FIVE

ACUTE MYELOID LEUKAEMIA, THE
MYELODYSPLASTIC SYNDROMES
AND HISTIOCYTIC NEOPLASMS

Acute myeloid leukaemia (AML) is a disease resulting from the neoplastic proliferation of a clone of myeloid cells, characterized by uncoupling of proliferation and maturation. The leukaemic clone may be derived from a pluripotent stem cell (capable of giving rise to both myeloid and lymphoid lineages), from a multipotent stem cell (capable of giving rise to more than one myeloid lineage) or from a committed precursor cell (for example, one capable of giving rise only to cells of granulocyte and monocyte lineages). Normal haemopoietic marrow is largely replaced by immature myeloid cells, mainly blast cells, which show a limited ability to differentiate into mature cells of the different myeloid lineages. Pancytopenia is common, as a result both of the replacement of normal bone marrow and of the defective capacity for maturation of the leukaemic clone.

The myelodysplastic syndromes (MDS) resemble AML in that normal polyclonal haemopoietic bone marrow is largely replaced by a neoplastic clone, usually derived from a multipotent stem cell. The neoplastic clone is characterized by defective maturation so that haemopoiesis is usually both morphologically dysplastic and functionally ineffective. In the great majority of patients with MDS, the bone marrow is hypercellular but there is increased intramedullary death of haemopoietic precursors leading to defective production of mature cells of one or more haemopoietic lineages; this process, which leads to various combinations of anaemia, neutropenia and thrombocytopenia, is designated ineffective haemopoiesis. In MDS, as in AML, there is imbalance between proliferation and maturation but the degree of abnormality is less than in AML so that the proportion of blast cells is lower. The neoplastic cells in MDS show a tendency to clonal evolution; emergence of a subclone with more ‘malignant’ characteristics may be manifested clinically as transformation to acute leukaemia. MDS may therefore be regarded as a preleukaemic condition.

Acute myeloid leukaemia

AML is a heterogeneous disease. In different patients the leukaemic clone shows differing patterns of differentiation and maturation. From 1976 onwards, an international co-operative group, the French–American–British (FAB) group, published a series of papers on the classification of AML. The FAB classification [1,2] became widely accepted and was subsequently incorporated into other systems of classification. It is based on the pattern of differentiation shown (for example: granulocytic, monocytic, erythroid, megakaryocytic) and the extent of maturation (for example: myeloblast, promyelocyte, granulocyte). Both differentiation and maturation are assessed and the predominant cell types in peripheral blood and bone marrow are determined. The FAB classification is summarized in Table 4.1.

The FAB classification was incorporated into the morphologic, immunologic, cytogenic (MIC) classification [3], the morphological, immunological, cytogenetic, molecular genetic (MIC-M) classification [4] and, most recently, the WHO classification [5]. The WHO classification is summarized in Table 4.2. The major aim of this classification is to recognize subtypes of AML that differ in their prognosis. The classification is hierarchical, so that therapy-related cases are first assigned to two specific categories. Cases falling into one of four cytogenetic subtypes are then categorized. Next, cases with multilineage dysplasia are assigned to a specific category. Finally, all remaining cases are categorized morphologically by a modification of the FAB classification. An important difference from the FAB classification is...
<table>
<thead>
<tr>
<th>Criteria for diagnosis of AML</th>
<th>FAB category</th>
<th>Criteria for classification as specific FAB subtype of AML</th>
<th>Equivalent name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts ≥30% of bone marrow NEC (non-erythroid cells)</td>
<td>M1</td>
<td>• Blasts ≥90% of bone marrow NEC • ≥3% of blasts MPO- or SBB-positive • Maturing monocytic component in bone marrow ≤10% • Maturing granulocytic component in bone marrow ≤10%</td>
<td>Acute myeloblastic leukaemia without maturation</td>
</tr>
<tr>
<td>BM maturing granulocytic component &gt;10% NEC</td>
<td>M2</td>
<td>• Blasts 30–89% of BM NEC • BM maturing granulocytic component &gt;10% NEC • BM monocytic component &lt;20% of NEC and other criteria of M4 not met</td>
<td>Acute myeloblastic leukaemia with maturation</td>
</tr>
<tr>
<td>Blast morphology</td>
<td>M3</td>
<td>• Characteristic morphology</td>
<td>Acute promyelocytic leukaemia</td>
</tr>
<tr>
<td>≥3% of blasts SBB- or MPO-positive†</td>
<td>M3v</td>
<td>• Characteristic morphology</td>
<td>Variant form of acute promyelocytic leukaemia</td>
</tr>
<tr>
<td>Blasts ≥30% of BM NEC • Granulocytic component ≥20% of BM NEC • Monocytic component ≥20% of BM NEC and either PB monocytes ≥5 × 10⁹/l or BM like M2 but PB monocytes ≥5 × 10⁹/l and cytochemical proof of monocytic differentiation</td>
<td>M4</td>
<td>Acute myelomonocytic leukaemia</td>
<td></td>
</tr>
<tr>
<td>Blasts ≥30% of NEC • BM monocytic component ≥80% of NEC • Monoblasts ≥80% of BM monocytic component</td>
<td>M5a</td>
<td>Acute monoblastic leukaemia</td>
<td></td>
</tr>
<tr>
<td>Blasts ≥30% of NEC • BM monocytic component ≥80% of NEC • Monoblasts ≥80% of BM monocytic component</td>
<td>M5b</td>
<td>Acute monoblastic leukaemia</td>
<td></td>
</tr>
<tr>
<td>Erythroid cells ≥50% of BM cells • BM blasts ≥30% of NEC</td>
<td>M6</td>
<td>Acute erythroleukaemia</td>
<td></td>
</tr>
<tr>
<td>Blasts shown to be predominantly megakaryoblasts</td>
<td>M7</td>
<td>Acute megakaryoblastic leukaemia</td>
<td></td>
</tr>
<tr>
<td>&lt;3% of blasts MPO- or SBB-positive • Lymphoid markers negative • Immunological or ultrastructural evidence of myeloid differentiation</td>
<td>M0</td>
<td>Acute myeloid leukaemia with minimal evidence of myeloid differentiation</td>
<td></td>
</tr>
</tbody>
</table>

* Except in some M3 and some M6.
† Except in M0 and some M5a.

that cases with between 20 and 30% of bone marrow blasts are classified as AML rather than as refractory anaemia with excess of blasts in transformation, a FAB category of poor prognosis MDS. In addition, cases with an even lower blast percentage are accepted as AML if they have one of the specific cytogenetic abnormalities listed—t(8;21), t(15;17), inv(16), t(16;16) or an 11q23 rearrangement. Since only an outline of the WHO classification has so far been published, we shall discuss AML mainly in terms of the FAB classification but will note where there are important differences from the WHO
AML occurs at all ages but becomes increasingly common with advancing age. The incidence rises from one to 10/100 000/year between the ages of 20 and 70 and is somewhat higher in men than in women.

The different categories of AML have certain haematological features in common although the morphological features of the predominant leukaemic cells differ. Normocytic normochromic anaemia, neutropenia and thrombocytopenia are common. The total white cell count is usually elevated, as a result of the presence of circulating leukaemic cells, but some patients have a normal or low total count with few circulating immature cells. A normal or low count is most often observed in M3 and M7 AML. In adults, M7 AML commonly presents with the features of acute myelofibrosis, that is with pancytopenia, few circulating immature cells and a bone marrow which, as a consequence of bone marrow fibrosis, cannot be aspirated. (However, it should be noted that not all cases of acute myelofibrosis are examples of M7 AML.)

The blood film and bone marrow aspirate features are of prime importance in the diagnosis of AML. The bone marrow biopsy is of secondary importance except in those cases in which an adequate aspirate cannot be obtained. Assignment to a FAB category is more readily done on the basis of the blood and bone marrow aspirate findings and is not always straightforward from tissue sections. It may also be impossible, in tissue sections, to distinguish M1 and M0 AML from acute lymphoblastic leukaemia (ALL) unless immunohistochemistry is employed. Antibodies reactive with CD68, CD117, lysozyme, neutrophil elastase and myeloperoxidase (MPO) are useful in distinguishing AML from ALL in trephine biopsy sections [6–8]. The antigen (calprotectin) detected by the MAC387 antibody is also myeloid-associated, being expressed by cells of the monocyte lineage and by mature cells of the granulocyte lineage. To distinguish AML with erythroid or megakaryocytic differentiation from ALL, it is necessary to use antibodies reactive with antigens such as glycophorin and spectrin (erythroid) or CD42b, CD61 and von Willebrand’s factor (megakaryocytic). Some cases of AML, particularly those belonging to the FAB M0 category, give negative reactions with some of the antibodies commonly used to detect myeloid differentiation (see Table 2.6). This and other problems in diagnosis will be discussed under ‘Problems and pitfalls’ (see page 166).

### Table 4.2 The WHO classification of the acute myeloid leukaemias [5].

<table>
<thead>
<tr>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AML with recurrent cytogenetic translocations</strong></td>
</tr>
<tr>
<td>AML with t(8;21)(q22;q22), AML1(CBFα)/ETO</td>
</tr>
<tr>
<td>Acute promyelocytic leukaemia (AML with t(15;17)(q22;q11–12) and variants, PML/RARα)</td>
</tr>
<tr>
<td>AML with abnormal bone marrow eosinophils (inv(16)(p13q22) or t(16;16)(p13;q22), CBFβ/MYH11)</td>
</tr>
<tr>
<td>AML with 11q23 (M7) abnormalities</td>
</tr>
<tr>
<td><strong>AML with multilineage myelodysplasia</strong></td>
</tr>
<tr>
<td>With prior myelodysplastic syndrome</td>
</tr>
<tr>
<td>Without prior myelodysplastic syndrome</td>
</tr>
<tr>
<td><strong>AML and myelodysplastic syndromes, therapy-related</strong></td>
</tr>
<tr>
<td>Alkylating agent-related</td>
</tr>
<tr>
<td>Epipodophyllotoxin-related</td>
</tr>
<tr>
<td>Other types</td>
</tr>
<tr>
<td><strong>AML not otherwise categorized</strong></td>
</tr>
<tr>
<td>AML minimally differentiated</td>
</tr>
<tr>
<td>AML without maturation</td>
</tr>
<tr>
<td>AML with maturation</td>
</tr>
<tr>
<td>Acute myelomonocytic leukaemia</td>
</tr>
<tr>
<td>Acute monocytic leukaemia</td>
</tr>
<tr>
<td>Acute erythroid leukaemia</td>
</tr>
<tr>
<td>Acute megakaryocytic leukaemia</td>
</tr>
<tr>
<td>Acute basophilic leukaemia</td>
</tr>
<tr>
<td>Acute panmyelosis with myelofibrosis</td>
</tr>
</tbody>
</table>

* Defined as dysplastic features in two or more cell lines.
Acute myeloblastic leukaemia (M1 and M2 AML)

The term acute myeloblastic leukaemia indicates leukaemia in which lineage commitment (differentiation) is to one of the granulocyte lineages, usually the neutrophil lineage. Cases can be divided between the FAB categories M1 and M2, depending on whether leukaemic cells are predominantly myeloblasts or whether, alternatively, further maturation to promyelocytes and later cells is occurring (see Table 4.1). Cases of AML assigned to FAB M1 and M2 categories would, in the WHO classification, fall into various categories including ‘t(8;21)-associated AML’, ‘AML with and without maturation’, ‘AML with multilineage dysplasia’ and ‘acute basophilic leukaemia’.

Peripheral blood

Anaemia, thrombocytopenia and a high white cell count are usual. Neutropenia is common in M1 AML but cases of M2 AML may have a normal or high neutrophil count. The majority of cases have considerable numbers of circulating myeloblasts. These are large cells, usually about twice the diameter of an erythrocyte. They have a high nucleocytoplasmic ratio. The nucleus has a diffuse chromatin pattern and one or more nucleoli. The cytoplasm is weakly or moderately basophilic and may contain scanty azurophilic granules. In some cases, particularly but not exclusively M2 AML, the blasts contain Auer rods; these are cytoplasmic crystals formed by fusion of primary granules with which they share staining characteristics. In M2 AML the peripheral blood may also contain morphologically abnormal promyelocytes and other maturing cells. Occasional patients with M2 AML have eosinophilia or basophilia. In AML associated with t(8;21)(q22;q22) the peripheral blood may show large blasts with basophilic cytoplasm and often a single long thin Auer rod, together with morphologically abnormal maturing cells. In AML associated with t(6;9)(p23;q34) there may be peripheral blood basophilia.

