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MYELODYSPLASTIC SYNDROMES IN THE AGE OF GENOMIC MEDICINE

Diagnosis and classification of myelodysplastic syndromes

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Myelodysplastic syndromes (MDSs) are neoplastic myeloid proliferations characterized by ineffective hematopoiesis resulting in peripheral blood cytopenias. MDS is distinguished from nonneoplastic clonal myeloid proliferations by the presence of morphologic dysplasia and from acute myeloid leukemia by a blast threshold of 20%. The diagnosis of MDS can be challenging because of the myriad other causes of cytopenias: accurate diagnosis requires the integration of clinical features with bone marrow and peripheral blood morphology, immunophenotyping, and genetic testing. MDS has historically been subdivided into several subtypes by classification schemes, the most recent of which are the International Consensus Classification and World Health Organization Classification (fifth edition), both published in 2022. The aim of MDS classification is to identify entities with shared genetic underpinnings and molecular pathogenesis, and the specific subtype can inform clinical decision-making alongside prognostic risk categorization. The current MDS classification schemes incorporate morphologic features (bone marrow and blood blast percentage, degree of dysplasia, ring sideroblasts, bone marrow fibrosis, and bone marrow hypocellularity) and also recognize 3 entities defined by genetics: isolated del(5q) cytogenetic abnormality, *SF3B1* mutation, and *TP53* mutation. It is anticipated that with advancing understanding of the genetic basis of MDS pathogenesis, future MDS classification will be based increasingly on genetic classes. Nevertheless, morphologic features in MDS reflect the phenotypic expression of the underlying abnormal genetic pathways and will undoubtedly retain importance to inform prognosis and guide treatment.

Introduction and general principles

Myelodysplastic syndromes (MDSs) are myeloid neoplasms characterized by morphologically abnormal and ineffective maturing hematopoiesis, resulting in peripheral blood cytopenias. Despite this clear conceptual definition, the diagnosis of MDS is often challenging because of the diverse nonneoplastic causes of cytopenia, the distinction between MDS and other cytopenic clonal proliferations, and the border between MDS and acute myeloid leukemia (AML).[1-4] Current diagnostic criteria establish a precise level of morphologic dysplasia (10% dysplastic cells) and maturation impairment (20% blasts) that separate MDS from nonneoplastic clonal hematopoiesis and AML, respectively.[5,6] However, these thresholds have been historically and artificially applied to what is in effect a disease continuum.[7] Moreover, assessment of morphologic dysplasia is subjective and associated with significant interobserver reproducibility.[8]

Once a patient is diagnosed with MDS, the disease is classified into one of several subtypes defined by morphologic and/or genetic features. MDS classification aims to identify subgroups with homogeneous genetic underpinnings and shared molecular pathogenesis, whereas MDS prognostic schemes aim to recognize disease groups with homogeneous outcomes irrespective of any underlying molecular or morphologic similarities. Although both classification and prognostic assessment can inform clinical decision-making in MDS, the adoption of distinct frameworks for classification and risk assessment is required given their different aims. Prognostic strata are relevant to select patients for generic risk-adapted therapies (such as stem cell transplantation), whereas defining disease classes with common pathophysiology is critical to inform targeted treatments (such as splicing modulators[9] and TP53 reactivators[10,11]), which could benefit patients across diverse risk categories. This review focuses on the diagnosis and classification of MDS in the adult population; MDS is rare in children and differs biologically from MDS affecting adults.[12]

Establishing the diagnosis

Initial diagnostic workup

Clinical suspicion for MDS typically arises when there is unexplained anemia or, less commonly, another cytopenia, bicytopenia, or pancytopenia. Therefore, the diagnostic approach should begin with the exclusion of the numerous secondary

Table 1. Blood tests of potential value in the diagnostic workup of suspected MDS

CBC, including WBC full differential count, red blood cell indices, and reticulocyte count; multiple measurements over time to document persistence/progression of any cytopenia is helpful
RBC-folate/S-folic acid, cobalamin, iron, total iron binding capacity, ferritin, copper, lactate dehydrogenase, bilirubin, haptoglobin, direct antiglobulin test (Coombs test), CRP, alanine transaminase, aspartate transaminase, alkaline phosphatase, albumin, uric acid, creatinine, S-protein electrophoresis (S-immunoglobulins), thyroid function tests, and hemoglobin electrophoresis
Serologic testing to rule out HIV, parvovirus B19 (hypoplastic MDS), cytomegalovirus, hepatitis B, and hepatitis C infections (immunosuppressed patients may require PCR testing for viral infections)
Somatic mutation (and CNV) analysis might be considered to identify clonal hematopoiesis and to inform selection of patient candidates for bone marrow examination
Germ line mutation analysis should be considered in a patient where a suspicion about an inherited bone marrow failure has been raised

CBC, complete blood count; CNV, copy number variation; CRP, C-reactive protein; PCR, polymerase chain reaction; RBC, red blood cell; WBC, white blood cell.

causes of cytopenias.[13-15] A careful family history should be taken and the possibility of an inherited bone marrow failure or predisposition disorder should be considered. Complete information should be collected for any prior chemo/radiotherapy and occupational or hobby environmental exposures (eg, benzene or pesticides), as well as on concomitant medications, alcohol intake, smoking, and tendency to bleeding/bruising or infection. Blood tests of value in the diagnostic workup of suspected MDS are summarized in Table 1. The median duration of reported cytopenias in newly diagnosed MDS is 4 months.[16] Although more severe and persistent cytopenia(s) increase the likelihood of MDS, some patients may present with mild cytopenia and/or cytopenia of relatively recent onset.[17]

