**Non-metastatic effects of cancer**

Patients with cancer but without bone marrow metastases may show a variety of haematological abnormalities.

**Peripheral blood**

Anaemia is common. Red cells may be normocytic and normochromic or microcytic and hypochromic. Rouleaux formation is often increased. Some patients have neutrophil leucocytosis, eosinophilia, monocytosis or thrombocytosis.

**Bone marrow cytology**

Erythropoiesis often shows the features of the anaemia of chronic disease. There may also be dyserythropoiesis. Granulopoiesis (neutrophil and/or eosinophil) may be increased and there may also be hypogranularity or some cells showing the acquired Pelger-Huët anomaly [1]. Megakaryocytes are often increased, as are macrophages, plasma cells and sometimes mast cells. Bone marrow necrosis may occur. It has been suggested that this may be mediated by tumour necrosis factor [2].

**Bone marrow histology**

There may be suppression of erythropoiesis, granulocytic hyperplasia and increased megakaryocytes (Fig. 9.1). Dyserythropoiesis, abnormal localization of immature granulocytes and dysplastic megakaryocytes are sometimes noted [1]. Macrophages, plasma cells and mast cells are sometimes increased. Stromal changes can include paratrabecular fibrosis, sinusoidal congestion, oedema and bone remodelling [1]. In patients with advanced disease, there may be gelatinous transformation, which is sometimes extensive. Patients with parathyroid...
hormone-secreting tumours may show changes of hyperparathyroidism. Iron stores may be increased.

**Bone marrow dysplasia with polyclonal haemopoiesis**

It is important to distinguish secondary bone marrow dysplasia from the myelodysplastic syndromes (MDS). The MDS are characterized by dysplastic and ineffective clonal haemopoiesis. They are neoplastic conditions which are potentially preleukaemic. In secondary myelodysplasia, haemopoiesis is polyclonal. The condition is neither neoplastic nor preleukaemic and, if the underlying cause can be removed, it is reversible. The commonest causes of secondary bone marrow dysplasia are infections (particularly HIV infection and tuberculosis), critical illness, often with multi-organ failure [3], exposure to drugs and toxins (particularly alcohol) and auto-immune diseases (such as systemic lupus erythematosus and juvenile rheumatoid arthritis). Dyserythropoiesis, possibly with an auto-immune basis, has also been reported in the auto-immune lymphoproliferative syndrome associated with Fas deficiency [4]. Certain anti-cancer drugs have the potential to induce MDS but, in addition, many anti-cancer drugs and related agents (e.g. azathioprine, mycophenolate mofetil and zidovudine) cause reversible dysplastic changes. Dysplastic changes have been reported following liver and other solid organ transplantation [5]. Macrocytosis and trilineage myelodysplasia have been reported in a significant minority of patients with large granular lymphocyte leukaemia (see page 290). Dyserythropoiesis is common in malaria. Anaemia with sideroblastic erythropoiesis has been observed in hypothermia [6]. The characteristic haematological effects of HIV infection (see page 123), anti-cancer and immunosuppressive chemotherapy (see page 393), excess alcohol intake (see page 399) and protein-calorie malnutrition (see page 413) are dealt with in detail elsewhere. The general features of secondary bone marrow dysplasia will be described here.

**Peripheral blood**

Anaemia is usual and thrombocytopenia is common. Some patients have leucopenia or pancytopenia. Red cells may show anisocytosis, macrocytosis or poikilocytosis. Neutrophils may show non-specific abnormalities such as cytoplasmic vacuolation, variable granulation, abnormalities of nuclear shape, binuclearity and detached nuclear fragments. Agranular neutrophils and the acquired Pelger–Huët anomaly are uncommon but do occur.

**Bone marrow cytology**

Dyserythropoiesis is common (Figs 9.2 and 9.3). Abnormalities seen include cytoplasmic bridging.
abnormal nuclear lobulation, binucularity and vacuolation. Erythropoiesis is sometimes megaloblastic. This is particularly common in intensive care ward patients who have been exposed to nitric oxide [3]. Ring sideroblasts may be present although they are usually less frequent than in MDS. Granulopoiesis may show abnormal chromatin clumping, hypolobulation, left shift, vacuolation, hypogranularity or variable granulation and the presence of giant metamyelocytes. Erythroid and granulocyte precursors are sometimes vacuolated. Multinucleated or non-lobulated megakaryocytes may be present. In contrast to MDS, very small mononuclear or binuclear megakaryocytes are uncommon in secondary dysplasia.

**Bone marrow histology**

The bone marrow may be hypercellular, normocellular or hypocellular. There is often a discrepancy between a hypercellular or normocellular marrow and peripheral cytopenia. Erythropoiesis is often decreased. Reactive changes (e.g. increased macrophages with haemophagocytosis, increased lymphocytes or increased plasma cells) are often present and some patients show gelatinous transformation. Marrow architecture may be disturbed and reticulin may be increased. A marked increase in reticulin deposition may be a feature of systemic lupus erythematosus. A very rare finding in this condition is that of lupus erythematosus (LE) cells in the bone marrow biopsy [7].

**Problems and pitfalls**

It is important not to over-interpret dysplastic features in the bone marrows of patients with severe illness. It is also important to distinguish dysplastic features that are a direct effect of chemotherapeutic agents from therapy-induced MDS. The former disappear on cessation of the causative agent whereas the latter do not.

**The haematological effects of anti-cancer and immunosuppressive chemotherapy**

The majority of anti-cancer and immunosuppressive chemotherapeutic agents are damaging to the bone marrow. Most cause hypoplasia, some cause megaloblastosis and some have other, more specific effects. The nature of the bone marrow damage depends on dose and duration of therapy. A drug may, for example, cause erythroid hyperplasia and megaloblastic erythropoiesis at a low dose and severe hypoplasia at a higher dose.

**Peripheral blood**

The most prominent effect of anti-cancer chemotherapy is pancytopenia. This is usual with all of the
commonly employed agents, the exceptions being vincristine and bleomycin. Neutropenia and thrombocytopenia are apparent well in advance of anaemia. Some degree of anisocytosis and poikilocytosis, together with basophilic stippling and Howell–Jolly bodies, occurs as a consequence of the dyserythropoiesis induced by chemotherapeutic agents. When megaloblastic change is induced, formation of Howell–Jolly bodies is more marked and macrocytosis is common. Dysplastic changes, including abnormalities of nuclear shape and nuclear inclusions within the cytoplasm, may also be apparent in neutrophils. A reversible acquired Pelger–Huët anomaly has been observed with a number of drugs including chlorambucil and mycophenolate mofetil (Fig. 9.4).

Platelets are small but do not show any specific morphological abnormality. Vincristine is unusual in occasionally causing thrombocytosis, although not when it is given in combination with other drugs which are highly toxic to the bone marrow.

Occasionally chemotherapy is followed by the development of micro-angiopathic haemolytic anaemia. This appears to be particularly a feature of therapy with mitomycin C.

**Bone marrow cytology**

The bone marrow aspirate shows a variable degree of hypoplasia. If bone marrow aspiration is performed after an episode of severe hypoplasia, early regeneration may produce appearances misinterpreted as ‘maturation arrest’ (Fig. 9.5). Erythropoiesis is dysplastic, often strikingly so. Drugs that cause megaloblastosis include methotrexate, cyclophosphamide, daunorubicin, adriamycin, cytosine arabinoside, hydroxycarbamide (hydroxyurea), azathioprine and zidovudine. The megaloblastosis induced by anti-cancer chemotherapeutic agents, with the exception of folate antagonists, differs from that due to vitamin B₁₂ or folate deficiency in that dyserythropoiesis is very striking and hypersegmented neutrophils and giant metamyelocytes are not usually a feature. Depending on drug dose, megaloblastosis may be associated with erythroid hyperplasia (Fig. 9.6) or hypoplasia. Other drugs cause dysplastic features without megaloblastosis. Erythroid dysplasia may be striking, both with megaloblastic and with normoblastic erythropoiesis. Vincristine and other spindle poisons cause mitotic arrest in quite a high proportion of erythroblasts; this is detected if a bone marrow aspirate is performed 1–2 days after the administration of one of these drugs (Fig. 9.7). Bone marrow aspirates taken shortly after the administration of chemotherapeutic agents may show increased apoptosis and increased numbers of macrophages containing cellular debris.

