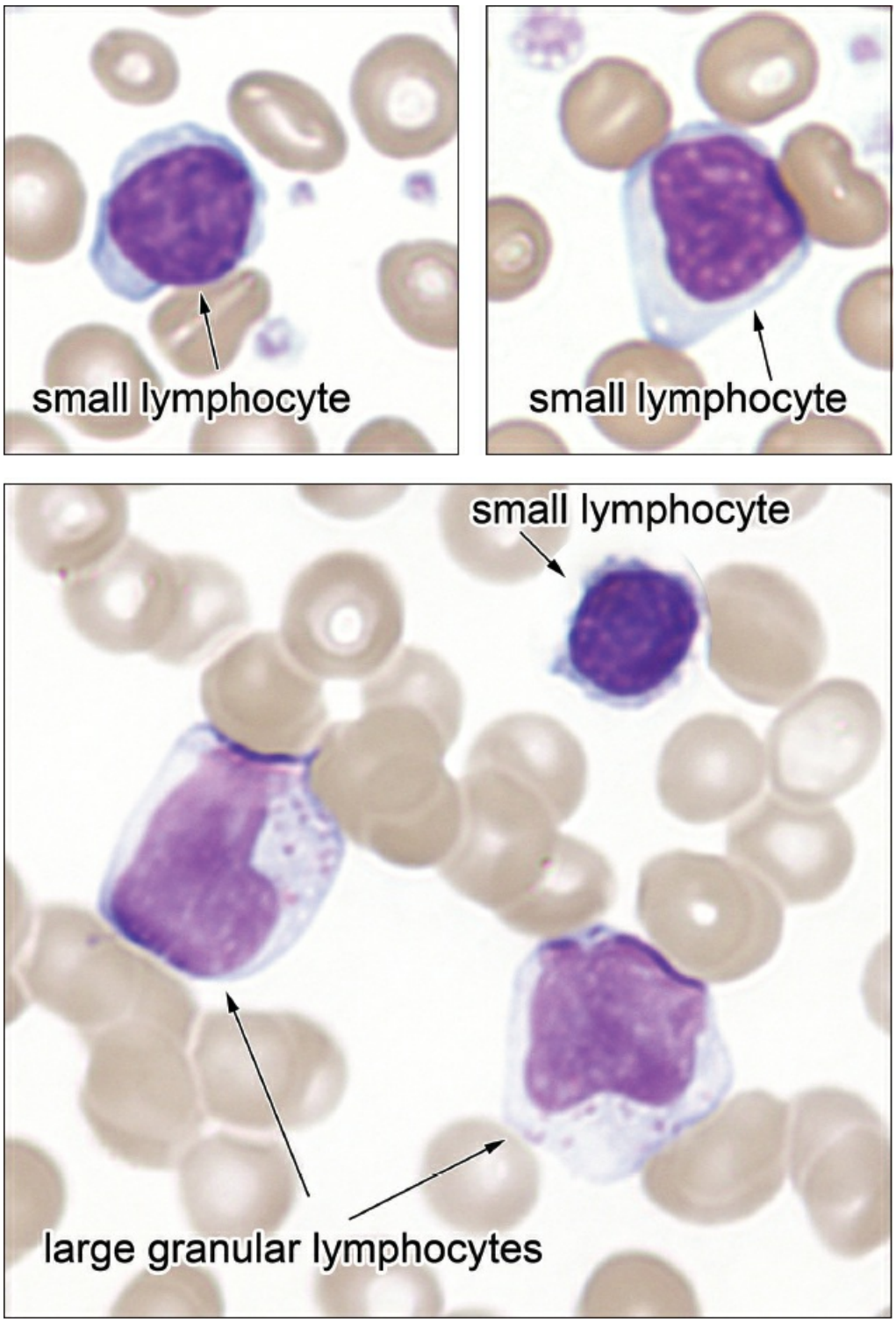

Figure 1.8. Lymphocyte and large granular lymphocyte (LGL) morphology. Normal small circulating lymphocytes are usually 10 to 15 μm (slightly bigger than normal red blood cells) and typically have high nuclear/cytoplasmic (N:C) ratios, with scant amounts of slightly basophilic staining cytoplasm. Lymphocyte nuclei are usually smoothly contoured with a mature (or "condensed") chromatin pattern and absent nucleoli. The appearance of LGLs shows more variable morphology, but they generally have moderate N:C ratios and more abundant neutral-staining cytoplasm containing purplish granules. The nuclei of LGLs are frequently irregular and folded and the chromatin is often slightly paler or more or "open" than that of small lymphocytes.

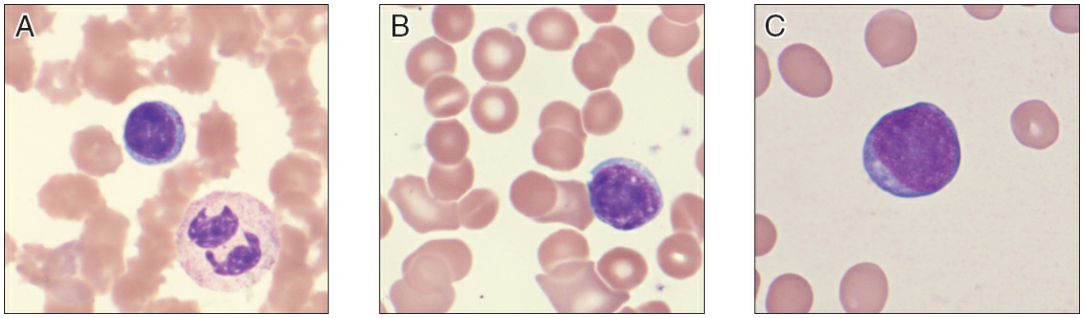

Figure 1.9. Morphology of lymphocytes in common neoplastic lymphoid disorders: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and acute lymphoblastic leukemia (ALL). The designation ALL includes both precursor B- and T-lymphoblastic leukemias. **A:** Normal lymphocyte with mature, clumped chromatin. **B:** CLL cells are variable in appearance and typically look very much like slightly larger versions of normal small lymphocytes and can display variably clumped or "soccer ball" chromatin. **C:** The ALL cell has a high nuclear/cytoplasmic (N:C) ratio with an immature or "open" chromatin.

