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23

Approach to the Diagnosis and Classification of Blood Cell Disorders

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CHAPTER OUTLINE

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COMMON PRESENTATIONS OF HAEMATOLOGICAL DISEASES

An abnormal blood count or blood cell morphology does not necessarily indicate a primary haematological problem because it may reflect an underlying nonhaematological 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 nonspecific symptoms characteristic of leukaemias and lymphomas such as malaise, sweats or weight loss.

As with any clinical problem, the first steps in determining the diagnosis include obtaining a careful clinical, travel and drug history and a 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.

INITIAL SCREENING TESTS

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 26) and examination of a peripheral blood film 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.

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, ethnic group 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 overproduction of cells originating in an early precursor cell. This occurs in myeloproliferative neoplasms in which one cell type may predominate, e.g. platelets in essential thrombocythaemia and red cells in polycythaemia vera.

Erythrocytosis

Patients with a persistently (e.g. >2 months) raised venous haematocrit (Hct) (>0.52 males, >0.48 females) should be assessed to determine the cause. Erythrocytosis can be relative or absolute and, if absolute, primary or secondary.

Relative: normal total red cell volume with reduced plasma volume (e.g. dehydration).

Absolute: males and females with Hct values above 0.60 and 0.56, respectively, can be assumed to have an absolute erythrocytosis and do not require confirmatory studies.[1] However, the reason for the elevation of the Hct must still be elucidated.

Primary: this is usually polycythaemia vera (PV), part of the spectrum of myeloproliferative neoplasms. The mutation *JAK2* V617F is present in approximately 95% of patients with PV and mutations affecting exon 12 occur in many of those who lack the V617F mutation.[2]

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 or high performance liquid chromatography (HPLC) plus an oxygen dissociation curve and abdominal ultrasound examination. If initial screening tests are negative for a *JAK2* mutation and there is no obvious secondary cause for the high Hct, then red cell mass studies are indicated (Chapter 17).

Leucocytosis

Neutrophilia. Neutrophils are commonly increased during pregnancy and in acute infections, inflammation, alcohol intoxication, corticosteroid therapy and acute blood loss or red cell destruction. Additional findings on the full blood count can be helpful to identify the cause of neutrophilia. The combination of anaemia and neutrophilia occurs in chronic infection or inflammation, and also in malignant conditions; a high Hct with neutrophilia suggests polycythaemia vera. Neutrophilia with an increased platelet count occurs in infectious or inflammatory processes or malignant conditions and during marrow recovery. Neutrophilia with thrombocytopenia is classically seen in sepsis and occasionally in microangiopathic haemolytic anaemia. Examination of the peripheral blood film also provides additional clues to confirm or exclude a particular diagnosis. For example, 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 myelogenous leukaemia (CML), and cytogenetic and molecular studies to look for t(9;22) and the *BCR–ABL1* fusion gene are indicated (Chapter 8).

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 (Table 23-1). 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 specimen (and if necessary other tissue biopsy) are used to classify these disorders and to give an indication of management and prognosis.[3] If lymph nodes are enlarged, a lymph node biopsy for histology and immunohistochemistry may be helpful in diagnosis. It is occasionally difficult to differentiate between a reactive and a neoplastic lymphocytosis. In this situation, immunophenotyping, to provide 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.

TABLE 23-1

CAUSES OF LYMPHOCYTOSIS

Infections
- Predominantly viral (commonest is infectious mononucleosis)
- Occasionally bacterial (e.g. pertussis and chronic infections like tuberculosis)
- Unusually parasites (e.g. babesiosis)

Stress and Postsplenectomy

Smoking

Hypersensitivity Reactions

Autoimmune Disorders

Thymoma

Clonal
- Monoclonal B cell lymphocytosis
- Lymphoproliferative disorders especially chronic lymphocytic leukaemia and lymphomas

Monocytosis. A slight to moderate monocytosis may be associated with some protozoal, rickettsial and bacterial infections including malaria, typhus and tuberculosis. Monocytosis associated with neutrophilia is suggestive of chronic myelomonocytic leukaemia. High levels of monocytes (monocyte count $>1\times10^9$/l) in an elderly patient suggest chronic myelomonocytic leukaemia or sometimes, atypical chronic myeloid leukaemia. These conditions fall into the myelodysplastic/myeloproliferative neoplasm group of disorders,[4] so the diagnosis is supported by finding splenomegaly, quantitative and qualitative abnormalities in other cell lines or a clonal cytogenetic abnormality.

Eosinophilia. Eosinophilia is typically associated with parasitic infections, skin diseases and allergic disorders. Eosinophils have a tendency to infiltrate and damage tissues such as the heart, lungs and gut, so in patients with eosinophilia, assessment of these organs is necessary. In most cases, the cause of the eosinophilia is indicated by the clinical history, which should include details of all medications and foreign travel, and by examination of the stool and urine for parasites, cysts and ova. Other causes of eosinophilia are given in Table 23-2.

Basophilia. Basophilia as an isolated finding is unusual. However, it is a common feature of myeloproliferative neoplasms, and basophils may be particularly prominent in CML. In this condition, an increasing basophil count may be the first indication of accelerated phase disease. Endocrinopathies such as myxoedema and oestrogen abnormalities, infections and allergic diseases – and rarely, other haematological malignancies – can also cause basophilia.

TABLE 23-2

CAUSES OF EOSINOPHILIA[13,14]

Parasitic Infections – Especially with Helminths

Neoplastic Diseases

- Primary (or neoplastic) hypereosinophilia, e.g. associated with *FIP1L1-PDGFRA* fusion gene (or occasionally *PDGFRB* or *FGFR1* rearrangement or *PCM1-JAK2* fusion)
- Other acute or chronic eosinophilic leukaemia
- Other myeloproliferative neoplasms such as chronic myeloid leukaemia and systemic mastocytosis
- Reactive to other neoplasms, e.g. to B- or T-cell lymphoma or leukaemia or solid tumour

Allergic Disorders

- Gastrointestinal disorders – may be associated with tissue eosinophilia rather than peripheral blood eosinophilia
- Drug reactions including the DRESS syndrome (drug reaction with eosinophilia and systemic symptoms)
- Allergic rhinitis, asthma and atopic dermatitis

Immunodeficiency Disorders

- Hyper IgE (Job) syndrome
- Autoimmune lymphoproliferative syndrome
- Graft-versus-host disease

Connective tissue/rheumatology disorders

Ig, immunoglobulin.

