Chapter 2
Morphological, Flow Cytometry, and Cytogenetic Diagnosis of MDS

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Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematological proliferations presenting with unremitting cytopenias and morphologic dyspoiesis in one or more hematopoietic cell lineages. The hallmark dyspoiesis can be appreciated morphologically in the peripheral blood and bone marrow, and is associated with aberrant patterns of antigen expression on hematopoietic cells detected by flow cytometry (FCM). FCM can be used as a part of the diagnostic algorithm in suspected cases of MDS; however, in the revised 4th edition WHO Classification of Myeloid Neoplasms published in 2017, the presence of FCM abnormalities alone in the absence of conclusive morphologic features is not considered sufficient to establish a diagnosis of MDS [1].

The term clonal signifies that the abnormal hematopoiesis is due to recurrent genetic abnormalities affecting the MDS stem cells, the proliferation of which overtakes normal hematopoiesis, leading to ineffective hematopoiesis and peripheral blood cytopenias. For nearly 50 years, these genetic abnormalities were detected by bone marrow karyotype, which provides a global view of the full chromosome complement. By conventional karyotyping, about 50% of MDS cases have cytogenetic abnormalities, which usually result in unbalanced losses or gains of genetic material. Specific chromosomal aberrations, along with the degree of cytopenias and percentage of blasts in bone marrow, represent a cornerstone of MDS risk stratification. The International Prognostic Scoring System (IPSS) was defined in 1997 for predicting the prognosis and overall survival in MDS cases and was subsequently

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revised in 2012 [2]. Due to rapid advances in the field of molecular genetics, subkaryotypic genetic abnormalities detected by single nucleotide polymorphism (SNP) arrays and next-generation sequencing (NGS) techniques have increased the percentage of MDS cases with detectable genetic aberrations to over 90%. This chapter addresses the use of morphology (in peripheral blood and bone marrow), FCM, and cytogenetics to diagnose and classify MDS.

Diagnosis and Classification of MDS

In the initial approach to a putative MDS case, the diagnostician must first determine if the basic underlying criteria of MDS are fulfilled and exclude possible non-neoplastic reactive mimics of MDS; the latter are discussed in another chapter. The basic prerequisites to establish a diagnosis of MDS are as follows: (1) the presence of at least one unexplained cytopenia, which is most often anemia (with or without thrombocytopenia and/or neutropenia) and is less commonly isolated neutropenia or thrombocytopenia; and (2) morphologic dysplasia (with or without an increase in blast cells) in at least one of the three hematopoietic lineages manifesting in the bone marrow and/or blood smear. MDS is known to be a clonal disease and this clonality can often be documented by abnormal bone marrow karyotype and/or specific mutations detected by NGS. Additionally, FCM often demonstrates phenotypic abnormalities in myeloblasts and maturing hematopoietic elements. However, while genetic evidence of clonality and abnormal FCM immunophenotype can be supportive of a diagnosis of MDS (and conversely, normal FCM and lack of detectable mutations on a large MDS-directed NGS panel tend to argue against MDS), these findings are insufficient to establish a diagnosis of MDS in the absence of the two prerequisites mentioned above. The only exception is in cases bearing certain MDS-defining cytogenetic abnormalities (discussed later), which can establish a diagnosis of MDS in a cytopenic patient even in the absence of sufficient morphologic dysplasia.

Once a primary diagnosis of MDS is established, the disease must be classified in order to help guide patient management according to the expected disease behavior. The classification of MDS has changed over the years. These hematological conditions were first described in the early 1900s and were labelled as “refractory anemia/preleukemia.” The French-American-British (FAB) co-operative group in 1976 gave them the name “dysmyelopoietic syndromes,” which comprised refractory anemia with excess blasts (RAEB) and chronic myelomonocytic leukemia (CMML). The term was modified to “myelodysplastic syndromes” in 1982, in order to acknowledge the wide range of morphologic findings in the peripheral blood and bone marrow seen in the disease. MDS according to the FAB comprised 5 entities: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), RAEB, refractory anemia with excess blasts in transformation to AML (RAEB-t), and CMML. This classification was widely used for the next 20 years by clinicians and pathologists and had prognostic value; however, there was broad variation in patient outcome in each category. The World Health Organization (WHO) classification in

2001 built upon the FAB system and added new entities to this category, including the distinction between single and multilineage dysplasia in refractory cytopenia with multilineage dysplasia (RCMD), blast count stratification splitting RAEB into two sub-categories, and the first use of a cytogenetic aberration, an isolated del(5q), to define a new MDS subtype [3]. Additionally, CMML was removed and placed in the MDS/myeloproliferative neoplasm (MDS/MPN) category and RAEB-t was reclassified as AML by reducing the AML-defining blast count from 30% to 20%. In 2008, the WHO also added a provisional category of MDS in children and an unclassifiable MDS (MDS-U) category and also allowed for patients with cytopenias other than anemia (diagnostic names changed to "refractory cytopenia" rather than "refractory anemia") [3].

The latest classification of MDS was published in 2017 by the WHO and is currently the most widely used classification system [1]. This classification removed the terms cytopenia, anemia, neutropenia, and thrombocytopenia from the MDS names and instead used the terms "single lineage dysplasia" or "multilineage dysplasia." Another major change was that cases with erythroid predominance (>50% bone marrow erythroids) and non-erythroid blast count of ≥20%, previously considered to be the erythroid/myeloid erythroleukemia subtype of AML, were re-classified as MDS based on the blast percentage of total bone marrow cells. The following are the MDS disease categories according to the 2017 WHO Classification [1]:

I. Myelodysplastic syndrome with single lineage dysplasia (MDS-SLD)
II. Myelodysplastic syndrome with multilineage dysplasia (MDS-MLD)
III. Myelodysplastic syndrome with ring sideroblasts (MDS-RS)
    (a) MDS-RS and single lineage dysplasia (MDS-RS-SLD)
    (b) MDS-RS and multilineage dysplasia (MDS-RS-MLD)
IV. Myelodysplastic syndrome with isolated del(5q)
V. Myelodysplastic syndrome with excess blasts (MDS-EB)
    (a) MDS-EB-1
    (b) MDS-EB-2
VI. Myelodysplastic syndrome, unclassifiable (MDS-U)
VII. Refractory cytopenia of childhood (provisional)
VIII. Therapy-related MDS (t-MDS)

Morphology in Peripheral Blood and Bone Marrow

Peripheral Blood

Examination of the peripheral blood smear is the first step in the diagnosis of MDS. By definition, all MDS patients have at least one peripheral cytopenia at presentation. The peripheral smear may show the presence of blasts as well as with

dyspoietic granulocytes, red cells, and abnormal platelets. In a five-part cell differential analyzer, certain parameters have been shown to be associated with an underlying MDS. These include NEUT-X and NEUT-Y, which are parameters for neutrophil structure and maturation. NEUT-X is the direct measurement of side scatter diffraction, corresponding to channel number, and is representative of the internal structure of the neutrophils. It correlates with hypogranularity of neutrophils and when taken into consideration with anemia, abnormalities of NEUT-X can be suggestive of an underlying MDS. NEUT-Y is the direct measurement of the fluorescence intensity. These parameters can allow a more detailed workup of cases with higher likelihood of MDS in places with limited resources and also aid in differentiating MDS from secondary causes of cytopenia(s), such as megaloblastic anemia [4]. In the latter condition, patients often present with pancytopenia and dyserythropoiesis, potentially mimicking MDS. However, unlike MDS, NEUT-X and NEUT-Y are noted to be high in these cases, helping differentiate them from MDS and facilitating early diagnosis and cost effectiveness [5].

