THREE

INFECTION AND REACTIVE CHANGES

Infection

The response of the bone marrow to infection is very variable, depending on the nature and chronicity of the infection, the age of the subject and the presence of any associated diseases. The response differs according to whether the infection is bacterial, rickettsial, viral or fungal. The peripheral blood and bone marrow responses to infection are non-specific and similar changes occur in many other conditions, including trauma and other tissue damage, administration of growth factors, carcinoma, Hodgkin's disease, non-Hodgkin's lymphoma and auto-immune disorders such as systemic lupus erythematosus. Only a minority of patients with an infection show peripheral blood or bone marrow changes suggestive of a particular micro-organism.

Bacterial and rickettsial infection

Peripheral blood

In adults, the usual haematological response to an acute bacterial infection is neutrophil leucocytosis with a left shift (an increase of band forms and possibly the appearance of neutrophil precursors in the peripheral blood) (Fig. 3.1). The neutrophils usually show toxic granulation and may show Döhle bodies and cytoplasmic vacuolation. When there is very severe bacterial infection and in neonates, alcoholics and patients with reduced bone marrow reserve, neutrophilia does not occur but there is a left shift with the above 'toxic' changes in neutrophils. Certain bacterial infections, specifically typhoid, paratyphoid and tularaemia, are characterized by neutropenia rather than neutrophilia. In

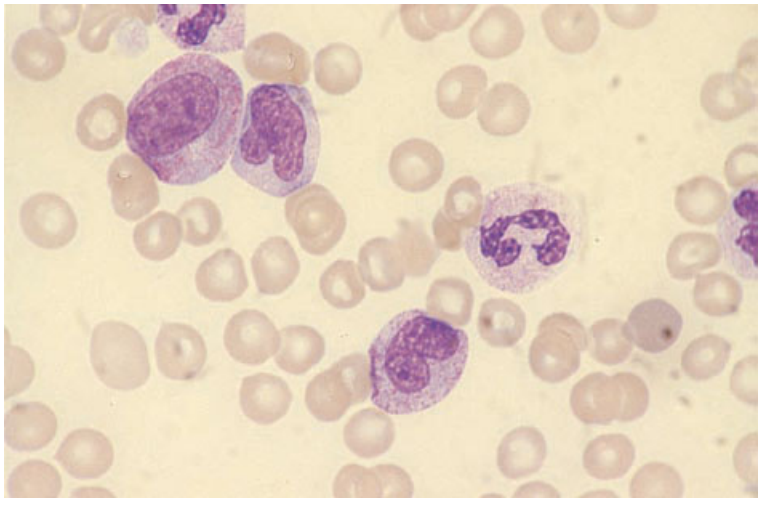


Fig. 3.1 PB, bacterial infection, left shift and toxic granulation. MGG ×940.

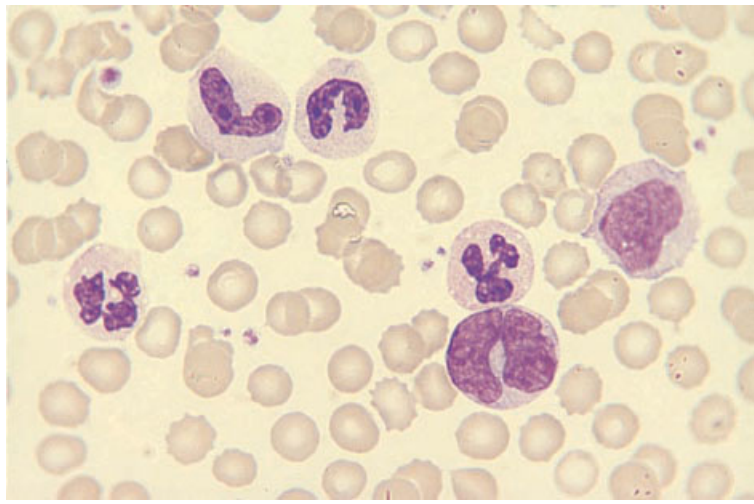


Fig. 3.2 PB, bacterial infection, monocytosis and neutrophilia. MGG ×940.

severe infection, particularly if there is shock or hypoxia, nucleated red blood cells may appear in the blood, the presence of both granulocyte precursors and nucleated red cells being referred to as leuco-erythroblastosis. The lymphocyte count is reduced but a few atypical lymphocytes, including plasmacytoid lymphocytes, may be present; plasma cells are sometimes seen. The eosinophil count is reduced during acute infection but eosinophilia can occur during recovery. Children may respond to bacterial infection with lymphocytosis rather than neutrophilia, and certain bacterial infections, particularly whooping cough and sometimes brucellosis, are characterized by lymphocytosis. In bacterial infection, the platelet count is often reduced but sometimes increased.

Certain bacterial infections can be complicated by haemolytic anaemia. Infection by *E. coli* or Shigella species can be followed by a micro-angiopathic haemolytic anaemia as part of a haemolytic uraemic syndrome. Sepsis due to *Clostridium welchii* can be complicated by acute haemolysis with spherocytic red cells. Mycoplasma infection is commonly associated with the production of cold auto-antibodies so that red cell agglutinates are present in blood films made at room temperature and haemolytic anaemia sometimes occurs.

Rarely, neutrophils contain phagocytosed bacteria. The presence of bacteria, either extracellularly or within neutrophils, is usually seen only in overwhelming infections, particularly when there is associated hyposplenism. In relapsing fever, however, the characteristic spiral organisms of Borrelia species appear episodically in the bloodstream and are seen lying free between red cells. In ehrlichial infections, organisms are often detectable within monocytes or granulocytes. They are detected in monocytes in human monocytic ehrlichiosis (caused by *Ehrlichia chaffeensis*) and in neutrophils in human granulocytic ehrlichiosis and human infection by *Ehrlichia ewingii* (the agent responsible for canine granulocytic ehrlichiosis) [1]. The detection of organisms within leucocytes is facilitated by examination of buffy coat films.

In more chronic infections, there may be anaemia, increased rouleaux formation, increased background staining and monocytosis (Fig. 3.2). Anaemia is initially normocytic and normochromic but, as the infection becomes increasingly chronic, the anaemia develops the characteristics of the anaemia of chronic disease with the cells produced being hypochromic and microcytic.

Pulmonary tuberculosis characteristically causes anaemia, increased rouleaux formation and, when severe, neutrophilia. Monocytosis occurs in a minority of patients. Monocytopenia is actually more common than monocytosis. In miliary tuberculosis, leucocytosis is uncommon and leucopenia is common. The lymphocyte count is reduced. A minority of patients have monocytosis. Pancytopenia

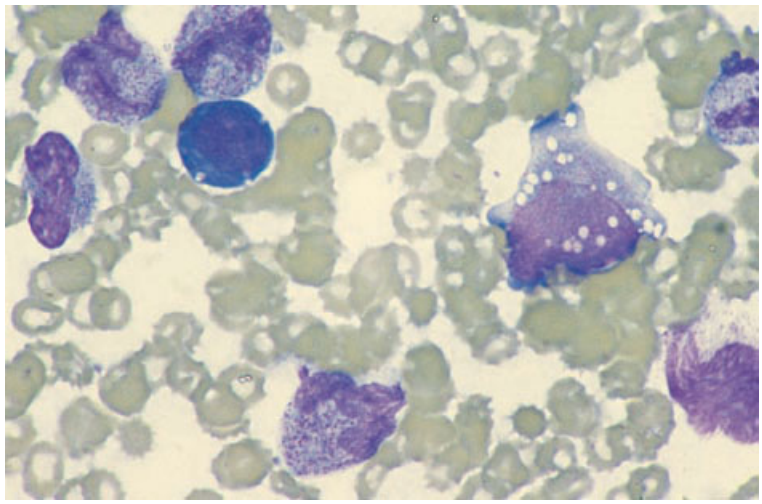


Fig. 3.3 BM aspirate film from a patient with severe infection showing heavy toxic granulation and vacuolation of neutrophil precursors. MGG ×940.

may occur, sometimes as a consequence of haemophagocytosis and, less often, in association with bone marrow necrosis.

Rickettsial infections cause varied haematological effects which may include neutrophilia, neutropenia, lymphocytosis, the presence of atypical lymphocytes and thrombocytopenia which is sometimes severe.

Occasionally, severe infections are associated with a haematological picture which simulates leukaemia, designated a leukaemoid reaction (see below).

Bone marrow cytology

In severe bacterial infection, the bone marrow features reflect those of the peripheral blood. There is granulocytic hyperplasia with associated toxic changes (Fig. 3.3). In overwhelming infection, the marrow sometimes shows an increase of granulocyte precursors but with few maturing cells. Erythropoiesis is depressed and erythroblasts show reduced siderotic granulation. When there is thrombocytosis, megakaryocytes may be increased. Macrophages are increased and, in a minority of patients with severe infection, prominent haemophagocytosis occurs (see page 119). When infection is chronic, an increase of iron stores is apparent.

Microscopy or bone marrow culture occasionally provides evidence of a specific infection. In Whipple's disease, the causative organisms may be seen within bone marrow macrophages [2]. In human monocytic ehrlichiosis, organisms can be detected in bone marrow monocytes, whereas in human granulocytic ehrlichiosis, caused by a related organism, they are present in granulocytes [3]. Bacteria visible within macrophages have also been reported in bacterial endocarditis. In typhoid fever, the bone marrow aspirate shows only non-specific features but bone marrow culture can be useful since it increases the detection rate by 50% in comparison with culture of the peripheral blood [4]. In brucellosis, the bone marrow is usually hypercellular with prominent haemophagocytosis and increased eosinophils and plasma cells [5]. Bone marrow cultures are occasionally positive when blood cultures are negative but, as the diagnosis can be made serologically, bone marrow aspiration is not indicated for this purpose.

In tuberculosis, the bone marrow aspirate shows non-specific features such as increased iron stores and an increase in macrophages, often with haemophagocytosis. Mycobacteria may be detected in a minority of cases using specific stains (Ziehl–Neelsen or auramine). Cultures should always be performed when this diagnosis is suspected. In lepromatous leprosy, bacilli may be apparent as negative images within bone marrow macrophages [6].

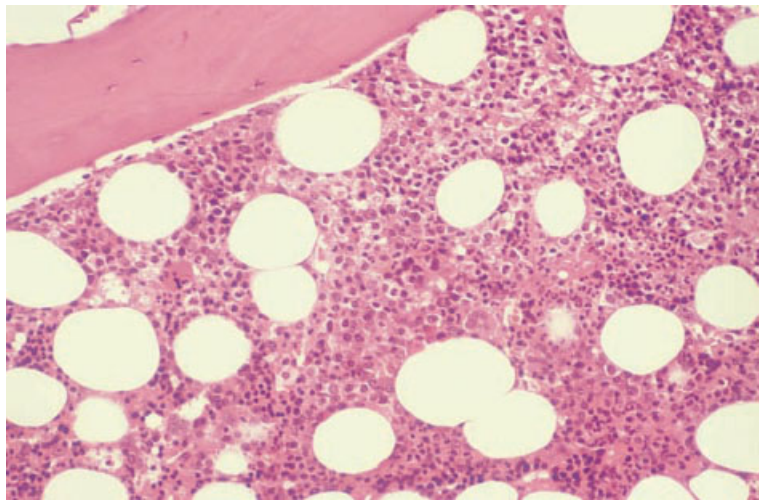


Fig. 3.4 BM trephine biopsy section, bacterial infection with leukaemoid reaction: there is increased cellularity and granulocytic hyperplasia with left shift; note emperipolesis of a mature neutrophil by a megakaryocyte. Paraffin-embedded, H&E ×195.

Bone marrow histology

Severe bacterial infection leads to an increase in marrow cellularity due to granulocytic hyperplasia. There is often left shift of the granulocytic series, i.e. an increase in the numbers of immature precursors (myelocytes and promyelocytes) in relation to mature polymorphonuclear neutrophils (Fig. 3.4). However, the normal topographical arrangement of granulopoiesis is retained, with the more immature cells (mainly promyelocytes) found predominantly in the paratrabecular region [7]. Megakaryocytes are often increased in number [8]; they are morphologically normal, but there may be an increase in 'bare' megakaryocyte nuclei and increased emperipolesis. Erythropoiesis is often reduced although morphologically normal.

In more chronic bacterial infections, changes in other lineages become apparent. Bone marrow plasmacytosis is a common but non-specific response [9]. Very rarely, there may be up to 50% plasma cells in the marrow in reactive conditions including infections. The differential diagnosis of increased numbers of plasma cells in the marrow is discussed on page 166. In infections, plasma cells are distributed through the marrow in an interstitial manner, often with focal pericapillary accentuation. This may be accompanied by plasma cell satellitosis, in which a central macrophage is surrounded by three or more plasma cells. The plasma cells have mature nuclear and cytoplasmic characteristics although there are often occasional binucleate forms and cells containing Russell bodies. Chronic infection is also associated with an increased frequency of reactive lymphoid aggregates (see page 144). Macrophages may be increased and commonly contain ingested granulocytes (Fig. 3.5). A wide variety of infectious agents, including bacteria, can cause a secondary haemophagocytic syndrome (see page 119). Stromal changes related to infection include prominent sinusoids, focal reticulin fibrosis and rarely, in very severe chronic infections, gelatinous change (see page 128). A decrease in marrow cellularity and loss of fat spaces usually accompany gelatinous transformation [10].

Mycobacterial infections can cause the same reactive changes as infection by other bacteria. However, haemophagocytosis and granuloma formation are particularly common. Whenever granulomas are present it is necessary to perform special stains such as a Ziehl–Neelsen stain or an auramine stain, in the latter instance mycobacteria being detected by fluorescence microscopy. In one study, immunohistochemistry with a polyclonal antibody to mycobacteria was found to be more sensitive than an acid-fast stain [11]. Occasional patients with mycobacterial infection have extensive necrosis which is indicative of poor prognosis. In those

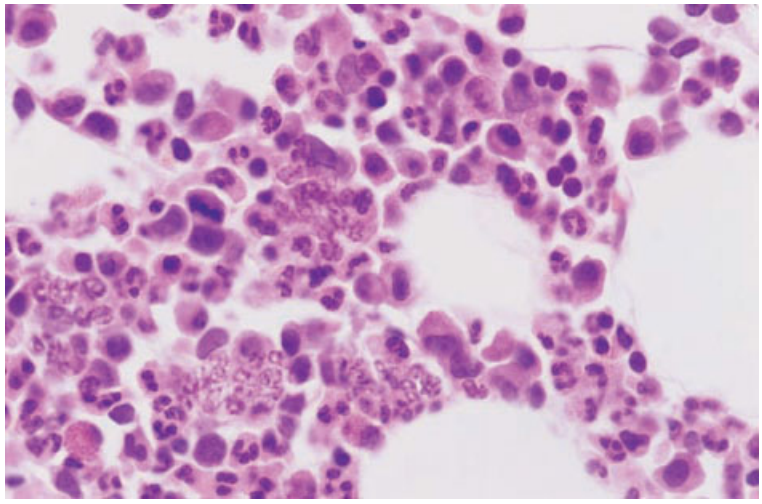


Fig. 3.5 Section of BM trephine specimen showing neutrophil shadows within macrophages in a patient with Hodgkin's disease; similar features are seen in bacterial infection. Paraffin-embedded, H&E ×940.

with impaired immunity, there may be diffuse macrophage hyperplasia rather than granuloma formation. Macrophages may appear foamy. *Mycobacterium leprae* infection may cause granuloma formation and, in lepromatous leprosy, proliferation of foamy macrophages. Patients have also been reported in whom interstitial lepra bacilli were found in bone marrow sections, stained with a Fite stain, in the absence of any macrophage proliferation or granulomas [12].

In ehrlichiosis, the bone marrow is often hypercellular with both granulocytic and megakaryocytic hyperplasia. Granulomas are often present. Ehrlichial inclusions can be identified in macrophages [13] (Fig. 3.6). Ehrlichiae can be detected immunocytochemically [13].

Granuloma formation can also occur in rickettsial infections such as Q fever (infection by *Coxiella burnetti*) and Rocky Mountain spotted fever (infection by *Rickettsia rickettsii*).

Problems and pitfalls

Mycobacteria are not always detectable with special stains in patients with haemophagocytosis or granulomas caused by miliary tuberculosis. Cultures are more sensitive than microscopy.

Acute myeloid leukaemia (AML) can be simulated by overwhelming infection with associated cytopenia. Leukaemoid reactions can also simulate chronic myelomonocytic leukaemia (CMML). In a leukaemoid reaction, anaemia and thrombocytopenia are common. The white cell count is sometimes low with neutropenia and sometimes high; the blood film may show granulocyte precursors or be leuco-erythroblastic. Leukaemoid reactions are most often seen in association with very severe bacterial infection, particularly when there is coexisting megaloblastic anaemia. The bone marrow response to miliary tuberculosis can also simulate leukaemia. Toxic changes such as toxic granulation and neutrophil vacuolation are useful clues to the correct diagnosis. If necessary, a bone marrow examination makes the distinction. In severe infection there may be 'maturation arrest' with increased promyelocytes but there is no significant increase in blasts and the promyelocytes differ from those of acute hypergranular promyelocytic leukaemia in having prominent Golgi zones and no Auer rods.

The blood and bone marrow features of severe chronic infection may be difficult to distinguish from those of CMML since 'toxic' changes may be lacking. Consideration of the clinical features facilitates the distinction. It is imprudent to make a diagnosis of CMML in a patient with a significant infection unless there are dysplastic features of a type showing a strong association with the myelodysplastic syndromes (see Chapter 4).

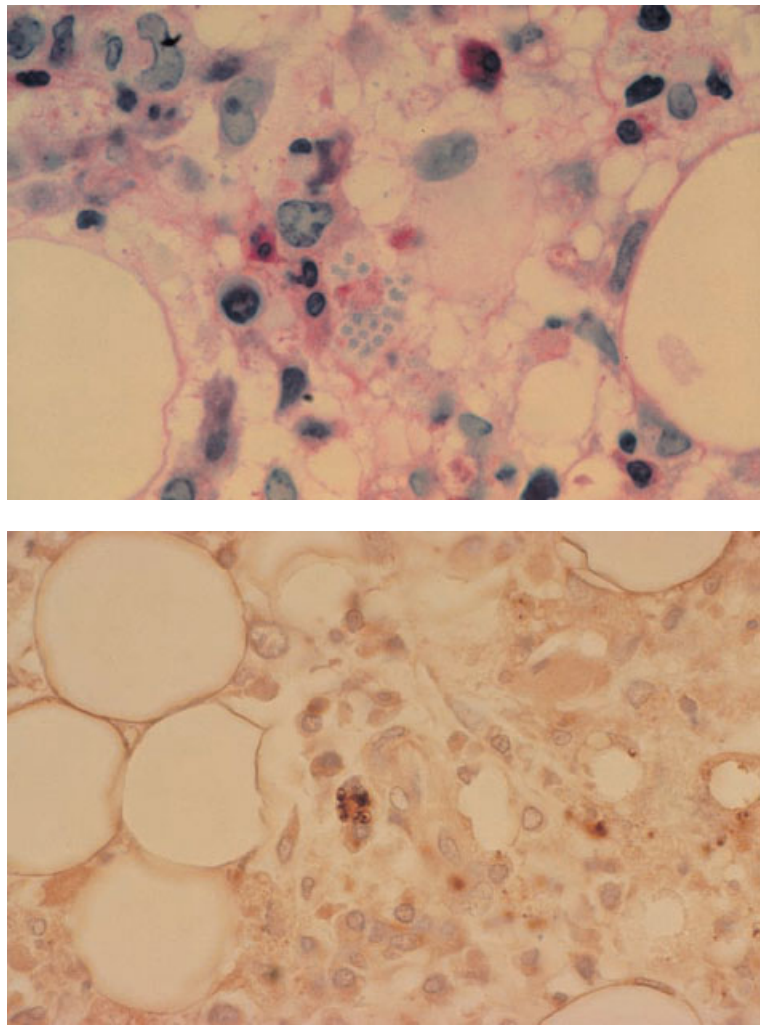


Fig. 3.6 Intracellular ehrlichiae in a BM trephine biopsy section: (a) PAS stain with haematoxylin counterstain; (b) immunoperoxidase stain with anti-ehrlichial IgG antibody. (By courtesy of Dr CD Paddock, Dr RL Kerschmann and Dr BG Herndier, San Francisco, reprinted by permission of the *New England Journal of Medicine*, **329**, 1165, 1993.)

