An abnormal blood count or blood cell morphology does not necessarily indicate a primary haematology problem because it may reflect an underlying non-haematological condition or may be the result of therapeutic interventions. Anaemia occurs in many conditions, but a primary blood disease should be considered when a patient has splenomegaly; lymphadenopathy; a bleeding tendency or thrombosis; and/or non-specific symptoms (malaise, sweats, or weight loss).

As with any clinical problem, the first steps in determining the diagnosis include obtaining a careful clinical and drug history and thorough physical examination. The result of these, in combination with the patient's age, sex, ethnic origin, social and family history, and knowledge of the locally prevalent diseases, will determine subsequent laboratory investigations.

Although the range of haematological tests available to support clinical and public health services is broad, it is often the simplest investigations that are most useful in indicating the diagnosis. Even poorly resourced laboratories are usually able to provide an initial panel of tests such as haemoglobin concentration (Hb), white blood cell count (WBC) and platelet count (Chapter 27), and examination of a peripheral blood smear for a differential leucocyte count (Chapter 3) and cellular morphology (Chapter 5). These screening tests will often enable the underlying pathological processes to be suspected promptly and point to a few key diagnostic tests.
The investigation of specific haematological problems is covered in detail in Chapters 7 (iron deficiency anaemia), 8 (megaloblastic anaemia), 9, 10, and 11 (haemolytic anaemias), 12 (haemoglobinopathies), and 16 and 17 (coagulation disorders).

**Interpretation of Screening Tests**
Results of laboratory screening tests should always be interpreted with an understanding of the limitations of the tests and the physiological variations that occur with sex, age, and conditions such as pregnancy and exercise. Physiological variations in cell counts are detailed in Chapter 2. Abnormalities of red cells, white cells, or platelets may be quantitative (increased or reduced numbers) or qualitative (abnormal appearance and/or function).

**Quantitative Abnormalities of Blood Cells**

**Increased Numbers of Cells**

**Increases Affecting More Than One Cell Line**
A simultaneous increase in the cells of more than one cell line suggests that the overproduction of cells originates in an early precursor cell. This occurs in myeloproliferative disorders in which one cell type may predominate, e.g., platelets in essential thrombocythaemia and red cells in polycythaemia vera (primary proliferative polycythaemia), but there are often increases in other cell lines. The diagnosis will depend on which cell line expansion is dominant.

**Erythrocytosis**
Increases in red cells may be one of the following:

- “Relative” (pseudopolycythaemia) owing to reduced plasma volume
- “Primary” (polycythaemia vera) as part of the spectrum of myeloproliferative disorders
- “Secondary” to chronic hypoxia (e.g., chronic lung disease, congenital heart disease, high-affinity haemoglobins) or aberrant erythropoietin production

Secondary polycythaemia can generally be excluded by the clinical history and examination, assessment of serum erythropoietin concentration and arterial oxygen saturation, haemoglobin electrophoresis, and abdominal ultrasound. The presence of splenomegaly is suggestive of polycythemia vera, and this diagnosis can be confirmed by demonstrating an absolute increase in total red cell volume and excluding other causes of erythrocytosis. Measurement of red cell and/or plasma volume (Chapter 15) will identify pseudopolycythaemia.

**Leucocytosis**

**Neutrophilia**
Neutrophils are commonly increased in number during pregnancy and in acute infections, inflammation, intoxication, corticosteroid therapy, and acute blood loss or destruction. Neutrophilia with the neutrophils showing heavy cytoplasmic granulation (“toxic” granulation) is a common finding in severe bacterial infections. In the absence of any underlying cause, a high neutrophil count with immature myeloid cells suggests chronic granulocytic leukaemia; cytogenetic and molecular studies to look for t(9;22) and the BCR–ABL fusion gene are indicated (Chapter 21).

**Lymphocytosis**
Lymphocytosis is a feature of certain infections, particularly infections in children. It may be especially marked in pertussis, infectious mononucleosis, cytomegalovirus infection, infectious hepatitis, tuberculosis, and brucellosis. Lymphocytosis is also a common transient reaction to severe physical stress. Elderly patients with lymphoproliferative disorders, including chronic lymphocytic leukaemia and lymphomas, often present with lymphadenopathy and a lymphocytosis. Morphology and immunophenotyping of the cells combined with histological examination of a bone marrow trephine biopsy are used to classify these disorders and to give an indication of management and prognosis. It is occasionally difficult to differentiate between a reactive and a neoplastic lymphocytosis. In this situation, immunophenotyping, immunophenotypic evidence of light chain restriction, and polymerase chain reaction for immunoglobulin or T-cell receptor gene rearrangements may indicate the presence of a monoclonal population of lymphocytes, thereby supporting a diagnosis of neoplastic, rather than reactive, lymphoproliferation. If lymph nodes are enlarged, a fine needle aspirate for cytology and immunocytochemistry or a lymph node biopsy for
histology and immunohistochemistry may be helpful in diagnosis.

**Monocytosis**
A slight to moderate monocytosis may be associated with some protozoal, rickettsial, and bacterial infections including malaria, typhus, and tuberculosis. High levels of monocytes (monocyte count >1 \(\times\) 10^9/l) in an elderly patient suggest chronic myelomonocytic leukaemia or, sometimes, atypical chronic myeloid leukaemia. Because these conditions fall into the myeloproliferative/myelodysplastic group of disorders, the diagnosis would be supported by finding splenomegaly, quantitative and qualitative abnormalities in other cell lines, and a clonal cytogenetic abnormality.

