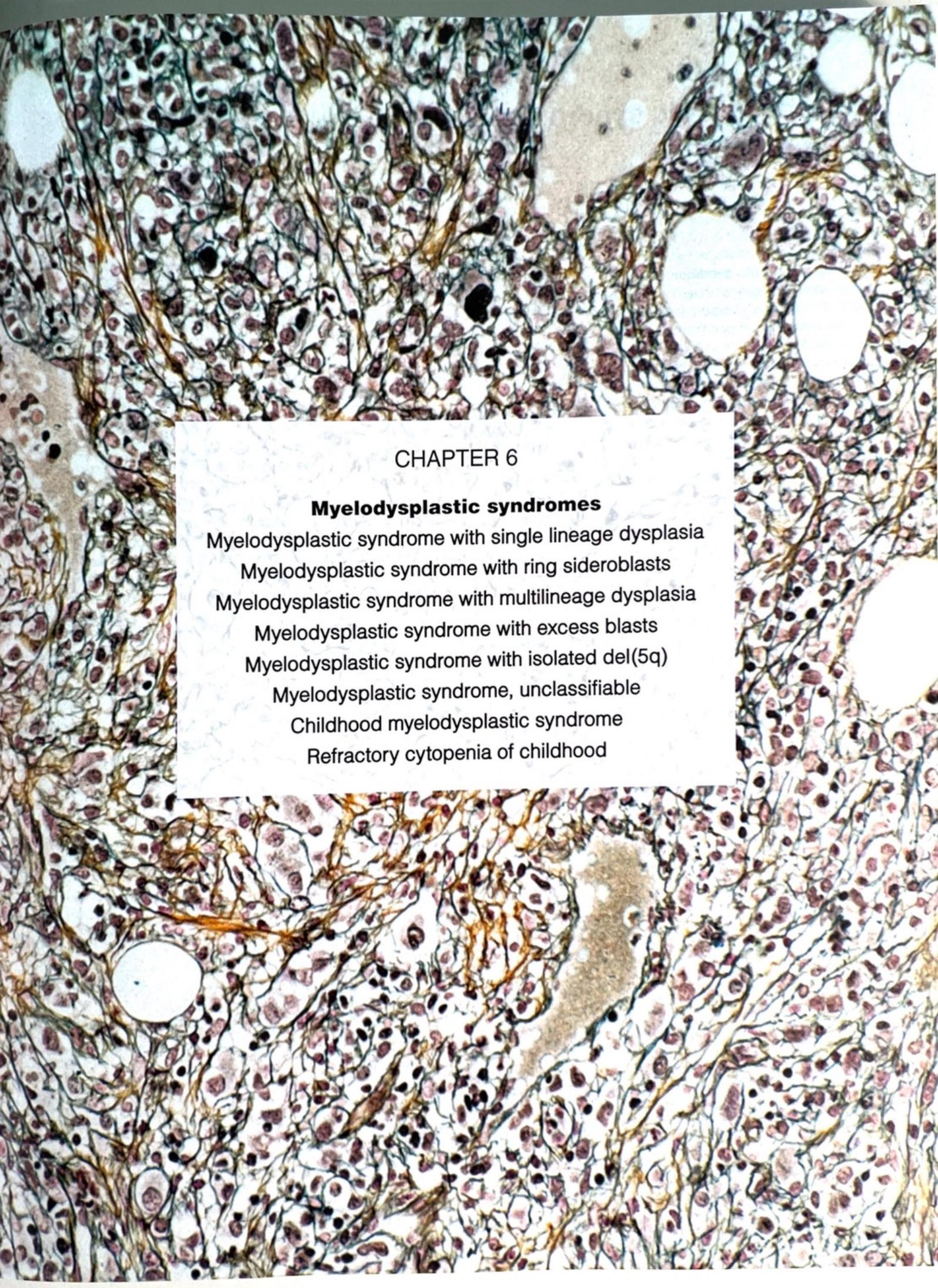

CHAPTER 6

Myelodysplastic syndromes

Myelodysplastic syndrome with single lineage dysplasia

Myelodysplastic syndrome with ring sideroblasts

Myelodysplastic syndrome with multilineage dysplasia

Myelodysplastic syndrome with excess blasts

Myelodysplastic syndrome with isolated del(5q)

Myelodysplastic syndrome, unclassifiable

Childhood myelodysplastic syndrome

Refractory cytopenia of childhood

Myelodysplastic syndromes: Overview

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Definition

The myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterized by cytopenia, dysplasia in one or more of the major myeloid lineages, ineffective haematopoiesis, recurrent genetic abnormalities and increased risk of developing acute myeloid leukaemia (AML) {340, 592,4151}. There is an increased degree of apoptosis within the bone marrow progenitors, which contributes to the cytopenias {439}. Cytopenia in at least one haematopoietic lineage is required for a diagnosis of MDS. The recommended thresholds for cytopenias established in the original International Prognostic Scoring System (IPSS) for risk stratification (haemoglobin concentration < 10 g/dL, platelet count $< 100 \times 10^9$/L, and absolute neutrophil count $< 1.8 \times 10^9$/L {1442,1442A}), have traditionally been used to define cytopenias for MDS diagnosis and most MDS patients will have a cytopenia below at least one of these thresholds. However, a diagnosis of MDS may still be made in patients with milder degrees of anaemia (haemoglobin < 13 g/dL in men or < 12 g/dL in women) or thrombocytopenia (platelets $< 150 \times 10^9$/L) if definitive morphologic and/or cytogenetic findings are present {1444A,4179}. In determining whether a patient is cytopenic, it is important to be cognizant of each laboratory's lower reference range and to take into account conditional variants of these values, such as due to ethnicity and sex. These are particularly important considerations in patients with a borderline low neutrophil count {229}. Persistent neutrophilia, monocytosis, erythrocytosis or thrombocytosis in a patient with cytopenias and dysplastic morphology generally warrants classification as a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) or myeloproliferative neoplasm rather than MDS. However, thrombocytosis (platelet count $\geq 450 \times 10^9$/L) is allowed in MDS with isolated del(5q) or with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2).

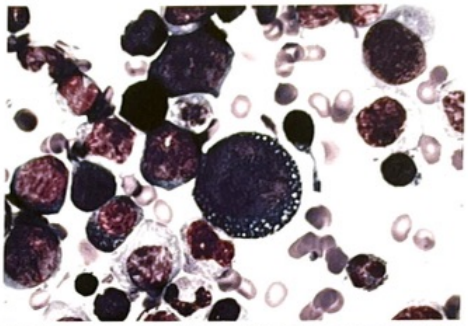

Fig. 6.01 Parvovirus B19 infection. Bone marrow smear shows marked erythroid hypoplasia, with occasional giant erythroblasts with dispersed chromatin and fine cytoplasmic vacuoles.

