The Challenging World of Cytopenias: Distinguishing Myelodysplastic Syndromes From Other Disorders of Marrow Failure

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ABSTRACT

Over the past decade, our understanding of bone marrow failure has advanced considerably. Marrow failure encompasses multiple overlapping diseases, and there is increasing availability of diagnostic tools to distinguish among the subtypes. Identification of genetic alterations that underlie marrow failure has also greatly expanded, especially for myelodysplastic syndromes. Molecular markers are increasingly

used to guide the management of myelodysplasia and may distinguish this diagnosis from other marrow failure disorders. This review summarizes the current state of distinguishing among causes of marrow failure and discusses the potential uses of multiple diagnostic and prognostic indicators in the management of myelodysplastic syndromes and other bone marrow failure disorders. *The Oncologist* 2014;19:735–745

Implications for Practice: Over the past decade, our understanding of the molecular mechanisms underlying bone marrow failure (BMF) conditions has expanded, especially with respect to molecular testing for somatic mutations in myelodysplastic syndromes (MDS). Applications of genomics include correctly diagnosing the BMF condition and incorporating prognostic markers to ensure correct therapy. This review aims to clarify the current state of diagnostic modalities in BMF and to examine the potential utility of an algorithmic approach using molecular markers distinguish MDS from other BMF conditions. In addition, the prognostic value of molecular markers in predicting the risk of disease aggressiveness, recurrence, and mortality is discussed.

INTRODUCTION

The bone marrow failure (BMF) syndromes are a heterogeneous group of clinically and pathologically distinct disorders associated with cytopenias and failure of normal hematopoiesis. Each of these overlapping diseases has a distinct underlying pathophysiology and may be indistinguishable in the early stages. BMF conditions can be acquired or inherited and can confer a significant risk of morbidity and death because of their progressive natural history, clonal evolution, and complications of suboptimal therapy [1–4].

Appropriate therapy and prognostication depends on accurate and early diagnosis. The disorders categorized as BMF include the myelodysplastic syndromes (MDS; including hypocellular MDS [hMDS]), aplastic anemia (AA), inherited BMF syndromes (IBMFS), large granular lymphocytosis (LGL), pure red cell aplasia (PRCA), paroxysmal nocturnal hemoglobinuria (PNH), and myeloproliferative neoplasms (MPNs) (Fig. 1). The heterogeneity of these disorders is emphasized by the spectrum of how they can be categorized: by cellular or hypocellular marrow, by neoplastic or nonclonal biology, by inherited or acquired etiology, by acute or chronic

manifestation, or as primary stem cell disorders versus stem cell toxic evolution. There is some degree of overlap among these syndromes; two disorders can coexist in the same patient, and this can complicate diagnoses. An example of this is MDS/MPN overlap syndromes. Refractory anemias or bilineage and trilineage cytopenias in the context of normocellular or hypercellular bone marrows, in particular, can present difficult diagnostic dilemmas. The diagnosis can remain unclear even after repeated bone marrow evaluations. Many of these disorders can evolve to acute myeloid leukemia (AML) and, at the very least, predispose to malignant potential. Consequently, the evaluation of BMF is often aimed at distinguishing treatable conditions for which therapies are readily available, such as MDS or PNH, from other causes of marrow failure so as to prescribe the correct therapy. This approach is due to the perceived status of MDS as a malignancy equivalent, the availability of drugs to treat MDS, and the necessity for future planning for potentially curative hematopoietic stem cell transplantation (HSCT). It is also essential to ensure that patients without MDS avoid the toxicity of MDS

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Figure 1. Overlap of bone marrow failure syndromes.

Abbreviations: AA, aplastic anemia; hMDS, hypocellular myelodysplastic syndromes; IBMFS, inherited bone marrow failure syndromes; LGL, large granular lymphocytosis; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasm; PNH, paroxysmal nocturnal hemoglobinuria; PRCA, pure red cell aplasia.

therapies and that those patients with lower-risk (and potentially self-limited) conditions are not unnecessarily rushed to HSCT. Furthermore, if a patient is likely to have a more aggressive course, early planning and human leukocyte antigen (HLA) typing can be initiated promptly. In the absence of diagnostic conviction, many of these patients are given a diagnosis of possible or probable MDS, although such a diagnosis can be imprecise in the absence of increased blasts or disease-defining karyotype abnormalities.