The dyspoiesis seen in MDS peripheral blood granulocytes is illustrated in Fig. 2.1. It includes pseudo-Pelger–Huët abnormality, hypogranularity, and abnormal nuclear lobation (typically hypolobation or non-lobated nuclei, but also less commonly hypersegmentation). There can be a mild left shift noted in the granulocytic series, with the presence of a variable number of blasts, which are usually myeloblasts (Fig. 2.1 G, H), but can also show features of monocytic differentiation. The percentage of circulating blasts in MDS is variable, but it is always <20%. Auer rods can be seen in the blasts or in immature circulating granulocytes, and if present in MDS, they upgrade the disease to MDS with excess blasts-2 (MDS-EB2). Dyspoietic features seen in the red cells (Fig. 2.2) include basophilic stippling, Howell–Jolly bodies, and poikilocytosis, as well as circulating nucleated RBCs. The platelets can show dyspoiesis in the form of giant platelets, vacuolated and hypogranulated platelets, and megakaryocytic fragments (Fig. 2.2).

Bone Marrow

The bone marrow in MDS usually shows increased cellularity relative to the patient's age; this finding, in the required setting of peripheral cytopenias, exemplifies the ineffective hematopoiesis inherent to MDS (Figs. 2.3, 2.4 and 2.5). However, in 10–20% of cases the marrow is normocellular or hypocellular; the latter is sometimes termed "hypoplastic MDS," although not a formal MDS subtype in the WHO Classification.

As mentioned above, morphologic dysplasia is a defining feature of MDS and there must be significant dyspoiesis in one or more hematopoietic lineages. The WHO suggests that at least 10% of a given lineage should be dyspoietic to consider it significant. However, due to inter-observer variations, dysplasia can be missed or overcalled. Moreover, the presence of dyspoiesis is not pathognomonic of MDS and can be seen in patients with non-MDS conditions. Since the

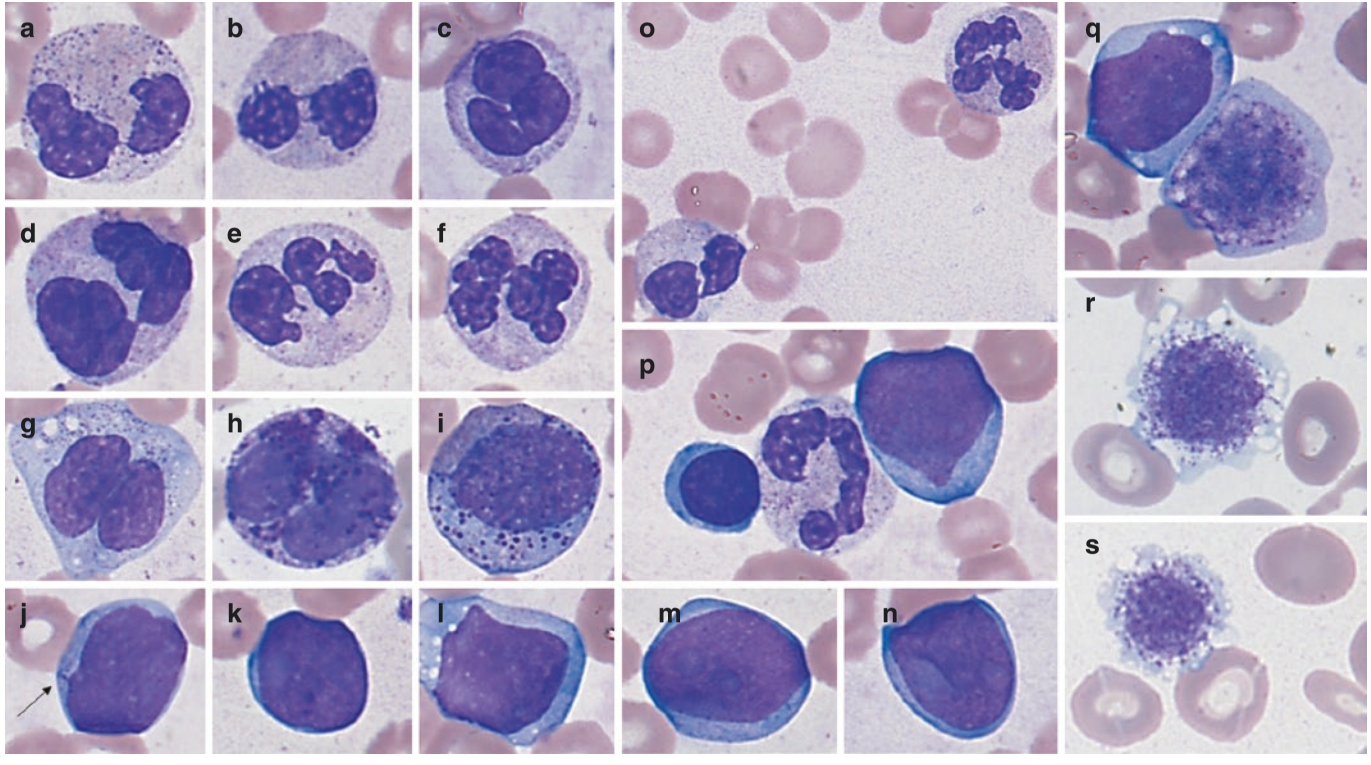

Fig. 2.1 Peripheral smears stained with Leishman and Giemsa (100×). (**a**–**i**) Dyspoietic changes in myeloid cells in MDS. Pseudo–Pelger–Huët cells (**a**, **b**); abnormal lobation of nuclei, including hypersegmentation, and hypogranulation of neutrophils (**c**–**f**); abnormal monocyte (**g**); hypogranular basophil (**h**); abnormally granulated eosinophilic myelocyte (**i**). (**j**–**n**) Circulating blasts in MDS, including small forms (**j**, **k**; arrow in **j** indicating an Auer rod); monoblast (**l**); large forms with prominent nucleoli (**m**, **n**). (**o**, **p**) Combined findings on peripheral smear in MDS. RBC anisocytosis with Pseudo–Pelger–Huët cell and hypersegmented neutrophil (**o**). Blast with a giant dyspoietic neutrophil and nucleated RBC (**p**). (**q**–**s**) Platelet abnormalities in MDS, including platelet giant forms with cytoplasmic vacuolation

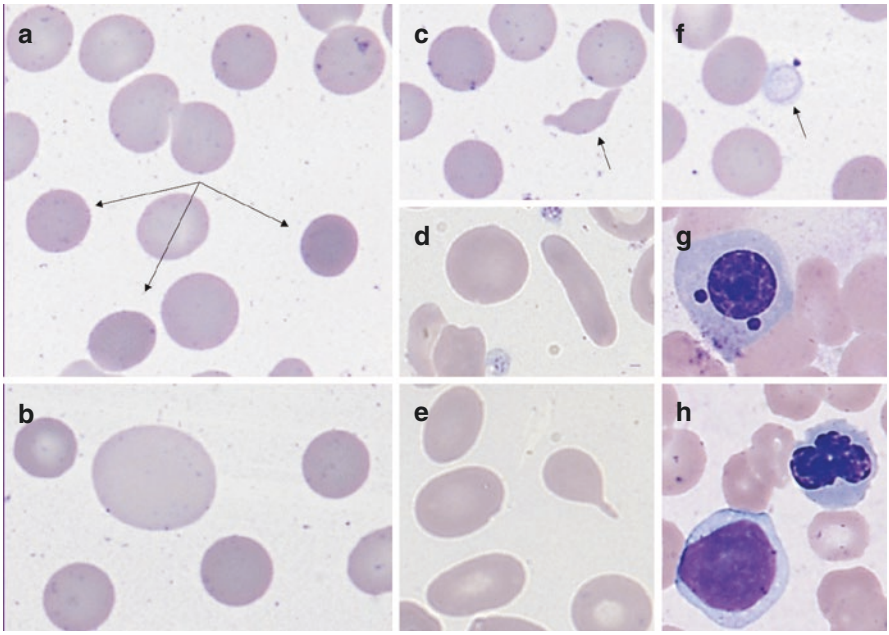

Fig. 2.2 Peripheral smears stained with Leishman and Giemsa (100×) showing abnormalities on red cells and erythroids in MDS, including anisopoikilocytosis with spherocytes (**a**); macrocytes (**b**); fragmented RBCs (**c**); elliptocytes (**d**); teardrop cells (**e**); cabot rings (**f**); basophilic stippling (**g**); and dyspoietic nRBCs (**g**, **h**)

