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Diagnostics and Prognostication of Myelodysplastic Syndromes

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MDS are a heterogeneous and complex group of clonal hematological neoplasms arising from a hematopoietic stem cell, and characterized by ineffective hematopoiesis, resulting in increased apoptosis in the bone marrow and peripheral cytopenia, which involves one or more lineages. Epigenetic changes are reported as 'founder' mutations in the case of MDS. Its incidence in the general population has been reported as five new MDS diagnoses per 100,000 people. It affects men more frequently than it does women, and its incidence increases with age. The diagnostic classification, now in use, is the one of the World Health Organization, revised in August 2016. It recognizes six distinct entities in addition to a provisional entity of childhood. In most of the cases, diagnosis is based on the morphologic quantitative and qualitative evaluation of the peripheral blood and bone marrow using basic hematological techniques. Bone marrow biopsy and flow cytometric immunophenotyping also offer support for further diagnostic elucidation, while cytogenetics and molecular genetics are presently fully integrated into prognostication, treatment processes, and decision-making.

Key Words: Myelodysplastic syndromes (MDS), WHO 2016 classification, Diagnosis, Prognostication

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DEFINITION AND ETIOLOGY

MDS are a heterogeneous and complex group of clonal hematological neoplasms arising from a hematopoietic stem cell (HSC). Epigenetic changes, such as DNA methylation/hydroxymethylation, histone demethylation/modifications, and transcription coregulation, are reported as 'founder' mutations in the case of MDS, and their key roles in the differentiation and aging of HSCs drive stable clonal changes in gene expression, thereby leading to maturation pathway dysfunctions [1]. MDS are characterized by ineffective hematopoiesis, resulting in increased apoptosis of the bone marrow (BM) and peripheral blood (PB) cytopenia involving one or more lineages. The common features of MDS are: i) morphological dysplasia in one or more lineages; ii) a blast percentage less than 20% in the PB and BM; iii) the presence of cytogenetic and molecular genetic abnormalities in up to 90% of *de novo* cases; and iv) the variable risk of evolution to acute leukemia, mainly in the absence of peripheral leukocytosis. In a majority of the cases, MDS are acquired diseases related to aging (*de novo* cases) or are secondary to environmental/occupational exposure to toxic compounds, benzene, smoking, ionizing radiation, or antineoplastic or immunosuppressive therapy (therapy-related MDS, t-MDS). Rare, inherited predispositions to primary MDS associated with BM failure syndromes, aplastic anemia, Fanconi anemia, dyskeratosis congenita, Diamond–Blackfan anemia, Shwachman–Diamond syndrome, and paroxysmal nocturnal hemoglobinuria are widely described in the literature, mainly in pediatric settings; these are not included within the MDS group. Multiple hereditary predispositions to MDS have been discovered (familial MDS) [2, 3]; a mutation in at least one

of seven well-defined single-gene loci is reported as predisposing one to an increased lifetime risk of primary MDS [4].

Due to the heterogeneity of the clinical presentation of this group of hematological neoplasms, particularly in the cases of lower-risk MDS, differential diagnosis should exclude drug-induced cytopenias, vitamin B_{12}/folate/zinc/copper deficiency, excessive alcohol intake, exposure to heavy metals (lead, arsenic), infections (HIV, Epstein-Barr virus, hepatitis C virus, parvovirus, leishmaniasis), hemophagocytic lymphohistiocytosis, anemia of chronic disorders (infection, inflammation, cancer), autoimmune cytopenia, and metabolic disorders (liver failure, kidney failure). The 2001 WHO classification [5] has recognized groups of hematological neoplasms with dysplasia that nevertheless are not classified as MDS; these include MDS/myeloproliferative neoplasms (MPN), AML with myelodysplasia/dysplasia-related changes, and therapy–related AML/MDS. Finally, it is noted that a low number of dysplastic erythroid, granulocytic, or megakaryocytic cells can be recognized in the BM of healthy subjects [6].

EPIDEMIOLOGY

The incidence of MDS in the general population is reported as five new MDS diagnoses per 100,000 people, with a higher incidence among men [7]. In Western countries, among individuals older than 70 yr, the incidence is reported as between 22 and 45 per 100,000 people, and this incidence further increases with age [8, 9]. The occurrences of MDS at a younger age have been more frequently reported in Asian countries, including Japan, China, Korea, India, Thailand, India, and Turkey, with the median age of patients reported between 40 and 50 yr; this is one to two decades younger than that of patients in Western countries. Environmental pollutions and/or other factors, including uncontrolled pesticide use, may contribute to these differences [10].