Viral infection

Peripheral blood

Viral infections usually provoke lymphocytosis. Often the cells that are produced are morphologically fairly normal but sometimes they have atypical features. Infectious mononucleosis consequent on infection by the Epstein–Barr virus (EBV) is characterized by the production of large numbers of atypical lymphocytes, often referred to as atypical mononuclear cells (Fig. 3.7). These are pleomorphic, usually large, and often have abundant basophilic cytoplasm and nuclei with a diffuse chromatin pattern and nucleoli. The presence of large numbers of atypical mononuclear cells is not specific for EBV infection; this may also be a feature of primary infection by other viruses (cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis A and adenovirus) and of toxoplasmosis, as well as being seen in hypersensitivity reactions to drugs. Smaller numbers of similar atypical lymphocytes are seen in a wider variety of infective (and non-infective) conditions. Viral infections which may be associated with marked lymphocytosis without many atypical features

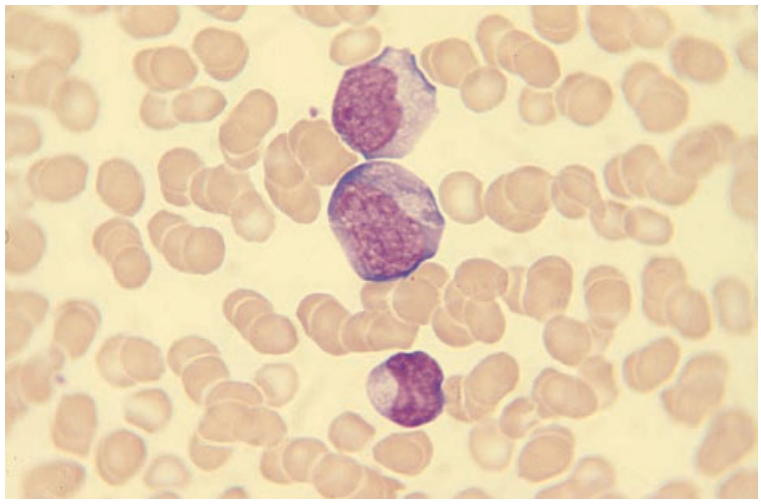


Fig. 3.7 PB, infectious mononucleosis, atypical lymphocytes. MGG ×940.

include those due to Coxsackie virus, various adenoviruses and HIV; other haematological abnormalities produced by HIV are discussed below (see page 124). In immunosuppressed hosts, e.g. following bone marrow transplantation, CMV infection can cause neutropenia, thrombocytopenia and pancytopenia. CMV infection in such patients may be associated with very large virus-infected cells, which are probably endothelial in origin, in the feathered edge of the film [14]. Viral infections, particularly those due to herpesviruses, may be associated with a haemophagocytic syndrome (see page 119) with the peripheral blood showing resultant pancytopenia. In a few individuals, viral hepatitis, particularly non-A non-B non-C hepatitis, is followed in a period of a few weeks or months by pancytopenia caused by aplastic anaemia. In certain subjects who are unable to mount a normal immune response to EBV, infection by this virus may also be followed by chronic pancytopenia due to bone marrow aplasia. Parvovirus commonly causes transient pure red cell aplasia but, unless red cell survival is reduced, this may go unnoticed; less often, it is a cause of neutropenia or thrombocytopenia.

Viral infections may be complicated by cytopenias consequent on either damage to cells by immune complexes or auto-antibody production. Rubella and, less often, other viral infections may be followed by transient thrombocytopenia caused by damage to platelets by immune complexes. Infectious mononucleosis may be complicated by either auto-immune thrombocytopenia or auto-immune haemolytic anaemia due to a cold antibody with anti-i specificity; in these cases, there are red cell agglutinates and occasional spherocytes. Rarely, viral infections, particularly measles, are followed by acute haemolysis due to an auto-antibody with anti-P specificity ('paroxysmal' cold haemoglobinuria); in these cases, the blood film usually shows only occasional spherocytes and subsequently polychromasia. Contrary to what might be anticipated from the name, there is only a single episode of haemolysis.

Chronic hepatitis C infection can be a cause of thrombocytopenia. Some patients develop mixed cryoglobulinaemia with associated haematological features (see page 355). A single case has also been reported of pure red cell aplasia associated with hepatitis C infection [15].

In patients with impaired immunity, EBV infection may be associated with a lymphoproliferative disorder which may evolve to lymphoma (see page 319).

If fresh tissue is available, molecular techniques can be used for the detection of viral infection. For example, in suspected EBV infection, reverse transcriptase polymerase chain reaction (RT-PCR) can be used to detect messenger RNA (mRNA) for EBNA-1 and -2, LMP-1, -2A and -2B, BZLF1 (ZEBRA 'protein') and BCRF1 (viral IL10).

Bone marrow cytology

In viral infection, the bone marrow shows an increase

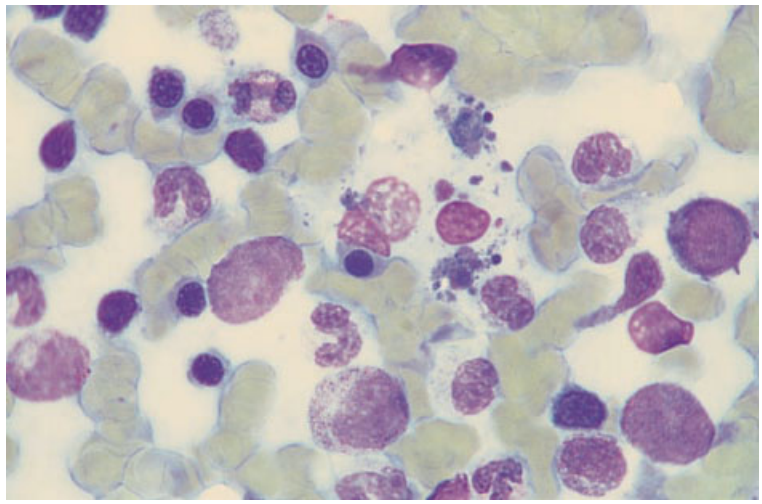


Fig. 3.8 BM aspirate. Platelets within a macrophage in a patient with severe thrombocytopenia during acute CMV infection. MGG ×960.

of lymphocytes, either typical or atypical. In some infections, particularly by herpesviruses, haemophagocytosis is prominent (see page 119). In immunosuppressed hosts, CMV infection can also cause marked bone marrow hypocellularity with suppression of all lineages [16]. When haemolytic anaemia occurs, e.g. in infectious mononucleosis, erythroid hyperplasia will be apparent. In pure red cell aplasia consequent on parvovirus B19 infection there are prominent, very large proerythroblasts with a striking lack of more mature cells. In one patient with parvovirus-induced pancytopenia there were also large atypical cells of granulocyte lineage which were shown to contain viral antigens [17]. Parvovirus infection can be confirmed by immunofluorescence with monoclonal antibodies to either a capsid antigen [17] or a nuclear antigen, or by *in situ* DNA hybridization. When viral infections are complicated by thrombocytopenia due to increased platelet destruction, megakaryocytes are present in normal or increased numbers. Occasionally, platelets are visible within bone marrow macrophages (Fig. 3.8).

Bone marrow histology

Viral infections may lead to an increase of bone marrow lymphocytes, plasma cells and macrophages, with or without haemophagocytosis. Atypical lymphoid cells, morphologically identical to those in the peripheral blood, can be seen in the marrow in EBV and CMV infection; in CMV infection, eosinophilic intranuclear inclusions may be seen, albeit rarely, in endothelial cells and in cells, probably macrophages, within granulomas or interspersed among haemopoietic cells [18]. Atypical lymphoid cells with intranuclear viral inclusions have also been detected in human herpesvirus 6 (HHV6) infection [19]. In the post-transplant setting, CMV [16] and HHV6 [20] infection can cause severe bone marrow hypoplasia. The presence of CMV (Fig. 3.9) and EBV can be confirmed by immunohistochemistry using monoclonal antibodies (Table 3.1). However, it should be noted that, even in patients with generalized CMV infection, it is unusual for virus-infected cells to be detectable in the bone marrow. Pure red cell aplasia is characteristically seen in infection by parvovirus B19 and is readily detected in trephine biopsy sections (see page 376). Very occasionally, it has been associated with erythroid hyperplasia, sometimes with marked dyserythropoiesis [25]. Parvovirus B19 capsid antigens may be detectable by immunohistochemistry although this technique is less sensitive than PCR [26]; curiously, in one study, the positive cells were among the few late erythroid cells while the giant proerythroblasts were negative [26]. Viral infections, particularly herpesvirus infections, can lead to formation of small non-caseating granulomas (see Table 3.3). Chronic hepatitis B and hepatitis C infection may be associated with the presence of reactive lymphoid nodules [27]. Hepatitis C infection can also be complicated by the development of low grade, B-cell lymphoma, often supervening in a patient with

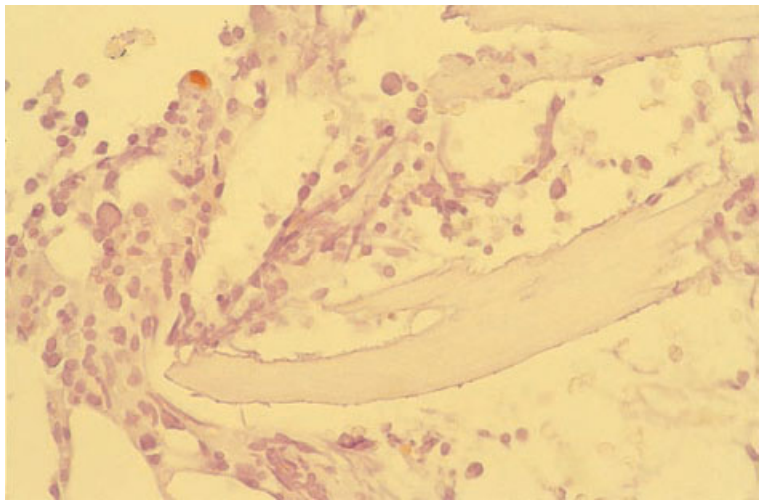


Fig. 3.9 BM trephine biopsy section from a renal transplant patient with CMV infection showing one positive cell (top left). Paraffin-embedded, immunoperoxidase with Dako-CMV McAb ×940.

Table 3.1 Techniques applicable to trephine biopsy specimens for confirmation of certain viral infections.

Virus	Technique	Monoclonal antibody or other specific reagent
Parvovirus B19	Immunohistochemistry with detection of a nuclear antigen or a viral capsid antigen	R92F6 (Novocastra) directed at capsid proteins VP1 and VP2
	In situ hybridization for detection of viral DNA	Digoxigenin-labelled parvovirus B19 DNA probe [21]
Cytomegalovirus (CMV)	Immunohistochemistry with detection of nuclear and cytoplasmic antigen	AAC10 (Dako) directed at CMV lower matrix protein pp65; QB1/06 (Novocastra) directed at CMV late antigens
	In situ hybridization for detection of viral DNA	CMV probe ISH kit (Novocastra) or CMV Bioprobe (ENZO Diagnostics) [22]
Epstein–Barr virus (EBV)	Immunohistochemistry for detection of latent membrane protein-1 (LMP-1)* or Epstein–Barr nuclear antigen-2 (EBNA-2) in latent infection	CS1-4 directed at LMP-1 (Dakopatts or Novocastra); PE2 directed at EBNA-2 (Dako and Novocastra)
	EBV early lytic antigens	G3-E31 (Novocastra) directed at 50–52 kD diffuse early antigen; W1-F2 (Novocastra) directed at 85 kD restricted early antigen
	Immunohistochemistry for detection of BZLF ('ZEBRA' protein) in lytic EBV infection	Clone βZ.1 directed at βZLF1 (Dako)
	In situ hybridization for detection of nuclear Epstein–Barr early RNA (EBER)	EBER1 PNA probe/FITC kit (Dako) or EBER-probe ISH kit (Novocastra)
	Southern blot analysis to detect EBV clonality (dependent on the number of terminal repeats) [24]	
Herpes simplex	Immunohistochemistry for detection of various shared and type-specific antigens	Rabbit polyclonal antiserum (Dako)

* LMP McAb is a cocktail of clones (CS1-4) raised against a fusion protein containing 189 amino acids of the terminus of LMP. Cross-reaction with normal early myeloid and erythroid precursors, leukaemic myeloblasts and leukaemic lymphoblasts has been reported [23] but this has not been our experience when using formalin fixation and EDTA decalcification; we have observed no false-positive staining in normal bone marrow or in AML, and in ALL we have observed weak nuclear staining only, whereas the specific product would be cytoplasmic.

mixed type II cryoglobulinaemia; the lymphoid infiltrate is then monoclonal and more extensive.

As already noted, immunohistochemistry is useful in the diagnosis of viral infections. The special techniques applicable to trephine biopsies for confirmation of infection by specific viruses are summarized in Table 3.1.

Problems and pitfalls

Lymphocytosis with atypical lymphocytes can be confused with a lymphoproliferative disorder, particularly with mantle cell lymphoma and the mixed cell type of chronic lymphocytic leukaemia in which neoplastic cells are pleomorphic. When there is diagnostic difficulty, immunophenotyping is indicated to demonstrate or exclude a clonal proliferation of B lymphocytes.

Virus-induced haemophagocytic syndrome can be confused with malignant histiocytosis. The latter is a very rare condition and the diagnosis should be made with caution. It is characterized by proliferation of very immature cells of monocyte–macrophage lineage which, in contrast to the cells of virus-induced haemophagocytic syndrome, usually show little phagocytic activity. Marked haemophagocytosis therefore suggests that macrophage activation is reactive. Virus-induced haemophagocytosis can also be confused with other reactive haemophagocytic syndromes, including those associated with some T-cell and NK-cell lymphomas.

Fungal infection

Peripheral blood

Fungal infections have no specific haematological features. Some fungal diseases, for example actinomycosis and coccidioidomycosis, are associated with neutrophilia. Aspergillus and other fungi may evoke an allergic response and be associated with eosinophilia. When systemic fungal infections occur in immunocompromised hosts, fungi such as candida or histoplasma are occasionally seen in the peripheral blood. Candida may also be seen in blood films when there is infection originating from a colonized indwelling venous line.

Bone marrow cytology

Fungi are rarely seen in the bone marrow aspirate in immunologically normal subjects. Histoplasma is an exception, organisms sometimes being detected within bone marrow macrophages. Fungi are much more often detected in bone marrow aspirates from severely immunocompromised patients, such as those with HIV infection or following bone marrow transplantation, when *Candida albicans*, *Aspergillus fumigatus*, *Histoplasma capsulatum* (Fig. 3.10), *Cryptococcus neoformans* (Fig. 3.11) and *Penicillium marneffei* (Fig. 3.12) [28] may be seen. Organisms may be within macrophages or lying free. Although fungi may be seen in bone marrow aspirate films, a trephine biopsy is usually more sensitive in the

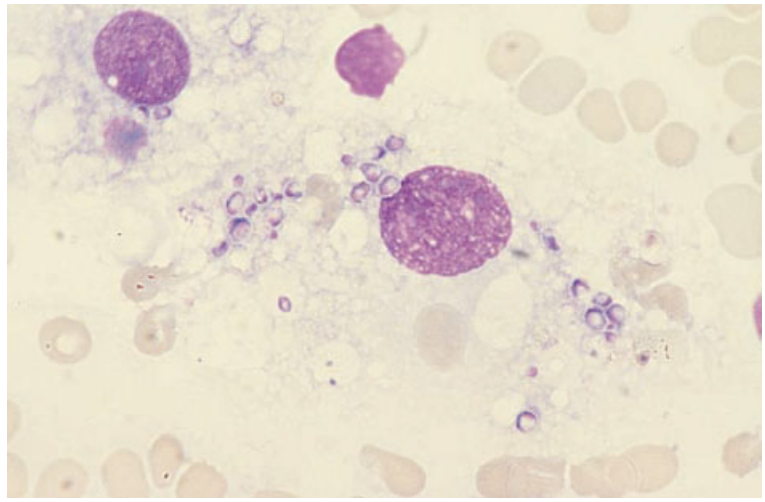
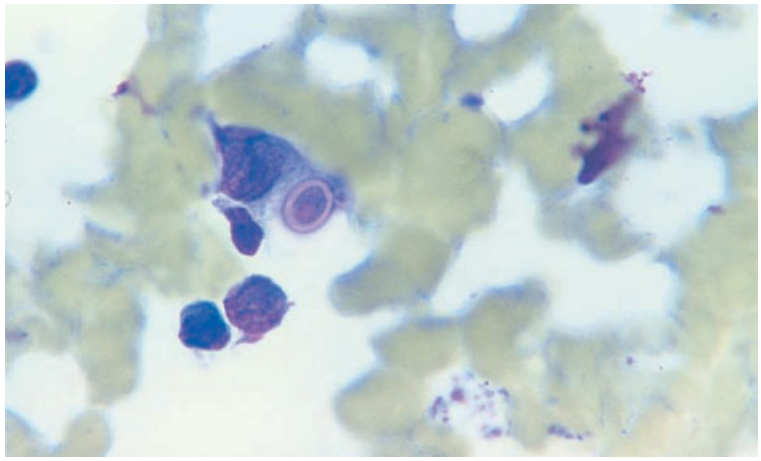


Fig. 3.10 BM aspirate. Histoplasmosis in a patient with AIDS, showing numerous organisms within a macrophage. MGG ×940.

(a)



(b)

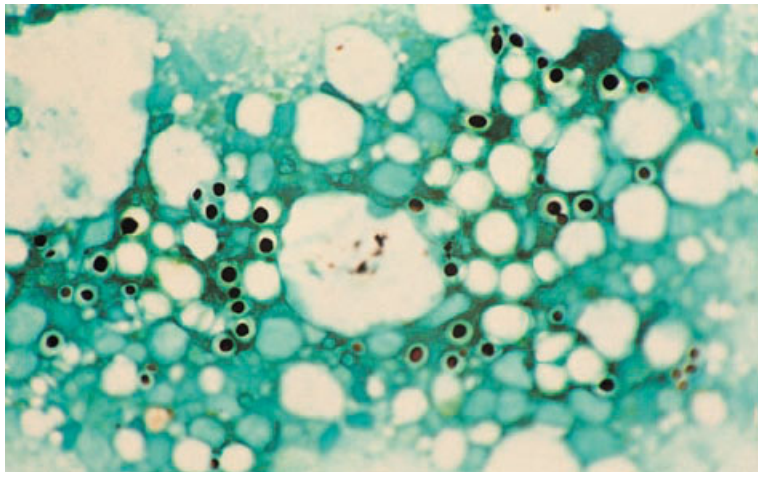


Fig. 3.11 (a) BM aspirate. Budding cryptococcus in an HIV-positive man. MGG ×960. (b) BM aspirate. Budding cryptococcus in another HIV-positive man. GMS ×960. (By courtesy of Dr Christine Costello, London.)

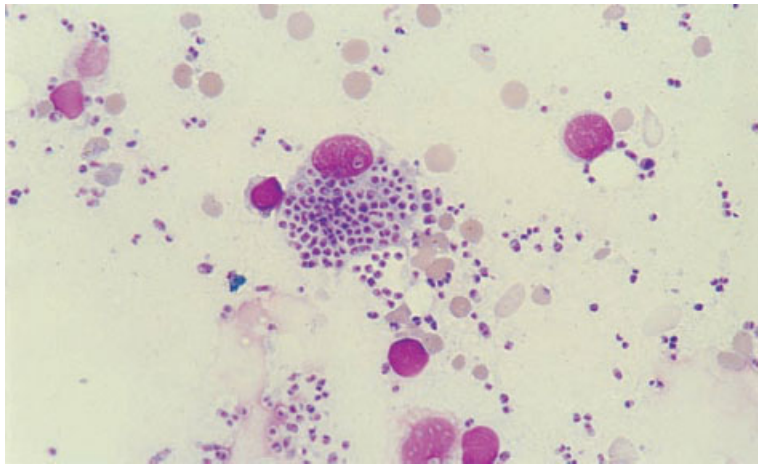


Fig. 3.12 BM aspirate. *Penicillium marneffei* in an HIV-positive man. MGG ×960. (By courtesy of Dr KF Wong, Hong Kong.)