mainstay of diagnosis of MDS is morphology, the various hematological disorders that may show dyspoiesis associated with cytopenias are close differentials. These disorders need to be ruled out before rendering a diagnosis of MDS. One of the most common differential in developing countries is megaloblastic anemia, which presents with macrocytic anemia (with or without other cytopenias) and often with significant dyserythropoiesis and megaloblastoid change, potentially mimicking MDS [4]. Marrow recovery from chemotherapy and infections such as parvovirus B19 can elicit variable dyspoiesis in myeloid and erythroid lineages. Another differential, which is seen in some parts of the world, is the intake of different forms of medicinal therapies that may include heavy metals such a lead, arsenic, or zinc as a constituent. These agents can induce significant trilineage dyspoiesis and sometimes an increase in blast percentage in the peripheral blood and bone marrow. Certain hereditary conditions such as autosomal dominant Pelger–Huët abnormality as well as paroxysmal nocturnal hemoglobinuria, autoimmune disorders, and lymphomas involving the marrow can also cause dyspoiesis [6]. A careful, complete history and laboratory evaluation, including drug history and ancillary microbiological studies, can help in most of the differentials.

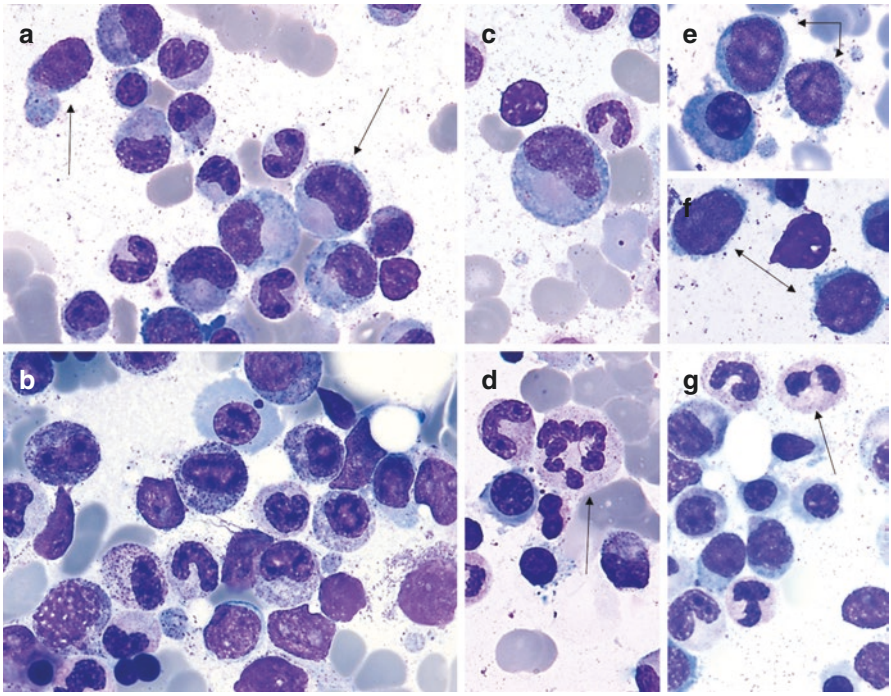

Fig. 2.3 Bone marrow aspirate in MDS showing dyspoiesis in the myeloid lineage, including hypogranulated, variably sized myelocytes (**a**); uneven granulation (**b**); abnormal nuclear lobation in large hypogranular myelocyte (**c**); abnormal nuclear hypersegmentation in neutrophil (**d**); blasts (**e** and **f**); and pseudo-Pelger–Huët cell (**g**). (Leishman and Giemsa, 100×)

Interobserver variations can result from differences in specimen quality, preparation, and staining between different institutions, and hence a high quality of staining is of utmost importance in the overall diagnosis of MDS. Bone marrow aspirate smears are stained by Wright–Giemsa or May–Grunwald–Giemsa and they optimally should contain spicules. Interpretation should be done in areas in which the cells are well spread (Figs. 2.3 and 2.4) rather than crowded. Staining by the Leishman–Giemsa stain enhances nuclear and cytoplasmic details [7]. An iron stain should also be performed on the bone marrow aspirate in any putative MDS case in order to allow for the identification of ring sideroblasts. The bone marrow biopsy should be of sufficient length to include several intertrabecular areas of active hematopoiesis and should be decalcified for as short a period of time as possible to permit sectioning while avoiding deleterious effects that many decalcifying agents have on the morphology and immunostaining results. Thin sectioning (2 to 3 microns) enhances evaluation of the cytology. In addition to Hematoxylin & Eosin staining, a reticulin stain is recommended, as a subset of MDS cases manifests increased reticulin fibrosis. Giemsa staining can be helpful to facilitate the identification of early erythroid elements and distinguish them from myeloblasts. A Perls iron stain