When mycophenolate mofetil causes the acquired Pelger–Huët anomaly, abnormal chromatin clumping and detached nuclear fragments
**Fig. 9.5** BM aspirate from a patient with severe methotrexate toxicity, showing ‘maturation arrest’; two promyelocytes and one proerythroblast are seen but maturing cells are severely diminished. MGG ×940.

**Fig. 9.6** BM aspirate from a patient taking hydroxycarbamide for psoriasis, showing erythroid hyperplasia, mild megaloblastosis and one dyserythropoietic cell. MGG ×940.

**Fig. 9.7** BM aspirate, performed about 24 h after administration of vincristine, showing a binucleate erythroblast and four erythroblasts arrested in mitosis. MGG ×940.
can be seen in granulocyte precursors in the bone marrow as well as in peripheral blood cells (Fig. 9.8).

**Bone marrow histology**

Cells exposed to chemotherapeutic agents show apoptosis. Dead cells degenerate to granular eosinophilic debris. With intensive chemotherapy, depletion of haemopoietic cells is severe and stromal elements become prominent. There are dilated sinusoids containing red cells and fibrin [8], and sometimes residual lymphocytes and plasma cells, the latter particularly along small blood vessels. Red cells may be extravasated from dilated sinusoids. In the acute phase of bone marrow damage there may be interstitial oedema; at this stage stains for stromal mucin are negative. Subsequently, typical features of gelatinous transformation may develop.

In the majority of patients treated with intensive chemotherapy for acute leukaemia [8,9], the marrow is almost completely emptied of haemopoietic cells, particularly when therapy is of the type used in acute myeloid leukaemia (AML). Varying degrees of stromal damage occur, including stromal necrosis. Subsequently, there may be collagen deposition, increased osteoblastic activity and focal appositional or intertrabecular bone formation [8]. Prominent residual plasma cells (Fig. 9.9) are more a feature of AML than of acute lymphoblastic leukaemia (ALL) [9]. Cellular depletion persists for 3–4 weeks, to be followed by regeneration of fat cells, which are initially multivesicular, then by regeneration of haemopoietic cells. Erythroid and megakaryocytic regeneration often occurs before granulocytic regeneration but this is variable. In the early stages of regeneration, clusters of haemopoietic precursors made up of cells from a single lineage (Fig. 9.10) are often seen. Topography may be abnormal with erythroid islands adjacent to trabeculae, abnormal localization of immature precursors (ALIP) and megakaryocyte clustering. Extensive bone remodelling may be seen following intensive chemotherapy.

**Problems and pitfalls**

Megakaryocyte clustering and ALIP are common features during recovery from intensive chemotherapy and may persist for many months. In this context these abnormalities should not be interpreted as evidence of MDS. It is important to know if chemotherapeutic regimens include growth factors such as granulocyte colony-stimulating factor (G-CSF), since this will complicate the interpretation of increased numbers of myeloblasts and promyelocytes. Following cessation of chemotherapy, particularly in children, there may be a rebound increase in immature lymphoid cells. This should not be confused with relapse of leukaemia.
The haematological effects of other drugs and chemicals

Anti-cancer and related drugs have predictable haematological toxicity. Other drugs more often cause idiosyncratic reactions with an immunological mechanism such as agranulocytosis (see page 381), immune haemolytic anaemia and aplastic anaemia (see page 401). There is also a small group of other drugs with predictable toxicity. Oxidant drugs and chemicals can cause haemolytic anaemia. Chloramphenicol, as well as causing severe idiosyncratic reactions, regularly causes mild bone marrow suppression with ring sideroblasts and vacuolation of erythroid and granulocyte precursors. A number of drugs including isoniazid cause sideroblastic erythropoiesis. Lead poisoning can cause basophilic stippling of erythrocytes, hypochromic microcytic anaemia, haemolytic anaemia and sideroblastic erythropoiesis. Arsenic can cause pancytopenia with dysplastic erythropoiesis including basophilic stippling and the presence of ring sideroblasts [10,11] (Fig. 9.11). Zinc toxicity can lead to copper deficiency with consequent anaemia, neutropenia...
**Fig. 9.11** BM aspirate, showing dyserythropoiesis induced by arsenic. MGG ×960. (By courtesy of Professor A Newlands, London.)

**Fig. 9.12** BM aspirate from a patient with copper deficiency caused by chelation therapy for Wilson’s disease showing: (a) hypocellularity; (b) vacuolation of granulocyte precursors. MGG ×188 and ×940. (By courtesy of Dr A Grigg, Melbourne.)
sideroblastic erythropoiesis and vacuolation of erythroid and myeloid precursors [12]. Similar features are seen with copper-depleting drugs such as penicillamine, trientine and ammonium tetranthiomolybdate used in the treatment of Wilson’s disease [13] (Figs 9.12 and 9.13).

**The effect of irradiation on the bone marrow**

Irradiation of a significant proportion of the bone marrow causes a fall in the neutrophil and platelet counts. Extensive irradiation causes pancytopenia. Monitoring of blood counts is therefore carried out during radiotherapy.

**Peripheral blood**

The blood film may show neutropenia, thrombocytopenia and the features of anaemia.

**Bone marrow cytology**

The initial change in irradiated bone marrow is pyknosis and karyorrhexis of haemopoietic cells followed by disappearance of haemopoietic and fat cells and replacement by areas of gelatinous transformation. Subsequently, at the site of irradiation, hypoplastic marrow is found, with haemopoietic cells being replaced by fat. Extensive high-dose irradiation of the bone marrow is followed by aplastic anaemia.

**Bone marrow histology**

Initially, there may be necrosis of the bone marrow within the field that has received high-dose radiation. Cell loss is initially greatest adjacent to trabeculae as more mature cells are more radio-resistant. There is endothelial cell swelling, sinusoidal dilation, interstitial haemorrhage and sometimes stromal necrosis. Haemosiderin-laden macrophages appear on a background of eosinophilic debris. Subsequently, gelatinous transformation may occur. Bone necrosis may occur during the acute phase and may be followed by bone remodelling and radiation-induced osteodysplasia (Fig. 9.14). Later, there is permanent replacement of haemopoietic marrow by fat or, less often, fibrous tissue.

**The haematological effects of alcohol**

Excess intake of ethanol is often complicated by dietary deficiency and liver disease. However, ethanol itself has well-defined haematological toxicity.

**Peripheral blood**

There is a normocytic or macrocytic anaemia with red cells being normochromic. Macrocyes differ from those of megaloblastic anaemia in that they are usually round rather than oval (Fig. 9.15). Stomatocytes are common and target cells are...
sometimes present. A dimorphic blood film has been reported but is not common. Heavy alcohol intake and acute alcoholic liver disease have been associated with haemolytic anaemia with hyperlipidaemia and with the blood film showing spherocytes or irregularly contracted cells; this is designated Zieve’s syndrome. The neutrophil count is usually normal but the capacity of the bone marrow to mount a neutrophil response to infection is reduced and infection may lead to neutropenia. The lymphocyte count may be reduced. Thrombocytopenia is common. If alcohol intake is suddenly stopped, a rebound thrombocytosis can occur.