If no cause of cytopenia(s) is identified based on history, physical examination, and screening blood tests, a bone marrow biopsy and aspirate are typically performed for diagnostic evaluation. Recent studies have explored the feasibility and reliability of somatic mutation analysis through next-generation sequencing (NGS) on peripheral blood to screen individuals with unexplained cytopenia for the likelihood of MDS, with the ultimate goal to avoid invasive procedures in patients with cytopenia lacking proof of a clonal disorder.[18] In individuals harboring somatic mutations in blood cells, the clone metrics (number of mutations, variant allele frequency [VAF], and mutation patterns) are predictive of the likelihood of a myeloid neoplasm.[1,18-21] Although these approaches show promising results, abnormal bone marrow morphology remains a major defining feature of MDS. Thus, examination of a bone marrow sample is necessary to establish a diagnosis of MDS as well as to classify and risk-stratify the disease according to current criteria.

Diagnostic criteria

The diagnosis of MDS requires a compilation of results of several different tests; no single criterion is pathognomonic for MDS. An absolute prerequisite for MDS is the presence of at least 1 cytopenia (hemoglobin [Hb] in females, <12 g/dL; Hb in males, <13 g/dL; platelets, <150 × 10^9/L; and/or absolute neutrophil count <1.8 × 10^9/L).[5,6] The presence of morphologic dysplasia affecting at least 10% of cells in at least 1 hematopoietic (erythroid, granulocytic, or megakaryocytic) lineage is also mandatory, with only rare exception made for certain genetic abnormalities considered to be MDS-defining in the International Consensus Classification (ICC) and revised fourth edition World Health Organization Classification (WHO) systems (see supplemental Table 1, available on the *Blood* website).[5] Importantly, morphologic dysplasia in hematopoietic cells is not specific for MDS and can be found in other myeloid neoplasms as well as in reactive conditions.[22] It is necessary to integrate the morphology findings with immunophenotyping and genetic studies and then, in turn, to interpret these comprehensive results in the context of the clinical presentation, including the type and tempo of blood count abnormalities.

A blast percentage of ≥20% in blood or bone marrow as well as certain AML-defining genetic abnormalities[5,6] excludes MDS and mandates a diagnosis of AML. Additionally, a sustained elevation of monocytes (≥0.5 × 10^9/L and ≥10% of leukocytes), platelets (≥450 × 10^9/L, in the absence of isolated del(5q) or inv(3)/t(3;3) cytogenetic aberrations), or leukocytes (≥13 × 10^9/L) tend to suggest an alternative diagnosis of an MDS/myeloproliferative neoplasm. However, mild thrombocytosis may be seen in some patients with MDS with *SF3B1* mutation, and in the absence of other myeloproliferative features, a diagnosis of MDS is appropriate in such cases. An algorithm for the suggested workup for a patient with suspected MDS is shown in Figure 1.

Diagnostic testing

Peripheral blood smear A full differential count of at least 200 nucleated peripheral blood leukocytes must be performed to quantify the proportion of blasts as well as monocytes (Table 2). Granulocytes should be examined for signs of dysplasia, including pseudo–Pelger-Hüet cells and agranular or hypogranulated forms. Platelets may show anisocytosis (variation in size) and include macrothrombocytes. Red cells may show basophilic stippling, nucleated precursors, dimorphic populations, megaloblastic cells, anisocytosis, and poikilocytosis; however, it should be noted that the aforementioned changes in platelets and red cells are not specific for MDS.[23,24] The majority of patients with MDS present with a normal to increased median corpuscular volume, but a small subset has microcytosis.[25,26] A lack of circulating blasts or dysplastic cells in the blood does not rule out MDS because dysplasia or blast increase in the bone marrow may not manifest in the blood.

Bone marrow aspirate The gold standard in the evaluation of bone marrow morphology for MDS is the aspirate smear. To ensure accurate morphologic evaluation, the aspirate must be collected, prepared, and stained correctly. Optimally, air-dried aspirate smears should be prepared as soon as possible from the first aspiration of 2 to 3 mL of liquid marrow. In the instance of a "dry tap" (inaspirable marrow), air-dried touch preparations may be made from the fresh bone marrow trephine biopsy. Suitable staining methods are May-Grünwald, Giemsa, or Wright.

A systematic description of bone marrow cytology includes a quantification of specific cell types and a description of any qualitative changes of the nucleated marrow cells[23] (Table 2). It

