

REVIEW ARTICLE

CURRENT CONCEPTS

Diagnosis from the Blood Smear

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N Engl J Med 2005;353:498-507.
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AN EXAMINATION OF THE BLOOD SMEAR (OR FILM) MAY BE REQUESTED by physicians or initiated by laboratory staff. With the development of sophisticated automated blood-cell analyzers, the proportion of blood-count samples that require a blood smear has steadily diminished and in many clinical settings is now 10 to 15 percent or less. Nevertheless, the blood smear remains a crucial diagnostic aid. The proportion of requests for a complete blood count that generate a blood smear is determined by local policies and sometimes by financial and regulatory as well as medical considerations. For maximal information to be derived from a blood smear, the examination should be performed by an experienced and skilled person, either a laboratory scientist or a medically qualified hematologist or pathologist. In Europe, only laboratory-trained staff members generally “read” a blood smear, whereas in the United States, physicians have often done this. Increasingly, regulatory controls limit the role of physicians who are not laboratory-certified. Nevertheless, it is important for physicians to know what pathologists or laboratory hematologists are looking for and should be looking for in a smear. In comparison with the procedure for an automated blood count, the examination of a blood smear is a labor-intensive and therefore relatively expensive investigation and must be used judiciously.

A physician-initiated request for a blood smear is usually a response to perceived clinical features or to an abnormality shown in a previous complete blood count. A laboratory-initiated request for a blood smear is usually the result of an abnormality in the complete blood count or a response to “flags” produced by an automated instrument. Less often, it is a response to clinical details given with the request for a complete blood count when the physician has not specifically requested examination of a smear. For example, a laboratory might have a policy of always examining a blood smear if the clinical details indicate lymphadenopathy or splenomegaly. The International Society for Laboratory Hematology has published consensus criteria (available at www.islh.org) for the laboratory-initiated review of blood smears on the basis of the results of the automated blood count. The indications for smear review differ according to the age and sex of the patient, whether the request is an initial or a subsequent one, and whether there has been a clinically significant change from a previous validated result (referred to as a failed delta check). All laboratories should have a protocol for the examination of a laboratory-initiated blood smear, which can reasonably be based on the criteria of the International Society for Laboratory Hematology. Regulatory groups should permit the examination of a blood smear when such protocols indicate that it is necessary.

WHEN PHYSICIANS SHOULD REQUEST A BLOOD SMEAR

There are numerous valid reasons for a clinician to request a blood smear (Table 1), and these differ somewhat from the reasons why laboratory workers initiate a blood-smear examination. Sometimes it is possible for a definitive diagnosis to be made from a blood smear. More often, the smear is an important tool in the provision of a differential di-

agnosis and the indication of further necessary tests. The blood smear can have an important part in the speedy diagnosis of certain specific infections. Otherwise, its major roles are in the differential diagnosis of anemia and thrombocytopenia and in the identification and characterization of leukemia and lymphoma.

ANEMIA

In patients with anemia, physician-initiated examinations of blood smears are usually performed in response to clinical features or to a previously abnormal complete blood count. The presence of unexplained jaundice, particularly if unconjugated hyperbilirubinemia is also present, is an additional reason for a blood-smear examination. Laboratory-initiated examinations of blood smears for patients with anemia are usually the result of a laboratory policy according to which a blood smear is ordered whenever the hemoglobin concentration is unexpectedly low. This policy should be encouraged, since the consideration of the blood smear and the red-cell indices is a logical first step in the investigation of any unexplained anemia.¹ Initiating a smear as a reflex test also means that a further blood sample does not have to be taken for this purpose.

Modern automated instruments impart valuable information about the nature of anemia. They provide not only a red-cell count, mean cell volume, mean cell hemoglobin (a measure of the average amount of hemoglobin in an individual red cell), and the mean cell hemoglobin concentration (a measure of the average concentration of hemoglobin in a cell) but also newer variables that give information that previously could be derived only from a blood smear. These variables usually include the red-cell-distribution width, which correlates on a blood smear with anisocytosis, and they may also include the hemoglobin-distribution width and the percentages of hypochromic and hyperchromic cells, which correlate with anisochromasia, hypochromia, and hyperchromia. A variety of histograms and scatterplots give a visual representation of red-cell characteristics. It may be possible to detect increased numbers of hyperchromic cells (spherocytes or irregularly contracted cells), small hyperchromic cells (microspherocytes), hypochromic microcytic cells, large normochromic cells (normally hemoglobinized macrocytes), and hypochromic macrocytes (either reticulocytes or dysplastic red cells).

Table 1. Clinical Indications for Examination of a Blood Smear.

Features suggestive of anemia, unexplained jaundice, or both
Features suggestive of sickle cell disease — dactylitis or sudden splenic enlargement and pallor in a young child or, in an older child or adult, limb, abdominal, or chest pain
Features suggestive of thrombocytopenia (e.g., petechiae or abnormal bruising) or neutropenia (e.g., unexpected or severe infection)
Features suggestive of a lymphoma or other lymphoproliferative disorder — lymphadenopathy, splenomegaly, enlargement of the thymus (a mediastinal mass on radiology) or other lymphoid organs, skin lesions suggestive of infiltration, bone pain, and systemic symptoms such as fever, sweating, itching, and weight loss
Features suggestive of a myeloproliferative disease — splenomegaly, plethora, itching, or weight loss
Suspicion of disseminated intravascular coagulation*
Acute or recent-onset renal failure or unexplained renal enlargement, particularly in a child
On retinal examination, hemorrhages, exudates, signs of hyperviscosity, or optic atrophy
Suspicion of a bacterial or parasitic disease that can be diagnosed from a blood smear
Features suggestive of disseminated nonhematopoietic cancer — weight loss, malaise, bone pain
General ill health, often with malaise and fever, suggesting infectious mononucleosis or other viral infection or inflammatory or malignant disease

* In acute disseminated intravascular coagulation, red-cell fragments may be absent.