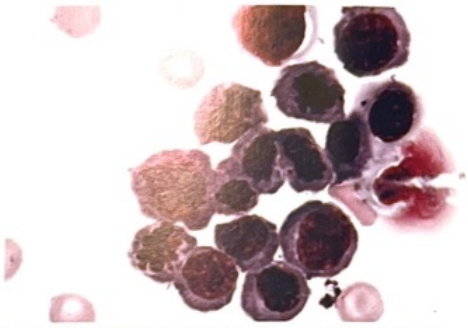

Fig. 6.02 Arsenic poisoning. Bone marrow smear from a 47-year-old man with pancytopenia being chronically exposed to arsenic; there is marked dyserythropoiesis.

The morphological hallmark of MDS is dysplasia in one or more myeloid lineages. Dysplasia may be accompanied by an increase in myeloblasts in the peripheral blood and/or bone marrow, but the blast percentage is always < 20%, which is the requisite threshold recommended for the diagnosis of AML. It is important to recognize that the threshold of 20% blasts distinguishing AML from MDS does not reflect a therapeutic mandate to treat cases with ≥ 20% blasts as acute leukaemia. Recurrent cytogenetic abnormalities are present in 40–50% of cases, whereas acquired somatic gene mutations are seen in the vast majority of MDS cases at diagnosis.

The MDS category encompasses several distinct subtypes, which are defined by the number of cytopenias at presentation, the number of myeloid lineages manifesting dysplasia, the presence of ring sideroblasts, and the blast percentages in the blood and bone marrow. In the current classification, only one cytogenetic abnormality, del(5q), is used in the definition of a specific MDS subtype. Mutation of one gene, *SF3B1*, is closely associated with MDS with ring sideroblasts as well as with one of the MDS/MPN subtypes: MDS/MPN with ring sideroblasts and thrombocytosis.

Although progression to AML is the natural course in many cases of MDS, the percentage of patients who progress varies substantially across the subtypes, with a higher probability of progression in subtypes with increased myeloblasts

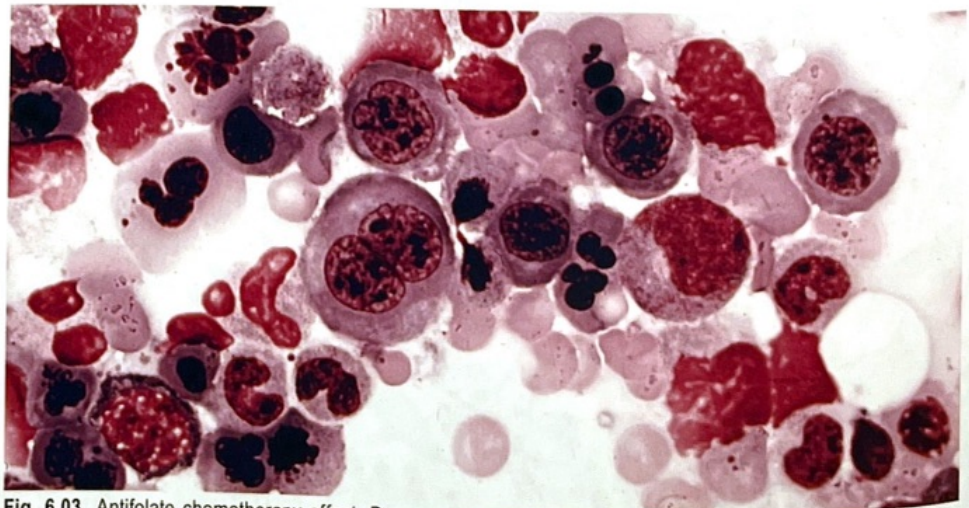

Fig. 6.03 Antifolate chemotherapy effect. Bone marrow smear from a 57-year-old woman who received several chemotherapeutic agents for breast carcinoma, including folic acid antagonists, showing transient marked dyserythropoiesis and megaloblastic changes.

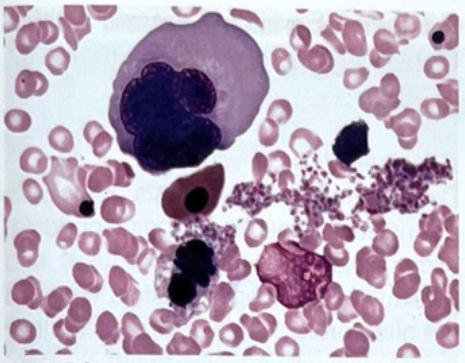

Fig. 6.04 Congenital dyserythropoietic anaemia, type III. Bone marrow smear shows marked dyserythropoiesis.

{1340,2467}. Most subtypes are characterized by progressive bone marrow failure, but the biological course of some subtypes is prolonged and indolent, with a very low incidence of evolution to AML {2462,4179}.

Epidemiology

MDS occurs principally in older adults (median patient age: 70 years), with a male predominance. The annual incidence is 3–5 cases per 100 000 population overall (non–age-corrected) and is at least 20 cases per 100 000 individuals aged >70 years. Due to underreporting of MDS in most cancer registries, the true annual incidence in patients aged >65 years may be closer to 75 cases per 100 000 population {199,777,1342}. Approximately 10 000 new cases of MDS are diagnosed annually in the USA, according to 2003–2004 data from the Surveillance, Epidemiology, and End Results (SEER) Program and the North American Association of Central Cancer Registries (NAACCR), but estimates based on Medicare claims for the same time period are as high as 45 000 cases diagnosed in individuals aged >65 years annually {1394,2421,3397}. Therapy-related myeloid neoplasms are discussed separately (see *Therapy-related myeloid neoplasms*, p. 153). MDS affecting children is rare and has unique characteristics and diagnostic criteria that differ from those of MDS in adults; therefore, childhood cases are also discussed separately (p. 116).

Etiology

Primary or de novo MDS occurs without a known history of chemotherapy or radiation exposure. Possible etiologies for primary MDS include benzene exposure (at levels well above the minimum allowed by most government agencies), cigarette smoking (at least in part also due to benzene in cigarette smoke), exposure to agricultural chemicals or solvents, and family history of haematopoietic neoplasms {3815}. Some inherited haematological disorders, such as Fanconi anaemia, dyskeratosis congenita, Shwachman–Diamond syndrome and Diamond–Blackfan anaemia, are also associated with an increased risk of MDS; familial syndromes predisposing to MDS and AML are discussed separately (see *Myeloid neoplasms with germline predisposition*, p. 121). Acquired aplastic anaemia is also associated with increased risk of development of MDS {222}.

Clinical features

The majority of patients present with symptoms related to cytopenia. Most patients are anaemic, whereas neutropenia and/or thrombocytopenia are less common; about one third of patients are dependent on red blood cell transfusions at diagnosis {1444,2511}. Organomegaly is infrequently observed.

Microscopy

The morphological classification of MDS is principally based on the percentage of blasts in the bone marrow and peripheral blood, the type and degree of dysplasia, and the percentage of ring sideroblasts (Table 6.01, p. 101). The myeloid lineages affected by cytopenias are not necessarily those that manifest dysplasia {1338, 2423,4179}. To determine blast percentage in the bone marrow and blood, a 500-cell differential count of all nucleated cells in a smear or trephine biopsy imprint is recommended for the bone marrow and a 200-leukocyte differential count for the peripheral blood. In patients with severe cytopenia, buffy coat smears of peripheral blood may facilitate the differential count. An accurate blast count in the peripheral blood is important, because patients with higher blast percentages in the blood than in the bone marrow (seen in ~13% of MDS cases) appear to have more aggressive disease {89}. The blast count in myeloid neoplasms is expressed as a percentage of all nucleated cells (always including nucleated erythroid cells) in the bone marrow and as a percentage of the leukocytes (excluding nucleated erythroid cells) in the peripheral blood.