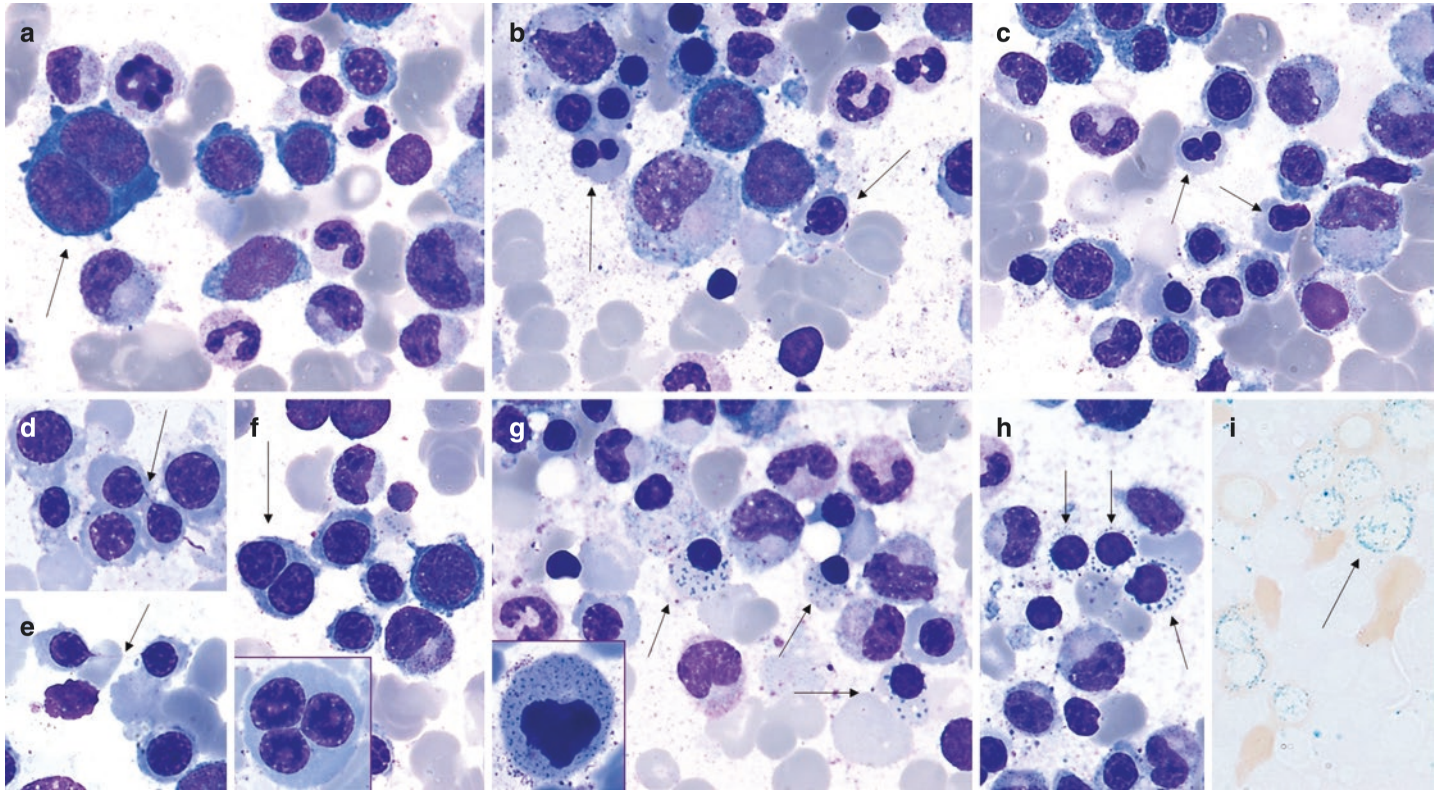

Fig. 2.4 Bone marrow aspirate in MDS showing dyspoiesis in the erythroid lineage, including megaloblasts (**a**, arrow shows a binucleate megaloblast); nuclear budding (**b** and **c**); nuclear bridging (**d** and **e**); binucleate and trinucleate erythroid precursors (**f**, with inset); basophilic stippling (**g**, with inset, and **h**). (Leishman and Giemsa, 100×) Perls Prussian blue stain highlights the presence of ring sideroblasts in a case of MDS-RS (**i; 100x**)

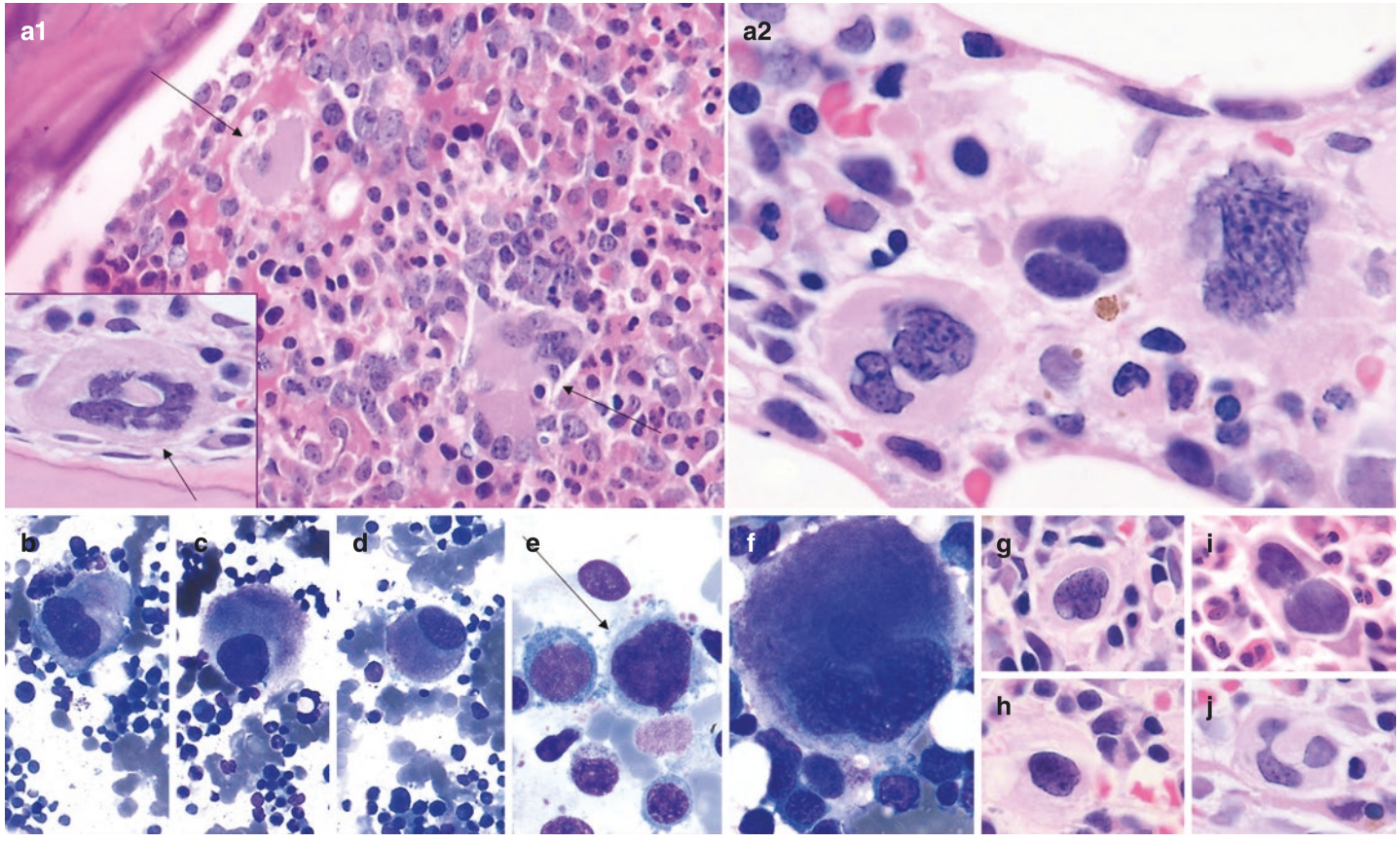

Fig. 2.5 Abnormal megakaryocyte findings in MDS. Bone marrow biopsy (Hematoxylin and Eosin) shows increased cellularity with paratrabecular location of megakaryocytes [**a**1; 40× and 100× (inset)] and loose megakaryocyte clustering (**a**1; 40× and **a**2; 100×). The bone marrow aspirate (Leishman and Giemsa) shows megkaryocytes with hypolobated and non-lobated nuclei (**b–d**; 40×); a megakaryoblast (**e**; 100×); and an abnormally lobated megkaryocyte nucleus (**f**; 100×). In the bone marrow biopsy there are variably sized megakaryocytes with non-lobated, hypolobated, and abnormally lobated nuclei (**g–j**; 100×)

on the core biopsy is not recommended, as the ring sideroblasts are usually not visible in decalcified, paraffin-embedded material.

Granulocytic dyspoiesis in the bone marrow (Fig. 2.3) includes all the features described above in the peripheral blood, as well as hypogranularity, uneven granulation, or abnormally shaped granules in early granulocytic forms. There may be abnormally prominent nucleoli in myelocytes and abnormal nuclear lobation in precursor cells, such as ring-shaped nuclei. The blasts can be normal or increased (≥5% of all cells) in number, and in the biopsy may show abnormal clustering away from the bone trabecular surface where blasts and early myeloid elements normally reside (see Fig. 2.6a1, a3–a4). Blasts can be abnormally large or small in size and show abnormal nuclear features (Fig. 2.3). Erythroid dyspoiesis includes both cytologic dyspoiesis and abnormal disruption of the topographic erythroid islands normally found in the biopsy (see Fig. 2.6b2). Erythroid elements are often left-shifted in MDS. Cytologic erythroid dysplasia is illustrated in Fig. 2.4 and includes megaloblastic changes; nuclear abnormalities such as budding, fragmentation, inter-nuclear bridging, bi-nucleation, and multi-nucleation; and cytoplasmic abnormalities such as blebbing and basophilic stippling. Perls stain for iron should be performed on all bone marrow aspirate smears of possible MDS cases in order to investigate for the presence of ring sideroblasts (Fig. 2.4i). Ring sideroblasts can help establish a diagnosis of MDS (since they are by definition dysplastic erythroids) and may also suggest the specific subcategory of MDS-RS-SLD or MDS-RS-MLD. Megakaryocytic dyspoiesis, illustrated in Fig. 2.5, includes abnormal clustering and paratrabecular localization of megakaryocytes in the bone marrow biopsy. Cytologically, the megakaryocytes usually show pleomorphism, with non-lobated or hypolobated nuclei, abnormal nuclear lobation, widely separated, rounded nuclear lobes, and abnormally small size, including so-called "micromegakaryocytes" (Fig. 2.6c2).