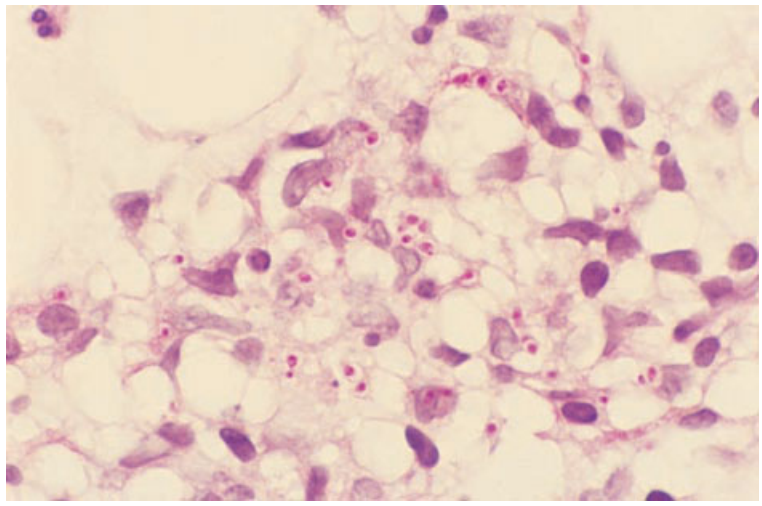


Fig. 3.13 BM trephine biopsy section from a patient with AIDS showing *Histoplasma capsulatum*. Paraffin-embedded, PAS-diastase ×960.

detection of fungal infection. Fungi may also be cultured from the bone marrow, sometimes when peripheral blood culture is negative.

Bone marrow histology

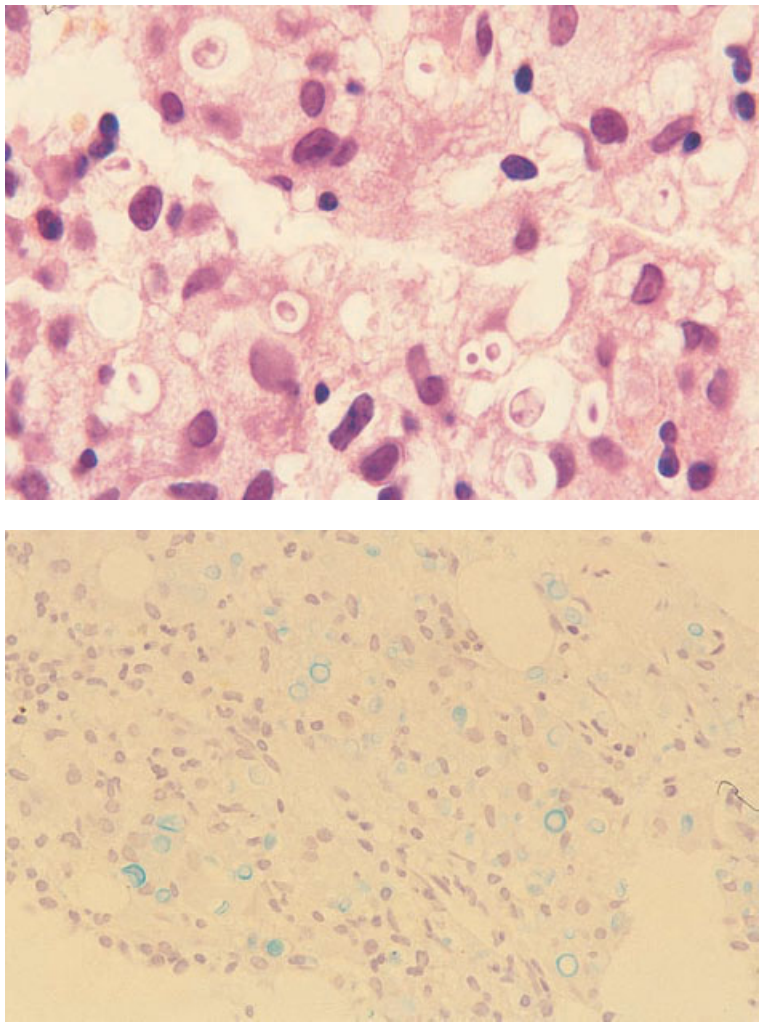
In systemic fungal infections, particularly in immunocompromised patients such as those with acquired immune deficiency syndrome (AIDS) (see page 123), organisms can sometimes be identified in the marrow (Figs 3.13–3.15). Usually fungi are within macrophages, including the altered macrophages comprising granulomas, or are associated with necrotic tissue. Rarely they are detectable only within megakaryocytes [29].

The histological features of the important fungi that can be seen in histological sections of bone marrow are summarized in Table 3.2. Cases of

Table 3.2 Differential diagnosis of fungal and protozoal pathogens.

Species	Tissue forms	Special stains
Fungi		
Candida albicans	Non-branching pseudohyphae and small (2–4 μm) budding yeast forms	PAS* and GMS
Cryptococcus neoformans	Yeast forms (5–10 μm), thick capsule, narrow-based unequal buds	PAS*, GMS and mucicarmine
Histoplasma capsulatum	Small (2–5 μm) yeast forms†	PAS* and GMS
Penicillium marneffei	Small (2–6 μm) round or oval to sausage shape containing reddish-purple dot-like structures and sometimes septae	PAS* and GMS
Protozoa		
Toxoplasma gondii	Tachyzoites (ovoid 3 × 6 μm, tiny nucleus, 'single-dot' appearance); occasionally cysts with numerous small bradyzoites	Giemsa, PAS‡, IHC
Leishmania donovani	Small (3 μm) intracellular amastigote, nucleus and paranuclear kinetoplast give 'double-dot' appearance	Giemsa

PAS, periodic acid–Schiff; GMS, Grocott's methenamine silver stain; IHC, immunohistochemistry.
* Pre-treatment with diastase to remove glycogen considerably reduces positive staining of neutrophils and megakaryocytes and facilitates the detection of micro-organisms.
† *Histoplasma capsulatum* var *duboisii*, which is the cause of African histoplasmosis, is a larger organism, 12–15 μm in diameter [30].
‡ Tachyzoites PAS-negative, cysts and bradyzoites usually PAS-positive [31].



(a)

(b)

Fig. 3.14 BM trephine biopsy section, *Cryptococcus neoformans* in a patient with AIDS. Paraffin-embedded: (a) H&E ×960; (b) alcian blue ×376.

disseminated infection caused by unusual fungi—not previously known to be pathogenic—are also being recognized in patients with AIDS [32].

Problems and pitfalls

Diagnosis of fungal infection does not present any difficulty when organisms are detectable in the bone marrow. However, when there are granulomas without detectable micro-organisms, or if there are only reactive changes, diagnosis can be difficult. Bone marrow culture is more sensitive than bone marrow microscopy for the detection of fungi and can be employed when bone marrow examination is done for investigation of a fever, particularly in immunosuppressed patients. For example, bone marrow cultures are positive in 90% of patients with disseminated histoplasmosis whereas the detection rate with microscopy of bone marrow aspirates is considerably lower and the proportion of patients with granulomas is lower still [33,34]. However, it should be noted that peripheral blood cultures for histoplasma have almost as high a success rate as bone marrow cultures [34]. Supplementary tests, such as testing urine or serum for cryptococcal or histoplasma antigens, can also be useful. The test for

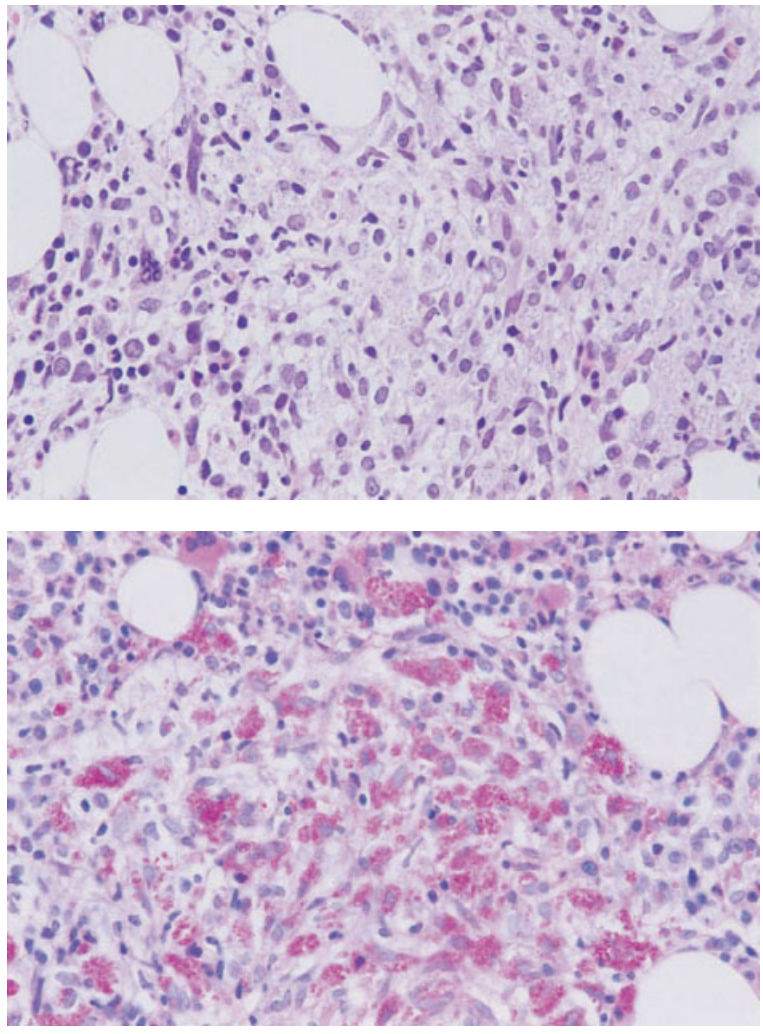


Fig. 3.15 Section of BM trephine biopsy specimen in a patient with AIDS with *Penicillium marneffei* infection. Paraffin-embedded: (a) H&E ×376; (b) PAS ×376.

histoplasma antigen in the urine is highly sensitive and in endemic areas this test can avoid the need for a bone marrow examination [35]. PCR for the detection of aspergillus DNA can also be useful.

Parasitic diseases

Peripheral blood

The presence of parasites within the bowel may lead to blood loss with consequent iron deficiency anaemia. Eosinophilia is also common in patients with helminth infections. In malaria and babesiosis, parasites are seen within red cells. Malaria is associated with haemolytic anaemia and sometimes thrombocytopenia. Atypical lymphocytes are present both in malaria and in some patients with hyper-reactive malarial splenomegaly. Toxoplasmosis can be associated with lymphocytosis and the presence of considerable numbers of atypical lymphocytes. Leishmaniasis can cause leucopenia and phagocytosed parasites are occasionally detectable within peripheral blood monocytes or neutrophils. Other parasites detectable free in the peripheral blood are trypanosomes and microfilariae.

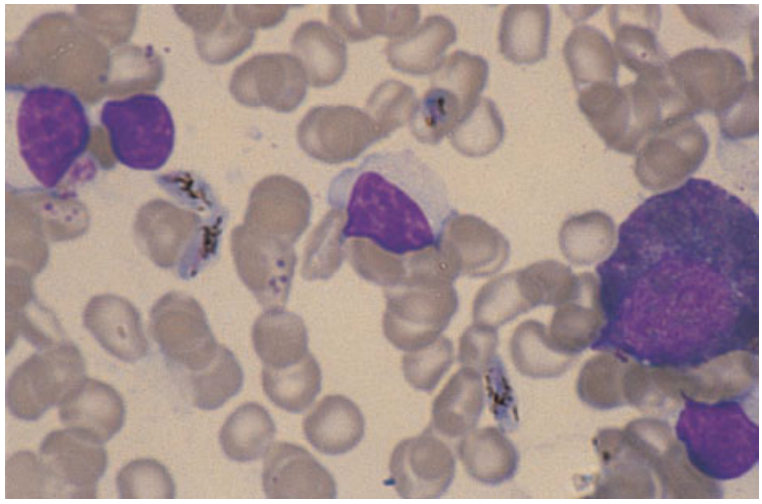


Fig. 3.16 BM aspirate from a patient with falciparum malaria showing gametocytes. MGG ×960. (By courtesy of Dr D Swirsky, Leeds.)

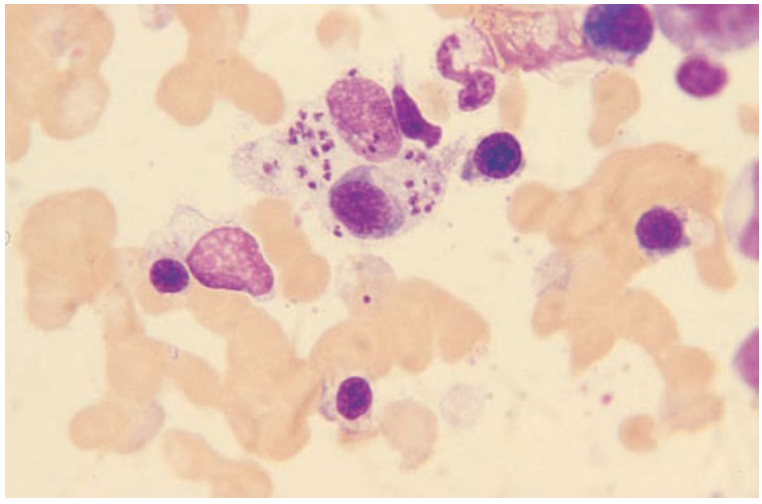


Fig. 3.17 BM aspirate, leishmaniasis, showing a macrophage containing numerous organisms which, in addition to a nucleus, have a small paranuclear kinetoplast giving them a characteristic 'double-dot' appearance. MGG ×940.

Bone marrow cytology

Malaria parasites are sometimes detected, in red cells or neutrophils, in a bone marrow aspirate (Fig. 3.16), although bone marrow aspiration is not a recommended diagnostic method if malaria is suspected. The bone marrow in malaria also shows hypercellularity due to erythroid and granulocytic hyperplasia, dyserythropoiesis, giant metamyelocytes and increased eosinophils, lymphocytes, plasma cells and macrophages [36,37]. There may be haemophagocytosis and macrophages can also contain malarial pigment. In hyper-reactive malarial splenomegaly there may be a marked increase in bone marrow lymphocytes [38]. A bone marrow aspirate is very useful in the diagnosis of leishmaniasis (Fig. 3.17) and is a recommended diagnostic method when this diagnosis is suspected. Trypanosomes are sometimes detected in the bone marrow, but less often than leishmania. Detection is more common in immunosuppressed patients [39]. Microfilaria are occasionally observed in a bone marrow aspirate in immunologically normal hosts (Fig. 3.18). Other parasites are rarely detected in the



Fig. 3.18 BM aspirate from a patient with filariasis showing a microfilaria. MGG ×470. (By courtesy of Mrs Seema M Zainal, Bahrein.)

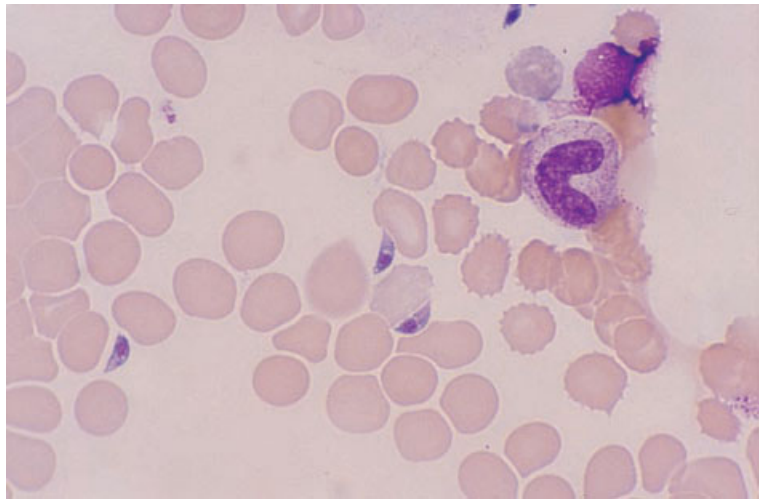


Fig. 3.19 BM aspirate showing *Toxoplasma gondii* in a patient who had had a renal transplant. MGG ×940. (By courtesy of Dr R Cobcroft, Brisbane.)

bone marrow, except in severely immunosuppressed patients in whom pneumocystis [40] and toxoplasma (Fig. 3.19) have sometimes been found. Features useful in identifying various protozoan parasites are shown in Table 3.2.

An increase of bone marrow eosinophils and their precursors is often apparent when there is helminth infection.

Bone marrow histology

A bone marrow biopsy in malaria usually shows increased cellularity and increased macrophage activity, often with haemophagocytosis. During acute attacks of malaria, sinusoids may be packed with parasitized red cells [37]. In patients who have suffered recurrent attacks of malaria, the bone marrow may be slate grey or black due to deposition of malaria pigment. It is important to distinguish malaria pigment (haemozoin) from formalin pigment.

In visceral leishmaniasis, there may be granuloma formation. In more severe cases there is a diffuse increase of macrophages. The organisms are

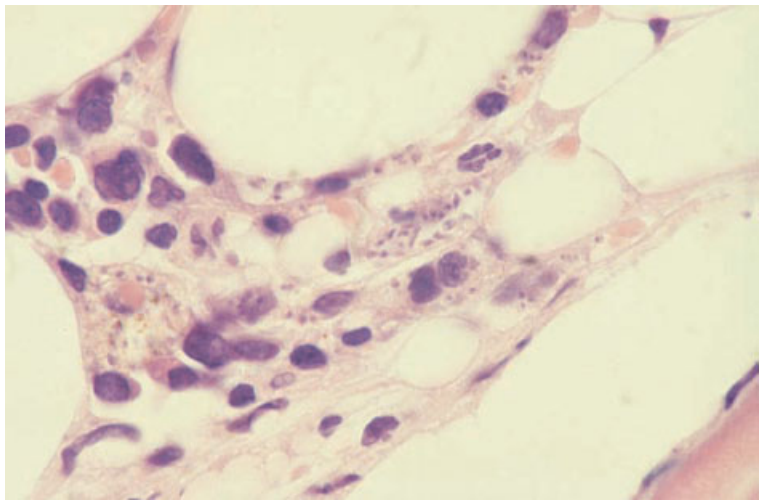


Fig. 3.20 BM trephine biopsy section from an HIV-positive man showing *Leishmania donovani* within macrophages. H&E ×960.

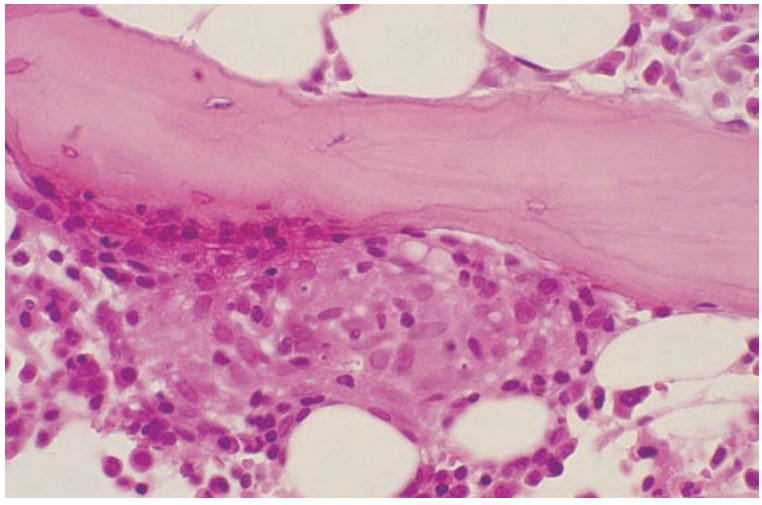


Fig. 3.21 BM trephine biopsy section, toxoplasmosis, showing a small granuloma in a paratrabecular position containing several small organisms with a single nucleus consistent with the tachyzoites of *Toxoplasma gondii*; fungal stains were negative and the patient had raised serum IgM antibodies to *Toxoplasma*. Plastic-embedded, H&E ×390.

often seen within macrophages (Fig. 3.20); their small size (3 μm) sometimes leads to their being confused with the fungus *Histoplasma capsulatum*. However, leishmania fail to stain with periodic acid–Schiff (PAS) or silver stains and a Giemsa stain will demonstrate a small paranuclear basophilic body, known as the kinetoplast, giving the organism a characteristic 'double-dot' appearance.