The number of dysplastic lineages (i.e. single lineage vs multilineage dysplasia) is relevant for distinguishing between the types of MDS (see Table 6.01, p. 101) and may be important for predicting disease behaviour {947,4179}. Assessment of the degree of dysplasia may be problematic, depending on the quality of the smear preparations and the stain. Poor-quality smears may result in misinterpretation of the presence or absence of dysplasia, particularly in assessing neutrophil granulation. Given the critical importance of recognizing dysplasia, the need for high-quality slide preparations for the diagnosis of MDS cannot be overemphasized. Slides for the assessment of dysplasia should be made from freshly obtained specimens; specimens exposed to anticoagulants for >2 hours are unsatisfactory. It should be noted that the determination of whether significant dysplasia is present (particularly in the erythroid lineage) and the distinction between single lineage and multilineage dysplasia have been found in some studies to be subject to significant interobserver variability {1233,3622}. This interobserver variability is more problematic for cases in which the degree of dysplasia is near the requisite 10% threshold, and some authors have reported individual lineage dysplasia exceeding the 10% threshold in non-cytopenic controls {947,3067, 3297}; consequently, it is essential to apply strict criteria for dysplasia and to evaluate high-quality and well-stained material.

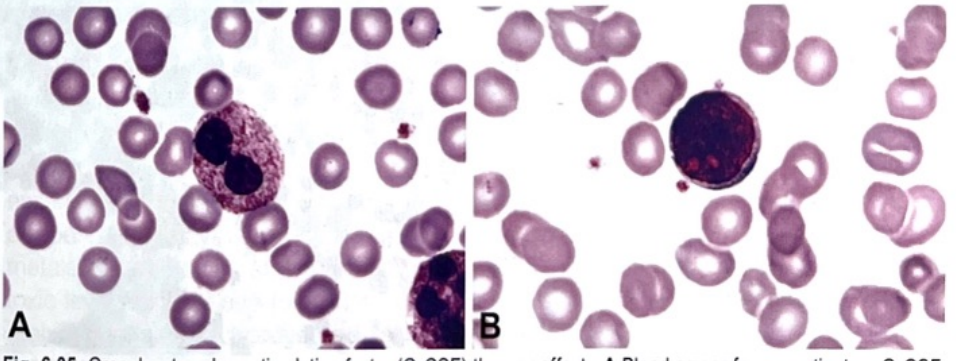

Fig. 6.05 Granulocyte colony-stimulating factor (G-CSF) therapy effect. **A** Blood smear from a patient on G-CSF, showing a neutrophil with a bilobed nucleus and increased azurophilic granulation and a myeloblast (**B**).

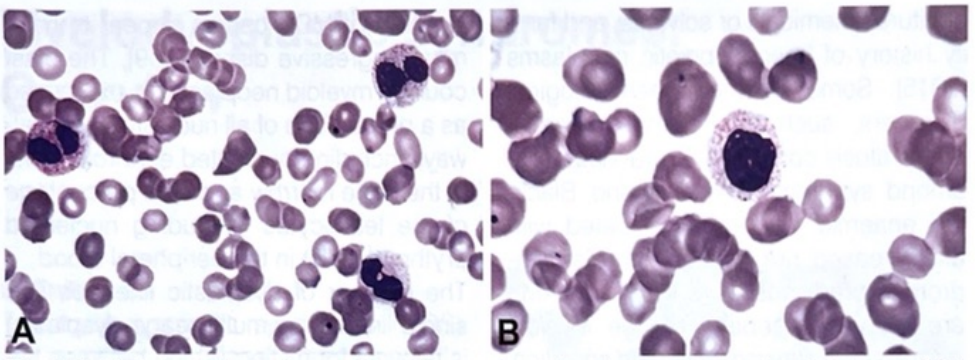

Fig. 6.06 Myelodysplastic syndrome with multilineage dysplasia and a complex karyotype including del(17p). Dysgranulopoiesis is evident on these blood smears, showing three neutrophils with bilobed nuclei (pseudo–Pelger–Huët anomaly) (**A**) and a neutrophil with a non-segmented nucleus (**B**).

As a general precaution, no patient should be diagnosed with MDS if the clinical and drug history is unknown, and no case of MDS should be reclassified while the patient is on growth factor therapy, including erythropoietin. Certain drugs, infections, metabolic deficiencies and immune disorders can cause both cytopenias and morphological dysplasia; these possible secondary etiologies must be carefully considered prior to rendering a diagnosis of MDS (see *Differential diagnosis*). Unexplained, persistent cytopenia in the absence of dysplasia should not be interpreted as MDS unless certain specific cytogenetic abnormalities are present (see *Genetic profile* below and Table 6.03, p. 104). Persistent cytopenia without dysplasia and without one of the specific cytogenetic abnormalities should be diagnosed as idiopathic cytopenia of undetermined significance, and the patient's haematological and cytogenetic status should be carefully monitored {4106,4336}. Patients with MDS-associated clonal gene mutations identified in haematopoietic cells but without significant dysplasia on bone marrow examination should not be diagnosed with MDS either; this condition has been termed 'clonal haematopoiesis of indeterminate potential' {3772}.

Cases of MDS without an increase in blasts are recognized as manifesting either single lineage dysplasia or multilineage dysplasia. In most cases of MDS with single lineage dysplasia, the dysplasia is confined to the erythroid lineage. Single lineage dysplasia can also affect the granulocytic lineage or megakaryocytes, but this is much less common than dysplasia isolated to erythroid cells {450,2423}. In MDS with multilineage dysplasia, significant dysplastic features are recognized in two or more lineages. The recommended requisite percentage of erythroid and granulocytic cells manifesting dysplasia to be considered significant is ≥10% {3404}. Significant megakaryocyte dysplasia is defined as ≥10% dysplastic megakaryocytes based on evaluation of ≥30 megakaryocytes in smears or sections; however, some studies suggest that a 30–40% threshold for megakaryocyte dysplasia may provide greater specificity {947,1309,2567}. Micromegakaryocytes and multinucleated megakaryocytes with separated nuclei are the most reliable dysplastic findings in the megakaryocyte series {947,2567, 4179}.