**Eosinophilia**
Eosinophilia is typically associated with allergic disorders including drug sensitivity, skin diseases, and parasitic infections. In most cases, the cause is indicated from the clinical history, which should include details of all medications and foreign travel, and by examination of the stool and urine for parasites and ova. A diagnosis of chronic eosinophilic leukaemia is made if there is an increase in blast cells in the blood or marrow or if there is cytogenetic or molecular evidence of an abnormal myeloid clone. Idiopathic hypereosinophilic syndrome is an unusual cause of eosinophilia in which release of the contents of eosinophil granules results in damage to the heart, lungs, and other tissues. This is a diagnosis of exclusion, made only when detailed investigations exclude all known causes. It is necessary to specifically exclude eosinophilic leukaemia and cytokine-induced eosinophilia resulting from the presence of a neoplastic clone of T cells before diagnosing a condition as the idiopathic hypereosinophilic syndrome.

**Basophilia**
Basophilia as an isolated finding is unusual. However, it is a common feature of myeloproliferative disorders and basophils may be particularly prominent in chronic granulocytic leukaemia. In this condition, an increasing basophil count may be the first indication of transformation to a more aggressive course.

**Thrombocytosis**
Thrombocytosis is often associated with infectious and inflammatory conditions such as osteomyelitis and rheumatoid arthritis. Haematological causes of thrombocytosis include chronic blood loss, red cell destruction, splenectomy, and rebound following recovery from marrow suppression. Under these circumstances, a moderately increased platelet count (e.g., 400–800 \(\times\) 10^9/l) does not usually have any pathological consequences. Primary (essential) thrombocythaemia belongs to the spectrum of myeloproliferative diseases and is characterized by a persistently high platelet count (often arbitrarily defined as greater than 600 \(\times\) 10^9/l) and thrombotic or haemorrhagic complications. Further investigations to confirm primary thrombocythaemia include bone marrow examination for increased and abnormal megakaryocytes and cytogenetic analysis.

**Reduced Numbers of Cells**

**Reductions in More Than One Cell Line**
A reduction in cell numbers occurs because of increased destruction, reduced production, or increased pooling in the spleen or other organ. Reduced production of cells may be the result of aplastic anaemia, a lack of haematinics such as folate or vitamin B_{12}, or interference with normal haemopoiesis by infiltration (e.g., leukaemia, lymphoma, multiple myeloma, metastatic carcinoma — often with secondary myelofibrosis), infection (e.g., human immunodeficiency virus [HIV] infection, tuberculosis, leishmaniasis), or exposure to toxins (e.g., alcohol) or myelosuppressive drugs (e.g., hydroxyurea* or busulphan). Certain myeloid neoplasms (e.g., “idiopathic” myelofibrosis) and the myelodysplastic syndromes (MDS) are characterised by cytopenias, and this is also sometimes a feature of acute myeloid leukaemia (AML). A relatively common cause of a global reduction in circulating cells is pooling of the cells in a grossly enlarged spleen (hypersplenism), which may be secondary to conditions such as myelofibrosis and portal hypertension. Examination of a bone marrow aspirate and trephine biopsy specimen is often helpful in

*Previously known as hydroxyurea.
determining the cause of bicytopenia or pancytopenia for which no obvious cause can be found.

**Anaemia**

There are many causes of anaemia, and a logical classification would be according to mechanism:

- Decreased production
- Reduced lifespan of red cells
- Blood loss
- Splenic pooling

In practice, if the cause is not readily apparent from the clinical circumstances and an automated blood count is available, classification according to cell size is more practicable. The choice of further investigations is then guided by the mean cell volume (MCV) and red cell morphology in addition to clinical features. Anaemia is thus broadly divided into three types:

- Microcytic (low MCV)
- Macrocytic (high MCV)
- Normocytic (normal MCV)

Low MCV may be associated with low mean cell haemoglobin (MCH). A low mean cell haemoglobin concentration (MCHC) is less common and correlates with hypochromia.

Figures 23.1–23.3 are flow charts that provide an orderly sequence of investigations for the different types of anaemia on the basis of these indices. Examination of a blood film will usually suggest the quickest route to the diagnosis; confirmation may require the more specific tests, which are given in the text. The presence of basophilic stippling in a patient with microcytic red cells suggests thalassaemia trait or lead poisoning. A dimorphic blood film is typical of congenital sideroblastic anaemia but is more often the result of iron deficiency responding to treatment. Pappenheimer bodies suggest that a microcytic anaemia is the result of sideroblastic erythropoiesis.

**Microcytic Anaemia (See Fig. 23.1)**

The most common cause of anaemia worldwide is iron deficiency (Fig. 23.1). It can be suspected from a low MCV and the presence of hypochromic, microcytic red cells. Laboratory confirmation of iron deficiency may include measurements of serum ferritin, serum iron plus total iron-binding capacity or transferrin assay, red cell protoporphyrin, and staining of bone marrow aspirates for iron (see Chapter 4). A diagnosis of iron deficiency requires a search for the cause. This should include specific questions relating to blood loss and dietary insufficiency and may require stool examination for parasites and occult blood, endoscopic examination.