Despite this wealth of information, there are still morphologic abnormalities that are critical in the differential diagnosis of anemia and that can be determined only from a blood smear. Particularly important is the detection of variations in cell shape and of red-cell inclusions, such as Howell-Jolly bodies (nuclear fragments), Pappenheimer bodies (hemosiderin-containing granules), and basophilic stippling or punctate basophilia (altered ribosomes).

HEMOLYTIC ANEMIA

In the hemolytic anemias, red-cell shape is of considerable diagnostic importance. Some types of hemolytic anemia yield such a distinctive blood smear that the smear is often sufficient for diagnosis. This is true of hereditary elliptocytosis (which is only infrequently associated with anemia) (Fig. 1A) (a slide show is included in the Supplementary Appendix, available with the full text of this article at www.nejm.org), hereditary pyropoikilocytosis (Fig. 1B), and Southeast Asian ovalocytosis, a distinctive type of inherited hemolytic anemia that is common in some parts of Southeast Asia and is

now also seen in Europe and North America as a result of immigration (Fig. 1C). The presence of spherocytes is not diagnostically specific, since this may result from hereditary spherocytosis, autoimmune hemolytic anemia, or alloimmune hemolytic anemia (e.g., hemolytic disease of the newborn or a delayed transfusion reaction). Nevertheless, consideration of the clinical features, together with the results of a direct antiglobulin test, in patients with spherocytes will generally indicate the correct diagnosis.

Microspherocytes (i.e., cells that are both hyperchromic and significantly reduced in size and therefore in diameter) may be present in low numbers in patients with a spherocytic hemolytic anemia but are also characteristic of burns and of microangiopathic hemolytic anemia. The detection of a microangiopathic hemolytic anemia (Fig. 1D) is

of considerable clinical significance, since this type of anemia may indicate pregnancy-associated hypertension, disseminated cancer, chronic disseminated intravascular coagulation, the hemolytic-uremic syndrome, or thrombotic thrombocytopenic purpura; the latter two conditions both require urgent diagnosis so that appropriate management can be initiated. In microangiopathic hemolytic anemia, examination of the blood smear is also important to validate the platelet count, since red-cell fragments and platelets may be of similar size. Most automated instruments cannot make this distinction. A minority of automated instruments that measure both the size and the refractive index of small particles in the blood sample can make this distinction and can be used to exclude red-cell fragmentation; however, although the fragment “flag” on such instruments is sensitive, it is not specific. Hence, a

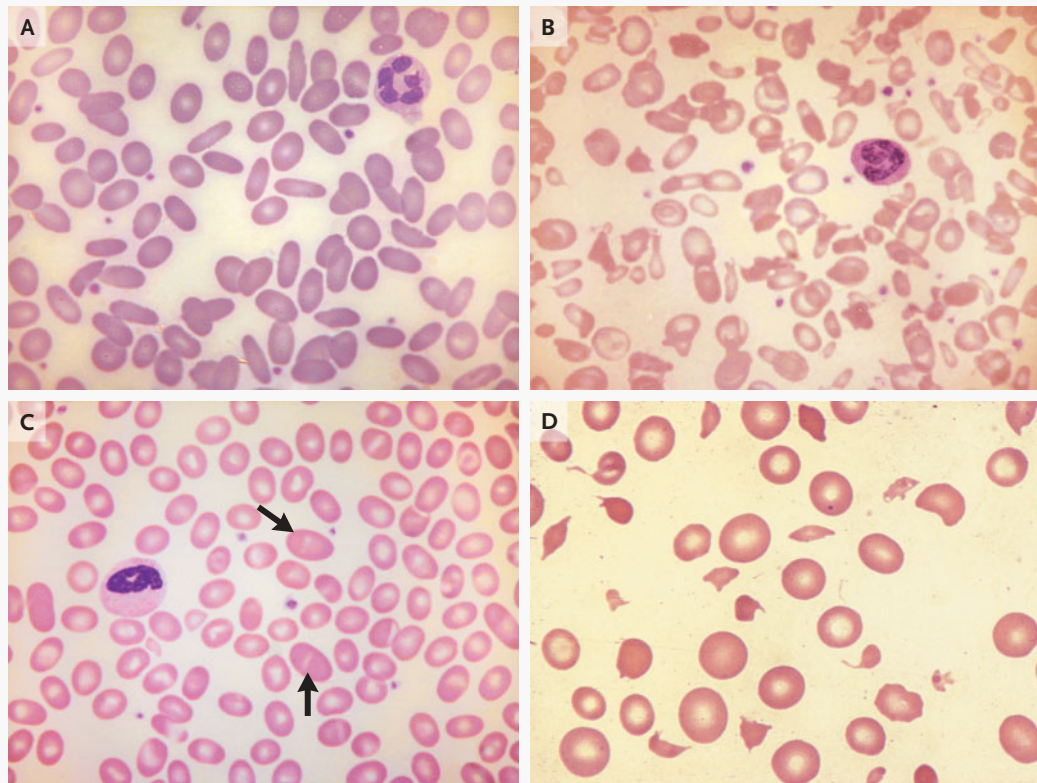


Figure 1. Hemolytic Anemias, Characterized by Different Types of Poikilocytes.