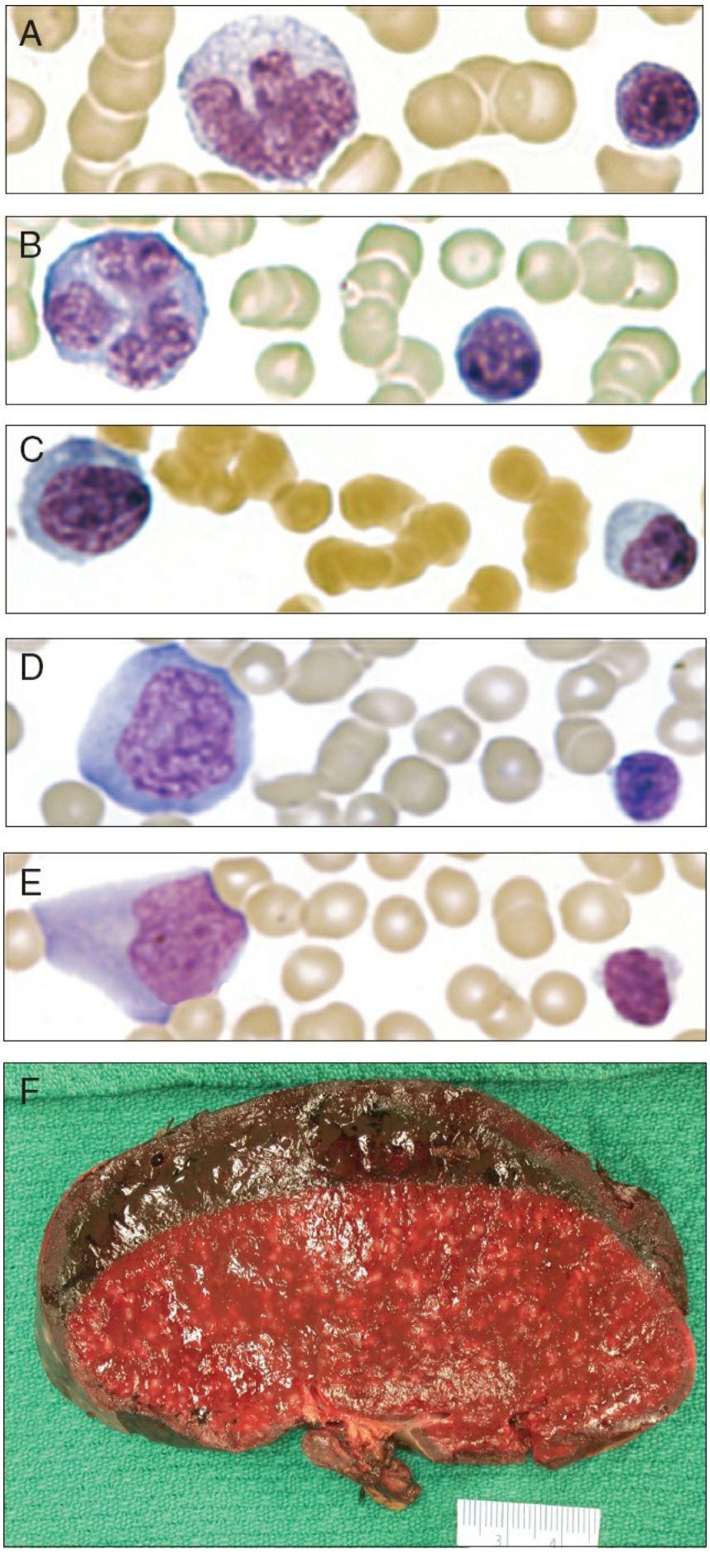
A
B
C
D
E
F

Figure 1.10. “Atypical” lymphocyte morphology in infectious mononucleosis. When referring to circulating lymphocytes, “atypical” is a confusing term that generally denotes benignity, despite the pleomorphic appearance of the cells, which can be confused with immature cells or monocytes. Characteristic features of atypical lymphocytes in infectious mononucleosis include large size, abundant basophilic and vacuolated cytoplasm, prominent nucleoli, diffuse or partially condensed chromatin, and large, often irregularly shaped nuclei. **A–E:** Samples from different patients with infectious mononucleosis. For comparison, normal lymphocytes are shown on the right-hand side of each panel. **F:** Gross image of an enlarged spleen with nodular areas in a red background, corresponding to markedly expanded white pulp follicles and red pulp sinusoids. Splenomegaly in patients with infectious mononucleosis can lead to rupture.

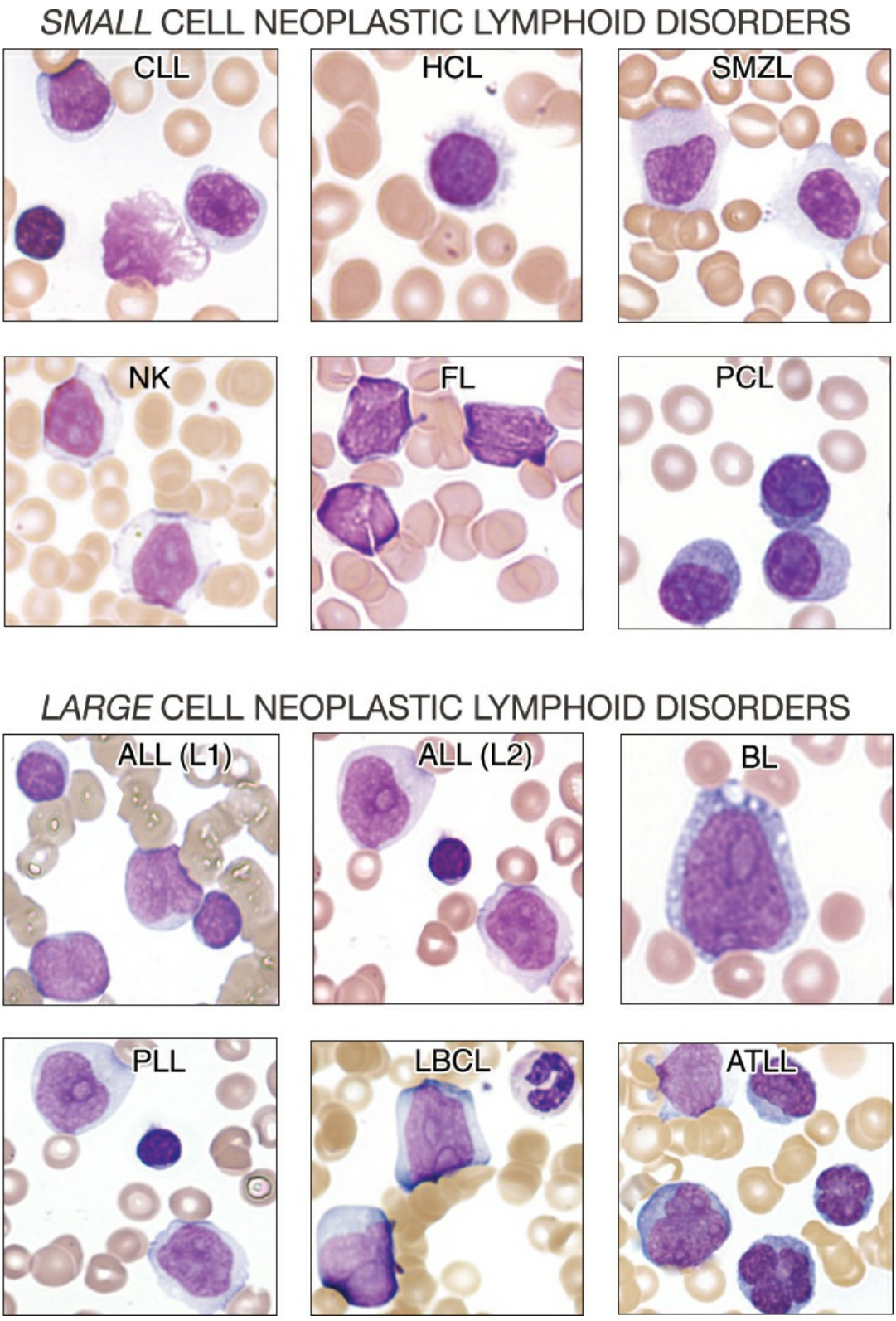

Figure 1.11. Appearance of circulating neoplastic cells in small and large cell lymphoproliferative disorders. Peripheral blood smears from patients with various lymphomas and leukemias that feature primarily small and large cells are shown in the top and bottom sets of panels, respectively. CLL, chronic lymphocytic leukemia/lymphoma. As described in Figure 1.9, these neoplastic cells are slightly larger than normal lymphocytes with clumped chromatin. HCL, hairy cell leukemia cells, are characterized by abundant pale blue cytoplasm with circumferential “hairy” projections and variable nuclear morphology. SMZL, splenic marginal zone lymphoma, cells have abundant pale cytoplasm that can be

characterized by short polar villi and relatively mature chromatin. NK, aggressive NK-cell leukemia cells show a range of morphologic features and can be similar to LGLs or atypical cells with irregularly folded nuclei, open chromatin, and prominent nucleoli. FL, follicular lymphoma, cells when found in the peripheral blood show cleaved nuclear morphology and scant cytoplasm. PCL, plasma cell leukemia, cells usually look similar to non-neoplastic plasma cells with eccentric nuclei, abundant blue cytoplasm with a perinuclear clear zone, and condensed “clock-face” chromatin; however, high nuclear to cytoplasmic ratio and prominent nucleoli can also be seen in these cells. ALL, acute lymphoblastic leukemia, as described in Figure 1.9 features immature chromatin and scant cytoplasm. The FAB classification, which has been superseded by the WHO classification, made morphologic distinctions of ALL blasts. L1 type blasts featured homogenous open chromatin, relatively regular nuclei, and absence of nucleoli. L2 type blasts showed heterogenous chromatin, irregular nuclear shape, prominent nucleoli, and variably abundant basophilic cytoplasm. L3 type blasts share morphologic features with leukemic Burkitt lymphoma, in that the blasts have fine chromatin with prominent nucleoli and are characterized by deeply basophilic cytoplasm with vacuoles. PLL, B-cell prolymphocytic leukemia, shows medium to large cells with a round nucleus, somewhat condensed chromatin, and a prominent central nucleolus, whereas the cytoplasm is abundant and slightly basophilic. LBCL, peripheralized diffuse large B-cell lymphoma, shows variable morphology with irregular nuclei, vesicular chromatin, and can show prominent nucleoli. ATLL, adult T-cell leukemia/lymphoma, features characteristic abnormal nuclear convolutions in the acute variant that are known as “flower cells,” and somewhat abundant basophilic cytoplasm. Although usual morphologic features are outlined pertaining to these entities, these findings alone cannot be used to establish these diagnoses.

