It has been known for many years that some leukemias smoulder for a long time before catching fire. The terms, smouldering leukemia, oligoblastic leukemia and preleukemia were applied. It had also been recognized that some obscure anemias were unresponsive to the usual hematinics. They were variously known as refractory anemia, sideroachrestic anemia and sideroblastic anemia. It was also known that some of these anemias accumulated blasts in the bone marrow and eventually transformed to acute leukemia. However, it needed an international classification from the French–American–British (FAB) Group[1] (Table 19.1) to prompt hematologists regularly to diagnose the syndrome and then to realize that it is relatively common.

Table 19.1 FAB group classification of MDS

| Type | Peripheral blood | Bone marrow |
|---|---|---|
| RA | < 1% blasts | Dyshemopoiesis in one, two or all three lineages; < 5% blasts |
| RARS | < 1% blasts | As RA with ring sideroblasts comprising > 14% erythroblasts |
| RAEB | < 5% blasts | As RA with 5–20% blasts |
| RAEBt | < 5% blasts | As RA with 20–30% blasts or as RAB with Auer rods |
| CMML | As any of above with > $10^9$/l monocytes | As any of the above plus promonocytes |

RA, refractory anemia; RARS, RA with ring sideroblasts; RAEB, RA with excess of blasts; RAEBt, RAEB in transformation; CMML, chronic myelomonocytic leukemia.
Variants that do not fit well into this classification include hypoplastic MDS, fibrotic MDS and juvenile myelomonocytic leukemia.

## Definition

Myelodysplastic syndrome (MDS) may be defined as a clonal disorder of hematopoietic stem cells that retain the ability to differentiate into end-stage cells, but do so in a disordered and ineffective manner. Consequently, the bone marrow is usually hypercellular in the face of peripheral blood cytopenias. The hallmarks of MDS are morphologic abnormalities of red cells, white cells and platelets and of their precursors, some of which may be quite subtle (Table 19.2). As time progresses there is a tendency for cells to lose the ability to differentiate so that blast cells build up. The syndrome may culminate in acute leukemia.

## Epidemiology

MDS is usually idiopathic, occuring predominately in those over 60 years. A Leukemia Research Fund (LRF) study put the annual incidence at 3.6 per 100 000 but this masks the fact that many cases remain undiagnosed.[2] One group has suggested a prevalence of 1 in 500 in those over 60 years.[3] In those over 85 years it represents about a quarter of all hematologic malignancies. Although some think it is getting commoner, this apparent increase in incidence simply reflects a greater willingness to perform bone marrow investigations in the elderly.[4]

Secondary MDS, which tends to occur in younger people, develops after exposure to certain chemicals, particularly benzene and its derivatives, and to cytotoxic drugs, especially alkylating agents. The younger age at

Table 19.2 Morphologic abnormalities of blood and bone marrow cells in MDS

| Lineage | Blood | Bone marrow |
|---|---|---|
| Red cells | Macrocytes<br>Aniso-poikilocytosis<br>Dimorphic picture<br>Polychromasia<br>Punctate basophilia<br>Normoblasts<br>Reticulocytopenia | Erythroid hyperplasia<br>Multinuclearity<br>Dyskaryorrhexis<br>Megaloblasts<br>Cytoplasmic vacuoles<br>Howell–Jolly bodies<br>Ringed sideroblasts |
| White cells | Hypogranular neutrophils<br>Unilobed or bilobed neutrophils (Pelger cells)<br>Hypersegmented neutrophils<br>Monocytosis (often with multiple, elongated nuclear lobes)<br>Promonocytes (with fine azurophil granules)<br>Degranulated eosinophils | Hypogranularity of myeloid precursors<br>Increased promonocytes<br>Increased blast cells (type I with scanty agranular cytoplasm and type II with sparse granules |
| Platelets | Agranular platelets<br>Giant platelets<br>Megakaryocyte fragments | Micromegakaryocytes<br>Large megakaryocytes with single round or oval nucleus<br>Large megakaryocytes with multiple small round nuclei<br>Megakaryoblasts |

presentation of idiopathic MDS in developing countries suggests a less rigorous control of noxious chemicals in these communities. Familial MDS is rare, but also occurs at a young age. Childhood MDS is very rare, but unusual diseases such as juvenile myelomonocytic leukemia (JMML) are often included within the classification.

## Classification

The FAB group recognized that those patients with more than 5% blast cells in their bone marrows had shorter survivals and were more likely to transform to acute leukemia. This formed the basis for their classification of the syndrome. Two other factors, the presence of monocytes and the presence of ringed sideroblasts, further defined the syndrome. Finally, a demarcation had to be made between MDS and acute leukemia. An arbitrary 30% of blast cells in the marrow became the threshold for acute myeloblastic leukemia. A category, refractory anemia with excess of blasts in transformation, for those patients with between 20 and 30% marrow blast cells became the transition category. The final classification (Table 19.1) also had prognostic value.

Because, only a third of patients die from leukemia, a third die from irrelevant causes and a third from their cytopenias, several prognostic scoring systems[5–9] have been introduced to take account of the prognostic importance of cytopenias. Others recognized that age, serum lactate dehydrogenase (LDH) and chromosomal aberrations also influenced survival, and that the effect of the blast count was subtler than the FAB group had appreciated, with patients with 5–10% blast cells doing better than those with 11–20%. A coming-together of all those who had designed scoring systems produced the International Prognostic Scoring System (IPSS)[10] (Table 19.3). This system has been independently validated in clinical practice.[11]

In 1997 the WHO group[12] reclassified MDS (Table 19.4). The refractory anemia with excess of blasts in transformation (RAEBt) group was eliminated and patients with > 20% blast cells were designated as acute myeloblastic leukemia. Refractory anemia and refractory anemia with sideroblasts should henceforth refer only to cases with unilineage dysplasia. There is little disagreement that 'pure sideroblastic anemia' is an entity that seldom, if ever, transforms to acute leukemia,[13] but 'pure refractory anemia' is less well defined. Stand-alone dyserythropoiesis is often found in both normal marrows and in inflammatory conditions. Without karyotypic abnormalities or another clonal marker this subtype will be very difficult to diagnose. Cases with dysplastic features in two or more lineages should be known as refractory cytopenias with multilineage dysplasia. There was disagreement about chronic myelomonocytic leukemia (CMML). The pathologists in the group proposed a new category of MDS with myeloproliferative features (alternatively myelodysplastic, myeloproliferative disease, MDS/MPD) to include CMML, JMML and atypical chronic myeloid leukemia (aCML).

Particular types of acute myeloblastic leukemia (AML) pass through an oligoblastic phase, often showing dysplastic features in granulocytic line. They have characteristic chromosomal translocations: t(8;21), t(15;17), and inv

Table 19.3 International Prognostic Scoring System for MDS

| Prognostic variable | Score value | | | | |
|---|---|---|---|---|---|
| | 0 | 0.5 | 1 | 1.5 | 2 |
| % bone-marrow blasts | < 5 | 5–10 | — | 11–20 | 21–30 |
| Karyotype | Good | Intermediate | Poor | | |
| Cytopenias | 0/1 | 2/3 | | | |

Good karyotype = –Y, del(5q) or del(20q)
Poor karyotype = 3 or more abnormalities (complex) or chromosome 7 abnormalities
Intermediate = all other abnormalities.
Risk groups: Low scores 0, Intermediate 1 scores 0.5–1, Intermediate 2 scores 1.5–2, High scores > 2.
Median survivals are age related:
For low-risk group, 11.8 years for patients < 60 years and 4.8 years for patients > 60 years.
For intermediate 1 group: 5.2 years for patients < 60 years and 2.7 years for patients > 60 years.
For intermediate 2 group, 1.8 years for patients < 60 years and 1.1 years for patients > 60 years.
For high risk group: 0.3 years for patients < 60 years and 0.5 years for patients > 60 years.

Table 19.4 WHO classification of MDS

**Myelodysplastic syndromes**
1. Refractory anemia:
   (a) with ringed sideroblasts (RARS)
   (b) without ringed sideroblasts
2. Refractory cytopenia with multilineage dysplasia
3. Refractory anemia with excess blasts
   (a) Type I with 5–9% blasts
   (b) Type II with 10–19% blasts
4. 5q- syndrome
5. Myelodysplastic syndrome, unclassifiable

**Myelodysplastic/myeloproliferative diseases**
1. Chronic myelomonocytic leukemia
2. Atypical chronic myeloid leukemia
3. Juvenile myelomonocytic leukemia

**Acute myeloblastic leukemia**
1. Acute myeloid leukemia with multilineage dysplasia
2. Acute myeloid leukemia and myelodysplastic syndromes, therapy related

16 abnormalities. These should not be categorized as MDS, but as distinct entities: varieties of AML. The 5q- syndrome is also a distinct entity and is so categorized as a variant of MDS. Refractory anemia with excess of blasts (RAEB) was separated into RAEB I with 5–9% blast cells and RAEB II with 10–19% blast cells. Some cases of AML may be recognized as having derived from MDS and this should be stated. Some of these cases will be what had been previously diagnosed as RAEB in transformation (RAEBt) and some will have been called AML. It is probable that a large proportion of cases of AML in the elderly will fall into this category.

Finally, it was recognized that some cases do not fit into this or any classification and should be categorized as MDS unclassifiable. Although the WHO classification encountered some opposition[14] it has been retrospectively evaluated by the Dusseldorf group and found to have prognostic value.[15]

## Diagnosis

The majority of patients with MDS are asymptomatic and are diagnosed because a blood test has been performed for an irrelevant reason. Some will present with the symptoms of anemia (tiredness, breathlessness and lassitude), thrombocytopenia (bruising or bleeding), or neutropenia (recurrent infections, mouth ulcers). CMML may have some features of a myeloproliferative syndrome with moderate degrees of hepatosplenomegaly. In patients with high monocyte counts the abnormal monocytes become tissue macrophages and may produce pathology remote from the bone marrow. Gum hypertrophy, pleural and pericardial effusion, painful swollen joints and skin deposits have all been reported on rare occasions.