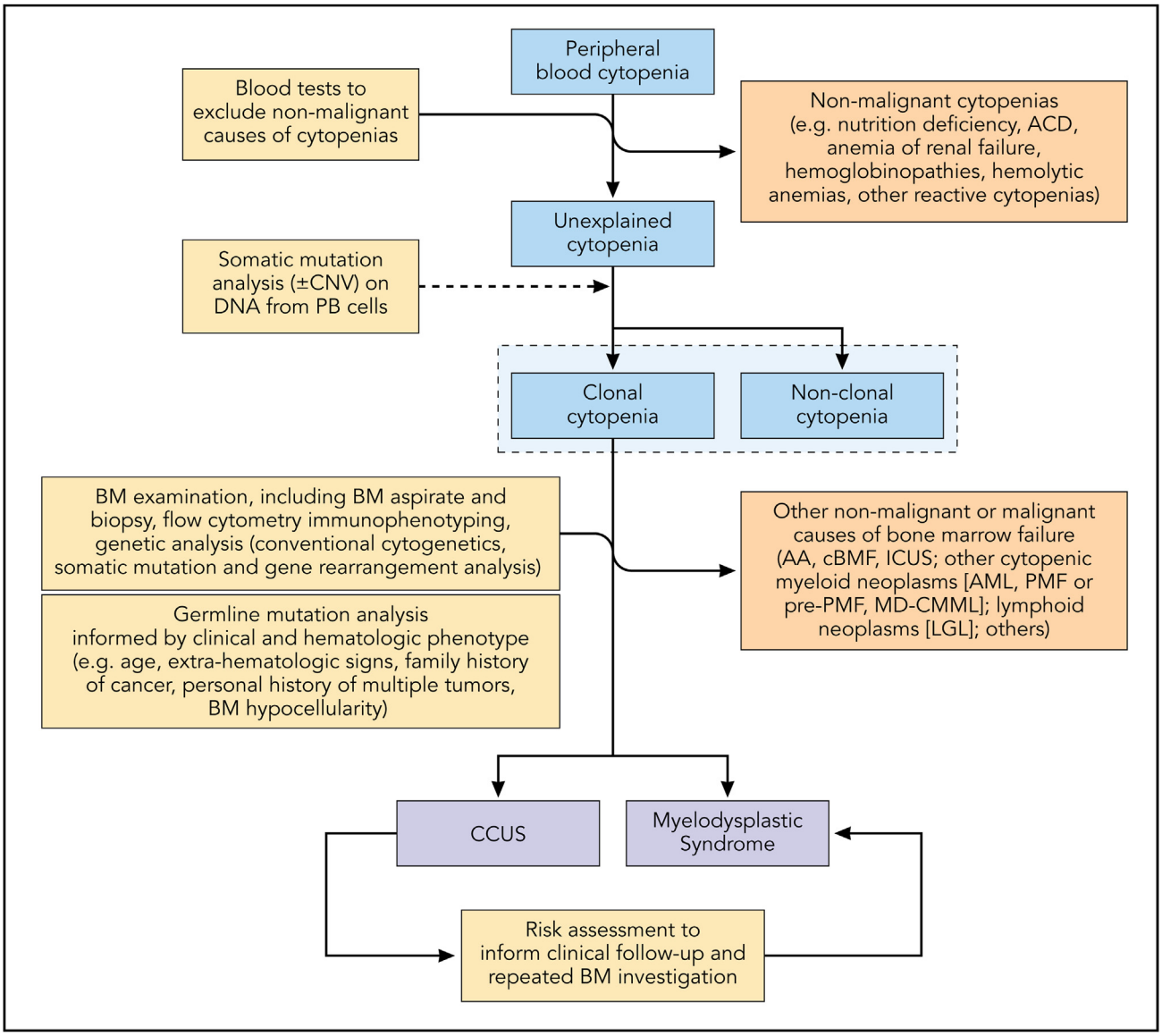

Figure 1. Diagnostic workup of suspected myelodysplastic syndromes. The diagnostic approach to suspected MDS begins with the exclusion of the secondary causes of cytopenias. If no cause for the cytopenia(s) is identified based on history, physical examination, and screening blood tests, a bone marrow examination is recommended for diagnostic evaluation. Recent studies have explored the feasibility and reliability of somatic mutation analysis on DNA from peripheral blood cells (dotted line) to screen individuals with unexplained anemia for the likelihood of MDS. The absence of somatic mutations has a high negative predictive value for ruling out a diagnosis of myeloid neoplasm, whereas in individuals harboring hematologic clone(s), the clone metrics, (number of mutations, VAF, and mutation patterns) are predictive of the likelihood of a myeloid neoplasm. AA, aplastic anemia; ACD, anemia of chronic disease; BM, bone marrow; cBMF, constitutional bone marrow failure; LGL, large granular lymphocytic leukemia; MD-CMML, myelodysplastic-type chronic myelomonocytic leukemia; PMF, primary myelofibrosis. Professional illustration by Somersault18:24.

is important to count at least 500 nucleated cells and quantify blasts, including any promonocytes, which are regarded as blast equivalents.[27] Granulocytic, erythrocytic, and megakaryocytic cells should be evaluated for dysplastic changes: a lineage is considered to be dysplastic if at least 10% of cells in that lineage manifest cytologic dysplasia. Characteristic dysplastic features of each lineage are summarized in the supplemental Text. The bone marrow blast percentage in MDS varies between 0% and 19% and must be reported as an actual number (not "<5%" or "5%-10%") to allow for the application of prognostic risk stratification and establish a precise baseline from which to measure treatment response.[28]

Bone marrow biopsy A bone marrow biopsy is essential for optimal disease classification (Table 2). Erythroid and granulocytic dysplasia are difficult to appreciate in the biopsy, whereas megakaryocyte dysplasia is often more easily identified in the biopsy compared with the aspirate smear. The biopsy can also reveal alternate pathologies that simulate MDS in their clinical presentation or may occur concomitantly with MDS, such as tumor metastases, granulomas, multiple myeloma, systemic mastocytosis, and lymphomas. The fifth edition WHO Classification (WHO5) recognizes 2 new MDS subtypes defined by bone marrow hypocellularity and fibrosis (see "Classification" section and Figure 2A), features assessed on the bone marrow biopsy.[6]

Table 2. Morphologic features in blood and bone marrow used in the diagnosis and classification of putative MDS