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**Abbreviations:** HbEP, Hb electrophoresis; HPLC, high performance liquid chromatography
of the gastrointestinal tract to exclude occult malignancy, and serological or other tests for coeliac disease. The differential diagnosis of iron deficiency anaemia includes anaemia of chronic disease. Clinical and laboratory features of inflammation may suggest this diagnosis, which is confirmed by demonstration of normal or high serum ferritin, low serum iron, and low transferrin and iron binding capacity.

The thalassaemias also cause microcytosis, but both \( \alpha \) and \( \beta \) thalassaemia are usually associated with an increased red blood cell count and a normal or near-normal Hb despite a considerable reduction of the MCV and MCH, whereas in iron deficiency the MCV and MCH do not fall until the Hb is significantly reduced. Further investigations, such as haemoglobin electrophoresis, high-performance liquid chromatography (HPLC), or measurement of \( \text{HbA}_2 \) and HbF usually confirm the diagnosis of \( \beta \) thalassaemia trait. The diagnosis of \( \alpha \) thalassaemia trait is more difficult; detection of infrequent HbH inclusions is usually possible in \( \alpha^0 \) thalassaemia trait, but definitive diagnosis requires DNA analysis. The diagnosis of \( \alpha^+ \) thalassaemia trait is of less clinical importance; HbH inclusions may not be detected, so DNA analysis is needed.

### Macrocytic Anaemia (See Fig. 23.2)

A high MCV with oval macrocytes and hypersegmented neutrophils suggests folate or vitamin B\(_{12}\) deficiency and is an indication for assays of these vitamins (see Chapter 8); subsequent investigations could include malabsorption studies, serological test for coeliac disease, and either tests for intrinsic factor antibodies or a Schilling test to detect pernicious anaemia (p. 180). If intrinsic factor antibodies are detected, a Schilling test is not necessary. A high MCV may also be associated with alcohol excess and liver disease or drugs such as hydroxycarbamide or zidovudine. Macrocytosis resulting from chronic haemolysis is associated with increased numbers of immature red cells, which appear slightly larger and more blue than normal red cells on a Romanowsky-stained peripheral blood film. Supravital staining of blood films (p. 40) or an automated reticulocyte count can be used to confirm reticulocytosis. Untreated anaemia associated with polychromasia is likely to indicate blood loss or haemolysis. The combination of red cell fragments, thrombocytopenia, and polychromasia indicates microangiopathic haemolytic anaemia and should trigger further tests such as a platelet count, coagulation studies, assessment of renal function, and a search for infection or neoplastic disease. This further assessment is urgent because these may be features of thrombotic thrombocytopenic purpura, which requires speedy treatment by plasma exchange.

### Normocytic Anaemia (See Fig. 23.3)

Normochromic, normocytic anaemia is frequently the result of an underlying chronic, non-haematological disease. Investigations should include screening for renal insufficiency, subclinical infections, autoimmune diseases, and neoplasia. In the presence of anaemia, a lack of polychromasia, confirmed by reticulocytopenia, points toward a primary failure of erythropoiesis or blood loss or haemolysis without compensatory red cell production. Examination of the bone marrow may be help-
ful in demonstrating haematological causes for the normochromic, normocytic anaemia such as aplastic anaemia or early myelodysplastic syndrome. Staining for iron may also show that there is a block in iron metabolism suggestive of anaemia associated with chronic inflammatory disease.

**Leucopenia**

*Neutropenia*

Once physiological variation, ethnicity, and familial or cyclic neutropenia have been excluded (p. 20), the non-haematological causes of isolated neutropenia to be considered include overwhelming infection, autoimmune disorders such as systemic lupus erythematosus, irradiation, drugs (particularly anticancer agents), and large granular lymphocyte leukaemia. Bone marrow examination may assist in determining whether the problem is the result of peripheral destruction (increased marrow myeloid precursors) or stem cell failure (lack of narrow myeloid precursors). Typical marrow appearances occur in drug-induced neutropenia, in which there is a relative paucity of mature neutrophils and in Kostmann’s syndrome (infant genetic agranulocytosis) where there is maturation arrest at the promyelocytic stage.

**Reduced Numbers of Lymphocytes, Monocytes, Eosinophils, and Basophils**

Lymphocytes, eosinophils, and basophils may all be reduced by stress such as surgery, trauma, and infection. Lymphopenia with neutrophilia is a common combination of haematological abnormalities in severe acute respiratory syndrome. Lymphopenia, especially affecting the CD4 cells, may occur in HIV.
infection and renal failure. Monocytopenia (<0.2 × 10⁹/l) is typically found in hairy cell leukaemia, which is also associated with pancytopenia, typical bone marrow histology, and lymphocytes with a characteristic cytology and immunophenotype.

**Thrombocytopenia**
Thrombocytopenia is a common isolated finding, and it is important to ensure that the laboratory result reflects a true reduction in platelet count before embarking on further diagnostic tests. Frequent causes of spurious thrombocytopenia include blood clots in the sample, platelet aggregation, and platelet satellitism. Platelet aggregation, which can be seen on the blood film, may occur *in vitro* as the result of a temperature-dependent or anticoagulant-dependent autoantibody. Small platelet aggregates are also seen in slides that have been made directly from a fingerprick sample. True thrombocytopenia is most frequently the result of anticancer chemotherapy, HIV infection, autoantibodies (“idiopathic” autoimmune thrombocytopenic purpura), other drugs (such as thiazide diuretics), alcohol excess, hypersplenism, and MDS (in the elderly). The clinical circumstances, together with bone marrow examination and relevant serological tests, should enable these conditions to be differentiated. Thrombocytopenia associated with other complications, such as thromboses, disturbed renal or hepatic function, and haemolytic anaemia, should prompt investigations for other diseases such as thrombotic thrombocytopenic purpura or the HELLP (Haemolysis + Elevated Liver enzymes + Low Platelet count) syndrome. A bone marrow examination is often carried out early in the investigation of thrombocytopenia because it is helpful in excluding conditions such as acute leukaemia, which occasionally present with isolated thrombocytopenia.