However, in a report from a single institution in Italy, about 10% of patients with MDS were younger than 50 yr (median age 43 yr), with a female predominance [11]. MDS may also affect children and adolescents, rarely, with an incidence of less than 5% of hematopoietic malignancies [12]. Familial cases of MDS are rare; remarkably, a recent increase in the reported cases in the literature testifies the higher knowledge and consciousness of clinicians in the investigation and identification process of familial cases of MDS [13]. Therapy-related myeloid neoplasms, including t-MDS, account for 10–20% of all the cases of AML, MDS, and MDS/MPN [14].

CLASSIFICATION

First described in 1900 by von Leube [15] as a 'leukanemia', on the basis of an alleged co-existence of pernicious anemia and leukemia, MDS were named and described in a variety of ways until 1976, when the French-American-British (FAB) classification named them 'dysmyelopoietic syndromes' and categorized them separately from AML [16]. In 1982, the FAB group refined the proposal, changed the designation to 'myelodysplastic syndromes', and provided the modern basis for the diagnosis and classification of this group of disorders [17]. Five subtypes were identified, on the basis of quantitative (peripheral cytopenia[s] involving one or more hematopoietic lineages, the blast percentage in PB and BM, monocytes in PB) as well as qualitative abnormalities, (ineffective hematopoiesis and morphological dysplasia affecting one to three lineages): refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), refractory anemia with an excess of blasts (RAEB), refractory anemia with an excess of blasts in transformation (RAEB-t), and chronic myelomonocytic leukemia (CMML). From this classification, the introduction of new diagnostic techniques, mainly cytogenetics and molecular genetics, has made the correlation between the subtypes of MDS and other new variables possible. This has been very useful for the prognostication and development of new therapies, as well as for the redefinition of subtypes. After the publication of the first FAB proposal, more than 20,000 scientific articles on the diverse diagnostic, prognostic, and therapeutic aspects of MDS have been published, attesting the enormous interest developed by the scientific community in studying these diseases. Morphology remains a cornerstone in the diagnosis of MDS. Nevertheless, in terms of the accuracy of diagnosis and the ability to compare different series of cases, a gray area still exists owing to the impossibility of experts reaching a unanimous opinion, particularly when the disease is in its early stages, mainly because of the lack of specificity of many morphological aspects of dysplasia in the case of MDS.

The diagnostic classification now in use is the one proposed by the working group of the WHO, starting from the first edition in 2001 [5], moving to the second edition in 2008 [18] which was an expanded and updated version of the previous one, and now using a further revised classification of myeloid neoplasms and acute leukemia which was published in August 2016 [19]. This updated classification introduces refinements in the nomenclature, morphologic interpretation, and cytopenia assessment. It also addresses the influence of rapidly accumulating genetic information in MDS diagnosis and classification; for the first time,

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the molecular test for *SF3B1* mutation has been included among the diagnostic tools [20]. Six distinct entities within the MDS group are nowadays recognized, and defined at diagnosis by precise criteria including i) the number of lineages presenting dysplastic features, ii) number of cytopenias in the PB, iii) presence/absence and percentage of ring sideroblasts, iv) percentage of blasts in the PB and BM, and v) karyotype and molecular genetics, when needed [19]. They are:

1) MDS with single lineage dysplasia (MDS-SLD): one dysplastic lineage, one or two PB cytopenias, less than 5% of blasts in BM and less than 1% blasts in PB, non Auer rods, less than 15% ring sideroblasts in BM or less than 5%, if *SF3B1* mutation is present, (includes the 2008 subtypes refractory cytopenia with unilineage dysplasia, or RCUD);
2) MDS with multilineage dysplasia (MDS-MLD): two or three dysplastic lineages, one to three PB cytopenias, less than 15% ring sideroblasts in BM or less than 5%, if *SF3B1* mutation is present, less than 5% of blasts in BM and less than 1% blasts in PB, no Auer rods (includes the 2008 subtype of refractory cytopenia with multilineage dysplasia, or RCMD);
3) MDS with ring sideroblasts (MDS-RS) (previously named as RARS). It includes the two subtypes i) with single lineage dysplasia (MDS-RS-SLD): one dysplastic lineage, one or two PB cytopenias, 15% or more ring sideroblasts in BM or 5% or more if *SF3B1* mutation is present, less than 5% of blasts in BM and less than 1% blasts in PB, no Auer rods; and ii) with multilineage dysplasia (MDS-RS-MLD): two to three dysplastic lineages, one to three PB cytopenias, 15% or more ring sideroblasts in BM or 5% or more if *SF3B1* mutation is present, less than 5% of blasts in BM and less than 1% blasts in PB, no Auer rods;
4) MDS with isolated del(5q): one to three dysplastic lineages, one or two PB cytopenias, less than 5% of blasts in BM and less than 1% of blasts in PB, no Auer rods, none or any ring sideroblasts, presence of del(5q) alone or with one additional abnormality except -7 or del(7q) (this last is a new compared to the 2008 WHO classification);
5) MDS with excess blasts (MDS-EB): none to three dysplastic lineages, one or three PB cytopenias, none or any ring sideroblasts; it includes the two subtypes MDS-EB-1 (5 to 9% blasts in BM and/or 2 to 4% blasts in PB, no Auer rods) and MDS-EB-2 (10 to 19% blasts in BM or 5 to 19% blasts in PB, and/or presence of Auer rods);
6) MDS, unclassifiable (MDS-U) including three categories: i) with 1% PB blasts, ii) with single lineage dysplasia and pancytopenia, and iii) with a defining cytogenetic abnormality related to myelodysplasia.