Features	Use in diagnosis and/or classification
Blood counts (Hb, ANC, Plt)	Essential to document at least 1 cytopenia. Essential to document absence of thrombocytosis (Plt ≥450 × 10^9/L) that would tend to exclude MDS and suggest an MDS/MPN, except if meeting criteria for MDS with isolated del(5q).
WBC	Essential to document absence of persistent leukocytosis (WBC ≥13 × 10^9/L, not due to lymphocytosis) that would tend to exclude MDS and suggest an MDS/MPN.
Monocytes in blood	Essential to document absence of persistent monocytosis (≥0.5 × 10^9/L and ≥10% of WBC) that would tend to exclude MDS and suggest CMML.
Blasts in blood and marrow	Blast percentage of nucleated cells essential to classify MDS and exclude AML (if blasts are ≥20%), optimally counting at least 500 cells in the bone marrow aspirate smear or touch preparation and 200 cells in blood. Blast estimation by CD34 staining of the bone marrow biopsy may substitute if the aspirate is hemodilute or unobtainable.
Auer rods	Essential to document, if present, for classification.
Megakaryocyte dysplasia	Essential to document and quantify (≥10%, present but <10%, or absent) in both aspirate smear and biopsy; at least 25 megakaryocytes should be evaluated.
Erythroid dysplasia	Essential to document and quantify (≥10%, present but <10%, or absent) in aspirate smear.
Granulocytic dysplasia	Essential to document and quantify (≥10%, present but <10%, or absent) in aspirate and blood smears.
Ring sideroblasts	Essential to evaluate and quantify (% of mature erythroid precursors) in an iron-stained aspirate smear.
Bone marrow cellularity	Essential to evaluate and quantify as percentage of marrow space in biopsy specimen for WHO5 classification.
Bone marrow fibrosis	Essential to evaluate and quantify (MF grade 0-3) in reticulin-stained biopsy specimen for WHO5 classification.

ANC, absolute neutrophil count; Hb, hemoglobin; MPN, myeloproliferative neoplasm; MF, myelofibrosis; Plt, platelet count; WBC, white blood cell count.

Immunohistochemistry of the biopsy specimen can aid in blast quantification, identification of specific cell types (particularly micromegakaryocytes, which can be difficult to visualize on routine histology), and evaluation of alternative etiologies for cytopenia.[29] Although the aspirate smear blast percentage is the standard for classifying MDS and separating it from AML, CD34 immunostaining of the biopsy specimen can provide a blast estimate and is particularly helpful if the aspirate is hemodilute, potentially leading to underestimation of the blast percentage. In cases in which there is discrepancy between the aspirate blast count and the blast estimation by CD34 immunohistochemistry, it is recommended to use the higher percentage for the purposes of classification and risk stratification.[30] A marked discrepancy should prompt careful rereview of the aspirate smear to assure that blasts have not been overcounted or undercounted.

Flow cytometry Flow cytometric methods can be useful to quantify the proportion of $CD34^+$ and $CD117^+$ myeloid progenitors, which usually correlate with the blast percentage obtained by visually counting the bone marrow aspirate smear. However, the blast percentage by flow cytometry is influenced by preanalytic variables and in situations of discrepancy, the manual blast count from the aspirate smear should be used, provided it is an adequate specimen. Flow cytometry also reliably identifies aberrant features in blast and nonblast cells that can help support a diagnosis of MDS. The European Leukemia Net/International MDS Flow Cytometry Working Group recently put forth recommendations for the application of flow cytometry to diagnose MDS.[31,32] According to these guidelines, a putative diagnosis of MDS can be supported with varying levels of certainty, depending on the score. Finally, flow cytometry of bone can reveal clonal B-cell populations or abnormal T-cell populations that suggest alternative diagnoses, such as hairy cell leukemia or large granular lymphocytic leukemia, which may be subtle and missed on initial morphologic review[33] (Table 3).

Cytogenetics Karyotyping remains the most widely used and unbiased method for assessing numerical and structural chromosomal abnormalities, which are observed in 50% to 60% of patients with MDS. Karyotype of bone marrow aspirate should be performed in all patients with suspected MDS undergoing bone marrow examination. At least 20 metaphases should be analyzed whenever possible and described according to the International System for Human Cytogenetic Nomenclature recommendations.[34] The most frequent single cytogenetic abnormalities include del(5q), monosomy 7, del(7q), trisomy 8, and del(20q).[35,36] Cytopenic cases with del(5q), −7/del(7q), or complex karyotype that lack qualifying dysplasia or excess blasts can be diagnosed as MDS according to the current ICC criteria[5] because they exhibit clinical behavior similar to overt MDS.[37]

Interphase fluorescence in situ hybridization to detect MDS-associated abnormalities is recommended in the case of repeated failure of standard G-banded karyotyping, but it is not required in the setting of an adequate karyotype.[38,39] Chromosomal microarrays can be used to identify small, unbalanced abnormalities or cryptic copy number alterations, and the application of single nucleotide polymorphism probes (SNP arrays) can detect loss of heterozygosity at key loci. For example, detection of copy-neutral loss of heterozygosity is