**Pancytopenia**
Pancytopenia (reduction in the white cell count, Hb, and platelet count) is most often the result of anticancer chemotherapy, HIV infection, hypersplenism, and bone marrow infiltration or failure. Careful examination of a blood film is important if the reason for the pancytopenia is not apparent from the clinical history. If this does not reveal the cause, bone marrow aspiration and trephine biopsy may be needed.

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**Qualitative Abnormalities of Blood Cells**
In health, only the most mature forms of cells appear in the peripheral blood. Earlier, less mature cells, such as nucleated red blood cells, polychromatic red cells, myelocytes, and metamyelocytes, may be released from the bone marrow in conditions where the bone marrow is overactive (e.g., acute haemolytic states or recovery after suppression) or functionally abnormal. Their presence in the peripheral blood indicates that active haemopoiesis is taking place.

**Abnormalities of All Cell Lines**
The combination of anisopoikilocytosis, mild macrocytosis, hypogranular neutrophils with abnormal nuclear morphology, and platelet anisocytosis, often with quantitative abnormalities, is virtually pathognomonic of a myelodysplastic syndrome. These features are reflected in the bone marrow with disturbance of the normal developmental pathway and nuclear:cytoplasmic asynchrony. Cytogenetic studies can confirm the diagnosis when cytological abnormalities are minor and also can assist in determining the prognosis.

**Abnormalities of Individual Cell Lines**

**Red Cells**
Congenital abnormalities of the red cell affecting the structure (e.g., spherocytosis, elliptocytosis) and content (e.g., haemoglobinopathies, enzymopathies) often produce typical morphological changes (see Chapter 5). The type of changes will guide further investigations toward analysis of structural proteins, haemoglobin electrophoresis, or HPLC and enzyme assays. Acquired red cell abnormalities may also help to indicate underlying pathology. For example, target cells may prompt investigation of liver function, whereas rouleaux may indicate the need for investigations for multiple myeloma or inflammatory conditions such as rheumatoid arthritis.

**White Cells**
Congenital abnormalities of neutrophils are unusual, but similar morphological abnormalities (e.g., Pelger–Huët cells) may be seen in acquired conditions such as myelodysplastic syndrome. Reactive
changes in lymphocytes including basophilic, faceted cytoplasm, are typically seen in infectious mononucleosis, which can be diagnosed using an appropriate screening test (p. 601) or, if this is negative, by demonstration of immunoglobulin M (IgM) antibodies to the Epstein–Barr virus. These atypical lymphocytes can sometimes be difficult to differentiate from circulating lymphoma cells. Bone marrow histology, combined with immunophenotyping studies and determination of lymphocyte clonality by demonstration of light chain restriction or by gene rearrangement studies, may be needed to reach a firm conclusion.

Platelets
Platelets that function poorly may not necessarily appear morphologically abnormal, although sometimes they are hypogranular or larger than normal. A normal platelet count with a prolonged bleeding time is characteristic of a disorder of platelet function, but some patients with abnormal platelet function are also thrombocytopenic. Hereditary disorders of platelet function are uncommon and usually present as a bleeding diathesis. When a qualitative disorder of platelets is suspected, platelets should be examined to assess size and to detect the cytological features of the grey platelet syndrome. They can broadly be divided into two categories: abnormalities of the platelet membrane (e.g., Bernard–Soulier syndrome, Glanzmann’s thrombasthenia) and of platelet secretory function (e.g., storage pool diseases). In comparison, acquired disorders of platelet function are common. Haematological conditions associated with platelet dysfunction include myeloproliferative and myelodysplastic disorders and dysproteinaemias. Many widely prescribed drugs can interfere with platelet function, including aspirin and non-steroidal anti-inflammatory agents, whereas systemic conditions, particularly chronic renal failure and cardiopulmonary bypass, are associated with a bleeding tendency as a result of qualitative platelet defects. Most of these acquired functional defects are not associated with any abnormality in platelet appearance but in the myelodysplastic and, to a lesser extent, in the myeloproliferative disorders there may be hypogranular and giant platelets.

SPECIFIC TESTS FOR COMMON HAEMATOLOGICAL DISORDERS

Common haematological disorders are outlined in the following sections with suggestions for investigations that may be helpful in confirming the diagnosis. The lists are not intended to be exhaustive because the range of tests provided locally will depend on the availability of expertise and technology. The investigations discussed are those that are likely to be available within a general haematology department.

Red Cell Disorders
Microcytic Hypochromic Anaemias
For more information, see Chapters 7 and 12.