As an adjunct to the morphology on routine stains of the bone marrow aspirate and biopsy, an immunohistochemical profile on the biopsy can also help in the final diagnosis of these conditions. CD34 aids in identifying blasts and their abnormal clustering and may help achieve a more accurate enumeration of blasts in cases in which the aspirate is compromised due to hemodilution or preparation artifacts (Fig. 2.6a1–a4). CD71, E-cadherin, glycophorin, and other erythroid markers can highlight abnormal disruption or localization of erythroid islands (Fig. 2.6b1, b2). CD61, CD42b, or Factor VIII highlight abnormal megakaryocyte topography and cytology, particularly very small forms (so-called micromegakaryocytes) that are often missed on routine stains (Fig. 2.6c1, c2). P53 can also be a diagnostic aid, since if strongly expressed in many hematopoietic cells, it supports a diagnosis of MDS and usually correlates with a *TP53* mutation and an adverse prognosis (particularly in the therapy-related setting).

Flow Cytometry

The clonal hematopoietic stem cells in MDS usually exhibit aberrant antigenic expression and thus show an abnormal pattern when interrogated by FCM, as highlighted below. In the WHO revised fourth edition, FCM is not a required diagnostic

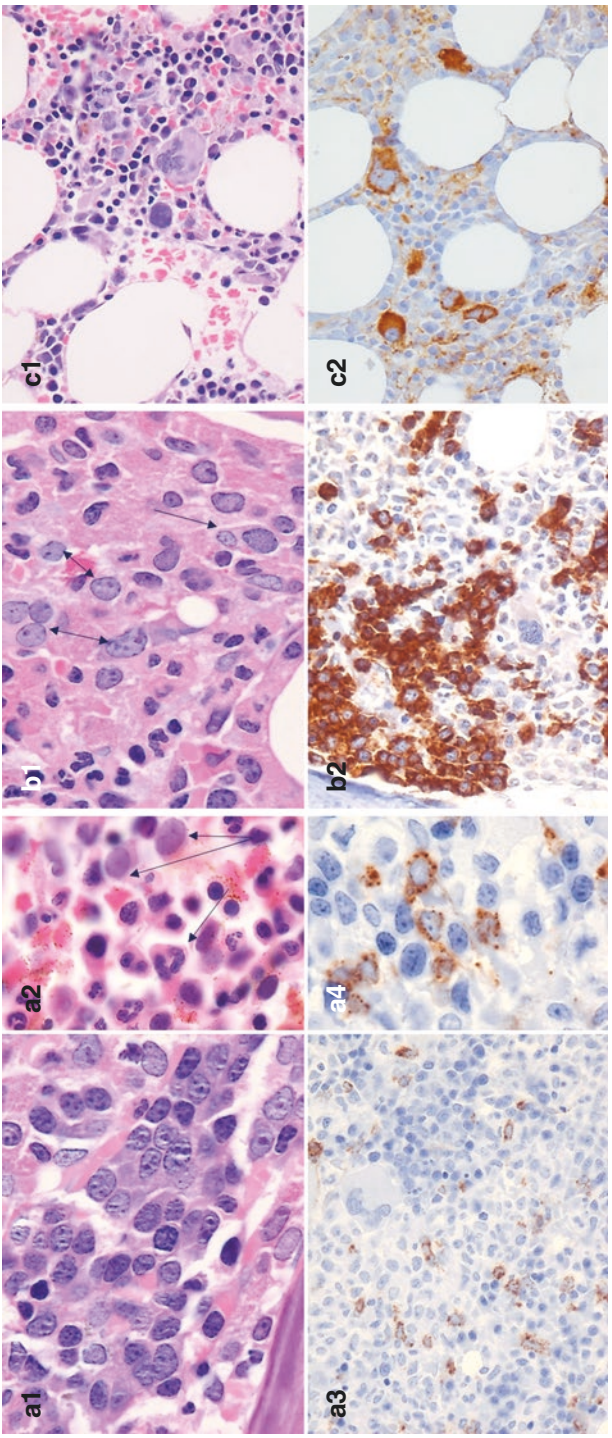

Fig. 2.6 Bone marrow biopsy (Hematoxylin and Eosin) findings in MDS. Increased blasts can be paratrabecular (**a1**; 100×) or interstitial (**a2** double arrow; 100×) in location; abnormal lobation in a neutrophil is also apparent in **a2** (single arrow). CD34 immunostain shows mildly increased interstitial blasts, scattered singly (**a3**; 40×) and in small clusters (abnormal localization of immature precursors, ALIP) (**a4**; 100×). The erythroid lineage shows singly scattered and small aggregates of pronormoblasts (**b1**; 100×). CD71 immunostain highlights an erythroid island in an abnormal paratrabecular location (**b2**; 40×). There are loose clusters of megakaryocytes with dysmegakaryopoiesis (**c1**; 40×). Factor VIII immunostain highlights the micromegakaryocytes, some of which are difficult to appreciate on routine histologic stains (**c2**; 40×)

procedure for MDS. However, multiple immunophenotypic abnormalities involving one or more myeloid linages can be considered as suggestive or supportive of MDS [8]. With the advancement to multi-color cytometers, the development of monoclonal antibodies to an increased number of antigens, and new fluorochromes, there has been significant improvement in use of FCM to support the diagnosis of MDS, particularly in early and lower-grade MDS subtypes, where the morphologic abnormalities may be subtle and the karyotype is often normal. Recent ELN (International/European LeukemiaNet Working Group) guidelines included FCM as a recommended diagnostic procedure for MDS, if performed according to the published guidelines. The ELN has recommended methods of cell sampling, handling, and processing in order to standardize the results obtained by FCM across laboratories, which remains a challenge [9].

There are different FCM scoring systems which have been developed in the past decade, based on the interpretation of the surface marker abnormalities and quantitative differences in immature progenitors versus normal counterparts. In the proposed guidelines of the ELN group, the "Ogata score" can be used as a screening test. It includes the percentage of CD34+ myeloid progenitor cells, the frequency of B-cell precursors within the CD34+ compartment, CD45 expression on myeloid progenitors compared to lymphocytes, and evaluation of neutrophil granularity by comparison to the light scatter pattern of lymphocytes. A score of ≥ 2 has been considered to be reasonably specific for MDS after various validation cohort studies [9]. However, some cases of reactive conditions can have high scores as well [9]. A more comprehensive immunophenotypic panel has been suggested by the ELN group, in which an aberrant finding in at least three tested features affecting at least two cell lineages has been associated with an MDS or MDS/MPN diagnosis in several studies [8]. Examples of flow cytometry aberrations in MDS in both the blast compartment and in maturing myeloid cells are shown in Fig. 2.7.

Abnormalities in Progenitor Myeloid Cells Progenitor myeloid cells in MDS may have an increased side scatter (SSC); decreased expression of CD45 and/or CD117; and increased expression of HLA-DR, CD11b, and CD13/33. The CD34+ blast compartment contains fewer CD19+ and CD38−/dim cells and CD34+/CD117+ cells may show abnormal expression of CD5, CD56, and/or CD7 [9]. However, while aberrant expression of CD5 and CD7 in blasts is relatively specific for MDS, the percentage of MDS cases showing these abnormalities is small (<2% and <10%, respectively). The antigens collectively showing abnormal expression patterns in more than 50% of MDS cases are HLA-DR, CD13, CD33, CD38, and CD117 [10].