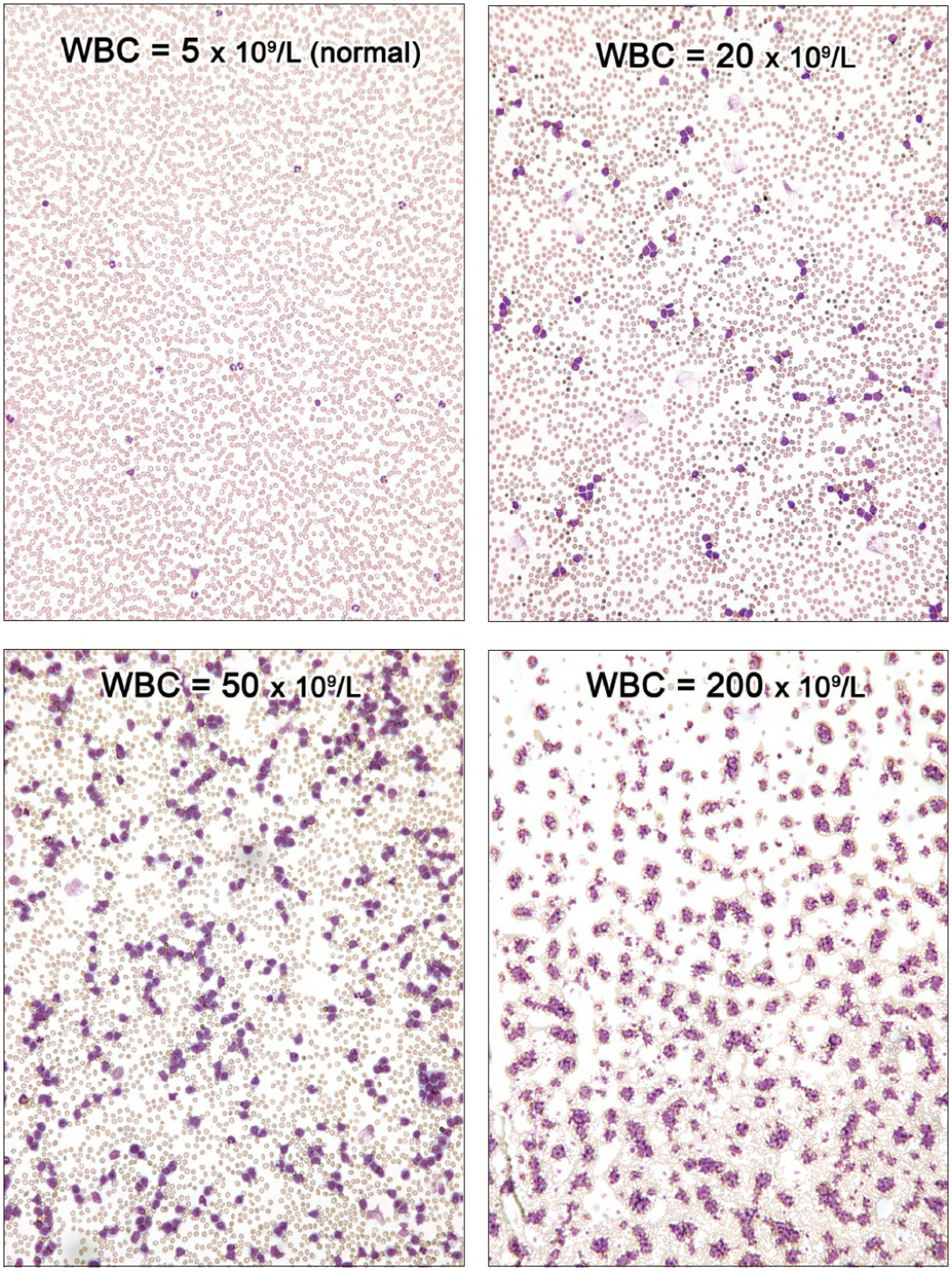

Figure 1.12. Estimating leukocyte counts at low power. Leukocytes are best counted by automated hematology analyzers, but an approximate estimate of white cell numbers can be done during screening of the peripheral blood smear at low magnification and by comparing the general ratio of white cells to erythrocytes (normal is approximately 1–500).

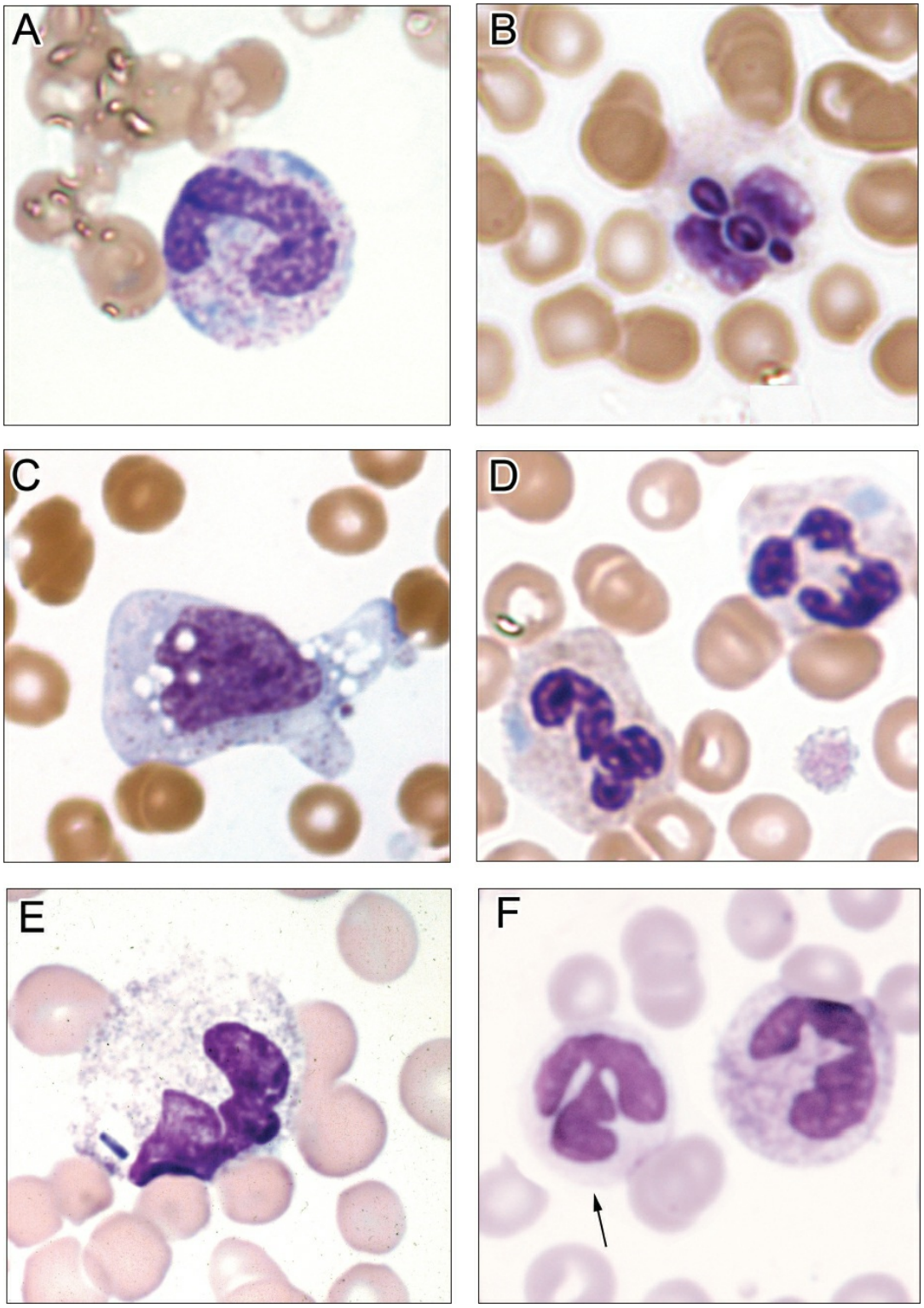

Figure 1.13. Granulocyte morphology: abnormal cytoplasmic features. **A:** Band showing "toxic changes," including increased cytoplasmic granulation and numerous bluish Döhle bodies. **B:** Circulating band ingesting a budding yeast (*Candida albicans*). **C:** Monocyte showing "toxic changes," including marked cytoplasmic granulation and vacuolization. **D:** May–Hegglin anomaly with large Döhle-like inclusions and giant platelets. **E:** Neutrophil-ingesting bacteria in a case of sepsis from *Clostridium perfringens.* (Courtesy of Dr. I. Quirt.) **F:** A dysplastic neutrophil with abnormal nuclear lobation and hypogranular cytoplasm (*arrow*) adjacent to a normal neutrophil.