**Bone marrow cytology**

Erythropoiesis is normoblastic, macronormoblastic or mildly megaloblastic. Siderotic granules are prominent and there may be ring sideroblasts; sometimes these are numerous. There are other dyserythropoietic features such as erythroid multinuclearity. Erythroid and granulocyte precursors are sometimes vacuolated (Fig. 9.16). Iron stores may be increased; sometimes haemosiderin inclusions are present in plasma cells [14] and in endothelial cells lining sinusoids [15]. The latter phenomenon may be noted in the absence of any
increase in macrophage iron [15]. In Zieve’s syndrome there may be an excess of iron-laden foamy macrophages (Fig. 9.17). Megakaryocytes are often increased [15]. Alcohol-induced reversible bone marrow hypoplasia has been reported [16].

**Bone marrow histology**

Trephine biopsy sections show dyserythropoiesis. There may be increased iron in macrophages and iron in plasma cells and endothelial cells.

**Problems and pitfalls**

It is important to be aware of the likelihood of excess alcohol intake in interpreting cytopenias and dysplastic features. Otherwise there may be a misdiagnosis of MDS.

**Aplastic anaemia**

Aplastic anaemia is a heterogeneous disorder characterized by pancytopenia and a hypocellular marrow without any apparent underlying neoplastic process. The name, although well established, is somewhat misleading since all haemopoietic lineages are involved. Aplastic anaemia is rare. In Europe and North America the incidence is of the order of 5–10/1 000 000/year but in various other parts of the world, for example in Asia, the disease is considerably more common. Although some cases of aplastic anaemia result from an inherited disorder and develop in infancy or childhood, the incidence, in general, increases with age.

The commonest inherited form of aplastic anaemia is Fanconi’s anaemia. This is an autosomal recessive condition in which sufferers have defective DNA repair mechanisms. The pancytopenia usually develops between the ages of 5 and 10 years. Without bone marrow transplantation many patients die from infection or bleeding but approximately 20% develop AML [17]. Other inherited disorders which may progress to aplastic anaemia include dyskeratosis congenita, the Hoyeraal–Hreidarsson syndrome (a severe variant of dyskeratosis congenita) [18], the Schwachman–Diamond syndrome and amegakaryocytic thrombocytopenia without physical defects [19]. In the Schwachman–Diamond syndrome, neutropenia often develops first; pancytopenia follows, resulting from aplastic anaemia.

Known causes of acquired aplastic anaemia include viral hepatitis, irradiation, auto-immune disease, drugs (such as chloramphenicol) and chemicals (such as benzene). Aplastic anaemia may be the initial presentation of systemic lupus erythematosus [20]. Pregnancy appears to be a rare cause of aplastic anaemia [21]. In many cases the cause is not apparent and the designation ‘idiopathic aplastic anaemia’ is then used.
The diagnosis of aplastic anaemia may be suspected from peripheral blood and bone marrow aspirate findings but a trephine biopsy is essential for diagnosis. This is because of the frequent difficulty in obtaining an adequate aspirate and the variable degree of hypoplasia in different areas of the marrow. If bone marrow examination does not confirm a strong clinical suspicion of aplastic anaemia, repeat examination at another site is indicated since the bone marrow may be affected in an uneven manner.

Aplastic anaemia has been categorized on the basis of peripheral blood and bone marrow features as severe, very severe or non-severe. Patients with severe aplastic anaemia have a platelet count less than $20 \times 10^9/l$, a granulocyte count less than $0.5 \times 10^9/l$ and bone marrow cellularity less than 25% [22]. Patients with very severe aplastic anaemia have a granulocyte count less than $0.2 \times 10^9/l$. Other cases are categorized as non-severe.

Prior to the development of stem cell transplantation and immunosuppressive therapy, the prognosis of aplastic anaemia was poor with severe cases having a median survival of less than a year. With immunosuppressive therapy (anti-lymphocyte globulin plus cyclosporin) or stem cell transplanta-

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Fig. 9.17 BM aspirate from a patient with Zieve’s syndrome showing: (a) foamy macrophages, MGG $\times 960$; (b) an iron-laden foamy macrophage, Perls’ stain $\times 960$. (By courtesy of Dr Sue Fairhead, London.)
tion from a histocompatible sibling, 5-year survivals of the order of 50–70% can be anticipated. Bone marrow or other stem cell transplantation may cure aplastic anaemia whereas, following immunosuppressive therapy, defective stem cells persist giving the possibility of evolution into paroxysmal nocturnal haemoglobinuria (PNH), MDS or AML.

**Peripheral blood**

Severe cases are characterized by pancytopenia and a low reticulocyte count. The lymphocyte count is also low. The anaemia may be normocytic or macrocytic and poikilocytes may be present. Neutrophils often have dark red granules and high alkaline phosphatase activity, even in the absence of any apparent infection. Platelets are of normal size, in contrast to the large platelets which are common when thrombocytopenia is the result of increased platelet destruction. Macrocytosis and borderline cytopenias may persist following remission induced by immunosuppressive therapy.

**Bone marrow cytology**

The bone marrow may be difficult to aspirate with the result being a ‘dry tap’ or ‘blood tap’. In the majority of patients a hypocellular aspirate is obtained with the fragments being composed largely of fat (Fig. 9.18). The cell trails are also hypocellular. Different lineages are affected to a variable extent so that the M:E ratio may be increased, normal or decreased. Dyserythropoiesis may be seen. Ring sideroblasts are not a feature but, otherwise, the changes seen can be similar to those observed in MDS [23,24]. Dysplastic changes in granulocytes are less common and pseudo-Pelger neutrophils are not a feature. There is no disproportionate increase in immature granulocyte precursors. Megakaryocytes are often so infrequent in the aspirate that it is difficult to assess their morphology.

In a minority of patients the aspirate is normocellular or even hypercellular [23,24]. Examination of trephine biopsy specimens from such patients shows that such ‘hot spots’ co-exist with extensive areas of hypoplastic marrow.

The bone marrow aspirate shows at least a relative increase in lymphocytes and sometimes an absolute increase. There may also be increased numbers of plasma cells, macrophages and mast cells. Foamy macrophages are sometimes present and macrophage iron is increased.

**Bone marrow histology**

Trephine biopsy is crucial in the diagnosis of aplastic anaemia. The bone marrow is usually hypocellular with a marked reduction of haemopoietic cells (Figs 9.19–9.21). Myeloid cells are mainly replaced
Fig. 9.19 BM trephine biopsy section, aplastic anaemia, showing marked hypocellularity. Plastic-embedded, H&E ×39.

Fig. 9.20 BM trephine biopsy section, aplastic anaemia, showing a marked reduction in haemopoietic precursors; many of the remaining cells are plasma cells. Plastic-embedded, H&E ×390.

Fig. 9.21 BM trephine biopsy section, Fanconi’s anaemia, showing large, poorly formed erythroblastic islands containing increased numbers of early erythroblasts. Plastic-embedded, H&E ×188.
by fat but there is a variable inflammatory infiltrate composed of lymphocytes, plasma cells, macrophages, mast cells and sometimes eosinophils [25] (Fig. 9.20). Lymphocytes, which are CD3-positive and either CD4- or CD8-positive, are preferentially increased in areas of residual haemopoiesis [26]. Reactive lymphoid aggregates are also increased. Necrotic cells and cellular debris may be present. Walls of sinusoids may be disrupted and there may be oedema and haemorrhage. In some patients the inflammatory infiltrate is so heavy that the marked reduction of haemopoietic cells is not immediately apparent. Sinusoids are reduced but arterioles and capillaries are normal or increased [27]. Residual erythroid cells show dysplastic features [25]. Macrophage iron is increased. A distinctive appearance has been observed in aplastic anaemia induced by acetazolamide. In many of these patients there is depletion of haemopoietic cells leaving abnormal stroma, lymphocytes and plasma cells but without replacement by fat.