Bone marrow cytology

The bone marrow is markedly hypercellular. Numbers of megakaryocytes and developing erythroid cells are usually reduced. In M1 AML the bone marrow is almost totally replaced by myeloblasts, some of which may contain scanty granules or Auer rods (Fig. 4.1). In M2 AML myeloblasts are relatively less numerous and there are considerable numbers of maturing cells (Fig. 4.2). Many of these are morphologically abnormal and often difficult to categorize; abnormalities include: (i) hypogranularity; (ii) bizarre nuclear shapes; and (iii) the presence of Auer rods, not only in blasts but sometimes also in promyelocytes, myelocytes and neutrophils. In some cases of M2 AML the maturing granulocytic

Fig. 4.1 BM aspirate, M1 AML. Note that some of the blast cells resemble lymphoblasts in that they are small and round with a high nucleocytoplasmic ratio and no granules. The presence of an agranular neutrophil and occasional blasts with granules suggests the correct diagnosis. MGG ×940.
component includes, or is composed of, basophils or eosinophils. In both M1 and M2 AML the erythrocytes and megakaryocytes may show dysplastic features.

In M2 AML associated with t(8;21) the bone marrow, in addition to maturing cells of neutrophil lineage, commonly shows increased eosinophils; these are usually cytologically normal. Erythroid cells and megakaryocytes do not show dysplastic features. In M2 AML associated with t(6;9), the bone marrow commonly shows not only blasts of basophil lineage but also increased numbers of mature basophils; there is often associated myelodysplasia and Auer rods are commonly present. In M2 AML associated with 12p-, blasts are also of basophil lineage but there is very little maturation so that they may appear undifferentiated by light microscopy.

Cytochemistry

The myeloid nature of M2 AML is evident from the features of differentiation observable in May–Grünwald–Giemsa (MGG)-stained films but in M1 AML cytochemical staining [9] is often necessary to confirm the diagnosis. Either MPO or Sudan black B (SBB) staining must be positive in at least 3% of blasts to satisfy the FAB criteria of AML [1]; usually both stains are positive. These stains are also very useful in identifying Auer rods and reveal their presence in some cases in which they are not identifiable in the MGG stain. A naphthol AS-D chloro-acetate esterase (chloro-acetate esterase) stain is also positive in myeloblasts in the majority of cases of M1 and M2 AML. Metachromatic staining with toluidine blue is useful in the diagnosis of cases with basophil differentiation but little maturation, for example cases of M2Baso AML associated with 12p-.

Bone marrow histology

The marrow is markedly hypercellular (greater than 95% cells) in most cases. Often the cellularity seen in biopsy sections is greater than that estimated from aspirated fragments in films [10]. The morphology of the neoplastic cells in tissue sections is different from that seen in films of aspirates. In tissue sections, myeloblasts have large round to oval nuclei, delicate chromatin, one or more small well-defined nucleoli and scant basophilic cytoplasm (Fig. 4.3). In a half to two thirds of all cases there is a dense homogeneous infiltrate of blasts whereas, in the remainder, there is a mixture of blasts, more mature haemopoietic cells and inflammatory cells such as plasma cells, lymphocytes and mast cells [10,11]. Evidence of maturation is often apparent in M2 AML (Fig. 4.4). Leder’s chloro-acetate esterase stain is usually positive in M2 AML (depending on the method of processing—see page 59) but is often negative in M1 AML. Dysplastic changes are
commonly detected in megakaryocytes and erythroid precursors (see page 172). In M2 AML associated with t(8;21) there is often a prominent infiltrate of eosinophils scattered among the blasts. Reticulin fibrosis is present in up to a third of cases [12] but collagen fibrosis is rare. Areas of bone marrow necrosis are sometimes present. Following chemotherapy, the marrow is hypoplastic and often shows necrosis, stromal oedema and gelatinous change. Residual leukaemic cells are often more apparent in tissue sections than in aspirate films. However, they can be difficult to distinguish from foci of immature regenerating granulocytic or erythroid precursors.

In M2 AML there are usually sufficient maturing granulocytic cells for a diagnosis of AML to be made from H&E-stained sections. However, a provisional diagnosis of M1 AML may require immunohistochemistry for confirmation if the diagnosis rests on a trephine biopsy specimen alone.

**Cytogenetics and molecular genetics**

Various subtypes of AML, characterized by specific chromosomal abnormalities, are included within the FAB M1 and M2 categories [8]. Of these, the commonest is M2 AML associated with t(8;21)(q22;q22)
and formation of an *AML1-ETO* fusion gene, which is recognized as a separate entity in the MIC, MIC-M and WHO classifications. A much less common subtype is that associated with t(6;9)(p23;q34) and formation of a *DEK-CAN* fusion gene.

**Acute (hypergranular) promyelocytic leukaemia**

The majority of cases of acute promyelocytic leukaemia have hypergranular promyelocytes and are therefore designated acute hypergranular promyelocytic leukaemia, or M3 AML. A minority of cases have abnormal promyelocytes which are either microgranular or hypogranular when examined by light microscopy; such cases are variously referred to as the variant form of acute promyelocytic leukaemia, M3 variant AML, acute hypogranular promyelocytic leukaemia or acute microgranular promyelocytic leukaemia. Acute promyelocytic leukaemia is recognized as a specific entity in all leukaemia classifications. Rapid, correct diagnosis of M3 and M3 variant AML is of critical importance if early death from haemorrhage is to be avoided.

**Peripheral blood**

In M3 AML, the peripheral blood white cell count is not usually greatly elevated and the number of circulating leukaemic cells tends to be low. There is usually anaemia. The platelet count may be disproportionately low as a consequence of complicating disseminated intravascular coagulation. The abnormal promyelocytes are large cells, usually two to three times the diameter of an erythrocyte. Their cytoplasm is packed with granules which stain bright pink or reddish-purple. Some cells contain bundles of Auer rods (‘faggot cells’) or giant granules. No Golgi zone is apparent. The nucleus is usually round or oval but cytoplasmic granulation is so marked that the nuclear outline is difficult to discern.

In M3 variant AML, the white cell count is usually elevated. Again, there is usually anaemia and marked thrombocytopenia. Abnormal promyelocytes are characteristically more frequent in the peripheral blood in M3 variant AML than in typical, hypergranular M3 AML. The promyelocytes may appear completely agranular or may have fine, dust-like reddish granules (Fig. 4.5). Some cells contain bundles of Auer rods or other crystalline inclusions. The nucleus is usually deeply lobed, often with two large lobes joined by a narrow bridge. The cytoplasm is usually weakly or moderately basophilic but some cases have promyelocytes with more marked basophilia and cytoplasmic protrusions or blebs. A careful search in cases of M3 variant AML often discloses a minor population of more typical, hypergranular promyelocytes, occasionally with multiple Auer rods.

**Bone marrow cytology**

Bone marrow aspiration is often difficult since the
hypercoagulable state leads to clotting of the specimen, even during aspiration. However, the bone marrow aspirate is important in diagnosis since, in M3 AML, there may be only infrequent leukaemic cells in the peripheral blood and, in M3 variant AML, the bone marrow often contains a higher proportion of typical hypergranular cells than does the peripheral blood.

The bone marrow aspirate is usually intensely hypercellular. The number of blasts is relatively low since the predominant cell is an abnormal promyelocyte (Fig. 4.6); in the majority of cases there are fewer than 30% of blasts in the marrow. In M3 AML the predominant cell is a hypergranular promyelocyte while, in M3 variant, the predominant cell is a hypogranular promyelocyte with a variable admixture of hypergranular forms. There is a marked reduction in the number of normal maturing granulocytes. Erythroid cells and megakaryocytes are also considerably reduced in number but are cytologically normal.

**Cytochemistry**

Cytochemical stains are unnecessary in typical hypergranular M3 AML but are important in confirming a diagnosis of M3 variant AML. Typically, there are positive reactions with MPO, SBB and chloroacetate esterase and negative reactions with nonspecific esterase.

**Bone marrow histology**

There is usually marked hypercellularity with a homogeneous infiltrate of abnormal promyelocytes (Fig. 4.7). These cells have a characteristic appearance; they have prominent large granules that fill the cytoplasm and often obscure the nucleus. The nucleus may be oval or bilobed and has a single prominent nucleolus. Faggot cells may be detectable. In M3 variant AML the granules are much smaller and may be inconspicuous; the nuclei are often bilobed. Occasional cases of M3 AML show little increase in cellularity at presentation, an unusual feature in de novo AML.

Because of their hypergranularity, the leukaemic cells of M3 AML can be readily recognized in H&E-stained sections of trephine biopsy specimens. A proportion of cases of M3 variant AML can also be recognized from cytological features. Other cases of M3 variant AML, with very infrequent hypergranular cells, require histochemistry (Leder’s stain) or immunohistochemistry (e.g. demonstration of neutrophil elastase) for confirmation if the diagnosis rests on the trephine biopsy specimen alone.

Collagen fibrosis may be observed at presentation in M3 AML. This is greatly increased by treatment with all-trans retinoic acid (ATRA) [13]. ATRA therapy leads to maturation of the leukaemic clone with the bone marrow remaining hypercellular in contrast to the hypocellular marrow that is seen.
when chemotherapy is administered. Extensive bone marrow necrosis has been reported in association with hyperleucocytosis caused by ATRA [14].

Cytogenetics and molecular genetics
M3 and M3 variant AML are uniformly associated with t(15;17)(q22;q21), a PML-RARα fusion gene or both.

Acute myelomonocytic leukaemia
Acute myelomonocytic leukaemia (M4 AML) shows significant evidence of both granulocytic and monocytic differentiation. The granulocytic differentiation is usually neutrophilic but, in some variants, it is eosinophilic (M4Eo) or basophilic (M4Baso). Cases of AML assigned to FAB M4 category would, in the WHO classification, fall into various categories including ‘AML with abnormal bone marrow eosinophils’, ‘AML associated with 11q23 rearrangements’ and ‘acute myelomonocytic leukaemia’.

Peripheral blood
There is usually anaemia and thrombocytopenia with an elevated white cell count and circulating leukaemic cells of both granulocytic and monocytic lineages. Myeloblasts and monoblasts show the usual cytological features of these lineages (see below). Maturation of leukaemic cells is usual so that the leukaemic cell population commonly includes both monocytes and neutrophils. However, some cases of M4 AML have no evidence in the peripheral blood of the significant monocytic component which is present in the bone marrow. In AML associated with inv(16)(p13q22) or t(16;16)(p13;q22), there are usually only occasional eosinophils in the peripheral blood and these are not morphologically very abnormal. In AML associated with t(6;9)(p23;q34), there may be basophilia with some dysplastic basophils.

Bone marrow cytology
The bone marrow is hypercellular and shows a variable mixture of cells of granulocytic and monocytic lineages (Fig. 4.8). In the majority of cases, maturation of leukaemic cells is occurring and mature monocytes as well as maturing cells of granulocytic lineage can be readily recognized. However, there are a minority of cases in which, despite a peripheral blood monocytosis, the bone marrow cannot be distinguished morphologically from that of M2 AML. In such cases the significant monocytic component can be confirmed either by cytochemistry (see below) or by assays of serum or urinary lysozyme. The explanation of such cases is probably twofold. Firstly, leukaemic monocytes may be infrequent in the bone marrow because of early migration to the peripheral blood. Secondly, promonocytes can
be difficult to distinguish morphologically from promyelocytes.

Megakaryocytes and erythroid precursors are usually reduced. Dysplastic features are sometimes present in these lineages.

In AML associated with inv(16)(p13q22) or t(16;16)(p13;q22), the bone marrow aspirate often shows a mixture of cells of monocyte and eosinophil lineages but cells of neutrophil lineage are relatively infrequent (Fig. 4.9). Some basophils may also be present. The cells of eosinophil lineage are a mixture of myelocytes and mature eosinophils. In the great majority of cases the eosinophil myelocytes are morphologically abnormal with prominent pro-eosinophilic granules, which are basophilic in their staining characteristics, mixed with typical eosinophilic granules. The mature eosinophils may show cytological abnormalities such as nuclear hyper- or hypolobulation or the presence of occasional pro-eosinophilic granules. Some cells of granulocyte lineage, mainly immature cells, show Auer rods. There is usually maturation in the monocyte lineage so that mature monocytes are present. Megakaryocytes and erythroid cells do not show dysplastic features. It should, however, be noted that not all cases of AML associated with
these chromosomal rearrangements have the cyto-
logical features of M4Eo; some cases fall into either
the M4 or M2 category of AML.

In AML associated with t(6;9)(p23;q34), the bone
marrow commonly shows, in addition to an increase
in monocytes and their precursors, an increase of
mature basophils and sometimes an increase of
neutrophils or eosinophils. There are associated
myelodysplastic features.

Cytochemistry

A double esterase stain, combining chloro-acetate
esterase and non-specific esterase, is important in
confirming a diagnosis of M4 AML. In AML associ-
ated with inv(16), the eosinophils may show aber-
rant chloro-acetate esterase positivity. In addition,
SBB and MPO stains may show Auer rods in occa-
sional eosinophils and their precursors as well as
in neutrophil precursors. A toluidine blue stain is
useful for confirming basophil differentiation in
M4Baso AML.