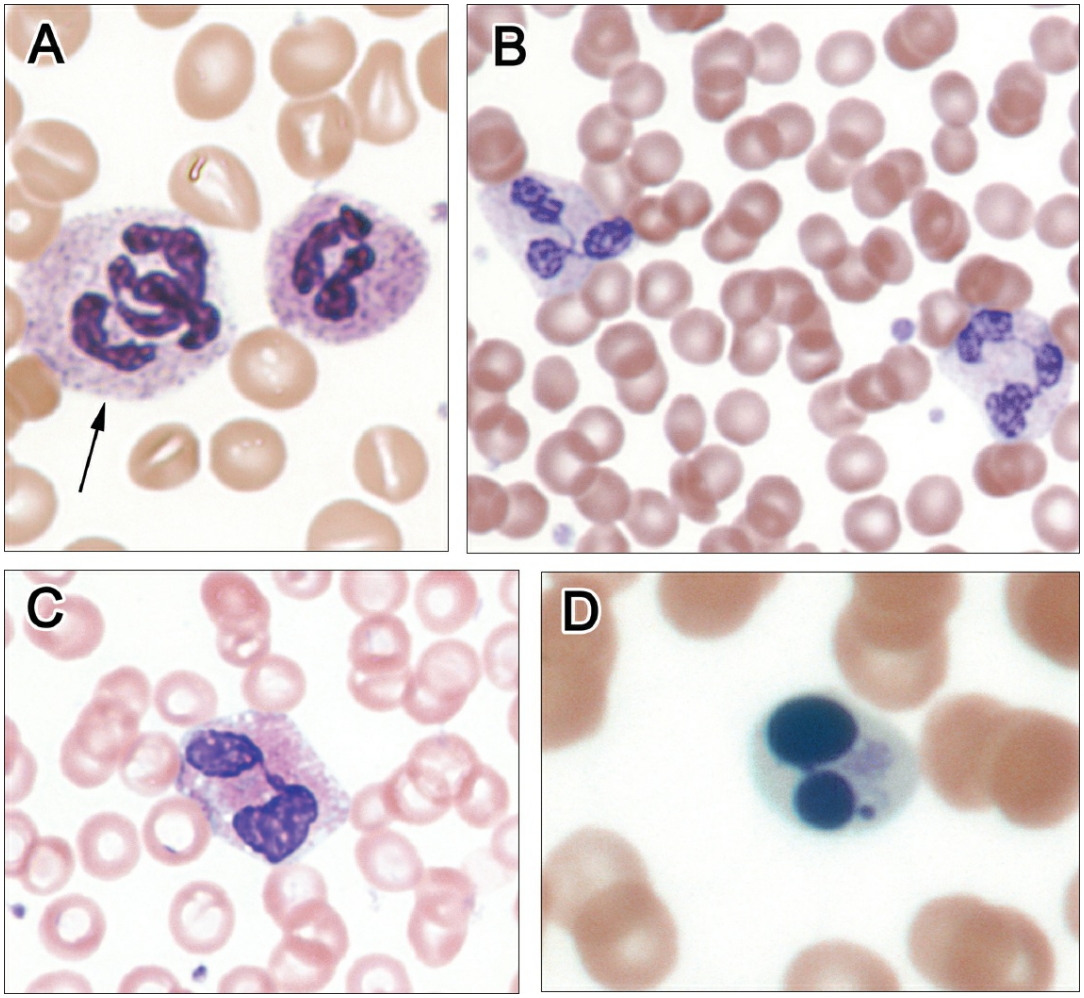

Figure 1.14. Granulocyte morphology: abnormal nuclear features. **A:** Giant hypersegmented neutrophil (*arrow*) from a patient with vitamin B_{12} deficiency. **B:** Hypersegmented neutrophils in a patient on antifolate chemotherapy. **C:** Dysplastic neutrophil with abnormal nuclear lobation (pseudopelgeroid) in a patient with myelodysplastic syndrome (MDS). This neutrophil with a bilobed nucleus connected by a thin filament resembles neutrophils in the hereditary Pelger–Huët anomaly. However, the Pelger–Huët-like morphology is acquired in MDS. **D:** Degenerating (apoptotic) neutrophil with marked nuclear hyperchromasia. Such apoptotic neutrophils may be an artifact of making a blood smear with old blood rather than fresh specimens, or may occur *in vivo*.

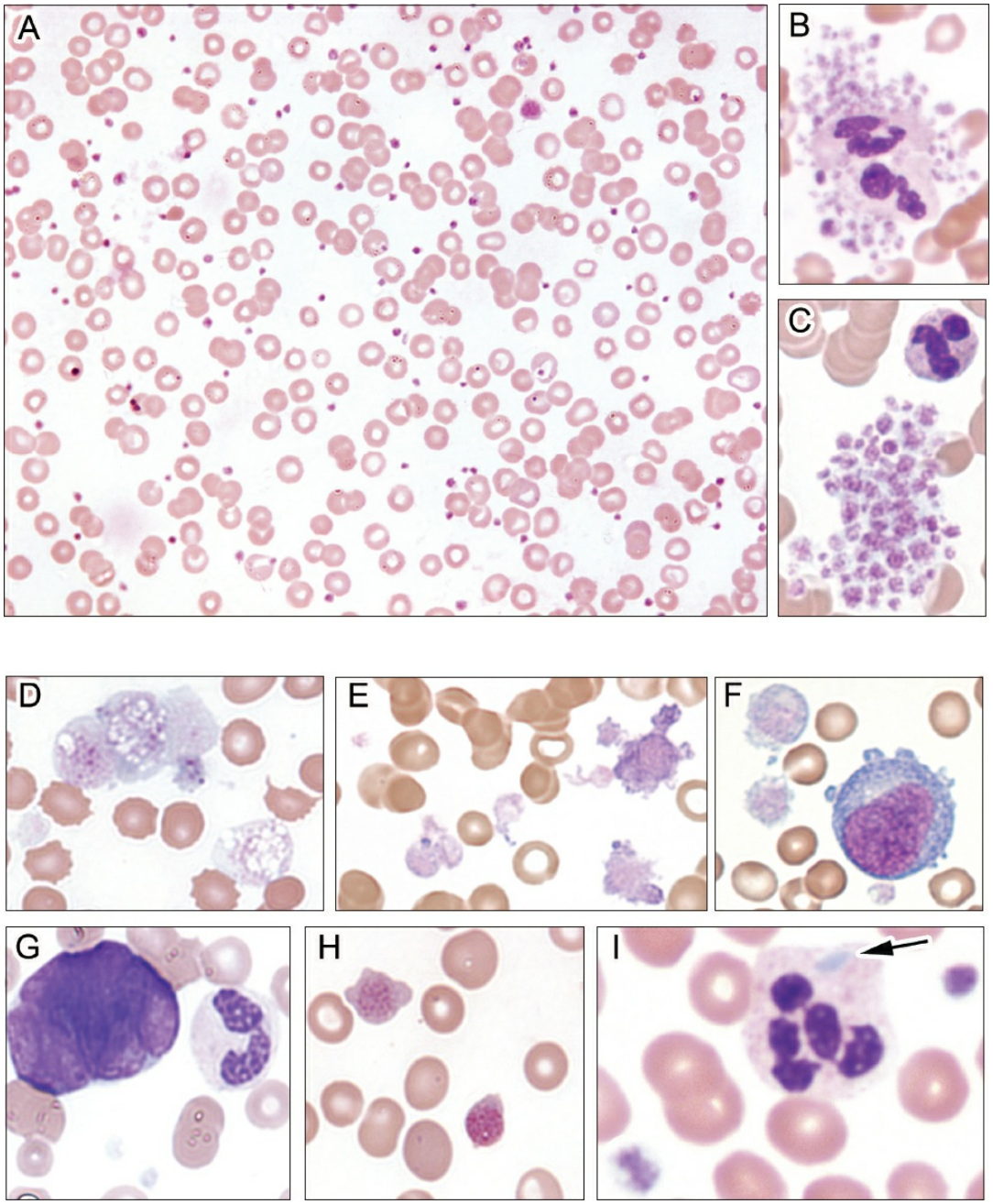

Figure 1.15. Abnormalities in platelet number and morphology. Platelet numbers are best measured using automated hematology analyzers, but generally microscopic visualization of 7 to 20 platelets per oil immersion field represents approximately normal platelet counts. **A:** Increased numbers of platelets in a patient with essential thrombocythemia and a platelet count of 500 × 10^9/L (normal range is between 150 and 450 × 10^9/L). Note several giant platelets. Elevated platelet counts also occur in reactive thrombocytosis associated with infection or postsurgery. Artifactual platelet satellitism **(B)** and aggregation **(C)** can occur from EDTA exposure in blood collection tubes and can lead to falsely low platelet counts by automated hematology analyzers. This phenomenon can be recognized by examining the peripheral blood smear and then re-drawing the blood specimen using heparin or citrate as anticoagulants in collection tubes. **D–I:** Abnormal platelet morphology. **D:** Giant platelets in primary myelofibrosis, cellular phase. **E:** Bizarre and giant platelets in essential thrombocythemia. **F:** Giant platelets and a megakaryoblast in a patient with acute megakaryoblastic leukemia. **G:** A naked nucleus of a megakaryocyte. **H:** Bernard–Soulier syndrome. This congenital bleeding disorder is characterized by thrombocytopenia and large platelets. (Courtesy of Dr. M. Abdelhaleem.) **I:** May–Hegglin anomaly is characterized by