Treatment algorithms depend both on correct diagnoses and accurate prognostication strategies. Within the past decade, significant advances have been made in identification of molecular abnormalities in disease. This is particularly true in MDS, for which anomalies have been demonstrated in approximately 90% of cases. An example in MDS is the spliceosome mutations, which can aid in identification of refractory anemia with ring sideroblasts [5]. This review will focus on distinguishing MDS from other BMF syndromes and discuss both established and newer techniques, with a particular emphasis on molecular diagnostics. This is a common clinical challenge, and the appropriate application and interpretation of cutting-edge diagnostic approaches can ensure correct and early diagnosis of BMF conditions and the most appropriate subsequent therapies.

DIAGNOSTIC APPROACH TO MARROW FAILURE

Although established approaches to the evaluation of cytopenias are often designed to be sensitive to the diagnosis of MDS [6], it can be a challenge to ensure that the “benign” BMF conditions are also appropriately triaged and evaluated. Diseases such as LGL or AA, although not considered overtly malignant, can confer significant morbidity and even mortality if inappropriately identified and treated as MDS.

BMF diagnostic criteria distinguish these disorders from both reactive cytopenias and dysplasia as well as from other clonal stem cell disorders [7]. After nonmalignant causes of cytopenias (e.g., vitamin deficiencies) are excluded, the diagnostic approach to the suspected BMF disorder begins with morphologic studies of peripheral blood and bone

marrow. Specialized tests should be performed at BMF specialty centers where testing infrastructure and a comparatively large volume of BMF patients lend experience to the evaluation.

Repeated bone marrow examinations a few weeks, months, or even years apart are sometimes required to establish a diagnosis and to identify patients with rapid disease progression. These should be repeated any time the clinical picture changes. Table 1 delineates appropriate testing in this patient population.

DISEASE-SPECIFIC DIAGNOSTIC EVALUATIONS

Myelodysplastic Syndromes

MDS is a collection of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis involving one cell lineage or more. For diagnosis, a bone marrow biopsy with aspirate to assess marrow cellularity, fibrosis, dysplasia, and topography is mandatory; flow cytometry and cytogenetics studies are also invaluable [6]. A finding of excess blasts ($\geq 5\%$), for example, is supportive of MDS or an evolving acute leukemia. If extensive fibrosis is present, it may be more indicative of an MDS overlapping with a myeloproliferative neoplasm. Furthermore, the degree of dysplasia and the presence or absence of specific types of dysplasia are sometimes useful in distinguishing AA from hMDS. The presence of pseudo-Pelger-Huet cells is rarely found in AA but can often be seen in MDS. In contrast, megaloblastoid erythroid maturation or megakaryocytic atypia is less diagnostic of either disorder. Cytogenetic studies can identify nonrandom chromosomal abnormalities. Some of these, such as del(5q), monosomy 7, or inversion 3, are considered virtually diagnostic of MDS or AML [8–10]. Deletion of Y, 20q, trisomy 8, and recurrent translocations and inversions involving chromosome 3q, among others, also have prognostic relevance [3]. Even once an MDS diagnosis has been established, morphologic discrepancies in the MDS subtypes have been reported to be as high as 12% between centers, with upstaging more commonly occurring at tertiary referral centers [11].

The diagnosis of MDS may be difficult in patients with a normal karyotype or noninformative cytogenetics who do not have robust morphologic markers such as ring sideroblasts or an excess of myeloblasts. Because these patients with possible MDS usually present with mild cytopenias, rapid disease progression is unlikely, and whether an MDS diagnosis is confirmed, therapeutic intervention is unlikely in the initial stages. Because an earlier MDS diagnosis will neither invoke therapy nor influence outcome, such patients can comfortably be followed with routine blood tests and repeated bone marrow examinations only on worsening of blood counts or no sooner than 6 months from initial examination [6].

CD34 quantification can aid in the diagnosis of MDS, especially in hypocellular cases. It can be performed two ways. One way is with multicolor flow cytometry of bone marrow aspirates, using a sequential gating strategy with CD45, CD34, and CD71 (to identify and include nucleated red cells) [12]. Alternatively, CD34 immunohistochemistry can be performed on bone marrow biopsy using commercially available CD34 avidin-conjugated monoclonal antibodies. Elevations in CD34 count in the marrow are consistent with MDS.