A minority of cases have some areas of normal or increased cellularity. Such cellular areas are commonly adjacent to sinusoids [27] and are composed of erythroid cells, all at the same stage of development and showing dysplastic features [24]. This finding is more common in Fanconi's anaemia (Fig. 9.21). In this condition the marrow is initially normocellular but becomes hypocellular. Megakaryocytes are often the first lineage to show reduction, followed by granulocytes and then erythroid cells.

Reticulin shows little if any increase. Various abnormalities of bone have been reported. Some studies have found osteoporosis and others increased osteoblastic and osteoclastic activity with irregular remodelling of bone [25].

When aplastic anaemia remits, for example following therapy with anti-thymocyte or anti-lymphocyte globulin, dysplastic features are very evident [28,29] and the inflammatory infiltrate often persists [29].

The presence of trilineage dysplasia and increased reticulin deposition is indicative of a worse prognosis [25,30] but this was not so in three large series of patients treated either with antithymocyte globulin or by bone marrow transplantation [29,31].

**Cytogenetics and molecular genetics**

At presentation, a significant proportion of patients with acquired aplastic anaemia are found to have a clonal cytogenetic abnormality, most often trisomy 6 or 8 or anomalies of chromosomes 5 or 7. Although this is indicative of the presence of a neoplastic clone, it is not predictive of progression to MDS or AML [32]. The abnormal clone may disappear following immunosuppressive therapy [32].

Clonal cytogenetic abnormalities appearing following a response to immunosuppressive therapy are of more significance. They are often present at the time of evolution to MDS or AML. Abnormalities observed have included monosomy 6 and monosomy or deletion of chromosome 7.

The molecular genetic abnormalities underlying some types of inherited aplastic anaemia have been defined. Dyskeratosis congenita is caused by mutation or deletion of the **DKC** gene at Xq28. Fanconi's anaemia is associated with chromosomal fragility and cytogenetic analysis following exposure to clastogenic agents is diagnostically useful.

**Problems and pitfalls**

A diagnosis of aplastic anaemia should not be based on a bone marrow aspirate alone. A trephine biopsy is essential in order both to assess cellularity of an adequate sample of marrow and to assess the cytological features of residual cells. A trephine biopsy is particularly important in distinguishing aplastic anaemia from hypoplastic MDS and AML and from conditions in which bone marrow fibrosis leads to a hypocellular uninformative aspirate. Abnormal cells such as blast cells or hairy cells may be present in the trephine biopsy although not detectable in a hypocellular aspirate.

An adequate clinical history is important in order to avoid performing a bone marrow biopsy at the site of previous radiotherapy; the bone marrow at such sites is hypocellular and histological features may be indistinguishable from those of aplastic anaemia (Fig. 9.22). It should be noted that subcor-
tical bone marrow is hypocellular (Fig. 9.23) so that a diagnosis of aplastic anaemia should never be based on an inadequate biopsy composed mainly of cortical bone and subcortical bone marrow.

The relationship of aplastic anaemia to hypocellular MDS is problematical since neoplastic clones arise in some cases of aplastic anaemia and may be predictive of subsequent MDS and AML. However, it should be noted that, although the detection of a clonal cytogenetic abnormality in a hypoplastic bone marrow is indicative of the presence of a neoplastic clone, it is not necessarily predictive of disease progression. Such clones sometimes disappear spontaneously. It may be that hypocellular MDS represents an intermediate stage of evolution of typical aplastic anaemia to MDS [19] or to AML. Aplastic anaemia can also progress through typical hypercellular MDS to AML [33]. Of the long-term survivors of aplastic anaemia, the number who develop MDS and AML may be as high as 10% [34]. In the differential diagnosis of hypoplastic MDS and aplastic anaemia the most important feature is the presence of clusters of blasts which are indicative of the former diagnosis. Other features which have been found, to some extent, to be predictive of progression to AML and which can therefore be
considered to favour a diagnosis of hypocellular MDS are: (i) trilineage atypia, particularly megakaryocyte atypia; (ii) increased numbers or clustering of megakaryocytes; and (iii) reticulin fibrosis [33]. In Fanconi’s anaemia the development of trilineage dysplasia and reticulin fibrosis may herald transformation to AML.

The relationship of aplastic anaemia to PNH is discussed below.

It should be noted that, in children, apparent aplastic anaemia may represent an aplastic presentation of ALL. Spontaneous recovery of haemopoiesis occurs, to be followed within a few months by frank ALL; increased reticulin is common in pre-ALL aplasia and a proportion of patients also have prominent bone marrow lymphocytes [35]. DNA analysis has shown that leukaemic cells are present in significant numbers in the hypoplastic stage [36].

Other causes of bone marrow aplasia and hypoplasia

Reversible aplasia follows intensive cytotoxic chemotherapy. In subjects unable to mount a normal immune response to the EBV, primary infection by the virus may cause bone marrow aplasia. Hypoplasia can also be a feature of CMV infection. Other infections, including toxoplasmosis, sometimes cause bone marrow aplasia [37]. Bone marrow aplasia is also one of the features of graft-versus-host disease (GVHD) (see below).

Other causes of bone marrow hypoplasia include starvation, anorexia nervosa (Fig. 9.24), severe hypothyroidism, copper deficiency (see Fig. 9.13) and arsenic toxicity.

Pearson’s syndrome and other mitochondrial cytopathies

Several congenital syndromes with mitochondrial inheritance cause anaemia and cytopenia with an onset during childhood [38]. There may be associated pancreatic dysfunction, metabolic disorder or developmental delay.

Peripheral blood

Normocytic normochromic anaemia, neutropenia and thrombocytopenia occur in variable combinations.

Bone marrow cytology

There is dyserythropoiesis with numerous ring sideroblasts and vacuolation of erythroid and granulocytic precursors (Fig. 9.25).

Other constitutional abnormalities associated with abnormal haemopoiesis

Down’s syndrome may be associated, in the neonatal period, with otherwise unexplained

**Fig. 9.24** BM trephine biopsy section in anorexia nervosa, showing marked hypocellularity. H&E×192.
polycythaemia or with transient abnormal myelopoiesis, which probably represents transient leukaemia [39]. Subsequently, there may be trilineage myelodysplasia. The incidence of AML, specifically acute megakaryoblastic leukaemia, is greatly increased.

Griscelli syndrome is a rare fatal disorder with abnormal pigmentation and variable cellular immune deficiency [40]. Pancytopenia is characteristic. The bone marrow may appear normal or there may be lymphohistiocytic infiltration with haemophagocytosis.

Thiamine-responsive anaemia is an autosomal recessive condition that can cause not only megaloblastic anaemia but also pancytopenia with trilineage myelodysplasia. Features include small and hypolobulated megakaryocytes, multinucleated megakaryocytes and hypolobulated neutrophils [41].

**Paroxysmal nocturnal haemoglobinuria**

PNH is a heterogeneous disease, the essential feature of which is abnormal complement sensitivity of red cells. PNH is a clonal disorder resulting from a somatic mutation in a multipotent myeloid stem cell. In the majority of cases, cells of the abnormal clone co-exist with normal polyclonal haemopoietic cells; in a minority the PNH clone constitutes virtually all haemopoietic tissue [42]. The causative mutation occurs in an X-linked gene, *PIG-A*, that encodes a protein essential for the biosynthesis of glycosyl phosphatidylinositol (GPI). GPI is an important component of the red cell membrane, providing an anchor for many proteins. GPI-anchored proteins include CD55 (a complement-regulatory protein) and CD59. The defect in the red cell membrane leads, *in vitro*, to lysis of cells when serum is acidified and, *in vivo*, to intravascular haemolysis which is often nocturnal.