Granulomas are also seen in toxoplasmosis; rarely, in immunosuppressed individuals, organisms are seen in the marrow (Fig. 3.21). These usually take the form of tachyzoites, which are 3–6 μm in diameter and have a tiny single nucleus. Occasionally, cysts containing numerous bradyzoites are present. These are of a similar size to tachyzoites and also have a single nucleus; the lack of a kinetoplast helps to distinguish them from leishmania. Toxoplasma can be identified with a polyclonal antibody.

Microsporidiosis rarely involves the bone marrow but involvement has been reported at autopsy in a patient with AIDS [41].

Extrapulmonary infection with *Pneumocystis carinii* is rare; it is invariably secondary to pulmonary disease, and appears to be more common in those patients who have received aerosolized pentamidine therapy. Approximately a third of patients with extrapulmonary infection have marrow involvement [42]. There are areas of 'frothy' exudate which is pink on an H&E stain; Grocott's methenamine silver stain demonstrates the cysts, which are 4–6 μm in diameter and often have a crumpled or cup-shaped appearance. Diagnosis is aided by immunohistochemistry using monoclonal antibody of the 3F6 clone (available commercially from Dako and Novocastra).

Bone marrow granulomas

A granuloma is a compact aggregate of macrophages. These may include a major component of epithelioid macrophages which have large amounts of pale pink cytoplasm and ovoid or elongated nuclei with a dispersed chromatin pattern. Often several epithelioid cells fuse to form a giant cell. Two major types of giant cell are recognized in granulomas: (i) Langhans' type, which has numerous nuclei arranged around the periphery of the cell; and (ii) foreign body type with nuclei scattered throughout the cell. Other cell types, including lymphocytes, plasma cells, neutrophils, eosinophils and fibroblasts, may be found within granulomas, but these are not a constant feature.

A wide range of aetiological agents are associated with marrow granulomas (Table 3.3). It should be noted that patients with immunodeficiency, such as AIDS, may fail to produce granulomas in response to infection with organisms that stimulate granuloma formation in normal individuals; this is probably consequent on the lack of important T-cell functions that facilitate formation of some types of granuloma.

Peripheral blood

There are no specific peripheral blood findings associated with the presence of bone marrow granulomas. The blood film may show features associated with the primary disease or, if bone marrow disease is extensive, there may be anaemia or pancytopenia with a leuco-erythroblastic blood film. Lymphopenia is also common [43].

Table 3.3 Bone marrow granulomas [43–46].

Infection
Tuberculosis
Atypical mycobacterial infection
Disseminated bacillus Calmette–Guérin (BCG) infection [47]
Brucellosis
Leprosy
Syphilis
Typhoid fever
Legionnaire's disease
Tularaemia [43]
Ehrlichiosis [44]
Q fever
Rocky Mountain spotted fever [43]
Leishmaniasis
Toxoplasmosis
Histoplasmosis
Cryptococcosis
Saccharomyces infection [43]
Blastomycosis
Coccidioidomycosis [48]
Paracoccidioidomycosis
Herpesvirus infection (EBV, CMV, herpes zoster)
Hantaan virus infection (Korean haemorrhagic fever) [49]
Cat scratch disease [49]

Sarcoidosis

Malignant disease
Hodgkin's disease*
Multiple myeloma [50]
Non-Hodgkin's lymphoma*
Chronic NK-cell lymphocytosis [51]
Mycosis fungoides [43]
Acute lymphoblastic leukaemia [43,46,49]
Myelodysplastic syndrome [46]
Metastatic carcinoma (breast or colon) [52,53]

Drug hypersensitivity
Phenytoin
Procainamide
Phenylbutazone [43]
Chlorpropamide
Sulfasalazine [54]
Ibuprofen [43]
Indometacin [43]
Allopurinol
Carbamazepine [55]

Associated with eosinophilic interstitial nephritis

Reaction to foreign substances
Anthracosis and silicosis [46,49,56]
Talc [57]
Berylliosis [58]

* With or without bone marrow infiltration.

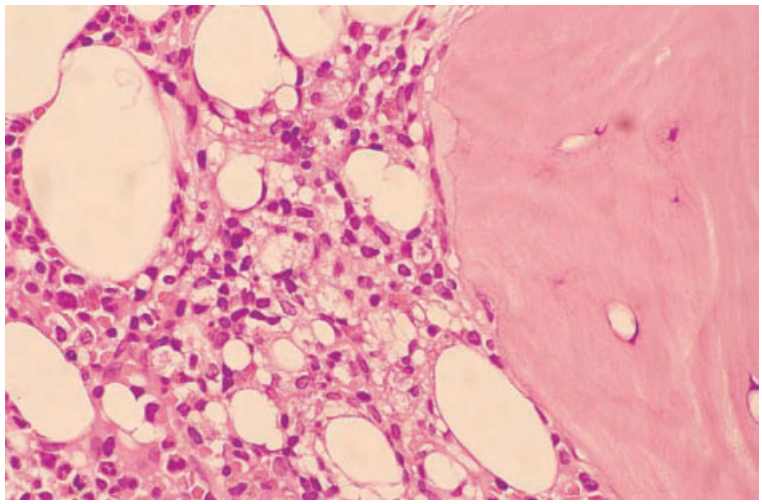


Fig. 3.22 BM trephine biopsy section, lipid granuloma. Paraffin-embedded, H&E ×390.

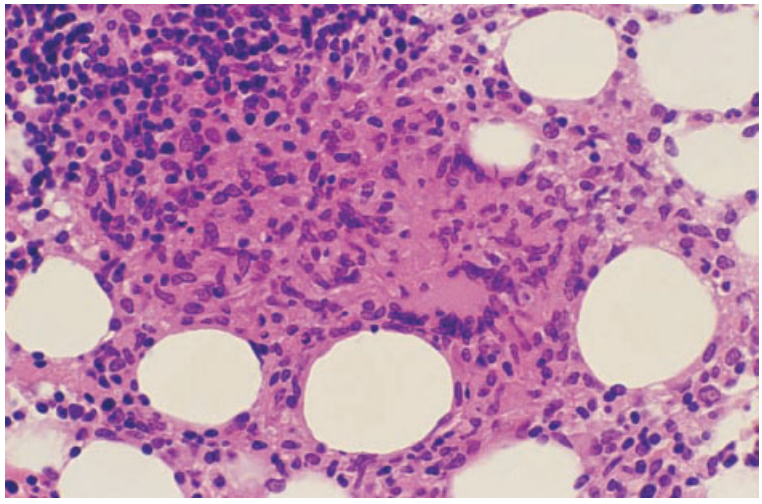


Fig. 3.23 BM trephine biopsy section, miliary tuberculosis, showing an epithelioid granuloma containing a Langhans' giant cell; there are numerous lymphocytes at the periphery of the granuloma. Paraffin-embedded, H&E ×390.

Bone marrow cytology

There are no specific features in the bone marrow aspirate in patients with bone marrow granulomas. Occasionally, it is possible to recognize epithelioid cells.

Bone marrow histology

Lipid granulomas

Lipid granulomas (Fig. 3.22) were previously reported to be the most common type of granuloma seen in the marrow, being present in up to 9% of biopsies [59]. In our experience, they are considerably less common than this. They are of no clinical importance and must be distinguished from epithelioid granulomas which they can sometimes resemble. Similar lesions may be seen in the liver, spleen and lymph nodes and some of these cases have been reported to be associated with the ingestion of mineral oil [60]. In the marrow they are usually located close to sinusoids or lymphoid nodules and measure from 0.2 to 0.8 mm in diameter. They contain fat

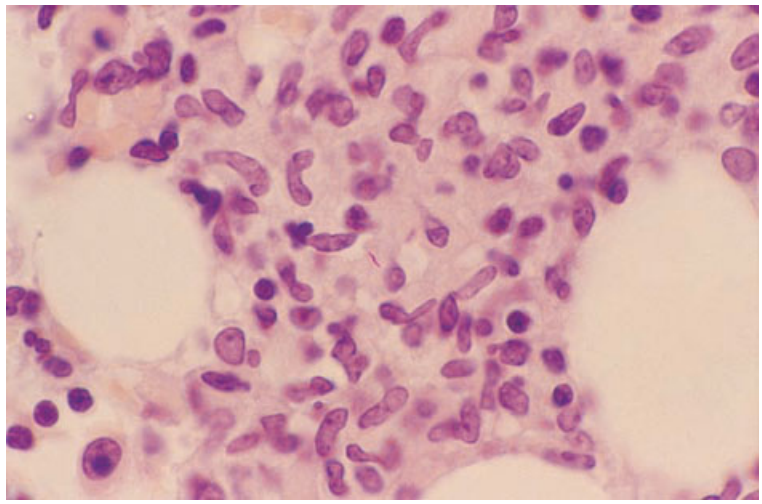


Fig. 3.24 BM trephine biopsy section, miliary tuberculosis, showing a granuloma containing an acid-fast bacillus. Paraffin-embedded, Ziehl–Neelsen stain ×970.

vacuoles which vary in size but are usually smaller than the vacuoles in marrow fat cells; these are found both within macrophages and extracellularly. Lipid granulomas usually have plasma cells, eosinophils and lymphocytes within them, and approximately 5% contain giant cells. Occasionally, the fat vacuoles may be small and easily overlooked, giving the granulomas a sarcoid-like appearance.

Other granulomas

Unless a specific organism can be demonstrated within a granuloma, there are usually no histological features that allow a definitive diagnosis to be made [61]. Because of this, it is important for the pathologist to be aware of all relevant clinical details, in order to be able to suggest an appropriate differential diagnosis. All biopsies with granulomas should have special stains for acid-fast bacilli and fungi performed. Ideally, in those cases in which marrow granulomas with an infective aetiology are possible, for example in patients with a pyrexia of unknown origin, this should be anticipated and part of the marrow aspirate should be cultured for mycobacteria and fungi.

Granulomas are found on marrow biopsy in 15–40% of patients with miliary tuberculosis (Fig. 3.23). Tuberculous granulomas usually contain Langhans'-type giant cells and caseation is present in approximately half the cases with marrow involvement [61]. Acid-fast bacilli cannot be demonstrated in most cases and, when seen, they are usually scanty (Fig. 3.24). Approximately 50% of patients with disseminated *Mycobacterium avium intracellulare* infection have marrow granulomas (Figs 3.25 and 3.26), ranging from small, ill-defined lymphohistiocytic aggregates to larger, more solid lymphohistiocytic lesions, and small, well-formed epithelioid granulomas [62]. Giant cells are only present in a minority of lesions and necrosis is not usually seen. When organisms are very numerous, the macrophages containing them may appear foamy (Fig. 3.26a) or may even resemble Gaucher's cells. Occasionally, organisms are recognizable on a Giemsa stain (Fig. 3.26b). Atypical mycobacteria may be demonstrated by special stains, sometimes in large numbers; they tend to be longer, more curved and more coarsely beaded than tubercle bacilli. They are PAS-positive, whereas *M. tuberculosis* is PAS-negative. Marrow granulomas containing foamy macrophages are occasionally seen in patients with leprosy; a Fite stain will demonstrate the acid-fast bacilli of *M. leprae* [6]. Foamy macrophages may also be a feature of granulomas due to typhoid. Small, poorly formed epithelioid granulomas are found in the bone marrow in most cases of brucellosis. Distinctive 'doughnut-type' granulomas may be seen in the marrow in Q fever [63], although they do not

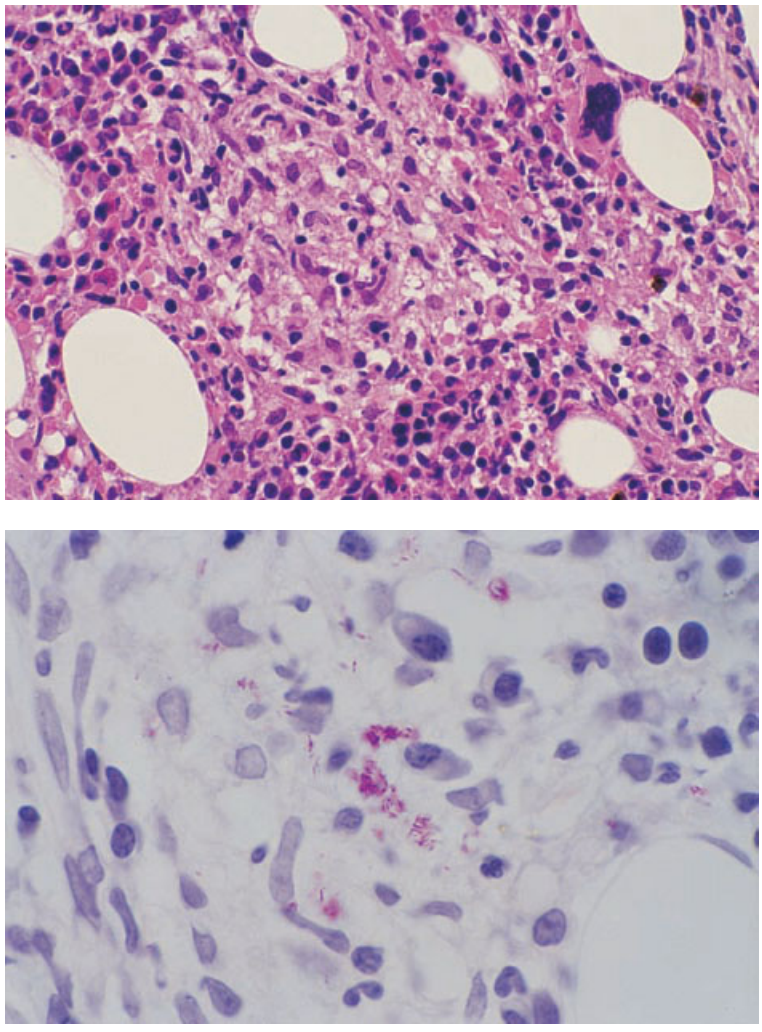
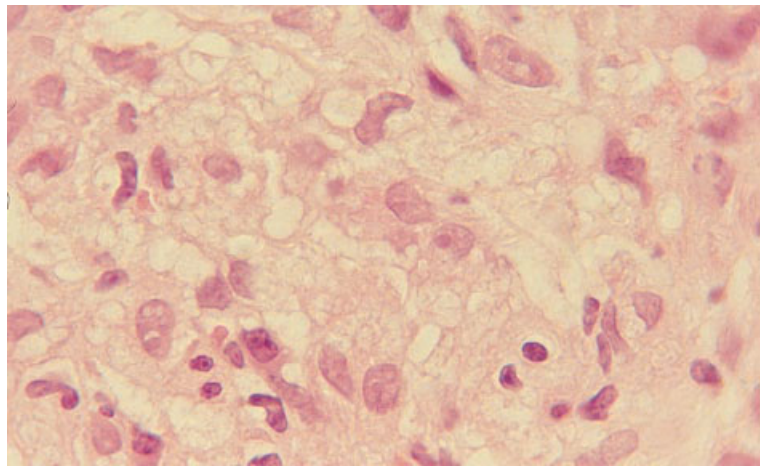


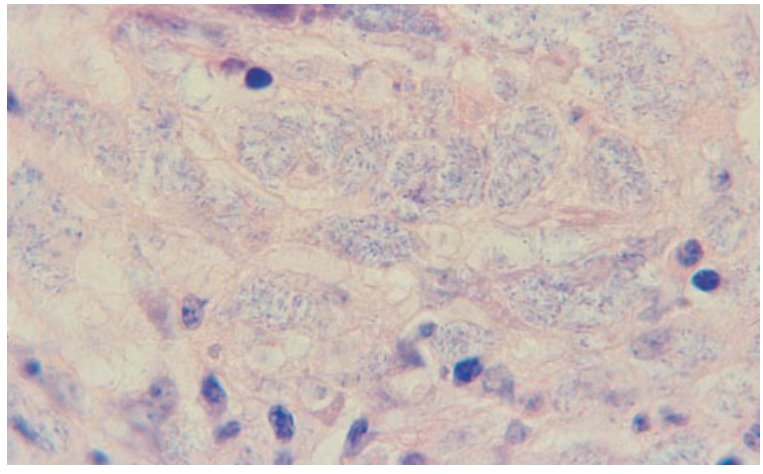
Fig. 3.25 BM trephine biopsy section from a patient with AIDS and disseminated *M. avium intracellulare* infection. (a) Poorly formed granuloma made up of epithelioid macrophages, many of which have vacuolated cytoplasm. Paraffin-embedded, H&E ×390. (b) Large numbers of acid-fast bacilli; note the coarse beading of the organisms. Paraffin-embedded, Ziehl–Neelsen stain ×970.

appear to be specific for this disease [18]. They have also been reported, for example, in CMV infection in immunodeficient subjects [64] and in Hodgkin's disease, infectious mononucleosis and typhoid fever. This type of granuloma often has a central empty space, surrounded by neutrophils, lymphocytes, histiocytes and concentrically arranged, laminated fibrinoid material; more haphazardly arranged lesions without a central space also occur, as do small areas of fibrinoid necrosis [63].