A full blood count may show anemia, neutropenia or thrombocytopenia. A raised mean cell volume (MCV) is frequently found and may be the only abnormality in the blood count. The differential diagnosis includes other causes of macrocytic anemia: B12 or folate deficiency, alcohol, liver disease, hypothyroidism and hemolytic anemia; and other causes of cytopenias: acute leukemia, aplastic anemia, drug-induced cytopenias, immune thrombocytopenia and marrow infiltration with various tumors. The morphologic abnormalities seen in AIDS have some similarities to those of MDS. Diagnosis of MDS will almost certainly depend on bone marrow examination although this may be legitimately resisted in the very elderly. Sometimes there are sufficient abnormalities on the blood film to make the diagnosis, and sometimes there are changes that make bone marrow aspirate and trephine biopsy imperative.

## Blood film examination

The ability to diagnose MDS crucially depends on the optimum staining of blood and bone marrow films. We prefer Jenner–Giemsa staining, but the stain used is a matter of personal preference. Staining varies enormously from laboratory to laboratory, so each observer should become familiar with his or her own laboratory's stain. Films sent for a second opinion should be sent unstained. It is important that the stain used picks up granularity in neutrophils well and also reveals basophilic stippling in red cells. Tired, end-of-the-day stains are treacherous.

### Red cells

Anemia is the most common feature of MDS but it is not necessarily present. Sometimes the only feature on the blood count suggestive of MDS is a raised MCV. Usually the red cells are large, but they may be of normal size and very occasionally small. Many textbooks list sideroblastic anemia among the causes of a low MCV. This is very misleading and only applies to the rare hereditary form. Refractory anemia with ring sideroblasts (RARS) is associated with a raised or normal MCV.

Frequently the red cells in MDS vary in size and shape (anisopoikilocytosis). Oval macrocytes are common. When these are accompanied by small hypochromic cells, so as to give a dimorphic picture, RARS should be suspected (Fig. 19.1). However, small hypochromic red cell fragments may be seen in all forms of MDS. A preponderance of small hypochromic red cells is rarely seen and should

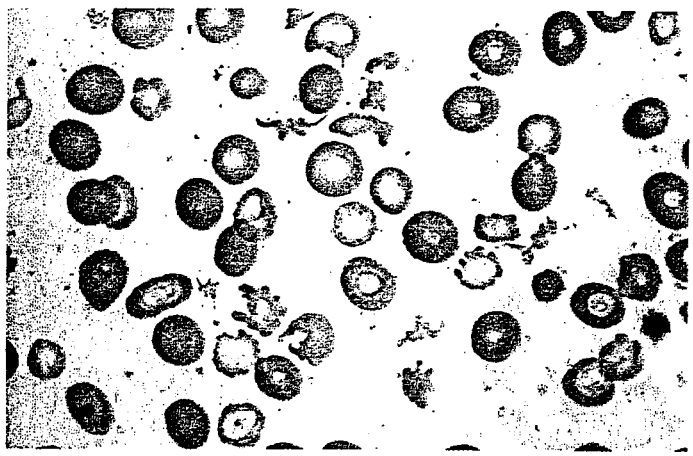

**Fig. 19.1** Blood film from patient with RARS showing anisocytosis, poikilocytosis, anisochromasia, basophilic stippling and irregularly shaped red cells.

suggest acquired haemoglobin H (HbH) disease (Fig. 19.2). This is a very rare finding in MDS, usually in elderly men, but may be revealed in the usual way with supravital staining (Fig. 19.3).

Variation is cell shape is common. Poikilocytosis with tear-drop cells, acanthocytes and cell fragments are all frequently seen. MDS is a rare cause of elliptocytosis. However, red cell size and shape may be normal. Howell–Jolly bodies are occasionally seen. Rather more common is basophilic stippling which may be fine or coarse (Fig. 19.4). Fine basophilic stippling confined to large misshapen hypochromic cells usually indicates RARS.

## White cells

Neutropenia is the rule in MDS. Although it may be severe, it is frequently mild. In CMML, the reverse is often true. In addition to the absolute monocytosis that defines the condition, there is usually a neutrophil leukocytosis. The neutrophil count is often $> 10 \times 10^9/l$ and we have seen cases with neutrophil counts $> 50 \times 10^9/l$.

The granularity of neutrophils is an important feature. Classically, there are few if any neutrophil granules (Fig. 19.5), but sometimes hypergranularity is seen (Fig. 19.6). Rarely, giant granules typical of those seen in Chediak–Higashi syndrome have been identified. Auer rods are sometimes seen, but they have no prognostic significance.

Neutrophil lobulation is the other feature to observe. Pseudo Pelger–Huët cells are neutrophils with unilobular or bilobed neutrophils with normal condensation of the nucleus (Fig. 19.7). It is important to distinguish these cells from myelocytes and metamyelocytes where the nucleus remains relatively uncondensed. Frequently, pseudo Pelger cells are seen together with hypogranularity.

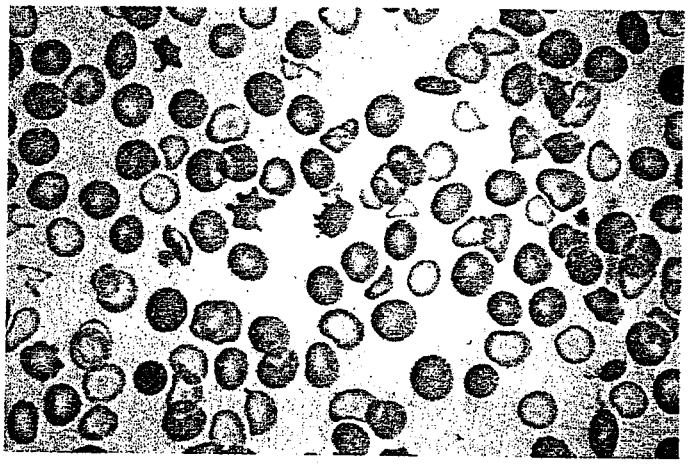

**Fig. 19.2** Blood film from a patient with acquired HbH disease showing very hypochromic population together with anisocytosis and poikilocytosis and irregularly shaped red cells.

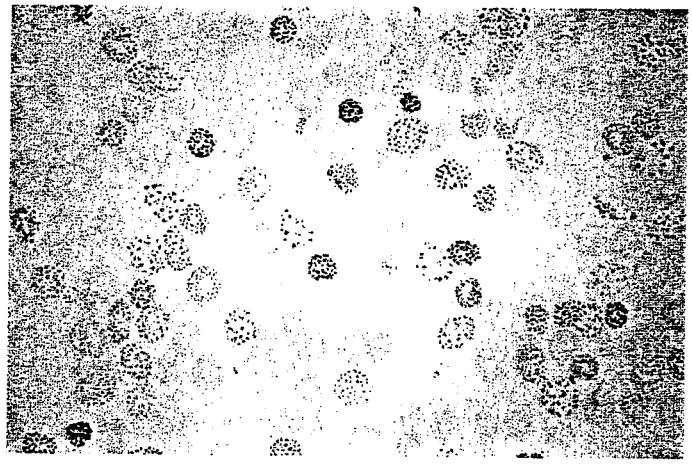

**Fig. 19.3** Blood film from a patient with acquired HbH disease showing 'golf ball' inclusions when stained with brilliant cresyl blue.

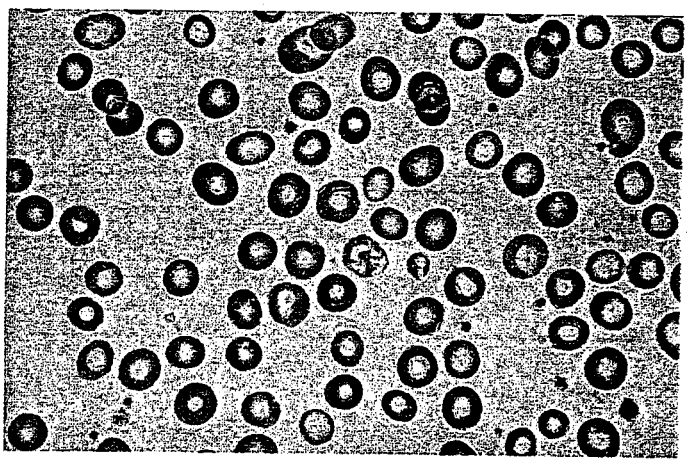

**Fig. 19.4** Blood film from a patient with RARS showing a Howell–Jolly body in a cell with coarse basophilic stippling.

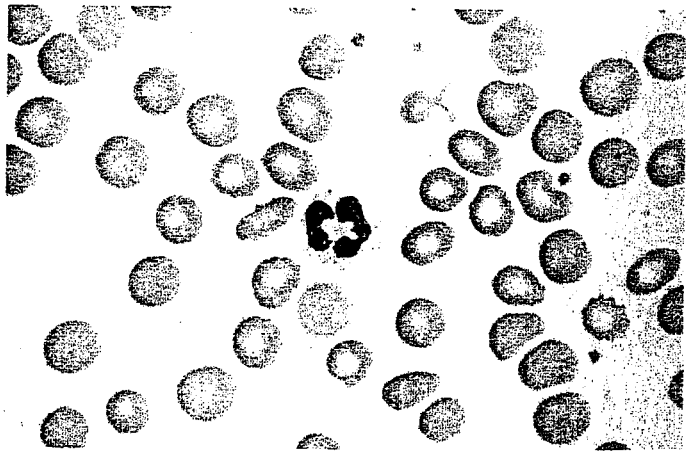

**Fig. 19.5** Blood film from a patient with RA showing a hypogranular neutrophil.

those of 17p affect *p53*. However, gene mappers have yet to isolate the responsible genes on 5q and 7q despite many years of effort.

The t(5:12) translocation dysregulates the ras signaling pathway,[53] and mutations of the *ras* gene have been a regular finding. Various authors have found mutations, mostly involving *N-ras*, in between 9 and 40% of cases of MDS,[54,55] especially CMML. Most mutations involve a G to A transition at codon 12 or 13. A rare association between juvenile myelomonocytic leukemia and neurofibromatosis is caused by a mutation of the *NF1* gene.[56] This gene product is a GTPase-activating protein that also interacts with *ras*, converting it from an active to an inactive form.