Abnormalities in Mature Myeloid Cells The morphometric parameter of hypogranularity in neutrophils in the "Ogata score" has a good specificity for MDS (nearly 90%) but can be discordant in hemodiluted aspirates. Abnormal expression patterns of CD13/CD16 and CD11b/CD16, aberrant expression of CD56, and lack of CD33 and CD64 expression can also be seen. The ELN Working Group has suggested a strong association of MDS with an asynchronous expression of CD34; aberrant pattern of CD11b/CD16; and abnormal expression of CD5, CD56, and

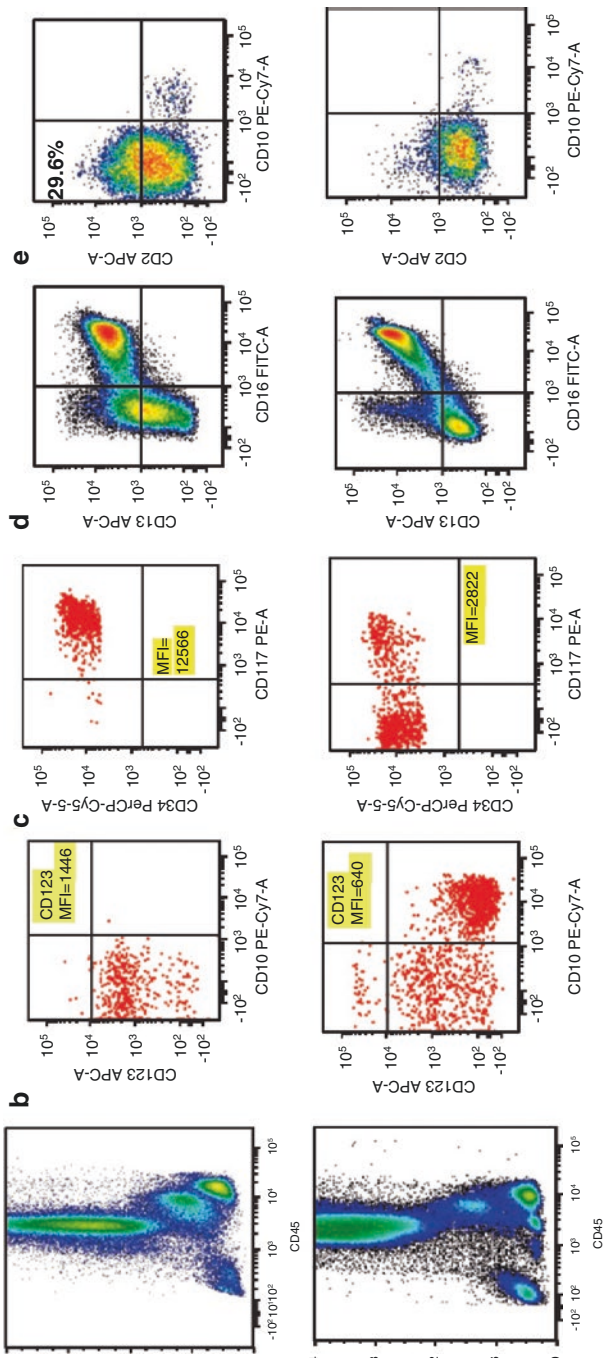

Fig. 2.7 Examples of flow cytometry abnormalities in MDS, with MDS cases shown in the upper panels and normal marrow in the lower panels. (**a**) In CD45 versus side-scatter (SSC), granulocytes show decreased SSC in MDS compared to the normal control. (**b**, **c**) The CD34+ blasts (red) in MDS show abnormally increased CD123 (**b**) and CD117 (**c**) expression compared to the normal control; also note the numerous CD10+ B-lymphoid precursors (hematogones) in Panel B in the normal control. (**d**) Maturing granulocytes in the MDS case show an abnormal pattern of CD16 versus CD13 expression in MDS, deviating from the normal "check-mark" pattern seen in the normal control. (**e**) Monocytes in this MDS case show abnormal expression of CD2, which is normally negative on monocytes. Figure reprinted (with permission) from *Diagnosis of Blood and Bone Marrow Disorders* (Wang SA and Hasserjian RP, eds, Springer Nature 2018.)

CD7 in maturing granulocytes. Additionally, they documented an increased expression of CD117, HLA-DR, CD36, and aberrant patterns of CD15/CD10 and HLA-DR/CD11b [9].

Abnormalities in Monocytes Maturing monocytes in MDS may have decreased SSC and decreased expression of CD45, HLA-DR, and CD11b; abnormal expression of CD36; and an aberrant pattern of CD36/CD14 and HLA-DR/CD11b. There is a strong association of MDS with asynchronous CD34 expression; abnormal CD16 expression in CD11b+ monocytes; and abnormal CD5, CD56, and CD7 expression.

Abnormalities in Erythroid Lineage The ELN group describes four major FCM abnormalities in erythroids in MDS: an increased percentage of CD117 positive erythroid precursors, abnormally heterogeneous and low expression of CD36 and CD71, and an aberrant pattern of CD71/CD235 expression [9]. Recently, increased expression of CD105 in immature erythroid precursors has also been suggested [10].

Cytogenetic Studies

MDS is characterized by recurrent genetic abnormalities which can manifest as gross chromosomal alterations, smaller chromosomal deletions or gains, or mutations in specific genes. The first recurrent genetic abnormality associated with MDS was reported in 1974 by geneticist Herman van den Berghe and his colleagues as deletion in the long arm of chromosome 5 (del5q), which was associated with anemia and thrombocytosis. The first point mutation reported in MDS was in the *NRAS* gene in 1987, followed by *KRAS* mutations [3]. Since then there has been rapid growth in this field of molecular biology from the traditional techniques such as karyotyping, to the use of FISH and SNP arrays, and finally sequencing, including next-generation sequencing (NGS), which has increased the rate of detection of the genetic abnormalities in MDS cases from 50% detected by conventional karyotyping to approximately 90% detected by conventional karyotype plus NGS [11, 12]. Cytogenetic abnormalities play a significant role in the diagnosis, including specific abnormalities defining particular disease subtypes within MDS and also strongly influence the prognosis. Thus, a conventional karyotype should be performed on bone marrow aspirate material in all putative MDS cases. FISH panels which interrogate for the most common MDS-associated cytogenetic abnormalities, do not substitute for a conventional karyotype, but can be used if the karyotype fails or is insufficient [13]. Previously, in the 2008 4th edition WHO Classification, the only genetic marker used in MDS classification was del(5q). In the recent 2017 revised 4th edition WHO classification, the presence of an *SF3B1* mutation (detected by molecular genetic methods rather than karyotype) can define MDS-RS even when the RS count is <15%, provided other diagnostic features are fulfilled and there are at least 5% RS [1]. The role of NGS in the diagnosis of MDS and its mutational landscape are discussed in a separate chapter.

The large majority of identified cytogenetic abnormalities in MDS consists of loss or gain of large segments of chromosomes, the most frequent being −7, del(5q), and +8. Deletions or losses of chromosomal material may also result from unbalanced translocations. These cytogenetic aberrations can be dynamic, increasing in complexity or with some abnormalities disappearing over time with the progression of the disease, with or without superimposed treatment [14–16]. The various cytogenetic abnormalities found in MDS strongly correlate with prognosis [17–20]. Thus, karyotypes have been used in various MDS prognostic systems over the years, as discussed in another chapter. Aside from their influence on prognosis, cytogenetic abnormalities can also be diagnostically useful: some abnormalities are considered to be pathognomonic for MDS in a cytopenic patient in the WHO classification (Table 2.1). Of note, the presence of a del(5q) cytogenetic abnormality, present

Table 2.1 MDS categories with peripheral blood counts, morphology, and cytogenetic abnormalities

MDS categories	Lineages with significant dysplastic changes	Lineages with Cytopenias	Blast (%)	Cytogenetic abnormalities
MDS-SLD	1	1–2	<5% BM; <1% PB	Up to 50%; usually simple karyotype
MDS-MLD	2–3	1–3	<5% BM; <1% PB	~50%; more frequent than in MDS-SLD and MDS-RS-SLD
MDS-RS	≥5% RS with *SF3B1;* ≥15% RS without *SF3B1*		<5% BM; <1% PB	
SLD	1	1–2		
MLD	2–3	1–3		
MDS with isolated del(5q)	1–2	1–2	<5% BM; <1% PB	Del(5q) only or any 1 additional abnormality except del(7q)
MDS-EB	Any	Any	<20% on BM/PB	Clonal abnormalities are more frequent in MDS-EB than in MDS-SLD/MLD; often complex/high-risk karyotype
EB-1	1–3	1–3	2–4% PB; 5–9% BM	
EB-2	1–3	1–3	5–19% PB; 10–19% BM; Auer rods	
MDS-U				
SLD with pancytopenia	1	3	<1% PB; <5% BM	Any
Any MDS category with 1% blasts	1–3	1–3	1 PB; <5% BM	Any
Defining cytogenetic abnormality	0	1–3	<1% PB; <5% BM	MDS-defining abnormality

Abbreviations: *BM* bone marrow; *PB* peripheral blood; Other abbreviations defined in text.

alone or with a single additional abnormality (that does not involve loss of chromosome 7) in a cytopenic patient is associated with a specific MDS subtype, MDS with isolated del(5q), discussed below and in a separate chapter. Illustrations of some of the common karyotype findings in MDS are shown in Fig. 2.8a.

