Peripheral blood

The peripheral blood shows initially a normocytic, normochromic anaemia and later, when the deficiency is more severe, a hypochromic, microcytic anaemia. Red cells also show anisocytosis, anisochromasia and poikilocytosis, particularly the presence of elliptocytes. Occasional patients show thrombocytosis, thrombocytopenia or the presence of occasional hypersegmented neutrophils.

Bone marrow cytology

Bone marrow cellularity is mildly increased as a result of a moderate degree of erythroid hyperplasia. Erythropoiesis is micronormoblastic with erythroblasts being smaller than normal with scanty or ragged cytoplasm or with cytoplasmic vacuolation (Fig. 8.1). There is a minor degree of dyserythropoiesis. An iron stain shows siderotic granules to be severely reduced or absent and there is a complete or virtual absence of the iron within macrophages which usually constitutes the body’s iron stores (see Fig. 2.1). Since iron is irregularly distributed in the marrow, a number of bone marrow fragments must be available for the performance of an iron stain before it can be concluded that storage iron is lacking. In iron deficiency, the bone marrow sometimes shows occasional giant metamyelocytes but granulopoiesis and thrombopoiesis are otherwise usually normal. Individuals whose bone marrow lacks storage iron but in whom erythropoiesis is normal should be regarded as iron depleted rather than as iron deficient; a significant proportion of healthy women fall into this group.

Iron deficiency anaemia

Iron deficiency anaemia results from inadequate iron intake, increased loss of iron from the body or a combination of the two. Peripheral blood features, supplemented by biochemical assays, are often sufficient for a definitive diagnosis. In more complicated cases bone marrow aspiration permits a definitive diagnosis. Trephine biopsy is of little importance and, if iron is leached out during decalcification, histological sections can be misleading.

Useful biochemical tests in the diagnosis of iron deficiency include estimations of serum ferritin and serum iron concentration, the latter only if combined with an estimate of either transferrin concentration or serum total iron binding capacity. Serum ferritin and serum iron concentrations are reduced, whereas serum transferrin concentration and total iron binding capacity are increased. The concentration of soluble serum transferrin receptors is also increased but this test is not very specific for iron deficiency since concentration is also increased if there is increased erythropoiesis.
**Bone marrow histology**

Trephine biopsy sections show mild hypercellularity, erythroid hyperplasia and absent iron stores. Megakaryocytes are sometimes increased (Fig. 8.2).

**Problems and pitfalls**

An iron stain performed on a plastic-embedded, non-decalcified trephine biopsy section permits reliable assessment of iron stores. However, it should be noted that the decalcification needed for paraffin-embedded biopsy specimens leads to leaching out of some or all of the iron. It is therefore possible to exclude a diagnosis of iron deficiency if stainable iron is present but it is not possible to state that iron stores are absent or reduced. A diagnosis of iron deficiency therefore cannot be made from a biopsy specimen that has been decalcified.

**Sideroblastic anaemia**

Sideroblastic anaemia as a feature of myelodysplastic syndromes (MDS)—refractory anaemia with ring
sideroblasts or primary acquired sideroblastic anaemia—has been discussed in Chapter 4. Sideroblastic anaemia may also be inherited or may be secondary to exogenous agents such as alcohol, chloramphenicol or certain drugs used in the treatment of tuberculosis. Congenital (inherited) sideroblastic anaemia occurs predominantly but not exclusively in males, most cases being caused by defects in synthesis of 5-amino-laevulinic acid. Other cases, in either males or females, are a feature of Pearson’s syndrome (see page 407) or of thiamine-responsive megaloblastic anaemia (see page 371). Sideroblastic anaemia is most readily diagnosed from a bone marrow aspirate but diagnosis is also possible from sections of plastic-embedded trephine biopsy specimens.

**Peripheral blood**

Congenital and secondary sideroblastic anaemias are associated with microcytosis and hypochromia (Fig. 8.3), in contrast to the macrocytosis which is usual when sideroblastic erythropoiesis is a feature of MDS. In some patients the peripheral blood film is dimorphic with a mixture of hypochromic microcytes and normochromic normocytes. Congenital sideroblastic anaemia varies in severity from moderate to severe. Secondary sideroblastic anaemia is of mild to moderate severity. In families in which males have sideroblastic anaemia, females may show a small population of hypochromic microcytes.

**Bone marrow cytology**

The bone marrow shows mild hypercellularity and mild erythroid hyperplasia. A proportion of the erythroblasts show micronormoblastic maturation and defective haemoglobinization with ragged or vacuolated cytoplasm (Fig. 8.4). An iron stain shows the presence of abnormal sideroblasts including frequent ring sideroblasts (Fig. 8.5). Iron stores are usually increased. Plasma cells may contain haemosiderin (see Fig. 2.8).

**Bone marrow histology**

Trephine biopsy sections show some degree of erythroid hyperplasia. Increased storage iron and ring sideroblasts are detectable in plastic-embedded sections but not in sections of decalcified paraffin-embedded biopsy specimens. Plasma cells may contain haemosiderin (see Fig. 2.8). A trephine biopsy is not indicated in the investigation of suspected congenital sideroblastic anaemia but is useful if acquired sideroblastic anaemia, particularly as a feature of MDS, is suspected.

**Problems and pitfalls**

Making a distinction between congenital and acquired sideroblastic anaemias and between primary and secondary sideroblastic anaemias is not always possible from the peripheral blood and bone
marrow features alone. In some cases a family history, drug history and supplementary tests are needed.

A diagnosis of sideroblastic anaemia cannot be made from decalcified trephine biopsy specimens.

**Thalassaemia trait and thalassaemia intermedia**

The various thalassaemic disorders, including thalassaemia trait, are most readily diagnosed from peripheral blood features but it is necessary for haematologists and pathologists to be aware of the bone marrow features to avoid misdiagnosis as other conditions. Bone marrow aspiration and trephine biopsy are not of any importance in the diagnosis.

Thalassaemia trait is the term used to describe an asymptomatic condition usually consequent on dysfunction of one of the two $\beta$ genes or lack of one or two of the four $\alpha$ genes. The term ‘thalassaemia intermedia’ denotes a symptomatic condition, more severe than thalassaemia trait, but in which blood transfusion is not generally necessary; the genetic basis is diverse.
Diagnosis of $\beta$ thalassaemia trait is based on a typical blood count and blood film together with demonstration of an elevated percentage of haemoglobin $A_\beta$. There may or may not be an elevated percentage of haemoglobin F. The diagnosis of $\beta$ thalassaemia intermedia is made on the basis of clinical and haematological features, haemoglobin electrophoresis and DNA analysis. A presumptive diagnosis of $\alpha$ thalassaemia trait is made when there is microcytosis that is not explained by other more readily diagnosable conditions such as iron deficiency anaemia or $\beta$ thalassaemia trait. A definitive diagnosis of $\alpha$ thalassaemia trait requires DNA analysis, most cases being caused by deletion of one or more of the $\alpha$ genes.