Other genetic abnormalities encountered in MDS include hypermethylation of the cyclin-dependent kinase inhibitor p15,[57] mutations in the gene coding for receptor for macrophage colony stimulating factor (*c-fms*)[58] and mutations of *p53*. In some cases of RARS mutations of the mitochondrial gene encoding mitochondrial cytochrome C oxidase have been seen.[59]

## Cell biology

The growth of marrow progenitor cells in short-term culture is abnormal in MDS.[60] In some cases there is no growth, but more commonly there is a reduction of myeloid colony-forming units (CFU-GM), erythroid burst-forming units (BFU-E) and mixed granulocyte-erythroid-macrophage-megakaryocytic colony units (CGU-GEMM), with an increase in clusters and defective maturation of cells within individual colonies. In general, the defects are worse in patients with greater numbers of blasts in the marrow. In CMML and JMML, CFU-GM growth is increased, due to over production of granulocyte-macrophage colony-stimulating factor (GM-CSF).

In dissecting the causes of these abnormalities, it appears that there is a defect of colony-forming cells in responding to growth factors by proliferation and differentiation. Increasing concentrations of these factors improves response, laying a basis for treatment of MDS with growth factors.[61] Growth in long-term marrow culture is poor in MDS and seldom sustained for longer than 2 weeks.[62]

Of great recent interest is the discovery of increased apoptosis in MDS,[63,64] which has been demonstrated by a variety of techniques. When $CD34^+$ cells are studied, it is most apparent in early MDS. Increased bone marrow blast cell counts are associated with a significant reduction of apoptosis.

Immune function is impaired in MDS.[65–68] A wide range of supposedly auto-immune diseases has been found in association with MDS. It is likely that these represent disorders of macrophage rather than lymphoid function. Although the susceptibility to infection is caused by defects in granulocyte or macrophage number and function there is considerable evidence that lymphocytes are affected by the disease (Table 19.5). One possible cause for this is that lymphocytes are involved in the dysplastic clone via a common progenitor. There is conflicting evidence for this (Table 19.6), but recent opinion favors the immune system being affected by abnormal activities of monocytes and dendritic cells, which certainly are part of the clone.

## Other abnormalities

A wide range of laboratory abnormalities may be found in MDS. These are largely epiphenomena that give little insight into the pathogenesis of MDS. They are detailed in Table 19.7.

## Pathogenesis

Most workers view MDS as the result of the cumulative acquisition of multiple genetic errors occurring over a long period. Some of these abnormalities may be congenital, but most are acquired. The acquired genetic abnormalities may be random errors, but some environmental insults increase the risk. Chief among these have been exposure to X-irradiation, alkylating agents and benzene and its derivatives. Some individuals may be more prone to this process because they lack effective detoxifying enzymes.[86–88]

**Table 19.5** Immunologic abnormalities associated with MDS

| Immunoglobulins | B-cells | T-cells | NK cells |
|---|---|---|---|
| Polyclonal hypergammaglobulinemia | Normal in number | T-cell lymphopenia | Reduced in number |
| Hypogammaglobulinemia | Functionally immature | Reduced $CD4^+$ cells | Functionally immature |
| Monoclonal gammopathy | | Impaired T-cell function | |
| Anti-red cell antibodies | | | |

difficult. Nothing replaces practice. Some of the morphologic abnormalities are very subtle and referral of the slides for expert opinion is sometimes necessary. However, even experts disagree. Most disagreements arise from the recognition of mild degrees of RA.

Typically, the marrow is hypercellular, but cellularity is better appreciated from trephine biopsy than from the aspirate films. This is one of the reasons that the two investigations should be seen as complementary.

## Erythropoiesis

All the features seen in the blood may be present, but the bone marrow aspirate gives the opportunity of examining the whole of erythropoiesis. In many cases there is erythroid hyperplasia, but pure red cell aplasia is a rare finding. Frequently, the red cell precursors appear megaloblastic. Multinuclear forms are common (Fig. 19.10), and megaloblastic anemia forms one of the chief differential diagnoses. Mitotic figures are common (Fig. 19.11) and there is frequently dyskaryorrhexis (literally, abnormal bursting of a cell nucleus). Pyknosis, nuclear budding and intranuclear bridging are frequently seen. There is often asynchrony between the maturation of nucleus and cytoplasm, with fully hemoglobinized cells with uncondensed nuclei.

Ringed sideroblasts are red cell precursors with an accumulation of iron in the mitochondria, which stains blue with Perls' stain. Older classifications, which counted the number of granules, have been superseded by a definition of a ring as one that extends at least a third of the way around the nucleus. Over half of cases of MDS have some sideroblasts. Arbitrarily, when ringed sideroblasts comprise > 15% of the total erythroblast population the disease is designated RARS (Figs 19.12 and 19.13).

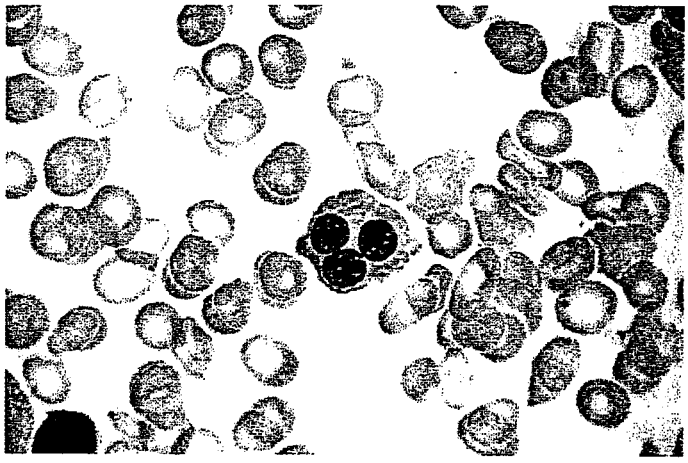

**Fig. 19.10** Bone marrow film from a patient with RA showing a trinucleate normoblast.

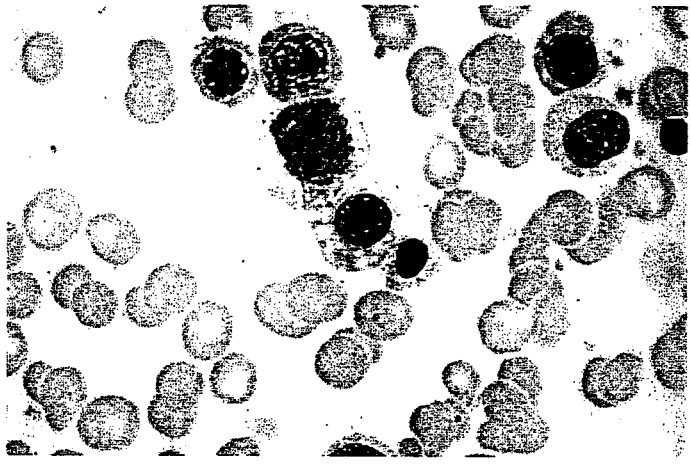

**Fig. 19.12** Bone marrow film from a patient with RARS showing vacuolated and stippled normoblasts.

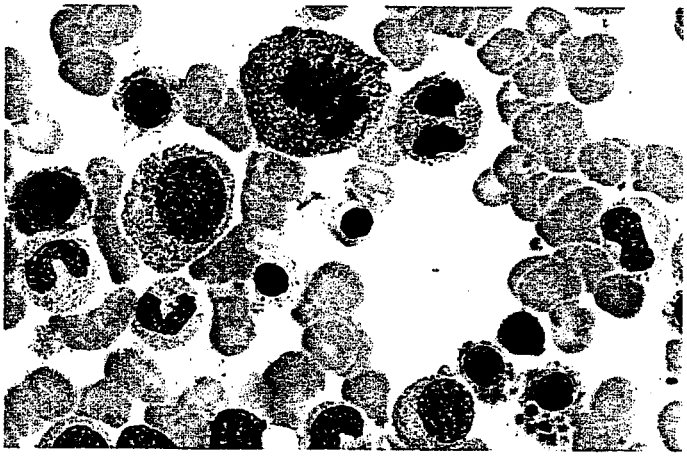

**Fig. 19.11** Bone marrow film from a patient with RARS showing mitotic figures, and vacuolated and stippled normoblasts.

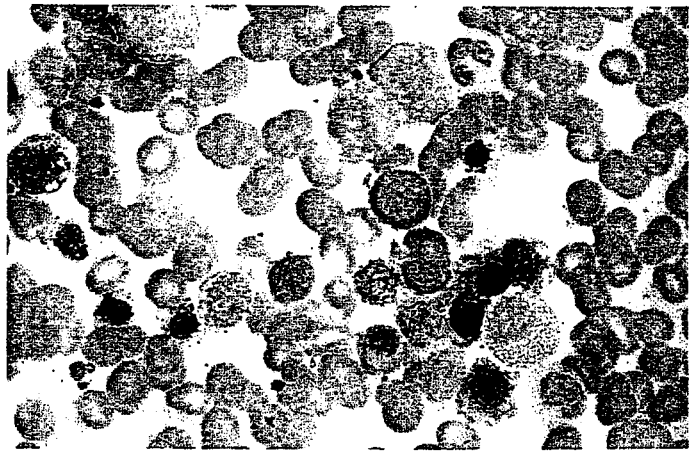

**Fig. 19.13** Bone marrow film from a patient with RARS stained with Perls' stain showing ringed sideroblasts.

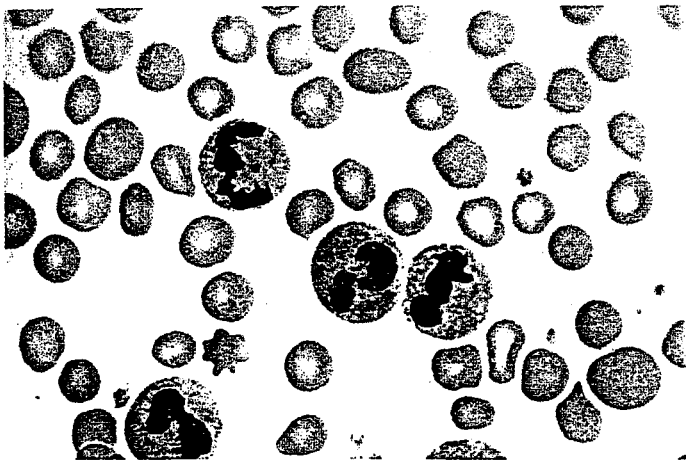

Fig. 19.6 Blood film from a patient with RA showing hypergranular neutrophils.