An additional provisional entity within this category is refractory cytopenia of childhood, characterized by one to three dysplastic lineages, one to three PB cytopenias, less than 5% blasts in BM and less than 2% blasts in PB.

DIAGNOSIS

1. Peripheral blood and bone marrow aspirate

The diagnosis of MDS is based on the quantitative and qualitative evaluation of the cytological composition of the PB and BM, using basic hematological techniques, such as hemocytometry, optical microscopy on PB and BM films, fixed and stained with panoptical stains, and cytochemistry for the detection of iron in the BM. The presence of at least one cytopenia is a "*sine qua non*" for any MDS diagnosis; the thresholds are hemoglobin <10 g/dL, platelets $<100\times10^9$/L, and absolute neutrophil count (ANC) $<1.8\times10^9$/L. PB monocytes must be less than 1×10^9/L. Different potential disorders should be accurately excluded as primary etiologies of cytopenia. Cytopenia should be stable for ≥six months, unless it is associated with a specific karyotype or bilineage dysplasia, in which case only two months of stable cytopenia are required [21]. It is to be noted that some ethnic groups may have a reference range of ANC$<1.8\times10^9$/L; caution should be exercised in interpreting neutropenia, if it is the only cytopenia [22].

2. Cytomorphology of dysplasia

On PB examination, the observation of the presence of morphological abnormalities in the red blood cells (RBC) is quite usual, including the occurrence of circulating nucleated RBC (NRBC) with morphological stigmata of dyserythropoiesis, which is not rare. Another characteristic finding at the time of diagnosis is the detection of two RBC populations, one of which is usually normal, while the other, being a direct expression of the anomalous neoplastic clone, is microcytic or macrocytic. Dysgranulopoiesis in neutrophils is variably observed, from absent to severe, and can involve both the nucleus and cytoplasm, and/or abnormalities in size. The nucleus can show abnormal lobulation and/or an abnormal chromatin pattern, as well as the presence of more than four nuclear projections, while the cytoplasm can show abnormalities in granule size (pseudo-Chédiak-Higashi) and/or content (reduction of two thirds to absence) [23]. The evaluation of granularity requires optimally stained smears. The visibility of granules, in fact, is the morphological characteristic that can most easily be compromised by an inadequate staining

technique, either manual or automated. Morphological alterations of the MDS cells at diagnosis are less frequent in eosinophils and rare in basophils. The presence of even <1% blast cells in the PB is a crucial feature in MDS diagnosis, and usually indicates cases with an unfavorable prognosis. In patients presenting with marked leukopenia, it can be very useful to prepare buffy coat PB smears after centrifugation.

3. Blast cell identification and count

The upper blast cell threshold for the diagnosis of MDS is <20% in the PB and/or BM, on a PB differential count performed on 200 nucleated cells, and/or on a myelogram performed on 500 nucleated cells. Blasts are generally small to medium in size, with a high nucleo-cytoplasmic ratio. The nucleus is usually nucleolated with a finely dispersed chromatin pattern. The cytoplasm is relatively scanty and basophilic. Primary (azurophilic) granules may be absent (agranular blasts), scanty or, sometimes, more in number (granular blasts); in the latter, the cytoplasm is more plentiful. The absence of a clear Golgi area is considered a key morphological feature to differentiate granular blasts from normal or dysplastic promyelocytes [24]. The recognition of Auer rods in the cytoplasm, either in circulating or BM blast cells, entails an unfavorable prognosis and leads to the classification of the patient as MDS-EB2. The cytochemical stain for peroxidase and the study of specific immunophenotypic markers are useful, when positive, to confirm the myeloid nature of blast cells, particularly when the blasts are agranular. The exclusion of an expansion of monocytic lineage should always be taken into account, in the diagnostic pathway of MDS [25].