Table 1. Diagnostic approach for bone marrow failure

Diagnostic tool	Diagnostic utility
Peripheral blood	
Complete blood count with differential	Diagnosis
Reticulocyte counts	Prognostic testing
Chemistries	Prognostic testing
Transaminases and bilirubin	Prognostic testing
Hepatitis serologies	Prognostic testing
Fluorescent aerolysin assay for PNH	Prognostic testing
Chromosome breakage tests	Prognostic testing
Telomere length and mutational analysis (if DKC suspected)	Prognostic testing
SNP array	Prognostic testing
Somatic mutation test	Prognostic testing
Bone marrow	
Aspirate and biopsy	Diagnosis
Flow cytometry (including quantitative CD34)	Prognostic testing
Fluorescence in situ hybridization	Prognostic testing
Cytogenetics	Prognostic testing
SNP array	Prognostic testing
Somatic mutation test	Prognostic testing
Colony-forming assays	Prognostic testing
Patient history	
Duration of cytopenias (are old records available?)	Distinguish acquired disease
Medications (prescribed and over-the-counter supplements)	Rule out reversible causes
Exposures	Note a treatment- or exposure-related MDS
Transfusions	Suggest severity of BMF phenotype
Family history	
Constitutional abnormalities	For use in younger patients suspected of IBMFS
Physical examination	
Height (in context of mean parental height)	For use in younger patients suspected of IBMFS
Limb abnormalities	For use in younger patients suspected of IBMFS
Skin and nail abnormalities (café au lait spots, nail dystrophy, pale patches)	For use in younger patients suspected of IBMFS

Abbreviations: AML, acute myeloid leukemia; BMF, bone marrow failure; DKC, dyskeratosis congenita; IBMFS, inherited bone marrow failure syndromes; MDS, myelodysplastic syndromes; PNH, paroxysmal nocturnal hemoglobinuria; SNP, single-nucleotide polymorphism.

Finally, novel diagnostic tools may make the diagnosis of MDS more accurate. These include flow cytometry immunophenotyping [13–18] and screening for recurrent molecular defects by using genome-wide and massive parallel genotyping technology [19–21]. These techniques can provide evidence of clonal abnormalities, given prognostic information as well as a documented mutation to follow over the course of one's disease and therapy.

Aplastic Anemia

AA is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis. As with MDS, a good-quality bone marrow biopsy to assess marrow cellularity and morphology is mandatory. Skepticism about a diagnosis of AA is warranted if a marrow is noted as being aplastic but the specimen is subcortical or of suboptimal quality. It should also be noted that the cellularity in AA is inherently patchy, and some sampling distribution may be present. From the aspirate, flow cytometry and cytogenetics studies are required. The reticulocyte count will be low, particularly given the degree of anemia. The modified Camitta criteria [22] are widely used for determining severity—defined as moderate, severe, or very severe—with degree of severity based on bone marrow cellularity of less than 30% and severe pancytopenia with at least two of the following peripheral blood count criteria: absolute neutrophil count $<0.5 \times 10^9/L$, platelets $<20 \times 10^9/L$, and corrected reticulocyte count $<1\%$. PNH clones are also considered standard at the time of initial diagnosis. These tests should be performed by multicolor flow cytometry [23].

Testing for the presence of PNH clones is routine in AA, both to exclude a PNH diagnosis and to identify potential for PNH evolution, as with syndromes meeting the International PNH Interest Group criteria for PNH [24]. The definition of a “positive” clone varies in the literature. The threshold for a PNH-positive clone should be 0.01% of cells deficient in glycosylphosphatidylinositol-anchored protein (GPI-AP; a reliable surrogate for the presence of a PNH clone) [25], ideally, using multiparameter fluorescent aerolysin [23, 26]. Populations of GPI-AP-deficient cells (usually 0.1%–15%) can also be found in most patients with acquired AA [27] and in as many as 40% of MDS patients at the time of diagnosis [28]. These are not necessarily clonal and may not meet the threshold for positivity as described. Although National Comprehensive Cancer Network guidelines suggest that presence of a PNH clone should be evaluated in MDS patients [29], it is not necessary to look at all MDS patients if the diagnosis of MDS is clear. Patients with a smaller PNH clone who are neither hemolyzing nor having thromboses should not be treated with drugs such as eculizumab. PNH clones are also followed over time in AA because their outgrowth may be a manifestation of the clonal evolution in AA after immunosuppression therapy (IST).

Large Granular Leukemia

LGL is a clonal lymphoid disorder characterized by peripheral lymphocytosis with concomitant other cytopenias. LGL represents a clonal expansion of either CD3-positive cytotoxic T lymphocytes or CD3-negative natural killer cells. Although LGL can be associated with other entities such as AA or MDS, it is a distinct clinical entity with a specific diagnostic pathway [30]. It is best diagnosed by flow cytometry from the peripheral blood (not the marrow) with the phenotype CD3 positive, dim CD5 positive, CD8 positive, and CD57 positive for T-LGL. T-cell gene rearrangements must also be identified by polymerase chain reaction to demonstrate clonality. In addition, up to 40% of patients with LGL will have a somatic *STAT3* mutation [31]. The presence of the mutation suggests that aberrant *STAT3* signaling underlies the pathogenesis of the disease [31].