Characteristics of dysplasia

Dyserythropoiesis manifests principally as nuclear alterations, including budding, internuclear bridging, karyorrhexis and multinuclearity. Megaloblastoid changes are often present in MDS, but alone they are insufficiently specific to firmly establish dyserythropoiesis. Cytoplasmic features include formation of ring sideroblasts, vacuolization and aberrant periodic acid–Schiff (PAS) positivity (either diffuse or granular). Dysgranulopoiesis is characterized primarily by nuclear hyposegmentation (pseudo–Pelger–Huët anomaly) or hypersegmentation, cytoplasmic hypogranularity, pseudo–Chédiak–Higashi granules and small size {1387}. Megakaryocyte dysplasia is characterized by micromegakaryocytes, non-lobated nuclei in megakaryocytes of all sizes, and multiple widely separated nuclei {1388}; however, the finding of multiple widely separated nuclei is of limited specificity for MDS, unless the nuclei are rounded and roughly similar in size. Megakaryocytic dysplasia is readily apparent in bone marrow sections, and both biopsy and aspirate specimens should be evaluated. The morphological manifestations of dysplasia in each lineage are summarized in Table 6.02. Auer rods are considered to be evidence of MDS with excess blasts regardless of the blast percentage. Cases of MDS with <5% blasts in the bone marrow and <1% in the peripheral blood may rarely have Auer rods, and such cases are associated with an adverse prognosis {4328}.

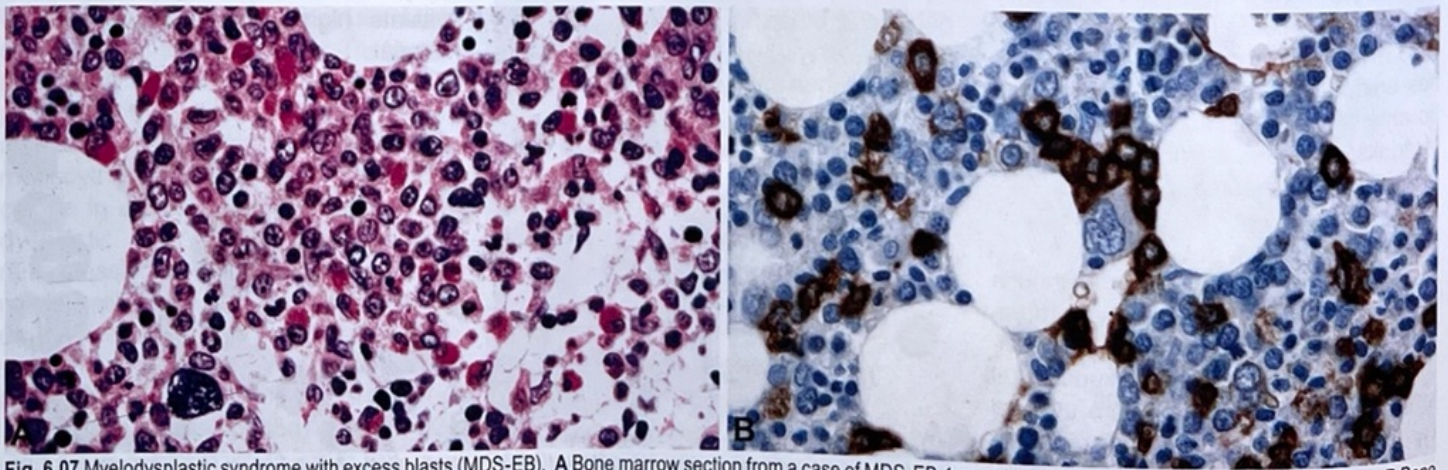

Fig. 6.07 Myelodysplastic syndrome with excess blasts (MDS-EB). **A** Bone marrow section from a case of MDS-EB-1 containing a focus of immature myeloid precursors. **B** Bone marrow biopsy from a case of MDS-EB-2 showing a focus of immature cells, most of which stain positively for CD34.

Table 6.01 Diagnostic criteria for myelodysplastic syndrome (MDS) entities

Entity name	Number of dysplastic lineages	Number of cytopenias[a]	Ring sideroblasts as percentage of marrow erythroid elements	Bone marrow (BM) and peripheral blood (PB) blasts	Cytogenetics by conventional karyotype analysis
MDS-SLD	1	1–2	<15% / <5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
MDS-MLD	2–3	1–3	<15% / <5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
MDS-RS					
MDS-RS-SLD	1	1–2	≥15% / ≥5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
MDS-RS-MLD	2–3	1–3	≥15% / ≥5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1–3	1–2	None or any	BM <5%, PB <1%, no Auer rods	del(5q) alone or with 1 additional abnormality, except loss of chromosome 7 or del(7q)
MDS-EB					
MDS-EB-1	1–3	1–3	None or any	BM 5–9% or PB 2–4%, BM <10% and PB <5%, no Auer rods	Any
MDS-EB-2	1–3	1–3	None or any	BM 10–19% or PB 5–19% or Auer rods, BM and PB <20%	Any
MDS-U					
with 1% blood blasts	1–3	1–3	None or any	BM <5%, PB = 1%[c], no Auer rods	Any
with SLD and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1–3	<15%[d]	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality[e]

MDS-EB, MDS with excess blasts; MDS-MLD, MDS with multilineage dysplasia; MDS-RS, MDS with ring sideroblasts; MDS-RS-MLD, MDS with ring sideroblasts and multilineage dysplasia; MDS-RS-SLD, MDS with ring sideroblasts and single lineage dysplasia; MDS-SLD, MDS with single lineage dysplasia; MDS-U, MDS, unclassifiable; SLD, single lineage dysplasia.

[a] Cytopenias defined as haemoglobin concentration <10 g/dL, platelet count <100 × 10^9/L and absolute neutrophil count <1.8 × 10^9/L, although MDS can present with mild anaemia or thrombocytopenia above these levels; PB monocytes must be <1 × 10^9/L.
[b] If *SF3B1* mutation is present.
[c] 1% PB blasts must be recorded on ≥2 separate occasions.
[d] Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia and are classified as MDS-RS-SLD.
[e] See Table 6.03, p. 104.

Differential diagnosis

A major difficulty in the diagnosis of MDS is the determination of whether the presence of morphological dysplasia and cytopenia is due to a clonal disorder or is the result of another factor. Dysplasia, even if prominent, is not in itself definitive evidence of a clonal process. Some dysplastic features, such as micromegakaryocytes, are strongly associated with MDS {947}, but several nutritional, toxic and other factors can also cause myelodysplastic changes in any of the haematopoietic lineages. These factors include vitamin B12 and folic acid deficiency, essential element deficiencies (such as copper deficiency), exposure to heavy metals (in particular arsenic, lead and toxic levels of zinc) and exposure to several commonly used drugs and biological agents {439}. Isoniazole treatment in the absence of vitamin B6 supplementation causes ring sideroblast formation. The antibiotic cotrimoxazole and the immunosuppressants tacrolimus and mycophenolate mofetil can cause marked neutrophil hyposegmentation, often indistinguishable from the changes seen in MDS. In some patients on multiple drugs or with multiple comorbidities, it may be difficult to identify the cause of dysplastic changes {1991,3867}. Dysplastic changes can also be encountered in otherwise

Table 6.02 Morphological manifestations of dysplasia

Dyserythropoiesis
- Nuclear
  - Nuclear budding
  - Internuclear bridging
  - Karyorrhexis
  - Multinuclearity
  - Megaloblastoid changes
- Cytoplasmic
  - Ring sideroblasts
  - Vacuolization
  - Periodic acid–Schiff (PAS) positivity

Dysgranulopoiesis
- Small or unusually large size
- Nuclear hyposegmentation (pseudo–Pelger–Huët)
- Nuclear hypersegmentation
- Decreased granules; agranularity
- Pseudo–Chédiak–Higashi granules
- Döhle bodies
- Auer rods

Dysmegakaryopoiesis
- Micromegakaryocytes
- Nuclear hypolobation
- Multinucleation (normal megakaryocytes are uninuclear with lobated nuclei)

normal marrow {3067}, such as in individuals with the hereditary autosomal dominant Pelger–Huët anomaly resulting from mutations in the *LBR* gene (encoding lamin B receptor) {1658}. Therefore, it is extremely important to correlate the morphological findings with the clinical presentation and any pertinent family history.