Thrombocytosis

Thrombocytosis can be primary or secondary (reactive) to surgery, infectious and inflammatory conditions, hyposplenism, blood loss and malignancy, and can occur as a rebound phenomenon following recovery from marrow suppression. Spurious thrombocytosis can also occur in severe burns and cryoglobulinaemia because the size of the red cell fragments or cryoglobulin particles is similar to that of platelets. A moderately increased platelet count (e.g. $450–800\times10^9$/l) often does not indicate a primary haematological disorder. When there is isolated persistent thrombocytosis in a myeloproliferative neoplasm the diagnosis is essential thrombocythaemia (providing that the presence of a *BCR–ABL1* fusion gene has been excluded). Thrombotic or haemorrhagic complications can occur with thrombocytosis but often the diagnosis is made incidentally.[5] Individuals with essential thrombocythaemia have been noted to have *JAK2* V617F (50%), *MPL* (10%) or *CALR* mutations, with the *JAK2* mutation being associated with an increased risk of thrombosis.

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 organs. Reduced production of cells may be the result of aplastic anaemia, a lack of haematinics such as folate or cobalamin or interference with normal haemopoiesis by infiltration (e.g. leukaemia), infection (e.g. human immunodeficiency virus (HIV) infection, tuberculosis, leishmaniasis) or exposure to toxins (e.g. alcohol) or myelosuppressive drugs (e.g. hydroxycarbamide or methotrexate). Certain myeloid neoplasms, e.g. primary myelofibrosis and myelodysplastic syndromes (MDS), are characterised by cytopenias, which are at least in part the result of ineffective haemopoiesis. Cytopenia is also sometimes a feature of acute myeloid leukaemia (AML), when it is due both to ineffective haemopoiesis and to replacement of normal haemopoietic stem cells by leukaemic cells. A relatively common cause of a global reduction in circulating cells is pooling of the cells in a markedly enlarged spleen (hypersplenism), which may be secondary to conditions such as primary myelofibrosis and portal hypertension. Examination of a bone marrow aspirate and trephine biopsy specimen is often helpful in determining the cause of cytopenias for which no obvious cause is apparent.

Anaemia

The mechanisms which result in anaemia are decreased production, reduced red cell lifespan, blood loss and splenic pooling. Anaemia is broadly divided into three types: microcytic (low mean cell volume (MCV)), macrocytic (high MCV) and normocytic (normal MCV). The choice of investigations is guided by the MCV and red cell morphology in

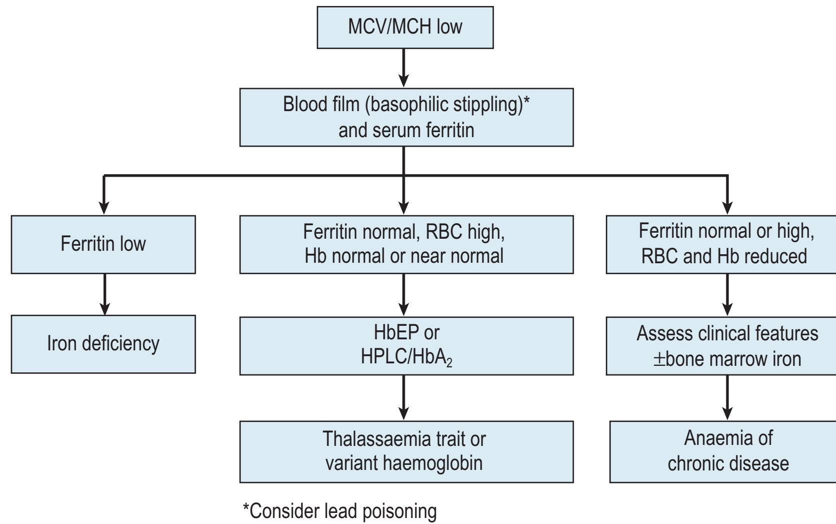

FIGURE 23-1 Investigation of a microcytic hypochromic anaemia. HbEP, haemoglobin electrophoresis; HPLC, high performance liquid chromatography.

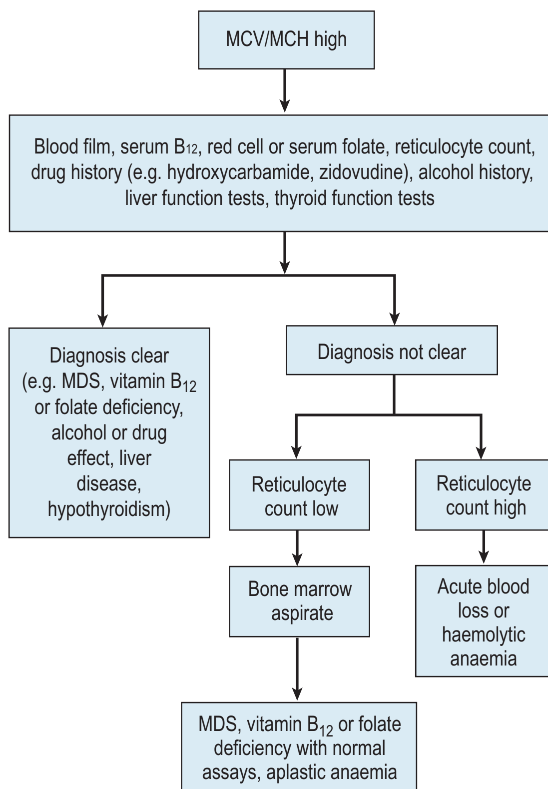

FIGURE 23-2 Investigation of a macrocytic anaemia. MDS, myelodysplastic syndrome.