The 2016 WHO classification introduces a change in the evaluation of blast percentage; now, it should simply be calculated as the percentage of all the nucleated cells of the BM, irrespective of the percentage of the erythroid precursors (EP). Originally introduced by the FAB group, the "50% rule" (the blast percentage in patients presenting with EP ≥50% <80% of BM nucleated cells, when less than 20%, should be recalculated and reported as the percentage of non-erythroid BM cells, excluding lymphocytes and plasma cells) was adopted till the 2008 WHO edition, to allow for the differential diagnosis between MDS presenting with erythroid hyperplasia versus acute erythroid/myeloid leukemia (AEL). Different potential disorders as a primary etiology of erythroid hyperplasia should be preliminarily excluded. There is evidence in the literature that suggests that the percentage of EP does not impact prognosis, overall survival or leukemia-free survival in these patients [26]. This change determines that cases previously diagnosed as erythroid/myeloid AML are now included in the MDS group [27]. It is to be noted that, in the literature, cases of MDS developing in patients with untreated CLL are reported [28, 29]. It is also important to come to an agreement on how to evaluate the myeloid blast percentage in suspect myeloid neoplasms presenting with the infiltration of lymphoma cells in the BM, using microscopy, to avoid the risk of the underestimation of myeloblasts in BM infiltrated by lymphoid cells and/or plasma cells. The increased heterogeneity of the platelet size and abnormalities in the granule content and/or size are common findings in the PB of MDS. The presence of micromegakaryocytes and/or bare megakaryocyte nuclei in the PB is associated with MDS, but is not specific, because these cells are also found in other hematology neoplasms. They are easier to find in buffy coat films and are more frequently observed in high-risk cases of MDS.

4. Bone marrow cellularity

BM cellularity is most often increased in cases of MDS, at diagnosis, with hyperplasia of the erythroid or granulocytic series, or both. In 30 to 40% of the cases, cellularity is quantitatively normal, while in about 10% of the patients, the BM aspirates appear hypocellular. In such cases, other diagnostic techniques should be used, such as histology, immunohistochemistry, and cytogenetic studies, to distinguish MDS from other hypoplastic myeloid disorders [30]. Finally, it is not unusual to find non-specific reactive alterations, such as increased lymphocytes, plasma cells, or mast cells or an increase in hemosiderin-laden macrophages with some hemophagocytosis in the BM of MDS cases, at diagnosis.

5. Quantification of dysplasia

Precise morphological criteria, both quantitative and qualitative, have been identified for each lineage for the definition of morphological dysplasia, with the aim of harmonizing microscopic diagnosis, in the form of the minimal criteria necessary for an unequivocal recognition of dysplasia; in particular, to recognize dysplasia within a specified lineage in the BM, it is necessary that dysplastic features are present in at least 10% of the EP (not taking into account mature erythrocytes) and/or 10% of the granulocytic cells (in this case, also including mature cells) out of a count of at least 200 cells of each lineage, and/or in a minimum of 10% of megakaryocytes out of at least 30 cells of the megakaryocyte lineage [31]. The characteristic morphologic features that allow the inclusion of the cells into the dysplastic group, according to the WHO classification, are listed and illustrated in Fig. 1 to 3.

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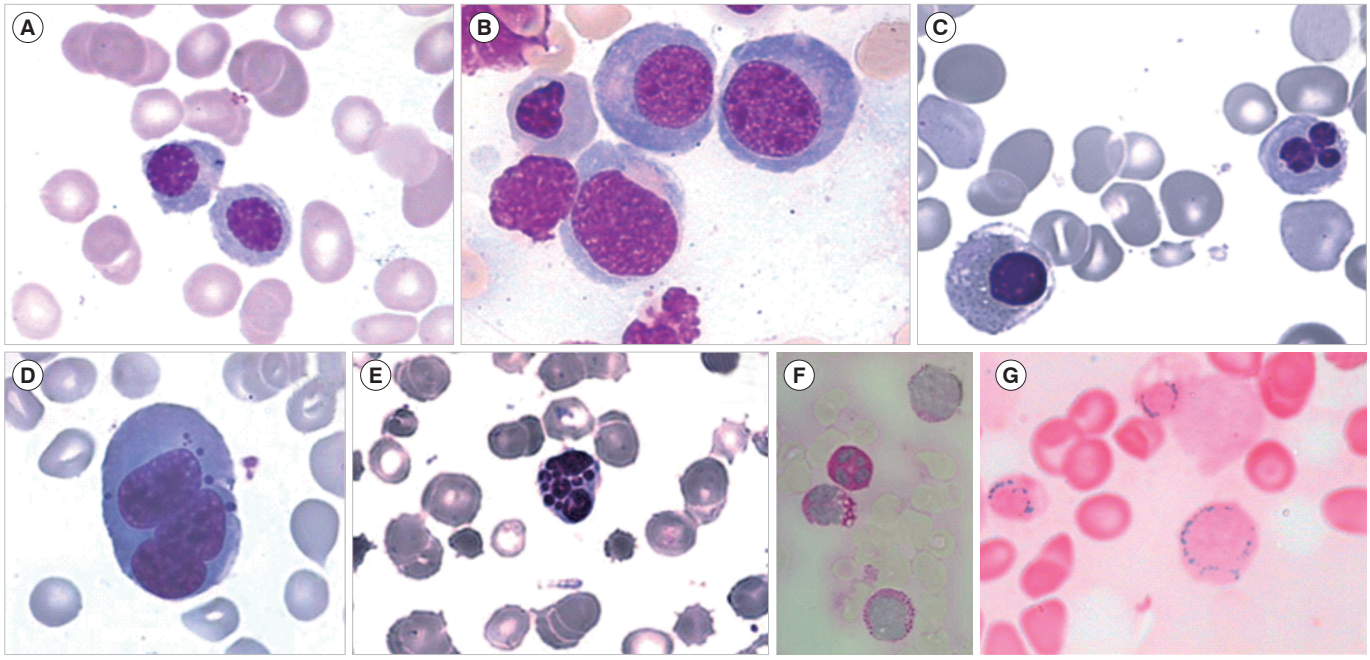