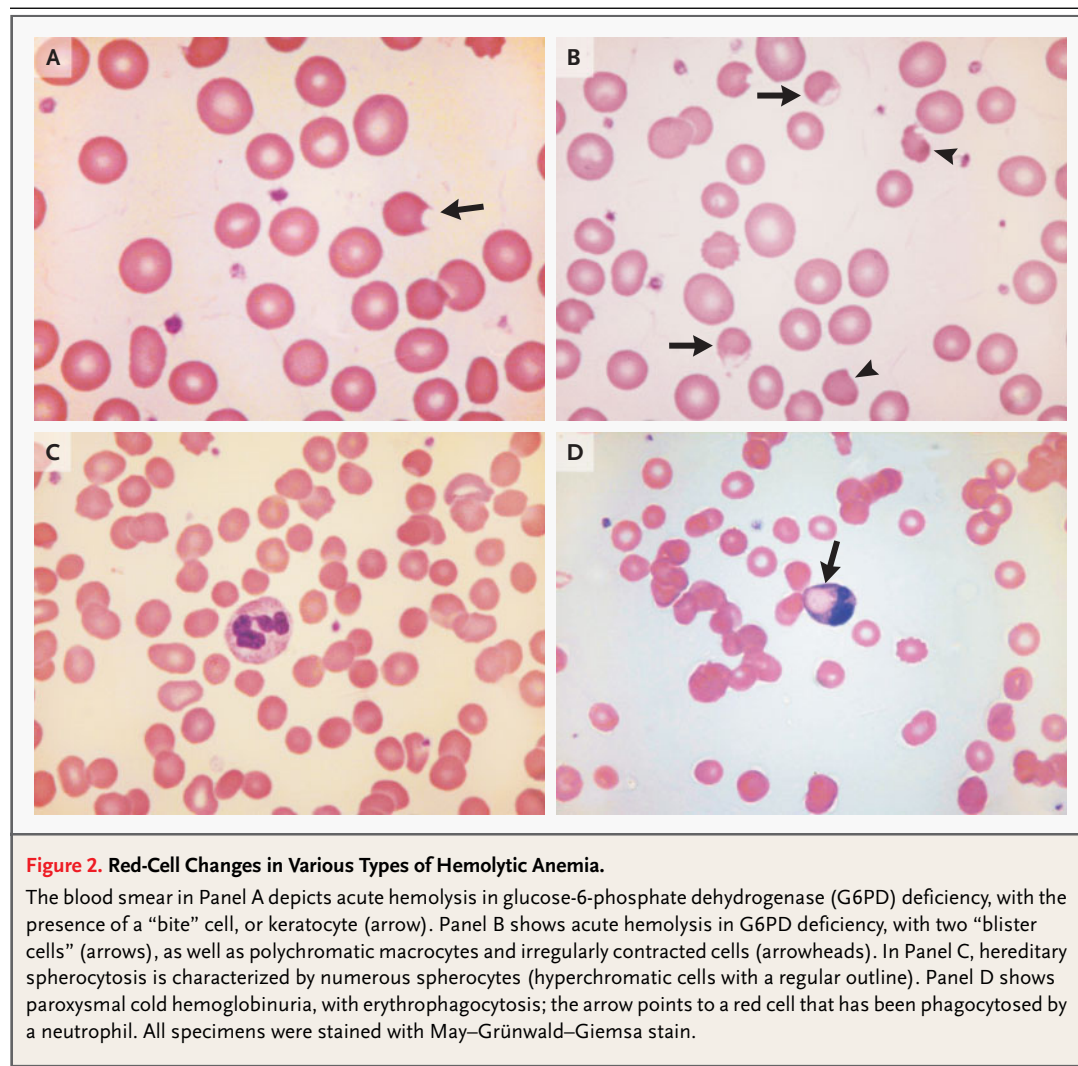
In Panel A, the blood smear shows hereditary elliptocytosis, with numerous elliptocytes and smaller numbers of ovalocytes. Panel B shows hereditary pyropoikilocytosis; there is striking poikilocytosis, with elliptocytes, ovalocytes, and fragments. In Panel C, Southeast Asian ovalocytosis shows moderate poikilocytosis, with the poikilocytes including several macro-ovalocytes (arrows). Panel D shows microangiopathic hemolytic anemia resulting from cyclosporine therapy, with numerous red-cell fragments. All specimens were stained with May-Grünwald-Giemsa stain.

blood smear is still advised for validation.² Blood-smear features similar to those seen in microangiopathic hemolytic anemia are also a feature of mechanical hemolytic anemia, such as that associated with a leaking prosthetic valve, and provide important evidence of this cause of hemolytic anemia.