Paroxysmal Nocturnal Hemoglobinuria

PNH is a clonal hematopoietic stem cell disorder with features including hemolytic anemia, marrow failure, and thrombosis. These patients have an acquired mutation of the *PIGA* gene on X(p22.1). Classical PNH, as defined by the International PNH Interest Group [24], requires a peripheral blood flow cytometric diagnosis [23]. Peripheral laboratories including elevated lactate dehydrogenase and reticulocyte count support the diagnosis. Given the hemolysis, a negative Coombs test is also useful. Bone marrow evaluation is also performed and will show a cellular marrow in classical PNH.

Pure Red Cell Aplasia

Pure red cell aplasia is a syndrome characterized by severe normocytic anemia, reticulocytopenia, and absence of erythroblasts from an otherwise normal bone marrow. This diagnosis is challenging to make without a bone marrow biopsy and often is a diagnosis of exclusion.

ADDITIONAL TESTS TO DISTINGUISH MDS FROM OTHER BMF CONDITIONS

The majority of the diagnostic testing described can be performed within a reasonable time frame and is considered standard at nearly all centers. Some specialty testing, as discussed below, requires referral to a more specialized center or the use of send-out laboratories.

Single-Nucleotide Polymorphism Array Karyotyping

Single-nucleotide polymorphism arrays (SNP-As) have emerged as an important tool in the identification of chromosomal defects undetected by metaphase cytogenetics (MC) in BMF disorders such as MDS and MPNs, offering improved measurement of unbalanced chromosomal defects and acquired copy-neutral loss of heterozygosity. Identification of chromosomal abnormalities by SNP-A can enrich the diagnostic evaluation of MDS, especially in cases of a normal karyotype, in aspirable marrow without an available sample on which to run MC, or in cases of noninformative karyotypes. These can identify clonal markers with which to follow a patient's disease and might also distinguish MDS from other BMF conditions, particularly in cases for which a clonal marker is found. In one study [21], combined MC/SNP-A karyotyping led to a higher diagnostic yield of chromosomal defects (74% vs. 44%, $p < .0001$) compared with MC alone, often through detection of novel lesions in patients with normal or noninformative (54%) and abnormal (62%) MC results. These defects detected by SNP-A contributed to poorer prognosis for patients stratified by current morphologic and clinical risk schemes. The presence and number of new SNP-A-detected lesions are independent predictors of overall and event-free survival when compared with other stratification schemas [21].

Molecular Genetic Testing

Given the novelty of genomic assessment, its clinical promise as a diagnostic and predictive tool has yet to be fully realized. As with SNP-As, genomic studies may be performed on peripheral blood and may be useful in distinguishing patients with suspected MDS from other BMF disorders. More than 90% of bone marrow cells have clonal somatic mutations at

the time of MDS diagnosis, even in those patients who do not have increased blasts [32, 33]. More than 30 recurrent MDS-associated somatic mutations have been identified and involve diverse biologic pathways. Several of these mutations have prognostic implications that are independent of existing risk-stratification systems based on clinical and pathologic parameters [32, 33]. Genomic analyses are also useful in making the diagnosis of IBMFS in the pediatric population (relevant genes are shown in Table 2) [34–43].

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Somatic mutations in MDS can be categorized by subtype (Table 3) [20, 44, 45]. They include transcription factors (e.g., *TP53*, *RUNX1*, *ETV6*, *CUX1*) [20, 46–48], epigenetic regulators (e.g., *TET2*, *DNMT3A*, *ASXL1*, *IDH1/2*, *EZH2*) [49–54], signaling molecules (e.g., *NRAS*, *JAK2*, *NPM1*) [20, 55, 56], and precursor mRNA splicing factors (e.g., *SF3B1*, *U2AF1*, *SRSF2*) [5, 57–60]. More than 70% of patients with MDS harbor somatic mutations or clonal cytogenetic abnormalities, and more than 50% of patients with MDS carry at least one somatic mutation [44]. Somatic mutations in *SF3B1*, *TP53*, *TET2*, and *ASXL1* are among the most commonly identified changes, whereas those in *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1* have been found to predict clinical phenotype and survival independent of other variables such as the International Prognostic Scoring System (IPSS) score [20]. This latter panel is starting to be used in clinical settings.

APPLICATION OF THESE TECHNIQUES TO DISTINGUISH MDS FROM OTHER BMF DISORDERS

Each of the techniques described has relevance in the diagnostic approach to marrow failure. Next we will discuss the utility and applicability of this testing in the context of the patient being evaluated for cytopenias. This algorithm is summarized in Figure 2.