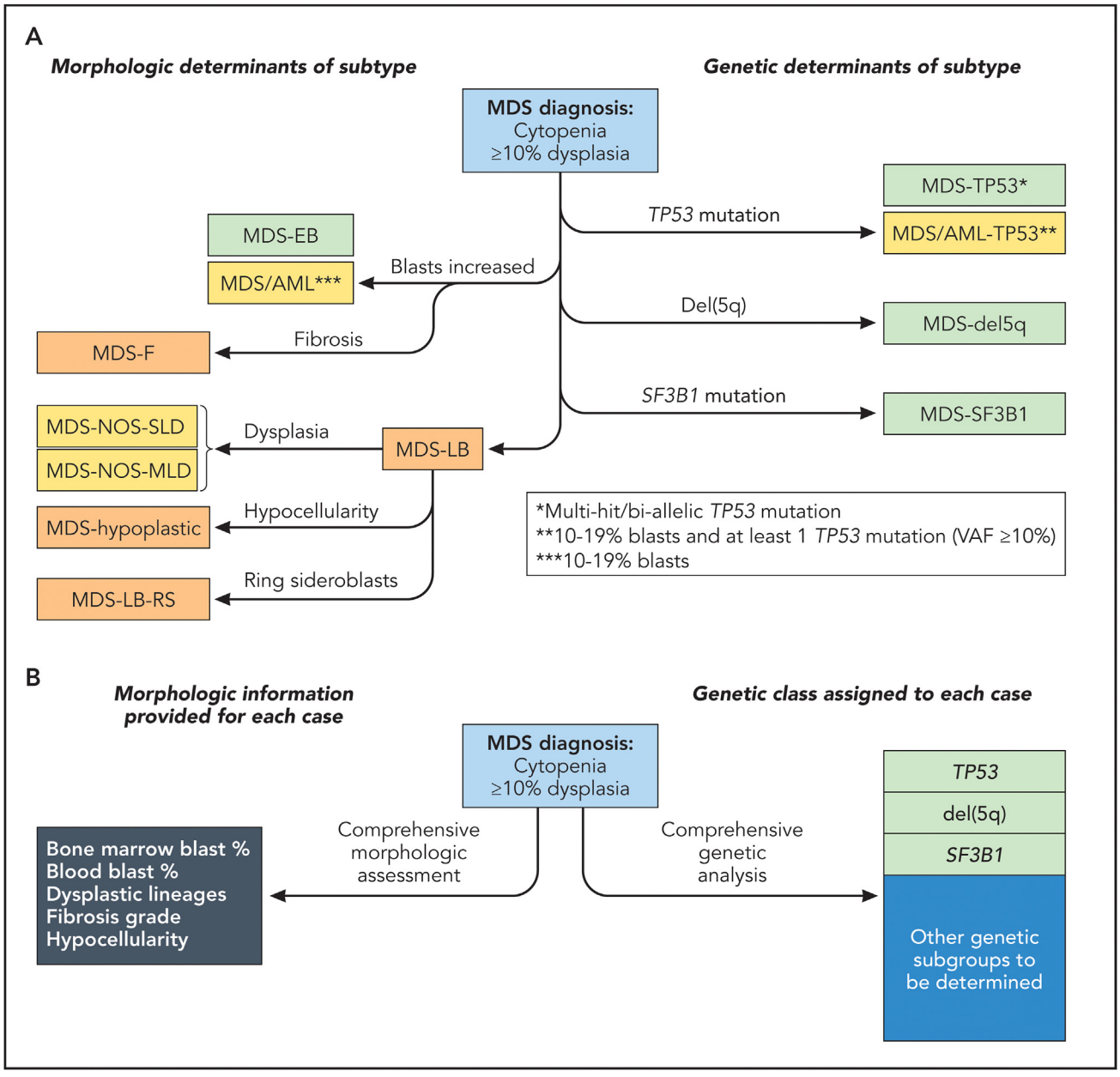

Figure 2. Current and possible future classification process of MDS. (A) MDS hierarchical classification process according to current ICC and WHO5. Morphologic features affecting classification are on the left and genetic determinants are on the right. Subtypes that are common to both ICC and WHO5 are shown in green, ICC subtypes are in yellow, and WHO5 subtypes are in orange. The detailed diagnostic criteria and exclusionary features of each entity are not shown on this diagram and are provided in separate publications.[5,6] (B) A possible future classification approach that would separate the current hierarchical interweaving of morphologic and genetic MDS subtypes. Genetic classes are shown on the left and would be based on molecular clustering analyses of MDS cohorts; each case would be assigned a genetic class at diagnosis. Independent of the genetic class, the diagnosis of each case would include morphologic information, shown on the left. These morphologic data would reflect disease stage and treatment response (blasts) and express phenotypic features that provide additional prognostic information (SLD vs MLD, fibrosis grade) or guide therapy (hypocellularity), depending on the specific genetic class and patient characteristics. Professional illustration by Somersault18:24.

particularly important to identify multihit *TP53* lesions, which affect MDS classification and risk stratification.[40,41]

Molecular genetic testing Detection of driver somatic mutations is of utmost relevance in the diagnosis and clinical management of MDS. Most driver mutations occur in a core set of ~50 genes, which are typically interrogated by targeted NGS panels. Key genes that should be assessed by NGS in putative MDS cases are summarized in Table 4, and targeted NGS testing is recommended for patients with known or suspected MDS.[34]

Given the high concordance between bone marrow and blood,[40,42-44] if NGS testing has been performed on a blood sample before the bone marrow biopsy, the testing does not necessarily need to be repeated on the bone marrow sample unless there is suspicion of a change in the disease status.