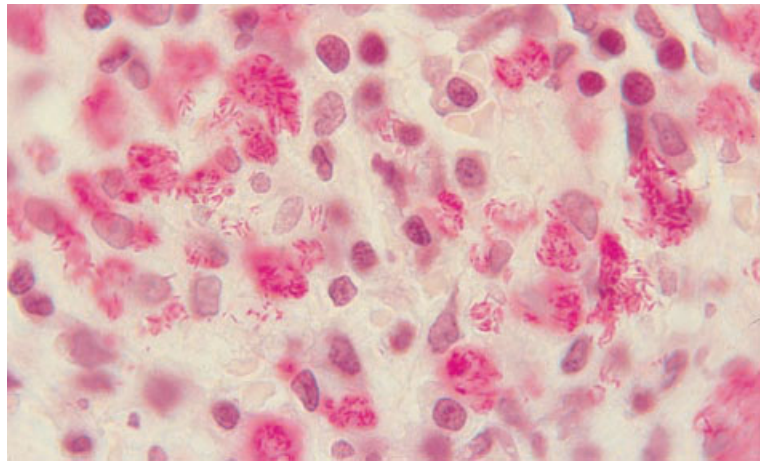
Disseminated infection by the fungus *H. capsulatum* usually involves the bone marrow; in normal hosts there are numerous granulomas, often with Langhans' giant cells and necrosis. Discrete granulomas are present in only a minority of immunodeficient hosts; more commonly such patients have ill-defined lymphohistiocytic aggregates or sheets of macrophages infiltrating between haemopoietic cells [65] (see Fig. 3.13). Tiny yeast forms, 2–5 μm in diameter, some of which show unequal budding, are present within macrophages. The organisms may be seen with an H&E stain, but are best visualized using Gomori's methenamine silver (GMS) stain or a PAS stain. *Cryptococcus neoformans* infection may cause granulomas. The organisms seen in tissue sections are yeasts (5–10 μm in diameter)



(a)

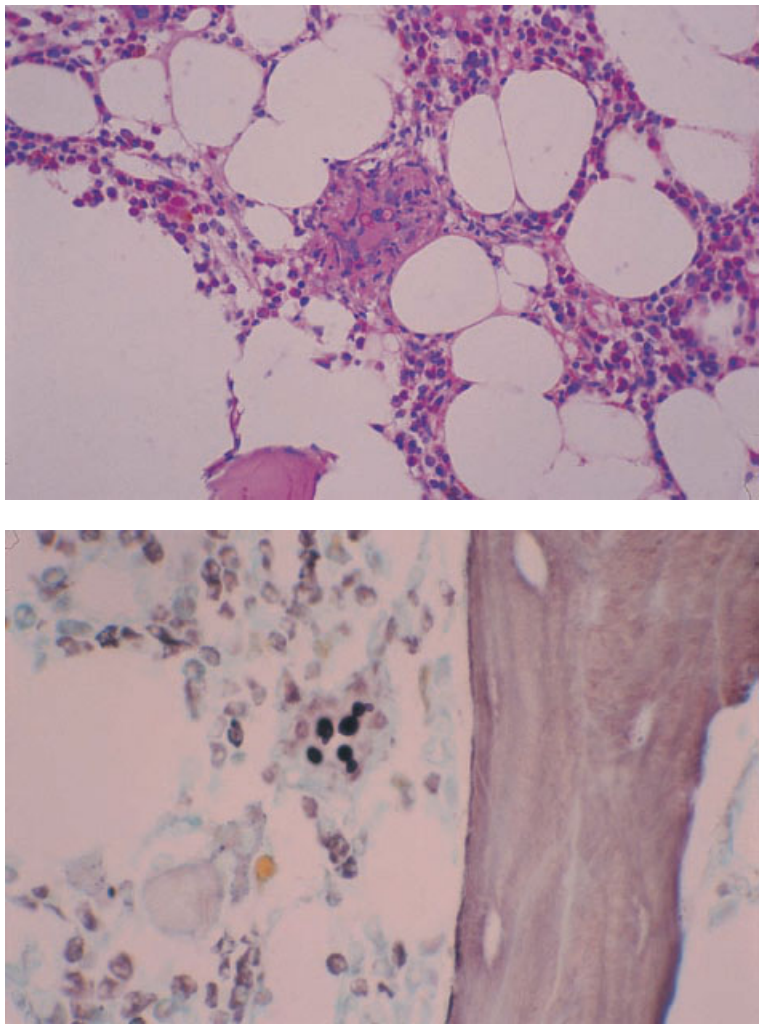


(b)



(c)

Fig. 3.26 BM trephine biopsy section from a patient with AIDS and *M. avium intracellulare* infection showing foamy macrophages packed with micro-organisms: (a) H&E; (b) Giemsa; (c) Ziehl–Neelsen stain. Paraffin-embedded, ×960.



(a)

(b)

Fig. 3.27 BM trephine biopsy sections from a patient with *C. neoformans* infection. Paraffin-embedded. (a) A small epithelioid granuloma containing large yeast forms with narrow-based unequal budding. PAS ×195. (b) Budding yeast forms. GMS ×390.

with a wide capsular halo and narrow-based, unequal budding (Fig. 3.27); with a mucicarmine stain the capsule of the yeast is red. The capsule is also well stained with alcian blue (see Fig. 3.14b) and with PAS (Fig. 3.27a). Infection with the protozoans *Leishmania donovani* and *Toxoplasma gondii* may involve the marrow and provoke granuloma formation (see Figs 3.20 and 3.21).

In up to 60% of patients with infectious mononucleosis, small epithelioid granulomas are seen in histological sections of bone marrow biopsy; Langhans' giant cells and necrosis are not seen. Marrow granulomas are seen less commonly in other viral infections.

Granulomas may be seen in Hodgkin's disease [66], non-Hodgkin's lymphoma (Fig. 3.28) and multiple myeloma [50] (Fig. 3.29) in association with neoplastic infiltration of the marrow. In both Hodgkin's disease and non-Hodgkin's lymphoma, granulomas also occur in the absence of marrow involvement; these are usually small, well-formed epithelioid granulomas, although larger, poorly formed lymphohistiocytic lesions have also been reported [67]. Since patients with lymphoma have

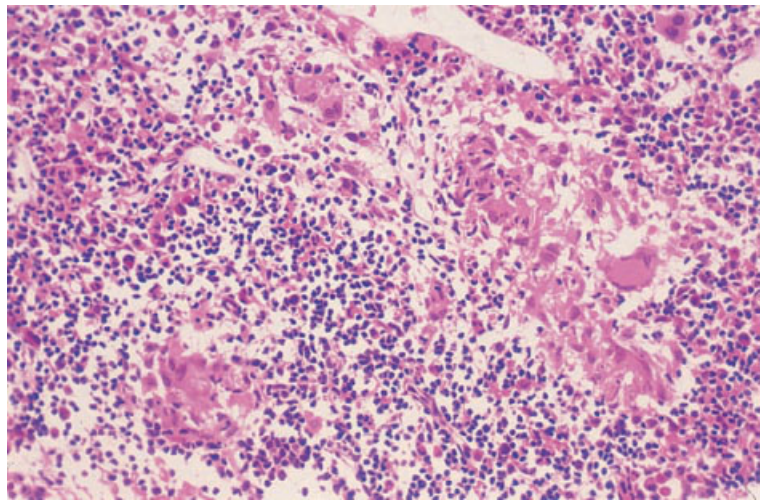


Fig. 3.28 BM trephine biopsy section showing a reactive granuloma in a patient with diffuse bone marrow involvement by low grade non-Hodgkin's lymphoma (follicular lymphoma). No evidence of an infective cause of the granuloma was found in this patient. Paraffin-embedded, H&E ×195.

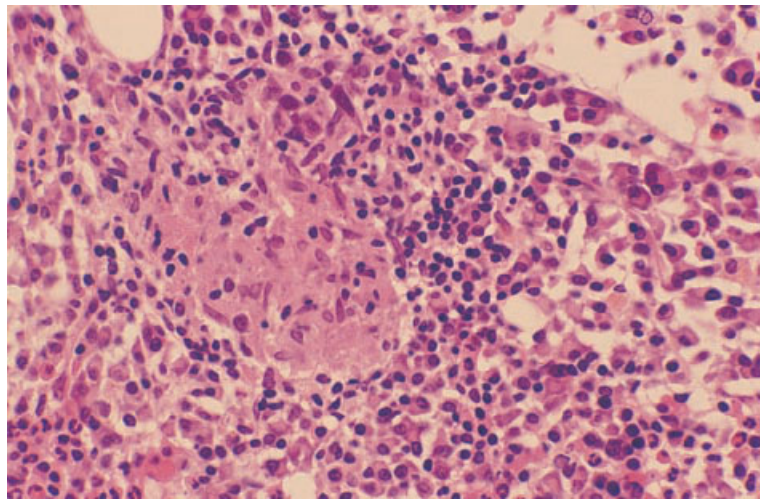


Fig. 3.29 BM trephine biopsy, multiple myeloma, showing large numbers of plasma cells and an epithelioid granuloma (centre). No evidence of an infective cause for the granuloma was found in this patient. Paraffin-embedded, H&E ×390.

an increased susceptibility to many of the infections associated with marrow granulomas, these should always be excluded before attributing the granulomas to the underlying neoplastic disease. Bone marrow granulomas, in the absence of any apparent infection, have also been reported in single cases of hairy cell leukaemia treated with 2-chlorodeoxyadenosine [68], AML treated with interleukin-2 and lymphokine-activated killer cells [69] and, in a total of four cases, in chronic NK-cell lymphocytosis [70,71].

The bone marrow is commonly involved in sarcoidosis; granulomas were seen in nine out of 21 patients in a biopsy series [61]. Often patients with marrow granulomas have evidence of multisystem involvement, such as hepatosplenomegaly, although chest radiography may be normal [72]. Typically there are numerous, well-formed epithelioid granulomas which, in approximately a third of cases, contain Langhans' giant cells; necrosis is seen very rarely. Sarcoid granulomas usually lack a surrounding zone of inflammatory cells. They are associated with prominent reticulin fibrosis and sometimes collagen formation. Giant cells in sarcoid granulo-

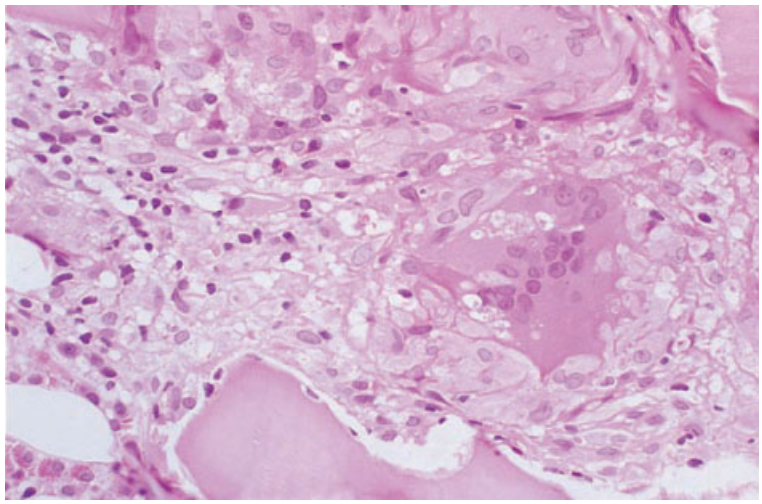


Fig. 3.30 BM trephine biopsy, sarcoidosis, showing a granuloma with a giant cell containing asteroid bodies. Plastic-embedded, H&E ×390.

mas may contain asteroid and Schaumann bodies (Fig. 3.30).

The granulomas associated with drug hypersensitivity are poorly circumscribed lymphohistiocytic lesions, often containing eosinophils. Rarely, they are accompanied by a systemic vasculitis which may involve the vessels in the marrow.

Reactive lymphoid aggregates and polymorphous lymphoid hyperplasia

Reactive lymphoid aggregates, often referred to as 'benign lymphoid aggregates', are commonly present in the bone marrow, their incidence increasing with age. An increased frequency has been reported in association with infection, inflammation, haemolysis, myeloproliferative disorders and autoimmune diseases such as rheumatoid arthritis and thyrotoxicosis. Their frequency appears to be increased in Castleman's disease [73]. Bone marrow lymphoid aggregates, assessed histologically as benign, have been associated with the subsequent development of low grade lymphoma [74].

Peripheral blood

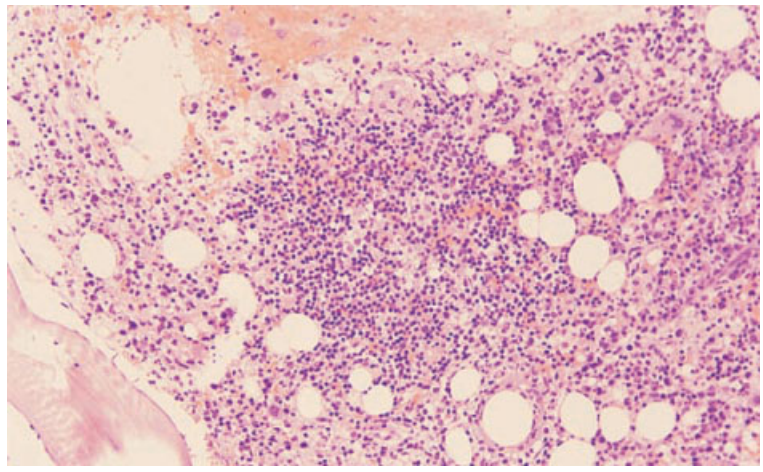
There are no specific peripheral blood features associated with the presence of reactive lymphoid aggregates in the bone marrow.

Bone marrow cytology

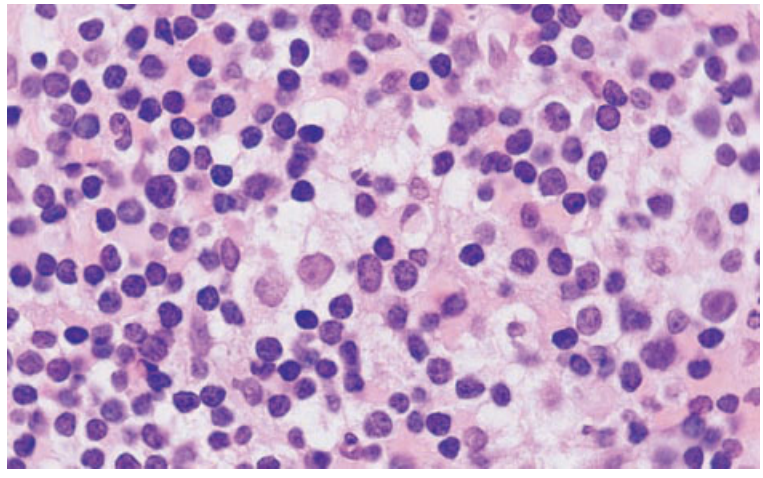
The bone marrow aspirate is usually normal but may show an increase in normal, mature lymphocytes.

Bone marrow histology

Reactive lymphoid aggregates are usually few in number, not paratrabecular in position, well circumscribed and composed of small mature lymphocytes interspersed with some plasma cells, macrophages and sometimes occasional eosinophils, mast cells and immunoblasts [74–76] (Fig. 3.31). Their presence is usually indicative of a reactive process, particularly auto-immune disease. Although occasional paratrabecular infiltrates have been reported in apparently healthy people [77], this is very rare and a paratrabecular infiltrate is unlikely to be reactive. We would advocate careful follow-up of such cases. Reactive lymphoid aggregates may be associated with small blood vessels. The lymphocytes show more pleomorphism than those in neoplastic lymphoid aggregates. Occasionally germinal centres are seen [78] (Fig. 3.32). Reticulin fibres are increased within the nodule. Bone marrow biopsies showing reactive lymphoid aggregates have an increased incidence of lipid granulomas and plasmacytosis. When lymphoid nodules are relatively



(a)



(b)

Fig. 3.31 Section of BM trephine biopsy showing: (a) a reactive lymphoid nodule and surrounding normal haemopoietic cells, H&E ×97; (b) the centre of a lymphoid nodule containing several macrophages and occasional immunoblasts, in addition to normal small lymphocytes; paraffin-embedded, H&E ×970.

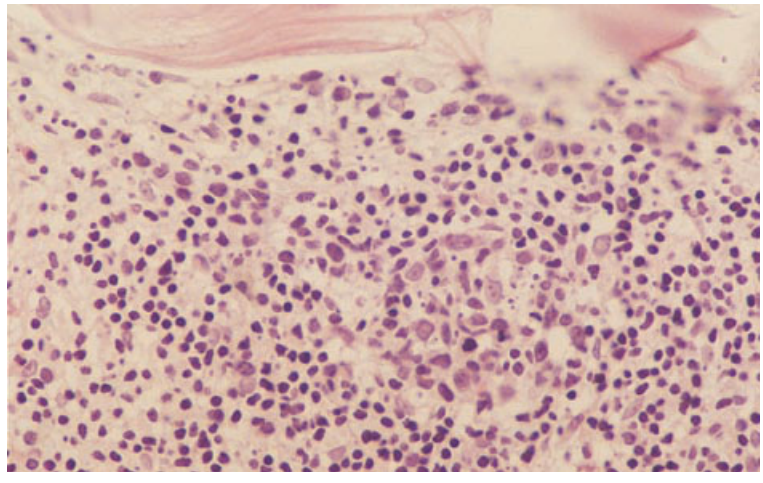


Fig. 3.32 Section of BM trephine biopsy showing a secondary lymphoid follicle containing a germinal centre in a patient with rheumatoid arthritis. Paraffin-embedded, H&E ×192. (By courtesy of Dr R Brunning, Minneapolis.)

large or numerous, immunohistochemical demonstration of a normal κ:λ ratio (approximately 2:1) or of a mixture of T and B cells may be helpful in confirming their reactive nature [76,79,80]. However, it should be noted that, although lymphoid aggregates composed of homogeneous B cells are likely to be neoplastic and most reactive lymphoid aggregates contain a mixture of T and B cells [79,80], there may also be admixed reactive T cells in infiltrates of low grade B-cell lymphoma.

Unusual causes of reactive lymphoid infiltrates are thymoma, which has been associated with a nodular and interstitial infiltrate of polyclonal T cells [81], and persistent polyclonal B-cell lymphocytosis of middle-aged, mainly cigarette-smoking, females which has been associated with a nodular infiltrate, presumably of B cells [82].

More extensive lymphoid infiltrates, often with admixed eosinophils, macrophages, epithelioid cells, endothelial cells, plasma cells and immunoblasts, are common in AIDS (see Figs 3.43 and 3.44) and may also be seen in congenital immunodeficiency, angioimmunoblastic lymphadenopathy, following bone marrow transplantation and occasionally in association with auto-immune diseases such as rheumatoid arthritis. It is sometimes not possible to distinguish this condition, sometimes designated polymorphous reactive lymphoid hyperplasia, from T-cell lymphoma on the basis of histology alone. An extensive lymphoid infiltrate can also be seen in the lymphoproliferative disorder associated with phenytoin therapy [83].

Plasmacytosis and cytological abnormalities in plasma cells

A reactive increase of polyclonal plasma cells is common and is associated with a variety of conditions including HIV and other infections, chronic inflammatory diseases (particularly rheumatoid arthritis), haemopoietic and non-haemopoietic malignant disease, angio-immunoblastic lymphadenopathy, systemic Castleman's disease, cirrhosis, diabetes mellitus, iron deficiency, megaloblastic anaemia and haemolytic anaemia [9,84]. A reactive increase in plasma cells needs to be distinguished from bone marrow infiltration by neoplastic plasma cells, such as occurs in multiple myeloma and in many cases of immunocyte-derived amyloidosis, systemic light chain disease and monoclonal gammopathy of undetermined significance (see Chapter 7).

Peripheral blood

Patients with reactive bone marrow plasmacytosis commonly show non-specific abnormalities in the peripheral blood consequent on the underlying disease. There is often anaemia which may have the features of the anaemia of chronic disease (either a normochromic, normocytic anaemia or, if the inflammatory process is severe, a hypochromic, microcytic anaemia). Rouleaux formation is commonly increased as a consequence of an increased concentration of polyclonal immunoglobulins and other reactive changes in plasma proteins. Occasional patients with reactive plasmacytosis have plasma cells in the peripheral blood, usually in small numbers.

Bone marrow cytology

In reactive plasmacytosis the bone marrow shows an increased number of plasma cells, not usually exceeding 10–20% of nucleated cells, but in rare cases up to 50%. The plasma cells are predominately mature (see Fig. 1.48) although occasional cells may have nucleoli, a diffuse chromatin pattern or some degree of nucleocytoplasmic asynchrony. Small numbers of bi- or trinucleated forms can be present. The plasma cells may contain cytoplasmic vacuoles or inclusions. Intranuclear inclusions have also been described but they are more characteristic of neoplastic plasma cells. Large homogeneous hyaline inclusions, often single, 2–3 μm in diameter and displacing the nucleus, are designated Russell bodies. They are occasionally identified in bone marrow aspirates (Fig. 3.33). Cells containing multiple vacuoles or inclusions are referred to as Mott cells, grape cells or morular cells. Cells may contain cytoplasmic crystals. Cells may have a pyknotic nucleus and voluminous pale-staining cytoplasm, as a result of greatly dilated endoplasmic reticulum; such cells are designated thesaurocytes indicating that they are storing the products they have synthesized. Cells that synthesize carbohydrate may have flaming pink cytoplasm ('flaming cells'). All these inclusions and unusual tinctorial qualities are consequent on increased immunoglobulin synthesis

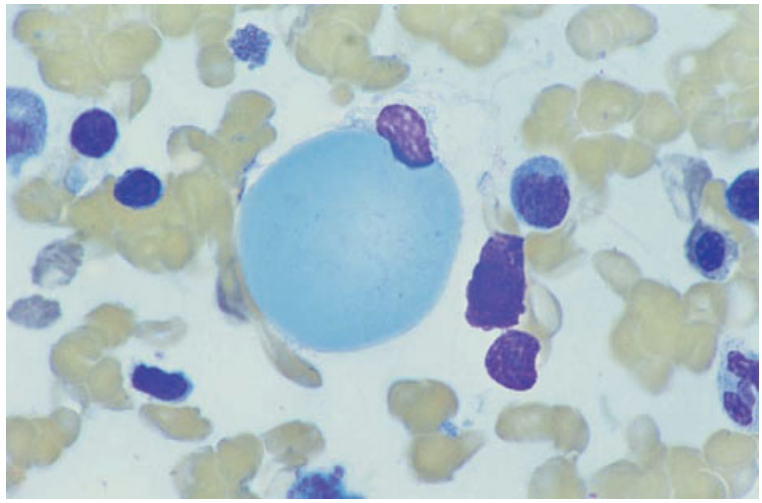


Fig. 3.33 BM aspirate film showing a Russell body in a plasma cell in a patient with reactive plasmacytosis. MGG ×940.

within the rough endoplasmic reticulum. Nuclear inclusions, known as Dutcher bodies, are consequent on invagination from the cytoplasm. Cells of these various types are characteristic of conditions with immune stimulation but neoplastic plasma cells often show similar features. Plasmacytic satellitism (a central macrophage surrounded by plasma cells) and increased mast cells, eosinophils and megakaryocytes favour reactive rather than neoplastic plasmacytosis [9].