Congenital haematological disorders such as congenital dyserythropoietic anaemia must also be considered as a possible cause of isolated dyserythropoiesis. Parvovirus B19 infection may be associated with erythroblastopenia with giant pronormoblasts; the immunosuppressive agent mycophenolate mofetil may also be associated with erythroblastopenia. Chemotherapeutic agents may result in transient marked dysplasia of all myeloid lineages. Granulocyte colony-stimulating factor therapy causes morphological alterations in the neutrophils, including a substantial left shift, marked hypergranularity and nuclear hyposegmentation {3572}. Additionally, blasts (usually <5%) may be observed transiently in the peripheral blood; the bone marrow blast percentage is generally normal in such cases, but is transiently increased in some cases. Hypothyroidism, infections, autoimmune disorders, paroxysmal nocturnal haemoglobinuria and bone marrow lymphomatous involvement (in particular large granular lymphocytic leukaemia and hairy cell leukaemia) may clinically mimic MDS.

Given all these possibilities, it is extremely important to be aware of the clinical history (including exposure to drugs or chemicals) and to always consider non-clonal disorders as possible etiologies of morphological dysplasia in haematopoietic cells, particularly in cases with no increase in blasts. Haematological follow-up over a period of several months, possibly including repeated bone marrow sampling, may be necessary for difficult cases.

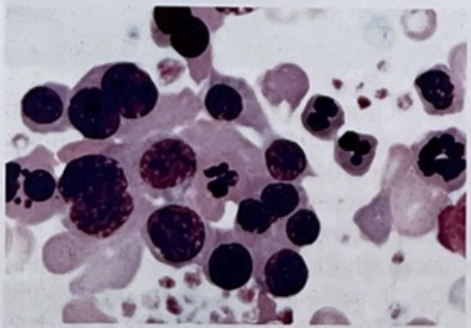

Fig. 6.08 Myelodysplastic syndrome with multilineage dysplasia and complex cytogenetic abnormalities, including del(17p) and del(5q). Dyserythropoiesis is evident on this bone marrow smear from an adult male patient.

Microscopy

The value of bone marrow biopsy in MDS is well established {2981}. It increases the diagnostic accuracy compared with examination of the aspirate smear alone and provides additional information about blast percentage and distribution within the marrow space {4180}. Bone marrow cellularity, megakaryocyte morphology and stromal fibrosis are important features revealed by the biopsy. The bone marrow in MDS is usually hypercellular and less commonly normocellular or hypocellular for age; cytopenias result from ineffective haematopoiesis despite the typically increased cellularity. Histologically, aggressive MDS subtypes can be characterized by the presence of aggregates (3–5 cells) or clusters (>5 cells) of immature myeloid cells in bone marrow biopsies, usually localized in the central portion of the bone marrow away from the vascular structures and endosteal surfaces of the bone trabeculae (so-called abnormal localization of immature precursors) {942}. Immunohistochemistry with an antibody to CD34 (an antigen expressed in the blasts in most MDS cases) can be used to confirm the blast nature of immature myeloid cells in the biopsy

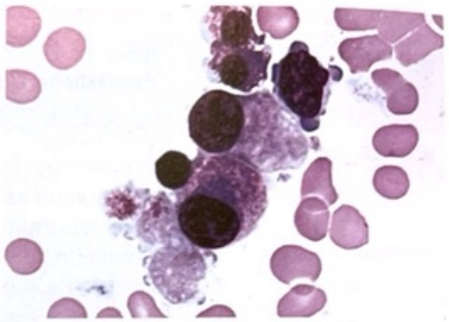

Fig. 6.09 Dysplastic megakaryocytes. Bone marrow aspirate smear from a 37-year-old man with pancytopenia, showing hypolobated megakaryocytes and micromegakaryocytes.

sections {3724,3895A}. Immunohistochemical analysis with CD34 is especially useful for assessing blast percentage in cases of MDS with fibrosis or a hypocellular marrow, in which blast percentages are often underestimated in the smear preparations. CD34 is positive in megakaryocytes in some cases of MDS, but may also stain megakaryocytes in megaloblastic anaemia {1761}. KIT (CD117) staining can be informative in MDS cases with CD34-negative blasts. However, KIT is expressed not only by myeloblasts but also by proerythroblasts, promyelocytes and mast cells. Megakaryocyte markers (e.g. CD42b and CD61) can facilitate identification of small megakaryocytes and micromegakaryocytes, although apoptotic megakaryocytes may superficially mimic micromegakaryocytes in the immunostained sections. Immunostaining for p53 can be useful {2983,2988}, because it correlates well with *TP53* mutation status and has important prognostic significance {769,3472}.

Hypoplastic myelodysplastic syndrome

In approximately 10% of MDS cases, the bone marrow is hypocellular for age. These cases have been referred to as hypoplastic MDS. This group does not constitute a specific MDS subtype in this classification. It can present with or without increased bone marrow blasts; some studies have suggested that hypocellularity may be an independent favourable prognostic variable in MDS {1723,4453}. Hypocellularity in MDS may lead to difficulties in the differential diagnosis with aplastic anaemia {1445,2982}; significant dysplasia (most often micromegakaryocytes), increased blasts identified by CD34 staining of bone marrow biopsy sections, and an abnormal karyotype (excluding trisomy 8, which may be seen

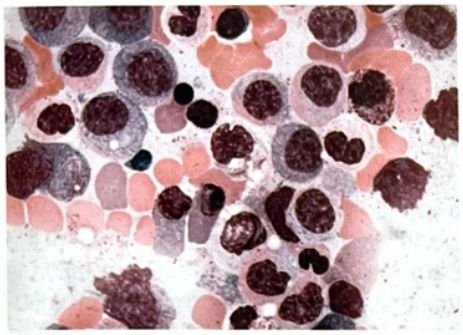

Fig. 6.10 Myelodysplastic syndrome with multilineage dysplasia. Dysgranulopoiesis is present in the bone marrow aspirate smear, including hypogranular neutrophils with pseudo–Pelger–Huët anomaly.

in some cases of aplastic anaemia) are helpful in this distinction {342,945,947}. MDS-associated somatic mutations have been reported to occur in as many as one third of patients with aplastic anaemia {4440}. Immunosuppressive therapies used to treat aplastic anaemia have been used with some degree of success in this MDS subgroup {439,2334,3698,4447, 4448}. When considering the diagnosis of hypoplastic MDS, it is important to exclude acute marrow injury due to a toxin, infection or an autoimmune disorder.