Somatic mutation information is a valuable complement to the diagnostic workup of patients with suspected MDS. A high number of somatic mutations, a mutant clone with high VAF (>20%), and certain mutation patterns show high predictive

Table 3. Flow cytometry features used in the diagnosis of putative MDS

Features	Use in diagnosis
Blast percentage and immunophenotype in bone marrow	Documents blast immunophenotype (myeloid); report should specify how blasts are quantified
Aberrant blast immunophenotypes	Support a diagnosis of MDS already suspected based on other features
Aberrant immunophenotypes of granulocytes, monocytes, and erythroids	Support a diagnosis of MDS already suspected based on other features
Clonality of B cells	Evaluates for a B-cell lymphoma involving the bone marrow if lymphoma is suggested by morphology
T-cell immunophenotype	Evaluates for large granular lymphocytic leukemia or other T-cell lymphomas involving the bone marrow
Paroxysmal nocturnal hemoglobinuria assessment (blood)	Useful in hypocellular cases when aplastic anemia or paroxysmal nocturnal hemoglobinuria are considered as alternate diagnoses

values for a diagnosis of MDS or another myeloid neoplasm, whereas the absence of any driver mutations has a high negative predictive value for myeloid neoplasia (but does not absolutely exclude MDS).[18,19,45] Supportive mutation information may be particularly helpful in cases with borderline morphologic dysplasia or comorbidities that complicate the use of cytopenia to establish an MDS diagnosis. Similar to the selected cytogenetic abnormalities discussed above, mutations in *SF3B1* or multihit *TP53* alterations (at minimum VAF levels of 10%) are considered to be MDS-defining in patients with cytopenia lacking qualifying dysplasia according to the ICC.[5,46] However, *SF3B1* mutation and multihit *TP53* are almost always associated with significant morphologic dysplasia.[1,2,19,47,48] Given that larger clones are more strongly associated with MDS than smaller clones, it is recommended to take VAF into account when using mutation information to support a diagnosis of MDS.[19]

Genetic germ line variants are increasingly recognized as predisposing patients to myeloid neoplasms, including MDS.[49-51] Identifying patients with underlying genetic predisposition has highly relevant clinical implications with regard to patient management, including the selection of related donors for stem cell transplantation, therapeutic conditioning, and genetic counseling for family members. Germ line mutations (eg, *CEBPA*, *GATA2*, *RUNX1*, and *TP53*) are often captured by routine somatic NGS panels, and their detection at VAF in the germ line range (40%-60%) may prompt confirming that the mutation is germ line by testing of nonhematopoietic cells (supplemental Table 2); rare germ line mutations may present with VAF levels <40%. Identifying characteristic nonhematologic signs on physical examination or eliciting a personal or family history of thrombocytopenia or cancer may also foster suspicion of a constitutional disorder and inform testing. However, many predisposition conditions present without symptoms or signs.[49,52-54] Expert guidance has suggested that younger patient age, bone marrow hypocellularity, a personal history of multiple tumors (at least one of which is a hematologic neoplasm), and/or a family history of cancer should prompt testing using a dedicated NGS platform that targets germ line predisposition mutations.[29,55-58] Some germ line predisposition disorders, such as *DDX41* mutation, may present in older patients within the typical age range of sporadic MDS.[52,59,60]

Integrative diagnosis and reporting

It is obligatory for the diagnostician to integrate the myriad of findings from the aforementioned testing to establish an MDS diagnosis or rule out MDS and consider other neoplastic or nonneoplastic causes for the cytopenia. The final diagnostic report should include a statement that clearly indicates whether a diagnosis of MDS is definitive and, if so, should provide the specific MDS subtype. If the data do not allow a definitive diagnosis, a descriptive diagnosis can be rendered, expressing the degree of suspicion of MDS. In this situation, a differential diagnosis should be provided, and optimally, further testing should be suggested that could clarify the diagnosis if cytopenias persist. It should be noted that cytopenias often remain unexplained even after a comprehensive workup; in the absence of diagnostic criteria, a persistent unexplained cytopenia does not "default" to a diagnosis of MDS and should be considered to represent *idiopathic cytopenia of undetermined significance* (ICUS) or *clonal cytopenia of undetermined significance* (CCUS) until diagnostic criteria for a myeloid neoplasm are met.[14] The required components of a diagnostic report of MDS are shown in Table 5.

Classification

History of MDS classification

The term "myelodysplastic syndrome" was established in the 1980s and first used by the French-British-American working group in 1982 to encompass a group of cytopenic myeloid neoplasms.[61] The aim of this first formal classification was to define the types of MDS based purely on morphologic criteria, which included the bone marrow blast percentage, ring sideroblasts, the presence of any Auer rods, and the presence and degree of monocytic proliferation in blood and marrow. This publication was a milestone in diagnostics in that clinicians and scientists were able to use a common nomenclature to enable the comparison of different patient subgroups and their disease course. After subsequent international studies,[62-65] the 2001 WHO working group subdivided MDS cases into those distinguished by unilineage dysplasias, multilineage dysplasias, and increased blasts.[66] Additionally, for the first time, a cytogenetic finding, the deletion of part of chromosome 5q as a sole finding on karyotype, was included as a disease-classifying feature.

Table 4. Genetic studies indicated for clinical testing in MDS

Conventional karyotype (at least 20 metaphases).* FISH for 17p deletion if *TP53* mutation is detected.	
Gene mutation panel for the following genes:	
ASXL1	*NF1*
BCOR	*NPM1*
BCORL1	*NRAS*
CBL	*PHF6*
CEBPA	*PPM1D*
CSF3R	*PRPF8*
DDX41	*PTPN11*
DMNT3A	*RAD21*
ETV6	*RUNX1*
ETNK1	*SAMD9*
EZH2	*SAMD9L*
FLT3-ITD	*SETBP1*
FLT3-TKD	*SF3B1**
GATA2	*SRSF2*
GNB1	*STAG2*
IDH1	*TET2*
IDH2	*TP53**
JAK2	*U2AF1*
KIT	*UBA1*
KRAS	*WT1*
KMT2A-PTD	*ZRSR2*

FISH, fluorescence in situ hybridization.