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

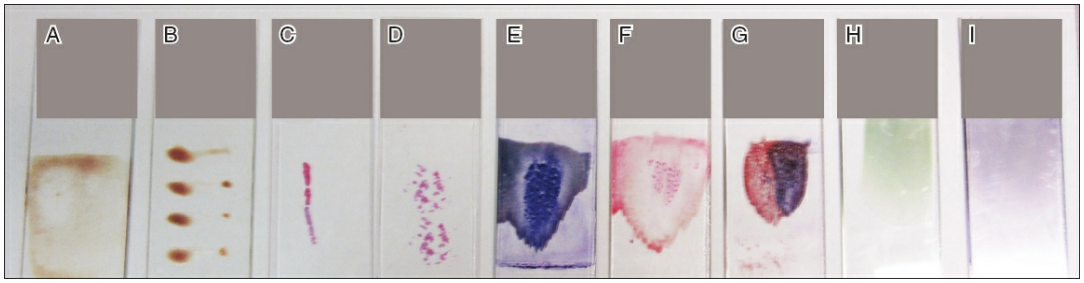

Figure 1.16. Routine evaluation of the bone marrow: staining and special studies. This figure shows a typical tray of slides for interpretation of a bone marrow sampling by a hematopathologist **(9 slides from left to right)**: **A:** An unstained air-dried aspirate smear that on close inspection shows granular white-gray particles surrounded by blood that represent bone marrow particles. **B:** An unstained touch preparation of a bone marrow biopsy that shows predominantly dried drops of blood in the shape of a core. Although a touch preparation is prone to artifacts, it can provide useful information in a "dry tap." **C** and **D:** H&E staining is routinely used for bone marrow core biopsy and clot sections after these specimens undergo routine histologic processing (see text for details). Additional studies such as immunohistology and special stains can be performed on the core or clot specimen by cutting additional sections as needed to complete the evaluation. **E:** Wright–Giemsa is used routinely to stain the aspirate smears and touch preparations for cytologic review of the marrow elements. **F:** Presence of iron stores is routinely evaluated by staining with Prussian blue, here performed on the aspirate smear. **G** and **H:** Although not routinely performed, with the use of flow cytometry, some laboratories still retain the ability to perform cytochemical staining (myeloperoxidase with counterstain and nonspecific esterase) on the aspirate smear in selected cases to further characterize blasts. **I:** Review of a peripheral blood smear, stained with Wright–Giemsa, is part of a complete bone marrow evaluation.

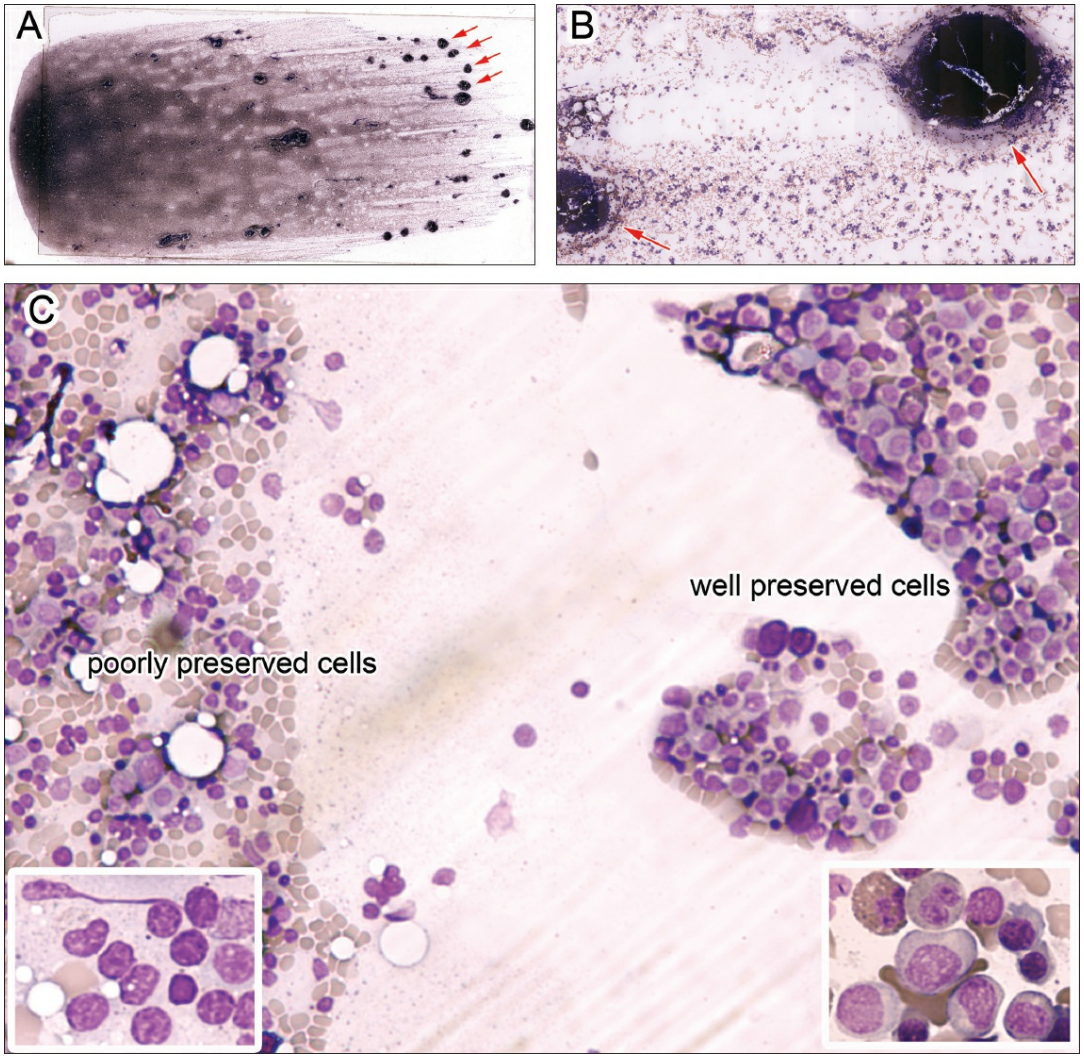

Figure 1.17. Microscopic approach to bone marrow aspirate smears. Selecting the correct area to examine is essential to properly assess marrow aspirate smears. **A** and **B:** The best regions are adjacent to the marrow particles (*arrows*) containing well-preserved clusters of cells that represent the actual cellular content of the marrow cavity. **C:** Areas of the aspirate where hematopoietic cells are well preserved and well spaced, almost touching each other but not overlapping are optimal **(right side)**. Areas where the cells are stripped of cytoplasm and/or display excessive air-dry artifact should be avoided and cannot be assessed for a manual differential count **(left side)**.