Myelodysplastic syndrome with fibrosis

Significant degrees of myelofibrosis (i.e. corresponding to grade 2 or 3 of the WHO grading scheme) {3975} are observed in 10–15% of MDS cases, and these cases have been referred to as MDS with fibrosis (MDS-F) {2201}. Significant fibrosis does not define a specific MDS subtype in this classification. However, many of the cases with fibrosis have an excess of blasts, and significant fibrosis is associated with an aggressive clinical course in MDS, independent of the blast count {942,1261}. MDS-F cases with excess blasts may erroneously be diagnosed as low-grade MDS based only on the blast count determined from the bone marrow aspirate, which is usually diluted with peripheral blood. In this fibrotic group, as in other cases of MDS with inadequate aspirates, accurate blast determination requires a bone marrow biopsy, and immunohistochemical studies for CD34 may prove invaluable. Unlike the myeloproliferative neoplasm entity primary myelofibrosis, MDS-F is usually not associated with splenomegaly, leukoerythroblastosis or intrasinusoidal haematopoiesis and typically exhibits MDS-type megakaryocyte morphology (i.e. micromegakaryocytes), other dysplastic changes and often increased blasts as revealed by CD34 immunostaining {947}.

Immunophenotype

The immunophenotypic abnormalities that have been described in MDS haematopoietic cells compared with normal haematopoiesis are abnormal quantity and aberrant phenotypes of progenitor cells; aberrant immunophenotypic profiles of maturing granulocytic, erythroid and monocytic cells; and a decrease of haematogones {63,1856,2555,2935, 3895}. Abnormal myeloid maturation patterns include asynchrony of CD15 and CD16 on granulocytes; altered expression of CD13 in relation to CD11b or CD16; and aberrant expression of CD56 and/or CD7 on progenitors, granulocytes or monocytes. Decreased side-scatter of granulocytes can also be seen. In erythroid cells, an increased coefficient of variation and decreased intensity of CD71 or CD36 expression are highly associated with MDS {2563}. There is generally good correlation between the percentage of blasts as determined by morphological examination of the bone marrow aspirate smear or touch imprint, immunohistochemistry of the bone marrow biopsy section and flow cytometry (CD34+ cells) {1994}. However, in some cases there may be significant discordance due to marrow fibrosis or haemodiluted samples; therefore, percentages of CD34+ cells as determined by flow cytometry cannot replace the morphological differential count. Nevertheless, the finding of CD34+ myeloid progenitors accounting for >2% of nucleated cells has been reported to be of adverse prognostic significance in MDS {2555A,2556}.

Flow cytometry findings alone are not sufficient to establish a primary diagnosis of MDS in the absence of definitive morphological and/or cytogenetic findings. A series of consensus guidelines has been published by the European LeukemiaNet (ELN) MDS working group regarding the use of flow cytometry in the diagnostic work-up of patients with MDS {3223, 4117,4118,4305}, including a summary of the reported aberrations associated with MDS and how to report the results {2463, 3223,4119}. Aberrant findings in at least three tested features and at least two cell compartments have been reported to be highly associated with an MDS or MDS/MPN diagnosis in several studies {3221,3223,4063,4119}. More limited

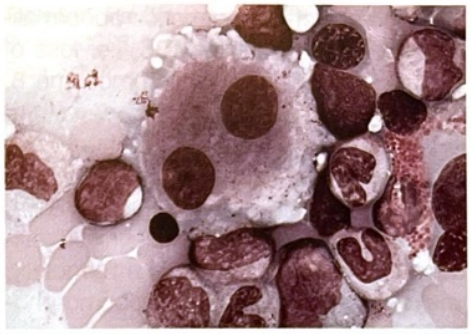

Fig. 6.11 Dysplastic megakaryocyte. Bone marrow smear from a case of myelodysplastic syndrome with single lineage dysplasia shows a binucleated megakaryocyte with separated round nuclei.

screening panels have also been applied {4,259,945,1856,2933,3287}, but may be less sensitive and less specific than larger panels.

Cell of origin

The postulated cell of origin is a haematopoietic stem cell.

Genetic profile

Cytogenetic studies play a major role in the evaluation of patients with MDS in regard to prognosis, determination of clonality {1442,3551,2971} and recognition of cytogenetic correlates with morphological and clinical features. MDS with isolated del(5q), i.e. either with a del(5q) alone or with one additional abnormality other than loss of chromosome 7 or del(7q), is a specific MDS subtype in this classification. It occurs more often in women and is characterized by megakaryocytes with non-lobated or hypolobated nuclei, macrocytic anaemia, normal or increased platelet count, and a favourable clinical course. Loss of 17p is associated with MDS or AML with pseudo–Pelger–Huët anomaly, small vacuolated neutrophils, *TP53* mutation and an unfavourable clinical course; it is most common in therapy-related MDS {2187}. Complex karyotypes (≥3 abnormalities) typically include abnormalities of chromosomes 5 and/or 7, such as del(5q) or t(5q), loss of chromosome 7, and del(7q); these are generally associated with an unfavourable clinical course. Several other cytogenetic findings appear to be associated with characteristic morphological abnormalities; for example, isolated del(20q) is associated with dysmegakaryopoiesis and thrombocytopenia, and inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) is associated with abnormal megakaryocytes and may be associated with thrombocytosis. {446,1500,2142,3392}.

Certain clonal cytogenetic abnormalities that often occur in MDS, i.e. loss of Y chromosome, gain of chromosome 8, and del(20q), have also been described in non-neoplastic conditions; when these occur as a sole abnormality in the absence of defining morphological criteria, they are not considered definitive evidence of MDS. In cases with refractory, unexplained cytopenia but no morphological evidence of dysplasia or increased blasts, the other cytogenetic abnormalities listed in Table 6.03 are considered presumptive evidence of MDS, and such cases are included in the category of MDS, unclassifiable. It is recommended that these patients be followed carefully for emerging morphological evidence of a more specific MDS subtype. The presence of MDS-type cytogenetic abnormalities may be used to support a diagnosis of MDS-EB in rare cases associated with excess blasts without clear-cut evidence of dysplasia.

In addition to recurrent cytogenetic abnormalities identified by conventional karyotyping, which are present in about 50% of MDS cases, recurrent somatic mutations in more than 50 genes have been identified in 80–90% of MDS cases. The genes found to be mutated in at least 5% of MDS cases are listed in Table 6.04. The most commonly mutated genes in MDS encode proteins that control RNA splicing (*SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2* in aggregate mutated in >50% of cases) or epigenetic regulation of gene expression via DNA methylation (*TET2*, *DNMT3A*, *IDH1* and *IDH2*) or histone modification (*ASXL1* and *EZH2*). Other commonly mutated genes are those encoding transcription factors (*RUNX1*, *NRAS*, *BCOR*), signalling proteins (*CBL*), the tumour suppressor p53 (*TP53*), and the cohesin complex (*STAG2*), which controls the cohesion of sister chromatids {1513,3050}. As with cytogenetic abnormalities, specific mutations have been associated with specific morphological features in MDS. For example, *SF3B1* mutation is associated with ring sideroblasts and mutations in *ASXL1*, *RUNX1*, *TP53* and *SRSF2* are associated with severe granulocytic dysplasia {947}.