**Peripheral blood**

In $\beta$ thalassaemia trait, and in cases of $\alpha$ thalassaemia trait in which two of the four $\alpha$ genes are lacking, the peripheral blood shows microcytosis and sometimes a degree of hypochromia. Some, but not all, cases of $\beta$ thalassaemia trait also have basophilic stippling and moderate poikilocytosis, including the presence of target cells. In cases of $\alpha$ thalassaemia trait in which only one of the four $\alpha$ genes is lacking, the haematological abnormalities are much less and the diagnosis may not be suspected. In $\beta$ thalassaemia intermedia, the haematological features are intermediate between those of thalassaemia trait and thalassaemia major.

**Bone marrow cytology**

In thalassaemia trait, the bone marrow aspirate shows moderate erythroid hyperplasia. Erythropoiesis is micronormoblastic and there is moderate dyserythropoiesis including nuclear lobulation and nuclei of irregular shape (Fig. 8.6). An iron stain shows increased siderotic granulation and occasional ring sideroblasts. Storage iron is commonly increased. In thalassaemia intermedia, erythroid hyperplasia and dyserythropoiesis are marked and storage iron is increased.

**Bone marrow histology**

Trephine biopsy sections show erythroid hyperplasia and dyserythropoiesis.

**Problems and pitfalls**

Misdiagnosis of $\beta$ thalassaemia intermedia as MDS can occur if the possibility of thalassaemia is not considered and if it is not appreciated that dysplastic features are confined to the erythroid lineage.

**Thalassaemia major**

Thalassaemia major indicates a transfusion-dependent thalassaemic condition, usually consequent on homozygosity or compound heterozygosity for $\beta$ thalassaemia.
Peripheral blood

The peripheral blood shows striking hypochromia, microcytosis, anisocytosis and poikilocytosis. Basophilic stippling, Pappenheimer bodies and dysplastic circulating erythroblasts are also present. If the patient has been transfused, the blood film is dimorphic.

Bone marrow cytology

The bone marrow shows very marked erythroid hyperplasia, severe erythroid dysplasia and poor haemoglobinization (Fig. 8.7). Some erythroblasts contain cytoplasmic inclusions, seen with difficulty in MGG-stained films, which represent precipitated α chains. There is an increase in macrophages which contain degenerating erythroblasts, cellular debris and haemosiderin. In some patients the increased cell turnover leads to the formation of pseudo-Gaucher cells and sea-blue histiocytes (see pages 416 and 420). An iron stain shows numerous abnormal sideroblasts and small numbers of ring sideroblasts. Storage iron is considerably increased. Plasma cells may contain haemosiderin.

Bone marrow histology

Bone marrow sections show marked erythroid hyperplasia with disappearance of fat spaces. Dyserythropoiesis is also very marked and iron stores are increased. Pseudo-Gaucher cells and sea-blue histiocytes may be present. Plasma cells and the endothelial cells lining sinusoids may contain haemosiderin.

Haemoglobin H disease

Haemoglobin H disease is a thalassaemic condition resulting from the lack of three of the four α genes or from a functionally similar defect. There is also a decreased red cell life span. Diagnosis rests on peripheral blood features and the results of haemoglobin electrophoresis; bone marrow examination contributes little. Haemoglobin electrophoresis shows a small percentage of haemoglobin H and haemoglobin H inclusions are seen within red cells that have been exposed to a suitable supravital dye. Occasionally, haemoglobin H disease is an acquired condition, occurring as a feature of MDS.

Peripheral blood

The peripheral blood shows marked hypochromia, microcytosis, anisocytosis and poikilocytosis. Because of the haemolytic component, there is also polychromasia and the reticulocyte count is elevated.

Bone marrow cytology

The bone marrow is hypercellular with marked ery-
throid hyperplasia, defective haemoglobinization and some dyserythropoietic features (Fig. 8.8).

**Bone marrow histology**

Bone marrow sections show hypercellularity due to erythroid hyperplasia.

**Problems and pitfalls**

It is important to distinguish acquired haemoglobin H disease from the much more common inherited condition. This is possible by examination of cells of other haemopoietic lineages.

**Haemolytic anaemias**

Haemolytic anaemia may be inherited or acquired. Aetiological factors, pathogenetic mechanisms and morphological features are very varied [1]. Examination of the peripheral blood is of great importance in the diagnosis but examination of the bone marrow adds little, except in detecting complicating megaloblastic anaemia or pure red cell aplasia or, occasionally, an associated lymphoma.

**Peripheral blood**

Haemolytic anaemias have in common polychromasia and an increased reticulocyte count. Macrocytosis is usual in those patients in whom haemolysis is chronic and severe. Other morphological features are very variable, depending on the precise nature of the condition [1].

**Bone marrow cytology**

The bone marrow is hypercellular as a consequence of erythroid hyperplasia (Fig. 8.9). The degree of hyperplasia reflects the extent to which the red cell life span is shortened. In some patients, fat cells are totally lost. Haemopoiesis is often macronormoblastic, i.e. the erythroblasts are increased in size but have nuclear and cytoplasmic characteristics similar to those of normoblasts. Some cases of haemolytic anaemia have quite marked dyserythropoiesis. This may occur transiently in autoimmune haemolytic anaemia [2] and has also been observed in haemolytic anaemia associated with the familial auto-immune lymphoproliferative disorder caused by FAS gene mutations [3]. Dyserythropoiesis is often very striking when severe haemolytic anaemia occurs in a neonate, e.g. in haemolytic disease of the newborn. A specific dyserythropoietic feature is associated with haemolytic anaemia due to haemoglobin C disease; normoblasts have irregular nuclear membranes (Fig. 8.10). Macronormoblastic erythropoiesis should be distinguished from mildly megaloblastic erythropoiesis which may occur in the haemolytic anaemias when there is complicating folic acid deficiency. When haemolysis is extravascular, bone marrow macrophages are increased and contain cellular debris. Iron stores are commonly increased,
except when there is severe intravascular haemolysis with consequent loss of iron from the body. Siderotic granulation is somewhat increased.