MDS Subtypes

The peripheral blood and bone marrow features described above are common to different subtypes of MDS. The key diagnostic points of each subtype, including the peripheral blood cytopenia(s), morphology, and defining genetic abnormalities, are described below along with a brief discussion of the overall clinical behavior (Table 2.2).

Myelodysplastic Syndrome with Single Lineage Dysplasia (MDS-SLD)

This entity strictly is defined by significant unilineage dysplasia with cytopenia in one or two lineages and no increase in bone marrow or blood blasts. The most common scenario is isolated anemia with isolated erythroid lineage dysplasia. However, of note the dysplastic lineage often does not coincide with the cytopenic lineage(s) (e.g., isolated anemia with megakaryocytic dysplasia but without significant erythroid lineage dysplasia still qualifies as MDS-SLD).

Cytogenetic abnormalities are present at diagnosis in up to 50% of patients and tend to be relatively simple. These characteristics are consistent with the relatively indolent behavior of MDS-SLD [21]. However, progression to AML can occur, particularly in cases with high-risk or complex karyotypes.

Myelodysplastic Syndrome with Multilineage Dysplasia (MDS-MLD)

MDS-MLD is one of the most common MDS subtypes. It is characterized by significant bilineage or trilineage dysplasia with variable cytopenias and no increase in blasts in bone marrow or blood. There is some degree of interobserver discordance in distinguishing MDS-MLD from MDS-SLD, as distinguishing single lineage from multilineage dysplasia is subjective [22].

Cytogenetic abnormalities are present in approximately 50% of patients and tend to be more frequent than in MDS-SLD or MDS-RS, but there are no specific or

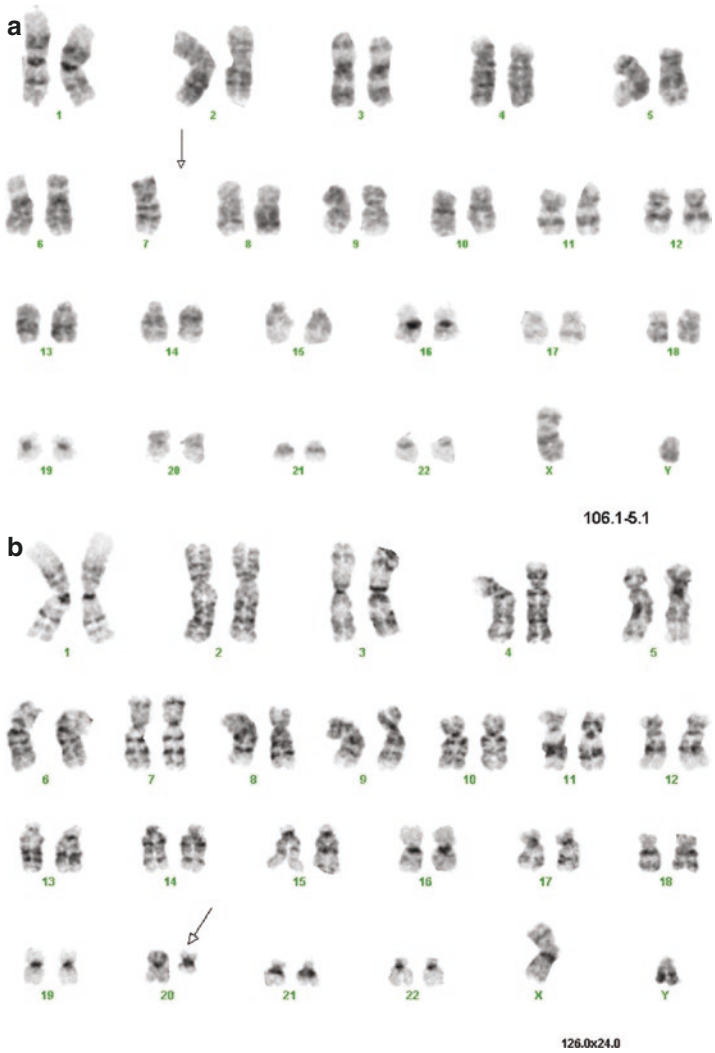

Fig. 2.8 Examples of common cytogenetic abnormalities in MDS. (**a**) Loss of the entire chromosome 7 (or just the long arm) is a relatively common finding in MDS and is considered to be a high-risk finding. (**b**) Deletion 20q is a common finding in MDS, but unlike the −7 abnormality illustrated in Panel **a**, it is not considered to be MDS-defining in isolation. (**c**) A complex karyotype in a case of MDS with excess blasts, illustrating numerical and structural abnormalities of multiple chromosomes and "marker chromosomes" (designated by "A" at the bottom left), which cannot be assigned to a specific chromosome number. Highly complex karyotypes in MDS (at least 4 independent cytogenetic aberrations, as in this case) are associated with very high risk. (**d**) If the bone marrow karyotype fails or is insufficient, interphase FISH studies can help confirm MDS-type cytogenetic abnormalities. In this case of MDS with excess blasts and marked bone marrow fibrosis, the presence of loss of 5q and 7q (red) signals indicated the presence of del(5q) and del(7q) abnormalities, supporting the diagnosis of MDS