*Essential to classify MDS according to ICC and WHO5 criteria.

Updated WHO Classifications in 2008 and 2017 made further changes in nomenclature and refined diagnostic criteria of individual entities, but aside from MDS with isolated del(5q), the MDS subgroups were defined entirely based on morphology.[67,68]

Current classification systems: WHO5 and ICC

WHO5, developed in 2022, renamed MDS "myelodysplastic neoplasms" (while retaining the "MDS" abbreviation) and introduced several significant changes in MDS nomenclature, entities, and diagnostic criteria.[6] Simultaneously, a Clinical Advisory Committee formed by members of the Society for Hematopathology and the European Association for Haematopathology published the ICC of MDS and other myeloid neoplasms in 2022.[5,69] Although the WHO5 and ICC classifications are overall similar, there are several differences in diagnostic criteria and nomenclature.[70-73] Importantly, both ICC

Table 5. Essential components of a report on a newly diagnosed MDS (or diagnosis of MDS before enrollment in a clinical trial)

Classification subtype according to WHO revised fourth edition, WHO fifth edition, and ICC schemes
Qualifying terms if the case is therapy-related/postcytotoxic therapy and/or in the background of a germ line predisposition condition
CBC and WBC differential from the date of the bone marrow sample, including peripheral blood blast percentage
Bone marrow blast percentage on bone marrow aspirate smear*
Presence of any significant (≥10%) dysplasia in each hematopoietic lineage (granulocytic, erythroid, and megakaryocytic) and any Auer rods
Presence or absence of ring sideroblasts on iron stain and their percentage of mature erythroid cells in the marrow
Bone marrow cellularity assessment (percentage)
Bone marrow fibrosis grade (MF 0-3)
Presence of any concomitant pathologies, such as metastasis, infection, lymphoma, mast cell disease, or multiple myeloma
Bone marrow karyotype (ISCN nomenclature) and any FISH results
Any pathogenic mutations identified on NGS panel, including VAF of each mutation

ISCN, International System for Human Cytogenetic Nomenclature.

*Blast estimation based on CD34 staining of the bone marrow biopsy should also be reported, if performed.

and WHO5 introduced 2 new MDS entities, defined by *SF3B1* mutation and multihit *TP53* mutation, expanding the role of genetics in MDS classification beyond MDS with isolated del(5q), which had previously been the sole genetically defined MDS subtype. All these genetically defined entities share key features: they are founding or early genetic lesions in the clonal ontogeny, major determinants of disease phenotype, and prognostically relevant. *SF3B1*-mutated and del(5q) MDS exhibit relatively indolent clinical behavior and lack increased blasts, whereas *TP53*-mutated MDS is a highly aggressive disease characterized by genetic instability and a rapidly progressive course. Additionally, both ICC and WHO5 reduced the absolute monocyte count defining chronic myelomonocytic leukemia (CMML) from 1×10^9/L to 0.5×10^9/L, which will result in some cases previously classified as MDS to be classified as CMML in the current schemes. The nomenclature and correspondence of the MDS entities in ICC, WHO5, and WHO revised fourth edition classification are shown in Table 6. The overall classification hierarchy of MDS according to current schemes is shown in Figure 2A.

The general features of the MDS subtypes in the ICC and WHO5 are provided in the supplemental Text, with detailed diagnostic criteria for each entity described in separate publications.[5,6,46]

Navigating the 2 classifications in practice

The classifications developed in parallel by the 2 international working groups in 2022 have led to the question of how to handle both within the context of daily practice as well as in

Table 6. Comparative MDS classification

WHO revised fourth edition	WHO fifth edition	ICC
MDS with RS (most *SF3B1*-mutated cases)	MDS with LB and *SF3B1* mutation	MDS with mutated *SF3B1*
MDS with RS (*SF3B1* wild-type cases)	MDS with LB and RS	Not included
MDS with isolated del(5q)	MDS with LB and isolated 5q deletion	MDS with del(5q)
MDS with SLD	MDS with LB	MDS-NOS with SLD
MDS with MLD	MDS with LB	MDS-NOS with MLD
MDS, unclassifiable (most cases)	CCUS	MDS-NOS without dysplasia
Not included	MDS, hypoplastic	Not included
MDS with EB1	MDS with IB1	MDS with EB
MDS with EB2	MDS with IB2	MDS/AML
Not included	MDS with fibrosis	Not included
Not included	MDS with bi-allelic *TP53* inactivation	MDS and MDS/AML with mutated *TP53*

CCUS, clonal cytopenia of undetermined significance; EB, excess blasts; IB, increased blasts; LB, low blasts; MLD, multilineage dysplasia; NOS, not otherwise specified; RS, ring sideroblasts; SLD, single lineage dysplasia.

research and clinical trials. The detailed criteria used to enroll patients with MDS in clinical trials are already complicated even when applying and interpreting a single classification system.[74] With this in mind, it is imperative that future study protocols are carefully designed to allow the entry of patients whether they have been classified by WHO5 edition or ICC; otherwise, patient enrollment may be unnecessarily limited.[70] Additionally, patients transferred from one institution to another could be mismanaged if their disease is classified according to different schemes. For these reasons, we strongly recommend that all diagnosticians provide both ICC and WHO5 classifications in diagnostic reports and in the clinical record, particularly when there are differences. It must be noted that that the key elements required to generate these categories are almost entirely overlapping, making the class assignments according to both ICC or WHO5 relatively straightforward (Figure 2A).