Bone marrow histology

The marrow is markedly hypercellular and there
is an infiltrate composed of variable numbers of
myeloblasts, monoblasts and maturing cells of both
lineages. Maturing granulocytes may be neu-
throphils, eosinophils or both. Monoblasts have large
irregular nuclei with delicate chromatin and pro-
minent nucleoli; cytoplasm is abundant and may be
vacuolated. The distribution of monoblasts is not
uniform: they are often seen in small clusters, par-
ticularly in the paratrabecular areas [10]. Dysplastic
changes are often seen in other haemopoietic lin-
eges and reticulin fibrosis may be present.

Trephine biopsy sections in AML associated with
inv(16) often show increased eosinophils and pre-
cursors (Fig. 4.10). However, M4Baso, associated
with t(6;9), cannot be distinguished from other
cases of M4 AML since basophil granules are
dissolved during processing. M4 and M4Eo AML
can usually be readily recognized in H&E-stained
sections so that histochemistry and immunohisto-
chemistry are not needed in most cases.

Cytogenetics and molecular genetics

M4 AML is preferentially associated with transloca-
tions having an 11q23 breakpoint and rearrangement
of the MLL gene. In addition, there are several other
subtypes within the M4 AML category defined by associated cytogenetic and molecular genetic
abnormalities. These are M4 and M4Eo AML asso-
ciated with inv(16)(p13q22) or t(16;16)(p13;q22)
and formation of a CBFβ-MYH11 fusion gene and
M4Baso associated with t(6;9)(p23;q34) and a CAN-
DEK fusion gene. The former is a WHO category but
the latter is not.
Acute monocytic/monoblastic leukaemia

AML showing predominantly or entirely monocytic differentiation is categorized as acute monocytic or acute monoblastic leukaemia—M5 AML—the former showing maturation of leukaemic cells to mature monocytes and the latter showing little maturation. The FAB group have assigned to these categories (designated M5) those cases of AML in which there is monocytic differentiation and in which less than 20% of bone marrow non-erythroid cells are granulocytes or their precursors. Cases are further divided into acute monoblastic leukaemia (M5a) and acute monocytic leukaemia (M5b) on the basis of whether or not the leukaemic clone is showing maturation (see Table 4.1). Cases of AML assigned to the FAB M5 category would, in the WHO classification, fall into various categories including ‘AML associated with 11q23 rearrangements’ and ‘acute monocytic leukaemia’.

Peripheral blood

The peripheral blood usually shows anaemia, thrombocytopenia and leucocytosis with circulating leukaemic cells which are variously monoblasts, promonocytes or monocytes. Monoblasts are larger than myeloblasts, usually with a diameter about three times that of an erythrocyte. Their shape varies from round to oval or irregular. The cytoplasm is voluminous and varies from weakly to strongly basophilic; it may contain very infrequent granules. The nucleus ranges from round to lobulated and is usually nucleolated; nucleoli vary from being large, single and prominent to being smaller and multiple. Promonocytes are large cells with an oval or lobulated nucleus and moderately basophilic cytoplasm containing fairly numerous azurophilic granules. Monocytes in M5 AML resemble normal monocytes in having lobulated nuclei and weakly basophilic, sometimes vacuolated, cytoplasm. They may show cytological abnormalities such as nuclei of bizarre shapes. In M5b AML, the peripheral blood contains monocytes and a variable number of promonocytes and monoblasts. In some cases of M5a AML the peripheral blood contains large numbers of monocytes and promonocytes, even though the predominant cell in the bone marrow is a monoblast. In other cases the peripheral blood leukaemic cells, like those in the marrow, are almost exclusively monoblasts.

Bone marrow cytology

The bone marrow is hypercellular and numbers of megakaryocytes and erythroid precursors are reduced. In M5a AML (Fig. 4.11) the great majority of cells are monoblasts. All cases in which at least 80% of bone marrow non-erythroid cells are monoblasts are classified as M5a, regardless of whether there are maturing cells in the peripheral blood. In M5b AML the marrow contains a mixture of monoblasts, promonocytes and monocytes (Fig. 4.12).
Cytochemistry

Cytochemical stains [9] are useful in confirming the nature of M5 AML, particularly in those cases of M5a with negligible maturation. Monoblasts are often negative for MPO and SBB, although positive results are obtained with promonocytes. The most useful stains are those for ‘non-specific’ esterases such as α-naphthyl acetate esterase or α-naphthyl butyrate esterase. An alternative is the demonstration of strong, fluoride-sensitive, positivity for naphthol-AS-acetate esterase, another ‘non-specific’ esterase. Monocyte differentiation can also be demonstrated by using a suspension of the bacterium Micrococcus lysodeikticus to show lysozyme activity. However, this test is now little used since the availability of monoclonal antibodies provides another means of confirming monocyte differentiation. Positive reactions with monoclonal antibodies, such as those of the CD11b and CD14 clusters, provide useful confirmation of the diagnosis of M5 AML in cases with negative reactions for non-specific esterases.

Bone marrow histology

The marrow is intensely hypercellular. In M5a AML (Fig. 4.13) there is a homogeneous infiltrate of monoblasts whereas in M5b AML (Fig. 4.14) there is a variable proportion of more mature cells of monocyte lineage. The monoblasts are similar to those seen in M4 AML. The more mature monocytic cells are smaller than monoblasts and have irregular nuclei that are often convoluted or lobulated, with delicate chromatin but without nucleoli.

Cytochemical stains to confirm the monocyte lineage are not generally applicable to tissue sections. Chloro-acetate esterase activity is usually absent. Immunohistochemistry is useful, particularly in M5a AML in which the diagnosis is less readily made from H&E-stained sections. Monoclonal antibodies that can be employed include antilysozyme, CD64, CD68, CD11b, CD14, CD15 and MAC387. Of CD68 monoclonal antibodies, the PG-M1 clone shows good specificity for M4 and M5 AML, while the KP1 clone is a sensitive marker of myeloid differentiation but does not discriminate between monocytic and granulocytic differentiation [15].

Cytogenetics and molecular genetics

Chromosomal rearrangements with an 11q23 breakpoint and with rearrangement of the MLL gene are strongly associated with M5 AML, particularly M5a AML. Other subtypes of M5 AML defined by the associated cytogenetic abnormality include M5a associated with t(8;16)(p11;p13), which is distinctive due to the frequent occurrence of haemophagocytosis by leukaemic cells (and, clinically, due to a relatively high incidence of coagulation abnormalities).
Acute erythroleukaemia

Acute erythroleukaemia (M6 AML) describes an AML in which erythroid cells represent a major part of the leukaemic population. The FAB group has recommended that cases be assigned to this category (M6) when at least 50% of bone marrow cells are erythroid and at least 30% of the remaining non-erythroid cells are blasts (see Table 4.1). Others have considered that a case can also reasonably be classified as erythroleukaemia if erythroid cells show prominent cytological abnormalities and constitute between 30 and 50% of bone marrow nucleated cells [16]. There is a problem in assigning to a FAB category cases with almost exclusively erythroid cells including many very primitive erythroid cells [17]. In such cases, fewer than 30% of non-erythroid cells may be blasts and consequently the FAB criteria for M6 AML may not be fulfilled; nevertheless this seems to be the category where they fit most naturally. We suggest a designation of M6 variant AML with criteria for diagnosis being that more than 50% of bone marrow cells are erythroid and more than 30% are immature erythroid cells resembling proerythroblasts or basophilic erythroblasts.
It appears likely that a very high proportion of cases of M6 AML represent transformation of underlying MDS.

Cases of AML assigned to FAB M6 category would, in the WHO classification, fall into various categories. Most would probably be designated ‘AML with multilineage dysplasia’ and others ‘acute erythroid leukaemia’.

Peripheral blood

The peripheral blood almost always shows anaemia, neutropenia and thrombocytopenia, usually with some circulating blasts. There may also be circulating erythroid precursors.

Bone marrow cytology

The bone marrow is hypercellular and shows both erythroid hyperplasia and, except in M6 variant, a significant population of blasts (Fig. 4.15). The morphology of erythroid cells varies between cases. In some patients, erythroid cells show striking cytological abnormalities which may include nuclear lobulation, karyorrhexis, multinuclearity, gigantism or megaloblastic or sideroblastic erythropoiesis. In other patients, cytological abnormalities are quite minor. The associated blasts are usually myeloblasts, which may contain Auer rods, or a mixed population including monoblasts or megakaryoblasts. Maturing granulocytes often show dysplastic features such as hypogranularity and nuclear hypolobulation. Megakaryocytes are also commonly dysplastic with features such as nuclear hypolobulation or the presence of micromegakaryocytes.

Cytochemistry

Cytochemical stains may show various abnormalities. SBB, MPO and chloro-acetate esterase methods can be employed to confirm the nature of myeloblasts; the first two stains will also demonstrate Auer rods. An α-naphthyl acetate esterase stain can be used to identify monoblasts. Erythroblasts may be positive with a periodic acid–Schiff (PAS) stain; such positivity is not shown by normal erythroid precursors but it is not confined to neoplastic erythroblasts. Erythroblasts in M6 AML may also show focal staining for α-naphthyl acetate esterase and acid phosphatase. An iron stain may show the presence of ring sideroblasts.

Bone marrow histology

There is marked hypercellularity of the marrow with intense erythroid hyperplasia (Figs 4.16 and 4.17). The erythroid precursors are usually markedly abnormal and may have bizarre appearances. They often show nuclear lobulation or fragmentation, marked variation in size or megaloblastic
change. They are arranged in sheets without the formation of normal erythroblastic islands. Megakaryocytic dysplasia is often seen. Non-erythroid blasts (myeloblasts or monoblasts) may be relatively inconspicuous although, by definition, they must make up more than 30% of the non-erythroid cells in the marrow except in the condition that we designate M6 variant AML.

The diagnosis of M6 AML can usually be readily made from H&E-stained sections. In cases with predominantly primitive erythroid cells, immunohistochemistry may be needed. Useful reagents are antibodies reactive with glycophorin, spectrin or haemoglobin A.

Cytogenetics and molecular genetics
M6 AML is often associated with abnormalities of chromosomes 5 and 7 and with complex chromosomal abnormalities.

Acute megakaryoblastic leukaemia
Acute megakaryoblastic leukaemia (M7 AML) has
blasts constituting at least 30% of bone marrow cells, with megakaryoblasts being the predominant form. There may be admixture with cells of other lineages, for example, myeloblasts. The diagnosis is often difficult, particularly in adults, because of the paucity of leukaemic cells in the peripheral blood and the difficulty in obtaining a bone marrow aspirate. A trephine biopsy is then of critical importance in making the diagnosis. In children, circulating blasts are often present and the bone marrow can more often be aspirated easily.

Cases of AML assigned to FAB M7 category would, in the WHO classification, fall into various categories including ‘acute megakaryocytic leukaemia’ and ‘acute panmyelosis with myelofibrosis’.

**Peripheral blood**

Commonly, the peripheral blood shows only pancytopenia with very infrequent or no circulating leukaemic blasts. Such patients usually lack organomegaly and have a fibrotic marrow; this condition has been described as ‘acute myelofibrosis.’ Other patients have features more typical of acute leukaemia, with hepatomegaly, splenomegaly and significant numbers of circulating blasts. Megakaryoblasts are similar in size to myeloblasts. They have a high nucleocytoplasmic ratio and agranular, moderately basophilic cytoplasm. In some cases there are distinctive features that suggest their nature, such as formation of peripheral cytoplasmic blebs or an association with circulating micromegakaryocytes, but in other cases there are no features to suggest lineage.

**Bone marrow cytology**

Aspiration may be impossible or a poor aspirate containing scanty blasts may be obtained (Fig. 4.18). In addition to megakaryoblasts, the aspirate may contain some micromegakaryocytes or other markedly dysplastic megakaryocytes. There may be admixture with myeloblasts. Erythroid precursors sometimes show dysplastic features.

**Cytochemistry**

Cytochemistry is often not very useful. SBB, MPO and chloro-acetate esterase stains are negative except in any associated myeloblasts. PAS, acid phosphatase and α-naphthyl acetate esterase stains may be positive in cells showing cytoplasmic maturation but not in more immature cells of megakaryocyte lineage. The PAS stain sometimes shows a distinctive pattern with positivity being confined to cytoplasmic blebs. The differential staining pattern with α-naphthyl acetate esterase (positive) and α-naphthyl butyrate esterase (negative) can be useful in distinguishing megakaryoblasts from monoblasts, since the latter cells give positive
reactions for both of these non-specific esterases. Immunocytochemistry with monoclonal antibodies directed at platelet glycoproteins is now the most practicable way to confirm the diagnosis.

**Bone marrow histology** [18,19]

The marrow histology is very variable. In those cases that present clinically as acute myelofibrosis, the marrow is largely replaced by fibrous tissue containing blasts and dysplastic megakaryocytes (Fig. 4.19). In other cases the marrow is very hypercellular with an infiltrate of blasts; in some cases the blasts are relatively small and monomorphic while, in others, they are large and pleomorphic. Cases with a hypercellular marrow usually show increased reticulin and scattered collagen fibres. Dyserythropoiesis is common.