A blood smear is particularly important in the diagnosis of acute hemolysis induced by oxidant damage. The characteristic feature is the presence of keratocytes, or “bite” cells (Fig. 2A and the Supplementary Appendix), “blister” cells (Fig. 2B), and irregularly contracted cells (Fig. 2B); the latter must be distinguished from spherocytes (Fig. 2C) because of the quite different diagnostic significance. These irregularly contracted cells share with spherocytes the lack of central pallor but differ in that they have an irregular outline. Oxidant-induced

hemolysis is most often seen in glucose-6-phosphate dehydrogenase (G6PD) deficiency but can also occur with other defects in the pentose shunt or in glutathione synthesis and when oxidant exposure overwhelms normal protective mechanisms. Oxidant damage may be exogenous, as in exposure to oxidant chemicals or drugs (most often dapsone), or endogenous, as in Wilson’s disease.³

G6PD deficiency affects millions of persons worldwide. A blood smear is important for the diagnosis of this condition, for two reasons. First, it is available far more rapidly than are the results of a G6PD assay and, when considered together with the patient’s ethnic origin and clinical history, permits a provisional diagnosis. Second, a blood smear can suggest the diagnosis of G6PD deficiency even if a G6PD assay is normal. Normal G6PD activity



may be found after acute hemolysis in G6PD-deficient persons in two situations. First, men of African or African-American ancestry who are hemizygous for the A- alloenzyme (which is present at normal levels in reticulocytes) can have normal G6PD levels because the reticulocyte count is high after hemolysis. Second, female carriers — for example, those who are hemizygous for the common Mediterranean variant of G6PD — can have a normal assay result after an acute hemolytic episode because the abnormal cells have lysed preferentially, leaving mainly the cells expressing the normal allele in the circulation. In both of these circumstances, the observation of a typical blood smear in an appropriate clinical setting is an indication to repeat the assay once the acute hemolytic episode is over.

Other features may aid in the differential diagnosis of hemolytic anemia. For example, the presence of red-cell agglutinates usually indicates the presence of a cold agglutinin, and erythrophagocytosis is often a feature of paroxysmal cold hemoglobinuria (Fig. 2D).

MACROCYTIC ANEMIA

The blood smear is of great importance in the differential diagnosis of macrocytic anemias. For patients in whom there is a deficiency of vitamin B₁₂ or folic acid, the blood smear shows not only macrocytes but also oval macrocytes and hypersegmented neutrophils (Fig. 3A and the Supplementary Appendix). When the anemia is more severe, there may be marked poikilocytosis, with teardrop poikilocytes and red-cell fragments. Although these deficiency states are now usually recognized on the basis of assays of vitamin B₁₂ and folic acid, the blood smear remains important for two reasons. First, it permits a speedy provisional diagnosis, and initiation of appropriate treatment in severely anemic patients while assay results are pending. Second, occasionally there are patients with a clinically significant vitamin B₁₂ deficiency despite a normal assay result. This discrepancy occurs because much of the vitamin B₁₂ that is measured in the assay is bound to haptocorrin, whereas the functional vitamin B₁₂, which is bound to transcobalamin, contributes much less to the assay of total B₁₂.

Similarly, acute folic acid deficiency sometimes develops in patients even though the total red-cell folate level remains normal. The observation of a blood smear that is typical of megaloblastic anemia despite normal assays is an indication that fur-

ther investigation and a trial of treatment are needed. Liver disease and excess ethanol consumption are common causes of macrocytosis, with the blood smear usually showing round rather than oval macrocytes and lacking hypersegmented neutrophils; target cells and stomatocytes may also be present.

In elderly patients, the myelodysplastic syndromes are an important cause of macrocytosis. Blood-smear features that may point to the diagnosis include hypogranular or hypolobulated neutrophils (Fig. 3B), blast cells (Fig. 3B), giant or hypogranular platelets, Pappenheimer bodies (Fig. 3C), and the presence of a minor population of hypochromic microcytic cells, leading to a dimorphic smear (Fig. 3C). Macrocytic anemia resulting from congenital dyserythropoietic anemia also yields a characteristic blood smear, with striking poikilocytosis (Fig. 3D). When macrocytosis is the result of hemolysis or recent blood loss, the blood smear shows polychromasia, which results from an increased reticulocyte count.

MICROCYTIC ANEMIA

The blood smear is generally less important in the differential diagnosis of the microcytic than the macrocytic anemias. Red-cell indices and serum ferritin levels, sometimes supplemented by markers of inflammation, that are interpreted in the context of clinical features, permit the diagnosis of the majority of cases. However, it is important to note that the presence of Pappenheimer bodies and red-cell dimorphism in the sideroblastic anemias and of basophilic stippling in cases of lead poisoning (Fig. 4A and the Supplementary Appendix) and in some types of thalassemia is diagnostically significant.

HEMOGLOBINOPATHY AND THALASSEMIA

A blood smear is useful in the diagnosis and differential diagnosis of sickle cell disease, particularly if there is an urgent need for diagnosis and if the results of hemoglobin electrophoresis or high-performance liquid chromatography are not instantly available. Patients with sickle cell anemia (in which there is homozygosity for hemoglobin S) have anemia, but those with compound heterozygosity for hemoglobin S and hemoglobin C may have a normal hemoglobin level, and the condition thus may be confused with sickle cell trait if a blood smear is not examined. Consideration of the blood-smear features, of the hemoglobin level, and of the results of a sickle cell solubility test usually permits an ac-