**Bone marrow histology**

The bone marrow is hypercellular with erythroid hyperplasia (Fig. 8.11) and a variable degree of dyserythropoiesis. The number of erythroid islands is increased and the central macrophage is large and prominent, often staining a dirty greenish colour with a Giemsa stain because of the presence of increased haemosiderin. An iron stain confirms increased storage iron.

**Problems and pitfalls**

Misinterpretation of erythroid hyperplasia with a variable degree of dyserythropoiesis, which is a consequence of haemolytic anaemia, is possible if a peripheral blood film is not examined as part of the assessment of a bone marrow aspirate and trephine biopsy. This may result in a failure to consider haemolysis as a diagnostic possibility.
Congenital dyserythropoietic anaemia

The congenital dyserythropoietic anaemias (CDAs) are a diverse group of inherited conditions [4,5], all of which are characterized by anaemia resulting from dysplastic and ineffective erythropoiesis. Splenomegaly and expansion of the bone marrow cavity are common. Three major types of CDA have been recognized but a considerable number of cases not conforming to these categories have also been described. Both peripheral blood and bone marrow aspirate features are important in making the diagnosis. In type II CDA, demonstration of a positive acidified serum lysis test, using a number of normal sera to exclude false-negative results, is required for confirmation. Trephine biopsy is not important in diagnosis.

Peripheral blood

Specific morphological features vary, depending on the category of CDA (Table 8.1). All are character-

| Table 8.1 Genetic, peripheral blood and bone marrow features of the congenital dyserythropoietic anaemias. |
|-----------------|-----------------|-----------------|-----------------|
| **Inheritance** | **Peripheral blood** | **Bone marrow** |
| Autosomal recessive | Mild to moderate anaemia, macrocytosis, marked anisocytosis and poikilocytosis including teardrop poikilocytes, basophilic stippling | Hyperplastic, megaloblastic, moderate binuclearity and internuclear chromatin bridges, nuclear budding and karyorrhexis |
| Autosomal recessive | Mild to severe anaemia, normocytic red cells, moderate anisocytosis and poikilocytosis, including teardrop poikilocytes, variable anisochromasia, irregularly contracted cells, basophilic stippling | Hyperplastic, normoblastic, marked binuclearity and multinuclearity |
| Autosomal dominant | Mild anaemia, macrocytosis, marked anisocytosis and poikilocytosis, basophilic stippling | Hyperplastic, megaloblastic, giant erythroblasts with single nuclei or marked multinuclearity—up to a dozen nuclei per cell, karyorrhexis |

* Hereditary erythroid multinuclearity with positive acidified serum test.
ized by anisocytosis and poikilocytosis (Fig. 8.12) which often includes the presence of fragments and irregularly contracted cells. Basophilic stippling is common. In all categories, the reticulocyte count is not elevated appropriately for the degree of anaemia.

**Bone marrow cytology**

Bone marrow features characteristic of the different categories of CDA are summarized in Table 8.1 and illustrated in Figs 8.13–8.15. In all types there is erythroid hyperplasia and dyserythropoiesis. In type II CDA the increase in cell turnover is such that pseudo-Gaucher cells may be present. Iron stores are commonly increased. Ultrastructural examination of bone marrow cells is diagnostically useful, showing a ‘Swiss cheese’ appearance of the nucleus in CDA type I, a double membrane parallel to the cell membrane in CDA type II and a variety of defects in CDA type III [5].

**Bone marrow histology**

Examination of trephine biopsy or bone marrow clot sections confirms erythroid hyperplasia and dyserythropoiesis (Fig. 8.16).
Fig. 8.14 BM aspirate, congenital dyserythropoietic anaemia, type II, showing one binucleate erythroblast and one erythroblast with a multilobulated nucleus. MGG ×940.

Fig. 8.15 BM aspirate, congenital dyserythropoietic anaemia, type III, showing giant, multinucleated erythroblasts. MGG ×940. (By courtesy of Professor SN Wickramasinghe, London.)

Fig. 8.16 BM clot section, congenital dyserythropoietic anaemia, type I, showing marked erythroid hyperplasia with large numbers of immature erythroid precursors and marked dyserythropoiesis. The chromatin pattern is very abnormal. Paraffin-embedded, H&E ×390.
Problems and pitfalls
Some cases of CDA present quite late in life. Misdiagnosis as MDS may occur if the possibility of CDA is not considered and if due consideration is not given to the fact that the abnormalities are essentially confined to the erythroid lineage.

Megaloblastic anaemia
Megaloblastic anaemia is usually consequent on a deficiency of vitamin B_{12} or folic acid. Less often, it is attributable to administration of a drug that interferes with DNA synthesis or, rarely, to a congenital metabolic defect. Some patients with acute myeloid leukaemia (AML) or MDS also have megaloblastic erythropoiesis. The presence of megaloblastic anaemia can usually be suspected from examination of the peripheral blood and, if features are totally typical, bone marrow aspiration is often not done. The ready availability of accurate assays for vitamin B_{12} and folic acid has lessened the importance of bone marrow examination. However, if typical peripheral blood features of megaloblastic erythropoiesis are lacking or if atypical features are present, bone marrow aspiration should be performed. Further tests indicated in patients with megaloblastic anaemia are assays of serum vitamin B_{12} and red cell folate followed, when appropriate, by tests for auto-antibodies and a Schilling test. If pernicious anaemia is suspected, tests for parietal cell and intrinsic factor antibodies are indicated; the former is more sensitive but less specific than the latter. If coeliac disease is suspected as a cause of malabsorption of folic acid or vitamin B_{12}, tests indicated include those for relevant auto-antibodies (antireticulin and antiendomysium antibodies), antigliadin antibodies and a small bowel biopsy.

Peripheral blood
In most cases there is a macrocytic anaemia, with oval macrocytes being particularly characteristic. The mildest cases have macrocytosis without anaemia. Some degree of anisocytosis and poikilocytosis is usual and, when anaemia is severe, there are striking morphological abnormalities including the presence of teardrop poikilocytes, fragments, basophilic stippling and occasional Howell–Jolly bodies and circulating megaloblasts. Hypersegmented neutrophils are usually present; they are highly suggestive of megaloblastic erythropoiesis although not pathognomonic. They persist for a week or more after commencement of vitamin B_{12} or folic acid therapy. There may also be increased numbers of macropolycytes (tetraploid neutrophils) but this feature is less strongly associated with megaloblastic erythropoiesis. In severe megaloblastic anaemia, leucopenia and thrombocytopenia also occur.