addition to clinical features. Figures 23-1 to 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, though confirmation may require more specific tests. The presence of basophilic stippling with microcytic red cells suggests thalassaemia trait or, much less often, 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

The most common cause of anaemia worldwide is iron deficiency, which can be suspected from a low MCV (Fig. 23-1) and the presence of hypochromic, microcytic red cells. Laboratory confirmation of iron deficiency can be based on measurement of (1) serum ferritin or (2) serum iron plus either total iron-binding capacity or transferrin or (3) red cell protoporphyrin or (4) staining of bone marrow aspirates for iron (see Chapter 4).[6] A diagnosis of iron deficiency must be followed by 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 of the gastrointestinal tract to exclude occult malignancy and tests for coeliac disease. The differential diagnosis of iron deficiency anaemia includes anaemia of chronic disease (also known as anaemia of inflammation). Clinical and laboratory features of inflammation or chronic infection may suggest this diagnosis, which is confirmed by demonstration of normal or high serum ferritin and reduced serum iron, transferrin and iron-binding capacity. Serum soluble transferrin receptors may be helpful in distinguishing between iron deficiency anaemia and anaemia of chronic disease when interpretation of ferritin levels is difficult, though additional research is needed to define the overall diagnostic accuracy of these tests.[7]

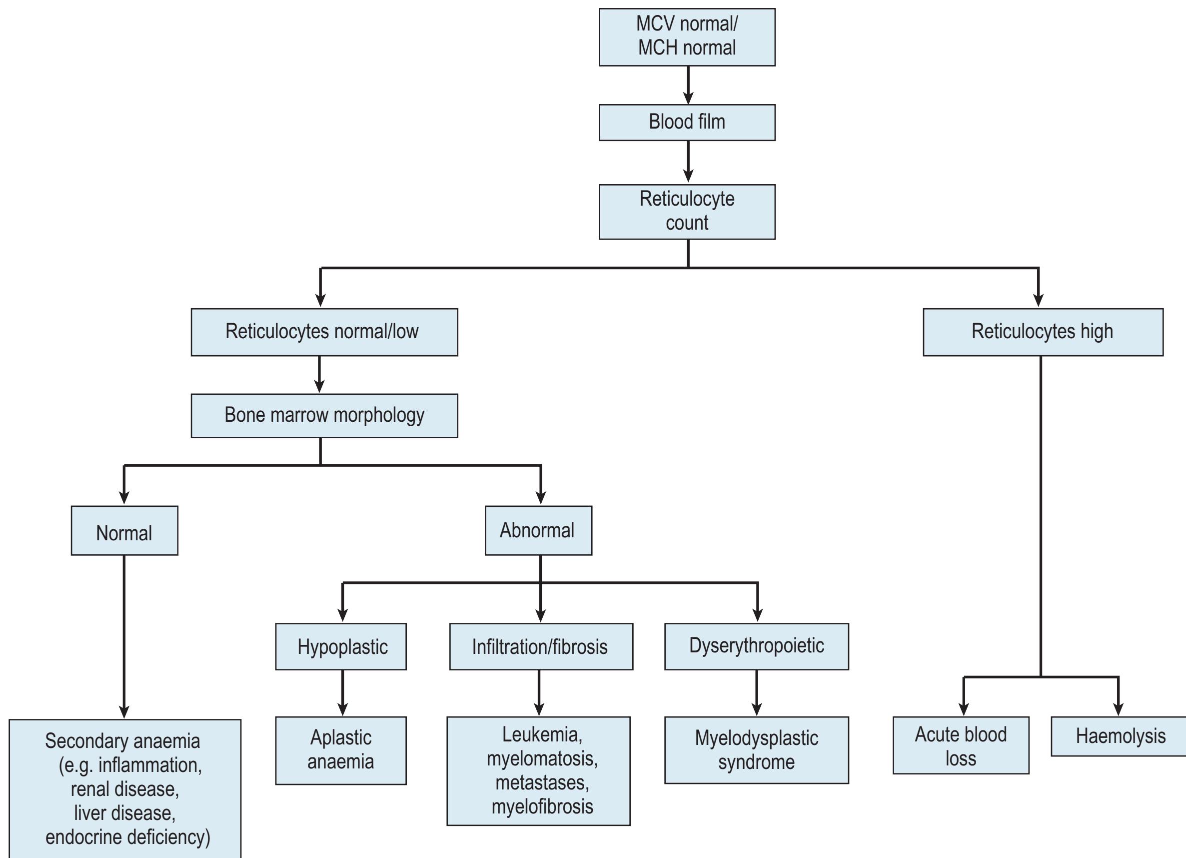

FIGURE 23-3 Investigation of a normocytic, normochromic anaemia.

The thalassaemias also cause microcytosis, but both α and β thalassaemia trait are usually associated with an increased red blood cell count (RBC) and a normal or near-normal Hb despite a considerable reduction of the MCV and mean cell haemoglobin (MCH). In contrast, in iron deficiency the MCV and MCH do not fall until the Hb is significantly reduced. Further investigations, such as HPLC or haemoglobin electrophoresis supplemented by measurement of haemoglobin A_2 and haemoglobin F usually confirm the diagnosis of β thalassaemia trait. The diagnosis of α thalassaemia trait is more difficult; detection of infrequent haemoglobin H inclusions is usually possible in α^0 thalassaemia trait, but definitive diagnosis requires deoxyribonucleic acid (DNA) analysis (see Chapter 8).[8] A diagnosis of α^0 thalassaemia heterozygosity can be clinically important for prediction of haemoglobin Bart's hydrops fetalis.