Distinguishing Cytopenias in Young Patients: Inherited From Acquired Disorders

Particularly in patients presenting at a young age (<30 years), the possibility of inherited cytopenias, as opposed to acquired ones, must be considered. AA and IBMFS entities can be difficult to distinguish because they both present with pancytopenia and hypocellular marrow [1, 2]. The history of a previously normal hemogram is uniquely helpful in identifying acquired disorders, whereas physical anomalies or family history may suggest that further investigation into constitutional abnormalities is required. Because there can also be phenotypic overlap, genotyping may be critical in establishing a diagnosis. IBMFS can be caused by germline mutations that result in impaired DNA repair (e.g., Fanconi anemia), abnormally short telomeres (e.g., dyskeratosis congenita [DKC]), ribosomopathies (e.g., Shwachman-Bodian-Diamond

Table 2. Mutations identified to date for inherited causes of BMF or MDS

Syndrome	Gene	Locus	Inheritance	Incidence of MDS, %
Myelodysplasia alone				
Familial platelet disorder/AML [34]	RUNX1	21q22	AD	20–60
Familial monosomy 7 [35]	Unknown	Unknown	Unknown	Unknown
Inherited BMF syndrome				
FA [36]	FANCA	FANCA/BRCA pathway	AD or X linked	50
Diamond Blackfan anemia [37]	RPS19	19q13	AD	1
	RPS24	10q22	AD	
	RPS17	15q25	AD	
	RPL5	1p22	AD	
	RPL11	1p35	AD	
	RPL35A	3q29	AD	
	RPS7	2p	AD	
Dyskeratosis congenita [38]	DKC1	Xq28	X linked	<5
	TERC	3q26	AD	
	TERT	5p15	AD	
	TINF2	14q11	AD	
	NOP10	15q14	AR	
	NPH2	5q35	AR	
	SBDS	7q11	AR	
SBDS [39]	MPL	1p34	AR	10–20
Congenital amegakaryocytic thrombocytopenia [40]				Unknown
Severe congenital neutropenia [41]	ELA2	19q13	AD	10
	GF11	1p22	AD	
	HAX-1	1q21	AR	
	GATA2	3q21	AD	
MonoMac [42, 43]				Unknown

Abbreviations: AD, autosomal dominant; AML, acute myeloid leukemia; AR, autosomal recessive; BMF, bone marrow failure; FA, Fanconi anemia; MDS, myelodysplastic syndromes; SBDS, Shwachman-Bodian-Diamond syndrome.

syndrome), abnormalities in the thrombopoietin gene (amegakaryocytic thrombocytopenia), or other rarer causes [61, 62]. (Table 2) The diagnosis of an IBMFS requires the presence of the noted gene mutations. The presence of a PNH clone is also thought to be a marker of acquired disease, not inherited, and can be used early in the diagnostic algorithm to move attention away from genotyping [63]. If inherited syndromes are considered, it may be necessary to refer patients to specialized centers for consultations on specialized genetic testing.

Telomere length is an important adjunct test used in diagnosing IBMFS, specifically DKC [64]. Short telomeres, however, can be found in other diseases [65]. The definition of “short” has not been established, and the method used to determine length has not been standardized. Below the first percentile is considered to be critical telomere shortening [65]. This is seen in inherited forms of AA such as DKC and is very useful for ruling in IBMFS [66]. Some groups have also used telomere lengths below the 25th percentile to define short telomeres in acquired AA [67]. This wide variation has

confounded efforts to use telomere length as a diagnostic tool. Methods to determine telomere length have also varied and have included polymerase chain reaction [67–69] and flow cytometry with fluorescence in situ hybridization (FISH), the only clinically validated technology [70].

Although studies have shown that a percentage of patients with acquired AA may also have shortened telomeres [65, 68, 71, 72], the role that telomeres play in this diagnosis of AA, especially acquired, is not clear. Telomere length is not a predictor of IST response [67]. Telomeres have also been investigated in MDS [73, 74]. Telomere length is shorter in MDS patients than in non-MDS patients without cytopenias ($p < .001$), and longer in lower-risk MDS patients (low, intermediate-1 IPSS, $p < .01$) than in higher-risk MDS patients (high, intermediate-2 IPSS, $p < .01$) [74]. It is a reasonable diagnostic test to send if there is a clear suspicion of DKC or a research question about the role of telomere length in other diseases such as acquired AA or MDS. DKC is the only true BMF disorder that will be fully distinguished if the telomere length is found to be below the first percentile.