Immunohistochemical staining for von Willebrand antigen or for platelet GpIIIa (CD61) or GpIb (CD42b) is useful in the identification of megakaryoblasts. Immunohistochemistry is necessary for diagnosis in those cases with predominantly megakaryoblasts with few megakaryocytes.

**Cytogenetics and molecular genetics**

M7 AML in infants is strongly associated either with constitutional trisomy 21 or with acquired t(1;22)(p13;q13).

**Acute myeloid leukaemia with minimal evidence of myeloid differentiation**

The availability of ultrastructural cytochemistry and of monoclonal antibodies detecting antigens specific for myeloid cells has revealed cases of acute leukaemia which are negative for markers of lymphoid lineages but have insufficient evidence of myeloid differentiation to meet the original FAB criteria for AML. Such cases have fewer than 3% SBB- or MPO-positive blasts; blasts are also negative for α-naphthyl acetate esterase and lysozyme activity. When such cases can be demonstrated to have peroxidase activity at ultrastructural level, or to be positive for myeloid antigens, such as CD13, CD14, CD33, CD117 or MPO, they should be classified as AML. The FAB group has suggested the designation AML M0 [20]. Cases of AML assigned to FAB M0 category would, in the WHO classification, fall into various categories including ‘AML minimally differentiated’.

**Peripheral blood**

The peripheral blood usually shows anaemia, neutropenia, thrombocytopenia and the presence of circulating blasts (Fig. 4.20). The blasts are agranular and have cytological features similar to those of the blasts of AML M1, ALL L2 or, more rarely, ALL L1 (see page 236). They tend to be large with prominent nucleoli and abundant, often basophilic.
cytoplasm but there are no constant features permitting the diagnosis from cytomorphology alone. The diagnosis may be suspected when apparently undifferentiated blasts are associated with dysplastic neutrophils or marked poikilocytosis.

**Bone marrow cytology**

The bone marrow features do not differ from those of M1 AML except that the blasts have scanty, if any, granules and have no Auer rods. Other lineages may show dysplastic features, suggesting that ALL is not the correct diagnosis.

**Cytochemistry**

Cytochemistry with all the stains that are usually used to identify cells of various myeloid lineages is either negative or is positive in fewer than 3% of blasts. Immunocytochemistry for the above antigens is therefore used to confirm the diagnosis.

**Bone marrow histology**

The trephine biopsy appearances do not differ from those of M1 AML. It is also often not possible to distinguish M0 AML from ALL, although the presence of dysplastic features suggests a diagnosis of AML. The diagnosis of AML cannot be confirmed by Leder’s stain, which is uniformly negative in M0 AML. Immunological markers, however, can permit the distinction. Lymphoblasts usually express CD45 and may express CD79a or CD3, whereas myeloblasts do not express CD79a or CD3 and are often CD45-negative or only weakly positive. Monoclonal or polyclonal antibodies reactive with myeloid antigens, including anti-MPO and CD15, may be positive in M0 AML.

**Cytogenetics and molecular genetics**

M0 AML is preferentially associated with abnormalities of chromosomes 5 and 7, trisomy 13 and translocations and deletions involving 12p [21], suggesting a relationship to M6 AML and to therapy-related and myelodysplasia-related AML.

**Acute panmyelosis with myelofibrosis**

The WHO expert group [5] has drawn attention to a rare entity in which there is trilineage differentiation associated with bone marrow reticulin fibrosis. This entity has some features in common with those cases of M7 AML that are associated with the clinicopathological features of ‘acute myelofibrosis’.

**Peripheral blood**

The blood film shows no specific features. There may be circulating blast cells.
Bone marrow aspirate

The bone marrow aspirate is often inadequate for diagnosis because of the associated reticulin fibrosis. Increased blast cells and multilineage dysplasia may be apparent.

Bone marrow histology

Bone marrow histology is critical in making the diagnosis, with immunohistochemistry being important in showing trilineage differentiation. Immature cells are increased and dysplasia may be prominent. Reticulin is increased.

Acute eosinophilic leukaemia

The term ‘eosinophilic leukaemia’ covers a heterogeneous group of disorders in which eosinophils are increased in the peripheral blood. Acute eosinophilic leukaemia falls into the FAB categories of M2 or M4 AML and can be designated more specifically as M2Eo and M4Eo. There is no precise percentage of eosinophils and precursors which leads to a case being categorized as M2Eo or M4Eo AML but cases of M4 AML with cytologically atypical eosinophils are usually categorized as M4Eo, even if the eosinophil percentage is relatively low. This latter group would not, however, usually be referred to as 'eosinophilic leukaemia'. Eosinophilic leukaemia, which is not synonymous with ‘AML with abnormal bone marrow eosinophils’, is not recognized specifically in the WHO classification. Cases without inv(16) would fall mainly into the WHO category ‘AML with maturation’. For a case to be classified as acute eosinophilic leukaemia, blast cells must constitute at least 20% of bone marrow cells. Cases of chronic eosinophilic leukaemia have peripheral blood and bone marrow eosinophilia and fewer than 20% bone marrow blasts. They are classified with the myeloproliferative disorders.

Peripheral blood

The peripheral blood shows mature eosinophils and eosinophil precursors. Eosinophils may be cytologically atypical but this is not diagnostically useful since atypical eosinophils are also seen in reactive eosinophilia. Eosinophil precursors often show nucleocytoplasmic asynchrony and immature granules with basophilic staining characteristics (Fig. 4.21).

Bone marrow cytology

The bone marrow shows an increase, often very marked, in eosinophils and their precursors (Fig. 4.22). Occasionally, Charcot–Leyden crystals are present, these being formed by crystallization of eosinophil granule contents. Other myeloid lineages (e.g. neutrophils or monocytes) may also be part of the leukaemic clone.

Fig. 4.21 PB film from a patient with acute eosinophilic leukaemia (M2Eo AML), showing eosinophil precursors with a mixture of primary and secondary granules. MGG x960. (By courtesy of Dr A Smith, Southampton.)
Cytochemistry

Eosinophils and precursors show strong peroxidase activity.

Bone marrow histology

Bone marrow trephine biopsy sections show an increase of eosinophils and their precursors (Figs 4.23 and 4.24).

Cytogenetics and molecular genetics

There is no specific cytogenetic association with acute eosinophilic leukaemia. Rare cases have had t(8;21)(q22;q22).

Acute basophilic leukaemia

Acute basophilic leukaemia is a rare form of AML which is not identified specifically in the FAB classification. Cases usually fall into the M2 or M4 categories and can be designated M2Baso and M4Baso. Some cases with little or no maturation fall into the M1 or M0 categories. Blasts in these cases can be identified as being of basophil lineage, either because they have basophil-type granules identifiable on light microscopy or by ultrastructural
examination. The WHO classification has a specific category for acute basophilic leukaemia.

Peripheral blood
The blood film usually shows both blast cells and maturing basophils. The latter may be hypogranular.

Bone marrow cytology
The bone marrow aspirate shows more than 20% blast cells, usually with maturation to dysplastic basophils.

Bone marrow histology
Trephine biopsy sections show increased blast cells but are of no use currently in the detection of basophilic differentiation since basophil granules are dissolved during processing.

Acute mast cell leukaemia
Acute mast cell leukaemia is a rare condition which can occur de novo or as a transformation of systemic mastocytosis. It is not included in the FAB classification. The WHO classification assigns it to a separate category of ‘mast cell diseases’ but, since the mast cell is derived from the haemopoietic stem cell, it seems to us more appropriate that it is classified as a form of AML.

Peripheral blood
The blood film shows immature mast cells which have round, oval or lobulated nuclei and a variable number of granules of mast cell type (Fig. 4.25).

Bone marrow cytology
The bone marrow aspirate shows immature cells of mast cell lineage. Immunocytochemistry for mast cell tryptase can be used to demonstrate mast cell differentiation if this is not certain from cytological features.

Cytochemistry
Mast cells stain metachromatically with toluidine blue. They are chloro-acetate esterase-positive.

Bone marrow histology
Trephine biopsy sections show effacement of the bone marrow by immature mast cells. These have more voluminous cytoplasm than most other blast cells so that, in an H&E-stained section, the round, oval or lobulated nuclei appear spaced apart.

Fig. 4.24 Section of BM trephine biopsy specimen from a patient with acute eosinophilic leukaemia (same case as in Fig. 4.21), showing eosinophils and their precursors and two Charcot–Leyden crystals. H&E ×960.
ACUTE MYELOID LEUKAEMIA

With Giemsa staining, granules may be apparent. Immunohistochemistry for mast cell tryptase confirms the lineage.

Hypoplastic AML

A minority of cases of AML have a bone marrow which is hypocellular rather than hypercellular. Hypoplastic AML is defined variously, for example, as AML with bone marrow cellularity less than 50% [22] or less than 40% [23]. Blasts constitute at least 20% of nucleated cells. Cases of hypoplastic AML may belong to various FAB categories but not M3. They may arise apparently de novo or be preceded by a hypoplastic variant of myelodysplasia. Clinical and haematological features differ from those of typical cases of AML with a hypercellular bone marrow. Hepatomegaly and splenomegaly are not commonly present. The median age is higher than that of AML in general [22]. The prognostic significance of hypoplastic AML is not yet clear. In one series, patients who were treated had a low remission rate and treated patients, overall, did not survive longer than those who received no specific treatment [22].
However, in another series, treated patients had a high remission rate and improved survival [23].

Hypoplastic AML is not identified specifically in the WHO classification. Some cases fall into the categories designated ‘AML with multilineage dysplasia’ and ‘AML, therapy-related’.

**Peripheral blood**

In contrast to cases of typical AML, pancytopenia is usual and peripheral blood blasts are often absent or infrequent. Blasts may contain Auer rods.

**Bone marrow cytology**

The bone marrow aspirate is often hypocellular and may therefore not be optimal for diagnosis. Blasts are increased and commonly show granulocytic rather than monocytic differentiation. There may be associated dysplastic features.

**Cytochemistry**

The blasts in hypoplastic AML have no specific cytochemical features.

**Bone marrow histology**

Because a poor aspirate is often obtained, trephine biopsy is usually important in diagnosis. The marrow shows irregular hypoplasia (Fig. 4.27) with small foci of blasts separated by fat cells (Fig. 4.28). Blasts make up more than 20% of bone marrow nucleated cells. There are often dysplastic changes in the other haemopoietic lineages. Reticulin may be increased.

**Cytogenetics and molecular genetics**

Cytogenetic abnormalities are non-specific but may include abnormalities of chromosomes 5 and 7.

**Cytochemistry in AML**

Cytochemistry permits the confirmation of a diagnosis of AML in all categories except M0 and M7. It is most important in M1 and M5a AML, which can be confused with ALL and high grade lymphoma respectively. Recommended cytochemical stains are either MPO or SBB (for identification of granulocytic differentiation) and a non-specific esterase stain such as α-naphthyl acetate esterase (for identification of monocytic differentiation).

**Immunocytochemistry and flow cytometry in AML**

Immunophenotyping [8] (Table 4.3) is of considerable importance in confirming the diagnosis of M0 and M7 AML, which may otherwise be confused
with ALL. It is also useful in the recognition of M6 variant AML since the primitive cells may be so immature that they are not readily recognizable from cytological features. Immunophenotyping is an alternative to cytochemistry for confirming the myeloid lineage of M1 and M5 AML. Other FAB categories are usually readily recognizable without recourse to immunophenotyping. Immunophenotyping is now generally performed by flow cytometry rather than by immunocytochemistry.