- Measurement of serum ferritin or iron plus either total iron-binding capacity or transferrin assay, red cell protoporphyrin
- Bone marrow aspirate with staining for iron
- Stool examination for occult blood; blood loss studies with ⁵¹Cr-labelled red cells
- Tests for malabsorption
- Serological tests for coeliac disease
- Endoscopic examination with biopsy
- Serum lead (if lead poisoning is suspected)

If thalassaemia is suspected:
- Haemoglobin electrophoresis plus Hb A₂ and Hb F measurements or HPLC
- Haemoglobin H preparation
- Family studies
- DNA analysis

Macrocytic Anaemias
Where macrocytic, megaloblastic erythroid maturation is demonstrated, further investigations should be undertaken as described in Chapter 8. If the blood film is typical of megaloblastic anaemia, relevant assays and further investigations are often performed without a bone marrow aspirate being done. Macrocytosis may also be secondary to common conditions such as alcohol excess, liver disease, myelodysplastic syndrome, hydroxyurea administration, and hypothyroidism. Reticulocytosis from any cause can also increase the MCV.
Aplastic Anaemia
- Bone marrow aspirate and trephine biopsy
- Acidified serum (Ham’s) test for paroxysmal nocturnal haemoglobinuria (urine examination for haemosiderin and neutrophil alkaline phosphatase if Ham’s test is positive)
- Vitamin B12 and folate assays
- Viral studies, particularly for Epstein–Barr and hepatitis viruses.

If Fanconi’s anaemia is suspected:
- Studies of sensitivity of chromosomes to breakage by DNA crosslinking agent
- Radiology of hands and forearms

Haemolytic Anaemias
A haemolytic process may be suspected by the presence of a falling haemoglobin, a reticulocytosis, and jaundice with an increase in unconjugated bilirubin level (see Chapters 9, 10, and 11).

White Cell Disorders
The blood may appear entirely normal in some patients with white cell disorders (e.g., lymphoma, myelomatosis, immune deficiency, neutrophil dysfunction). Changes in white cell numbers or morphology may occur rapidly in response to local or systemic disorders. The investigation of white cell disorders is more likely to require marrow examination than investigation of red cell disorders, especially when a primary marrow disorder is suspected. In chronic leukaemias, bone marrow examination may add little to the diagnosis, but the pattern of infiltration may have prognostic significance, e.g., in chronic lymphocytic leukaemia. The distribution of white cells is better appreciated in trephine biopsies, which are particularly important in lymphomas.

Acute Leukaemia
- Bone marrow aspirate
- Bone marrow trephine biopsy if an adequate bone marrow aspirate is not obtained
- Cytochemical stains
- Blood or marrow immunophenotyping, unless obviously myeloid
- Cytogenetic analysis
- Molecular studies (e.g., fluorescence in situ hybridization) for rearrangements of specific oncogenes

Neutropenia
- Serial neutrophil counts for cyclical neutropenia
- Tests for antineutrophil antibodies
- Bone marrow aspirate and trephine biopsy
- Autoantibody screen and investigations for systemic lupus erythematosus
- Vitamin B12 and folate assays
- Acidified serum (Ham’s) test

Chronic Granulocytic Leukaemia
- Bone marrow aspirate
- Cytogenetic analysis
- Molecular studies (e.g., fluorescence in situ hybridization) for BCR–ABL rearrangement
- Neutrophil alkaline phosphatase score, if cytogenetic and molecular genetic analysis are not available

Chronic Lymphoproliferative Disorders/ Lymphadenopathy
- Serological screening for infectious mononucleosis, cytomegalovirus infection, HIV infection, and toxoplasmosis (if infectious cause suspected)
- Bone marrow aspirate and trephine biopsy (for detection of the presence and distribution of abnormal lymphocytes)
- Immunophenotyping
- Serum protein electrophoresis and immunoglobulin concentrations
- Serum urate, calcium, and lactate dehydrogenase (LDH)
- Lymph node biopsy (aspiration or surgical)
- Cytogenetic or molecular genetic analysis including investigation for immunoglobulin heavy chain or T-cell receptor gene rearrangement if the diagnosis of lymphoma is in doubt
- Radiological studies (X-ray, ultrasonography, computed tomography scan, magnetic resonance imaging)

Myelomatosis
- Bone marrow aspirate
- Bone marrow trephine biopsy if a cellular aspirate is not obtained
- Serum protein electrophoresis and immunoglobulin concentrations
• Serum albumin and calcium measurements
• β₂-microglobulin
• Urine (random and 24 hours) for Bence–Jones protein detection and quantitation
• Tests of renal function
• Radiological skeletal survey
• Serum free light chain quantification and ratio

Other Disorders

Myeloproliferative Disorders
• Blood volume, red cell mass, and plasma volume (for polycythaemia) measurements
• Bone marrow aspirate and trephine biopsy
• Arterial oxygen saturation and carboxyhaemoglobin level
• Abdominal ultrasound examination
• Neutrophil alkaline phosphatase
• Vitamin B₁₂ (or B₁₂-binding capacity)
• Serum urate
• JAK2 analysis

Myelodyplasia
• Bone marrow aspirate and trephine biopsy
• Cytogenetic analysis

“Idiopathic” Myelofibrosis
• Bone marrow trephine biopsy
• Red cell folate assay
• Urate

If splenectomy is contemplated:
• Ferrokinetic and red cell survival studies
• Spleen scan and red cell pool measurement.

Pancytopenia with Splenomegaly
• Bone marrow aspirate and trephine biopsy
• Bacterial culture of marrow for tuberculosis
• Marrow examination for amastigotes of *Leishmania donovani*
• Biopsy of palpable lymph nodes (aspiration or surgical)
• Vitamin B₁₂ and folate assays
• Liver biopsy
• Splenic aspirate
• Acidified serum (Ham’s) test
• Serum rheumatoid factor and autoantibody screen
• Laparotomy and splenectomy.