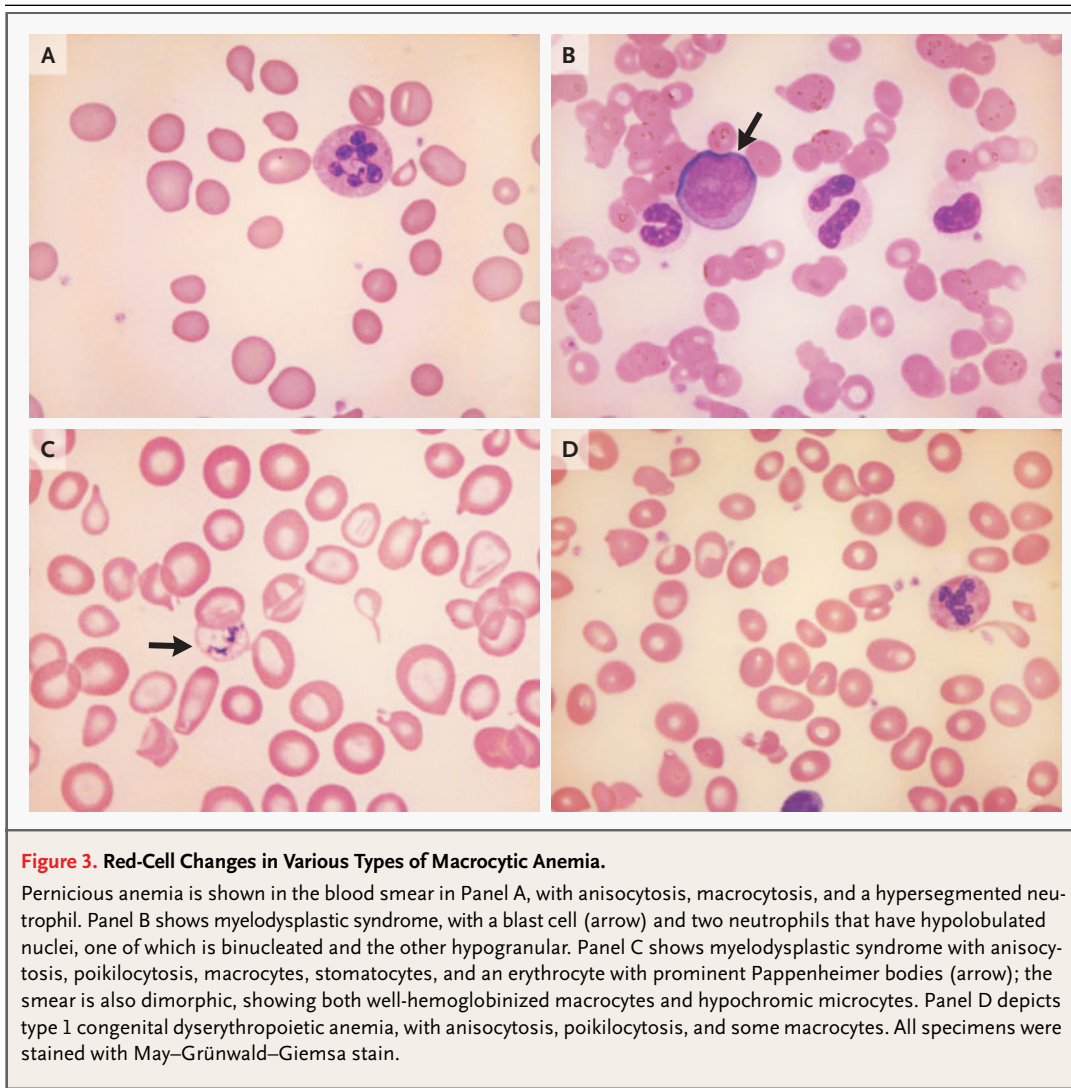


Figure 3. Red-Cell Changes in Various Types of Macrocytic Anemia.

Pernicious anemia is shown in the blood smear in Panel A, with anisocytosis, macrocytosis, and a hypersegmented neutrophil. Panel B shows myelodysplastic syndrome, with a blast cell (arrow) and two neutrophils that have hypolobulated nuclei, one of which is binucleated and the other hypogranular. Panel C shows myelodysplastic syndrome with anisocytosis, poikilocytosis, macrocytes, stomatocytes, and an erythrocyte with prominent Pappenheimer bodies (arrow); the smear is also dimorphic, showing both well-hemoglobinized macrocytes and hypochromic microcytes. Panel D depicts type 1 congenital dyserythropoietic anemia, with anisocytosis, poikilocytosis, and some macrocytes. All specimens were stained with May–Grünwald–Giemsa stain.

curate diagnosis^{4,5} (Fig. 4B and 4C). The blood smear of a compound heterozygote usually shows target cells, irregularly contracted cells, and boat-shaped cells but few classic sickle cells; typical hemoglobin SC poikilocytes (formed only when hemoglobin S and hemoglobin C are both present) are often seen. Sometimes the blood smear of a compound heterozygote shows only target cells and irregularly contracted cells and cannot be distinguished from the smear in hemoglobin C homozygosity; a positive sickle cell solubility test permits these conditions to be distinguished in an emergency situation (e.g., preoperatively). A blood smear is also important in the diagnosis of an unstable hemoglobin, with irregularly contracted cells and macrocytosis being characteristic of this

condition (Fig. 4D); sometimes there is coexisting thrombocytopenia.