Macrocytic anaemia

A high MCV (Fig. 23-2) with oval macrocytes and hypersegmented neutrophils suggests folate or cobalamin deficiency and is an indication for assays of these vitamins (see Chapter 10). Plasma methylmalonic acid assays may be a useful second-line test to help clarify uncertainties of underlying biochemical or functional cobalamin deficiencies. Serum folate is the first-line test to assess folate status and has equivalent diagnostic capability to red cell folate.[9] Subsequent investigations could include malabsorption studies, tests for coeliac disease and screening for intrinsic factor antibodies. In patients with these blood film findings and normal folate and cobalamin assays, haematinic deficiency cannot be completely excluded and further investigations are indicated (see Chapter 10). As there is no definitive test to define cobalamin deficiency, treatment should be started if there is a strong clinical suspicion of deficiency irrespective of the test results to avoid neurological impairment. In the absence of intrinsic factor antibodies, the diagnosis of pernicious anaemia may be presumptive. Pernicious anaemia is commonly associated with autoimmune thyroid disease and other autoimmune disorders, such as diabetes mellitus.

A high MCV may also be the result of alcohol excess and liver disease or the use of drugs such as hydroxycarbamide. Macrocytosis resulting from chronic haemolysis is associated with increased numbers of immature red cells, which appear slightly larger and bluer than normal red cells (polychromatic macrocytes) on a Romanowsky-stained peripheral blood film. An automated reticulocyte count or supravital staining of blood films (see p. 27) 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 a microangiopathic haemolytic anaemia. This is a medical emergency because these may be features of thrombotic thrombocytopenic purpura, which requires immediate treatment, usually by plasma exchange. These features should therefore trigger further tests such as a platelet count, coagulation studies, assessment of renal function, measurement of ADAMTS13 concentration and a search for infection or neoplastic disease.

Normocytic anaemia

Normochromic, normocytic anaemia (Fig. 23-3) is frequently the result of an underlying chronic, nonhaematological 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 lack of compensatory increased red cell production in blood loss or haemolysis. Examination of the bone marrow may be helpful in demonstrating haematological causes for a normochromic, normocytic anaemia such as MDS or aplastic anaemia.[10] 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 (see p. 15), the nonhaematological 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 lymphocytic 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 marrow myeloid precursors). Typical marrow appearances occur in drug-induced neutropenia, in which there is a relative paucity of mature neutrophils and in infant genetic agranulocytosis (Kostmann syndrome) in which 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 physical stress such as surgery, trauma and infection. Lymphopenia with neutrophilia is a common combination of haematological abnormalities in severe acute respiratory syndrome and in many other patients with acute illness or trauma. Lymphopenia, especially affecting the CD4 cells, may occur in HIV infection and renal failure. Monocytopenia (monocyte count $<0.2 \times 10^9/l$) 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 clumping and platelet satellitism. Platelet clumping, which is seen on the blood film, can occur *in vitro* as the result of a temperature-dependent or anticoagulant-dependent autoantibody or on slides that have been made directly from a finger prick sample. True thrombocytopenia is most frequently the result of autoantibodies (i.e. immune thrombocytopenia), HIV infection, anticancer chemotherapy, other drugs (such as thiazide diuretics), alcohol excess, hypersplenism and MDS. Heparin-induced thrombocytopenia and thrombosis is a particularly important syndrome to recognise (see Chapter 20).

The first step in the assessment of patients with thrombocytopenia is the examination of a blood film. The clinical circumstances, together with blood film and bone marrow examination, usually enable the various causes of thrombocytopenia to be differentiated. An association with thrombosis, disturbed renal or hepatic function and haemolytic anaemia should prompt investigations for other diseases, such as thrombotic thrombocytopenic purpura and, in a pregnant woman, the HELLP (haemolysis, elevated liver enzymes, low platelet count) syndrome. The presence of thrombocytopenia with atypical features on the blood film may prompt a bone marrow examination to exclude conditions such as acute leukaemia, especially in children.

Pancytopenia

Pancytopenia means a reduction in the WBC, Hb and platelet count and is most often the result of anticancer chemotherapy, HIV infection, hypersplenism and bone marrow infiltration or failure. Reduction of two cell lineages is referred to as bicytopenia and has similar causes to pancytopenia. Careful examination of a blood film is important if the reason for the cytopenia is not apparent from the clinical history. If this does not reveal the cause, bone marrow aspiration and trephine biopsy may be needed.

Qualitative abnormalities of blood cells

In health, only the most mature forms of cells appear in the peripheral blood. Cells at various stages of immaturity, 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 from 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 MDS. These features are reflected in the bone marrow with disturbance of the normal developmental pathway and sometimes nuclear:cytoplasmic asynchrony. Cytogenetic studies are helpful for confirming the diagnosis, especially when cytological abnormalities are minor, and can also assist in determining the prognosis.[11]

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 such as analysis of structural proteins, haemoglobin electrophoresis or HPLC, or enzyme assays. The type of red cell abnormality may also help to indicate underlying pathology. For example, target cells may prompt investigation of liver function, whereas increased rouleaux formation may indicate the need for investigations for multiple myeloma or inflammatory conditions.

White cells. Congenital abnormalities of neutrophils are unusual, but similar morphological abnormalities (e.g. pseudo-Pelger–Huët cells) may be seen in acquired conditions such as MDS (see Figs. 5-83 and 5-84). Reactive changes in lymphocytes, including increased size, irregular shape and basophilic cytoplasm, are typically seen in infectious mononucleosis (see Fig. 5-90), which can be diagnosed using an appropriate serological screening test or, if this is negative, by demonstration of immunoglobulin M (IgM) antibodies to the Epstein–Barr virus (EBV). These atypical lymphocytes can sometimes be difficult to differentiate from circulating lymphoma cells. 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 an abnormal *in vitro* platelet function test is characteristic of a disorder of platelet function, but some patients with abnormal platelet function also have thrombocytopenia. 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 platelet alpha-granule deficiency (i.e. grey platelet syndrome). Neutrophils should also be examined for inclusions indicative of *MYH9*-related disorders such as the May–Hegglin anomaly. Qualitative disorders of platelets can broadly be divided into two categories: abnormalities of the platelet membrane glycoproteins (e.g. Bernard–Soulier syndrome, Glanzmann thrombasthenia) and abnormalities of platelet secretory function (e.g. storage pool diseases).