Bone marrow cytology
The bone marrow is hypercellular, often markedly so. Erythropoiesis is hyperplastic and is characterized by the presence of megaloblasts (Fig. 8.17). These are large cells with a chromatin pattern more primitive than is appropriate for the degree of maturation of the cytoplasm. Late megaloblasts may be fully haemoglobinized and lack any cytoplasmic basophilia. They may therefore be described as orthochromatic, a term which is not really appropriate in describing normal erythropoiesis, in which the most mature erythroblasts are polychromatic. Erythropoiesis is ineffective so that early erythroid cells are over-represented in comparison with mature cells; macrophages are increased and contain defective red cell precursors and cellular debris. An iron stain shows abnormally prominent siderotic granules and sometimes occasional ring sideroblasts. Storage iron is usually increased. Plasma cells may contain iron. The mitotic count is increased and examination of cells in metaphase may show that chromosomes are unusually long.

Granulopoiesis is also hyperplastic although less so than erythropoiesis. Giant metamyelocytes are usually present (Fig. 8.17). They are twice to three times the size of a normal metamyelocyte and often have nuclei of unusual shapes, e.g. E- or Z-shaped rather than U-shaped. Myelocytes and promyelocytes are also increased in size but this abnormality is less obvious and less distinctive than the abnormality of metamyelocytes. When megaloblastic features in erythroblasts are partly or largely masked by co-existing iron deficiency, the detection of giant metamyelocytes is diagnostically important.

Megakaryocytes are hypersegmented and have more finely stippled chromatin than normal megakaryocytes.
Bone marrow histology

There is a variable hypercellularity with loss of fat cells. In some cases this can be so severe that it may resemble the ‘packed marrow’ appearance seen in acute leukaemia on examination at low power. There is erythroid hyperplasia with predominance of immature precursors (Figs 8.18 and 8.19). The early erythroid cells have large, round-to-oval nuclei with one or more basophilic nucleoli which often appear to have rather irregular margins and often abut on the nuclear membrane (Fig. 8.19); there is usually a moderate amount of intensely basophilic cytoplasm. Small Golgi zones may be seen. The later erythroid cells show asynchrony of nuclear and cytoplasmic maturation with cells having immature nuclei but haemoglobinized cytoplasm. Granulocytic precursors are increased but may appear relatively inconspicuous in the presence of profound erythroid hyperplasia. Giant metamyelocytes are usually easily seen (Fig. 8.19). Megakaryocyte numbers may be normal or decreased.
Problems and pitfalls

It is critically important that a bone marrow aspirate in severe megaloblastic anaemia is not interpreted as AML. The likelihood of these errors has probably increased in recent years as haematologists have had less experience in interpreting bone marrows from patients with straightforward megaloblastic anaemia. An appearance of ‘maturation arrest’ and gross dyserythropoiesis may suggest acute leukaemia but these are also features of severe megaloblastosis. Confusion with M6 AML should not occur since the bone marrow in megaloblastic anaemia does not have any increase in myeloblasts. However, confusion may occur with M6 variant AML in which the primitive cells present are all erythroid (see page 154). It is important that the diagnosis of megaloblastic anaemia is always considered in any such patient. Hypersegmented neutrophils and giant metamyelocytes should be sought since they are not a feature of AML. If there is any real doubt as to the correct diagnosis, a trial of vitamin B12 and folic acid therapy should be given.

Examination of a trephine biopsy specimen is rarely useful in the diagnosis of megaloblastic anaemia but it is important for pathologists to be able to recognize the typical histological features so that misdiagnosis, particularly as acute leukaemia, does not occur. Megaloblastic change in biopsy sections may be mistaken for acute leukaemia if the biopsy is reported without referring to the blood film and marrow aspirate findings and if the possibility of megaloblastic anaemia is not considered. Less often, there may have been failure to obtain an aspirate or the presence of immature cells in the peripheral blood in a patient with complicating infection may have given rise to the clinical suspicion of leukaemia; in these circumstances misdiagnosis of leukaemia is more likely [6].

Erythroid islands composed of early megaloblasts are also sometimes mistaken for clusters of carcinoma cells or for ‘abnormal localization of immature precursors’ in MDS (see Fig. 4.41). If there is any real doubt as to their nature, immunohistochemistry can be used.

Anaemia of chronic disease

The anaemia of chronic disease is characterized by a normocytic, normochromic anaemia or, when more severe, by a hypochromic, microcytic anaemia. Such anaemia is secondary to infection, inflammation or malignancy. Diagnosis is usually based on peripheral blood features and biochemical assays. Serum iron and transferrin are reduced whereas serum ferritin is normal or increased. Serum transferrin receptor concentration tends to be normal. A bone marrow aspirate is sometimes
necessary to confirm or exclude co-existing iron deficiency in a patient who has features of anaemia of chronic disease. A bone marrow biopsy does not usually give diagnostically useful information.

**Peripheral blood**

In addition to the possible occurrence of hypochromia and microcytosis, the peripheral blood usually shows increased rouleaux formation and sometimes increased background staining due to a reactive increase in various serum proteins. The erythrocyte sedimentation rate is increased.

**Bone marrow cytology**

The bone marrow is usually of normal cellularity. Erythropoiesis may show no specific abnormality or may be micronormoblastic with defective haemoglobinization. An iron stain shows storage iron to be increased, often markedly so when the condition is very chronic. Erythroblasts show reduced or absent siderotic granulation. The bone marrow often shows non-specific inflammatory changes including increased plasma cells, mast cells and macrophages.

**Bone marrow histology**

Sections of bone marrow trephine biopsy cores usually show normal cellularity. There may be increased lymphoid nodules, plasma cells, mast cells and macrophages. An iron stain shows increased storage iron.

**Problems and pitfalls**

An iron stain may be falsely negative if a trephine biopsy specimen has been decalcified, leading to a mistaken assumption that the patient has iron deficiency anaemia.

**Sickle cell anaemia and other sickling disorders**

Sickle cell anaemia denotes the disease resulting from homozygosity for the $\beta^S$ genes and the consequent replacement of haemoglobin A by haemoglobin S. Diagnosis of sickle cell anaemia is dependent on peripheral blood features and haemoglobin electrophoresis or an equivalent technique. Haemoglobin S comprises almost all the total haemoglobin, haemoglobin A being absent. Bone marrow aspiration is usually only indicated to detect suspected complications such as megaloblastic anaemia, pure red cell aplasia or bone marrow necrosis. Trephine biopsy is not often indicated. The clinical features consequent on sickling of red blood cells can also be found in various compound heterozygous states, such as sickle cell/haemoglobin C disease and sickle cell/\(\beta\) thalassaemia trait.