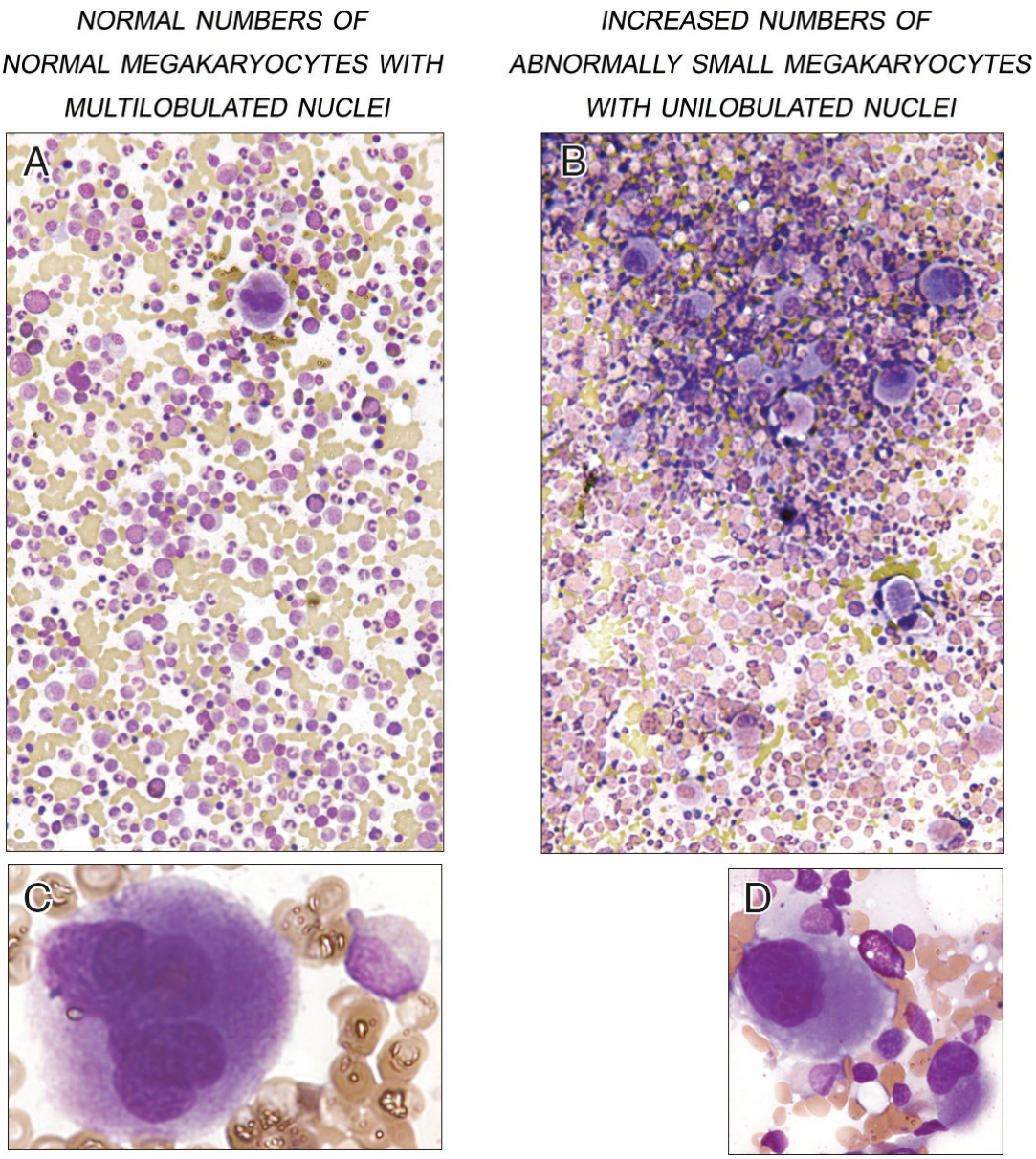

Figure 1.18. Assessing megakaryocytes in the aspirate smear. Megakaryocytes are relatively rare in normal bone marrow specimens, representing approximately 1% of all nucleated cells and are usually found in or near marrow spicules. **A** and **B:** Because of the large size of megakaryocytes, their numbers are best assessed at low magnification. **C** and **D:** The presence of morphologic atypia should be evaluated and quantified at higher power; however, megakaryocytes are normally at least twice as large as a promyelocyte and have polylobated nuclei. Presence of small megakaryocytes with nuclear hypolobation may be seen in neoplastic conditions.

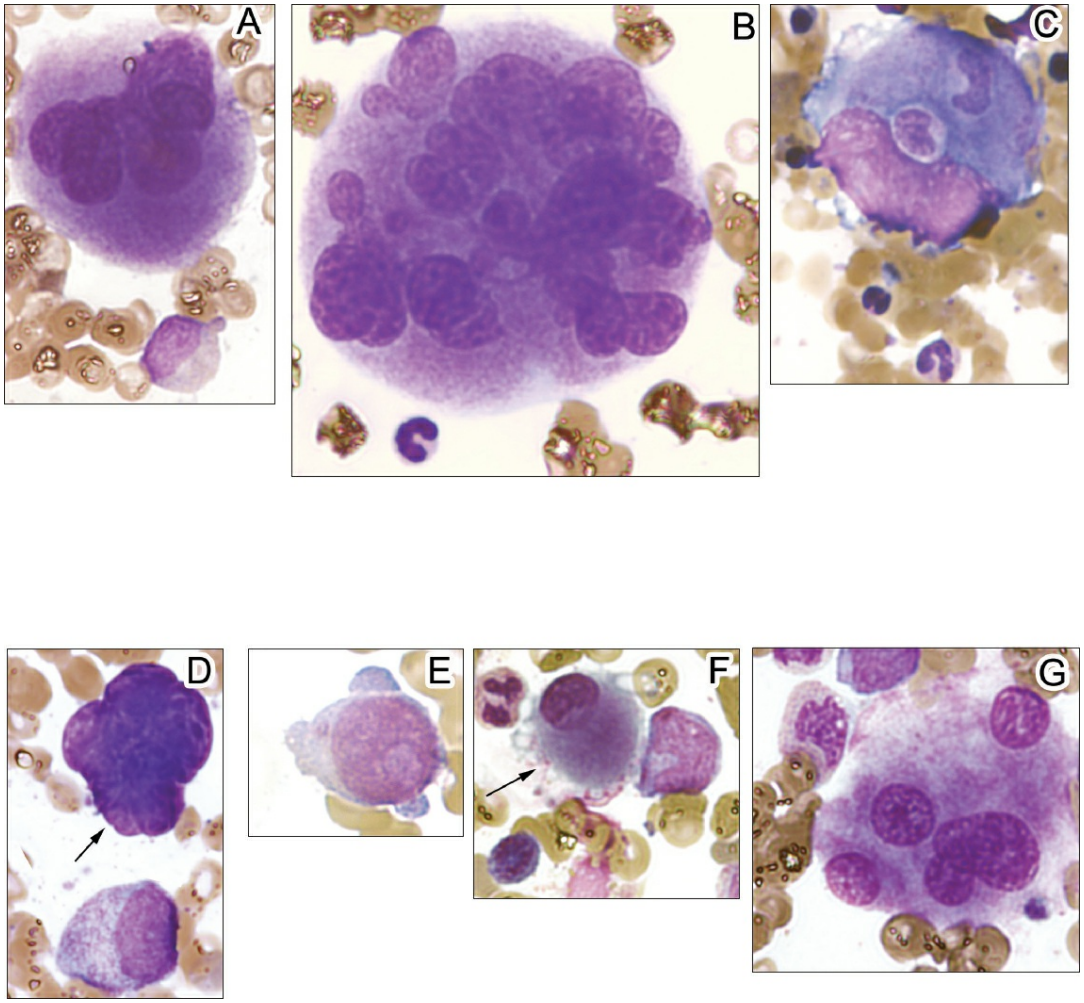

Figure 1.19. Variations in megakaryocyte morphology. **A:** Normal megakaryocyte with multiple contiguous nuclear lobes. **B:** Markedly enlarged megakaryocyte with nuclear hyperlobation from a patient with essential thrombocythemia. **C:** An example of emperipolesis (the present of an intact leukocytes within a megakaryocyte). **D:** A stripped "naked" megakaryocytic nucleus (*arrow*). **E-G:** Dysplastic megakaryocytes. **E:** A megakaryocyte with a prominent nucleolus, open chromatin and cytoplasmic blabbing. **F:** A micromegakaryocyte with a monolobated nucleus (*arrow*). **G:** A megakaryocyte with separated nuclear lobes.