The mutational landscape of MDS is complex and dynamic. Multiple mutations can be present (most often in a spliceosome gene plus an epigenetic regulator); distinct mutation profiles can be present in two or more subclones; and the relative proportions of these subclones can shift over the course of treatment and disease progression {4232}. Acquired clonal mutations identical to those seen in MDS (affecting genes such as *ASXL1*, *TP53*, *JAK2*, *SF3B1*, *TET2* and *DNMT3A*) can also occur in the haematopoietic cells of apparently healthy older individuals without MDS {1326,1830,3772}. Therefore, MDS-associated somatic mutations alone are not considered diagnostic of MDS in this classification, even in patients with unexplained cytopenia. Rare cases of familial MDS are associated with germline mutations, which can be investigated by sequencing non-MDS tissue (e.g. normal lymphocytes). These cases and their associated mutations are discussed separately (see *Myeloid neoplasms with germline predisposition*, p. 121). In the current classification, *SF3B1* mutation is the only genetic abnormality that influences MDS subtype assignment, as part of the diagnostic criteria for MDS with ring sideroblasts.

Table 6.03 Recurrent chromosomal abnormalities and their frequencies in myelodysplastic syndrome (MDS) at diagnosis

Chromosomal abnormality	Frequency	
	MDS overall	Therapy-related MDS
Unbalanced		
Gain of chromosome 8[a]	10%	
Loss of chromosome 7 or del(7q)	10%	50%
del(5q) or t(5q)	10%	40%
del(20q)[a]	5–8%	
Loss of Y chromosome[a]	5%	
Isochromosome 17q or t(17p)	3–5%	25–30%
Loss of chromosome 13 or del(13q)	3%	
del(11q)	3%	
del(12p) or t(12p)	3%	
del(9q)	1–2%	
idic(X)(q13)	1–2%	
Balanced		
t(11;16)(q23.3;p13.3)		3%
t(3;21)(q26.2;q22.1)		2%
t(1;3)(p36.3;q21.2)	1%	
t(2;11)(p21;q23.3)	1%	
inv(3)(q21.3q26.2)/ t(3;3)(q21.3;q26.2)	1%	
t(6;9)(p23;q34.1)	1%	

[a] As a sole cytogenetic abnormality in the absence of morphological criteria, gain of chromosome 8, del(20q) and loss of Y chromosome are not considered definitive evidence of MDS; in the setting of persistent cytopenia of undetermined origin, the other abnormalities shown in this table are considered presumptive evidence of MDS, even in the absence of definitive morphological features.

Prognosis and predictive factors

The subtypes of MDS included in this classification can be generally categorized into three risk groups on the basis of survival time and incidence of evolution to AML. The low-risk group contains MDS with single lineage dysplasia, MDS with ring sideroblasts and single lineage dysplasia, and MDS with isolated del(5q). The intermediate-risk group contains MDS with multilineage dysplasia and MDS with ring sideroblasts and multilineage dysplasia. The high-risk group consists of MDS with excess blasts. The category of MDS, unclassifiable, encompasses cases with heterogeneous clinical behaviour. Patients with bicytopenia despite single lineage dysplasia have been reported to have shorter survival times than patients with one cytopenia; conversely, patients with one cytopenia and multilineage dysplasia have longer survival times than patients with bicytopenia {4179}.

The importance of cytogenetic features as prognostic indicators in MDS was codified by the International MDS Risk Analysis Workshop in 1997 {1442}, and this cytogenetic risk categorization was updated in 2012 {3551}. The current Comprehensive Cytogenetic Scoring System (CCSS) for MDS contains five prognostic subgroups (Table 6.05). The original IPSS risk stratification scheme for MDS {1442} was also updated in 2012. The Revised IPSS (IPSS-R) {1444} incorporates the percentage of bone marrow blasts, CCSS cytogenetic risk group, and degree of cytopenia in each lineage to predict survival and risk of evolution to AML (Table 6.06). The blast percentage thresholds used in the IPSS-R differ from those in the current WHO classification, and include a 0–2% blast category that is not included in this classification; therefore, it is important to note the actual

Table 6.04 Common gene mutations in myelodysplastic syndromes (i.e. found in at least 5% of cases) {311,312,1513,3050,3988,4478}

Gene mutated	Pathway	Frequency	Prognostic impact
SF3B1[a]	RNA splicing	20–30%	Favourable
TET2[a]	DNA methylation	20–30%	See footnote[b]
ASXL1[a]	Histone modification	15–20%	Adverse
SRSF2[a]	RNA splicing	~15%	Adverse
DNMT3A[a]	DNA methylation	~10%	Adverse
RUNX1	Transcription factor	~10%	Adverse
U2AF1[a]	RNA splicing	5–10%	Adverse
TP53[a]	Tumour suppressor	5–10%	Adverse
EZH2	Histone modification	5–10%	Adverse
ZRSR2	RNA splicing	5–10%	See footnote[b]
STAG2	Cohesin complex	5–7%	Adverse
IDH1/IDH2	DNA methylation	~5%	See footnote[b]
CBL[a]	Signalling	~5%	Adverse
NRAS	Transcription factor	~5%	Adverse
BCOR[a]	Transcription factor	~5%	Adverse

[a] These genes are also reported to be mutated in clonal haematopoietic cells in a subset of healthy individuals (clonal haematopoiesis of indeterminate potential).
[b] Either neutral prognostic impact or conflicting data.

Table 6.05 The Comprehensive Cytogenetic Scoring System (CCSS) for myelodysplastic syndromes. From: Schanz J, et al. {3551}

Prognostic subgroup	Defining cytogenetic abnormalities
Very good	Loss of Y chromosome del(11q)
Good	Normal del(5q) del(12p) del(20q) Double, including del(5q)
Intermediate	del(7q) Gain of chromosome 8 Gain of chromosome 19 Isochromosome 17q Single or double abnormalities not specified in other subgroups Two or more independent non-complex clones
Poor	Loss of chromosome 7 inv(3), t(3q) or del(3q) Double including loss of chromosome 7 or del(7q) Complex (3 abnormalities)
Very poor	Complex (> 3 abnormalities)

bone marrow blast percentage in all MDS diagnoses, so that the IPSS-R can be applied. Five IPSS-R risk groups are defined, on the basis of the total score of the parameters listed in Table 6.06: very low, low, intermediate, high and very high. The IPSS-R is significantly better at predicting survival and evolution to AML than the original IPSS {4219}. Consideration of patient age further improves survival prediction in the IPSS-R {1444}. Another risk-stratification scheme used to predict outcome in MDS is the WHO Classification–based Prognostic Scoring System (WPSS), which incorporates additional variables of transfusion requirement and morphological dysplasia (single lineage vs multilineage) that are not included in the IPSS-R. The WPSS may be particularly useful when applied to lower-risk cases and at time points after the initial diagnosis {2462}.