Fig. 1. Erythroid series: morphologic dysplastic features. (A) Internuclear bridging (May-Grünwald-Giemsa stain, ×50), (B) Nuclear budding and megaloblastic changes (May-Grünwald-Giemsa stain, ×100), (C) Nuclear lobation and cytoplasmic vacuolization (May-Grünwald-Giemsa stain, ×50), (D) Multinuclearity (May-Grünwald-Giemsa stain, ×100), (E) Karyorrehexis (May-Grünwald-Giemsa stain, ×50), (F) Abnormal Periodic acid-Schiff positivity (PAS stain, ×50), and (G) Ring sideroblasts (Perls' Prussian Blue Stain, ×50).

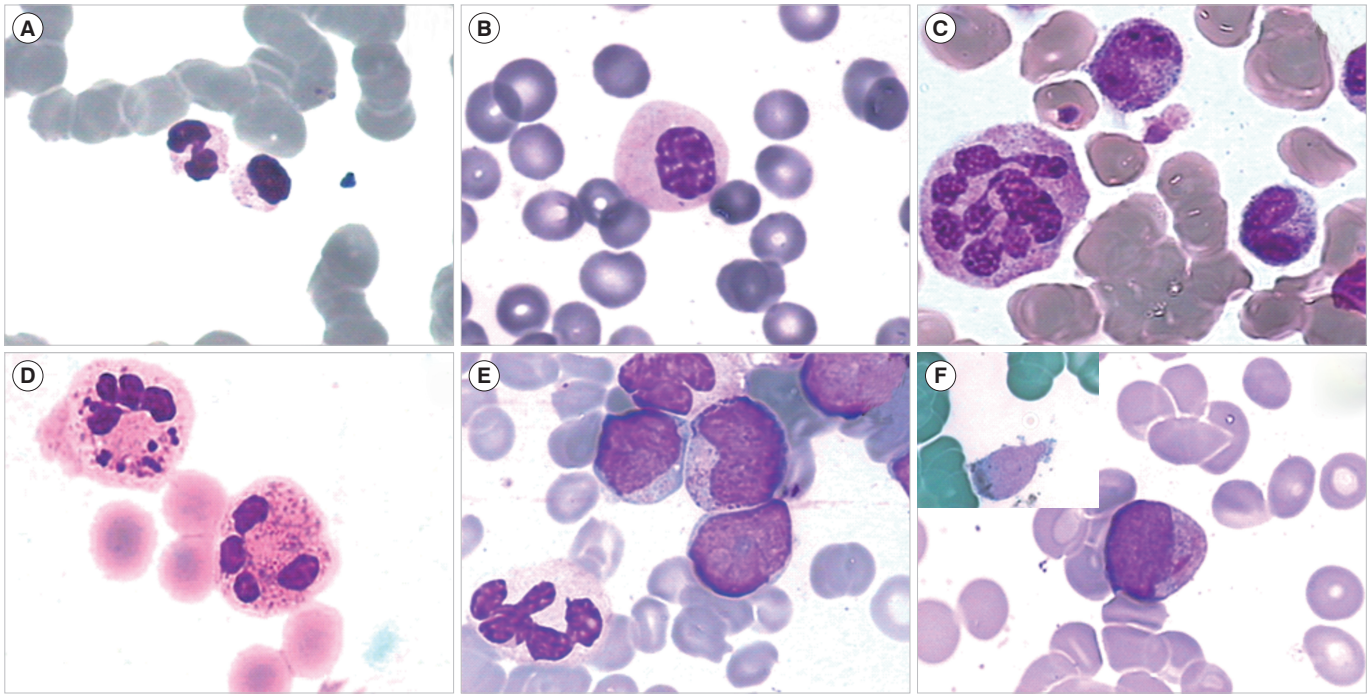

Fig. 2. Granulocytic series: morphologic dysplastic features. (A) Small size, nuclear hypolobulation/pseudo-Pelger and decreased granules/agranularity (May-Grünwald-Giemsa stain, ×100), (B) Pseudo-Pelger in 17p del (May-Grünwald-Giemsa stain, ×100), (C) Nuclear hypersegmentation (May-Grünwald-Giemsa stain, ×100), (D) Coalescent granules Pseudo Chédiak-Higashi (May-Grünwald-Giemsa stain, ×100), (E) Unusual large size and agranularity (May-Grünwald-Giemsa stain, ×100), and (F) Auer rods (May-Grünwald-Giemsa stain, ×100) (in the box: myeloperoxidase stain positivity).

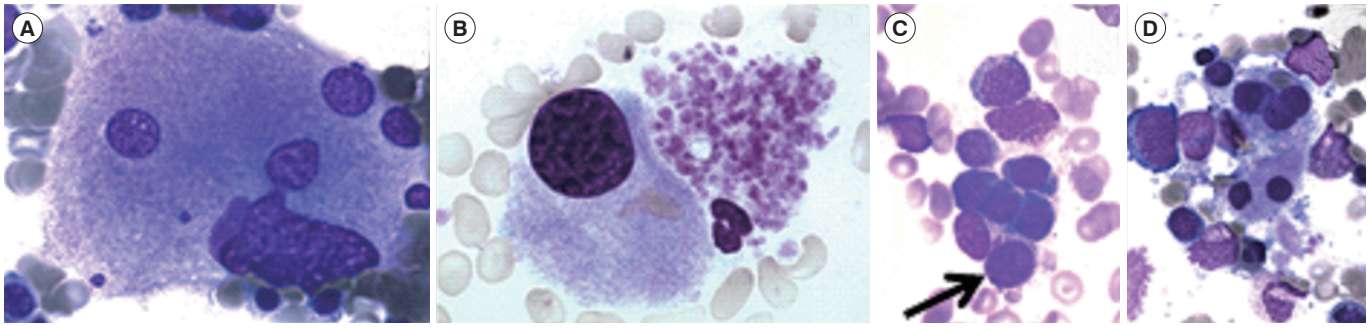

Fig. 3. Megakaryocytic series: morphologic dysplastic features (May-Grünwald-Giemsa stain, ×50). (A) Multinuclearity, (B) Nuclear hypolobulation, (C) Mononucleated micromegakaryocyte (arrow), and (D) Binucleated micromegakaryocyte.

6. Histopathology