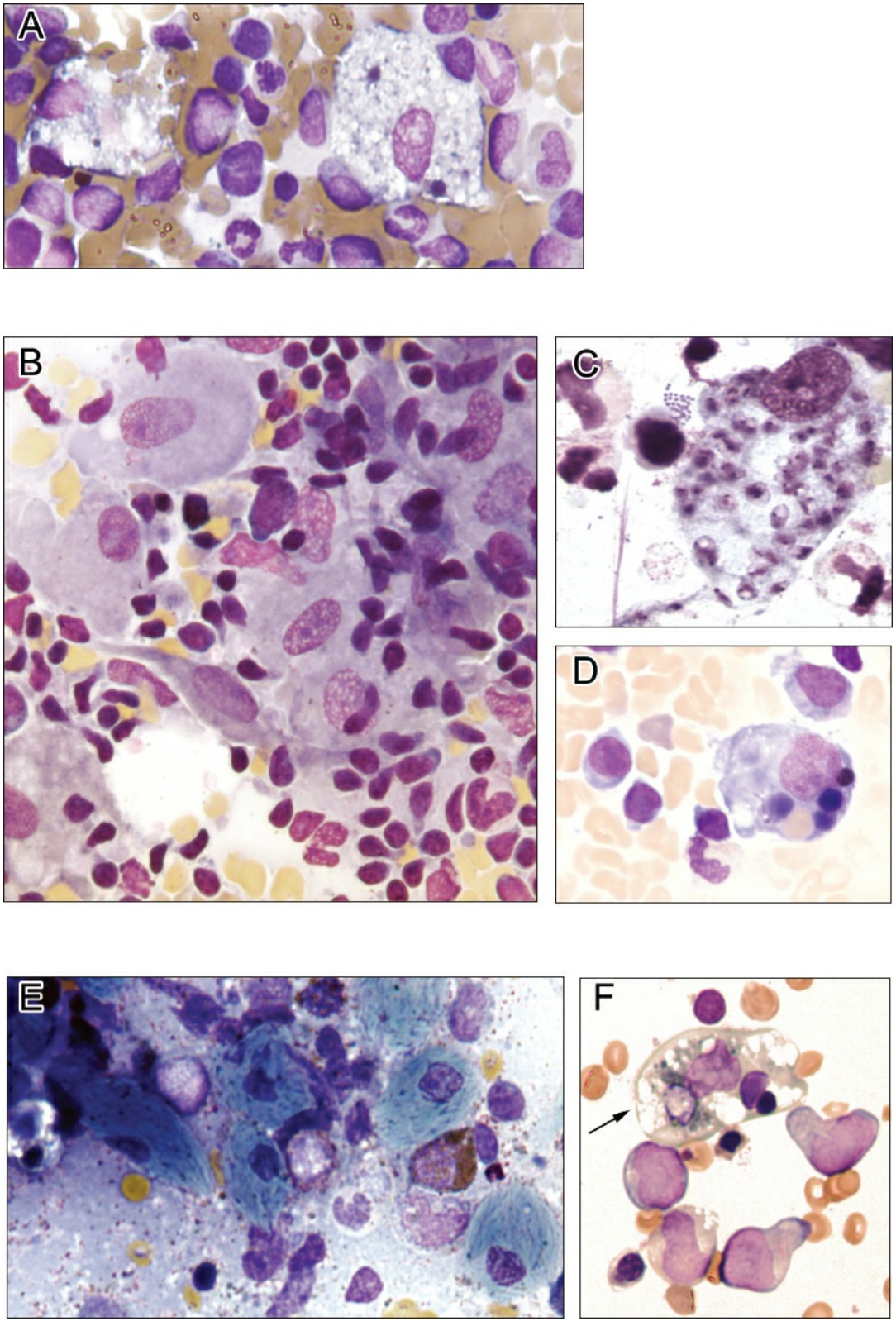

Figure 1.20. Histiocytes in bone marrow aspirate smears. **A:** Histiocytes are not usually prominent in smear aspirates, but an occasional histiocyte with abundant, pale, and granular cytoplasm, with tingible (stained) bodies can be seen in the aspirate smear, often in patients with excess iron stores. The engulfed material in such smears can be shown with appropriate staining to represent siderotic granules. **B–F:** Histiocytosis associated with infections and hematopoietic malignancies. **B:** Granulomas on aspirate smears show clusters of histiocytes with elongated nuclei, vesicular chromatin, and small inconspicuous nucleoli admixed with

lymphocytes in a patient with tuberculosis. **C:** An accumulation of characteristic intracellular amastigotes inside of a marrow histiocyte helps to establish this rare diagnosis in a patient with visceral leishmaniasis. **D:** Hemophagocytosis can be associated with a variety of neoplastic, reactive, and infectious conditions. This image of a histiocytic cell engulfing RBCs and lymphocytes is from a patient with fatal Epstein–Barr virus–associated hemophagocytic syndrome. **E:** Sea-blue or Gaucher-like histiocytes are often seen in patients with chronic myelogenous leukemia before treatment. **F:** Hemophagocytosis (*arrow*) associated with a case of T-cell lymphoproliferative disorder involving the bone marrow.

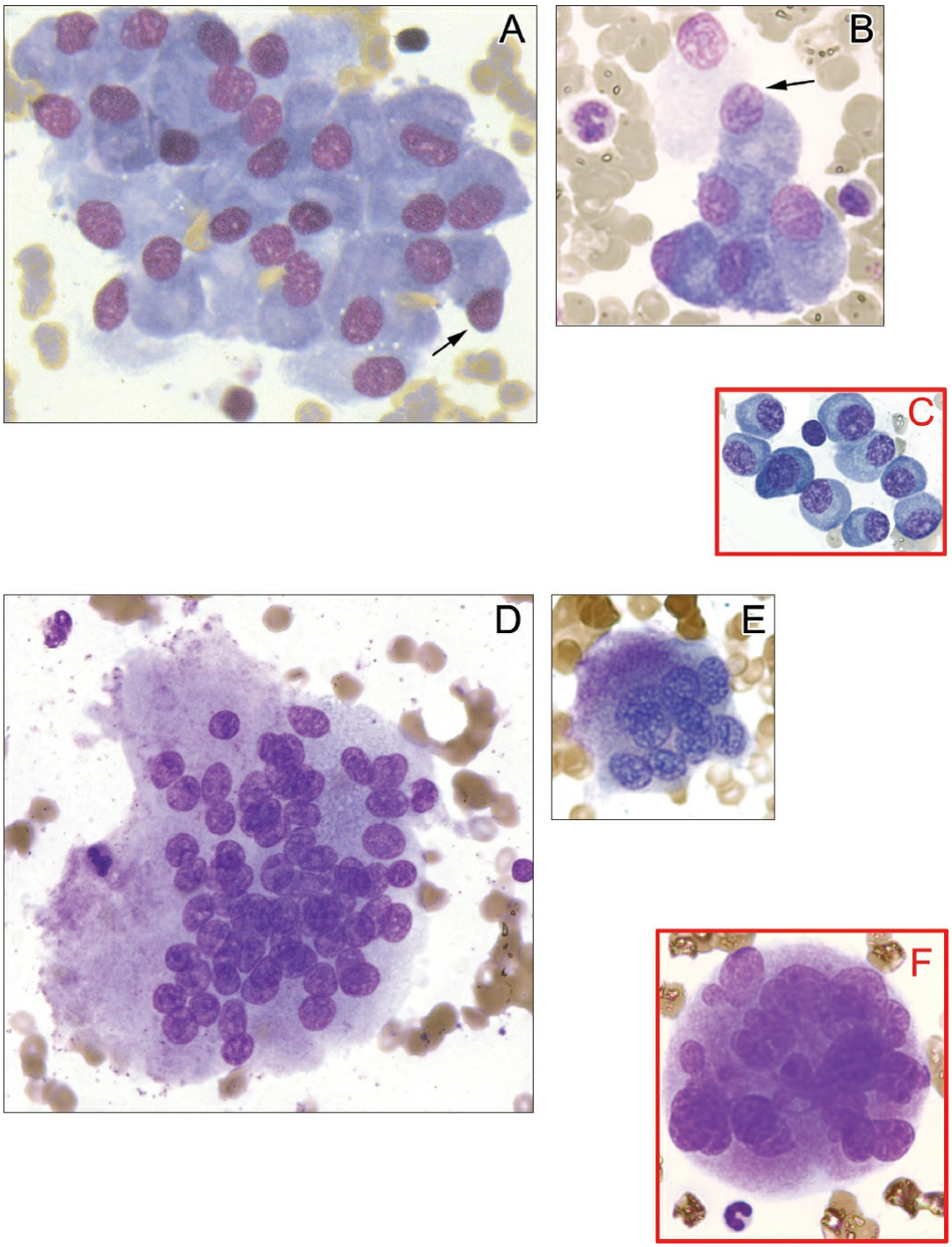

Figure 1.21. Bone cells in aspirate smears. **A** and **B:** Clusters of osteoblasts with the characteristic extruding or "pouting" nuclei (*arrows*). **C:** Plasma cells, shown here for comparison, are smaller than osteoblasts and do not have extruding nuclei, although their

nuclei are characteristically eccentric. Note prominent perinuclear clear areas (Golgi) in the plasma cells. **D** and **E:** Osteoclasts with numerous well-separated and uniformly sized nuclei resembling "pennies on a plate." (Courtesy of Dr. J. Lazarchick.) **F:** Megakaryocytes, one shown here for comparison, have variably sized, continuous nuclear lobulations.