**Peripheral blood**

The peripheral blood shows anaemia, usually with a haemoglobin concentration of 6–10 g/dl. There are variable numbers of sickle cells and, in addition, target cells and polychromasia; nucleated red blood cells may be present. After the age of 6 months, features of hypospplenism start to appear, particularly Howell–Jolly bodies and Pappenheimer bodies. The neutrophil count may be increased, particularly during episodes of sickling. The blood film in compound heterozygous states is often similar to that of sickle cell anaemia, although patients with sickle cell/haemoglobin C disease may have occasional cells containing haemoglobin C crystals and those with sickle cell/\(\beta\) thalassaemia have microcytosis.

**Bone marrow cytology**

The bone marrow aspirate shows hypercellularity due to erythroid hyperplasia. Iron stores are often increased and sickle cells are usually present. Sometimes they are much more elongated than is usual for sickle cells in the circulating blood. When there are complicating conditions, such as megaloblastic anaemia, pure red cell aplasia or bone marrow necrosis, the appropriate morphological features are superimposed on those of the underlying disease. Bone marrow macrophages may contain occasional or numerous sickle cells (Fig. 8.20). Macrophages and various storage cells are sometimes increased (Figs 8.20 and 8.21) as a consequence of increased cell turnover and episodes of bone marrow infarction. In sickle cell/\(\beta\) thalassaemia, erythropoiesis is hyperplastic and micronormoblastic (Fig. 8.22).
**Fig. 8.20** BM aspirate, sickle cell anaemia, showing a foamy macrophage and a macrophage containing a sickled cell. MGG ×940. (By courtesy of Professor Sally Davies, London.)

**Fig. 8.21** BM aspirate, sickle cell anaemia, showing erythroid hyperplasia, a partially sickled cell and a sea-blue histiocyte. MGG ×940.

**Fig. 8.22** BM aspirate, sickle cell/β thalassaemia compound heterozygosity, showing erythroid hyperplasia and erythroblasts with scanty cytoplasm and defective haemoglobinization; several sickle cells are present. MGG ×940.
Bone marrow histology

Trephine biopsy sections show hypercellularity due to erythroid hyperplasia. During episodes of sickling, sickle cells may be seen within bone marrow sinusoids (Fig. 8.23). As for bone marrow aspirate films, sickle cells in bone marrow sections may be much more elongated than the typical sickle cells that are seen in blood films (Fig. 8.24). Infarcted bone and bone marrow may be present in patients who are experiencing a sickling crisis, and foamy macrophages and small fibrotic scars may mark the sites of previous bone marrow infarction.

Problems and pitfalls

It should be noted that at autopsy sickle cells may be present in histological sections of bone marrow, not only in patients with sickle cell disease but also in those with sickle cell trait.

Pure red cell aplasia (including Blackfan–Diamond syndrome)

Pure red cell aplasia has been defined as severe anaemia with the reticulocyte count being less than 1% and mature erythroblasts in a normocellular
ERYTHROPOIESIS, GRANULOPOIESIS, THROMBOPOIESIS

bone marrow being less than 0.5% [7]. Pure red cell aplasia may be either constitutional or acquired and either acute or chronic. Constitutional pure red cell aplasia, also known as the Blackfan–Diamond syndrome, is a chronic condition which usually becomes manifest during the first year of life. It appears to be a trilineage disorder, consequent on an inherited stem cell defect, rather than a purely erythroid disorder. It shows some responsiveness to corticosteroids. Inheritance is usually autosomal dominant, with variable penetrance, but some cases are autosomal recessive or occur sporadically. A significant proportion of cases are due to a mutation in the gene encoding ribosomal protein S19 at 19q13 [8]. A small percentage of patients with the Blackfan–Diamond syndrome subsequently develop bone marrow aplasia [9], myelodysplasia [9] or AML. Infants, usually but not always over 1 year of age, may also suffer from acute pure red cell aplasia designated transient erythroblastopenia of childhood [10,11]; in this condition the aplasia, which is consequent on infection by human herpesvirus 6 [12], lasts only a matter of months and does not require specific treatment. In older children and adults, the most commonly recognized cause of acute aplasia is parvovirus infection; the aplasia is usually of brief duration and therefore causes symptomatic anaemia only in subjects with a pre-existing red cell defect. In children and adults, the most commonly recognized cause of acute aplasia is parvovirus infection; the aplasia is usually of brief duration and therefore causes symptomatic anaemia only in subjects with a pre-existing red cell defect. In children and adults, the most commonly recognized cause of acute aplasia is parvovirus infection; the aplasia is usually of brief duration and therefore causes symptomatic anaemia only in subjects with a pre-existing red cell defect. In children and adults, the most commonly recognized cause of acute aplasia is parvovirus infection; the aplasia is usually of brief duration and therefore causes symptomatic anaemia only in subjects with a pre-existing red cell defect.

Peripheral blood

The peripheral blood shows no specific abnormality. There is a complete absence of polychromatophilic cells and the reticulocyte count is zero or virtually zero. Associated features differ according to the cause of the red cell aplasia. Macrocytosis is usual in the Blackfan–Diamond syndrome and the red cells have some characteristics similar to those of fetal red cells; occasionally, there is mild neutropenia and the platelet count may be somewhat elevated [10]. In transient erythroblastopenia of childhood, the red cells are of normal size and lack fetal characteristics; neutropenia, which may be moderately severe, occurs in about a quarter of cases and thrombocytosis in about a third [10,11]. Since symptomatic anaemia following parvovirus-induced aplasia is largely confined to patients with an underlying red cell defect, the blood film usually shows features of an associated disease, most often hereditary spherocytosis or sickle cell anaemia. In such cases the absence of polychromatophilia, despite marked anaemia, is diagnostically important and should lead to a reticulocyte count being performed. Neutrophil and platelet counts are only occasionally reduced in patients with parvovirus-induced red cell aplasia. Patients with red cell aplasia associated with thymoma or with auto-immune disease sometimes also have neutropenia or thrombocytopenia. In patients with red cell aplasia as the dominant feature of MDS it is sometimes possible to detect dysplastic features in cells of other lineages.

Bone marrow cytology

Bone marrow cellularity is usually somewhat reduced. There is a striking reduction of maturing erythroid cells. Proerythroblasts are present in normal numbers and sometimes they may be increased (Fig. 8.25). Other lineages are usually normal. In Blackfan–Diamond syndrome (Fig. 8.26) there are scattered proerythroblasts and sometimes minimal evidence of maturation; haematogones and lym-
Fig. 8.25 BM aspirate, chronic idiopathic pure red cell aplasia, showing increased proerythroblasts and a lack of maturing erythroblasts. MGG ×940.