THROMBOCYTOPENIA AND THROMBOCYTOSIS

A blood smear should always be examined for patients with thrombocytopenia, both to confirm the thrombocytopenia and to look for the underlying cause. Falsely low platelet counts may be the result of small clots, platelet clumping (Fig. 5A and the Supplementary Appendix), platelet satellitism (Fig. 5B), or abnormally large platelets. Fibrin strands (Fig. 5C) indicate that thrombocytopenia is likely to be factitious. Underlying causes that may be revealed by the blood smear include the May–Hegglin

anomaly (Fig. 5D), microangiopathic thrombopathies, and leukemias and lymphomas. High platelet counts should be confirmed microscopically with a blood smear; falsely high counts may be the result of other particles (red-cell fragments, fragments of leukemic cells, or fungi) being counted as platelets.⁶⁻⁹ Examination of the blood smear is also important in patients with thrombocytosis to look for evidence of a myeloproliferative disorder, such as giant platelets, or an increase in the basophil count; the latter is not reliably detected by automated counters. A sudden, unexpected improvement in the platelet count also should be confirmed by blood-smear examination, since such an improvement may be factitious⁷ (Fig. 5E).

LEUKEMIA, LYMPHOMA, OR BONE MARROW FAILURE

Blood smears must always be examined when there is unexplained leukocytosis, lymphocytosis, or monocytosis or when the flagging system of an automated instrument suggests the presence of blast cells. Depending on the instrument and the practice of the local laboratory, a flag for atypical or variant lymphocytes may also be an indication for examination of a blood smear, since this flag is sometimes indicative of the presence of blast cells. Low rather than high counts likewise are an indication for a smear, since they may be indicative of aplastic anemia, acute leukemia, hairy-cell leuke-

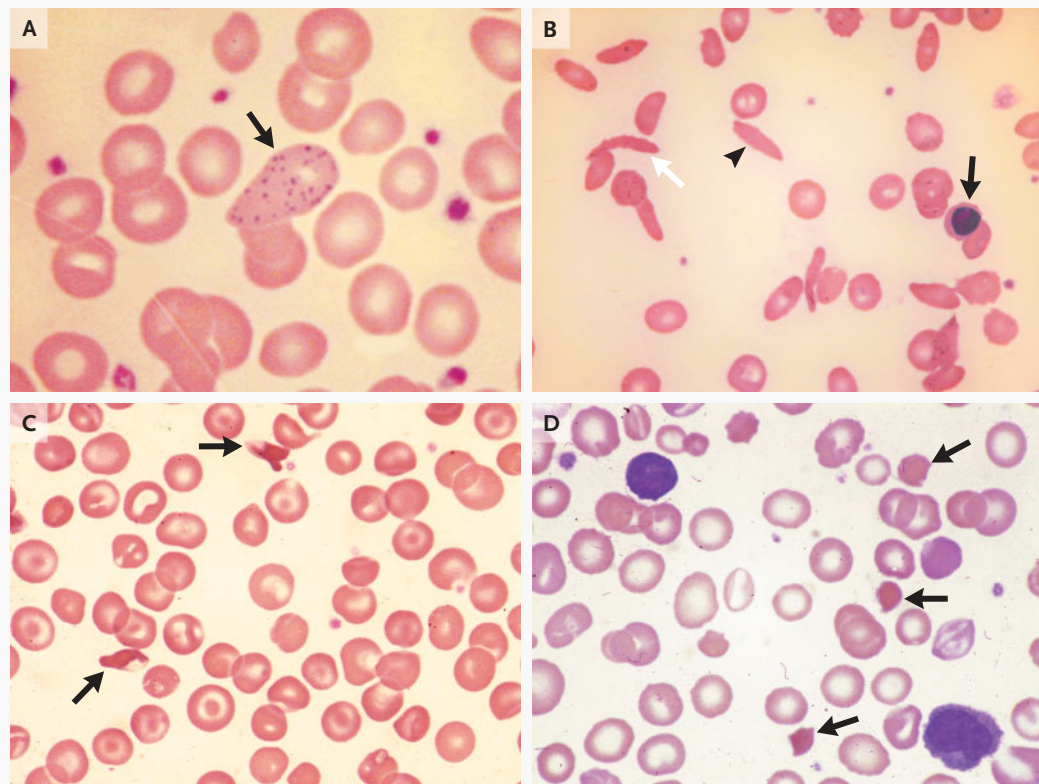


Figure 4. Red-Cell Changes with Lead Poisoning and in Hemoglobinopathies.

Panel A shows an erythrocyte with prominent basophilic stippling (arrow), a result of lead poisoning. Panel B shows sickle cell anemia, with a nucleated red cell (black arrow), sickle cells (white arrow), and boat-shaped cells (arrowhead). Panel C shows sickle cell-hemoglobin C disease, with target cells, irregular contracted cells, and two hemoglobin SC poikilocytes (arrows). Panel D demonstrates heterozygosity for hemoglobin Hammersmith (an unstable hemoglobin), with irregularly contracted cells (arrows). All specimens were stained with May-Grünwald-Giemsa stain.

mia, or infiltration of nonhematopoietic malignant cells into the bone marrow. The role of the blood smear in the diagnosis of leukemia and lymphoma is to suggest a likely diagnosis or range of diagnoses, to indicate which additional tests should be performed, and to provide a morphologic context without which immunophenotyping and other sophisticated investigations cannot be interpreted. For two conditions, Burkitt's lymphoma (Fig. 6A and the Supplementary Appendix) and acute promyelocytic leukemia (Fig. 6B), a blood smear is of particular importance because it facilitates rapid diagnosis and specific treatment.