Acquired disorders of platelet function are more common than inherited disorders. Haematological conditions associated with platelet dysfunction include myeloproliferative neoplasms, MDS and dysproteinaemias (in plasma cell neoplasms). Many widely prescribed drugs, including aspirin and nonsteroidal anti-inflammatory agents, interfere with platelet function. Systemic conditions, particularly chronic renal failure and cardiopulmonary bypass, are also 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 MDS and, to a lesser extent, in the myeloproliferative neoplasms, 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 indicative and are not intended to be exhaustive because the protocols and 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 9 and 14.

- Measurement of serum ferritin or iron plus either total iron-binding capacity or transferrin assay, red cell protoporphyrin or soluble transferrin receptors
- Bone marrow aspirate with staining for iron
- Stool examination for occult blood
- Gastrointestinal imaging and endoscopy, with biopsies if appropriate; rarely, blood loss studies with ^{51}Cr-labelled red cells
- Tests for malabsorption
- Serological tests for coeliac disease (e.g. tissue transglutaminase antibodies)
- Serum lead (if lead poisoning is suspected)

If thalassaemia is suspected:

- HPLC or haemoglobin electrophoresis plus haemoglobin A_2 and F measurements
- Haemoglobin H preparation
- Family studies
- DNA analysis (when the diagnosis is clinically important).

Macrocytic anaemias

If macrocytic, megaloblastic erythroid maturation is demonstrated, further investigations should be undertaken as described in Chapter 10. If the blood film is typical of megaloblastic anaemia, relevant assays and further

investigations can often indicate the diagnosis without the need for a bone marrow aspirate. Macrocytosis may also be secondary to conditions such as alcohol excess, liver disease, MDS, hydroxycarbamide administration and hypothyroidism. Reticulocytosis from any cause can also increase the MCV.

Aplastic anaemia[10]

- Cobalamin and folate assays (although bone marrow hypoplasia is rare)
- Viral studies, particularly for EBV, HIV and hepatitis viruses
- Bone marrow aspirate and trephine biopsy including cytogenetic analysis
- Flow cytometry for glycosylphosphatidylinositol-anchored proteins to detect a paroxysmal nocturnal haemoglobinuria (PNH) clone, followed by urine examination for haemosiderin if positive
- Peripheral blood gene mutation analysis for dyskeratosis congenita if there are relevant clinical features or lack of response to immunosuppressive therapy.

If Fanconi anaemia is suspected:

- Studies of sensitivity of chromosomes to breakage by DNA cross-linking agents.

Haemolytic anaemias

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

White cell disorders

The blood film is often of critical importance in the differential diagnosis of white cell disorders though it may sometimes be normal (e.g. in some patients with lymphoma or neutrophil functional defects). Changes in white cell numbers or morphology may occur rapidly in response to local or systemic disorders. In chronic leukaemias, bone marrow aspiration may add little to the diagnosis, but the pattern of infiltration of neoplastic cells seen on trephine biopsy can have diagnostic value or prognostic significance (e.g. in lymphoma and chronic lymphocytic leukaemia).

Acute leukaemia

- Full blood count and peripheral blood film
- Bone marrow aspirate and trephine biopsy
- Blood or marrow immunophenotyping for monitoring minimal residual disease (cytochemical stains can be used if immunophenotyping is not readily available)
- Cytogenetic analysis
- Molecular studies (e.g. fluorescence *in situ* hybridisation (FISH) analysis) for identification of acute lymphoblastic leukaemia (ALL) with hyperdiploidy or *ETV6–RUNX1* fusion, detection of *BCR–ABL1* fusion in adults with ALL and detection of other mutations of specific oncogenes (e.g. *NPM1*, *CEBPA* and possibly *FLT3* in AML).

Neutropenia

- Cobalamin and folate assays
- Autoantibody screen including rheumatoid factor and investigations for systemic lupus erythematosus
- Serial neutrophil counts for cyclical neutropenia
- Tests for antineutrophil antibodies
- Bone marrow aspirate and trephine biopsy
- Flow cytometry for PNH (see aplastic anaemia above)
- Consider the need for clonality studies for investigation for an abnormal T-cell population.

Chronic myelogenous leukaemia

- Full blood count and peripheral blood film
- Bone marrow aspirate
- Cytogenetic analysis
- Molecular studies (e.g. real-time quantitative reverse transcriptase or FISH) for *BCR–ABL1* transcripts
- Neutrophil alkaline phosphatase score using cytochemistry (only if cytogenetic and molecular genetic analysis are not available).

Chronic lymphoproliferative disorders and/or lymphadenopathy

Various specimens can be used for investigations including lymph nodes, bone marrow aspirates, trephine biopsy cores and peripheral blood and other fluids such as cerebrospinal fluid, ascitic fluid and pleural aspirates.[3]

- Full blood count and peripheral blood film
- Serum protein electrophoresis and immunoglobulin concentrations
- Plasma uric acid, calcium and lactate dehydrogenase (LDH)
- Serological screening for infectious mononucleosis, cytomegalovirus infection, HIV infection and toxoplasmosis (if infectious cause suspected) and human T-cell lymphotropic virus, when clinically relevant
- Bone marrow aspirate and trephine biopsy (to demonstrate the presence and distribution of abnormal lymphocytes) and/or lymph node or other tissue biopsy
- Flow cytometry immunophenotyping or immunohistochemistry of biopsy specimens
- 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
- Imaging (plain radiographs, ultrasonography, computed tomography scan, magnetic resonance imaging).