Fig. 8.26 BM aspirate, Blackfan–Diamond syndrome, showing a single intermediate erythroblast but no maturing cells. MGG ×940. (By courtesy of Dr R Brunning, Minneapolis.)

Fig. 8.27 BM aspirate, chronic pure red cell aplasia caused by parvovirus B19 infection in an HIV-positive child. MGG ×940.
ERYTHROPOIESIS, GRANULOPOIESIS, THROMBOPOIESIS

**Fig. 8.28** BM trephine biopsy section, pure red cell aplasia, showing absence of erythroblastic islands and late erythroblasts; only occasional early and intermediate erythroblasts are present. Paraffin-embedded. H&E ×390.

**Fig. 8.29** BM trephine biopsy section, Blackfan–Diamond syndrome, showing prominent proerythroblasts and early erythroblasts but very few maturing erythroid cells (same case as in Fig. 8.26). H&E ×940. (By courtesy of Dr R Brunning, Minneapolis.)

Phocytes may be increased [20]. In transient erythroblastopenia of childhood, granulopoiesis may be left shifted and there may be an infiltrate of immature lymphocytes [12]. In parvovirus-induced aplasia, giant proerythroblasts with prominent nucleoli are often noted (Fig. 8.27). Iron stores are commonly increased since the iron normally in erythroid cells has been deposited in the stores.

**Bone marrow histology**

The overall bone marrow cellularity is somewhat reduced. There is a striking lack of erythroid islands and of maturing erythroblasts (Figs 8.28 and 8.29). Large proerythroblasts with strongly basophilic cytoplasm are readily apparent. Occasionally, there is a striking increase in proerythroblasts (Fig. 8.30). In parvovirus infection, the giant proerythroblasts may show intranuclear eosinophilic degeneration with peripheral condensation of chromatin (Fig. 8.31). In immunocompetent patients the bone marrow is hypercellular and megakaryocytes are increased [20]. Immunohistochemistry can be used to show parvovirus antigens.

**Problems and pitfalls**

Since red cell aplasia may be the major manifestation
of MDS, it is important to examine other lineages carefully for dysplastic features.

Occasionally, patients with pure red cell aplasia show a striking increase of proerythroblasts which can cause diagnostic confusion (see Fig. 8.30).

**Fig. 8.30** BM trephine biopsy section, chronic pure red cell aplasia, probably auto-immune in nature, showing a striking increase of proerythroblasts. H&E ×940. (By courtesy of Dr Haley, Vancouver.)

**Fig. 8.31** BM trephine biopsy section in chronic parvovirus B19-induced pure red cell aplasia in an HIV-positive child (same case as in Fig. 8.27), showing several apoptotic cells and a proerythroblast with an eosinophilic intranuclear inclusion. Paraffin-embedded, H&E ×960.

**Congenital neutropenia**

Severe congenital neutropenia (Kostmann’s syndrome) is a heterogeneous group of disorders with either autosomal dominant or autosomal recessive inheritance. Isolated congenital neutropenia may also be mild with a benign clinical course. Congenital neutropenia may be cyclical with variation over a period of 3 weeks or more from very low to normal or above normal levels. Congenital neutropenia also occurs as a feature of other congenital syndromes.

**Peripheral blood**

In Kostmann’s syndrome, the peripheral blood shows severe neutropenia and often monocytosis, eosinophilia and the effects of chronic or recurrent infection such as anaemia and increased rouleaux formation.
Bone marrow cytology and histology

Most cases of Kostmann’s syndrome show an arrest at the promyelocyte stage of differentiation (Fig. 8.32). Haematogones may be increased. Some cases show a severe reduction of all granulopoietic cells with residual cells sometimes being morphologically atypical. The latter pattern may be predictive of failure of response to granulocyte colony-stimulating factor (G-CSF) therapy [21]. An association between Kostmann’s syndrome and osteoporosis has been observed [22].

In Schwachman’s syndrome (congenital neutropenia with exocrine pancreatic insufficiency), the bone marrow may show apparent maturation arrest [23]. In cyclical neutropenia there is myeloid hypoplasia during the neutropenic phase but, when the neutrophil count is normal, the bone marrow appears normal. In neutropenia associated with Cohen’s syndrome bone marrow examination shows left-shifted granulopoiesis [24].

Agranulocytosis

Agranulocytosis is an acute, severe, reversible lack of circulating neutrophils consequent on an idiosyncratic reaction to a drug or chemical. At least some cases result from the development of antibodies against the causative drug with destruction of neutrophils being caused by the interaction of the antibody and the drug. However, some cases may result from abnormal metabolism of a drug so that toxic levels develop when normal doses are administered. Clinical features are due to sepsis consequent on the neutropenia.

Peripheral blood

The neutrophil count is greatly reduced, usually to less than $0.5 \times 10^9$/l. Residual neutrophils may be morphologically normal but often they show toxic changes consequent on superimposed sepsis. During recovery there is an outpouring of immature granulocytes into the peripheral blood, constituting a leukaemoid reaction.

Bone marrow cytology

The bone marrow aspirate shows a marked reduction in mature neutrophils. Sometimes myelocytes are also greatly reduced. The degree of granulocyte compartment depletion is predictive of speed of recovery; if promyelocytes and myelocytes are present, recovery usually occurs in 4–7 days, without administration of growth factors, whereas if promyelocytes and myelocytes are absent recovery takes 14 days or more [25]. In severe cases with superimposed sepsis the majority of cells of granulocytic lineage may be promyelocytes with very heavy granulation. This appearance has been confused with hypergranular promyelocytic leukaemia. Useful points allowing differentiation of the
two conditions are the prominent Golgi zone in the promyelocytes of agranulocytosis and the absence of Auer rods and giant granules.

**Bone marrow histology**
Bone marrow sections show a lack of mature granulocytes and, often, superimposed changes due to infection.

**Auto-immune neutropenia**
Auto-immune neutropenia may occur as an isolated phenomenon or be one manifestation of an auto-immune disease such as systemic lupus erythematosus. It may also occur in association with thymoma and as a complication of T-cell granular lymphocytic leukaemia (with or without associated rheumatoid arthritis). Neutropenia associated with T-cell granular lymphocytic leukaemia may be cyclical [15].

**Peripheral blood**
There is a reduction in neutrophils but those present are cytologically normal.

**Bone marrow cytology**
Granulopoiesis appears normal or hyperplastic with a reduced proportion of mature neutrophils. An uncommon observation is phagocytosis of neutrophils by bone marrow macrophages (Fig. 8.33) [26]. In agranulocytosis associated with thymoma, the bone marrow may show either apparent arrest at the promyelocytic stage or a total absence of myelopoiesis [27].