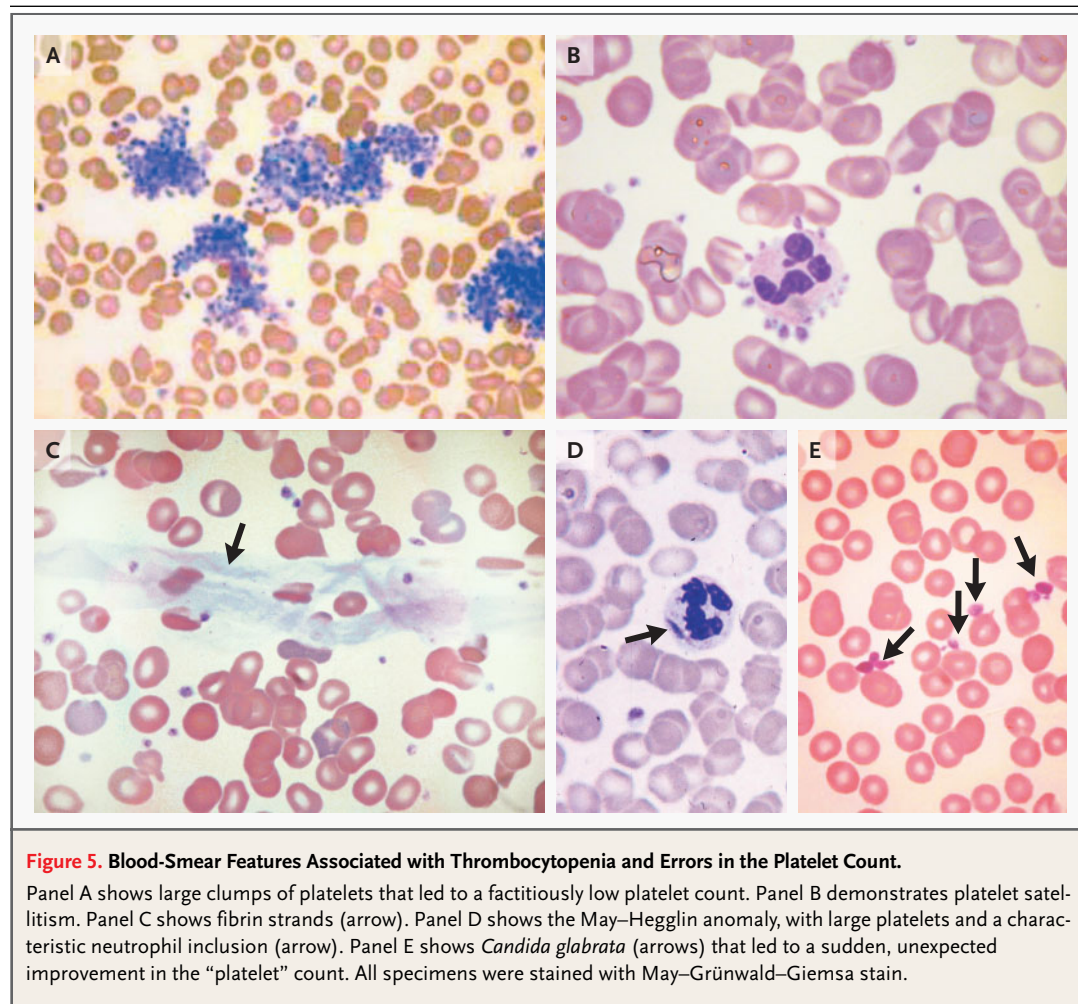
produces a highly improbable result. Such results may be factitious, resulting from the accidental freezing or heating of the blood, from hyperlipidemia, or from the presence of cold agglutinins, a cryoglobulin (Fig. 6C), bacteria, or fungi. Factitious results also may stem from unusual characteristics of the blood cells or the plasma, such as a pseudoneutropenia caused by a myeloperoxidase deficiency that occurs when the automated instrument employs a peroxidase reaction for the identification of neutrophils, eosinophils, and monocytes. Falsely low counts also may result from neutrophil or platelet clumping or from platelet satellitism.

PROBABLE FACTITIOUS RESULTS

Members of the laboratory staff should always initiate a blood smear if an automated instrument

SERENDIPITY

Occasionally, a blood smear leads to a fortuitous diagnosis that can be very important to the patient