Table 3. Summary of MDS somatic mutations

Gene	Cases, %	Effect on OS	Comments
Signaling molecules			
<i>BRAF</i>	<1	↓	Associates with 10%–15% ET and MF, not PV or erythrocytosis
<i>MPL</i>	<1	↔	
<i>PTEN</i>	<1	↔	
<i>CKDN2A</i>	<1	↔	
<i>GNAS</i>	<1	↔	
<i>PTPN11</i>	<1	↔	May affect use of tyrosine kinase inhibitors
<i>c-KIT</i>	1	↔	
<i>KRAS</i>	2	↔	
<i>CBL</i>	1	↓	Associated with CMML; also present in ~10% of higher grade MPNs
<i>JAK2</i>	3	↔	Often in RARS-T may affect use of ruxolitinib
<i>FLT3</i>	3	↔	Poor prognostic factor seen in 30% of AML
<i>NRAS</i>	10	↑	Independent prognostic significance for lower risk MDS
Epigenetic regulators			
<i>ATRX1</i>	<1	↔	Also seen specifically in MF Independent prognostic significance in MDS; Also associated with MF
<i>UTX</i>	1	↔	
<i>EZH2</i>	6–10	↓	
<i>DNMT3A</i>	10	↓	
<i>IDH1/2</i>	12	↓	
<i>ASXL1</i>	10–15	↓	Independent prognostic significance in MDS; Prevalent in 30% cases with MF, post et, post PV or de novo MF
<i>TET2</i>	19–24	↓	Often seen in CMML; Present in 10%–15% MPN (ET, PV or MF)
Transcription factors			
<i>WT1</i>	<1	↔	Independent prognostic significance Independent prognostic significance
<i>PHF6</i>	<1	↔	
<i>CUX1</i>	1–2	↔	
<i>BCOR</i>	1–2	↔	
<i>ETV6</i>	3	↓	
<i>RUNX1</i>	10–15	↓	
RNA splicing			
<i>SF1</i>	1	↔	Seen in 75% of patients with RARS or RCMD-RS
<i>SF3A1</i>	1	↔	
<i>ZRSR2</i>	6	↔	
<i>U2AF1</i>	8	↓	
<i>SRSF2</i>	15	↓	
<i>SF3B1</i>	15–24	↑	
Other			
<i>STAG2</i>	2–3	↔	Independent prognostic significance
<i>NPM1</i>	3	↔	
<i>TP53</i>	10–14	↓	

Formulated from mutational specific references noted in text, with emphasis on Bejar et al. [20], Bejar et al. [44], and Papaemmanuil et al. [45]. Abbreviations: ↔, unknown or no impact on overall survival; ↓, decreased overall survival; ↑, increased overall survival; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; ET, essential thrombocytosis; MF, myelofibrosis; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasm; OS, overall survival; PV, polycythemia vera; RARS, refractory anemia with ringed sideroblasts; RARS-T, refractory anemia with ringed sideroblasts and thrombocytosis; RCMD-RS, refractory cytopenias with multilineage dysplasia with ringed sideroblasts.

Distinguishing Hypocellular Disorders: AA and hMDS

Although the majority of MDS patients have normal to increased bone marrow cellularity at diagnosis, 5%–20% will present with hypocellular marrows [75, 76]. Patients with

hMDS may initially be difficult to distinguish from those with AA, given the low cellularity in the marrow. Although erythroid dysplasia is a hallmark of MDS, for example, it can also occur in AA [75]. Clonal cytogenetic abnormalities are usually

To Distinguish MDS from Other Syndromes of Marrow Failure

Figure 2. Algorithm for diagnosing bone marrow failure conditions.

Abbreviations: BFU-E, burst forming unit erythroid; flow-FISH, flow cytometry with fluorescence in situ hybridization; IBMFS, inherited bone marrow failure syndromes; LGL, large granular lymphocytosis; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasm; PNH, paroxysmal nocturnal hemoglobinuria; SNP, single-nucleotide polymorphism.

considered diagnostic for MDS, but these defects are typically found in only half of all MDS cases, and cytogenetic analyses may be less reliable when the bone marrow is hypocellular. Moreover, an accurate morphologic analysis may be difficult to perform because of inadequate diagnostic material from hypoplastic specimens. Despite the difficulty in distinguishing hMDS from AA, an accurate diagnosis has important clinical implications because patients with hMDS have greater risk of neoplastic progression [77, 78], and treatment for the two disorders can differ.