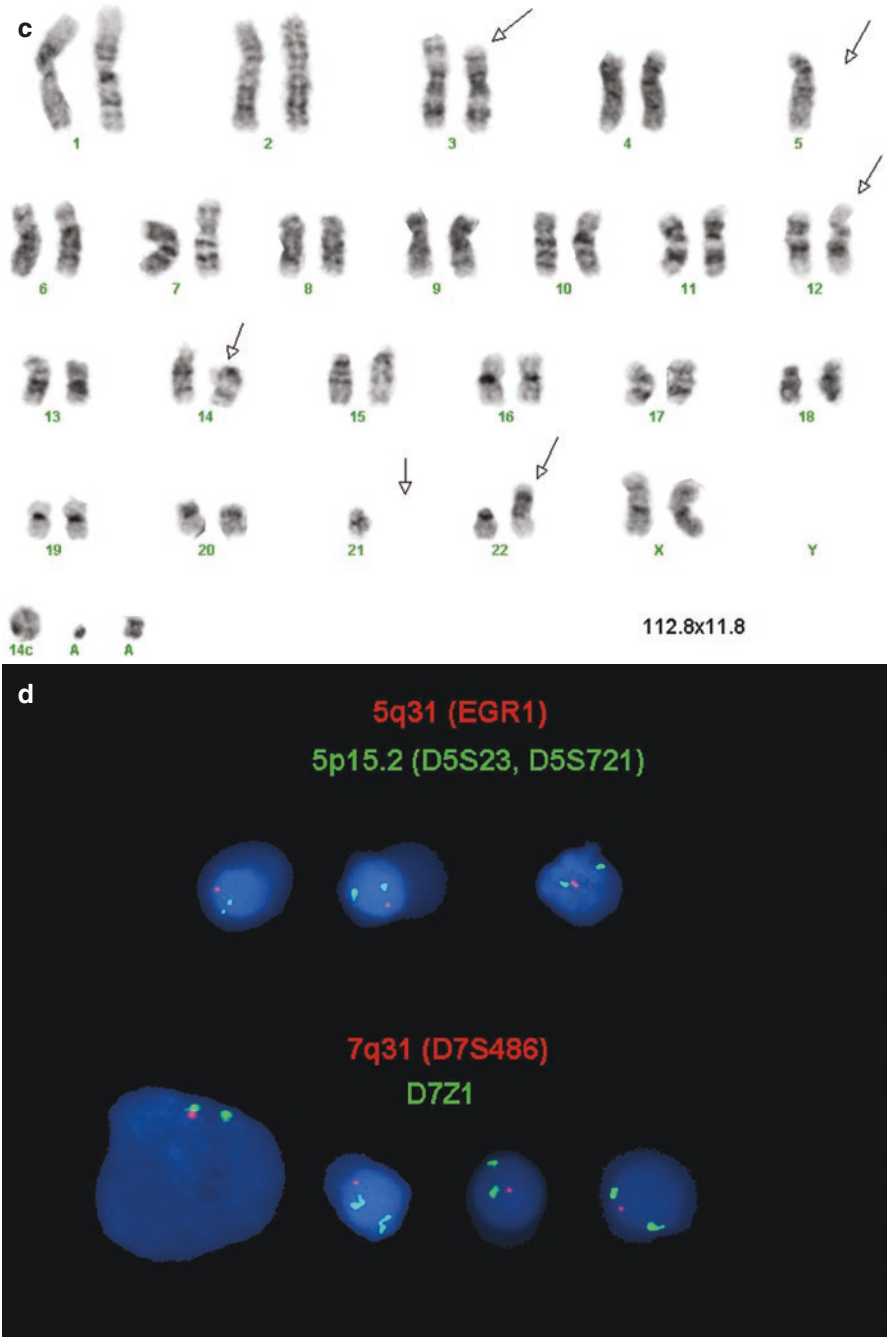

Fig. 2.8 (continued)

Table 2.2 Recurrent cytogenetic abnormalities in myelodysplastic syndrome (MDS)

I. Gain or loss of chromosomal material (relatively common)
–7/del(7q)
del(5q)
+8[a]
+21, −21
–17 and unbalanced translocations at 17p
–20/del(20q)[a]
del(11q)
–Y[a]
del(9q)
+6
del(12p) and unbalanced translocations at 12p
-13/del(13q)
II. Other translocations and inversions (relatively uncommon)
t(3;3)(q21;q26), inv3(q21q26), t(3;21)(q26;q22), and other 3q21 and 3q26 translocations
t(1;7)(p11;p11)
t(2;11)(p21;q23)
t(11;16)(q23;p13)
t(6;9)(p23;q34)
t(2;11)(p21;q23)
i(17q)

[a]Del (20q), +8, and –Y abnormalities, although common findings in MDS, are not considered MDS defining and cannot in isolation be used to make a diagnosis of MDS

defining cytogenetic abnormalities [23, 24]. The prognosis is inferior to MDS-SLD [21, 23, 25–29].

Disease morbidity is usually due to evolving peripheral cytopenias and not to the development of AML.

Myelodysplastic Syndrome with Ring Sideroblasts (MDS-RS)

MDS-RS is a category of MDS which shows ring sideroblasts (RS) on Perls stain for iron. It is subcategorized into MDS-RS with single lineage dysplasia (MDS-RS-SLD) and MDS-RS with multilineage dysplasia (MDS-RS-MLD), with similar dysplasia criteria as MDS-SLD and MDS-MLD, respectively.

In the latest WHO classification, the presence of *SF3B1* mutation is considered to be supportive of this diagnosis and is associated with favorable prognosis. Morphologically, on iron stain, the presence of ≥15% RS with or without *SF3B1* mutation and ≥5% RS accompanied by an *SF3B1* mutation is diagnostic of this entity [1]. However, if there are excess blasts in bone marrow or blood, then the case is classified under MDS-EB.

It is noted that patient survival of MDS-RS-SLD is similar to MDS-SLD with a low rate of progression to AML [23, 25, 26, 30]. The prognosis of MDS-RS-SLD is better than MDS-RS-MLD, which may have *TP53* and *ASXL1* mutations and more aggressive clinical behavior [31].

Myelodysplastic Syndrome with Isolated del(5q) (MDS-del5q)

This entity is defined by the presence of macrocytic anemia and variable dyspoiesis in the erythroid lineage and prominent megakaryocytic dysplasia. The myeloid series is usually relatively unaffected, with <10% dyspoiesis and no neutropenia. The platelet count may be normal or increased and there is usually an increase in megakaryocytes in the bone marrow with predominantly non-lobated forms. There are no increased blasts in bone marrow or blood.

Patients presenting with these classic features usually show an isolated del(5q) abnormality, which as mentioned previously was among the first cytogenetic abnormalities to be detected in MDS [3]; in the most recent WHO classification, a single additional cytogenetic abnormality is allowed, except for those involving deletion of chromosome 7 [1].

These patients generally have a favorable prognosis (although worsened if there is a concomitant *TP53* mutation) and are more likely to respond to the drug lenalidomide than MDS patients lacking del(5q) [23, 25, 26].

Myelodysplastic Syndrome with Excess Blasts (MDS-EB)

This category is defined by the presence of increased blasts in the bone marrow and/or blood in a background of variable degree of dyspoiesis and any number of cytopenias. Based on the blast count of all nucleated cells and the presence of Auer rods, MDS-EB is further classified as EB-1 and EB-2. Due to variable distribution of blasts, CD34 estimation on the bone marrow biopsy can be done to corroborate the aspirate smear blasts count.

The presence of any Auer rods in blasts classifies the disease as MDS-EB-2 irrespective of the blast count, superseding all other MDS categories mentioned above.

Previously in the 2008 4th edition WHO classification, the entity acute erythroid leukemia, erythroid/myeloid subtype encompassed cases with >50% bone marrow erythroid cells in which the blasts comprised ≥20% of the non-erythroid cells, even if they were <20% of all nucleated cells. These cases are now classified as MDS-EB in the 2017 revised 4th edition WHO classification, with the blast count being taken from all nucleated cells [1]. This change has been made on the basis that such cases of erythroid leukemias did not always have an aggressive clinical course and the cytogenetic and mutation profile was more akin to MDS than to de novo AML [32–35].

Clonal cytogenetic abnormalities are more frequent in MDS-EB than in MDS-SLD or MDS-RS and more often show complex or high-risk karyotype abnormalities. The median survival is shorter and disease progression to AML is higher in EB-2 when compared to EB-1.

Myelodysplastic Syndrome, Unclassifiable (MDS-U)

This entity encompasses three specific scenarios which do not fit into the above categories. This is based on the prognostic differences with the above entities.

1. Cases with features of MDS-SLD or MDS-RS-SLD with pancytopenia. Prognostically, these cases have a more aggressive behavior, akin to MDS-MLD, and are placed in the MDS-U category [36, 37].

2. MDS-SLD, MDS-MLD, MDS-RS, or MDS-del(5q) with exactly 1% blasts in the blood, confirmed independently on two separate occasions. These cases appear prognostically similar to MDS-EB and are placed in the MDS-U category [38].

3. The presence of MDS-defining cytogenetic abnormalities on karyotype in the absence of significant dysplasia in any lineage in a patient with persistent unexplained cytopenia. These cases are placed in the MDS-U category because their clinical behavior is uncertain.

Refractory Cytopenia of Childhood (RCC)

This is a provisional entity in the most recent WHO classification, encompassing cases of MDS in the pediatric population that lack excess bone marrow or blood blasts and typically show a hypocellular marrow [1].

The main differential diagnosis is with aplastic anemia.

Therapy-Related MDS (t-MDS)

Any of the above MDS subtypes occurring in patients with prior exposure to cytotoxic chemotherapy (for a neoplastic or non-neoplastic condition) and/or significant bone marrow radiation exposure is considered to be therapy related.

Compared to non-therapy-related cases, t-MDS has a poorer prognosis, mainly due to a much higher incidence of *TP53* mutations and complex karyotypes.

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