BM biopsy should be integrated with the aim of excluding reactive and secondary myelodysplasia [32]. This provides a precise evaluation of cellularity, BM architecture, distribution and localization of various cellular components, degree of fibrosis, the presence of anomalous localization of granulocyte precursors (ALIP) in intertrabecular areas and/or in the central zones of hemopoietic tissue, the presence of clusters of megakaryocytes, and the presence of micromegakaryocytes [33]. A pathological accumulation of blast cells can be confirmed by immunohistochemistry, using an anti-CD34 antibody [34]. All the editions of the WHO classification, including the last one, specify that, in the cases of MDS, not all the blasts are CD34-positive, and that positivity for this marker is also a feature of endothelial cells. Analysis of CD117 can be useful for a final assessment. Histological examination is particularly important when there is a 'dry tap'. Further useful information may concern the presence of lymphoid nodules, which can disclose the presence of a coexisting lymphoproliferative disorder [35].

7. Flow cytometry (FC)

Immunophenotyping of the blast population can be useful to detect minimal disease after therapy, but not to prove a quantitative blast percentage. In fact, according to the 2016 WHO classification, the reference method for the diagnosis of MDS remains the blast percentage, estimated by the morphological assessment of a BM aspirate. FC can also be useful to study the characteristics of the maturation of the precursors, looking for the anomalous expression of immunophenotypic markers as possible indicators of dysplasia of a particular lineage. The European LeukemiaNet, in 2013, published a position paper on the assessment of dysplasia by FC in cases of MDS [36].