Accumulating data indicate that both number and type of individual gene mutations are strongly associated with disease outcome in MDS. The addition of mutation data improves the ability of existing risk-stratification schemes such as the IPSS to predict prognosis in MDS {311,312}. Many commonly mutated

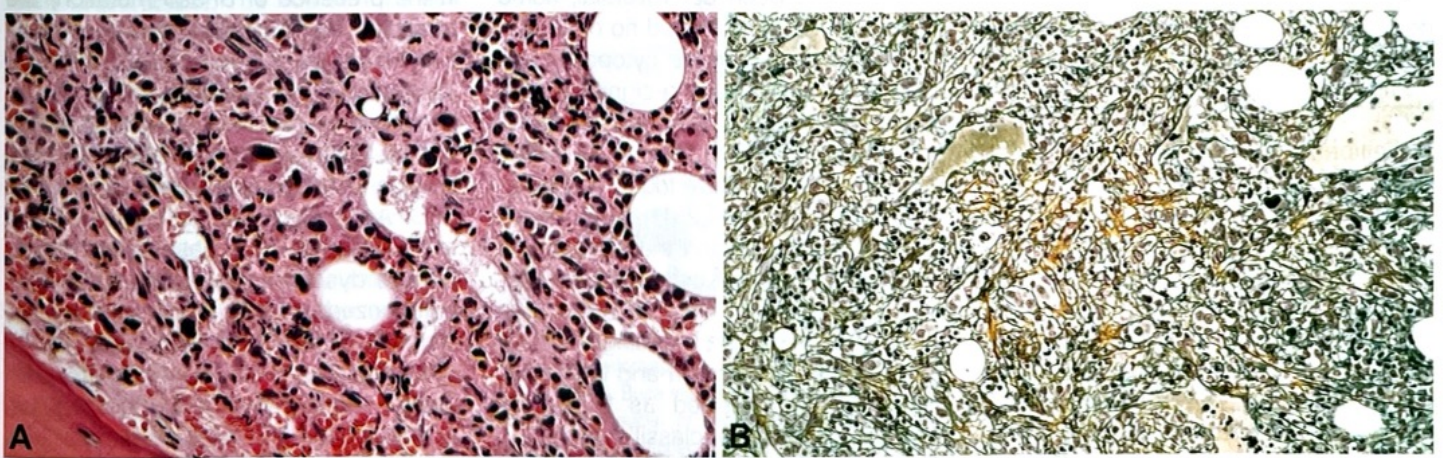

Fig. 6.12 Myelodysplastic syndrome with fibrosis (MDS-F). **A** Bone marrow biopsy from a case of MDS with excess blasts and fibrosis shows several micromegakaryocytes. **B** Reticulin of a marrow biopsy from a case of MDS with fibrosis reveals a marked increase in reticulin fibres.

genes have been associated with an unfavourable prognosis in MDS; whereas mutation in *SF3B1* is associated with a more favourable prognosis (Table 6.04, p. 105). Certain mutations may also be associated with responses to specific therapies. For example, *TET2* and *DNMT3A* mutations appeared to affect the therapeutic response of patients with MDS to hypomethylating agents in one study {4041}, and *TP53* mutation in MDS with del(5q) may predict a poorer response to lenalidomide {2474}. *TP53* mutation in MDS is associated with very aggressive disease, and predicts shorter survival in patients undergoing stem cell transplantation {769,313}. Sensitive sequencing techniques should optimally be applied in MDS mutation analysis for prognosis, because even small subclones present at the initial diagnosis may show mutations in relevant genes, such as *TP53*, and can later expand to confer therapeutic resistance {1894,4232}.

Table 6.06 The Revised International Prognostic Scoring System (IPSS-R) score values for myelodysplastic syndromes. From: Greenberg PL, et al. {1444}

Prognostic variable	Score values						
	0	0.5	1	1.5	2	3	4
Karyotype (CCSS group [a])	Very good	—	Good	—	Intermediate	Poor	Very poor
Bone marrow blast percentage	≤2%	—	>2% to <5%	—	5–10%	>10%	—
Haemoglobin concentration (g/dL)	≥10	—	8 to <10	<8	—	—	—
Platelets (× 10^9/L)	≥100	50 to <100	<50	—	—	—	—
Absolute neutrophil count (× 10^9/L)	≥0.8	<0.8	—	—	—	—	—

Five risk groups are defined, on the basis of the total score of the parameters listed above:

Very low:	≤1.5
Low:	>1.5 to 3
Intermediate:	>3 to 4.5
High:	>4.5 to 6
Very high:	>6

— Indicates not applicable

[a] The Comprehensive Cytogenetic Scoring System (CCSS) group definitions are listed in Table 6.05, p. 105.

Myelodysplastic syndromes

Myelodysplastic syndrome with single lineage dysplasia

Brunning R.D.
Hasserjian R.P.
Porwit A.
Bennett J.M.
Orazi A.
Thiele J.
Hellström-Lindberg E.
List A.F.

Definition

The category of myelodysplastic syndrome (MDS) with single lineage dysplasia (MDS-SLD) encompasses the MDS cases that present with unexplained cytopenia or bicytopenia, with ≥10% dysplastic cells in one myeloid lineage. Most patients present with persistent unexplained anaemia or bicytopenia; some present with persistent unexplained neutropenia or thrombocytopenia {2423}.

In the 2008 edition of this classification, MDS-SLD was called refractory cytopenia with unilineage dysplasia, and was divided into three subtypes: refractory anaemia, refractory neutropenia and refractory thrombocytopenia. This subclassification has been controversial; some studies have demonstrated no clear correlation between lineage cytopenia and lineage dysplasia and no significant differences in survival between the three subtypes {1503,2423,2568,4179}. However, other studies have found some survival differences {449,2511}. Given these conflicting findings and the inconsistencies between lineage cytopenia and lineage dysplasia, we recommend that cases of MDS presenting with single lineage cytopenia or bicytopenia and unilineage dysplasia be classified as MDS-SLD, without additional subclassification.

The presenting lineage dysplasia and cytopenias(s) should be noted in the diagnostic conclusion. The defining feature of this type of MDS is ≥10% dysplastic cells in one myeloid lineage. Cases with erythroid dysplasia only and ≥15% ring sideroblasts (or ≥5% ring sideroblasts in the presence of *SF3B1* mutation) are classified as MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD). If *SF3B1* mutation status is unknown, it is recommended that cases with 5–14% ring sideroblasts and single lineage dysplasia be classified as MDS-SLD. As in the 2008 classification, it is recommended that cases with single lineage dysplasia and pancytopenia be categorized as MDS, unclassifiable.

As noted in the *Overview* section (p. 98), the recommended thresholds for defining cytopenias are haemoglobin concentration <10 g/dL, absolute neutrophil count $<1.8 \times 10^9$/L and platelet count $<100 \times 10^9$/L, as per the risk