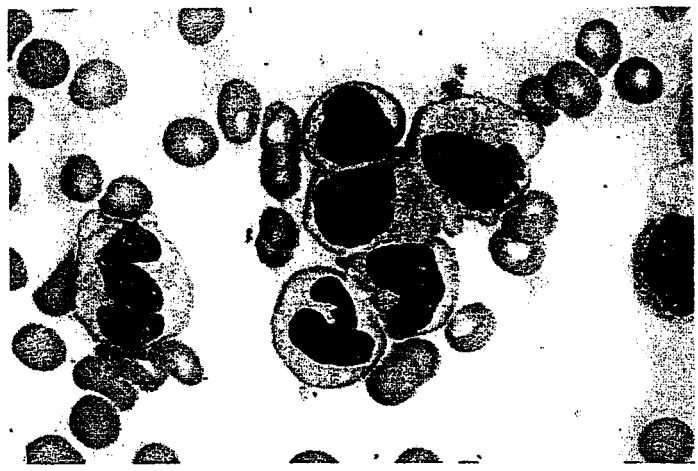

Fig. 19.8 Blood film from a patient with CMML showing abnormal monocytes.

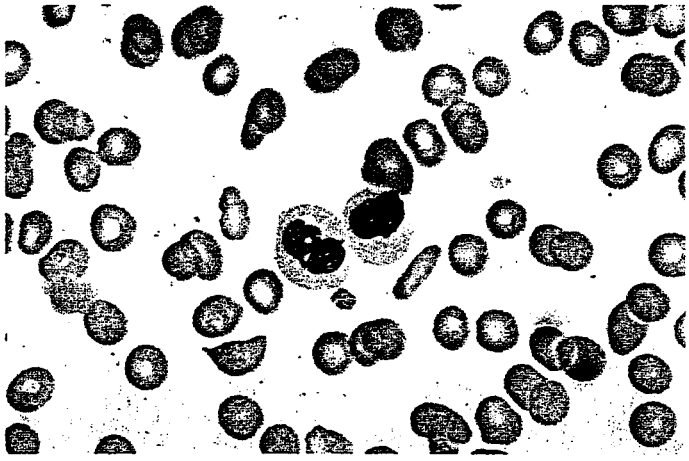

Fig. 19.7 Blood film from a patient with RA showing pseudo-Pelger–Huët cells.

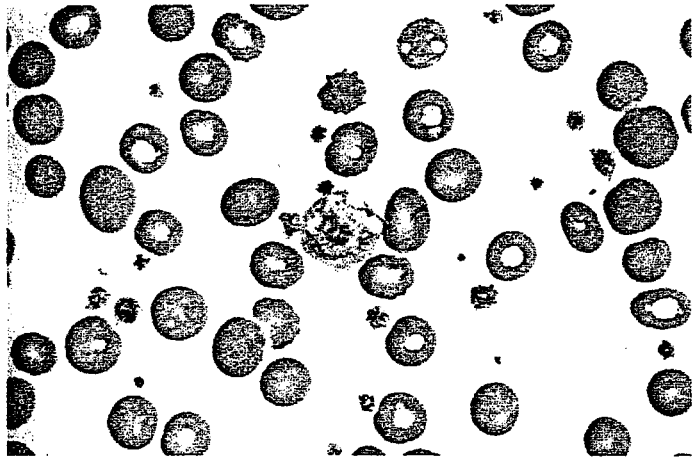

Fig. 19.9 Blood film from a patient with RA showing a giant platelet.

Occasionally, hyperlobulated polymorphs are seen. An unusual feature is arachnoid lobulation, where the nuclear lobules look like the segments of a spider's leg.

Monocytes are increased in CMML (Fig. 19.8). One of the difficulties with the FAB classification is the magic number of $10^9$/l monocytes. Some cases skip between refractory anemia (RA) and CMML from week to week as their monocyte count hovers either side of this number. Monocytes often have multiple, elongated nuclear lobes. Promonocytes, with fine azurophil granules, may also be seen. The diagnosis of CMML may be difficult to make because the monocytosis may be slight, and the predominant feature of the blood film is a granulocytosis. These granulocytes may have minimal dysplastic features. The differentiation between CMML and chronic myeloid leukemia is discussed below.

Degranulated eosinophils are sometimes a feature. Eosinophils with basophilic granules and vice versa are sometimes seen.

## Platelets

Thrombocytopenia is common. Thrombocytosis is rare, but occurs in the 5q– syndrome. It is important to distinguish myeloproliferative thrombocythemia from the thrombocytosis of MDS. The former should not have dysplastic features. Sometimes thrombocytosis accompanies the finding of ring sideroblasts.

Giant platelets or megakaryocte fragments may circulate in MDS (Fig. 19.9). Agranular platelets may also be seen.

# Bone marrow aspirate

A well-prepared, freshly stained bone marrow aspirate is the key to the diagnosis of MDS. Which is the best Romanowsky stain continues to be a matter of argument. For most of us the answer is – the one you are used to. Interpreting bone marrow aspirates in MDS can be

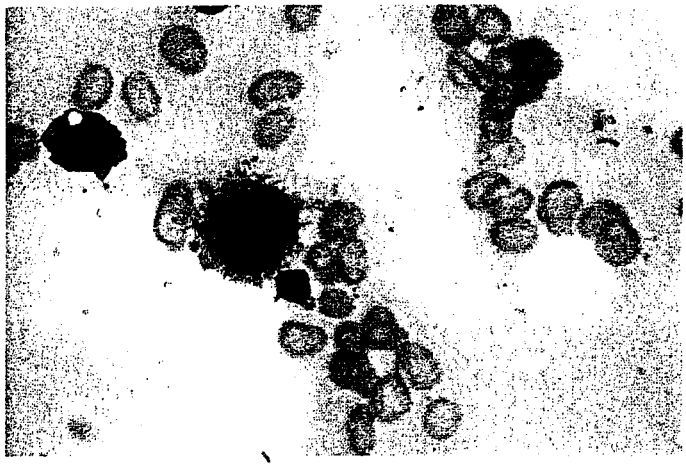

Fig. 19.18 Bone marrow film from a patient with RA showing micromegakaryocytes.

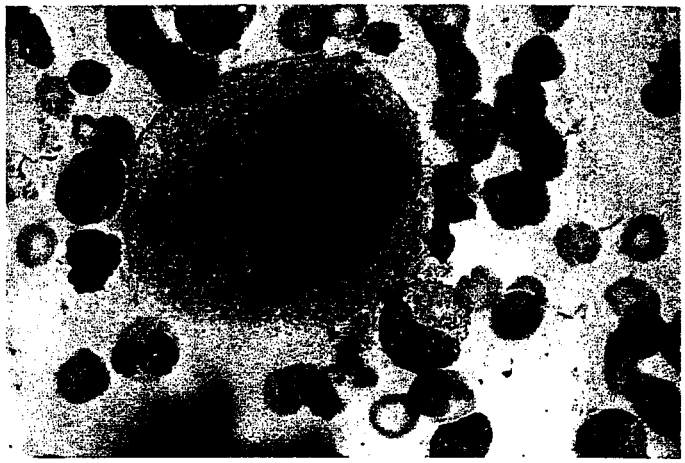

Fig. 19.20 Bone marrow film from a patient with 5q-syndrome showing a mononuclear megakaryocyte.

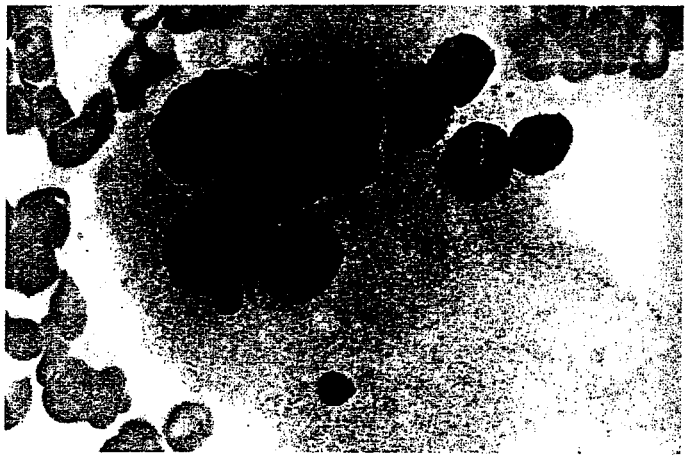

Fig. 19.19 Bone marrow film from a patient with RA showing a giant megakaryocyte with multiple dispersed nuclei.

There are characteristically three types of abnormal megakaryocyte seen: micromegakaryocytes (Fig. 19.18), giant megakaryocytes with multiple dispersed nuclei (Fig. 19.19), and moderate-sized megakaryocytes with a single round eccentric nucleus (Fig. 19.20). This last type is characteristic of the $5q^-$ syndrome, but is not confined to it.

## The role of trephine biopsy in the assessment of myelodysplastic syndrome

Trephine biopsy is rarely essential for the diagnosis of MDS but offers useful information in addition to that obtained from cytologic assessment of peripheral blood and aspirated bone marrow.[17–19] The particular value of trephine biopsy in this context is for the demonstration of spatial disturbance of hemopoietic tissue within the marrow, which cannot be appreciated in samples obtained by aspiration. Cytologic abnormalities in the various hemopoietic cell lineages can also be seen in histologic sections of bone marrow, although aspirate films show these features in more subtle detail and are the preferred preparation in which to make assessment of cytologic features of dysplasia. In any patient in whom aspiration proves difficult, trephine biopsy is recommended as it can supply much of the information that may be lacking from suboptimal aspirate films.

## General histologic features of myelodysplastic syndrome

Histologic sections in most patients with MDS will show hypercellularity of hemopoietic tissue but, in some cases, will appear normocellular or hypocellular. It is important to remember the expected range of cellularity is wide in the older age group within which most cases of MDS arise, but is generally lower as age increases. When relatively young patients present with suspected MDS, what might appear to be increased cellularity should not be overestimated.

Stromal components of the bone marrow usually appear relatively normal in MDS; there may be a mild increase in reticulin but collagen fibrosis is uncommon[20] and disturbances of trabecular bone remodeling, including new bone formation, are rare. Marked stromal abnormalities such as edema or fibrosis should raise suspicion of secondary myelodysplasia or an overlap myeloproliferative/myelodysplastic syndrome.