Therapy-relatedness and germ line predisposition

Approximately 10% of patients with MDS have a history of bone marrow exposure to cytotoxic therapies: chemotherapy, radiation exposing hematopoietic marrow, or combined modality therapies. Although prior classifications combined all such therapy-related cases into a single category of "therapy-related myeloid neoplasms," both ICC and WHO5 recommended subclassifying these cases similar to other MDS. This is based on evidence showing significant prognostic differences in therapy-related diseases based on their subtype and genetic risk category.[40,75] The WHO5 introduced the term "postcytotoxic therapy" to express the presumed disease ontogeny more cautiously, whereas the ICC retained the term "therapy-related" as a qualifier to the specific MDS disease category.[5,6] As discussed above, a subset of MDS cases occur in the setting of a germ line predisposition syndrome, and the ICC recommends that any known inherited germ line predisposition condition also be applied as a qualifier to the MDS subtype. Germ line mutations may be associated with dysplastic features (particularly in megakaryocytes), irrespective of whether the patient has a myeloid malignancy.[50,76] Thus, conventional morphologic criteria alone may be inadequate to diagnose MDS in this setting, emphasizing an integrative diagnosis based on bone marrow findings and genetic studies.[38,49]

Future directions

An increasing understanding of the molecular pathophysiology of MDS, including gene-gene associations, will likely further refine defined entities with homogeneous molecular basis. Recent applications of machine learning approaches have enabled the recognition of molecular subgroups with the potential to inform classification. Besides the already recognized genetic MDS entities and newly AML-defining genetic groups (see supplemental Text), molecular subgroups that may inform future MDS classification include other splicing factors (*SRSF2*, *U2AF1*, and *ZRSR2*) and other AML-like mutations (*WT1*, *FLT3-ITD*, and *KMT2A-PTD*).[77-80] A fully molecular MDS classification is expected to offer several advantages compared with the current largely morphology-based system: (1) improved reproducibility; (2) more accurate class assignment through robust biomarkers; (3) more accurate discrimination between premalignant (clonal cytopenia of undetermined significance) and early malignant myeloid disorders; (4) predicted pathways of disease progression, thus enabling more precise disease monitoring; and (5) therapeutic guidance, by offering therapeutic targets and optimizing clinical trial design and drug development. However, it should be acknowledged that a purely genetic MDS classification requires further validation and has limitations and caveats: (1) mutation patterns are complex and may overlap; (2) even within currently defined and well-established genetic subgroups, evidence-based treatment decisions depend on clinical parameters such as blood counts and transfusion dependency; and (3) full-scale NGS panels are not widely available across all settings.

With the above caveats in mind, an important question is what role morphology might play in an MDS classification based fully on genomic categories. The current classification strategy for MDS integrates genetic and morphologic parameters, yet it applies them mutually exclusively in a defined hierarchy (Figure 2A). Recent evidence has shown that some well-defined genetic MDS subgroups show a wide spectrum of blast percentages, suggesting that the blast percentage may function more as a "stage" of disease rather than defining a specific MDS subtype as it does currently.[77,80] One future approach could be to divorce the currently interwoven genomic and morphologic MDS categories and instead assign every MDS case to a genomic category and provide morphologic information in parallel (Figure 2B). With such an approach, morphologic features such as blast percentage could be expressed as continuous variables and accompanied by descriptions of the degree of dysplasia, marrow cellularity, and fibrosis in each unique case. This would allow for the simultaneous incorporation of the MDS genetic class with its phenotypic expression and facilitate the monitoring of changes in disease morphology after application of treatment. The current classification of central nervous system tumors follows a similar "layered" approach, assigning independent genetic and morphologic categories while also providing an integrated overarching diagnostic category.[81]

Continually advancing genetic technologies are expected to have highly relevant impact on the diagnosis and classification of MDS. Broad genomic sequencing (whole exome and whole genome sequencing) and transcriptomic sequencing (RNA sequencing) allow for robust detection of different types of genomic alterations. Although currently WGS and WES entail higher costs and more extensive data analysis pipelines, these approaches can detect a full range of genomic alterations, fostering clinical application to the diagnosis of diverse myeloid neoplasms.[82,83] Transcriptome-wide sequencing detects both chromosomal rearrangements and changes in messenger RNA,[84] facilitating the identification of unique disease subtypes.

Single-cell techniques are also emerging as highly informative approaches in clinical research, with potential for future clinical implementation. MDS is characterized by high genetic complexity, including the coexistence of multiple clones evolving over time.[85] Recent studies of clonal architecture at a single-cell level offer unique insights into the dynamic relationship of MDS subclones during the natural history of the disease or under the selection pressure of therapies[86] and, in the future, could help predict the trajectory of disease progression. For example, a small but highly aggressive or genetically unstable subclone could be intercepted by early therapeutic intervention, thus preventing clonal evolution, or multiple subclones could be targeted by combined therapies. In a clonally complex disease such as MDS and in the setting of increasing therapeutic options, more precise classification has the potential to transform the current approach that focuses on treating high-risk disease: instead, class-specific targeted therapies could alleviate progression early in the course of disease.

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Footnotes

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