**Idiopathic hypereosinophilic syndrome**
The idiopathic hypereosinophilic syndrome is a condition of unknown aetiology characterized by sustained hypereosinophilia and damage to tissues, usually including the heart and central nervous system, by eosinophil products. The clinical features are due to this tissue damage. The idiopathic hypereosinophilic syndrome has been arbitrarily defined as requiring the eosinophil count to be greater than $1.5 \times 10^9/l$ for greater than 6 months and for tissue damage to have occurred [28]. Diagnosis of the idiopathic hypereosinophilic syndrome is mainly dependent on peripheral blood and clinical features and on the exclusion of other diagnoses. A bone marrow aspirate and trephine biopsy are of importance in excluding eosinophilic leukaemia and lymphoma, the latter being an important cause of reactive eosinophilia. Some cases of idiopathic hypereosinophilic syndrome represent a myeloproliferative disorder (MPD) and subsequently transform to AML despite initially displaying no

**Fig. 8.33** BM aspirate in auto-immune neutropenia showing neutrophil shadows within macrophages. MGG ×940.
specific evidence of the nature of the underlying disorder. Some cases of otherwise unexplained eosinophilia result from cytokine secretion by aberrant, sometimes monoclonal, T cells [29].

**Peripheral blood**

The eosinophil count is considerably elevated and eosinophils usually show some degree of hypogranularity and cytoplasmic vacuolation; completely agranular eosinophils are sometimes present. Eosinophil nuclei may be non-segmented or hypersegmented or occasionally ring-shaped. Neutrophils may show heavy granulation. In contrast to eosinophilic leukaemia, there are usually only occasional if any granulocyte precursors in the peripheral blood. There may be a mild anaemia and thrombocytopenia with red cells showing anisocytosis and poikilocytosis. Nucleated red cells are sometimes present.

**Bone marrow cytology**

The bone marrow shows an increase of eosinophils and their precursors (Fig. 8.34). Some eosinophil myelocytes show granules with basophilic staining.
characteristics but this feature is much less striking than in AML of M4Eo type (see page 150). There is no increase in blast cells. Macrophages may contain Charcot–Leyden crystals [30].

**Bone marrow histology**

Eosinophils and their precursors are increased (Fig. 8.35). It is important to exclude marrow infiltration by lymphoma since this may be easily overlooked. However, it should be noted that benign lymphoid aggregates may be associated with the idiopathic hypereosinophilic syndrome [31].

**Fig. 8.36** Chediak–Higashi syndrome. (a, b) BM aspirates, showing giant granules and vacuolation of granulocyte precursors: (a) MGG; (b) Sudan black B ×960.

**Problems and pitfalls**

The idiopathic hypereosinophilic syndrome is a diagnosis of exclusion. The bone marrow aspirate should be examined for any increase of blast cells, indicative of a diagnosis of eosinophilic leukaemia. Bone marrow aspirate films and trephine biopsy sections should be examined carefully for features of systemic mastocytosis, which occasionally presents with striking eosinophilia. Bone marrow cytogenetic analysis is indicated since demonstration of a clonal abnormality means that the condition is not ‘idiopathic’ but represents a chronic eosinophilic
leukaemia [32,33]. Immunophenotyping of peripheral blood lymphocytes is indicated and, when an aberrant population is found, T-cell receptor gene analysis can be used to identify a monoclonal T-cell proliferation. If such a clonal population is demonstrated, the eosinophilia should be regarded as secondary to a T-cell neoplasm rather than idiopathic.

Despite thorough investigation, some patients with apparently idiopathic hypereosinophilic syndrome can be recognized as having chronic eosinophilic leukaemia only in retrospect when they subsequently develop a granulocytic sarcoma or AML.

**Chediak–Higashi syndrome**

The Chediak–Higashi syndrome is a fatal inherited condition characterized by a defect in formation of lysosomes in multiple cell lineages. Patients suffer from albinism, neurological abnormalities and recurrent infections. Haematological abnormalities are most apparent in the granulocyte series although anaemia and thrombocytopenia also occur.

**Peripheral blood**

All granulocyte lineages show striking abnormalities.
Granules are very large and also have abnormal staining characteristics. Lymphocytes and monocytes may also have abnormally prominent granules. With disease progression, there is development of anaemia, neutropenia and thrombocytopenia.

**Bone marrow cytology**

Granulocyte precursors as well as mature granulocytes show giant granules with unusual staining characteristics (Fig. 8.36a,b). Sometimes there is also vacuolation. A secondary haemophagocytic syndrome may occur; it is likely to be consequent on immune deficiency and superimposed infection.

**Bone marrow histology**

Giant granules may be apparent in granulocyte precursors (Fig. 8.36c,d) but, in general, detection is easier in bone marrow aspirate films. Marked haemophagocytosis may be seen during the terminal phase (see page 119).

**Congenital thrombocytopenias**

Congenital thrombocytopenia may be inherited or may be secondary to intra-uterine infection, mutagen exposure or platelet destruction by maternal anti-platelet antibodies.

**Peripheral blood**

Morphological features are dependent on which specific defect is responsible for thrombocytopenia [1]. In inherited thrombocytopenia, the platelets may be of normal size, increased in size as in Bernard–Soulier syndrome or decreased in size as in the Wiskott–Aldrich syndrome. In the grey platelet syndrome they are increased in size and lack normal azurophilic granules. In the May–Hegglin anomaly, and in several other rare inherited defects, thrombocytopenia and giant platelets are associated with weakly basophilic cytoplasmic inclusions in neutrophils. When thrombocytopenia is secondary to intra-uterine platelet destruction or damage to megakaryocytes, the platelets are usually normal in size and morphology.

Other lineages are generally normal but infants with the thrombocytopenia-absent radii (TAR) syndrome have been noted to be prone to leukaemoid reactions.

**Bone marrow cytology**

In inherited thrombocytopenia, megakaryocytes may be present in normal numbers, as in Bernard–Soulier syndrome, or may be severely reduced in number, as in constitutional amegakaryocytic thrombocytopenia. In the TAR syndrome megakaryocytes are greatly reduced in number and are small with
poorly lobulated nuclei; eosinophilia is common. In
the Jacobsen syndrome, associated with a constitut-
ional deletion of the long arm of chromosome 11,
there are increased numbers of micromegakary-
ocyes [34]. In other rare syndromes characterized
by familial thrombocytopenia, megakaryocyte num-
bers are variously increased, normal or decreased
and megakaryocyte size may likewise be increased,
normal or decreased [35].