Occasionally, plasma cells contain haemosiderin inclusions which are irregular in shape, relatively large and stain greenish-black with a May–Grünwald–Giemsa (MGG) stain (see Fig. 2.4). Their presence is associated with iron overload (e.g. haemochromatosis and transfusion siderosis) and with chronic alcoholism [85].

Bone marrow histology

A trephine biopsy section in reactive plasmacytosis shows an interstitial infiltrate of plasma cells, particularly adjacent to capillaries (see Fig. 1.49). Plasma cells are sometimes clustered around macrophages. A minority of cases have small clusters of plasma cells but large homogeneous nodules, which are a feature of multiple myeloma, are not seen. The variety of inclusions described above may also be apparent in histological sections (Fig. 3.34) and some degree of cellular immaturity may be noted. Russell bodies and Dutcher bodies stain pink with an H&E stain and show variable PAS staining. In reactive plasmacytosis immunocytochemistry shows that κ- and λ-expressing plasma cells are present in a ratio of approximately 2:1. About half the plasma cells express γ heavy chain, about a third α and the remainder μ [86].

Plasmacytosis may be associated with other reactive changes such as granulocytic hyperplasia, lymphoid aggregates and increased numbers of macrophages. The macrophages may have enhanced haemophagocytic activity and an increased iron content.

Mast cells

Small numbers of mast cells are present in normal bone marrow aspirates. However, it should be noted that the normal number of mast cells in the trephine biopsy of healthy subjects has not been defined. Mast cells are increased as a reactive change in association with a variety of pathological processes. Increased numbers have been noted in infection, inflammation, renal failure, lymphoproliferative disorders and reactive lymphocytosis, aplastic anaemia, paroxysmal nocturnal haemoglobinuria, myeloproliferative disorders and the myelodysplastic syndromes [87–89]. Mast cells accumulate in areas of connective tissue proliferation, for example in fracture callus and in zones of osteitis fibrosa in patients

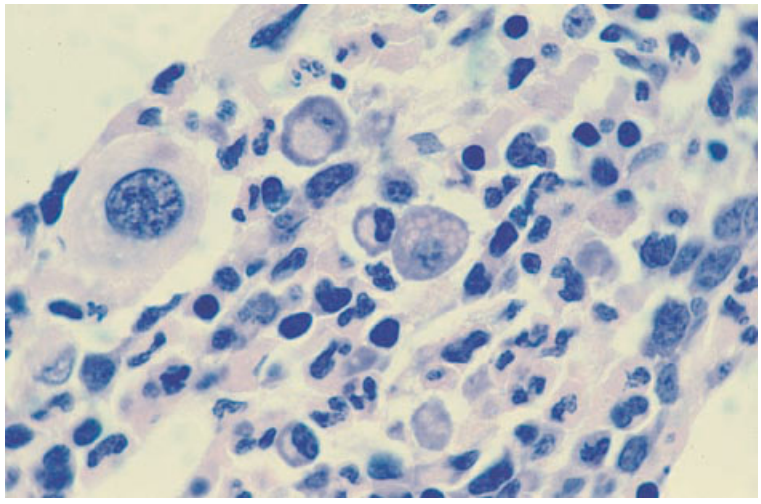


Fig. 3.34 BM trephine biopsy section, myelodysplastic syndrome, showing prominent plasma cells including one with multiple cytoplasmic vacuoles (Mott cell). Plastic-embedded, H&E ×940.

with renal failure. Increased numbers of mast cells are present also in systemic mastocytosis but these neoplastic mast cells are usually morphologically abnormal (see page 221).

Peripheral blood

There are no specific peripheral blood features associated with a reactive increase of bone marrow mast cells.

Bone marrow cytology

The cytological features of bone marrow mast cells have been described on page 25. Lymphocytosis and plasmacytosis may co-exist with a reactive increase in mast cells.

Bone marrow histology

The characteristics of mast cells in histological sections have been described on page 26 and the features of systemic mastocytosis will be described on page 223. In histological sections, mast cells may be confused with fibroblasts or macrophages because of their elongated shape, oval nuclei and the poor staining of their granules with H&E. The lesions associated with drug hypersensitivity, previously designated 'eosinophilic fibrohistiocytic lesions' [90], are now known to represent proliferation of eosinophils and mast cells, mainly in patients with systemic mastocytosis.

When mast cells are increased, there may be an associated increase in plasma cells and lymphocytes.

Histiocytosis

An increase in macrophages (histiocytes) is common in a variety of infective and inflammatory conditions and whenever there is bone marrow hyperplasia, ineffective haemopoiesis or increased breakdown of blood cells. All of the conditions capable of causing granuloma formation and haemophagocytic syndromes can also cause an increase of macrophages without granuloma formation or prominent haemophagocytosis. Macrophages present may range from relatively immature cells with limited phagocytic capacity to mature cells which may be foamy or contain cellular debris, haemosiderin or a few haemopoietic cells, erythrocytes, neutrophils or platelets.

Increased bone marrow macrophages may be seen after granulocyte–macrophage colony-stimulating factor (GM-CSF) therapy when they may constitute as many as 90% of bone marrow cells [91]. They have been prominent in patients with failure to engraft after bone marrow transplantation particularly, but not only, when GM-CSF has been given [92]. A marked increase in macrophages has been reported at presentation of acute lympho-

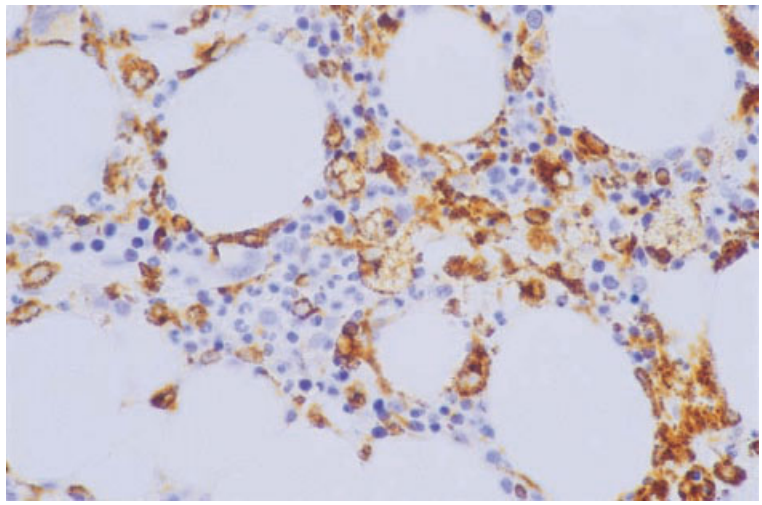


Fig. 3.35 BM trephine biopsy section showing a reactive increase in macrophages. Paraffin-embedded, immunoperoxidase, CD68 (McAb PG-M1) ×276.

blastic leukaemia in a patient without any apparent infection [93].

Macrophages can be identified by immunohistochemical staining (Fig. 3.35).

Haemophagocytic syndromes

Haemophagocytic syndromes result from increased numbers of haemophagocytic macrophages with resultant cytopenias. Common clinical features are hepatomegaly, splenomegaly and fever. The proliferating macrophages are reactive in most cases but rarely they are part of a neoplastic clone (malignant histiocytosis) (see page 184) (Table 3.4). Reactive haemophagocytic syndromes are commonly caused by bacterial or viral infection, occurring either in previously healthy subjects or as a terminal complication in patients with a defective immune response. Haemophagocytic syndromes are relatively common when viral or mycobacterial infections occur in patients with AIDS or with haemopoietic or other malignancy. Haemophagocytosis may also be prominent in patients with lymphoma, particularly T-cell lymphoma, when there is no evidence of infection; it is likely that in these cases there is increased proliferation of macrophage precursors and enhanced phagocytic activity in response to lymphokines secreted by the lymphoma cells. Familial haemophagocytic lymphohistiocytosis is an autosomal recessive condition of early childhood with helper T-cell and macrophage activation [128]; the condition is genetically heterogeneous. Since the cytological and histological features cannot be distinguished from those of infection-induced haemophagocytosis, the diagnosis must rest on clinical differences [127].

Malignant histiocytosis (see page 184) may be regarded as the tissue equivalent of acute monocytic leukaemia (AML of M5b type); with disease progression there is increasing bone marrow infiltration by malignant cells and increasing numbers of malignant cells appear in the peripheral blood. Phagocytosis is minor in comparison with that seen in reactive conditions.

It should be noted that the term 'erythrophagocytosis' refers to phagocytosis of mature erythrocytes, whereas haemophagocytosis refers to phagocytosis of nucleated cells. Erythrophagocytosis can occur in haemophagocytic syndromes but can also be consequent on the presence of antibody-coated or abnormal erythrocytes.

Peripheral blood

The haemophagocytic syndromes are characterized by pancytopenia. Phagocytic macrophages are rarely present in the peripheral blood although in malignant histiocytosis there may be small numbers of monoblasts. The blood film may also show the features of the primary condition, for example,

Table 3.4 Conditions associated with a haemophagocytic syndrome [45,94–127].

Malignant histiocytosis

Reactive haemophagocytic syndromes

Induced by viral infection
- Herpesviruses
  - EB virus (including fatal infectious mononucleosis in X-linked lymphoproliferative syndrome)
  - Herpes simplex
  - Herpes zoster
  - Human herpesvirus 6 [94]
  - Human herpesvirus 7 [95]
  - Cytomegalovirus
- Other viruses
  - Adenovirus
  - Measles (vaccine virus)
  - Influenza A [96]
  - Para-influenza
  - Vaccinia
  - Rubella (congenital)
  - Parvovirus B19 [97]
  - Respiratory syncytial virus [98]
  - Kyasanur forest disease
  - Dengue [99]
  - Hepatitis A virus [100]

Induced by bacterial infection
- *Salmonella typhi*
- Brucellosis
- Staphylococcal, streptococcal, *E. coli*, *H. influenzae*, *Acinetobacter* species, *Bacteroides fragilis*, *Pseudomonas* species and *Klebsiella* species infections [99,100–104]
- Legionnaire's disease (*Legionella pneumophila*)
- *Mycobacterium tuberculosis*
- Atypical mycobacterial infection
- Psittacosis [99]
- *Mycoplasma pneumoniae* infection [105]
- Human granulocytic and monocytic ehrlichiosis [106,107]

Induced by rickettsiae
- Rocky Mountain spotted fever
- Q fever [108]
- *Rickettsia tsutsugamushi* infection [99]

Induced by protozoan and other parasites
- Toxoplasmosis
- Leishmaniasis
- Malaria [109]
- Babesiosis [110]

Induced by fungi
- Histoplasmosis
- Candidiasis [103]
- Trichosporonosis [111]

Associated with certain lymphomas, particularly T-cell lymphomas [112–114], including T-cell granular lymphocytic lymphoma [115] but occasionally natural killer (NK) lymphoma [116], B-cell lymphoma [117] and Hodgkin's disease [118]

Kawasaki's disease

Kikuchi's disease [119]

Anticonvulsant lymphadenopathy

Sarcoidosis [114]

Systemic lupus erythematosus [99] and other autoimmune disease [120]

Graft-versus-host disease (GVHD) [121]

Following GM-CSF [122] or IL-3 [123] administration

Cytophagic histiocytic panniculitis [124]

Lysinuric protein intolerance [125]

Griscelli syndrome [126]

As a terminal complication in patients with various immunodeficiencies and malignant conditions (ALL, CLL, HD, NHL, hairy cell leukaemia, AML, carcinoma, Chediak–Higashi syndrome)—probably as a complication of infection

Familial haemophagocytic lymphohistiocytosis [127]

atypical lymphocytes in patients with familial lymphohistiocytosis (Fig. 3.36) or EBV or other viral infection.

Bone marrow cytology

In the secondary haemophagocytic syndromes there are increased numbers of macrophages and haemophagocytosis is usually prominent, with many macrophages having ingested numerous cells of various haemopoietic lineages (Figs 3.37 and 3.38). The macrophages are mainly mature and lack atypical features. The bone marrow aspirate may also show other abnormalities due to the primary disease. For example, in viral infections there is usually an increase of lymphocytes, which may be immature or atypical, and in bacterial infection there is granulocytic hyperplasia with toxic changes in the neutrophil lineage. In familial haemophagocytic lymphohistiocytosis, the bone marrow findings (Fig. 3.39) are identical to those of infection-induced haemophagocytosis. Repeated bone marrow exam-

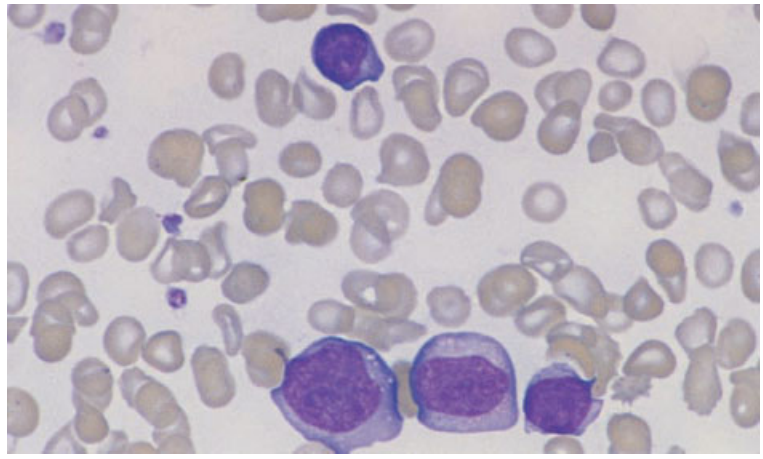


Fig. 3.36 PB film from a child with familial lymphohistiocytosis showing atypical lymphoid cells. MGG ×940.

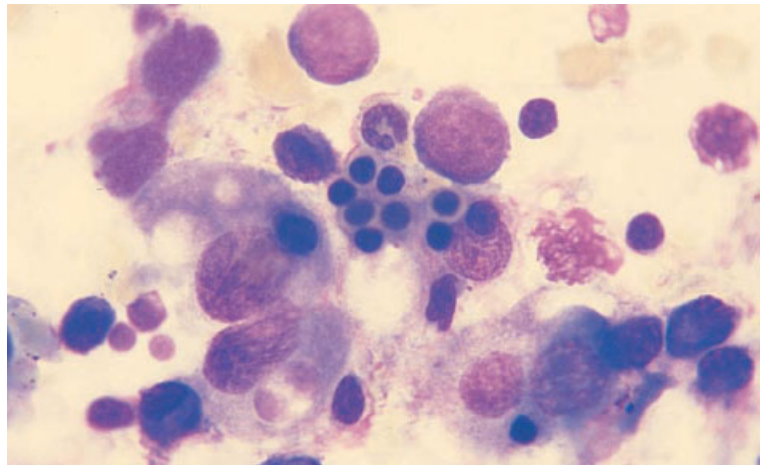


Fig. 3.37 BM aspirate, reactive haemophagocytosis in a patient with AIDS and miliary tuberculosis. MGG ×940.

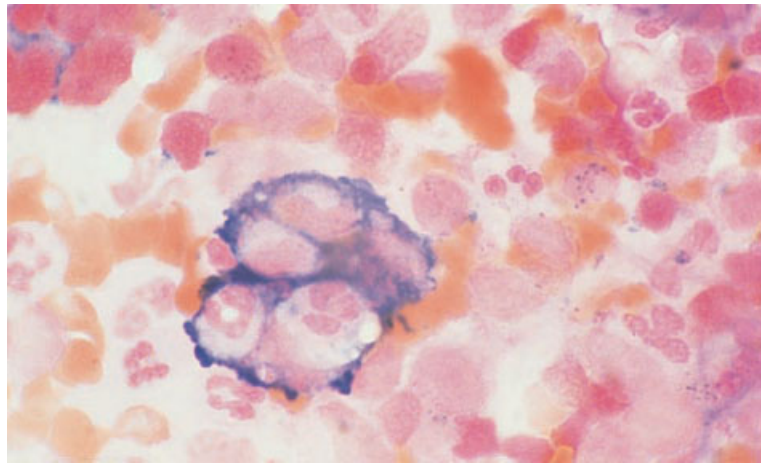


Fig. 3.38 BM aspirate, reactive haemophagocytosis, showing an iron-laden macrophage that has ingested neutrophils and neutrophil precursors. Perls' stain ×940.

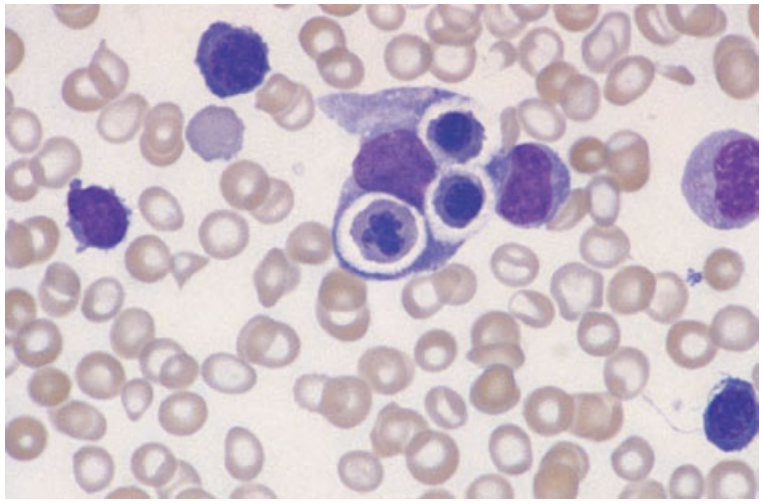


Fig. 3.39 BM aspirate, familial lymphohistiocytosis, showing haemophagocytosis and atypical lymphoid cells. MGG ×940.

ination may be needed to establish the diagnosis, since abnormalities are detected at presentation in less than a third of patients [127]. When haemophagocytosis is secondary to a T-cell lymphoma the marrow may show closely intermingled lymphoma cells and macrophages but cases have also been reported in which the bone marrow shows only haemophagocytosis with the lymphomatous infiltrate being confined to other tissues [113].

In malignant histiocytosis, the bone marrow infiltrate may initially be very scanty. The abnormal population includes a much larger proportion of monoblasts and promonocytes while mature histiocytes are relatively less common. Phagocytic activity is not prominent.

Bone marrow histology

In early cases of reactive haemophagocytic syndrome (e.g. virus-induced) there is a hypercellular bone marrow with few macrophages [103]. Later there are more macrophages with hypoplasia of erythroid and myeloid lines; megakaryocyte numbers are either normal or increased. The degree of macrophage infiltration is variable (Fig. 3.40); in some cases it is inconspicuous, while in others there is diffuse replacement of the marrow by mature macrophages with a low nucleocytoplasmic ratio, dispersed chromatin, inconspicuous nucleoli and abundant cytoplasm which is often vacuolated. Haemophagocytosis is often less apparent than in marrow films [103,129]; however, in some cases it is striking (Fig. 3.41). Features of the underlying disease may be present. Atypical lymphoid cells are seen in some cases of EBV infection. Lymphoma cells may be detected (see Fig. 6.61) but it should be noted that there may be no detectable lymphoma cells in the bone marrow in cases of lymphoma-associated haemophagocytic syndrome. Granulomas may be present in tuberculosis [130] and in a variety of other infections associated with a haemophagocytic syndrome (see Table 3.4).