**Immunohistochemistry in AML**

Monoclonal and polyclonal antibodies that are useful in confirming a diagnosis of AML are shown in Table 4.4. Their use is most important in M0, M1, M5a and M7 categories. In M2, M3, M4 and M5b subtypes it is usually possible to make a diagnosis of AML from H&E-stained sections. It is our practice to perform immunohistochemical stains whenever there is diagnostic difficulty, for example, when bone

---

**Table 4.3** Monoclonal and polyclonal antibodies useful for flow cytometry immunophenotyping in the diagnosis of AML.

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panmyeloid</td>
<td>CD13, CD33, CD65, CD117, anti-myeloperoxidase</td>
</tr>
<tr>
<td>Markers of maturation</td>
<td>CD15, CD11b</td>
</tr>
<tr>
<td>Markers of monocytic differentiation</td>
<td>CD14, CD11b, CD64</td>
</tr>
<tr>
<td>Erythroid markers</td>
<td>Anti-glycophorin</td>
</tr>
<tr>
<td>Megakaryocytic markers</td>
<td>CD41, CD42a, CD42b, CD61</td>
</tr>
<tr>
<td>Markers of immaturity</td>
<td>CD34, anti-terminal nucleotidyl transferase</td>
</tr>
</tbody>
</table>

**Table 4.4** Monoclonal and polyclonal antibodies useful in immunohistochemistry for the diagnosis of AML.

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytic and/or monocytic markers</td>
<td>Anti-myeloperoxidase, CD64, CD68, anti-neutrophil elastase</td>
</tr>
<tr>
<td>Megakaryocyte/platelet markers</td>
<td>CD42b, CD61, anti-von Willebrand’s factor</td>
</tr>
<tr>
<td>Erythroid markers</td>
<td>Anti-glycophorin, anti-spectrin</td>
</tr>
<tr>
<td>Mast cell marker</td>
<td>Mast cell tryptase</td>
</tr>
<tr>
<td>Markers of immaturity</td>
<td>CD34, anti-terminal deoxynucleotidyl transferase</td>
</tr>
</tbody>
</table>
Cytogenetics and molecular genetics in AML

It is the somatic mutation that occurred in the stem cell giving rise to the leukaemic clone that determines the clinical and pathological features of AML. The molecular genetic abnormality is therefore one of the most fundamental characteristics of any case of AML and it is appropriate that it be incorporated into classification of this disease. The MIC classification sought to do this, when cytogenetic analysis was added to morphology and immunophenotyping in classifying acute leukaemia [3]. With the advent of molecular genetic analysis, it became possible to identify genes involved in the oncogenic event and a MIC-M classification, incorporating the results of molecular genetic analysis [4], became possible. The MIC-M categorization is open-ended, new categories being added when the oncogenic events have been defined (Table 4.5).

Some cases of AML following topoisomerase-II-interactive drugs have the same clinical features and cytogenetic and molecular genetic abnormalities as are seen in de novo AML. They can be described by the term ‘de novo-type’ AML. The WHO classification incorporates the four numerically most important cytogenetic categories of de novo AML, associated with simple chromosomal rearrangements and a single major genetic event, but categorizes separately cases with the same cytogenetic abnormality that follow therapy with topoisomerase-II-interactive drugs. The MIC-M classification categorizes primarily by molecular event. It is applicable to cases of de novo and de novo-type AML in which there is one major oncogenic event. It is not applicable to many cases of AML in the elderly, myelodysplasia-related AML and therapy-related AML following alkylating agents and related drugs, all of which are consequent on an accumulation of multiple genetic events. This type of AML shows a different range of cytogenetic abnormalities. The most characteristic are monosomy 5, del(5)(q), monosomy 7, del(7)(q), trisomy 8, trisomy 9, del(11)(q), del(12)(p), monosomy 18, monosomy 19, del(20)(q), trisomy 21 and complex chromosomal rearrangements.

Problems and pitfalls in the diagnosis of AML

It is sometimes difficult to distinguish cases of M0 and M1 AML from ALL on the basis of examination of H&E-stained sections. Some histological features are useful. ALL is usually associated with effacement of the bone marrow whereas, in AML, there may be residual myeloid cells showing dysplastic features. ALL blasts tend to have more scanty cytoplasm and more chromatin condensation. If difficulty is experienced in making the distinction and if diagnosis depends on the trephine biopsy sections, immunohistochemistry should be used. However, it should be noted that immunohistochemistry for MPO and other myeloid antigens is less sensitive for the detection of myeloid differentiation than cytochemistry and immunophenotyping by flow cytometry. For this reason, there are some cases of M0 and M1 AML that cannot be distinguished from ALL histologically, even with the help of immunohistochemistry.

Other diagnostic problems relate mainly to M6 AML, M7 AML and hypoplastic AML. M6 AML and megaloblastic anaemia can be confused with each other in trephine biopsy sections or, less often, in a bone marrow aspirate. This is a very serious error which must be avoided. It arises mainly because the diagnosis of AML is not considered or because a case of M6 has megaloblastic erythropoiesis. Diagnostic error can be avoided by careful attention to cytological details (see page 371) supplemented, when necessary, by assays of vitamin B₁₂ and folic acid. A therapeutic trial of haematologic agents is sometimes useful.

M7 AML and acute panmyelosis with myelofibrosis, when accompanied by dense fibrosis, can be confused with chronic idiopathic myelofibrosis (see page 204). The clinical and haematological features of these three conditions differ. Marked splenomegaly is usual in idiopathic myelofibrosis but not in M7 AML or acute panmyelosis with fibrosis. A leuco-erythroblastic anaemia with marked poikilocytosis is likewise usual in idiopathic myelofibrosis but not in M7 AML or acute panmyelosis. Bone marrow aspiration usually fails or yields inadequate material for diagnosis in all these conditions. However, histology shows increased blast cells in M7 AML and acute panmyelosis and not in idiopathic myelofibrosis. AML with myelofibrosis can also be
confused with bone marrow infiltration by a non-
haemopoietic tumour with secondary myelofibrosis.
Dysplastic megakaryocytes and metastatic tumour
cells can be distinguished by immunohistochemistry.

Hypoplastic AML must be distinguished from
aplastic anaemia and from hypoplastic MDS. In-
creased reticulin deposition and dysplastic mega-
karyocytes suggest a diagnosis of AML or MDS

### Table 4.5
A morphological, immunophenotypic, cytogenetic, molecular genetic (MIC-M) classification of acute myeloid

<table>
<thead>
<tr>
<th>Cytogenetic abnormality</th>
<th>Molecular genetic abnormality</th>
<th>FAB category</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(1;11)(p32;q23)*</td>
<td>MLL-AF1p fusion</td>
<td>M5</td>
</tr>
<tr>
<td>t(1;11)(q21;q23)*</td>
<td>MLL-AF1q fusion</td>
<td>M4</td>
</tr>
<tr>
<td>t(1;11)(q23;p15)</td>
<td>NUP98-PMX1</td>
<td>M2</td>
</tr>
<tr>
<td>t(2;11)(q35;p15)</td>
<td>NUP98-HOXD13</td>
<td>Therapy-related</td>
</tr>
<tr>
<td>inv(3)(q21q26) or t(3;3)(q21;q26)</td>
<td>EVI1 dysregulation</td>
<td>Various</td>
</tr>
<tr>
<td>t(3;5)(q25.1;q34)</td>
<td>NPM-MLF1 fusion</td>
<td>Various</td>
</tr>
<tr>
<td>t(3;12)(q26;p13)</td>
<td>Fusion of various genes at 3q26 with TEL at 12p13</td>
<td>Various</td>
</tr>
<tr>
<td>t(3;21)(q26;q22)</td>
<td>Heterogeneous, mainly AML1-EAP, AML1-EV1 and AML1-MDS1</td>
<td>Variable</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)*</td>
<td>MLL-AF4 fusion</td>
<td>M5</td>
</tr>
<tr>
<td>t(4;12)(q11–12;p13)</td>
<td>BTV-TEL (BTV-ETV6)</td>
<td>M0</td>
</tr>
<tr>
<td>t(5;17)(q32;q21)</td>
<td>NPM-RARα fusion</td>
<td>M3-like</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>DEK-CAN fusion</td>
<td>M2Baso</td>
</tr>
<tr>
<td>t(6;11)(q27;q23)*</td>
<td>MLL-AF6 fusion</td>
<td>M4 or M5</td>
</tr>
<tr>
<td>t(7;11)(p15;p15)</td>
<td>NUP98-HOXA9 fusion</td>
<td>M2</td>
</tr>
<tr>
<td>inv(8)(p11q13)</td>
<td>MOZ-TIF2 fusion</td>
<td>M7*</td>
</tr>
<tr>
<td>t(8;16)(p11;p13)</td>
<td>MOZ-CBP fusion</td>
<td>M4 or M5</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)†</td>
<td>AML1-ETO fusion</td>
<td>M2</td>
</tr>
<tr>
<td>t(9;11)(p21–22;q23)*</td>
<td>MLL-AF9 fusion</td>
<td>M5</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABL fusion</td>
<td>M0, M1 or M2</td>
</tr>
<tr>
<td>t(10;10)(p12;q23)*</td>
<td>MLL-AF10 fusion</td>
<td>M5</td>
</tr>
<tr>
<td>t(10;11)(p11.2;q23)*</td>
<td>MLL-ABI1 fusion</td>
<td>AML</td>
</tr>
<tr>
<td>t(10;11)(p13;q14)</td>
<td>CALM-AF10 fusion</td>
<td>M4</td>
</tr>
<tr>
<td>ins(11;9)(q23;p22p23)*</td>
<td>MLL-AF9 fusion</td>
<td>M5</td>
</tr>
<tr>
<td>inv(11)(p15q22)</td>
<td>NUP98-DDX10 fusion</td>
<td>Variable</td>
</tr>
<tr>
<td>t(11;16)(q23;p13)</td>
<td>MLL-CBFβ fusion</td>
<td>Variable</td>
</tr>
<tr>
<td>t(11;17)(q23;q21)*</td>
<td>MLL-AF17 fusion</td>
<td>M5</td>
</tr>
<tr>
<td>t(11;17)(q23;q21)</td>
<td>PLZF-RARα fusion</td>
<td>M3-like</td>
</tr>
<tr>
<td>t(11;19)(q23;p13.1)*</td>
<td>MLL-ELL fusion</td>
<td>M4 or M5</td>
</tr>
<tr>
<td>t(11;19)(q23;p13.3)*</td>
<td>MLL-ENL fusion</td>
<td>M4 or M5</td>
</tr>
<tr>
<td>t(11;20)(p15;q11)</td>
<td>NUP98-TOP1 fusion</td>
<td>t-MDS or AML</td>
</tr>
<tr>
<td>t(11;22)(q23;q13)*</td>
<td>MLL-p300 fusion</td>
<td>AML</td>
</tr>
<tr>
<td>+11, 11q+ or normal*</td>
<td>Partial tandem duplication of MLL</td>
<td>AML</td>
</tr>
<tr>
<td>t(12;13)(p13;q12)</td>
<td>ETV6(TEL)-CDX2 fusion</td>
<td>AML</td>
</tr>
<tr>
<td>t(12;22)(p13;q11)</td>
<td>MN1-TEL fusion</td>
<td>Variable</td>
</tr>
<tr>
<td>t(15;17)(q22;q21)†</td>
<td>PML-RARα fusion</td>
<td>M3</td>
</tr>
<tr>
<td>inv(16)(p13q22)† or t(16;16)(p13;q22)†</td>
<td>CBFβ-MYH11 fusion</td>
<td>M4 or M5</td>
</tr>
<tr>
<td>t(16;21)(p11;q22)</td>
<td>FUS-ERG fusion</td>
<td>Variable</td>
</tr>
<tr>
<td>t(X;11)(q13;q23)‡</td>
<td>MLL-AFX fusion</td>
<td>M4 or M5</td>
</tr>
</tbody>
</table>

* Grouped together in the WHO classification as ‘AML with 11q23 (MLL) abnormalities.’
† Specific WHO categories of AML.
rather than aplastic anaemia. In addition, in hypoplastic AML and hypoplastic MDS there may be some circulating blasts and the bone marrow aspirate, although inadequate for diagnostic purposes, may also show blast cells. There is no increase in blast cells in aplastic anaemia. Accurate differential diagnosis of these three conditions requires good quality sections of trephine biopsy specimens, which need to be examined carefully with high power magnification so that the proportion of blast cells can be estimated. If there are more than 20% blast cells, the diagnosis is AML and, if blasts are increased but less than 20%, the diagnosis is MDS.

AML of M5a subtype can sometimes be confused with large cell non-Hodgkin’s lymphoma. If monocytes are very immature, they may lack peroxidase and non-specific esterase activity. Flow cytometry immunophenotyping or immunohistochemistry is then very important in making the correct diagnosis.

The myelodysplastic syndromes

As discussed at the beginning of this chapter, the MDS are diseases consequent on a clonal haemo poetic disorder characterized by dysplastic, ineffective haemopoiesis. There is thus often a discrepancy between a hypercellular bone marrow and peripheral cytopenia. In any one patient, different haemopoietic lineages are not necessarily affected to the same degree and there may be defective production of cells of one lineage while in another lineage normal numbers of cells are produced. There may even be increased production of cells of one or more lineages—for example, neutrophils, monocytes or platelets—despite other features typical of MDS (see page 215).

The MDS are predominantly diseases of the elderly with an incidence of the order of 70 cases/100 000/year.

Clinical features of MDS result from the various cytopenias; there may be haemorrhage, susceptibility to infections and symptoms of anaemia. Some patients have hepatomegaly and splenomegaly. MDS show a tendency to evolve into more severe forms of MDS and into acute leukaemia. Most cases of MDS are apparently primary but a minority are secondary to exposure of the bone marrow to known mutagens, such as alkylating agents. There are some differences in laboratory and clinical features between primary and secondary MDS.