Histologic features seen in trephine biopsy sections in MDS do not correlate well with individual subtypes as defined by the FAB or WHO classification systems. In

## Granulopoiesis

Again, all the features seen in the blood may be present. Absence of secondary granules may be a feature of all myeloid precursor cells (Fig. 19.14), but primary azurophil granules are usually present in promyelocytes and some myeloblasts. Interestingly, it is very unusual for the myeloid cells in MDS not to stain with myeloperoxidase albeit less densely than in normals. On the other hand the cells are usually negative for leukocyte alkaline phophatase. Especially in CMML, an apparently hybrid cell with both myeloid and monocytic characteristics is seen. These paramyeloblasts stain positively for both chloro-acetate esterase (granulocyte specific) and α-naphthyl acetate esterase (monocyte specific).

An accurate blast cell count is important prognostically. It may be difficult to distinguish myeloblasts from promyelocytes. The FAB group recognized two types of blast cells. Type I blast cells are myeloblasts of variable size without granules or Auer rods (Fig. 19.15). The nuclear chromatin is uncondensed and there are usually one or two nucleoli. A type II blast cell is usually larger with rather more cytoplasm, and contains a few azurophil granules. They are distinguishable from promyelocytes, which have a slightly eccentric nucleus, which is rather more condensed with less distinct nucleoli, and an obvious Golgi zone (Fig. 19.16). Abnormal promyelocytes may also have excessive granules (more than six) resembling those seen in M3 AML though without the bilobed or monocytoid nucleus. Goasguen and colleagues[16] later described a type III blast cell with more than 20 azurophil granules but without a Golgi zone (Fig. 19.17).

## Thrombopoiesis

Both very few and very many megakaryocytes are sometimes seen in MDS, but usually the numbers are normal.

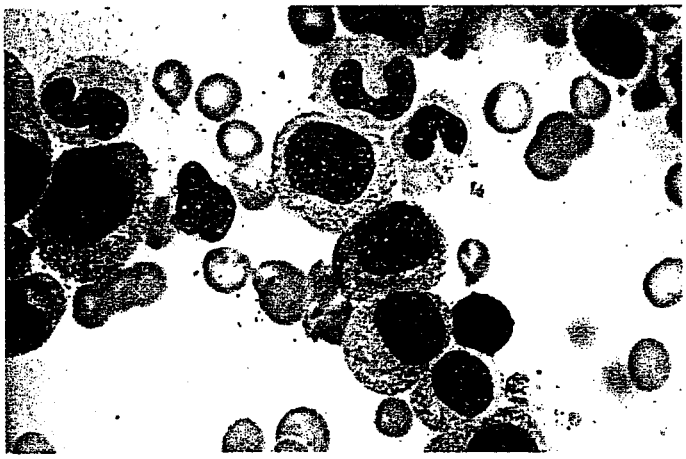

**Fig. 19.14** Bone marrow film from a patient with RA showing hypogranular myeloid cells at different stages of maturation.

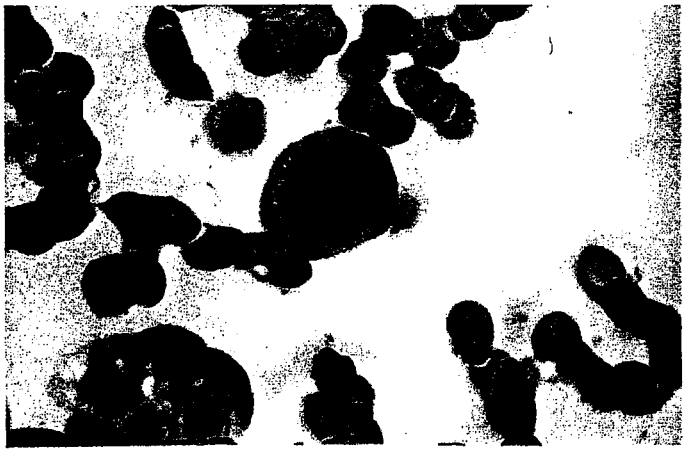

**Fig. 19.15** Bone marrow film from a patient with RAEB showing a type I myeloblast.

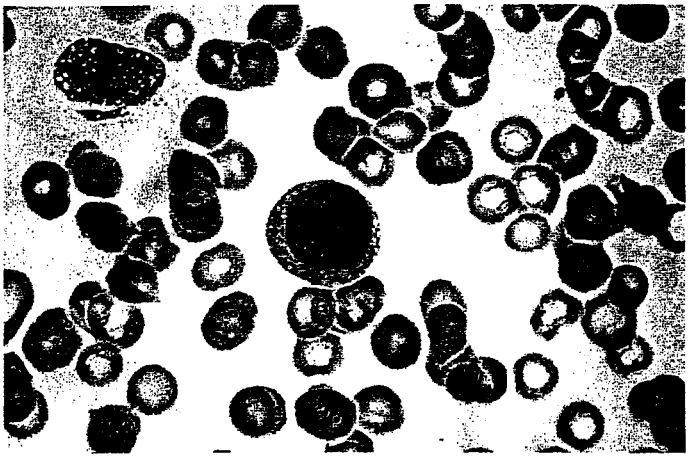

**Fig. 19.16** Bone marrow film from a patient with RAEB showing a type II myeloblast.

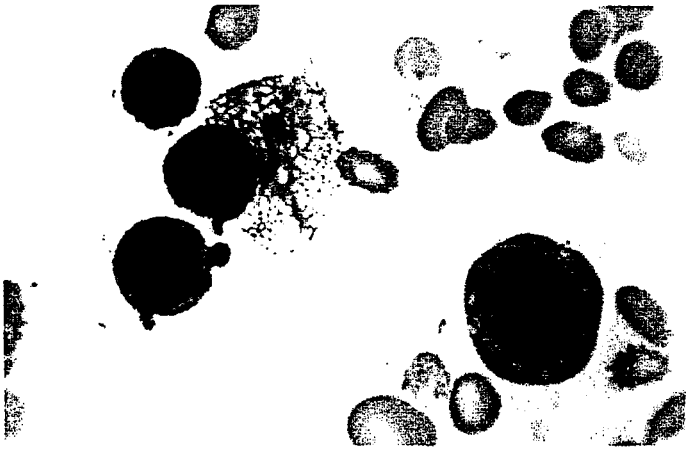

**Fig. 19.17** Bone marrow film from a patient with RAEB showing a type III myeloblast.

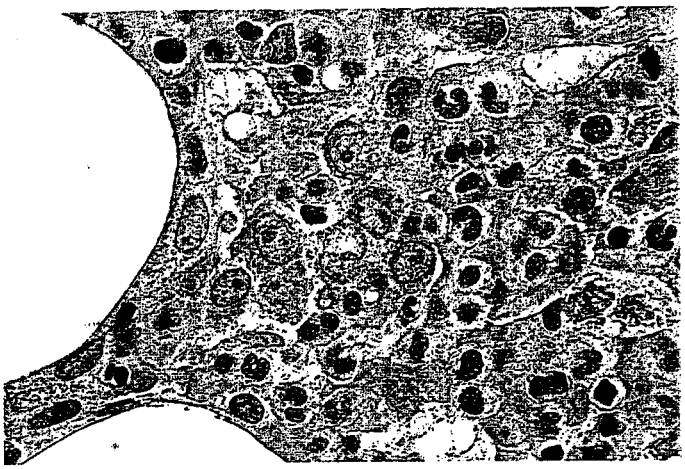

Fig. 19.23 Immature myelomonocytic cells in the center of an intertrabecular space; so-called 'ALIP'. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 100.

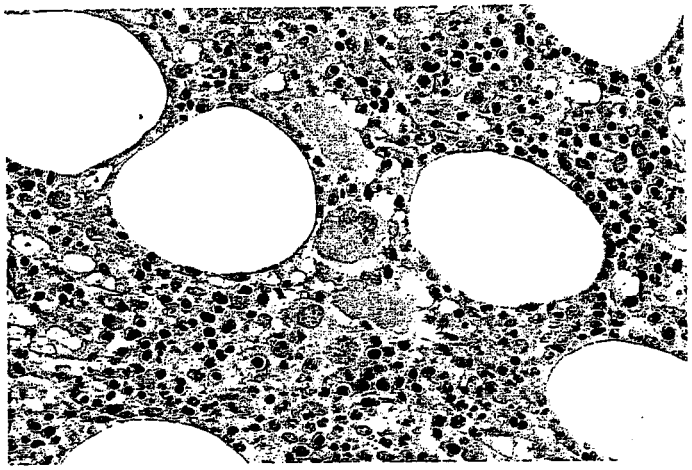

Fig. 19.25 Cluster of atypical, small megakaryocytes in MDS. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 40.

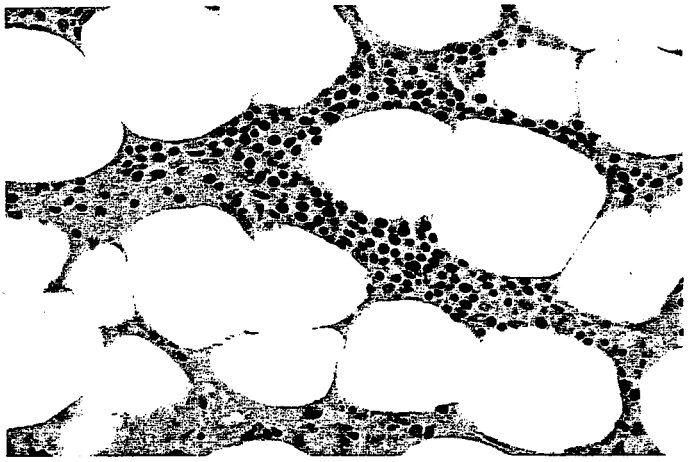

Fig. 19.24 Dyplastic erythropoiesis with irregular clustering of erythroid precursor cells, each cell at a similar (in this example, relatively late) stage of maturation to its neighbors; in normal erythroid cell clusters, a mixture of maturational stages would be present. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 40.