CD34⁺ hematopoietic progenitors are central to the pathogenesis of both MDS and AA and may be a good tool with which to distinguish the two disorders [75]. CD34⁺ cells normally comprise 1%–2% (range: 0.5%–5%) of the bone marrow cellularity but are significantly decreased in AA bone marrows [79] because they are the target of autoimmune destruction [80]. In contrast, CD34⁺ cells appear to be the cells from which MDS originates [81] and thus may be increased as a result of neoplastic clonal expansion. The potential quantitative difference between CD34⁺ bone marrow cells in hMDS and AA suggest that this parameter may be used to distinguish between these two disorders. A single-center study showed that patients with a normal or increased percentage of CD34⁺ cells were ultimately diagnosed with hMDS based on the detection of clonal cytogenetic abnormalities or progression to acute leukemia, whereas patients with low marrow CD34⁺ cell numbers met standard clinical criteria for AA and were responsive to IST [14].

SNP-As may also be used in distinguishing AA and hMDS. An analysis of patients with AA ($n = 93$) and hMDS ($n = 24$) from the Cleveland Clinic applied SNP-As along with MC. Combined metaphase and SNP-A karyotyping improved detection of chromosomal lesions: 19% of AA cases and 54% of hMDS cases harbored clonal abnormalities, including copy neutral loss of heterozygosity. In hMDS, additional clonal lesions were

detected in 5 of 14 patients (36%) with normal or non-informative routine cytogenetics. In a subset of AA patients studied at presentation, persistent chromosomal genomic lesions were found in 10 of 33, suggesting that the initial diagnosis may have been hMDS. Similarly, using SNP-As, earlier clonal evolution was found in 4 of 7 AA patients followed serially. SNP-As can identify cryptic clonal genomic aberrations in AA and hMDS, leading to improved distinction between these two diseases [82].

Distinguishing Cellular Disorders: MDS From LGL and PRCA and Cytopenias From Inflammation

The differential diagnosis for patients who have cytopenias and cellular (or hypercellular) marrows but without increased blasts or cytogenetic abnormalities is the broadest differential in the marrow failure world. The differential diagnosis includes PRCA, LGL, marrow suppression associated with systemic inflammation, immune-mediated cytopenias, or clonal BMF disorders such as MDS and PNH. Although classical PNH is easily ruled out with a negative PNH clone test, it remains difficult to differentiate among MDS, PRCA, LGL, and inflammatory or autoimmune diseases [83].

Although still predominantly performed in the research setting, colony-forming assays can be performed on the bone marrow aspirate or the peripheral blood [25, 84–87]. The colonies are enumerated by microscopy after 12–14 days of incubation. Based on morphologic appearance and size and according to published criteria, colonies are classified as “colony-forming unit granulocyte, erythrocyte, monocyte, megakaryocyte”; “burst forming unit erythroid” (BFU-E); and “colony-forming unit granulocyte, monocyte.” These can be used for diagnosis [87] of diseases such as PRCA as well as prediction of response to immunosuppressive therapy in this disease [85, 88, 89]. It is a useful tool for excluding the diagnosis of MDS because growth over the normal value of 30 BFU-E per

10^5 marrow mononuclear cells is very specific for ruling out MDS [87].

Although not routinely available at all centers, progenitor cell assays can also provide a sensitive screening parameter for undiagnosed cytopenias [90, 91] in the context of a cellular marrow. They may also have prognostic significance in MDS, with low growth corresponding to worse prognosis [84, 92]. Growth above the normal BFU-E value with a median growth of 40 BFU-E per 10^5 mononuclear cells excludes MDS and is associated with response to IST [87].

Dysplasia, somatic mutations, and karyotypic abnormalities by metaphase karyotype or SNP-A are all helpful if present to distinguish MDS from the other syndromes. There are limitations to this approach because there are patients with clonal hematopoiesis who do not have MDS. These patients may have genetic anomalies that are not disease related. Furthermore, the mutational testing in the context of something like an isolated ASXL1 mutation must be interpreted with caution because these tests cannot provide real diagnostic utility in isolation without appropriate context.

Distinguishing Diseases Responsive to Immunosuppressive Therapy

It is important to distinguish immune-responsive diseases from classic MDS because the former will be less responsive to the standard MDS paradigm of demethylating agents. However, hypoplastic MDS is an important exception because it is thought to possibly be more responsive to IST, and using a paradigm of upfront IST in treatment-naïve patients is reasonable [93, 94]. Some markers of immune responsiveness in MDS (as well as AA) include PNH cells, human leukocyte antigen-DR15 (DR2) positivity [95], and bone marrow stem cell expression of the tumor necrosis factor receptor [96, 97].