Diagnosis of MDS requires consideration of clinical, peripheral blood, bone marrow and cytogenetic features. Peripheral blood and bone marrow aspirate findings are most important and, in a straightforward case, may be all that is required for diagnosis. Bone marrow trephine biopsy in general offers only supplementary information; however, sometimes it is necessary for confirmation of the diagnosis, for example, when an excess of blasts or an abnormal localization of blasts is detected in a patient who has other features suggestive but not diagnostic of MDS. Bone marrow trephine biopsy is particularly important in patients with secondary MDS, in whom a hypocellular bone marrow with increased fibrosis often leads to a non-diagnostic aspirate. In some patients, a firm diagnosis cannot be made on cytological and histological features alone, but diagnosis is possible when these are supplemented by cytogenetic analysis or other tests giving information about clonality. An iron stain should be performed in all patients with suspected myelodysplasia; this demonstrates any ring sideroblasts as well as permitting an assessment of iron stores. An MPO or SBB stain should be performed at least in all patients with any increase in blasts; this will facilitate the detection of Auer rods, which are of importance both in diagnosis and in classification.

The MDS are a heterogeneous group of disorders with very variable prognoses. They can be divided into various disease categories which have more uniform clinicopathological characteristics. Until now the most widely used categorization has been that proposed by the FAB group [1,2,26]. The FAB classification is based on the presence or absence of significant sideroblastic erythropoiesis, on the numbers of monocytes in the peripheral blood and on the number of blasts in the peripheral blood and bone marrow (Table 4.6). The FAB categories are:

1. refractory anaemia (RA) or refractory cytopenia;
2. refractory anaemia with ring sideroblasts (RARS);
3. refractory anaemia with excess of blasts (RAEB);
4. chronic myelomonocytic leukaemia (CMML); and
5. refractory anaemia with excess of blasts in transformation (RAEB-T).

More recently, a WHO classification of MDS has been proposed (Table 4.7) [5]. The most important difference from the FAB classification is that the
ACUTE MYELOID LEUKAEMIA 169

RAEB-T category has been abolished. Cases with more than 20% of bone marrow blasts are classified as AML. In addition, CMML has been classified as a mixed myelodysplastic/myeloproliferative disease rather than as one of the MDS. The WHO group has also made a distinction between cases of RA and RARS with and without dysplasia of other lineages, since this is of prognostic significance.

Some morphological abnormalities are characteristic of MDS, without being specific for them, while others show sufficient specificity to be useful in confirming the diagnosis. Although the MDS are heterogeneous they also have many features in common. We will therefore describe these syndromes as a group before discussing specific categories of disease. CMML will be discussed mainly in the next chapter (see page 215). The other FAB categories of MDS will be discussed in this chapter, retaining the FAB definitions since the WHO classification has been published only in outline.

**Peripheral blood**

Anaemia is seen in the great majority of patients. Red cells are usually normochromic and either normocytic or macrocytic. In patients with sideroblastic erythropoiesis there is commonly a dimorphic blood film with a mixture of a minority population of hypochromic microcytes and a majority population of normochromic cells which are either normocytic or, more commonly, macrocytic; Pappenheimer bodies, the nature of which can be confirmed with an iron stain, may be present. Microcytosis is seen in certain rare variants including acquired haemoglobin H disease. Some patients have occasional circulating erythroblasts which may include dysplastic forms such as megaloblasts and, in patients with sideroblastic erythropoiesis, ring sideroblasts.

Neutropenia is common, particularly in RAEB and RAEB-T. Neutrophils often show dysplastic features

---

**Table 4.6** The FAB classification of the myelodysplastic syndromes [1,2,8,26].

<table>
<thead>
<tr>
<th>Category</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory anaemia (RA) or refractory cytopenia*</td>
<td>Anaemia*</td>
<td>Blasts &lt;5%, ringed sideroblasts ≤15% of erythroblasts</td>
</tr>
<tr>
<td>Refractory anaemia with ringed sideroblasts (RARS)</td>
<td>Anaemia, Blasts ≤1%</td>
<td>Blasts &lt;5%, ringed sideroblasts &gt;15% of erythroblasts</td>
</tr>
<tr>
<td>Refractory anaemia with excess of blasts (RAEB)</td>
<td>Anaemia, Blasts ≤1%</td>
<td>Blasts ≥5%</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukaemia (CMML)</td>
<td>Blasts ≤5%</td>
<td>Blasts up to 20% of promonocytes often increased</td>
</tr>
<tr>
<td>Refractory anaemia with excess of blasts in transformation (RAEB-T)</td>
<td>Blasts ≥5% or Auer rods in blasts in blood or marrow</td>
<td>Blasts &gt;20% but &lt;30%</td>
</tr>
</tbody>
</table>

* Or in the case of refractory cytopenia, either neutropenia or thrombocytopenia.

**Table 4.7** The WHO classification of the myelodysplastic syndromes [5].

- Refractory anaemia
  - With ringed sideroblasts
  - Without ringed sideroblasts
- Refractory cytopenia with multilineage dysplasia
  - 5q- syndrome
- Refractory anaemia with excess blasts
  - Myelodysplastic syndrome, unclassifiable
  - Myelodysplastic syndromes, therapy-related
  - Alkylating agent-related
  - Epipodophyllotoxin-related
including reduced granulation and the acquired or pseudo-Pelger–Huët anomaly. Hypogranular and agranular neutrophils (Fig. 4.29) are consequent on defective formation of secondary granules; agranular neutrophils are highly specific for the MDS [27]. The acquired Pelger–Huët anomaly refers to hypolobulation of nuclei associated with dense chromatin clumping (Fig. 4.29); nuclei of mature neutrophils may be completely non-lobed, dumb-bell or peanut-shaped, or bilobed with the shape resembling a pair of spectacles. This abnormality resembles the inherited Pelger–Huët anomaly, hence its name. The acquired anomaly is highly characteristic of the MDS and almost pathognomonic [27]. Eosinophil and basophil counts are commonly reduced but, in a small minority of patients, are increased; dysplastic forms with abnormalities of either nuclear shape or cytoplasmic granularity can occur. Monocytosis is sometimes present and monocytes may show cytological abnormalities such as increased cytoplasmic basophilia or nuclei of unusual shape. Blast cells may be present in the peripheral blood in all categories of MDS but particularly in RAEB and RAEB-T. They usually have the cytological features of myeloblasts with scanty cytoplasm and few granules. Auer rods are sometimes present. Other
granulocyte precursors are quite uncommon in the peripheral blood.

The platelet count is usually either normal or reduced. In a minority of patients it is increased. Dysplastic features which may be noted in platelets include hypogranular and agranular forms ('grey' platelets) and giant platelets.

Patients with MDS have an increased incidence of auto-immune thrombocytopenia [28].

**Bone marrow cytology**

The bone marrow is hypercellular in the majority of patients but is sometimes normocellular and in about 10% of patients is hypocellular. Hypercellularity may be due to hyperplasia of erythroid or granulocytic series or both.

Erythropoiesis may be normoblastic, macronormoblastic or megaloblastic. In patients with sideroblastic erythropoiesis there are some erythroblasts with poorly haemoglobinized or vacuolated cytoplasm. Other dysplastic features may include: (i) binuclearity and multinuclearity; (ii) internuclear bridges; (iii) nuclear lobulation; (iv) irregularity or fragmentation of nuclei; (v) gigantism; (vi) increased pyknosis; and (vii) basophilic stippling. The bone marrow erythroid component (calculated from cellularity and the percentage of erythroblasts) has been found to be predictive of response to therapy with erythropoietin plus GM-CSF [29].

Granulopoiesis is usually hyperplastic. Defects of granulation may be apparent from the promyelocyte stage onwards and defects of nuclear lobulation may also be present.

Megakaryocyte numbers are usually normal or increased but sometimes decreased. One of the features most specific for the MDS is the presence of micromegakaryocytes [27], cells of about the size of a myeloblast with one or two small round nuclei (Fig. 4.30). Megakaryocytes may also be of normal size but have a large non-lobulated nucleus (Fig. 4.31); this abnormality is less specific for MDS but is characteristic of cases with 5q- as an acquired chromosomal abnormality [30]. Other megakaryocyte abnormalities include bizarre nuclear shapes and the presence of multiple separate nuclei. Poor granulation of megakaryocyte cytoplasm is also common in the MDS and has been found to be highly specific [31].

The bone marrow aspirate may show non-specific abnormalities such as increased numbers of macrophages, sea-blue histiocytes, lymphocytes, plasma cells or mast cells.

**Cytochemistry**

The cytochemical stain of most value is an iron stain. This should be performed in all cases of suspected MDS, in order to quantify iron stores and to detect and enumerate ring sideroblasts and other abnormal
sideroblasts. Ring sideroblasts have iron-positive granules in a circle close to the nuclear membrane (Fig. 4.32). Other abnormal sideroblasts have scattered iron-positive granules which are both larger and more numerous than those of normal siderocytes. Ring sideroblasts are highly suggestive of MDS if the other known causes of sideroblastic erythropoiesis (see page 36) can be eliminated. Abnormal sideroblasts, other than ring sideroblasts, are common both in MDS and in other disorders of erythropoiesis so are not useful in the differential diagnosis of suspected MDS. Other cytochemical stains are of use in identifying abnormal cells of megakaryocyte lineages, in characterizing blasts and in detecting Auer rods. MPO and SBB stains will identify myeloblasts and may also show cells of the neutrophil lineage to have defective primary granules. Non-specific esterase stains are useful for identifying monoblasts; non-specific esterase and PAS stains are valuable for identifying abnormal megakaryocytes.

**Bone marrow histology**

In the majority of cases the marrow is hypercellular (Fig. 4.33), but a significant minority have a hypo-
cellular marrow [32,33]. There may be considerable variation of the cellularity between adjacent intertrabecular spaces [34]. In addition to cytological evidence of dysplasia, there is derangement of normal architecture. In histological sections, dysplasia is most obvious in the erythroid precursors and megakaryocytes; however, the acquired Pelger–Huët anomaly can be detected in good quality sections of paraffin-embedded material and, in good plastic-embedded material, Auer rods may also be identified. Disturbance of normal architecture results in groups of granulocytic precursors being found in the central parts of intertrabecular spaces (Fig. 4.34) and erythroid precursors and megakaryocytes in the paratrabecular regions. Erythroblastic islands may be poorly formed or very large and erythroid precursors may be multinucleated or show nuclear budding or fragmentation, megaloblastic change or cytoplasmic vacuolation (Fig. 4.35). Megakaryocytic dysplasia is present in the vast majority of cases and is usually more apparent in histological sections than in marrow films. They are usually increased in number and clustering is often seen (Fig. 4.36). Typically, they have hypolobulated nuclei which are often hyperchromatic; the small dysplastic megakaryocytes are usually referred to as
micromegakaryocytes [33,35]. Emperipolesis is increased [36]. Immunohistochemical staining with anti-GpIIa (CD61) and anti-GpIb (CD42b) may be used to accentuate the abnormal megakaryocytes (Fig. 4.37) [37]. Increased numbers of apoptotic erythroid and granulocytic precursors are commonly seen in MDS consequent on ineffective haemopoiesis (see Fig. 4.33) [38]. In a minority of cases, haemopoietic cells, particularly megakaryocytes, are present within sinusoids [36]. Reticulin fibrosis has been reported in a fifth [39] to almost a half [33] of cases of MDS, as defined by the FAB group. It is more common in chronic myelomonocytic leukaemia (now designated a myelodysplastic/myeloproliferative disorder) than in other subtypes [33,39]. The presence of reticulin fibrosis correlates with megakaryocyte numbers and atypia [39]. Severe collagen fibrosis is rare in all subtypes [33,39]. It is seen most often in secondary MDS. Reticulin fibrosis correlates with unfavourable cytogenetic abnormalities and is indicative of a worse prognosis. Other non-specific reactions are commonly seen including oedema, ectasia of sinusoids, increased numbers of plasma cells and increased

Fig. 4.36 BM trephine biopsy section, RA, showing an aggregate of dysplastic megakaryocytes. Paraffin-embedded, H&E ×192.

Fig. 4.37 BM trephine biopsy section, therapy-related MDS, showing dysplastic small megakaryocytes. Paraffin-embedded, immunoperoxidase with a CD61 monoclonal antibody ×970.
numbers of lymphoid follicles. Haemosiderin-laden macrophages are a frequent finding, particularly in patients who have received transfusions.

One feature that has been the subject of much debate is the significance of small groups of immature granulocytic precursors (promyelocytes and myeloblasts) in a central position within intertrabecular spaces (see Fig. 4.34). This has been termed abnormal localization of immature precursors (ALIP). Some studies have found this phenomenon to be an independent predictor of prognosis and to be associated with an increased incidence of leukaemic transformation [40]. Although ALIP is more frequent in the subtypes of myelodysplasia with increased numbers of blasts in the marrow, several recent studies have failed to confirm any independent influence on prognosis [33,34,41]. Others, however, have confirmed prognostic significance independent of the blast percentage in an aspirate [36] or in histological sections [42]. It should be noted that it can be difficult, particularly in paraffin-embedded sections, to distinguish between small groups of immature erythroid precursors and the clusters of immature cells of granulocytic lineage that are seen in ALIP. Aggregates of immature erythroid cells, which can mistakenly be taken as evidence of ALIP, can be distinguished by their positive reactions with antibodies to glycophorin or spectrin, whereas the immature cells in ALIP may react positively with CD68, ant myeloperoxidase and antineutrophil elastase antibodies. Other histological features found to be of poor prognostic significance, in one study using multivariate analysis, were an elevated blast percentage, increased haemosiderin, megakaryocyte atypia and reduced erythropoiesis, while increased mast cells were of good prognostic significance [42].