About a quarter of cases of PNH evolve to aplastic anaemia [19]. Conversely, 5–10% of patients with aplastic anaemia acquire a PNH clone during the course of their illness, often with associated clinical improvement [19,42]. In a small percentage of cases of PNH there is evolution to AML. The specific PNH defect of red cells leading to a positive acid lysis test has also been observed, occasionally, in patients with other clonal disorders of haemopoiesis including MDS (sideroblastic anaemia and refractory anaemia with excess of blasts) and myeloproliferative disorders (MPD) (myelofibrosis and unclassified MPD). Recovery of PNH can occur with the abnormal clone disappearing and being replaced by normal polyclonal haemopoietic cells.

The diagnosis of PNH is confirmed by an acid lysis (Ham) test or sugar–water test showing complement sensitivity of red cells. Alternatively, the diagnosis can be confirmed by flow cytometry, using monoclonal antibodies to demonstrate a deficiency of GPI-linked proteins such as CD59.
Peripheral blood

PNH is characterized by some degree of chronic haemolysis with episodes of more severe haemolysis. Red cells do not show any morphological abnormalities other than polychromasia associated with an elevated reticulocyte count. Some patients have neutropenia, thrombocytopenia or both. Neutrophil alkaline phosphatase activity is typically low or absent.

Bone marrow cytology

The most characteristic bone marrow abnormality is hypercellularity due, at least in part, to erythroid hyperplasia (Fig. 9.26); there is often also granulocytic and megakaryocytic hyperplasia. However, in some patients the specific red cell abnormality of PNH occurs when there is bone marrow hypoplasia. Mast cells may be increased.

Bone marrow histology

Trephine biopsy sections may show erythroid hyperplasia or generalized hypoplasia.

Bone marrow and other haemopoietic stem cell transplantation

Allogeneic haemopoietic stem cells suitable for transplantation may be obtained by bone marrow aspiration from volunteer donors. Alternatively, they may be obtained from cord blood or may be harvested from peripheral blood, following stimulation by growth factors such as G-CSF. Since stem cell transplantation necessitates prior immunosuppression, and often also ablative chemotherapy, the haematological features of bone marrow aplasia precede the signs of stem cell engraftment. Stem cell transplantation may be complicated by a variety of pathological processes [43] including sepsis, rejection and GVHD. Infection with CMV [44] and human herpesvirus 6 [45] post-transplant can cause bone marrow hypoplasia leading to pancytopenia. EBV-triggered lymphoproliferative disease (see page 319) occurs but is uncommon, in comparison with the incidence following solid organ transplantation; it occurs in about 1% of stem cell transplant recipients. Chronic parvovirus B19-induced red cell aplasia may develop as a consequence of post-transplant immune deficiency. In the early post-transplant period there is hypoplasemia. Post-transplant there is also an increased incidence of auto-immune thrombocytopenic purpura (often associated with chronic GVHD), auto-immune neutropenia, auto-immune haemolytic anaemia and Evans syndrome [46]. Microangiopathic haemolytic anaemia is also observed in some patients, occurring as a result of endothelial damage caused by cyclosporin A or other agents.

Autologous stem cell transplantation may lead to some of the same pathological processes that follow...
allogeneic stem cell transplantation, since there is a period of bone marrow aplasia and immune deficiency, but GVHD does not occur.

Post-transplantation, a bone marrow biopsy is generally more informative than the peripheral blood film or bone marrow aspirate.

**Peripheral blood**

Initially, there is a period of 2–3 weeks of severe pancytopenia, followed by a gradual rise of white cell and platelet counts as engraftment occurs. If there is failure of engraftment or if rejection occurs, there is a failure of counts to rise or a subsequent fall. Features of hyposplenism may be present. Those who develop auto-immune complications or micro-angiopathic haemolytic anaemia show the expected peripheral blood features. If patients develop EBV-triggered lymphoproliferative disease following transplantation, the peripheral blood film may be leuco-erythroblastic and show atypical immature lymphoid cells.

**Bone marrow cytology**

The bone marrow aspirate is initially severely hypoplastic. Subsequently, haemopoietic cells gradually reappear. Dysplastic features may be present. In the months following transplantation an appreciable increase may occur in haemato-gones, lymphoid cells which morphologically and immunophenotypically resemble lymphoblasts of L1 ALL [47]; with prolonged follow-up these are no longer apparent. If rejection occurs the abnormalities noted include lymphocytosis, plasmacytosis, increased macrophages and increased iron stores [43]. If chronic parvovirus infection occurs, the bone marrow aspirate shows a lack of erythroid cells beyond the proerythroblast stage. EBV-triggered lymphoproliferative disease is associated with bone marrow infiltration by highly atypical immature lymphoid cells including bizarre plasmacytoid lymphocytes. Patients with failure to engraft, particularly but not exclusively those treated with granulocyte–macrophage colony-stimulating factor (GM-CSF), may have an increase of foamy histiocytes in a hypocellular marrow [48]. When auto-immune complications occur, the expected erythroid or megalakaryocytic hyperplasia may be seen but this is dependent on adequate haemopoietic reconstitution.

**Bone marrow histology** [43,49–51]

The speed of haemopoietic regeneration depends on the type of transplantation; engraftment is much more rapid after transplantation of autologous peripheral blood stem cells, least rapid after allografting from unrelated donors and intermediate with allografts from related donors. In general, during the first 2 weeks cellularity is very low. Thereafter, clusters of proliferating cells appear at a variable rate (Fig. 9.27). In the early stages of engraftment, foci of regenerating cells commonly contain cells of only one lineage and cells may be all at the same stage of development. The topography may be abnormal, with foci of granulocyte precursors present in the central intertrabecular area rather than in a paratrabeular position. Megakaryocytes are often clustered. Haemopoietic cells may be dysplastic. Often there are stromal changes such as oedema, the presence of foamy macrophages, formation of small granulomas, sinusoidal ectasia and extravasation of red cells into the interstitium; these abnormalities are probably a result of damage caused by the ablative therapy employed prior to grafting and are more marked in patients transplanted for leukaemia. There may also be lymphoid foci, sometimes with associated eosinophils. Plasma cells may be increased in patients who have had a stem cell transplant for acute leukaemia. In patients with increased reticulin or collagen, there is gradual stromal remodelling with a return to normal or near normal appearances. If rejection occurs, the trephine biopsy may show oedema and fat necrosis, in addition to the features apparent in the aspirate, which have been mentioned above. There may be small foci of lymphoblast-like cells. A hypoplastic bone marrow biopsy post-transplant may result from failure of engraftment, infection by herpesviruses or stromal damage resulting from GVHD. Selective loss of maturing red cells is seen in parvovirus B19-induced chronic pure red cell aplasia.

**Problems and pitfalls**

Patients who have had an autologous stem cell transplant show an increased incidence of MDS as a
result of damage to stem cells by preceding chemotherapy. However, the diagnosis of MDS should be made with circumspection since disturbed architecture and dysplastic features are common in the early post-transplant period. Cytogenetic and molecular genetic analysis can be useful in making the distinction.

In patients transplanted for ALL, a post-transplant excess of haematogones must be distinguished from relapse. Immunophenotyping and cytogenetic and molecular genetic analysis can be useful in making the distinction.

In patients transplanted for multiple myeloma, increased monoclonal plasma cells are often present in the first 1–2 months after transplantation. These represent residual myeloma cells rather than relapse and are not predictive of disease progression [52].