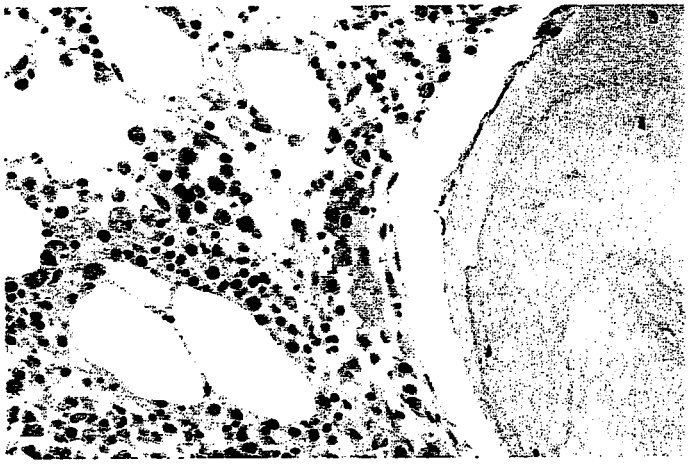

Fig. 19.26 Displacement of atypical megakaryocytes to the paratrabecular region in MDS. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 40.

of the marrow and also by loss of the normal organization of individual clusters. The clusters are frequently enlarged, with increased numbers of proerythroblasts and early normoblasts. The cells in some clusters may appear synchronous, rather than reflecting a spectrum of maturational stages of erythropoiesis (Fig. 19.24). In some patients, erythropoiesis may appear dispersed, with absent or infrequent formation of cell clusters. It is curious that, despite striking cytologic findings in aspirated bone marrow, erythropoiesis is frequently hyperplastic but otherwise apparently normal in many patients with MDS-RARS, in whom trephine biopsy histology may be remarkable for its lack of dysplastic features.

Assessment of megakaryocyte morphology and distribution is critical to the interpretation of bone marrow trephine biopsy histology in MDS.[26–29] Cytologic features are discussed below but spatial distribution of megakaryocytes, as for other hemopoietic lineages, is often highly abnormal. Because of their relatively large size and distinctive morphology, it is usually easy to detect clustering of megakaryocytes (Fig. 19.25) and their displacement from perisinusoidal to paratrabecular locations (Fig. 19.26). Occasional clusters of 2–3 megakaryocytes may be found in normal or reactive bone marrow but larger groupings are highly atypical and indicate pathology such as myelodysplasia or a chronic myeloproliferative disorder. Paratrabecular location of megakaryocytes has similar significance and is extremely

general, it is possible to determine that an individual example of MDS has 'low-grade' features, consistent with FAB categories myelodysplastic syndrome – refractory anemia (MDS-RA), RARS, other refractory cytopenias or the 5q-syndrome, or 'high-grade' features in keeping with MDS-RAEB or RAEBt.[21,22] This distinction can be made on the basis of prevalence of early myelomonocytic cells (myeloblasts, promyelocytes and promonocytes) within sections (Figs 19.21 and 19.22). Recognition of myeloblasts may be difficult but increased numbers of promyelocytes, usually accompanied by disproportionately reduced numbers of metamyelocytes and neutrophil polymorphs, are generally easy to recognize. It should be noted that in chronic myelomonocytic leukemia (discussed in Chapter 21), the presence of large numbers of immature myelomonocytic cells does not correlate well with the biologic aggressiveness of the disease in individual patients.

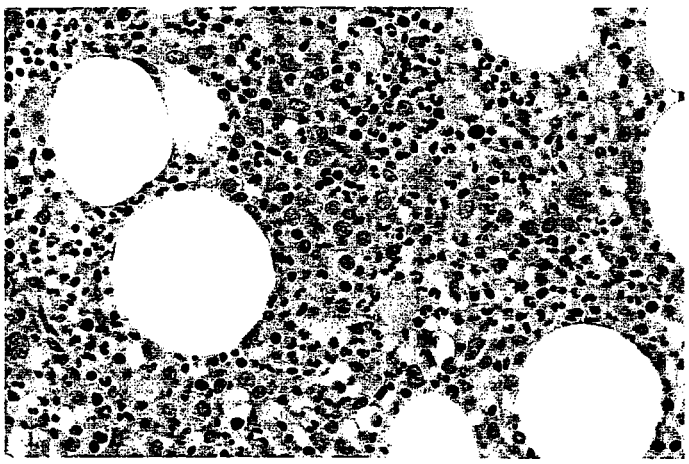

**Fig. 19.21** Histology of 'low-grade' myelodysplasia in MDS-RA. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 40.

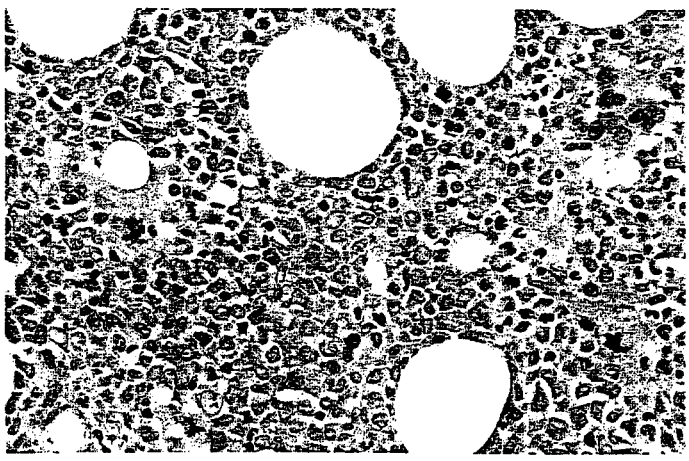

**Fig. 19.22** Histology of 'high-grade' myelodysplasia in MDS-RAEB. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 40.

## Assessment of spatial distribution of hemopoiesis in MDS

As mentioned above, trephine biopsy sections have the particular value in MDS of allowing assessment of the spatial distribution of hemopoietic cells. In normal bone marrow, promyelocytes and myelocytes are localized preferentially at the margins of bone trabeculae and around the adventitial aspects of arterioles and venules coursing through intertrabecular spaces.[23] Metamyelocytes and neutrophil polymorphs are found in increasing numbers with progression towards the centers of intertrabecular marrow spaces. Developing monocytes are more randomly distributed and are difficult to distinguish from early granulocytic cells in histologic sections.[24] Erythroid cells form orderly clusters in non-paratrabecular areas of the marrow spaces, with mid- and late normoblasts predominating. Megakaryocytes are usually found singly, in the central parts of intertrabecular spaces, and sometimes the plane of section allows their location at the edge of sinusoids to be seen.

In the MDS, and in secondary forms of myelodysplasia, this topographic arrangement is disturbed to a greater or lesser extent. Displacement of early granulocyte precursors from trabecular margins is a frequent finding and, accompanying this, scattered metamyelocytes and neutrophil polymorphs may be found immediately adjacent to trabecular margins. The presence of myeloblasts and promyelocytes in groups in the central parts of intertrabecular spaces is seen less commonly (Fig. 19.23).[25] The latter phenomenon has been a source of debate for many years, since some authors have claimed that such abnormal localization of immature precursors (ALIP) is of adverse prognostic significance in patients whose blast cell counts in blood and aspirated marrow are low. However, it has proved difficult to define objective criteria for the recognition and quantification of ALIP. In most cases where ALIP is seen in daily practice, it is present in the context of a blood or bone marrow blast cell count consistent with FAB categories of MDS-RAEB, RAEBt or even AML; in this context it has no independent prognostic value. It should also be noted that ALIP is not a phenomenon specific to MDS; it can be seen in reactive conditions and myeloproliferative disorders, in which it is of no known significance.

Spatial disorganization of erythropoiesis is represented by erythroid cell clusters occupying paratrabecular areas

## Histologic assessment of myelodysplasia in hypoplastic bone marrow

Assessment of myelodysplasia in hypocellular bone marrow specimens poses particular difficulties due to the paucity of hemopoietic tissue available for assessment.[32,33] Aspiration may have been unsuccessful or may have yielded a suboptimal sample, so that trephine biopsy has an important role to play. The differential diagnosis includes primary MDS, secondary myelodysplasia (see below) and disorders such as hypoplastic/aplastic anemia, paroxysmal nocturnal hemoglobinuria and hypoplastic acute myeloid leukemia. The same criteria should be applied in assessing hemopoietic cell distribution and cytologic features as in normocellular or hypercellular trephine biopsy sections. Even with very little hemopoietic tissue to evaluate, it should be possible to determine whether the biopsy shows: (1) hypoplastic normal hemopoiesis; (2) hypoplastic dysplastic hemopoiesis with evidence of at least partial maturation within each hemopoietic lineage; or (3) hypoplastic acute leukemia with blast cells and minimal or no evidence of maturation.

## Histologic features of secondary myelodysplasia

Dysplastic hemopoiesis may occur in response to a variety of, often poorly characterized, systemic diseases and toxic insults to the bone marrow. A familiar example is the myelodysplasia associated with infection by the human immunodeficiency virus (HIV).[34] Hemopoietic recovery following cytotoxic chemotherapy is also often transiently dysplastic,[35] in addition to the predictable megaloblastosis caused by use of folate antagonists.

It is not always possible to determine by bone marrow examination whether dysplasia is primary or secondary but trephine biopsy histology provides important clues to indicate the likelihood of one versus the other. The main features that indicate a secondary origin for myelodysplasia are abnormalities of the bone marrow stroma, reflecting toxic or inflammatory injury of stromal cells, occurring in addition to hemopoietic cell damage. At the least, increased stromal reticulin (grade 2–3) is usually present. In addition, there is often stromal edema, indicated by separation of hemopoietic cells in the interstitium and widening of sinusoidal lumens (Figs 19.28 and 19.29). In more severe injury, gelatinous change occurs; evidence for this may be found in aspirate films as well as in histologic preparations, with irregular

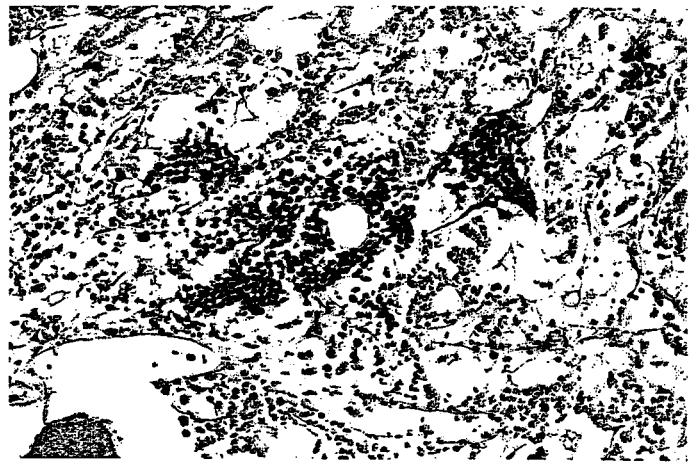

**Fig. 19.28** Stromal edema and red cell extravasation, presumed to represent stromal responses to inflammatory or toxic injury. Atypical, cohesive-appearing, 'synchronous' clusters of erythropoietic cells are prominent and other hemopoietic cells are widely separated in the background. Non-nucleated red cells are present throughout the edematous interstitium and a distended sinusoidal lumen can be seen (bottom left) adjacent to the end of a bony trabecula. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification x 20.