If haemophagocytic lymphohistiocytosis is suspected, a trephine biopsy is recommended because clusters or sheets of macrophages may be detected in cases in which the bone marrow aspirate shows no significant abnormality [131].

In malignant histiocytosis, the marrow biopsy is often normal in the early stages of the disease. Later, infiltration may occur; infiltration is initially focal then diffuse.

Problems and pitfalls

The major problems in assessing haemophagocytic syndromes are: (i) distinguishing malignant histiocytosis from reactive conditions; and (ii), in reactive haemophagocytosis, determining whether the

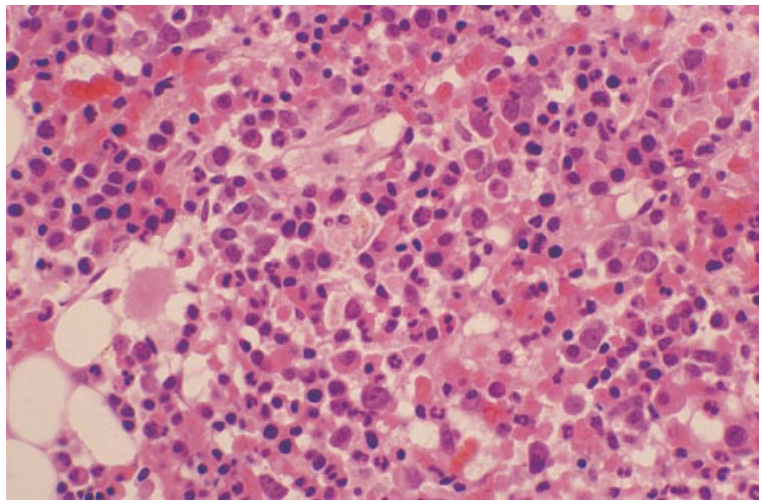


Fig. 3.40 BM trephine biopsy section, AIDS, showing increased cellularity, dyserythropoiesis and numerous macrophages, some of which contain erythrocytes and apoptotic normoblasts (haemophagocytosis). Paraffin-embedded, H&E ×390.

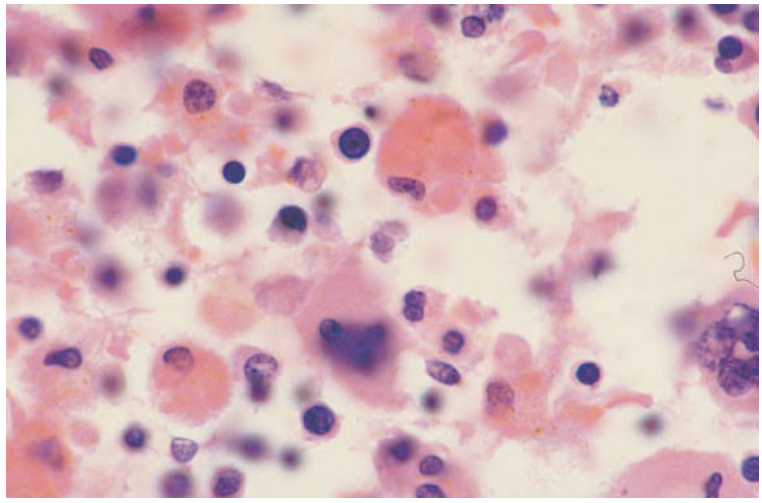


Fig. 3.41 BM section from an autopsy on a child with terminal haemophagocytic syndrome as a complication of Chediak–Higashi syndrome showing macrophages stuffed with red cells. Paraffin-embedded, H&E ×916. (By courtesy of Dr Wendy Erber and Dr L Matz, Perth, Western Australia.)

underlying condition is an infection or, less often, a lymphoid neoplasm. Malignant histiocytosis is rare whereas reactive haemophagocytosis is relatively common. Demonstration of rearrangement of immunoglobulin heavy chain (*IGH*) or T-cell receptor (*TCR*) genes provides presumptive evidence of an underlying neoplasm but does not give definite evidence of the lineage involved. Molecular evidence of clonality is lacking when the associated neoplasm is of NK lineage. In malignant histiocytosis, cytogenetic analysis occasionally shows an abnormality characteristic of neoplasms of monocyte lineage, thus confirming the diagnosis. In general, marked haemophagocytosis suggests a reactive rather than a neoplastic condition. Vigorous efforts should be made to identify an infective cause. *In situ* hybridization techniques (see Table 3.1) are useful for demonstrating underlying CMV or EBV infection [132], and stains and cultures for mycobacteria may be positive. Most childhood cases of haemophagocytic lymphohistiocytosis, both sporadic and familial, are infection-related. However, some

sporadic cases in children have atypical large granular lymphocytes; since T cells in such cases are sometimes shown to be clonal, it is possible that there is an underlying T-cell neoplasm [133].

HIV infection and the acquired immune deficiency syndrome

In HIV infection, the main indications for examination of the bone marrow are pyrexia of unknown origin and various cytopenias. It may also be required as a staging procedure in patients with lymphoma. A biopsy is very often useful, particularly in patients with a hypocellular aspirate. Part of any aspirate from a febrile patient should be submitted for microbiological culture. In addition to routine stains, biopsied tissues should be stained, when appropriate, for acid-fast bacilli and fungi.

Peripheral blood

The earliest haematological manifestations of HIV infection occur at the time of primary infection when atypical lymphocytes appear in the peripheral blood, often in association with a febrile illness which clinically can resemble infectious mononucleosis. Primary HIV infection also occasionally causes transient pancytopenia. Patients with established infection may have lymphocytosis, consequent on an increase in CD8-positive lymphocytes, or isolated thrombocytopenia resulting from peripheral destruction of platelets. Thrombocytopenia may also be a feature of a syndrome resembling thrombotic thrombocytopenic purpura and, in such cases, red cell fragments are present [134]. Late in the course of the disease, there is usually pancytopenia with very marked lymphopenia. Red cells may show anisocytosis and poikilocytosis. The reticulocyte count is reduced. Neutrophils may be dysplastic. Because of the frequency of opportunistic infections, the peripheral blood may also show non-specific reactive changes such as increased rouleaux formation, left shift and toxic changes in neutrophils, and the presence of immature monocytes and reactive lymphocytes. The haematological effects of the HIV infection itself, and the associated opportunistic infections, may be compounded by the effects of therapy; patients taking zidovudine, particularly in the higher doses used in single-agent anti-retroviral therapy, usually have marked macrocytosis and there may be marked dysplastic changes in blood cells.

Bone marrow cytology

Early in the course of HIV infection the bone marrow is of normal cellularity or, during the course of intercurrent infection, shows granulocytic hyperplasia. When there is immune thrombocytopenia, megakaryocyte numbers are normal or increased. Common non-specific changes are infiltration by lymphocytes and plasma cells and an increase of macrophages or eosinophils. Erythropoiesis may show mild dysplastic features such as nuclear irregularity and fragmentation. In patients taking zidovudine, erythropoiesis is megaloblastic and dyserythropoiesis is more marked; dysplastic changes such as nuclear fragmentation are also noted in the granulocytic series. Giant metamyelocytes are quite common, their presence correlating with the occurrence of detached nuclear fragments in granulocytes [135]. Apoptosis is increased. With disease progression, the bone marrow becomes progressively more hypocellular and aspiration becomes difficult due to increased reticulin deposition. Gelatinous transformation is very common, being greatest in cachectic patients. Bone marrow necrosis is sometimes seen. In patients with advanced disease, the bone marrow aspirate may provide evidence of miliary tuberculosis, atypical mycobacterial infection or disseminated fungal or parasitic infection. Because of the deficient host response, mycobacterial infection may be associated with the presence of numerous bacteria within macrophages, which may be foamy or may morphologically resemble Gaucher's cells. Haemophagocytic syndromes (see page 119) secondary to tuberculosis or other infections are relatively common in patients with AIDS (see Fig. 3.37). Bone marrow involvement is common when lymphoma, particularly Burkitt's lymphoma or Hodgkin's disease, complicates AIDS. The former is often detected in a bone marrow aspirate but in Hodgkin's disease the aspirate is often negative, even when the bone marrow is infiltrated.

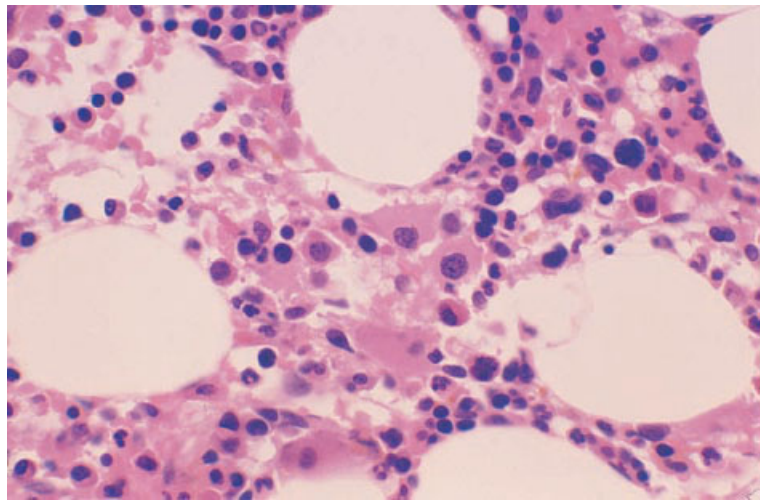


Fig. 3.42 BM trephine biopsy section, AIDS, showing clustering of megakaryocytes and a bare megakaryocyte nucleus. Paraffin-embedded, H&E ×390.

Bone marrow histology

A wide variety of non-specific reactive changes are commonly seen in HIV infection [136–138]. The cellularity is increased in approximately 40% of cases, and decreased in 20–40%; hypocellularity is more common in patients on zidovudine therapy. Frequently the haemopoietic marrow has an oedematous appearance with the cells being separated by clear spaces. Focal gelatinous transformation (see page 128) is seen in up to 20% of cases. Dyserythropoiesis is common; the erythroblastic islands are often large and poorly organized, and megaloblastic change may be present, particularly in patients taking zidovudine. Granulocytic hyperplasia with left shift is seen in some patients, usually in response to infection. Megakaryocytes are usually present in normal or increased numbers, bare nuclei are a frequent finding and, occasionally, dysplastic forms are seen. Clustering of megakaryocytes may occur (Fig. 3.42). Haemopoietic cells may be present within vessels [139]. Plasma cells are increased in 50–60% of biopsy specimens and lymphoid aggregates are seen in approximately a third. The lymphoid aggregates are often large and poorly circumscribed. They consist largely of small lymphocytes, with variable numbers of plasma cells, macrophages and eosinophils (Figs 3.43 and 3.44); sometimes there is proliferation of small vessels within the lesion. In some cases with a mixed infiltrate of lymphocytes and inflammatory cells the lesions may resemble a peripheral T-cell lymphoma [18]. Increased reticulin is seen in the majority of cases (Fig. 3.45). As a consequence there may be 'streaming' of haemopoietic cells and open sinusoids (in paraffin-embedded specimens), both features being apparent in H&E-stained sections. Rarely, the combination of marked marrow hypercellularity, severe reticulin fibrosis and increased numbers and clustering of megakaryocytes may closely resemble the appearance of a myeloproliferative disorder.

In all cases, a careful search should be made for evidence of opportunistic infections [140], in particular tuberculosis and atypical mycobacterial infection (see page 109), fungal infection (see Table 3.2), *P. carinii* infection and protozoal diseases (leishmaniasis and toxoplasmosis) (see page 105). Approximately 15% of biopsy specimens contain granulomas: the commonest cause is atypical mycobacterial infection; other causes include tuberculosis, histoplasmosis, cryptococcal infection and leishmaniasis (see page 105). In patients with AIDS, granuloma formation, in response to fungal and protozoal infections, is often poor. Associated changes in toxoplasmosis include interstitial oedema and focal

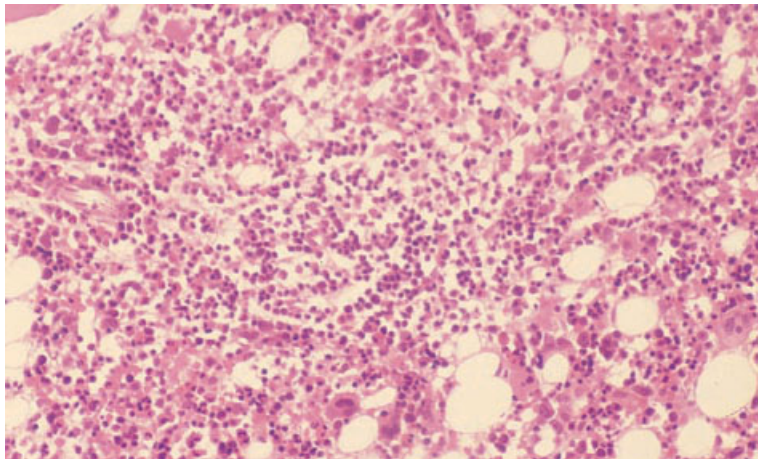


Fig. 3.43 BM trephine biopsy section, AIDS, showing increased cellularity and a polymorphous lymphoid aggregate (centre). Paraffin-embedded, H&E ×195.

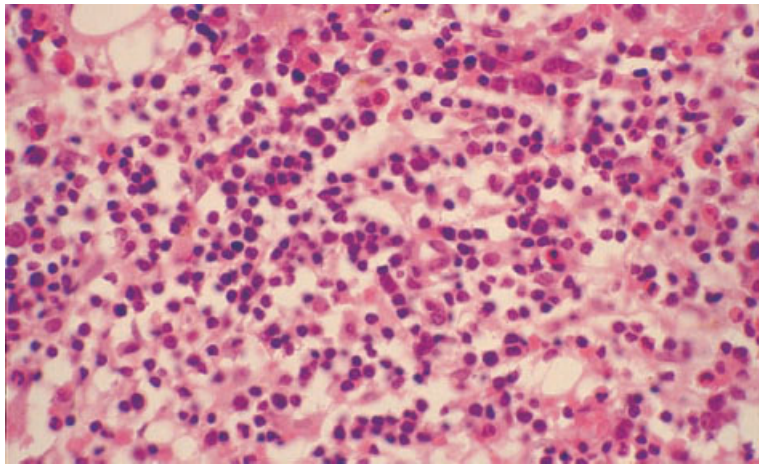


Fig. 3.44 BM trephine biopsy section, AIDS, showing a polymorphous lymphoid aggregate, composed of lymphocytes and small numbers of macrophages, eosinophils and plasma cells. Paraffin-embedded, H&E ×390.

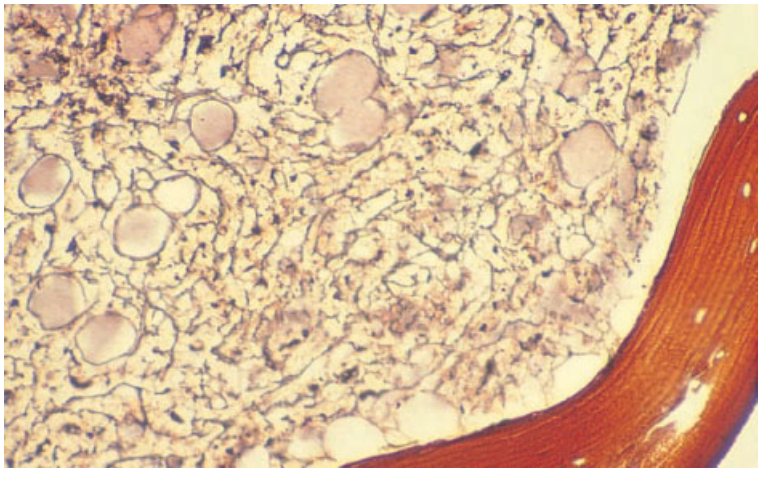


Fig. 3.45 BM trephine biopsy section, AIDS, showing reticulin fibrosis (grade 3). Paraffin-embedded, Gordon and Sweet stain ×195.

necrosis with both free forms and pseudocysts being found within granulocytes, macrophages and megakaryocytes [31]. Organisms can be identified using a polyclonal antibody [31]. Atypical mycobacteria can be cultured from the marrow in up to 20% of cases of AIDS. Granulomas are present in approximately 50% of culture-positive cases and acid-fast bacilli can be seen with special stains in 60%. Atypical mycobacteria may be seen in biopsies in the absence of granulomas, usually within macrophages scattered throughout the marrow; occasionally cells which morphologically resemble Gaucher's cells (see page 109) are seen. Culture of the bone marrow and examination of sections for acid-fast bacilli has been found to be less sensitive than peripheral blood culture in the diagnosis of atypical mycobacterial infection in AIDS but, when bacilli can be found in a stained film, diagnosis is much more rapid than when it is dependent on culture [141]. Focal areas of epithelioid angiomatosis have been observed in the marrow of HIV-positive patients with bacillary angiomatosis or disseminated cat scratch disease, infective conditions consequent on infection by organisms of the *Rochalimaea* genus or the related *Afipia felis* [142,143]; a Warthin–Starry stain may demonstrate the causative organisms [143].

Bone marrow involvement by multicentric Castleman's disease has been reported in several HIV-positive patients who were also positive for HHV8 [144].

The incidence of non-Hodgkin's lymphoma is greatly increased in HIV infection [145]. Marrow involvement is common in non-Hodgkin's lymphoma associated with AIDS. These are almost always high grade lymphomas, either Burkitt's or Burkitt-like lymphoma, or diffuse large B-cell lymphoma (immunoblastic or anaplastic large cell subtypes). Bone marrow infiltration is much more common in Burkitt's lymphoma than in diffuse large B-cell lymphomas. Infiltration is usually diffuse (see Fig. 6.54).

Hodgkin's disease is now also recognized as an AIDS-related lymphoma. There is a propensity to involve the bone marrow early in the course of the illness so that a trephine biopsy may provide the first evidence of the disease [146].

Plasma cell tumours also occur as AIDS-related neoplasms. Although initial presentation is often at extramedullary sites, the bone marrow may also be infiltrated [147].

Kaposi's sarcoma has a greatly increased incidence in AIDS and, when spread to the bone marrow occurs, may be detected by trephine biopsy.

Bone marrow necrosis

The term 'bone marrow necrosis' is conventionally used to describe necrosis of haemopoietic cells or necrosis of neoplastic cells that have replaced normal bone marrow cells. Bone marrow stromal cells may also be necrotic. There may or may not be associated death of bone cells and of bone. Bone marrow necrosis results from impairment of the blood supply, often in association with a hypercellular marrow. A high concentration of tumour necrosis factor in the blood may act as a mediator of necrosis [148]. Anti-phospholipid antibodies may also cause, or contribute to, bone marrow necrosis [149,150]. Causes are multiple (Table 3.5). Necrosis is followed either by haemopoietic regeneration, with or without small fibrotic scars, or occasionally by extensive fibrosis. Bone marrow necrosis commonly occurs at multiple sites. Clinical features include bone pain and fever. Fatal fat embolism can occur [159].

Peripheral blood

If bone marrow necrosis is extensive, pancytopenia occurs. The blood film shows leuco-erythroblastic, and sometimes micro-angiopathic [160], features. Recovery is associated with a rise in the reticulocyte count and recovery of the haemoglobin concentration and platelet and white cell counts.

Bone marrow cytology

A bone marrow aspirate is often macroscopically abnormal. It is sometimes opaque and whitish and sometimes reddish-purple. The stained film shows amorphous pink-staining material in which the faint outlines of necrotic cells can be seen (Fig. 3.46). Necrotic nuclei appear as darker-staining smudges. Some intact cells may also be present.

Bone marrow necrosis is more often noted in bone marrow trephine sections than in aspirates. This may be partly because a larger volume is sampled and partly because necrotic cells mixed with

Table 3.5 Causes of bone marrow necrosis [45,151–156].