Graft-versus-host disease (including the effects of donor-lymphocyte infusion)

GVHD occurs not only in the setting of stem cell transplantation but also when viable, immunocompetent, histo-incompatible lymphocytes have been transferred to an immuno-incompetent host. This may occur in utero, when there is transfer of maternal lymphocytes to a fetus with severe combined immune deficiency. Following birth, it can occur following blood transfusion in congenital and certain acquired immune deficiency states. It has been recognized in patients being treated for Hodgkin’s disease and in patients with low grade lymphoproliferative disorders who have received treatment with nucleoside analogues such as fludarabine.

GVHD can also occur in immunologically normal hosts when blood for transfusion was derived from a donor who was homozygous for a human leucocyte antigen (HLA) haplotype identical to one of the host’s haplotypes; the host is then unable to recognize the recipient’s lymphocytes as foreign and so cannot destroy them, whereas the transfused lymphocytes are capable of recognizing and attacking host tissues. GVHD in immunologically normal hosts has most often resulted from transfusions from closely related family members.

Donor-lymphocyte transfusion, increasingly practised for post-transplant relapse of chronic granulocytic leukaemia (CGL) or other haemopoietic neoplasms, can also be complicated by GVHD.

GVHD has resulted from inadvertent transfer of donor lymphocytes following solid organ transplantation [53].

The bone marrow features of GVHD differ depending on whether bone marrow has been transplanted or not. When viable lymphocytes only have been transferred, the host’s bone marrow will be among the tissues which come under immunological attack and bone marrow aplasia results. In patients who have received donor bone marrow containing viable lymphocytes, other tissues are attacked but, since the bone marrow is donor in
origin, it will not be recognized as foreign by donor lymphocytes. The haemopoietic marrow may, however, be indirectly damaged by the immunological reaction between donor cells and host cells including the bone marrow stroma.

It was previously suggested that Omenn’s syndrome, a condition of infants characterized by combined immunodeficiency and signs suggestive of GVHD, may represent GVHD consequent on transplacental passage of lymphocytes [54], but this condition is now known to be an inherited disorder resulting from a mutation in one of the recombination activating genes, \( RAG1 \) and \( RAG2 \) [55].

**Peripheral blood**

In patients who have received histo-incompatible donor lymphocytes the consequent bone marrow hypoplasia is reflected in peripheral blood pancytopenia. In bone marrow transplant recipients there are no specific peripheral blood features that indicate the occurrence of GVHD but there is a delay in the appearance of signs of engraftment.

**Bone marrow cytology**

The bone marrow aspirate is usually hypocellular.

**Bone marrow histology**

When donor lymphocytes have been transferred without donor bone marrow, histological sections of trephine biopsies show aplasia. In GVHD in the setting of bone marrow transplantation, histological abnormalities include a decrease in haemopoietic cells, increased macrophages, erythrophagocytosis, oedema and perivascular lymphoid infiltrates [49].

**Effects of haemopoietic growth factors and other cytokines**

An increasing number of haemopoietic growth factors and other cytokines are being administered to patients. Haematological effects are often profound.

**Peripheral blood**

G-CSF and GM-CSF cause neutrophilia and monocytosis with a marked left shift, ‘toxic’ granulation, neutrophil vacuolation and a variety of dysplastic changes in neutrophils including abnormal neutrophil lobulation and the presence of macrophages. Blast cells may appear in the blood following G-CSF therapy [56]. GM-CSF causes more marked monocytosis than G-CSF and can also cause eosinophilia. In patients with MDS, the administration of G-CSF can be associated with the appearance of significant numbers of myeloblasts in the peripheral blood [57]. Neutrophilia is induced by various interleukins (IL1, IL2, IL3 and IL6) and by stem cell factor [58]. Eosinophilia is induced by IL2, IL3 and IL5. Lymphocytosis is induced by IL2, IL3, IL6, IL11 and thrombopoietin [59]. Thrombocytosis is induced by IL1, IL3, IL6 and thrombopoietin. The administration of IL2 leads to anaemia and thrombocytopenia and IL6 and IL11 [60] also cause anaemia. Erythropoietin administration raises the haemoglobin concentration and leads to erythroid hyperplasia.

**Bone marrow cytology**

Administration of G-CSF and GM-CSF causes a marked left shift of granulopoiesis. This is particularly prominent when these cytokines are administered to patients with suppressed bone marrow function. Myeloblasts may reach 20–40% and promyelocytes 12–60% leading to possible confusion with M2 and M3 categories of AML [61]. In haematologically normal subjects, G-CSF causes a marked increase in cellularity and an increase of all cells of neutrophil lineage [62]; the greatest increase is in promyelocytes and myelocytes. Morphological alterations include increased granularity, particularly of early cells, and an increased prevalence of ring neutrophils [62]. GM-CSF can cause a marked increase in macrophage numbers and its administration has been associated with development of a haemophagocytic syndrome [63]. The administration of IL5 causes an increase in bone marrow eosinophils. Stem cell factor causes some increase in cellularity with increased promyelocytes and, in some cases, increased basophils and mast cells [58]. Administration of thrombopoietin (in the form of pegylated recombinant human thrombopoietin) leads to increased numbers of megakaryocytes of increased size and nuclear lobularity [59].
Bone marrow histology

The bone marrow following administration of G-CSF and GM-CSF may be hypocellular, normocellular or hypercellular, depending on the underlying disease and prior therapy. There is granulocytic hyperplasia, left-shifted granulopoiesis and expansion of the paratrabecular zone of neutrophil precursors (Fig. 9.28). There may be aggregates of granulocyte precursors [61] resembling ALIP seen in MDS. Administration of thrombopoietin increases megakaryocyte numbers, size and nuclear lobularity [59] and leads to megakaryocyte clustering with increased reticulin deposition (Fig. 9.29).

Problems and pitfalls

When blast cells appear in the peripheral blood in response to G-CSF therapy, there are usually other granulocyte precursors present and maturing cells show ‘toxic’ changes such as heavy granulation. These blast cells also show some immunophenotypic differences from leukaemic myeloblasts [56]. They express CD34 but not terminal deoxynucleotidyl transferase, co-express CD19, and express CD13 and CD33 weakly [56].

In patients receiving G-CSF following induction therapy for AML, an increased blast cell percentage may be misinterpreted as persisting leukaemia [64]. G-CSF can also increase the blast cell percentage in MDS so that AML is simulated [57,65].

An adequate clinical history should prevent ALIP resulting from G-CSF therapy leading to a misdiagnosis of MDS and, similarly, should prevent the changes induced by thrombopoietin from being misinterpreted as an MPD.

Protein-calorie malnutrition and calorie deficiency

Peripheral blood

Protein-calorie malnutrition (kwashiorkor or marasmus) is not usually associated with deficiency of specific haematinics such as iron, vitamin B₁₂ or folate but, nevertheless, anaemia occurs. Red cells are normocytic and normochromic. The white cell and platelet counts may also be reduced. Severely reduced calorie intake, as in anorexia nervosa, is associated with mild anaemia, lymphopenia, neutropenia and thrombocytopenia; the blood film may show small numbers of acanthocytes.

Bone marrow cytology

The bone marrow in protein-calorie malnutrition usually shows reduced cellularity with normoblastic but dyserythropoietic haemopoiesis (Fig. 9.30). Giant metamyelocytes (Fig. 9.31) are common, even in the majority of cases which have normoblastic erythropoiesis. There is vacuolation of erythroid and granulocyte precursors.
Fig. 9.29  BM trephine biopsy specimen from a patient receiving thrombopoietin, showing:
(a) a marked increase in megakaryocytes which are pleomorphic and forming clusters;
(b) increased reticulin deposition; both abnormalities reversed on cessation of therapy. H&E and reticulin stain ×192.

Fig. 9.30  Bone marrow aspirate, showing dyserythropoiesis in protein-calorie malnutrition. MGG ×960. (By courtesy of Professor SN Wickramasinghe, London.)
Dysmegakaryopoiesis is uncommon. Iron stores are normal or increased. There may be abnormal sideroblasts including ring sideroblasts. In anorexia nervosa, the bone marrow is hypocellular and may show gelatinous transformation.