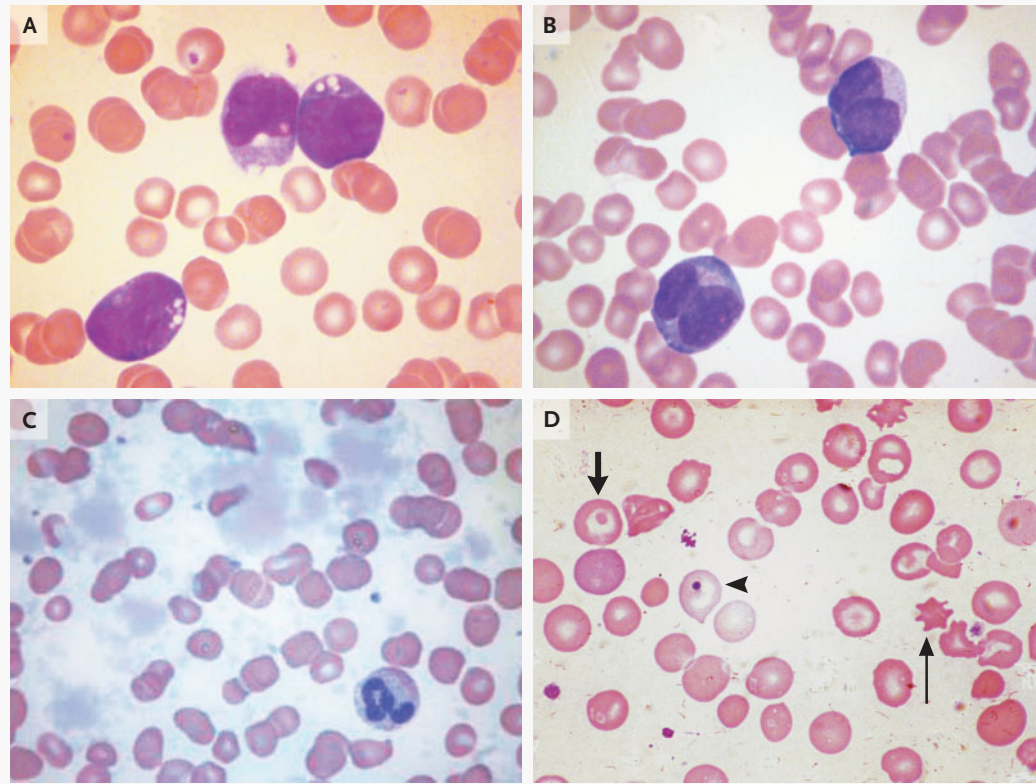


Figure 6. Miscellaneous Conditions in Which the Blood Smear Can Be Diagnostically Important.

Panel A shows Burkitt's lymphoma, with three basophilic vacuolated lymphoma cells. Hypogranular promyelocytic leukemia is shown in Panel B, with two characteristic bilobed leukemic promyelocytes. Panel C depicts cryoglobulin deposition in a blood sample from a patient with hepatitis C virus infection. Panel D shows target cells (short arrow), acanthocytes (long arrow), and a Howell-Jolly body (arrowhead) — all features of hyposplenism — in a blood smear from a patient with iron-deficiency anemia and splenic atrophy as features of celiac disease. All specimens were stained with May-Grünwald-Giemsa stain.

(Table 2). As an example, the detection of features of unexpected hyposplenism (Fig. 6D) may suggest a congenital absence of the spleen, splenic atrophy, deposition of amyloid in the spleen, infiltration of neoplastic cells (e.g., in leukemia, lymphoma, or carcinoma) in the spleen, previous splenic infarction, or even a splenectomy of which the patient was unaware — in each case putting the patient at risk for complications of hyposplenism. Conversely, the failure to observe expected hyposplenism in a blood smear from a patient who has undergone splenectomy for the treatment of autoimmune thrombocytopenic purpura may indicate that there is functioning residual splenic tissue, either from splenosis or from accessory spleens, that may be responsible for a relapse of the disease.

THE BLOOD SMEAR AS PART OF THE MEDICAL RECORD

Sometimes the blood smear provides the primary or the only evidence of a specific diagnosis, such as myelodysplastic syndrome, leukemia, lymphoma, or hemolytic anemia. It is important that, if possible, such blood smears be stored over the long term, just as a tissue that provides a histologic diagnosis is stored over the long term. In practice, such storage is easily achieved if a patient has also had a bone marrow aspirate (since a blood smear should always be stored with an aspirate), but it is harder to achieve if the blood smear alone has provided the diagnosis. Individual laboratories should have a mechanism to make possible the retention of such

smears or an image derived from them. Some laboratories retain all smears that have been reviewed by a laboratory hematologist or pathologist; this can create a storage problem, and it is likely that, increasingly, digital images of important abnormal smears will be stored.

THE FUTURE

The continuing importance of the blood smear is highlighted by the recent introduction of photographs of blood smears as a regular feature in both the journal *Blood*¹⁰ and the *British Journal of Haematology*, by ongoing efforts to develop image-recognition technology for the automated examination of blood smears, and by the development of telehematology to permit the remote interpretation or second opinions of blood smears.^{11,12}

CONCLUSIONS

Even in the age of molecular analysis, the blood smear remains an important diagnostic tool. Physicians should request a blood smear when there are clinical indications for it. Members of the laborato-

Table 2. Fortuitous Observations That May Be of Diagnostic Importance.

Red-cell fragmentation
Hyposplenism
Cryoglobulinemia (may indicate hepatitis C virus infection or a plasma-cell neoplasm)
Red-cell agglutinates (may indicate cold agglutinins, as in mycoplasma infection, infectious mononucleosis, or lymphoproliferative disorder)
Dysplastic features typical of human immunodeficiency virus infection
Presence of leukemic blasts or lymphoma or myeloma cells
Malaria or other parasitic infections (usually malaria but occasionally babesiosis, leishmaniasis, African trypanosomiasis, Chagas' disease, or filariasis)
Fungal infection (e.g., candidiasis, histoplasmosis)
Bacteria (relapsing fever, ehrlichiosis, meningococcal or pneumococcal infection)

ry staff should make and examine a blood smear whenever the results of the complete blood count indicate that a blood smear is essential for the validation or the further elucidation of a detected abnormality. If error is to be avoided, sophisticated modern investigations of hematologic disorders should be interpreted in the light of peripheral-blood features as well as the clinical context.

I am indebted to Dr. Bernadette Garvey of Toronto and Dr. LoAnne Peterson of Chicago for their helpful comments on the manuscript.

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