Myelomatosis (plasma cell myeloma)[12]

- Full blood count and peripheral blood film
- Serum protein electrophoresis, immunofixation and quantification of immunoglobulins and any paraprotein
- Urine electrophoresis and immunofixation for Bence–Jones protein (early morning urine sample and, if positive, quantification on 24 h collection)

- Serum free light chain quantification and ratio
- Serum albumin, tests of renal function, plasma uric acid, calcium, phosphate and alkaline phosphatase measurements
- β_2 microglobulin quantification
- Plasma viscosity
- Bone marrow aspirate (with cytogenetic or FISH analysis if results will influence treatment decisions, and flow cytometry immunophenotyping or DNA analysis if these analyses are to be used for monitoring minimal residual disease)
- Trephine biopsy
- Radiologic skeletal survey.

Other disorders

- Thrombocytopenia
- Full blood count and blood film
- Reticulocyte count
- Rh blood group
- Direct antiglobulin test
- HIV test
- Hepatitis screen
- *Helicobacter pylori* test
- Antinuclear antibodies
- Lupus anticoagulant
- Immunoglobulin profile

Myeloproliferative neoplasms

- Full blood count and blood film
- Cobalamin (or B_{12}-binding capacity)
- Uric acid assay
- *JAK2* and *CALR* mutation analysis if PV, ET or primary myelofibrosis is suspected
- Arterial oxygen saturation and carboxyhaemoglobin level (selected patients only)
- Abdominal ultrasound examination
- Cytogenetic analysis
- Bone marrow aspirate and trephine biopsy
- Serum erythropoietin assay
- Red cell and plasma volume (selected patients only).

If splenectomy is contemplated:

- Ferrokinetic and red cell survival studies
- Spleen scan and red cell pool measurement.

Myelodysplastic syndromes

- Full blood count and blood film
- Bone marrow aspirate and trephine biopsy
- Cytogenetic analysis.

Pancytopenia with splenomegaly

- Cobalamin and folate assays
- Serum rheumatoid factor and autoantibody screen
- Bone marrow aspirate and trephine biopsy
- Examination of bone marrow or splenic aspirate for amastigotes of *Leishmania donovani* and bacterial culture of marrow for infectious agents including mycobacteria
- Biopsy of palpable lymph nodes
- Liver biopsy
- Tests for PNH (see p. 271).

The rationale behind these tests and details of specialised investigations can be found in comprehensive haematology textbooks, in electronic databases and on websites.

CLASSIFICATION OF HAEMATOLOGICAL NEOPLASMS

Classifications for haematological neoplasms are based on World Health Organisation publications[3,13,14] which outline the international standards for assessment and diagnosis of haematological neoplasms. Application of the WHO criteria depends on the clinical history and physical examination, morphology (cytology or histology), immunophenotyping, cytogenetic analysis and, in some circumstances, molecular genetic analysis. The previous French–American–British (FAB) group classifications may be used (1) when these techniques are not all available and (2) in making a provisional morphological diagnosis (e.g. in acute leukaemia), while awaiting the results of further tests. Whichever classification is used, the criteria should be strictly observed so that there is consistency between different centres and countries. The WHO classification of haematological neoplasms has several major categories (Table 23-3).

Classification of acute myeloid leukaemia and related neoplasms

The WHO classification categorises cases as AML (Fig. 23-4) 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*

TABLE 23-3

WHO CLASSIFICATION OF MAJOR CATEGORIES OF MYELOID NEOPLASMS AND ACUTE LEUKAEMIAS
Myeloproliferative neoplasms (MPN)
Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of *PDGFRA, PDGFRB* or *FGFR1* or with *PCM1-JAK2*
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Myelodysplastic syndrome (MDS)
Acute myeloid leukaemia and related neoplasms
Acute leukaemias of ambiguous lineage
B lymphoblastic leukaemia/lymphoma
T lymphoblastic leukaemia/lymphoma

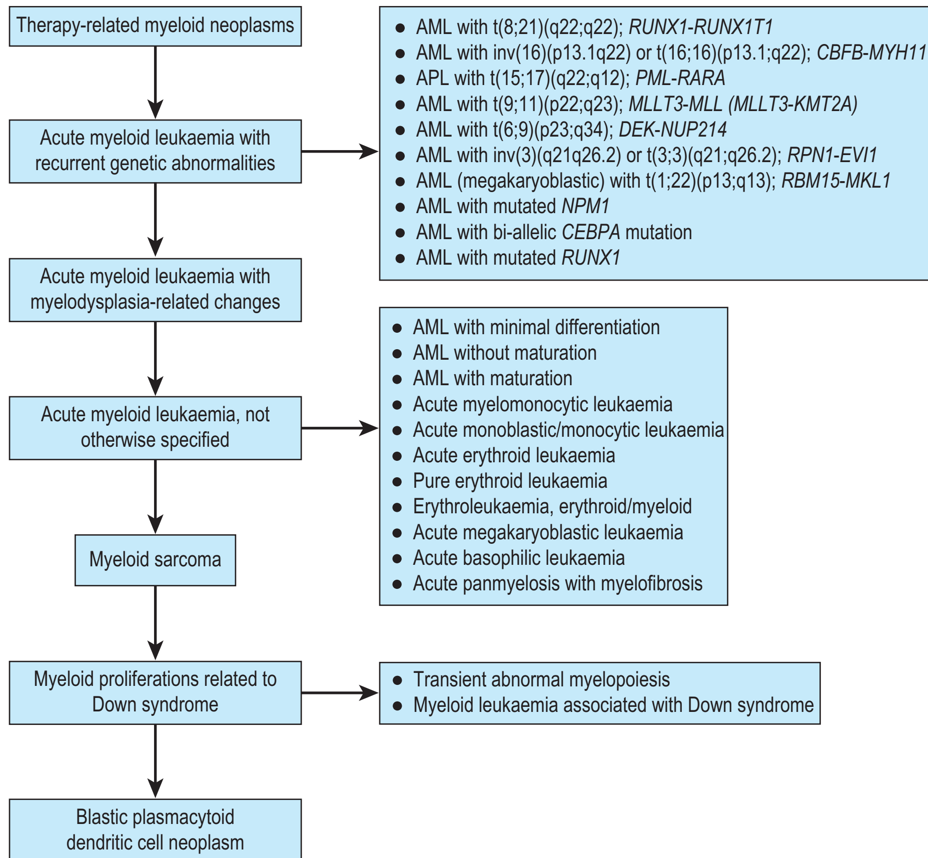

FIGURE 23-4 Hierarchical classification of acute myeloid leukaemia and related neoplasms. *Data from J. Vardiman, J. Thiele, D. Arber, et al. 2009. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes.* Blood *114 (5), 937–951.*

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.