The rationale behind these tests, and details of investigations outside general haematology practice, can be found in comprehensive haematology textbooks, in electronic databases, and on Web sites.

**CLASSIFICATION OF HAEMATOLOGICAL NEOPLASMS**

Since the late 1970s, acute leukaemia and the MDS have usually been defined and further classified according to the proposals of the French–American–British (FAB) group. These classifications are now being supplanted by the World Health Organization (WHO) classifications, published in definitive form in 2001. The WHO classifications are much broader in their aims and include myeloproliferative and chronic lymphoproliferative disorders.¹,²

Application of the WHO criteria requires the results of immunophenotyping and cytogenetic analysis. The FAB classifications therefore continue to have a place (a) when these techniques are not available and (b) in making a provisional morphological diagnosis while awaiting the results of further tests. It may be necessary to issue a provisional report, so that treatment can commence, with a supplementary report following when all diagnostic procedures have been completed. It is important that, whichever classification is used, the criteria are strictly observed so that there is consistency between different centres and countries. To avoid any possibility of confusion, FAB terminology (e.g., M1, M2) should not be applied if the WHO classification is being used.

**Classification of Acute Myeloid Leukaemia**

The FAB criteria for a diagnosis of AML (Table 23.1) are as follows:

1. Blast cells must constitute at least 30% of all bone marrow cells or
2. When erythroid cells are at least 50% of bone marrow cells and blasts cells must be at least 30% of non-erythroid cells (lymphocytes, plasma cells, macrophages, and mast cells also being excluded from the count) or
3. The characteristic cytological features of acute hypergranular promyelocytic leukaemia or its variant form are present and
4. Blast cells are shown to be myeloid by either there being at least 3% of blast cells positive for Sudan black B, myeloperoxidase, or nonspecific esterase or by demonstration of myeloid antigens on immunophenotyping.

The WHO classification categorises cases as AML if the following criteria are met:

1. There are at least 20% of blast cells of myeloid lineage in the blood or bone marrow or
2. If the erythroid cells are at least 50% of bone marrow cells, blast cells are at least 20% of nonerythroid cells, or
3. Primitive erythroid cells constitute at least 80% of bone marrow cells or
4. There is a myeloid sarcoma (granulocytic sarcoma) or
5. One of a number of specified chromosomal rearrangements is present (Table 23.2).

In the FAB classification, AML is further classified as shown in Table 23.1. In the WHO classification, AML is further categorised as shown in Table 23.2. It should be noted that the WHO classification is hierarchical. If appropriate, cases are first assigned to the category of therapy-related leukaemia. Next, cases are assigned, if appropriate, to the category of AML with recurrent genetic abnormalities. Cases continue to be assigned to successive categories in the order shown in Table 23.2 with remaining cases finally being categorised as “AML not otherwise categorized.” This final group is further subdivided into categories resembling those of the FAB classification (but defined in quite a different manner). The “not-otherwise-categorized” group includes several entities that are either newly defined (acute panmyelosis with myelofibrosis and pure erythroid leukaemia) or were not specifically mentioned in the FAB classification (acute basophilic leukaemia and myeloid sarcoma, either granulocytic or monocytic).

| Table 23.1 The French–American–British (FAB) classification of acute myeloid leukaemia |
|---------------------------------------------|---------------------------------------------|
| Category | Criteria |
| M0 | <3% of blasts MPO or SBB positive, lymphoid markers negative, immunological or ultrastructural features of myeloid differentiation |
| M1 | Blasts ≥90% of bone marrow NEC, ≥3% of blasts MPO or SBB positive, maturing monocytic component in bone marrow ≤10%, maturing granulocytic component in bone marrow ≤10% |
| M2 | Blasts 30–89% of BM NEC, maturing granulocytic component >10% NEC, BM monocytic component <20% of NEC and other criteria of M4 not met |
| M3 | Characteristic morphology |
| M4 | Blasts ≥30% of BM NEC, granulocytic component ≥20% of BM NEC, monocytic component ≥20% of BM NEC and either PB monocytes ≥5 × 10⁹/l or BM like M2 but PB monocytes ≥5 × 10⁹/l and cytochemical proof of monocytic differentiation |
| M5a | Blasts ≥30% of NEC, BM monocytic component ≥80% of NEC, monoblasts ≥80% of BM monocytic component |
| M5b | Blasts ≥30% of NEC, BM monocytic component ≥80% of NEC, monoblasts <80% of BM monocytic component |
| M6 | Erythroid cells ≥50% of BM cells, BM blasts ≥30% of NEC |
| M7 | Blasts shown to be predominantly megakaryoblasts |

MPO, myeloperoxidase; SBB, Sudan black B; BM, bone marrow; NEC, non-erythroid cells; PB, peripheral blood.
Classification of the Myelodysplastic Syndromes