Storage diseases and storage cells in the bone marrow [66–70]

In various inherited diseases the deficiency of an enzyme leads to accumulation of a metabolite in body cells, often in macrophages. The morphologically abnormal bone marrow macrophages, containing an excess of the relevant metabolite, are referred to as storage cells. Storage cells may also result from an abnormal load of a metabolite such that the enzymes of normal cells are unable to cope. Both bone marrow aspiration and trephine biopsy are useful in the detection of storage diseases. Peripheral blood cells may show related abnormalities [66,71].

Gaucher’s disease

Gaucher’s disease (hereditary glucosyl ceramide lipidosis) is an inherited condition in which glucocerebrosides accumulate in macrophages including those in the liver, spleen and bone marrow. Although Gaucher’s disease can be diagnosed readily by bone marrow aspiration and by trephine biopsy, it has been pointed out that this is unnecessary when assays for the relevant enzyme, β-glucocerebrosidase, are available [72]. Gaucher’s disease can be transferred to graft recipients by bone marrow transplantation [73].

Peripheral blood

There are usually no specific peripheral blood features although very occasionally Gaucher cells may be seen in the peripheral blood, particularly after splenectomy. Pancytopenia develops slowly, as a consequence of hypersplenism. The monocytes of patients with Gaucher’s disease are positive for tartrate-resistant acid phosphatase (TRAP) activity, whereas normal monocytes are not [70].

Bone marrow cytology

Gaucher cells are large, round or oval cells with a small, usually eccentric nucleus and voluminous weakly basophilic cytoplasm with a wrinkled, fibrillar or ‘onion-skin’ pattern (Fig. 9.32). The cells stain with Sudan black B (SBB) and periodic acid–Schiff (PAS). They are non-specific esterase- and TRAP-positive and may be positive for iron, particularly in older children and adults. Patients with Gaucher’s disease may also have an increase in foamy macrophages and in cells which resemble typical Gaucher cells but also contain more strongly basophilic granules.

Fig. 9.31 Bone marrow aspirate, showing a giant metamyelocyte in a patient with protein-calorie malnutrition. MGG ×960. (By courtesy of Professor SN Wickramasinghe, London.)
Bone marrow histology

Gaucher cells may be isolated or appear in clumps or sheets, sometimes replacing large areas of the marrow (Fig. 9.33). The cells have abundant pale-staining cytoplasm with a texture that has been likened to watered silk or crumpled tissue paper. The fibrillar pattern is accentuated by PAS staining. There may be an increase in reticulin and collagen deposition [70]. In advanced disease, osteolytic lesions occur [74]. Gaucher cells are strongly positive with an immunohistochemical stain for TRAP [74].

Pseudo-Gaucher cells

Cells resembling Gaucher cells, but not identical to them on ultrastructural examination [67], are seen in the bone marrow in a variety of haematological conditions [68,69] in which they result from an abnormal load of glucocerebroside presented to macrophages. They are seen in chronic granulocytic leukaemia (Fig. 9.34), acute leukaemia, occasional cases of MDS [75], thalassaemia major and congenital dyserythropoietic anaemia (particularly type II). They have also been recognized in occasional
patients with Hodgkin’s disease, non-Hodgkin’s lymphoma and a variety of other conditions [68,76]. They have been reported as a consequence of repeated platelet transfusions [77].

**Problems and pitfalls**

If there is any difficulty distinguishing pseudo-Gaucher cells from Gaucher cells, it is possible to assay β-glucosidase in peripheral blood leucocytes. Cells resembling Gaucher cells have been seen in multiple myeloma and in lymphoplasmacytic lymphoma but the macrophages in these cases may contain material derived from immunoglobulin rather than glucocerebrosidase [76]. Cells considered to resemble Gaucher cells have also been reported in the bone marrow of a patient with atypical mycobacterial infection complicating AIDS [78]; in this case it appears that the abnormal morphology was consequent on large numbers of mycobacteria packing the macrophage cytoplasm rather than on storage of a breakdown product. Similar appearances have been reported in an immunosuppressed patient who had received a renal transplant [79]. The distinction is easily made with a stain for acid-fast bacilli.
Niemann–Pick disease
Niemann–Pick disease is an inherited condition (sphingomyelin lipidosis) caused by reduced sphingomyelinase activity (type I), or a related ill-defined defect of cholesterol esterification (type II). It is characterized by the presence of foamy lipid-containing macrophages in the bone marrow and other tissues.

Peripheral blood
Lipid-containing monocytes and lymphocytes may be present in the peripheral blood. Anaemia and various cytopenias may occur as a consequence of hypersplenism.

Bone marrow cytology
The foamy macrophages of Niemann–Pick disease are large cells (exceeding 50 µm in diameter) with a nucleus which is usually central. They stain pale blue with Romanowsky stains (Fig. 9.35) and variably with PAS and lipid stains. There are also increased numbers of sea-blue histiocytes (see below), possibly reflecting slow conversion of sphingomyelin to ceroid [68].

Fig. 9.35 BM aspirate, Niemann–Pick disease, showing foamy macrophages. MGG ×375. (By courtesy of Dr SG Davis, Birmingham.)

Fig. 9.36 BM trephine biopsy section in Niemann–Pick disease, showing foamy macrophages. Paraffin-embedded, H&E ×960.
Bone marrow histology

Foamy macrophages may appear yellow–green when stained with Giemsa and are light brown or pink in H&E-stained sections (Fig. 9.36); they are PAS-positive and may be positive for iron [70].

Other causes of foamy macrophages [69]

Other metabolic defects which can lead to the presence of increased numbers of foamy macrophages in the bone marrow include hypercholesterolaemia (e.g. Zieve’s syndrome—see Fig. 9.17), hyperchylomicronaemia, Wolman’s disease (Fig. 9.37), late-onset cholesteryl ester storage disease, Fabry’s disease, neuronal lipofuscinosis (Batten’s disease) and Tangier disease. In Fabry’s disease the storage cells have small globular inclusions which appear weakly basophilic with Romanowsky stains and lightly eosinophilic with H&E; they are PAS-negative and SBB-positive [80].

Foamy cells are also increased as a result of damage to fat cells (Fig. 9.38) including trauma, fat necrosis, bone marrow infarction, infection, pancreatitis and
recent performance of a bone marrow biopsy at the same site [81]. Acquired diseases which have been associated with an increase of foamy macrophages include Langerhans cell histiocytosis (Hand–Schüller–Christian disease, Letterer–Siwe disease and eosinophilic granuloma), bone marrow metastases (Fig. 9.39), sickle cell disease (see Fig. 9.38) and a variety of other conditions [68]. Foamy cells have been noted in subjects who have, in the past, received polyvinyl pyrrolidine as a plasma expander; they contain amorphous grey–blue material and are PAS-, mucicarmine- and Congo red-positive [82–84]. In trephine biopsy sections, reticulin is increased [84]. Occasionally, the foamy cell infiltration is so heavy that bone marrow failure occurs [84]. Foamy ceroid-containing macrophages (see below) are seen in patients receiving prolonged intravenous nutrition with lipid emulsions [85].

Macrophages containing cholesterol crystals

Bone marrow macrophages may contain cholesterol crystals in various hyperlipidaemic conditions, both congenital and acquired. Such conditions include alpha-lipoprotein deficiency, hyperbeta-lipoproteinaemia, poorly controlled diabetes mellitus and hypothyroidism [86]. The cholesterol crystals are soluble and thus give rise to unstained needle-like clefts within the macrophages.