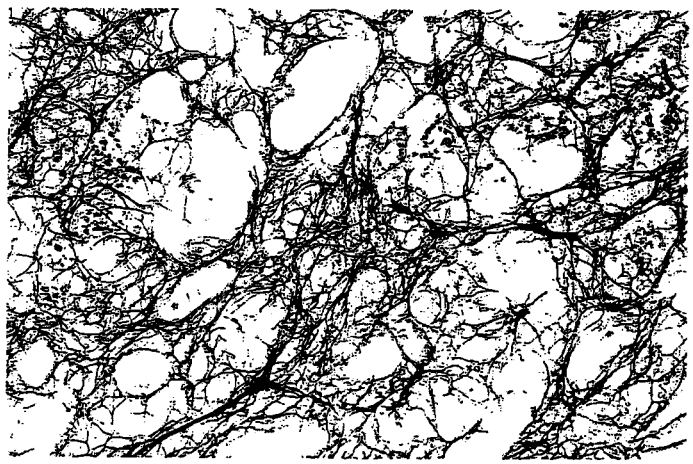

**Figure 19.29** Reticulin staining of the same case shows diffuse, grade 3 increase in interstitial reticulin fibers. Silver-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification x 20.

masses eosinophilic, periodic acid–schiff (PAS)-positive material in particles and trails. In histologic sections, distinction between severe edema and gelatinous change can be confirmed by alcian blue staining; edema fluid remains unstained but, in gelatinous change, the stroma stains turquoise/blue. Stromal injury is occasionally sufficiently severe to cause collagen fibrosis, particularly if necrosis has occurred. Evidence of previous necrosis may be found in the form of dead bone trabeculae or fragments of amorphous debris in the fibrotic stroma showing dystrophic calcification. Recent severe systemic

rare in other contexts, unless the bone marrow is severely hypoplastic for other reasons. Even in the latter situation, the possibility of hypoplastic MDS should be considered carefully.

## Assessment of cytologic features in trephine biopsy sections in MDS

Cytologic features of myelodysplasia in developing hemopoietic cells are best seen in bone marrow aspirate films. In decalcified trephine biopsy sections it is usually not possible to detect hypogranularity in granulocytic cells and nuclear abnormalities such as pseudo-Pelger changes can be seen only with difficulty. Failure of nuclear condensation and lobulation in terminally differentiated neutrophil polymorphs can be seen in occasional cases. Abnormalities of granulation may be visible in high-quality sections of plastic-embedded trephine biopsies. Since these are generally thinner than those cut from paraffin-embedded specimens (1–2 mm compared with 4–5 mm), familiarity with the normal degree of granularity visible in thin sections is essential. More readily appreciable are abnormalities in the proportions of developing granulocytes present representing different stages of maturation; there are frequently increased promyelocytes and myelocytes accompanied by reduced numbers of metamyelocytes and neutrophils. It is even more difficult in trephine biopsy sections than aspirate films to determine precisely which cells among the immature granulocytes are truly myeloblasts.

In the erythroid series, dysplastic cytology is frequently represented by a megaloblast-like appearance of individual nucleated red cell precursors. As with granulopoiesis, however, it is usually easier to appreciate the imbalance in relative numbers of cells at different stages of maturation. Increased numbers of dysplastic proerythroblasts and reduced numbers of later cells alter the composition of erythroid nests; absence of the familiar late normoblasts, abundant in normal bone marrow, is an important clue to the presence of erythroid dysplasia in trephine biopsy sections. The megaloblast-like proerythroblasts may be confused with early myelomonocytic precursors, and may even suggest ALIP, but staining reveals their basophilic cytoplasm, often with a perinuclear halo, allowing their distinction from other immature hemopoietic cells.

Cells of megakaryocytic lineage demonstrate the most readily appreciable dysplastic cytologic features in bone marrow trephine biopsy sections. Because megakaryocytes tend to remain adherent to particles in aspirate films, trephine biopsy is often superior for their assessment. Megakaryocyte numbers are frequently increased, with striking spatial abnormalities as described above, and their size is often variable but generally smaller than normal. True micromegakaryocytes, similar in size to promyelocytes, are difficult to see without specific immunohistochemical staining, but small megakaryocytes are usually easy to recognize. In addition to their reduced size, these cells typically have reduced numbers of nuclear lobules; the mean number of nuclear lobules per megakaryocyte in normal bone marrow is between 6 and 10. Monolobular small megakaryocytes are particularly associated with the 5q-syndrome (Fig. 19.27) but are also present, together with a heterogeneous population of less-distinctive dysplastic megakaryocytes, in other forms of myelodysplasia.

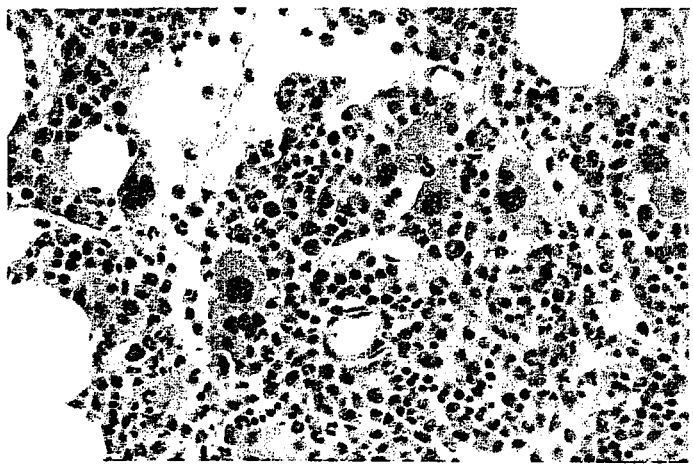

**Fig. 19.27** Small, monolobular megakaryocytes typical of the 5q-syndrome. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 40.

One aspect of cytology in myelodysplasia is often only appreciable in bone marrow trephine biopsy sections and not in aspirate films. This is the increased apoptotic activity that contributes to ineffective hemopoiesis in patients with MDS.[30,31] Apoptotic nuclei, recognizable by their characteristic patterns of nucleic acid condensation, can be seen scattered throughout the hemopoietic tissue and, sometimes, clustered inside the cytoplasm of stromal macrophages. However, increased apoptotic activity also occurs in other conditions involving increased cell turnover in the bone marrow, including hyperplastic states (e.g. associated with septicemia or in response to malignant disease elsewhere in the body), chronic myeloproliferative disorders and acute leukemias. Undue significance should not be attributed to finding increased apoptotic activity in bone marrow trephine biopsy sections if other features of dysplasia are absent.

An interesting recent application of immunohistochemistry in MDS has been for the enumeration of $CD34^+$ hemopoietic stem cells in bone marrow trephine biopsy sections. It has been reported that the $CD34^+$ count assessed in this way has prognostic value, with higher counts predicting increased likelihood of leukemic transformation.[37,38] To date, CD34 immunohistochemistry has not been put into widespread use, at least in the UK, but it may become more widely practiced if the predictive value is confirmed in larger studies. Evaluation of CD34 positivity in bone marrow trephine biopsy sections must be undertaken with care to exclude capillary endothelial cells. Endothelial cells express this antigen strongly and, particularly when cut in cross-section, cannot always be seen to be associated with a vascular lumen. With experience, hemopoietic cells can be recognized by their characteristic granular immunohistochemical staining pattern with monoclonal antibody QBEnd10, reactive with class I CD34 (Fig. 19.32).

As understanding of the genetic basis of hemopoietic disorders, including primary MDS, increases, interest in demonstrating cytogenetic abnormalities *in situ* in bone marrow trephine biopsy sections is growing. Despite the limitations of visualizing signals in sectioned nuclei, in which only part of the chromosomal complement of any cell is represented, methodology has been developed for successful demonstration of numerical chromosomal abnormalities and translocations by fluorescent DNA *in situ* hybridization (FISH) in trephine biopsy sections.[39,40] Applications of FISH to histologic preparations representing MDS have, as yet, been very limited but the prospect of further studies in this area is exciting.

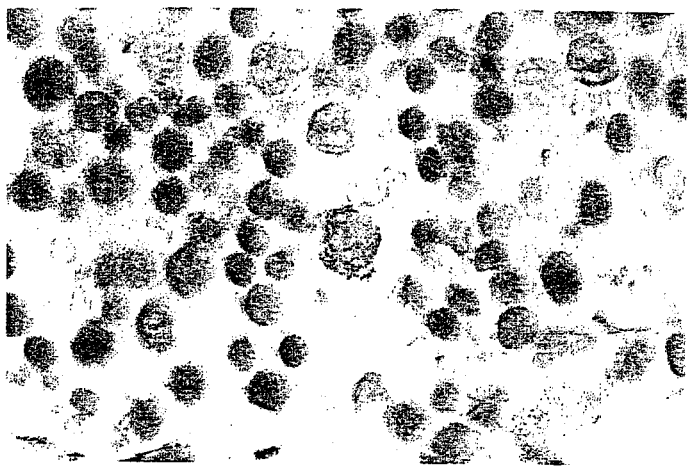

**Fig. 19.32** Primitive hemopoietic cell expressing CD34 with a characteristic granular distribution, as shown by immunohistochemistry using monoclonal antibody QBEnd10 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK). Streptavidin-biotin immunoperoxidase method using section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 100.