The FAB criteria for a diagnosis of MDS are that there is evidence for a myeloid neoplasm with ineffective haemopoiesis but the criteria for AML are not met. Blast cells must be less than 30% in the bone marrow. In the WHO classification, there must be evidence for a myeloid neoplasm with ineffective haemopoiesis and blasts must be less than 20% in both blood and bone marrow. In addition, the specific cytogenetic abnormalities shown in Table 23.2 must be absent (or the case is categorized as AML, regardless of the blast count). There is a further major difference between the two classifications, specifically that chronic myelomonocytic leukaemia is classified as MDS in the FAB classification, whereas in the WHO classification it is assigned to a new category of disorder designated myelodysplastic/myeloproliferative diseases. The details of the FAB classification of MDS are shown in Table 23.3 and of the WHO classification in Table 23.4. It will be noted that cytogenetic analysis is essential for the application of the WHO classification because cases of the 5q- syndrome cannot otherwise be recognized. Like the WHO classification of AML, this is a hierarchical classification. Therapy-related MDS is categorized with therapy-related AML. Remaining cases are then assessed as to whether they meet the criteria for the 5q- syndrome. If they do not, they are assigned to the remaining categories, depending on the number of lineages showing dysplasia, the percentage of ring sideroblasts, the presence or absence of Auer rods, and the percentage of blast cells in the blood and marrow. The details of the WHO classification of the myelodysplastic/myeloproliferative diseases are shown in Table 23.5.

Table 23.2 The World Health Organization classification of acute myeloid leukaemia (AML)

<table>
<thead>
<tr>
<th>Classification of AML and myelodysplastic syndrome*</th>
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<tr>
<td>Alkylating agent related</td>
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<tr>
<td>Topo-isomerase II inhibitor related</td>
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<td>Other types</td>
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<td>AML with recurrent genetic abnormalities*</td>
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<td>AML with t(8;21)(q22;q22)/AML1-ETO fusion</td>
</tr>
<tr>
<td>AML with abnormal bone marrow eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22)/CBFB-MYH11 fusion</td>
</tr>
<tr>
<td>Acute promyelocytic leukaemia with t(15;17)(q22;q12)/PML-RARA fusion, and variants</td>
</tr>
<tr>
<td>AML with 11q23 rearrangement and MLL abnormality</td>
</tr>
<tr>
<td>AML with multilineage dysplasia†</td>
</tr>
<tr>
<td>Following a myelodysplastic syndrome or a myelodysplastic/myeloproliferative syndrome</td>
</tr>
<tr>
<td>Without antecedent myelodysplastic syndrome</td>
</tr>
<tr>
<td>AML not otherwise categorized</td>
</tr>
<tr>
<td>AML, minimally differentiated (resembles FAB M0)</td>
</tr>
<tr>
<td>AML without maturation (resembles FAB M1)</td>
</tr>
<tr>
<td>AML with maturation (resembles FAB M2)</td>
</tr>
<tr>
<td>Acute myelomonocytic leukaemia (resembles FAB M4)</td>
</tr>
<tr>
<td>Acute monoblastic and acute monocytic leukaemia (resembles FAB M5a, M5b)</td>
</tr>
<tr>
<td>Acute erythroid leukaemia</td>
</tr>
<tr>
<td>Erythroleukaemia (resembles FAB M6)</td>
</tr>
<tr>
<td>Pure erythroid leukaemia</td>
</tr>
<tr>
<td>Acute megakaryoblastic leukaemia (resembles FAB M7)</td>
</tr>
<tr>
<td>Acute basophilic leukaemia</td>
</tr>
<tr>
<td>Acute panmyelosis with myelofibrosis</td>
</tr>
<tr>
<td>Myeloid sarcoma (granulocytic or monocytic)</td>
</tr>
</tbody>
</table>

*If therapy-related cases have recurrent cytogenetic abnormalities, this is noted.
†Defined as having at least 50% of cells dysplastic in at least 2 lineages.
globulin, and the condition represents a leukaemic presentation of Burkitt’s lymphoma. The WHO classification categorizes such cases as Burkitt’s lymphoma. This is more appropriate than their being categorized as ALL because the treatment of these cases differs very considerably from the treatment of ALL.

Classification of Acute Lymphoblastic Leukaemia

Both the FAB and WHO classifications require that an acute leukaemia be positively shown to be lymphoid before it is categorized as acute lymphoblastic leukaemia (ALL) so as to avoid inadvertently categorising FAB M0 and M7 AML as ALL. The WHO classification groups together ALL and lymphoblastic lymphoma, using the designations precursor B lymphoblastic leukaemia/lymphoblastic lymphoma and precursor T lymphoblastic leukaemia/lymphoblastic lymphoma. These designations are clearly too cumbersome to use in clinical practice, and undoubtedly haematologists will continue to refer to “acute lymphoblastic leukaemia.” The FAB group classified ALL into three morphological categories, designated L1, L2, and L3 ALL. It is of little significance whether a case falls into the L1 or L2 category, and this distinction can be dropped. However, L3 morphology—the presence of “blast cells” with basophilic cytoplasm and vacuolation—is of considerable clinical significance. In most, but not all, of these cases the cells are immunologically mature, expressing surface membrane immuno-