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 23-4, with remaining cases finally being categorised as 'AML not otherwise specified.' Blastic plasmacytoid dendritic cell neoplasm and myeloid neoplasms associated with Down syndrome are recognised as specific entities.

The WHO classification of acute leukaemia[15] lists cytogenetic abnormalities that, in combination with ≥20 blasts, indicate a diagnosis of AML with myelodysplasia-related changes; assignment to this category can also be based on a previous history of MDS or on morphological evidence of dysplasia.

Classification of the myelodysplastic syndromes

The WHO classification of MDS (Table 23-4) requires evidence for a myeloid neoplasm with ineffective and, generally, dysplastic haemopoiesis; blasts must be <20% in both blood and bone marrow (Table 23-5). 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 recognised. Like the classification of AML, this is a hierarchical classification. Therapy-related MDS is categorised with therapy-related AML. Remaining cases are then assessed to ascertain whether they meet the criteria for the 5q-syndrome. If they do not, they are assigned to one of 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.

Classification of acute lymphoblastic leukaemia

The WHO classification requires that acute leukaemia must be shown to be lymphoid before it is categorised as ALL. This classification groups together ALL and lymphoblastic lymphoma, using the designations B lymphoblastic leukaemia/lymphoma and T lymphoblastic leukaemia/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 classification of ALL is now redundant except that FAB L3 morphology (i.e. the presence of 'blast cells' with basophilic cytoplasm and vacuolation) is of considerable clinical significance and should be recognised. In most, but not all, of these cases the cells are

TABLE 23-4

WHO CLASSIFICATION OF THE MYELODYSPLASTIC SYNDROMES (MDS)

2008 WHO classification	Terminology proposed for the 2016 update of the WHO classification
Refractory cytopenia with unilineage dysplasia	MDS with single lineage dysplasia
Refractory anaemia	
Refractory neutropenia	
Refractory thrombocytopenia	
Refractory anaemia with ring sideroblasts	MDS with single lineage dysplasia and ring sideroblasts
Refractory cytopenia with multilineage dysplasia (with or without ring sideroblasts)	MDS with multilineage dysplasia MDS with multilineage dysplasia and ring sideroblasts
Refractory anaemia with excess blasts-1	MDS with excess blasts-1
Refractory anaemia with excess blasts-2	MDS with excess blasts-2
Myelodysplastic syndrome with isolated del(5q)	Myelodysplastic syndrome with isolated del(5q)
Myelodysplastic syndrome, unclassifiable	Myelodysplastic syndrome, unclassifiable
Childhood myelodysplastic syndrome	Childhood myelodysplastic syndrome
Provisional entity: refractory cytopenia of childhood	Provisional entity: refractory cytopenia of childhood

(Updated from Vardiman J, Thiele J, Arber D, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009;114:937–951)
Arber DA, Hasserjian RP (2015) Reclassifying myelodysplastic syndromes: what's where in the new WHO and why? *Hematology 2015*:294-298

TABLE 23-5

WHO DIAGNOSTIC CRITERIA FOR THE MYELODYSPLASTIC SYNDROMES (MDS)

Disease	Blood Findings	Bone Marrow Findings
Refractory cytopenia with unilineage dysplasia (RCUD): (refractory anaemia [RA]; refractory neutropenia [RN]; refractory thrombocytopenia [RT])	Unicytopenia or bicytopenia* No or rare blasts (<1%)**	Unilineage dysplasia: ≥10% of the cells in one myeloid lineage <5% blasts <15% of erythroid precursors are ring sideroblasts
Refractory anaemia with ring sideroblasts (RARS)	Anaemia No blasts	≥15% of erythroid precursors are ring sideroblasts† Erythroid dysplasia only <5% blasts
Refractory cytopenia with multilineage dysplasia with or without ring sideroblasts (RCMD)	Cytopenia(s) No or rare blasts (<1%)** No Auer rods $<1\times10^9$/l monocytes	Dysplasia in ≥10% of the cells in ≥2 myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) <5% blasts in marrow No Auer rods ±15% ring sideroblasts
Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts** No Auer rods $<1\times10^9$/l monocytes	Unilineage or multilineage dysplasia 5–9% blasts** No Auer rods
Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5–19% blasts‡ Auer rods ±‡ $<1\times10^9$/l monocytes	Unilineage or multilineage dysplasia 10–19% blasts‡ Auer rods ±‡

Continued

TABLE 23-5

WHO DIAGNOSTIC CRITERIA FOR THE MYELODYSPLASTIC SYNDROMES (MDS)—CONT'D

Disease	Blood Findings	Bone Marrow Findings
Myelodysplastic syndrome – unclassified (MDS-U)	Cytopenias ≤1% blasts**	Unequivocal dysplasia in <10% of cells in one or more myeloid lineages when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS (see Table 23-6) <5% blasts
MDS associated with isolated del (5q)	Anaemia Usually normal or increased platelet count No or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts Isolated del(5q) cytogenetic abnormality¶ No Auer rods

*Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U.
**If the marrow myeloblast percentage is <5% but there are 2–4% myeloblasts in the blood, the diagnostic classification is RAEB-1. Cases of RCUD and RCMD with 1% myeloblasts in the blood should be classified as MDS-U.
†It is proposed that, if an *SF3B1* mutation is present, cases with at least 5% ring sideroblasts be included in this category, and should similarly be included in the newly proposed category of MDS with multilineage dysplasia and ring sideroblasts (Arber 2015).
‡Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB-2. Although the finding of 5–19% blasts in the blood is, in itself, diagnostic of RAEB-2, cases of RAEB-2 may have <5% blasts in the blood if they have Auer rods or 10–19% blasts in the marrow or both. Similarly, cases of RAEB-2 may have <10% blasts in the marrow but may be diagnosed by the other two findings, Auer rods and/or 5–19% blasts in the blood.
¶It is proposed that one additional cytogenetic abnormality (excluding monosomy 7) be accepted in this category (Arber 2015).
(From Vardiman J, Thiele J, Arber D, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009;114:937–951. It is anticipated that terminology will be altered in the 2016 update of the WHO classification.)

TABLE 23-6

MYELOPROLIFERATIVE NEOPLASMS (MPN) AND RELATED CONDITIONS[13-16]

Myeloproliferative neoplasms
Chronic myelogenous leukaemia/chronic myeloid leukaemia
Polycythaemia vera
Essential thrombocythaemia
Primary myelofibrosis
Chronic neutrophilic leukaemia
Chronic eosinophilic leukaemia, not otherwise categorised
Mast cell disease
MPN, unclassifiable
Lymphoid and myeloid neoplasms associated with rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1* or with *PCM1-JAK2*
Lymphoid and myeloid neoplasms associated with rearrangement of *PDGFRA*
Lymphoid and myeloid neoplasms associated with rearrangement of *PDGFRB*
Lymphoid and myeloid neoplasms associated with rearrangement of *FGFR1*
Lymphoid and myeloid neoplasms associated with *PCM1-JAK2*

immunologically mature, expressing surface membrane immunoglobulin and the condition represents a leukaemic presentation of Burkitt lymphoma. The WHO categorisation of such cases as lymphoma is more appropriate than their being categorised as ALL and is clinically important because the treatment is urgent and differs considerably from the treatment of ALL.

Classification of myeloproliferative neoplasms and related conditions

The WHO classification of myeloproliferative neoplasms (previously called 'disorders') and related conditions (Table 23-6) is increasingly taking account of cytogenetic or molecular genetic analyses.

WHO criteria for a diagnosis of essential thrombocythaemia are: platelet count $\geq 450 \times 10^9$/l; megakaryocyte proliferation with large and mature megakaryocytes on examination of the bone marrow with little or no granulocyte or erythroid proliferation; not meeting WHO criteria for CML, PV, primary myelofibrosis, MDS or other myeloid neoplasm; demonstration of *JAK2* V617F or other clonal marker or no evidence of reactive thrombocytosis.[16]

WHO criteria for a diagnosis of primary myelofibrosis are divided into major criteria (e.g. megakaryocyte proliferation and atypical megakaryocytes accompanied by reticulin and/or collagen fibrosis; demonstration of *JAK2* V617F or other clonal marker; no evidence of reactive marrow fibrosis; not meeting WHO criteria for CML, PV, MDS or other myeloid neoplasm) and minor criteria (e.g. leucoerythroblastosis; increased serum LDH; anaemia; palpable splenomegaly).

WHO criteria for a diagnosis of systemic mastocytosis are highly complex.[17] A trephine biopsy with a mast cell

TABLE 23-7

SUMMARY OF THE WORLD HEALTH ORGANISATION CATEGORIES OF MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

Category	Criteria
Chronic myelomonocytic leukaemia (CMML)	A Ph-negative, *BCR–ABL1*-negative disorder with monocyte count $>1\times10^9$/l Fewer than 20% blasts plus promonocytes in PB or 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)
Atypical chronic myeloid leukaemia (aCML)	A Ph-negative, *BCR–ABL1*-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 <2% of white cells Monocytes <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
Juvenile myelomonocytic leukaemia (JMML)	A Ph-negative, *BCR–ABL1*-negative disorder with monocyte count $>1\times10^9$/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 $>10\times10^9$/l Clonal chromosomal abnormality (monosomy 7 not excluded) GM-CSF hypersensitivity of myeloid precursors *in vitro*
Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS-RS-T)	Ring sideroblasts $\geq 15\%$ Platelet count $\geq 450\times10^9$/l Blast cells $<1\%$ in peripheral blood and $<5\%$ in bone marrow
Myelodysplastic/myeloproliferative neoplasm, unclassifiable	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 $\geq450\times10^9$/l or a white cell count of $\geq13\times10^9$/l) The condition has developed *de novo* The criteria for other MDS/MPN (CMML, aCML and JMML) are not met There is no Philadelphia chromosome, *BCR–ABL1* fusion gene, 5q−, inv(3)(q21q26) or t(3,3)(q21;q26)

aCML, atypical chronic myeloid leukaemia; BM, bone marrow; CMML, chronic myelomonocytic leukaemia; GM-CSF, granulocyte-macrophage colony-stimulating factor; JMML, juvenile myelomonocytic leukaemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; PB, peripheral blood; Ph, Philadelphia; WBC, white blood cell count.

tryptase stain is often crucial in the diagnosis. Molecular analysis for a *KIT* mutation can also be important.

Recognition of lymphoid and myeloid neoplasms associated with rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1* and *PCM1-JAK2* requires cytogenetic and molecular analyses. Appropriate molecular analysis may be either FISH or reverse transcription polymerase chain reaction (RT-PCR). The diagnosis of chronic eosinophilic leukaemia, not otherwise specified, requires exclusion of the above-specified molecular abnormalities.

The categorisation of neoplasms with features of both myelodysplasia and myeloproliferation and their diagnostic criteria are summarised in Table 23-7.

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