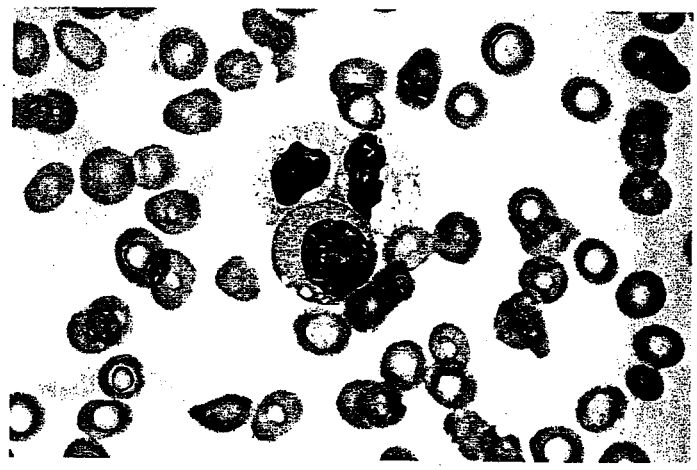

**Fig. 19.33** Blood film from a patient with RA showing vacuolated pseudo-Pelger cells characteristically seen in association with abnormalities of chromosome 17p.

## Karyotype

If possible, direct chromosomal analysis of the bone marrow should be performed in MDS. Clonal cytogenetic abnormalities are found in approximately 50% of cases of primary MDS[41,42] and more than 90% of cases of secondary MDS.[43,44] The more advanced the disease, the greater the incidence of karyotypic abnormality. Even when a normal karyotype is found, monosomies and trisomies are sometimes detected by FISH.[45] An abnormal karyotype gives important prognostic information. Common chromosomal abnormalities include del (5q), −7, +8, del (20q) and −Y. Some specific associations are recognized: for example, isolated del (5q)[46,47] commonly occurs in older women with a macrocytic anemia, normal or raised platelet count, monolobated megakaryocytes and a good prognosis. Del (5q) is not confined to this group; it may be found in other forms of MDS that do not have such a benign prognosis, but then the breakpoint on chromosome 5 is different (being telomeric to 5q31).

Deletions of 17p are characterized by small vacuolated pseudo-Pelger cells;[48,49] rearrangements of 3q26 are associated with raised platelet counts and micromegakaryocytes;[50] and a rare t(5:12) translocation is found in some cases of CMML.[51]

## Genetic abnormalities

In general it is not known what genetic effects of the commonest chromosomal abnormalities produce. Abnormalities at 3q23 cause activation of the *EVI-1* gene[52] while

illness or exposure to cytotoxic agents may leave a distinct cement line around many bone trabeculae, reflecting transient inhibition of normal bone remodeling (Fig. 19.30). Stromal injury may also lead to new bone formation; this is usually only focal and minor in extent but rare patients, for unknown reasons, respond to toxic bone marrow injury with florid neo-osteogenesis.

Other features suggestive of a secondary origin for dysplasia are the presence of inflammatory cells, particularly plasma cells, in increased numbers in the stroma and the finding of reactive lymphoid nodules or granulomas.

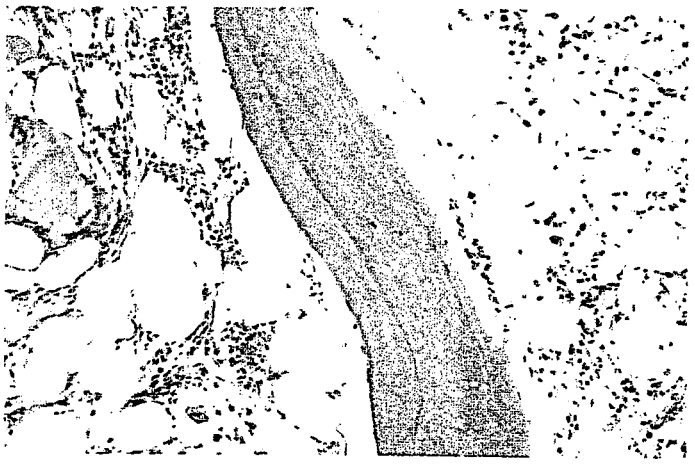

**Figure 19.30** Distinct cement line around a bone trabecula representing a recent period of decreased bone remodeling, as a response to cytotoxic therapy, followed by recovery. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 10.

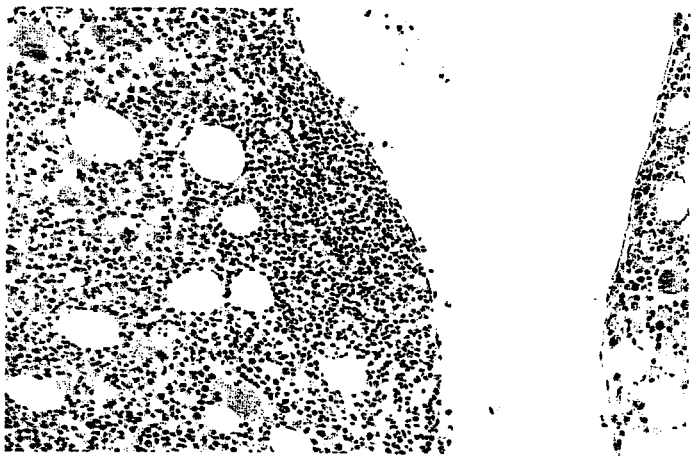

**Fig. 19.31** Poorly defined aggregate of small lymphoid cells abutting the margin of a dilated sinusoid in a case of MDS-RA; the site and organization of this infiltrate are atypical and, considered in isolation, would raise suspicion of a low-grade lymphoid neoplasm. Hematoxylin and eosin-stained section of decalcified, paraffin-embedded bone marrow trephine biopsy core; original magnification × 20.

If lymphoid nodules are seen, it should be remembered that such aggregates, sometimes with atypical features, might also occur in association with primary MDS (Fig. 19.31). The differential diagnosis must include, in addition, bone marrow involvement by lymphoma provoking secondary myelodysplasia (see Chapter 22). Distinguishing between these alternatives can be extremely difficult and requires integration of all clinical, hematologic, cytogenetic and molecular genetic information available for the individual patient under consideration. Even with such information, it may be necessary to follow the patient's subsequent progress, including re-biopsy of their bone marrow, to establish the nature and clinical significance of such abnormalities.

## Use of immunohistochemistry and fluorescent *in situ* hybridization in trephine biopsy sections in myelodysplasia

Immunohistochemistry can be very helpful in interpreting trephine biopsy histology in myelodysplastic conditions when cytologic abnormalities cause difficulty in the recognition of cells belonging to the various hemopoietic lineages.[36] Cells of the granulocytic series, at all stages of maturation, can be demonstrated by immunostaining for muramidase (lysozyme) or the CD68 variant recognized by monoclonal antibody KP1. Use of neutrophil elastase as a target antigen for immunohistochemistry may be unreliable in MDS if hypogranularity of granulocytes is a feature; otherwise, it can be useful to demonstrate the distribution of promyelocytes and myelocytes. Later granulocytes (metamyelocytes and neutrophil polymorphs) express CD15 and the calprotectin molecule recognized by monoclonal antibody Mac387. Numbers of eosinophil, basophil and monocyte precursors are not usually sufficient to cause problems in interpreting the results of these immunostains. However, caution is needed in any patient who does have a significant increase in any of these cell types in their blood or aspirated bone marrow, since the expression by such cells of the antigens described above is incompletely characterized at present.

The identity and distribution of dysplastic erythroid cells can be confirmed by immunohistochemistry to demonstrate glycophorin A or C; glycophorin C is expressed slightly earlier in erythropoiesis than is glycophorin A. Megakaryocytes are usually easily recognizable from their cytologic features but atypical forms, micromegakaryocytes and megakaryoblasts can be highlighted by immunostaining for CD61 (platelet glycoprotein IIIa) or CD42b (platelet glycoprotein Ib).

Table 19.6 Evidence of lymphoid involvement in myelodysplastic syndrome

Transformation of MDS to acute lymphoblastic leukemia
*Occurs but very rare*

Co-existent MDS and lymphoid malignancies
*Commoner than would be expected by chance, but mainly involves myeloma and chronic lymphocytic leukemia (CLL)*

Inactivation of same X chromosome in lymphoid and myeloid cells
*Most frequently not found but well reported index cases*

Same cytogenetic abnormality in lymphoid and myeloid cells
*Occurs but very rare*

Same oncogene mutations in lymphoid and myeloid cells
*Few case reports only*

Table 19.7 Other abnormal pathologic findings in MDS

| Investigation | Abnormality |
|---|---|
| Serum B12[69] | Often high, but coincident pernicious anemia has been reported |
| Serum LDH[70] | High levels carry poor prognosis |
| Ferrokinetics[71] | Shortened red cell survival; ineffective erythropoiesis |
| HbF[72] | Often raised in JMML |
| Ham's test[73] | Positive rarely |
| Red cell enzymes[74] | Raised levels of several enzymes on glycolytic pathway |
| Direct antiglobulin test[75] | Positive in 8% |
| Serum lysozme[76] | Raised in CMML |
| Granulocyte function[77] | Reduced motility, adherence, phagocytosis and bacterial killing |
| NAP score[78] | Often low |
| Platelet function[79] | Prolonged bleeding time, reduced aggregation with adrenaline and collagen |
| Monocyte function[80–85] | Increased cytokine production and receptors for immunoglobulin and complement; decreased cytoplasmic enzymes and phagocytosis |

CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; NAP, neutrophil alkaline phosphatase.

## Natural history

Although some individuals may follow an indolent course, in most the condition will progress. Cytopenias will become worse and bone marrow blast cell counts will increase. In this elderly group, around a third will develop acute leukemia, a third will die of the consequence of cytopenias and a third will die from an unrelated cause.[5]

## Management

Treatment for MDS is unsatisfactory. The keystone is good supportive care. Even in patients who develop acute myeloblastic leukemia, the course is often indolent and no attempt at aggressive therapy should be initiated until the pace of the disease is established. Many patients require no treatment beyond the psychological support of a concerned physician. However, cytopenias lead to infections bleeding and bruising and the symptoms of anemia. Judicious use of red cell transfusions and appropriate antibiotics is essential. Some patients will require platelet transfusions. Those patients expected to live for a considerable time on red cell transfusion will need to consider iron chelation therapy with desferrioxamine.

Some authorities recommend the use of hemopoietic growth factors. Both granulocyte colony stimulating factor (G-CSF) and GM-CSF raise the neutrophil count, but neither has been shown to improve survival in MDS.[89] In patients with a low transfusion requirement and a serum eythropoietin level lower than 200 µg/l, recombinant eythropoietin therapy will raise the hemoglobin in between 30 and 50% of patients with RA or RAEB.[90] In RARS the addition of G-CSF to the eythropoietin is synergistic and necessary to raise the hemoglobin.[91]