8. Genetics

Conventional karyotyping at diagnosis remains a cornerstone for the prognostic stratification of *de novo* MDS patients. Chromosomal and molecular abnormalities, often multiple, are found in 50% of patients affected by *de novo* MDS. With the availability of new diagnostic platforms, such as gene expression profiling (GEP), single nucleotide polymorphism (SNP)-array, and next-generation sequencing (NGS), genetic and/or molecular lesions are reported in more than 90% of MDS patients [37]. Some clonal cytogenetic abnormalities are associated with specific morphological anomalies affecting the megakaryocyte and erythroid series [38, 39]: i) the isolated deletion of 5q is particularly found in women with hyposegmented and non-segmented megakaryocytes, macrocitic anemia, a normal to increased platelet count, and good prognosis. These patients are classified within a specific entity of MDS [MDS with isolated del(5q)]. According to the revised 2016 WHO classification, the presence of an additional cytogenetic abnormality, except for monosomy 7 or del(7q), allows for the inclusion of patients in this category; del(5q) remains the only cytogenetic abnormality included in the diagnostic pathway of MDS; ii) the inversions and translocations involving chromosome 3 are found in AML and MDS, with an increase of abnormal megakaryocytes; iii) the deletion of 11q is associated with increased iron deposition; iv) the isolated deletion of 20q is associated with marked dyserythropoiesis and dysmorphic megakaryocytes; v) monosomy 7 is associated with micromegakaryocytes and has a negative prognostic significance; vi) the deletion of 17p is generally associated with small neutrophils with pseudo-Pelger nuclei and a vacuolated cytoplasm, and a very poor prognosis; vii) the deletion of 20q as an isolated cytogenetic abnormality is reported in association with thrombocytopenia. Different cytogenetic abnormalities are now considered MDS-defining [18]. The presence of one or more of the following unbalanced chromosomal abnormalities allows for the diagnosis of MDS even

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in the absence of morphologic dysplasia (within the subgroup of MDS-U): monosomy 5, 7, or 13; 5q, 7q and 13q deletions; i(17p) and t(17p); 11q deletion; 9q or 12p deletion or t(12p), idic(X)(q13). The presence of trisomy 8, Y deletion, or (20q) deletion as the sole anomaly is not considered to be MDS-defining, in the absence of the diagnostic morphological features of MDS. Several balanced cytogenetic abnormalities (translocations or inversions) are also MDS-defining [18]. The presence of a complex karyotype, with three or more abnormalities in a single patient usually including anomalies of chromosome 7 and/or 5, is associated with an unfavorable prognosis.

9. Molecular genetics

For MDS prognostication, the most important recurrently mutated genes involved in epigenetic regulation are *TET2, IDH1, IDH2, ASXL1, DNMT3A* and *EZH2*, while *TP53* and *SF3B1* are involved in the mechanisms of DNA repair and RNA splicing, respectively [40]. According to the 2016 WHO classification, *SF3B1* mutation analysis is the sole gene analysis included within the diagnostic pathway of MDS, because it identifies a distinct subset of MDS that correlates with MDS-RS. Cases of MDS-RS presenting with *SF3B1* mutations have more favorable prognoses than cases of MDS-RS lacking the mutation, even if the role of multilineage dysplasia vs the *SF3B1* mutation in influencing outcomes of MDS-RS is still controversial [41, 42]. Mutations in the *TP53, EZH2, ETV6, RUNX1*, and *ASXL1* are reported as independently associated with decreased overall survival in cases of MDS [43], although the prognostic significance of *TET2* mutations is not clear. *TET2* mutations, through altered DNA methylation, have been found to be an independent prognostic indicator with a high response rate to hypomethylating agents [44]. It is to be noted that the *DNMT3A*, *TET2*, and *ASXL1* mutations in normal elderly individuals are not sufficient by themselves for cancer development, and that acquired clonal mutations identical to those seen in cases of MDS can occur in the hematopoietic cells of apparently healthy older individuals without MDS-so called "clonal hematopoiesis of indeterminate potential" (CHIP) [45], as well as in patients with unexplained cytopenia [46]. Even if some individuals harboring a CHIP later developed MDS, there is limited clarity on the scenario, and further data are required; therefore, nowadays, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS.