**Immunocytochemistry and flow cytometry in MDS**

Immunophenotyping can be used to confirm the lineage of any blast cells present but, in general, immunophenotyping is of little value in MDS.

**Immunohistochemistry**

The value of immunohistochemistry in MDS can be summarized as follows:

1. Abnormal topography can be detected, e.g. the presence of ALIP or the presence of erythroid cells or megakaryocytes in a paratrabecular position;
2. Immature cells in ALIP can be distinguished from clusters of immature erythroid cells (Fig. 4.38);
3. Abnormally large erythroid islands can be identified;
4. Micromegakaryocytes can be identified and the presence of megakaryocyte clustering or dysplasia highlighted (see Fig. 4.37);
5. Prognostic information can be gained by the use of CD34 antibodies (Fig. 4.39)—the presence of more than 1% of CD34-positive cells is indicative of a worse prognosis in MDS as a whole and within the
RAEB category [43]: clusters of CD34-positive cells are predictive of leukaemic transformation; and making a distinction between hypocellular MDS and aplastic anaemia can be facilitated (see below).

**Cytogenetics and molecular genetics**

The cytogenetic abnormalities associated with MDS are heterogeneous. The most characteristic abnormalities are monosomies, deletions and unbalanced translocations. Abnormalities often observed include monosomy 5, monosomy 7, trisomy 8, del(5)(q), del(7)(q), del(9)(q) and del(20)(q). In MDS secondary to alkylating agents, monosomies and deletions of chromosomes 5 and 7 are common, whereas MDS secondary to topoisomerase-II-interactive drugs is characterized by balanced translocations with 3q26, 11q23 and 21q22 breakpoints.

Clonal cytogenetic abnormalities can confirm the diagnosis of MDS. The type of abnormality found is also of prognostic significance.

MDS is typically associated with multiple oncogenic events, formation of fusion genes, mutations of oncogenes and both mutation and loss of cancer suppressing genes. Genes that may be mutated include N-RAS, p53, IRF1, BCL2, p15

**Problems and pitfalls**

Diagnostic errors can result from a failure to assess clinical features, peripheral blood and bone marrow cytology, bone marrow histology and the results of cytogenetic analysis in all cases. Cytology and histology are complementary in the investigation of MDS since sometimes one will give information that could not be gained from the other. For example, ring sideroblasts and neutrophil dysplasia are best detected in an aspirate, whereas ALIP is detected only by means of trephine biopsy. Similarly, it is sometimes cytogenetic analysis that permits an unequivocal diagnosis when other features have been suggestive of MDS but not pathognomonic.

Some cases of MDS have pathological features that are strongly suggestive of the diagnosis. In other patients the diagnosis of MDS is a presumptive one, based on the presence of features that are characteristic but not diagnostic. In the latter group the exclusion of other diagnoses is particularly important. A careful clinical assessment is essential, in order to exclude relevant systemic illness and exposure to drugs, alcohol, heavy metals and growth factors. Some of the non-neoplastic causes of bone marrow dysplasia are discussed on page 392. Important pitfalls are relevant drug exposure that has not been disclosed to the pathologist and unexpected HIV positivity.

If dysplastic features are confined to the erythroid lineages, it is important to consider alternative causes of dyserythropoiesis such as the congenital dyserythropoietic anaemias and various thalassaemic conditions. Unstable haemoglobins are also sometimes associated with quite marked dyserythropoiesis.
It can sometimes be difficult to distinguish megaloblastic erythropoiesis as a consequence of MDS from that attributable either to a deficiency of vitamin B\textsubscript{12} or folic acid or to the administration of drugs that interfere with DNA synthesis (Fig. 4.40). A useful feature is the lack of associated white cell changes—giant metamyelocytes and hypersegmented neutrophils—when megaloblastosis is a feature of MDS. However, it should be noted that white cell abnormalities may be lacking in megaloblastic erythropoiesis caused by drug exposure. Occasionally cohesive clumps or sheets of erythroid cells, all at a similar stage of maturation, can be confused with infiltration by carcinoma cells (Fig. 4.41).

The differential diagnosis of RARS includes sideroblastic erythropoiesis secondary to drugs or heavy metals (see page 397), copper deficiency (see page 397), the mitochondrial cytopathies (see page 407) and thiamine-responsive anaemia with diabetes mellitus and sensorineural deafness. The latter condition may show granulocytic and megakaryocytic dysplasia in addition to ring sideroblasts [44].

The differential diagnosis of hypoplastic AML and hypoplastic MDS (Fig. 4.42) has been discussed on
Immunohistochemistry has been found to be of some value in making a distinction between hypoplastic MDS and aplastic anaemia [45,46]. Cases of hypocellular MDS have been found to have higher numbers of CD34-positive cells and cells expressing proliferating cell nuclear antigen [45].

Lymphoid aggregates are sometimes present in MDS, giving rise to a differential diagnosis with non-Hodgkin’s lymphoma, particularly T-cell lymphoma, with associated dysplastic features. It should also be remembered that some patients with lymphomatous infiltration of the bone marrow have secondary dysplastic changes resembling those of MDS (Fig. 4.43).

In the rare patients with MDS who present with isolated thrombocytopenia it is sometimes difficult to make a distinction from auto-immune thrombocytopenic purpura. Dysplastic features may be very minor.

It should be noted that, even with a careful assessment of clinical features and use of all available diagnostic methods, it may still not be possible to make a firm diagnosis of MDS. In such patients follow-up with regular review of the diagnosis is necessary.
The FAB categories and other identified subtypes of MDS

Refractory anaemia

RA is characterized by ineffective erythropoiesis, with or without ineffective granulopoiesis and thrombopoiesis but, as defined by the FAB group, there are insufficient monocytes, blast cells or ring sideroblasts for the case to qualify for inclusion in other categories of MDS (see Table 4.6). RA is usually either an incidental diagnosis in the elderly or is diagnosed because of symptoms of anaemia. Many cases belonging to the FAB category of RA fall into the WHO category of ‘refractory anaemia without ringed sideroblasts’ but others are designated ‘5q-syndrome’ (see below) or ‘refractory cytopenia with multilineage dysplasia’.

Peripheral blood

Often morphological and numerical abnormalities are confined to the erythroid series but some patients, particularly those with secondary MDS, manifest anomalies of other lineages. A minority of patients show marked erythroid hypoplasia, sometimes with an apparent arrest of erythropoiesis at the proerythroblast stage. Erythropoiesis usually shows dysplastic features but in some patients erythropoiesis is ineffective although dysplastic features are quite minor. Ring sideroblasts may be present but constitute no more than 15% of erythroblasts.

In RA the granulocyte series and megakaryocytes may be apparently normal or may be hyperplastic or dysplastic (see Figs 4.30 and 4.31).

Bone marrow histology

There are often no histological features of diagnostic importance in trephine biopsies of patients with RA. The marrow is usually hypercellular but hypocellular forms do occur. Erythroid hyperplasia and dyserythropoiesis are usually present and are easily seen in tissue sections (see Fig. 4.33). The granulocytic series may appear relatively normal. Dysplastic megakaryocytes are found in most cases; however, they are not universally present and, in their absence, the diagnosis of myelodysplasia may be easily overlooked if the clinical and cytological features are not taken into account.

Bone marrow cytology

The bone marrow is usually hypercellular as a result of erythroid hyperplasia (Fig. 4.44). A minority of patients show marked erythroid hypoplasia, sometimes with an apparent arrest of erythropoiesis at the proerythroblast stage. Erythropoiesis usually shows dysplastic features but in some patients erythropoiesis is ineffective although dysplastic features are quite minor. Ring sideroblasts may be present but constitute no more than 15% of erythroblasts.

Refractory cytopenia

A small proportion of patients with MDS are not anaemic but have refractory neutropenia or
thrombocytopenia. The FAB group has recommended that, if such cases lack the features of the other categories of MDS, they should be grouped with refractory anaemia and be designated refractory cytopenia.

Patients with refractory neutropenia show abnormalities predominantly of the neutrophil lineage, while patients with refractory thrombocytopenia have increased, dysplastic megakaryocytes and often dysplastic platelets.

**Refractory anaemia with ring sideroblasts**

RARS is also referred to as primary acquired sideroblastic anaemia. The FAB group criteria for this diagnosis are the presence of more than 15% of ring sideroblasts among bone marrow erythroblasts, with monocytes and blast cells being insufficiently increased to permit assignment to other MDS categories (see Table 4.6). In the WHO classification the majority of cases fall into the category ‘refractory anaemia with ring sideroblasts’ and a minority into the category ‘refractory cytopenia with multilineage dysplasia’. Sideroblastic anaemia is usually either an incidental diagnosis in the elderly or is diagnosed because of symptoms of anaemia.

**Peripheral blood**

There is anaemia which is sometimes normocytic but more often macrocytic. The film is dimorphic, consequent on the presence of a minor population of hypochromic and microcytic red cells. Occasional cells contain Pappenheimer bodies. There may be a small number of circulating erythroblasts, among which may be some ring sideroblasts. Abnormalities of neutrophils and platelets can occur but are uncommon. A significant minority of patients have thrombocytosis.

**Bone marrow cytology**

The bone marrow is usually hypercellular and shows erythroid hyperplasia. Erythropoiesis is usually normoblastic or macronormoblastic. A proportion of erythroblasts, which correspond to the ring sideroblasts, are micronormoblastic or show defective haemoglobinization or cytoplasmic vacuolation (Fig. 4.45). Other dysplastic features in red cells are uncommon. Abnormalities may occur in other lineages but they are uncommon except when the MDS is secondary.

By definition, in RARS an iron stain shows that more than 15% of erythroblasts are ring sideroblasts (see Fig. 4.32). They may be as frequent as 70 or 80% of erythroblasts and may be associated with other abnormal sideroblasts. Iron stores are commonly increased.

**Bone marrow histology**

Trephine biopsy is not usually very useful in the diagnosis of RARS. Bone marrow histology may be
relatively normal with the only abnormality being erythroid hyperplasia with large, poorly formed erythroid islands. There is often an increase in stainable iron within macrophages. Ring sideroblasts can be seen in plastic-embedded sections of trephine biopsies and occasionally in paraffin-embedded marrow clot sections (Fig. 4.46); they are not visible in sections of paraffin-embedded or other decalcified trephine biopsy specimens. The granulocytic series is usually normal. Dysplastic megakaryocytes are present in a minority of cases.

**Refractory anaemia with excess of blasts**

RAEB as defined by the FAB group (see Table 4.6) has:

- *either* an increase of peripheral blood blasts to more than 1% but less than 5%;
- *or* an increase of bone marrow blasts to at least 5% but not more than 20%;
- *and* no Auer rods;
- *and* a monocyte count of less than $1 \times 10^9/l$ (or the case falls into the CMML category) (see page 215).
Diagnosis of RAEB usually follows the development of symptoms of anaemia or the occurrence of bruising, bleeding or infection. This FAB category corresponds to the WHO category ‘refractory anaemia with excess blasts’.

**Peripheral blood**

The peripheral blood shows normocytic or macrocytic anaemia and may also show some hypochromic microcytic cells. In addition, there may be neutropenia, mild monocytosis or thrombocytopenia. Dysplastic features in neutrophils and platelets are commonly present. There may be some circulating blasts which are usually, but not necessarily, myeloblasts.

**Bone marrow cytology**

The bone marrow is usually hypercellular. Any or all lineages may be hyperplastic and trilineage dysplasia is common. The percentage of blasts is usually increased, although a case may qualify to be categorized as RAEB on the basis of increased peripheral blood blasts alone. Erythropoiesis may be sideroblastic but, because of the excess of blasts, the case is categorized as RAEB rather than as RARS.

An iron stain may show ring sideroblasts, other abnormal sideroblasts and increased iron stores. Either an MPO or a SBB stain should be performed routinely both to confirm the lineage of the blasts and to exclude the presence of Auer rods, which would lead to the case being categorized as RAEB-T (see below).

**Bone marrow histology**

Bone marrow biopsy is not usually essential for diagnosis but can give useful supplementary information. The majority of cases have increased or normal cellularity with only a small number of cases being hypocellular. Dyserythropoiesis and megakaryocytic dysplasia are seen in almost all cases (Fig. 4.47). Blasts are increased in number but it is not uncommon for the percentage of blasts seen in the biopsy sections to be less than that observed in marrow aspirates taken at the same time [33]. ALIP is seen in most cases.