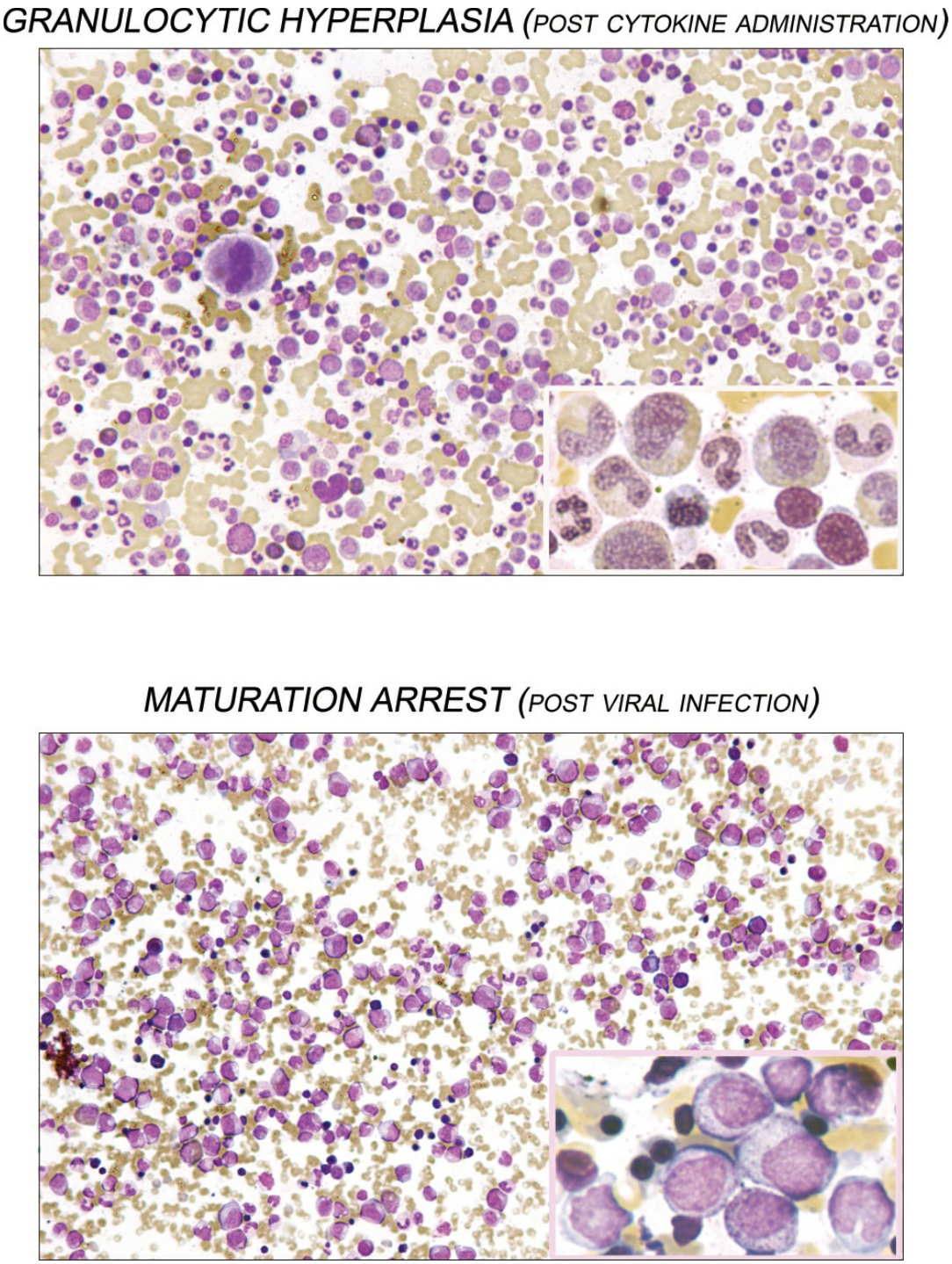

Figure 1.22. "Granulocytic pattern" in an aspirate smear may be due to diverse etiologies. The administration of cytokines or postviral infection maturation arrest may give similar morphologic findings, and clinical correlation is indispensable for the proper pathologic diagnosis. Both aspirates show predominant numbers of immature granulocytic precursors, but in the maturation arrest the early myeloid forms predominate. Granulocytic maturation arrest can also be immunologic or toxic in origin, resulting in the absence of later stages of myeloid maturation. In agranulocytosis, the most severe form, no myeloid precursors are

seen.

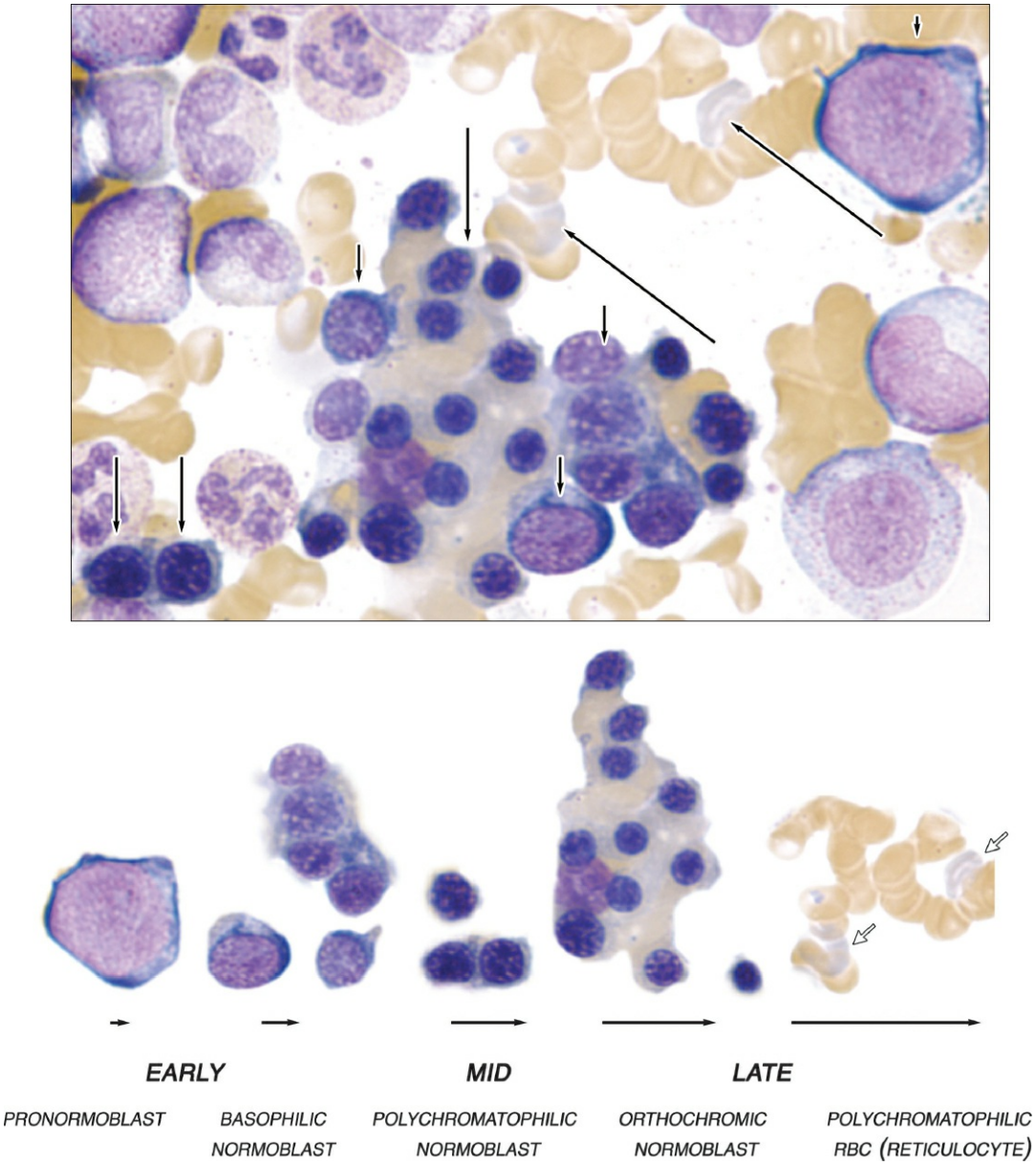

Figure 1.23. Morphology of erythroid maturation in bone marrow aspirates. In this composite figure, the spectrum of immature to mature erythroid precursors is designated by a series of lengthening arrows. Pronormoblasts are generally distinguished by having a scant basophilic cytoplasm and a more rounded nucleus and prominent nucleolus. As the cells mature, they become smaller but maintain a very rounded nuclear shape, the chromatin condenses, and nucleolus disappears, signaling features of basophilic normoblasts. Further decrease in size and further clumping of the chromatin, as well as changes in cytoplasmic staining, indicate transition to polychromatophilic normoblasts. Hemoglobinization of the cytoplasm and further nuclear maturation leads to orthochromatophilic normoblasts, which transition to a nucleated RBC before shedding the nucleus and finally becoming mature RBC.