PROGNOSTICATION OF UNTREATED PATIENTS

The clinical heterogeneity of MDS has led to the development of prognostic scoring systems to estimate the overall and leukemia-free survival, and to drive clinical and therapeutic decisions. Once the diagnosis has been established, patients should be accurately risk stratified by using a prognostic scoring system, to provide them with the best timing and therapeutic choice. Several prognostic scoring systems have been proposed in the past, based on the blast percentage, which carries an intrinsic prognostic value, associated with clinical, hematological, histological, and cytogenetic parameters, such as cytopenia, fibrosis, ALIP, and lactate dehydrogenase (LDH) [47-49]. The International Prognostic Score System (IPSS) for MDS, proposed by Greenberg in 1997 [50], was defined on more than 800 newly diagnosed untreated MDS patients (*de novo* MDS, and all FAB subgroups, except for CMML): the IPSS became immediately essential, predominantly because of its reproducibility and manageability; therefore, it was adopted worldwide, even in the context of the design and analysis of clinical trials. In this system, based on three variables, four risk groups are stratified to predict survival and AML evolution: low (score 0), intermediate-1 (score 0.5-1.0), intermediate-2 (score 1.5-2.0), and high (score ≥2.5). The prognostic variables are: *blast percentage in BM* (<5% [score 0], 5-10% [score 0.5], 11-19% [score 1.5], 20-30% [score 2]), *karyotype* (defined as Good [normal, -Y, del(5q), del(20q)], Poor [complex (≥3 abnormalities) or chromosome 7 anomalies], Intermediate [other anomalies]), and *number of cytopenias* (0-1 [score 0], 2-3 [score 0.5]), defined as hemoglobin <10 g/dL, platelets $<100\times10^9$/L, and neutrophils $<1.8\times10^9$/L. The different risk categories correlate with median survival (from 5.7 yr in patients with low score to 3.5 yr in those with intermediate-1, 1.2 yr in those with intermediate-2, and 0.4 yr in those with high score) and median time of leukemic transformation in 25% of patients (9.4 yr in low score cases, 3.3 yr in intermediate-1, 1.1 yr in intermediate-2, and 0.2 yr in high score cases) [50]. The degree of anemia, the transfusion dependency, and the presence of ALIP are negative prognostic factors [51]. In 2007, the WHO classification-based prognostic scoring system (WPSS) was published; it was able to classify patients into five risk groups with different survivals (median survival from 12 to 103 months), the most important variables being the WHO subgroups, karyotype according to the IPSS genetic categories, and transfusion requirement. The WPSS was shown to predict survival and leukemia progression at any time during follow-up; it is a dynamic, time-dependent prognostic scoring system [52]. The revised-IPSS (IPSS-R) [53] for MDS, based on the analysis of data from over 7,000 patients with *de novo* untreated MDS, identifies five cytogenetic prognostic subgroups (very good: -Y, del(11q); good:

normal, del(5q), and del(20q) as sole or double abnormalities including del(5q); intermediate: del(7q), +8, +19, i(17q), or any other not listed in the other risk groups; poor: -7, inv(3)/ t(3q)/ del(3q), double abnormalities including -7/del(7q) or complex with 3 abnormalities; very poor: complex with more than three abnormalities) and splits the low marrow blast percentage value into four groups (2%, >2 but <5%, 5 to 10%, >10%) and depth of cytopenias (anemia into three groups, thrombocytopenia in three, neutropenia in two). The different IPSS-R risk categories also show a strict correlation with median survival (from 8.8 yr in patients with very low score, 5.3 yr in those with low score, 3.0 yr in those with intermediate score, 1.6 yr in those with high score, to 0.8 yr in those with very high score), as well as with the median time of leukemic transformation in 25% of patients (more than 14.5 yr in patients with very low score, 10.8 yr in those with low score, 3.2 yr in those with intermediate score, 1.2 yr in those with high score, and 0.7 yr in those with very high score) [53]. All these scoring systems have been developed on data from MDS *de novo* untreated patients.

Global prognostication

The MD Anderson Cancer Center group, in 2008 [54], proposed the Global MDACC-MDS prognostic model- a new risk model applicable to cases of *de novo* MDS, treated MDS, t-MDS, and CMML. In this, the age, BM blast percentage, PB anemia, presence of thrombocytopenia and leukocytosis, prior treatment(s) for and transfusions of platelets and/or RBC, adverse cytogenetic abnormalities, and performance status are the evaluated prognostic variables. The same group developed the MDACC-LR Prognostic Scoring System [55], analyzing 856 patients, to identify patients classified as having a lower risk for MDS but having a poor prognosis; patients with CMML and t-MDS were also included. Unfavorable cytogenetics, Hb levels, platelet counts, and BM blasts percentages, together with higher ferritin and β2-microglobulin levels, are the analyzed variables that allow for the stratification of patients into three categories, thereby identifying patients with a median survival of 14.2 months, who require early treatment.

CONCLUSION

Owing to the availability of high-throughput molecular techniques over the last decade, a significant number of studies have demonstrated that new diagnostic pathways are fundamental for the comprehension of the aberrant mechanisms, which underlie the pathogenesis and development of MDS. The newly discovered molecular aberrations do have a great impact on the diagnosis, risk stratification, and choice of treatment approach. Several classification and prognostic scoring systems have been developed in recent years to incorporate new information and data. Each discovery leads to further diagnostic and prognostic refinements, thereby leading to improved knowledge and more informed attempts to treat this heterogeneous group of diseases.

Author's Disclosure of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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