When thrombocytopenia results from intra-
uterine damage to megakaryocytes, these cells are
usually reduced in number. When platelets have
been destroyed by exposure to maternal antia-
platelet antibodies, megakaryocytes are present in
normal or increased numbers.

**Bone marrow histology**

A bone marrow biopsy is not often needed in
determining the cause of congenital thrombocy-
topenia but it can be useful in permitting an
accurate assessment of megakaryocyte numbers
and morphology. In the grey platelet syndrome
there may be associated myelofibrosis, probably
resulting from intramedullary release by megakary-
ocyes of granular contents capable of stimulating
fibroblasts.

**Acquired thrombocytopenias**

Isolated acquired thrombocytopenia is commonly
due to peripheral destruction of platelets, caused by
anti-platelet antibodies, drug-dependent antibodies
or immune complexes; the latter may attach to
platelets both in auto-immune diseases and during
or after viral infections, including infection by HIV.
Thrombocytopenia may also be consequent on
platelet consumption, as in thrombotic thrombocy-
topenic purpura or in disseminated intravascular
coagulation. Less often, acquired thrombocytopenia
results from megakaryocytic hypoplasia, such as
that induced by thiazide diuretics, or a failure of
megakaryocytes to produce platelets, as in some
patients with MDS who present with isolated
thrombocytopenia. Antibody-mediated amega-

karyocytic thrombocytopenia is a rare cause [36].
Auto-immune thrombocytopenia and, rarely,

amegakaryocytic thrombocytopenic purpura may
be associated with LGLL [15].

**Peripheral blood**

When thrombocytopenia is caused by a sustained
increase in the peripheral destruction or consump-
tion of platelets, there is usually an increase in
platelet size with some giant platelets being present.
When thrombocytopenia is due to failure of pro-
duction, as in sepsis or during chemotherapy, the
platelets are small. When thrombocytopenia is con-
sequent on MDS, platelets often show increased
variation in size and hypogranular or agranular
forms may be present.

**Bone marrow cytology**

When thrombocytopenia resulting from peripheral
destruction or consumption of platelets has devel-
oped acutely, the bone marrow may show no rele-
vant abnormality, megakaryocytes being present in
normal numbers. With sustained thrombocytopenia,
there is an increase in megakaryocyte numbers
(Fig. 8.37) and a reduction in average size. There is
often very little morphological evidence of platelet
production despite the increased platelet turnover
which can be demonstrated by isotopic studies.

When thrombocytopenia results from ineffective
thrombopoiesis, for example in MDS, megakary-
ocyes may be present in normal or increased
numbers and may show dysplastic features. In
acquired megakaryocytic hypoplasia, for example
as an adverse drug effect, megakaryocytes are
usually morphologically normal although reduced
in number. Antibody-mediated amegakaryocytic
thrombocytopenia may be cyclical. In these cases,
megakaryocyte numbers are reduced and mega-

karyocytes are small when the platelet count is
falling. When the count is rising, they are normal
or increased in number and cytologically normal
[36].

**Bone marrow histology**

Trephine biopsy is not usually necessary in the in-
vestigation of suspected immune thrombocytopenia
but is useful in confirming megakaryocytic hypo-
plasia and in investigating suspected myelodysplasia.

In idiopathic (auto-immune) thrombocytopenia,
the bone marrow is normocellular with increased
numbers of megakaryocytes (Fig. 8.38). Mean
megakaryocyte diameter is decreased. There is increased variation in size so that, although small megakaryocytes predominate, there are also increased numbers of giant forms. There is no abnormal localization of megakaryocytes and clusters are not usually seen [37]. In cyclical, antibody-mediated amegakaryocytic thrombocytopenia, megakaryocyte numbers are reduced when the platelet count is falling and normal when it is rising [36].

**Problems and pitfalls**

The differential diagnosis of isolated thrombocytopenia in children includes ALL. Leukaemia is unlikely if the haemoglobin concentration and white cell count are normal. However, a small but significant proportion of children in whom a presumptive diagnosis of auto-immune or post-viral thrombocytopenia is made turn out to have ALL. This has led to controversy as to whether a bone marrow aspirate is required in children with isolated thrombocytopenia [38]. There is concern that administration of corticosteroids without a pretreatment bone marrow examination may lead to inadvertent suboptimal treatment of undiagnosed ALL. For this reason, UK guidelines suggest that bone marrow aspiration should be performed before corticosteroid therapy is given, whereas this is not considered essential prior to high-dose immunoglobulin therapy or if no treatment is required. USA guidelines, however, do not regard a bone marrow examination as necessary if there are no atypical clinical or haematological features.

**Familial thrombocytosis**

Familial thrombocytosis has been reported in at least 17 individuals in eight families [39]. Inheritance appears to be autosomal dominant. In some, but not all, families the cause appears to be a mutation in the thrombopoietin gene. A minority of individuals have had splenomegaly.

**Peripheral blood**

The blood film and count show thrombocytosis as an isolated abnormality. Platelet morphology has sometimes been reported as abnormal [39].

**Bone marrow cytology and histology**

Megakaryocytes are increased in number and have sometimes been considered to be cytologically abnormal [39]. Bone marrow cellularity is sometimes increased.

**Problems and pitfalls**

Use of the term ‘essential thrombocythaemia’ to describe familial thrombocytosis is not recom-
mended since the condition clearly differs from the MPD that is usually intended by this term. MPD is rare in children with a significant proportion of cases of primary thrombocythaemia being found to be familial. Investigation of parents and siblings is therefore indicated when persistent unexplained thrombocytosis is found in a child or adolescent.

**Reactive thrombocytosis**

The platelet count may increase in response to infection, inflammation and malignant disease. In reactive thrombocytosis it is uncommon for the platelet count to exceed $1000 \times 10^9/l$.

**Peripheral blood**

In contrast to MPD, there is no increase in platelet size when thrombocytosis is reactive. The blood film may show other reactive changes including leucocytosis and neutrophilia but the presence of basophilia suggests MPD.

**Bone marrow cytology**

The bone marrow aspirate shows increased numbers of megakaryocytes of normal morphology.

**Bone marrow histology**

Megakaryocyte numbers are increased. The average megakaryocyte diameter is increased in comparison with normal and there is increased variation in size. There is no clustering or abnormality of distribution [37].

**Problems and pitfalls**

The differential diagnosis of reactive thrombocytosis includes hyposplenism and essential thrombocythaemia. Changes of hyposplenism should therefore be sought in the blood film. An increased basophil count, increased reticulin deposition in the bone marrow and clustering of megakaryocytes favour a diagnosis of essential thrombocythaemia.

**References**