Relatively common associations
Sickle cell anaemia and haemoglobin S/C disease (particularly during pregnancy)
Acute myeloid leukaemia (sometimes following administration of G-CSF [157])
Acute lymphoblastic leukaemia
Metastatic carcinoma
Caisson disease

Less common associations
Chronic granulocytic leukaemia
Essential thrombocythaemia [151]
Lymphoma (mainly high grade non-Hodgkin's lymphoma but also Hodgkin's disease)*
Chronic lymphocytic leukaemia
Multiple myeloma
Malignant histiocytosis
Myelofibrosis
Other haemoglobinopathies (S/D, S/β thalassaemia and sickle cell trait)
Megaloblastic anaemia plus infection
Acute haemolytic anaemia
Embolism of the bone marrow, e.g. by vegetations from cardiac valves or tumour embolism [152]
Disseminated intravascular coagulation
Hyperparathyroidism
Systemic lupus erythematosus [153]
Primary anti-phospholipid syndrome [149]
Infections
- Typhoid fever
- Gram-positive infections, e.g. streptococcal and staphylococcal infection
- Gram-negative infections, e.g. *E. coli*
- Diphtheria
- Miliary tuberculosis
- Cytomegalovirus infection [154]
- Q fever
- Mucormycosis
- Histoplasmosis
- *Fusobacterium necrophorum* infection [155]
- Parvovirus infection [156]
- HIV infection (AIDS)

* Necrosis of low grade lymphoma has been observed following fludarabine therapy [158].

intact cells in an aspirate are often dismissed as an artefact.

Bone marrow histology

The appearances depend to some extent on the condition underlying the necrosis. In cases in which the necrosis is secondary to infiltration by leukaemia or lymphoma (Fig. 3.47), low power examination may reveal hypercellularity and loss of fat cells. In the early stages there is nuclear pyknosis and the cells have granular cytoplasm with indistinct margins. Later, there is nuclear karyorrhexis and complete loss of cell outlines. Finally, all that remains is amorphous eosinophilic debris. Marrow necrosis is often accompanied by necrosis of the adjacent bone. In some patients, the necrosis is very extensive and biopsy of several different sites may be necessary before a diagnostic sample is obtained. Necrotic marrow is eventually repopulated with haemopoietic cells. The only signs of previous necrosis may be small areas of fibrous scarring around bony trabeculae that have lost osteocytes [161]. Occasionally there is extensive fibrosis (Fig. 3.48). Necrosis of haemopoietic and stromal cells may be followed by metaplastic new bone formation

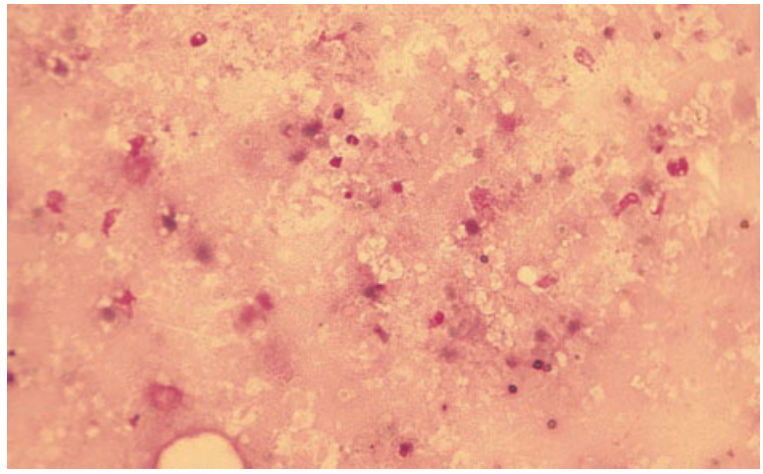


Fig. 3.46 BM aspirate, bone marrow necrosis, showing amorphous debris containing karyorrhectic nuclei. MGG ×940.

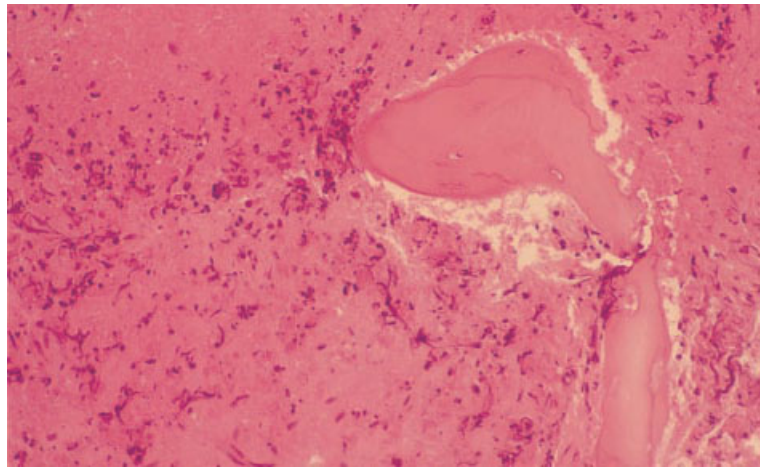


Fig. 3.47 BM trephine biopsy section, necrosis in a marrow infiltrated by high grade lymphoma. Note the necrosis of both the lymphoma cells infiltrating the marrow and of osteocytes within the bone trabecula. Paraffin-embedded, H&E ×195.

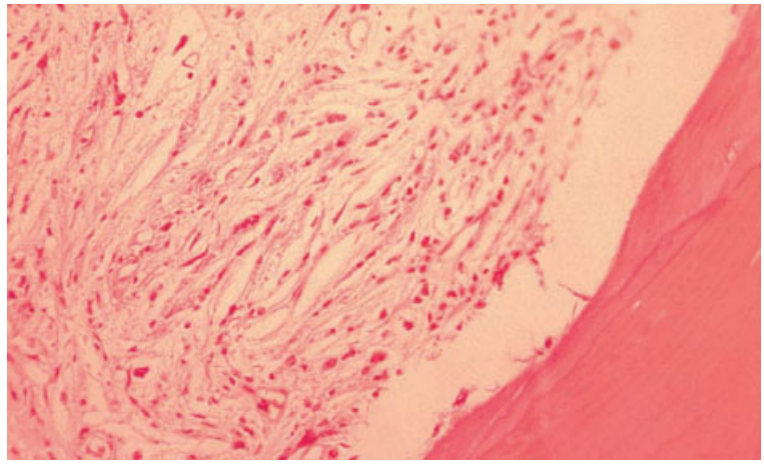


Fig. 3.48 BM trephine biopsy section, repair following marrow necrosis, showing collagen fibrosis. Paraffin-embedded, H&E ×188.

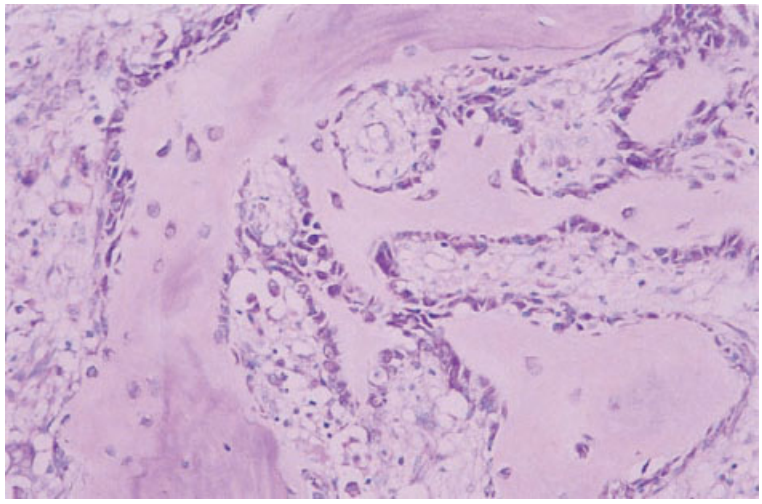


Fig. 3.49 BM trephine biopsy section showing new bone formation in an area of previous bone marrow necrosis. Paraffin-embedded, Giemsa ×188.

(Fig. 3.49), initially woven bone which is subsequently remodelled into structurally normal bone.

Problems and pitfalls

Immunohistochemistry may be very misleading if used in an attempt to identify neoplastic cells within necrotic areas, as a consequence both of the tendency of antibodies to adhere in a non-specific way to necrotic tissue and of loss of some antigens by necrotic cells.

Gelatinous transformation

Gelatinous transformation, also known as serous degeneration or serous atrophy, is a condition in which there is loss of fat cells and haemopoietic cells from the bone marrow with replacement by an increased amount of ground substance. Common causes include anorexia nervosa and cachexia due to chronic debilitating illnesses such as carcinoma and tuberculosis or other chronic infection. The condition is also observed in AIDS, which is now the commonest cause of this abnormality. It has also been reported in association with renal failure, coeliac disease [162] and severe hypothyroidism [163]. It occurs at sites exposed to high-dose X-irradiation. Gelatinous transformation can also develop rapidly; it is seen in acute illnesses with multiple organ failure [10] and in severe acute infections.

Peripheral blood

The peripheral blood shows variable cytopenia, often pancytopenia. In patients with anorexia nervosa, acanthocytes are seen but their presence has not been noted in other patients with gelatinous transformation.

Bone marrow cytology

The aspirate may not spread normally when a film is prepared. It contains amorphous material, sometimes fibrillar or finely granular, which is composed of acid mucopolysaccharide with a high content of hyaluronic acid. With Romanowsky stains it is pink or pinkish-purple (Fig. 3.50). This abnormal ground substance is positive with a PAS stain, the reaction being diastase resistant. Positive staining also occurs with alcian blue; this reaction is stronger at pH 2.5 than pH 1.0 [10]. A toluidine blue stain is weakly positive.

Bone marrow histology

The changes are usually focal; less commonly the whole of a biopsy section is affected. There is atrophy of fat cells, which are both reduced in number

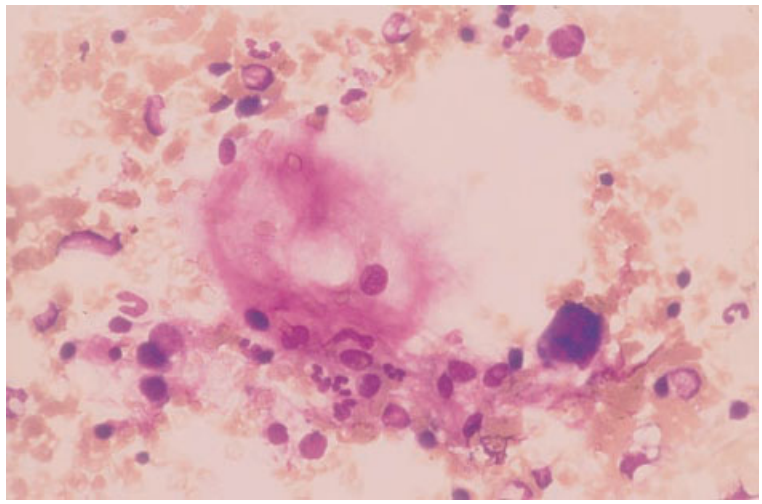


Fig. 3.50 BM aspirate, gelatinous transformation, showing amorphous purplish-pink material. MGG ×377.

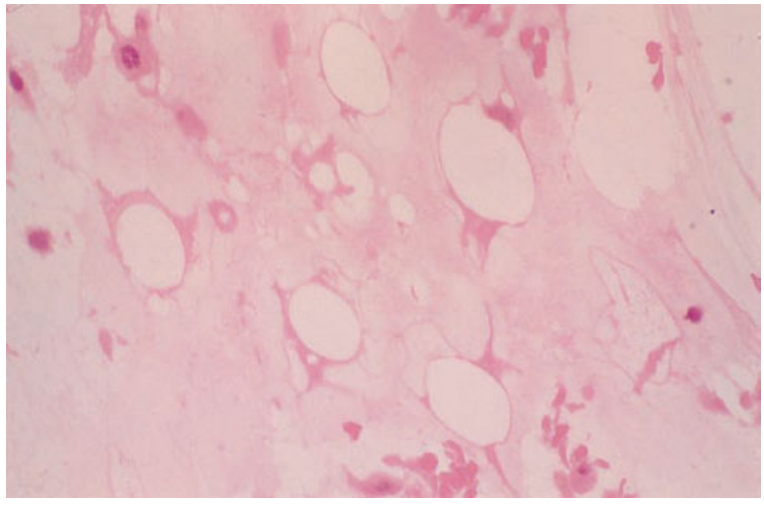


Fig. 3.51 BM trephine biopsy, gelatinous transformation, showing replacement of haemopoietic marrow and fat cells by amorphous pink material. H&E ×390.

and are of variable size. In the affected areas there is a mild to marked hypoplasia of haemopoietic cells. Both fat and haemopoietic cells are replaced by amorphous material which, with H&E staining, has a light blue to pale pink colour and a finely granular appearance (Fig. 3.51). Its other staining characteristics are identical to those seen in marrow films (see above).

Bone marrow fibrosis

Bone marrow fibrosis indicates an increase of reticulin or of reticulin and collagen in the bone marrow. Such fibrosis may be focal or generalized. Reticulin and collagen deposition are graded 0–4 as shown in Table 2.2. Some patients with grade 4 fibrosis also have osteosclerosis. Increased reticulin formation and even collagen deposition can revert to normal if the causative condition is amenable to treatment.

An increase in bone marrow reticulin is common and is a useful non-specific indication that the marrow is abnormal. It is also of some use in differential diagnosis; for example, it may be increased in

Table 3.6 Causes of grade 4 bone marrow fibrosis [45,164].

Generalized myelofibrosis

Malignant disease
Primary (idiopathic) myelofibrosis* (myelofibrosis with myeloid metaplasia)
Myelofibrosis secondary to essential thrombocythaemia or polycythaemia rubra vera*
Chronic granulocytic leukaemia*
Transitional myeloproliferative syndrome
Acute megakaryoblastic leukaemia*
Other acute myeloid leukaemias
Acute lymphoblastic leukaemia [165]
Systemic mastocytosis*
The myelodysplastic syndromes (particularly secondary MDS)
Paroxysmal nocturnal haemoglobinuria
Hodgkin's disease
Non-Hodgkin's lymphoma
Multiple myeloma
Waldenström's macroglobulinaemia
Secondary carcinoma*

Bone and connective tissue diseases
Marble bone disease—osteopetrosis
Primary and secondary hyperparathyroidism
Nutritional and renal rickets (vitamin D deficiency)
Osteomalacia
Primary hypertrophic osteoarthropathy [166]

Miscellaneous
Tuberculosis
Other granulomatous diseases
Grey platelet syndrome
Systemic lupus erythematosus
Systemic sclerosis
Prior thorium dioxide administration

Focal or localized

Osteomyelitis
Paget's disease
Following bone marrow necrosis
Following irradiation of the bone marrow
Adult T-cell leukaemia/lymphoma
Healing fracture
Site of previous trephine biopsy

* Osteosclerosis may also occur.

hypoplastic AML and hypoplastic myelodysplastic syndromes but is not increased in aplastic anaemia. Conditions characteristically associated with increased reticulin deposition, but with little if any collagen formation, include hairy cell leukaemia and HIV infection. Reticulin deposition may also be a feature of myeloproliferative disorders, such as polycythaemia rubra vera and essential thrombocythaemia, and occurs in some patients with acute leukaemia (lymphoblastic as well as myeloid), myelodysplastic syndromes and multiple myeloma. The increase in reticulin in uncomplicated polycythaemia rubra vera and essential thrombocythaemia is not marked.

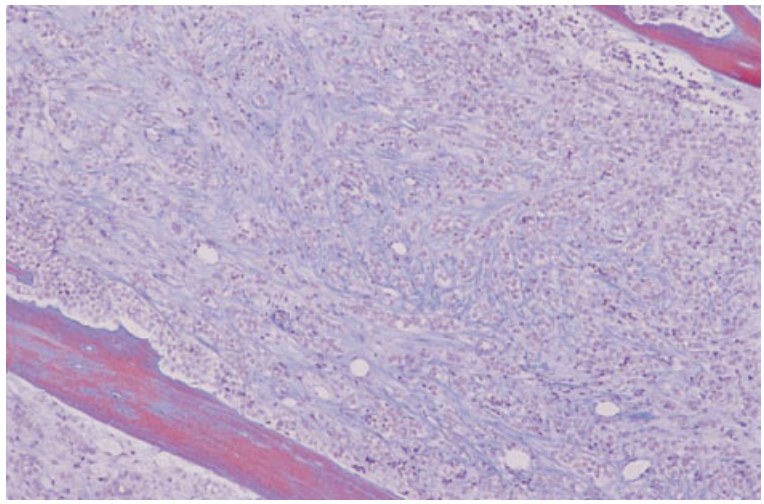


Fig. 3.52 BM trephine biopsy section showing marrow infiltrated by breast cancer with associated stromal fibrosis. Collagen is evident as blue strands when demonstrated by Martius Scarlet Blue trichrome stain. MSB ×94.

A focal increase in reticulin may have a different significance from a general increase, often indicating the presence of a focal infiltrate.

Collagen deposition is uncommon and of greater diagnostic significance than an increase in reticulin. It is therefore useful to distinguish between these two degrees of abnormality, either by grading the fibrosis or by using the terms 'reticulin fibrosis' for a grade 3 abnormality and 'fibrosis' or 'myelofibrosis' for a grade 4 abnormality. Causes of bone marrow fibrosis are shown in Table 3.6.

Peripheral blood

Bone marrow fibrosis is commonly associated with a leuco-erythroblastic anaemia; red cells show anisocytosis and poikilocytosis with teardrop poikilocytes often being prominent. When fibrosis is extensive, there may also be thrombocytopenia and leucopenia. The blood may also show abnormalities related to the primary disease that has caused the fibrosis. When bone marrow fibrosis has developed acutely, as in acute megakaryoblastic leukaemia, there may be little anisocytosis and poikilocytosis and the blood film is not necessarily leuco-erythroblastic.

Bone marrow cytology

Bone marrow fibrosis often leads to failure to aspirate marrow or to aspiration of peripheral blood or a diluted marrow specimen. Otherwise the aspirate may show carcinoma cells or the specific features of the primary disease that has led to the fibrosis. If there is associated osteosclerosis, the aspirate may contain increased numbers of osteoblasts and osteoclasts.

Bone marrow histology

The appearances of primary myelofibrosis (myelofibrosis with myeloid metaplasia) are described on page 204. In secondary fibrosis, findings range from a mild increase of fibroblasts and the presence of scattered collagen fibres to a dense fibrosis which obliterates normal haemopoietic tissue. An increase of reticulin can be suspected from an H&E stain by the distortion and angularity of fat cells and by the 'streaming' of haemopoietic cells held between parallel reticulin fibres. Fibrosis may also be suspected when sinusoids, which are normally collapsed in paraffin-embedded specimens, are held open. Increased reticulin deposition is confirmed by a silver impregnation technique such as the Gomori stain. Collagen fibres are detected as eosinophilic fibres which may be in bundles. The oval nuclei of fibroblasts are apparent in relation to the collagen fibres. It is important not to confuse either endothelial cells of collapsed capillaries or mast cells with fibroblasts. The presence of collagen is confirmed by a trichrome stain such as a Martius Scarlet Blue stain (Fig. 3.52). The distribution of reticulin

and collagen within the marrow depends on the causative condition. In renal osteodystrophy and primary hyperparathyroidism, fibrosis is usually paratrabecular. In Paget's disease, it is often preferentially paratrabecular. When associated with a myeloproliferative disorder or with the grey platelet syndrome, fibrosis may be spatially related to megakaryocytes.

Problems and pitfalls

When the bone marrow is very fibrotic, any bone marrow aspirate is likely to be very unrepresentative. No attempt should be made to assess cellularity. However, it is always worth looking for tumour cells or dysplastic haemopoietic cells, regardless of the quality of the aspirate. If no aspirate can be obtained, a trephine biopsy imprint can yield useful information as to the nature of any abnormal cells present.

Due weight must be given to the presence of focal reticulin deposition and the corresponding area in the H&E-stained sections should be carefully examined. A generalized increase in reticulin is an indication of an abnormal bone marrow and an explanation should be sought by reviewing of clinical, haematological and histological features.

When fibrosis is very pronounced, it may be difficult to distinguish residual haemopoietic cells, particularly megakaryocytes, from tumour cells. Immunohistochemistry is useful in this situation.

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