PNH clones are often seen in these diagnoses, usually once a diagnosis has been determined, to better predict response to therapy. They can also be helpful in guiding clinicians down a particular path of marrow failure versus another in challenging cases. Specifically, PNH cells are associated with immune pathophysiology [98–100]. Up to 70% of patients with acquired AA have a small PNH clone at diagnosis [101, 102]. The deficiency of GPI-AP-anchored proteins (PNH cells) on the surface of mutated stem cells renders them relatively resistant to immune attack; consequently, the cells do not have an inherent proliferative advantage but rather a conditional advantage because they escape immune destruction [102]. This is believed to be the basis for the high incidence of PNH clones in AA. The presence (as defined above) of PNH cells may predict therapeutic response [27, 101, 103]. BFU-E and other hematopoietic progenitor assays have also been shown to predict response to IST in PRCA, specifically [88]. Again, colony-forming assays can be used to distinguish disease with PRCA from LGL and autoimmune cytopenias growing robustly and more likely to respond to IST, whereas MDS and PRCA not caused by LGL are less likely to respond [87].

Distinguishing LGL From Its Associated Conditions

As shown in Figure 1, LGL can overlap with PRCA, MDS, and AA. The meaning of LGL cells in the setting of these other

confirmed diagnoses is unclear. It should be determined whether these cells are driving the cytopenias or are only partnered to the true disease. When LGL populations are present, looking for other markers of MDS (as described above) is important. Using *STAT3* mutations to distinguish pure LGL is not helpful because these mutations are also detected in AA and MDS when associated with LGL [104] and in classical LGL Felty's phenotype of isolated neutropenia and rheumatoid arthritis [31]. Consequently, it is also not surprising that *STAT3* mutations have been reported in PRCA (specifically, Y640F and D661Y). In patients with LGL associated with MDS (10% of cases) [105], the response to IST is lower than in pure LGL. In these cases, it is more clinically appropriate to assume the LGL is paraneoplastic and to focus on treating the underlying MDS [105]. The opposite is true in LGL-associated PRCA, which often is more immune responsive than PRCA from other causes [87, 88]. The identification of a population of LGL cells is important in all of these entities, although directed therapy may not actually be required.

Distinguishing Somatic Mutations in MDS

A number of recurrently mutated genes have been identified in MDS. The total number of mutations present in a patient with MDS has important prognostic value independent of current clinically used prognostic information [20, 45]. In particular, the most commonly mutated genes, including mutations in *ASXL1*, *SRSF2*, *RUNX1*, and *TP53*, are associated with worse outcome and decreased leukemia-free survival [44]. Certain mutational combinations are also likely critical in MDS pathogenesis. Specifically, *SF3B1* mutations have become pathognomonic of MDS subtypes with ring sideroblasts, are associated with favorable prognosis [5, 59, 60, 106], and have a unique spectrum of mutational overlap.

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Papaemmanuil et al. examined the mutational allele frequency of each of the commonly mutated genes to estimate the temporal order of mutation acquisition in MDS and to understand the impact of subclonal mutations (defined as mutations not present in the entire proportion of malignant cells) on disease course [45]. They found that the detection of these mutations had a significant impact on outcome, even when not present in the major clone. From a purely practical standpoint, this suggests that serial sequencing might simultaneously provide both mutational data and copy-number data capable of supplanting recurrent FISH and cytogenetic testing in MDS, for which balanced translocations are rare. There are always limitations and caveats to this type of testing. Mutations in isolation without appropriate clinical context must be interpreted with some caution, and their clinical utility has not been fully elucidated. It is only the patient

with MDS and a clonal abnormality and a mutation in ETV6 or TP53 to whom consideration should be given for very aggressive therapy for high-risk disease. Although SNP-As or genomic analyses are useful in detecting genetic alterations that contribute to disease phenotype, they are not disease defining in and of themselves because bone marrow failure syndromes and categories of myeloid and lymphoid neoplasms contain multiple subgroups that share genetic pathways en route to disease manifestation. Consequently, these should be used with caution in isolation in diagnosing a bone marrow failure condition.

CONCLUSION

Distinguishing MDS from other BMF disorders can be challenging, particularly in the setting of unilineage compromise or hypocellular marrow. A suggested diagnostic algorithm is presented in Figure 2. Novel bone marrow assessments and

genomic analyses can assist in making such clinical distinctions and are now used with increasing frequency. Older tests are also still quite useful to distinguish these overlapping disorders. Although some of the newer platforms (SNP and molecular gene testing) have yet to be incorporated formally into the currently accepted risk-stratification scoring systems [107–110], they likely will be in the near future.

AUTHOR CONTRIBUTIONS

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DISCLOSURES

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