**Refractory anaemia with excess of blasts in transformation**

As defined by the FAB group, the diagnosis of RAEB-T requires that:

- either peripheral blood blasts are at least 5%;
- or bone marrow blasts are greater than 20% but less than 30%;
- or there are Auer rods in blasts, either in the bone marrow or in the peripheral blood (see Table 4.6).

In practice, most cases have more than 20% blasts
in the bone marrow whether or not they show the other features. However, occasional cases are categorized as RAEB-T on the basis of one of the other criteria alone. In the WHO classification, cases with more than 20% bone marrow blasts would be categorized as AML rather than as MDS.

Patients with RAEB-T are almost always symptomatic at diagnosis with bruising, bleeding, infection or symptoms of anaemia. Pallor, bruising, hepatomegaly and splenomegaly are common.

**Peripheral blood**

Most patients have anaemia, neutropenia and thrombocytopenia and show morphological abnormalities of all lineages. Some circulating blasts are usually present and they may contain Auer rods. There may be monocytosis, particularly in those patients in whom the disease represents a transformation of CMML rather than RAEB.

**Bone marrow cytology**

The bone marrow is usually hypercellular with trilineage myelodysplasia, an increase of blasts and sometimes Auer rods in the blasts. Sideroblastic erythropoiesis is not inconsistent with a diagnosis of RAEB-T.

**Bone marrow histology**

Trephine biopsy is not usually essential for diagnosis but can give useful supplementary information. In addition to trilineage dysplasia there is an increase in numbers of blasts, often with ALIP. Reticulin fibrosis has been reported to be less common than in the other subtypes [33].

**The 5q- syndrome**

Among patients with MDS a group of patients can be delineated who have what is designated the 5q- syndrome. This entity is a specific category in the WHO classification. Patients tend to be middle-aged or elderly women with a relatively good prognosis. Haemopoietic cells show an interstitial deletion of the long arm of chromosome 5 as a single acquired chromosomal anomaly. Such patients most often have a refractory anaemia, often macrocytic, with or without ring sideroblasts and also have characteristic megakaryocytes; these are more than 30 µm in diameter but have non-lobulated nuclei (see Fig. 4.31). The platelet count is usually normal or even increased. The disease usually falls into the RA category of the FAB classification but sometimes into the RARS category.

**Refractory macrocytosis**

Occasional patients with myelodysplasia have refractory macrocytosis but are not anaemic and lack features which would lead to their being assigned to any of the FAB categories of MDS. When erythropoiesis is clonal, they should be recognized as having MDS and can reasonably be grouped with RA. With long-term follow-up, anaemia and other features of overt MDS develop.

**Refractory sideroblastic erythropoiesis**

Occasional patients are seen with primary acquired sideroblastic erythropoiesis who lack anaemia and other features which would allow them to be assigned to one of the FAB categories of MDS [47]. Nevertheless, erythropoiesis is clonal and such patients should be recognized as having MDS. They can reasonably be grouped with RARS. With disease progression, anaemia occurs.

**Secondary myelodysplasia**

Myelodysplasia may be secondary to deliberate or accidental exposure to mutagenic agents such as cytotoxic chemotherapy, benzene and irradiation. Such cases can be categorized according to the FAB recommendations but, because they have distinctive features, it is useful to consider them separately. Secondary MDS usually occurs at a younger age than primary MDS and has a much worse prognosis. Bone marrow failure and evolution to acute leukaemia occur much earlier. Many cases of secondary MDS fall into the WHO category of ‘AML and myelodysplastic syndromes, therapy-related’.

**Peripheral blood**

Abnormalities in the peripheral blood are usually more marked than in primary MDS. Even cases
which fall into the RA and RARS categories commonly show neutropenia, thrombocytopenia, monocytosis and evidence of trilineage dysplasia.

**Bone marrow cytology**

The bone marrow may be hypercellular but is often hypocellular. A poor aspirate may be obtained because of associated bone marrow fibrosis. Obvious trilineage myelodysplasia is common, even in cases which meet the criteria for RA and RARS. There are usually at least some ring sideroblasts. Hypogranular neutrophils, pseudo-Pelger–Huët neutrophils and micromegakaryocytes are also common.

**Bone marrow histology**

Because of the frequent difficulty experienced in obtaining a good aspirate, trephine biopsy is often important in diagnosis. The cellularity is more variable than in primary myelodysplasia and stromal injury may be prominent, with disruption of fat cells and extravasation of non-nucleated red cells into the interstitium. There is often a marked increase in reticulo and, in contrast to primary myelodysplasia, there may be collagen fibrosis. There is often a severe degree of dysplasia affecting all three lineages with numerous micromegakaryocytes.

**Malignant histiocytosis**

Malignant histiocytosis is a disease caused by the proliferation in tissues of a neoplastic clone of cells of monocyte/macrophage lineage: the abnormal cells show variable phagocytic activity. This disease may be regarded as the tissue counterpart of acute monocytic leukaemia. It differs from monocytic sarcoma in that the cells of the neoplastic clone are widely distributed in peripheral tissues rather than forming localized tumours. This disease is not specifically recognized by the WHO classification and would fall into the category ‘histiocytic neoplasms, unclassifiable’.

It appears likely that, in the past, a significant proportion of diagnoses of malignant histiocytosis [48,49] or of histiocytic medullary reticulosis [50] (usually regarded as a form of the same disease) were actually misdiagnoses [51–55]. The majority of cases misinterpreted as malignant histiocytosis were either reactive histiocytosis consequent on viral or other infections or reactive histiocytosis occurring as a response to large cell anaplastic lymphoma and other T-lineage lymphomas. A less common cause of confusion is a T-cell lymphoma in which the lymphoma cells are themselves phagocytic [56]. It is important that the term malignant histiocytosis be restricted to cases in which neoplastic cells are of monocyte lineage. The term histiocytic medullary reticulosis is probably best abandoned since the recent availability of immunophenotyping and other techniques has led to the recognition that, in the great majority of cases, the histiocytic proliferation and florid haemophagocytosis were secondary to a T-cell lymphoma [54,55] or a viral infection [53]. The reactive haemophagocytic syndromes are discussed further on pages 119–123.

The diagnosis of malignant histiocytosis rests on clinical, histological, cytochemical and immunophenotypic grounds. Neoplastic cells are primitive and, although phagocytosis occurs, it is not prominent [51]. Neoplastic cells can be demonstrated, by cytochemical staining or immunophenotyping, to belong to the monocyte lineage and not to the T-lymphocyte lineage whereas, in haemophagocytic syndromes consequent on a T-cell lymphoma, there is an admixture of reactive mature phagocytic histiocytes and immature neoplastic cells of lymphoid lineage [54].

Common clinical features of malignant histiocytosis are hepatomegaly, splenomegaly, lymphadenopathy, skin infiltration and systemic symptoms such as malaise, fever and weight loss.

**Peripheral blood**

Pancytopenia is common. Small numbers of immature cells of monocyte/macrophage lineage may be present in the blood (Fig. 4.48).

**Bone marrow cytology**

At the onset of disease, the bone marrow may show minimal or no infiltration by neoplastic cells. With more advanced disease there may be heavy infiltration (Fig. 4.49). The majority of neoplastic cells have the morphological features of monoblasts or, in older terminology, ‘reticulum cells’. Cells are large and usually have a round nucleus with nucleoli and...
a diffuse chromatin pattern. Cytoplasm is plentiful and moderately basophilic. A variable number of maturing cells with kidney-shaped nuclei and more abundant cytoplasm are also present [51]. Some cells are phagocytic and are seen to have ingested granulocytes and their precursors, erythroblasts and platelets; however, phagocytosis is much less marked than in reactive haemophagocytosis.

**Bone marrow histology**

Bone marrow infiltration has been reported to be more commonly detected by trephine biopsy than by bone marrow aspiration [57]. The bone marrow may appear normal at the time of diagnosis or may show a mild focal infiltrate of neoplastic cells. There may be intrasinusoidal neoplastic cells [58]. In the later stages of the disease diffuse replacement of haemopoietic tissue commonly occurs (Fig. 4.50) [49,59,60]. The infiltrate is largely composed of immature cells with large pleomorphic nuclei which may be lobulated and contain prominent nucleoli; there are moderate amounts of basophilic cytoplasm. Mitoses are usually numerous. A variable component of more mature cells of monocytic lineage may be present.
**Genetics and cytogenetics**

Some cases of malignant histiocytosis have been associated with translocations that are also associated with AML with monocytic differentiation, such as t(9;11)(p22;q23) [60] and t(8;16)(p11;p13).

**Problems and pitfalls**

The diagnosis of malignant histiocytosis is fraught with pitfalls and should be made with great circumspection. A minor degree of haemophagocytosis may be seen but marked haemophagocytosis suggests an alternative diagnosis. In children, familial or sporadic lymphohistiocytosis is likely and investigation for herpesvirus infection is indicated. In adults, reactive haemophagocytosis is often caused by mycobacterial or herpesvirus infection or by a T-cell lymphoma. Molecular analysis to demonstrate T-cell receptor gene rearrangement can be useful. Cytogenetic analysis is indicated if malignant histiocytosis appears a likely diagnosis, since it may confirm the diagnosis.

**True histiocytic lymphoma**

True histiocytic lymphoma initially presenting at extramedullary sites may subsequently infiltrate the bone marrow. The patterns of infiltration reported are interstitial, patchy focal and diffuse [61]. In the WHO classification the designation ‘histiocytic sarcoma’ is used.

**Langerhans’ cell histiocytosis**

Langerhans’ cell histiocytosis, previously known as histiocytosis X, is a heterogeneous disease or group of diseases characterized by proliferation of Langerhans’ cells [62]. Recent evidence suggests that the proliferation is clonal and neoplastic [63]. Localized and disseminated forms occur. Haematological involvement occurs in the disseminated forms of the disease, which in the past have been referred to by the eponymous terms Letterer–Siwe disease of infants and Hand–Schüller–Christian disease. Bone marrow infiltration is seen mainly in infants and children.

**Peripheral blood**

The peripheral blood may be normal or there may be pancytopenia as a consequence either of hypersplenism or of bone marrow infiltration. Rarely, a leukaemia of Langerhans’ cells occurs.

**Bone marrow cytology**

The bone marrow aspirate may show Langerhans’ cells together with a mixed population including eosinophils, monocytes, lipid-laden macrophages,
lymphocytes and plasma cells [64,65]. Haemophagocytosis may occur. Langerhans’ cells are large and slightly irregular in shape. The nucleus is somewhat irregular and sometimes grooved with delicately clumped chromatin and inconspicuous nucleoli (Fig. 4.51). The cytoplasm is weakly basophilic with occasional azurophilic granules. Ultrastructural examination demonstrates Birbeck granules.

**Cytochemistry**

Langerhans’ cells show tartrate-resistant acid phosphatase activity [66] and are negative for nonspecific esterase.

**Immunophenotyping**

Langerhans’ cells are positive for CD1a and HLA-DR.

**Bone marrow histology**

In those cases with marrow involvement, the bone marrow contains clusters or sheets of Langerhans’ cells together with eosinophils, neutrophils, lymphocytes, plasma cells, monocytes, phagocytic macrophages, lipid-laden macrophages and giant cells (Fig. 4.52). Xanthomatous transformation and fibrosis may occur [64,65]. Langerhans’ cells have a characteristic appearance; the nuclei are usually convoluted or twisted and longitudinal grooves may be present.

**Immunohistochemistry**

Immunohistochemical staining shows Langerhans’ cells to express S100 protein, although only a small proportion of cells may stain positively in some cases. Staining with the lectin, peanut agglutinin, gives a characteristic staining pattern of a cytoplasmic halo with a paranuclear dot. Langerhans’ cells are positive for vimentin and CD1a. Positivity for CD1a is currently the most reliable immunophenotypic marker of Langerhans’ cells; the value of new anti-TRAP monoclonal antibodies remains to be established.

**Genetics and cytogenetics**

A familial tendency to the development of Langerhans’ cell histiocytosis has been described [67]. A clonal cytogenetic abnormality, t(7;12)(q11.2;p13), has been reported in a single case of eosinophilic granuloma [68].

**Problems and pitfalls**

It should be noted that Langerhans’ cell histiocytosis often causes focal lesions, even when widespread.
throughout the skeleton, and a targeted biopsy of a radiologically suspicious lesion may be more informative than a standard iliac crest trephine biopsy.

Langerhans’ cell histiocytosis has been confused with systemic mastocytosis, hairy cell leukaemia and malignant melanoma. Careful assessment of cytological features, supplemented by immunohistochemistry, will resolve difficulties.

References


Fig. 4.52 BM trephine biopsy section in Langerhans’ cell histiocytosis, showing an admixture of Langerhans’ cells and normal haemopoietic cells (same case as in Fig. 4.51). Paraffin-embedded, H&E ×188. (By courtesy of Dr R Brunning, Minneapolis.)