Classification of Myeloproliferative Disorders

The WHO classification of the myeloproliferative disorders is summarized in Table 23.6. Most of these conditions are defined in accordance with established haematological practice. However, the method of distinguishing between essential thrombocythaemia and idiopathic myelofibrosis differs from previous practice with many cases that would previously have been categorized as essential thrombocythaemia now being classified as the cellular phase of myelofibrosis; whether this classification will be widely accepted remains to be seen. The WHO classification of myeloproliferative disorders takes little account of cytogenetic or molecular genetic analysis, only Ph-positive chronic myeloid leukaemia being defined...
### Table 23.4 Summary of World Health Organization classification of the myelodysplastic syndromes (MDS)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Peripheral blood findings</th>
<th>Bone marrow findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS associated with isolated del(5q)</td>
<td>Anaemia, platelet count usually normal or elevated, &lt;5% blasts</td>
<td>Megakaryocytes normal or increased but with hypolobated nuclei, &lt;5% blasts, no Auer rods</td>
</tr>
<tr>
<td>Refractory anaemia (RA)</td>
<td>Anaemia, no or rare blasts</td>
<td>Dysplasia confined to erythroid lineage, &lt;5% blasts, &lt;15% ringed sideroblasts</td>
</tr>
<tr>
<td>Refractory anaemia with ringed sideroblasts (RARS)</td>
<td>Anaemia, no blasts</td>
<td>Dysplasia confined to erythroid lineage, &lt;5% blasts, ≥15% ringed sideroblasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
<td>Cytopenias (bicytopenia or pancytopenia), no or rare blasts, no Auer rods, &lt; 1 × 10⁹/l monocytes</td>
<td>Dysplasia in ≥10% of the cells of two or more myeloid cell lineages, &lt;5% blasts, &lt;15% ringed sideroblasts, no Auer rods</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)</td>
<td>Cytopenias (bicytopenia or pancytopenia), no or rare blasts, no Auer rods, &lt; 1 × 10⁹/l monocytes</td>
<td>Dysplasia in ≥10% of the cells of two or more myeloid cell lineages, &lt;5% blasts, ≥15% ringed sideroblasts, no Auer rods</td>
</tr>
<tr>
<td>Refractory anaemia with excess blasts-1 (RAEB-1)</td>
<td>Cytopenias, &lt;5% blasts, no Auer rods, &lt;1 × 10⁹/l monocytes</td>
<td>Unilineage or multilineage dysplasia, 5–9% blasts, no Auer rods</td>
</tr>
<tr>
<td>Refractory anaemia with excess blasts-2 (RAEB-2)</td>
<td>Cytopenias, 5–19% blasts, Auer rods sometimes present, &lt;1 × 10⁹/l monocytes</td>
<td>Unilineage or multilineage dysplasia, 10–19% blasts, Auer rods sometimes present</td>
</tr>
<tr>
<td>Myelodysplastic syndrome-unclassifiable (MDS-U)</td>
<td>Cytopenias, no or rare blasts, no Auer rods</td>
<td>Unilineage dysplasia (megakaryocytic or granulocytic), &lt;5% blasts, no Auer rods</td>
</tr>
</tbody>
</table>
### Table 23.5 Summary of the World Health Organization categories of myelodysplastic/myeloproliferative diseases

<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myelomonocytic leukaemia (CMML)</td>
<td>A Ph-negative, BCR–ABL-negative disorder with monocyte count $&gt; 1 \times 10^9/l$ Fewer than 20% blasts plus promonocytes in peripheral blood (PB) or bone marrow (BM) Either dysplasia of one or more myeloid lineages or alternative criteria met (acquired clonal cytogenetic abnormality or monocytosis persisting for at least 3 months and alternative causes of monocytosis excluded)</td>
</tr>
<tr>
<td>Atypical chronic myeloid leukaemia (aCML)</td>
<td>A Ph-negative, BCR–ABL-negative disorder with leucocytosis resulting from an increase in neutrophils and their precursors, the precursors (promyelocyte to metamyelocytes) constituting a least 10% of PB white cells Basophils less than 2% of white cells Monocytes less than 10% of white cells Hypercellular BM with granulocytic hyperplasia and dysplasia, with or without dysplasia of other lineages Fewer than 20% blasts plus promonocytes in peripheral blood or bone marrow</td>
</tr>
<tr>
<td>Juvenile myelomonocytic leukaemia (JMML)</td>
<td>A Ph-negative, BCR–ABL–negative disorder with monocyte count $&gt; 1 \times 10^9/l$ Fewer than 20% blasts plus promonocytes in peripheral blood or bone marrow Plus two or more of the following Haemoglobin F increased for age Immature granulocytes in the PB WBC greater than $10 \times 10^9/l$ Clonal chromosomal abnormality (monosomy 7 not excluded) GM-CSF hypersensitivity of myeloid precursors in vitro</td>
</tr>
<tr>
<td>Myelodysplastic/myeloproliferative disease, unclassifiable</td>
<td>A myelodysplastic/myeloproliferative disorder in which the criteria of one of the myelodysplastic syndromes are met There are prominent proliferative features (e.g., a platelet count of greater than $600 \times 10^9/l$ or a white cell count of greater than $13 \times 10^9/l$) The condition has developed de novo The criteria for other MDS/MPD (CMML, aCML, and JMML) are not met There is no Philadelphia chromosome, BCR–ABL fusion gene, 5q–, inv(3)(q21q26) or t(3;3)(q21;q26)</td>
</tr>
</tbody>
</table>
on this basis. It should be noted that the WHO classification defines a case with hypereosinophilia as eosinophilic leukaemia, rather than as the idiopathic hypereosinophilic syndrome, when there is evidence of clonality. The 4q12 syndrome, resulting from a cryptic deletion at 4q12 with formation of a FIP1L1-PDGFRα fusion gene, is therefore categorized as eosinophilic leukaemia.

REFERENCES