Sea-blue histiocytosis

The terms ‘sea-blue histiocytosis’ [87] and ‘ceroid lipofuchsinosis’ [88] encompass an inherited group of conditions characterized by the presence of ‘sea-blue histiocytes’—distinctive macrophages containing ceroid or lipofuchsin—in the bone marrow, liver, spleen and other organs. The designation of the disease derives from the staining characteristics of the storage cells with Romanowsky stains. In unstained films, ceroid is brown. The inherited conditions causing increased numbers of sea-blue histiocytes include the Hermansky–Pudlak syndrome (oculo-cutaneous albinism with a bleeding diathesis).

Bone marrow cytology

Sea-blue histiocytes stain blue or blue–green with Romanowsky stains. They are SBB-, PAS- and oil-red-λ-positive, and are sometimes positive for iron. With ultraviolet illumination they exhibit yellow–green autofluorescence.

Bone marrow histology

Sea-blue histiocytes are brownish-yellow in H&E-stained sections and blue with a Giemsa stain. They are PAS-positive and may be positive for iron. Their cytoplasmic contents are acid-fast and exhibit autofluorescence.
Other causes of sea-blue histiocytes

Increased numbers of sea-blue histiocytes are seen in the bone marrow in a great variety of conditions [87] including many of the same disorders in which pseudo-Gaucher cells are present or foamy macrophages are increased (Figs 9.38, 9.40 and 9.41). Most of these conditions are characterized by increased turnover of bone marrow cells. Less often there is an exogenous cause, as in prolonged intravenous nutrition with fat emulsions [85].

Cystinosis

Peripheral blood

There are no specific abnormalities in the peripheral blood.

Bone marrow cytology

Bone marrow histiocytes are packed with almost colourless, refractile crystals of various shapes. They
are best seen under polarized light when they are birefringent but are also readily apparent with normal illumination (Fig. 9.42). Bone marrow aspiration has sometimes confirmed a provisional diagnosis of cystinosis when other diagnostic measures were negative [89].

**Bone marrow histology**

Crystals dissolve out of histological sections leaving a negative image.

**Hyperoxaluria** [90–92]

Hyperoxaluria or oxalosis is a metabolic disorder in which oxalate is deposited in various tissues including the bone, bone marrow, liver, spleen and kidneys. Renal deposition leads to renal failure. The introduction of haemodialysis has prolonged life in these patients and has permitted advanced bone marrow lesions to become apparent.

**Peripheral blood**

There is anaemia as a consequence of renal failure. Hypersplenism also contributes to anaemia and may cause pancytopenia. Deposition of oxalate in the bone marrow further aggravates anaemia and other cytopenias and causes a leuco-erythroblastic blood film.

**Bone marrow biopsy**

Central areas of intertrabecular bone marrow are extensively replaced by needle-like crystals arranged in a radial pattern (Fig. 9.43). There are variable numbers of epithelioid cells and multinucleated cells, including foreign body giant cells, present at the periphery of the crystalline deposits and engulfing crystals. The surrounding paratrabecular bone marrow shows mild fibrosis.

**Mucopolysaccharidoses**

The mucopolysaccharidoses are inherited diseases characterized by the storage of various mucopolysaccharides [66]. They are consequent on a deficiency of one of the lysosomal enzymes needed to degrade mucopolysaccharide.

**Peripheral blood**

Peripheral blood neutrophils may show the Alder–Reilly anomaly [66,71]. Lymphocytes may either be vacuolated or contain abnormal granules which stain metachromatically with toluidine blue.
Bone marrow cytology

Bone marrow granulocytes may contain inclusions similar to those observed in the peripheral blood. Similar inclusions have been observed in plasma cells. Bone marrow macrophages also contain abnormal metachromatic granules (Fig. 9.44) [66].

Bone marrow histology

In histological preparations, macrophages appear foamy since mucopolysaccharides are water-soluble. Abnormal histiocytes may be scattered between haemopoietic cells or in small clusters.

Deposition of foreign substances

Foreign substances may be deposited in the bone marrow, principally in bone marrow macrophages. Such substances may be apparent in bone marrow aspirates and in trephine biopsy sections. There are not usually any associated peripheral blood abnormalities.

In anthracosis there is widespread deposition of
anthracotic pigment in body macrophages including those of the bone marrow. Large aggregates of dense black particles are apparent [93]. Silica and anthracotic pigment are often co-deposited. Silica crystals are detected by their birefringence. There may be consequent granuloma formation.

Occasional patients are still seen who have, in the past, been exposed to Thorotrast (thorium dioxide) as a radiographic medium. Thorotrast within macrophages appears as a pale grey refractile material (see Fig. 10.28). Bone marrow abnormalities associated with the presence of Thorotrast include hypoplasia, hyperplasia, fibrosis and the development of MDS, acute leukaemia and haemangioendothelioma [94]. The peripheral blood film may be bizarre because of the combined effects of bone marrow fibrosis and Thorotrast-induced splenic atrophy.

Vascular and intravascular lesions [69,95]

The bone marrow vasculature may be altered as a consequence of bone marrow diseases but, in addition, the blood vessels within the marrow, particularly arterioles and capillaries, may be involved in a variety of generalized diseases. The peripheral blood film may show related abnormalities but in general the bone marrow aspirate does not give relevant information and a trephine biopsy is necessary to show the lesion.

Peripheral blood

The peripheral blood shows red cell fragments in patients with thrombotic thrombocytopenic purpura or with micro-angiopathie haemolytic anaemia as a consequence of disseminated malignancy. Eosinophilia may be a feature of some types of vasculitis and also of cholesterol embolism which may involve the marrow as well as other tissues. Leucocytosis and an elevated erythrocyte sedimentation rate have also been associated with cholesterol embolism. The peripheral blood film may show pancytopenia and leuco-erythroblastic features in patients with bone marrow necrosis as a consequence of vascular occlusion.

Bone marrow cytology

There are no specific abnormalities in the bone marrow aspirate in patients with vascular lesions.

Bone marrow histology

In patients with generalized atherosclerosis the bone marrow arterioles may show atherosclerotic changes. Embolism of atheromatous material to bone marrow vessels may occur; the embolus may be acellular or composed of hyaline material or cholesterol crystals (Fig. 9.45) [96,97]. Bone marrow
emboli are present at autopsy in about 10% of patients with generalized cholesterol embolism [96]. Vessels are partly or totally occluded by this material and by the granulomatous tissue which develops as a reaction to it. Any cholesterol crystals appear as empty clefts. There may be foreign body giant cells in addition to proliferating histiocytes, together with fibrosis and new bone formation simulating bone marrow metastases [98]. Vasculitic lesions are seen in polyarteritis nodosa, with fibrinoid necrosis being a feature. In patients with hypersensitivity reactions to drugs, a granulomatous vasculitis may occur. Patients with vasculitis may show granulocytic hyperplasia with both neutrophils and eosinophils being increased. Intravascular and subendothelial hyaline deposits may be seen in bone marrow capillaries in thrombotic thrombocytopenic purpura. In patients with microangiopathic haemolytic anaemia as a consequence of disseminated carcinoma, the bone marrow capillaries, like other capillaries, may contain tumour thrombi. Thrombi may also be seen in patients with thrombophilia, e.g. in patients with anti-cardiolipin antibodies (Fig. 9.46). In patients with sickle cell disease, sickle cells are usually present in sinusoids. During sickling crises there may also be thrombotic...
lesions and associated areas of bone marrow necrosis. Sickle cells may also be present in autopsy specimens from patients with sickle cell trait; their presence does not have any particular significance. Thrombi may also be noted in vessels in other patients with bone marrow necrosis. In amyloidosis, there may be deposition of amyloid in bone marrow vessels (see Fig. 7.29). Abnormal vessels encircled by mast cells may be seen in systemic mastocytosis (see Fig. 5.41).

References

Chapter Nine


