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I walk the line: How to tell MDS from other bone marrow failure conditions

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Abstract

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by peripheral cytopenias and ineffective hematopoiesis. MDS is an example of an age-related malignancy and its increasing prevalence and incidence can be attributed to a greater life expectancy in developed countries. Although frequently encountered in hematology/oncology clinics, MDS may constitute a diagnostic challenge especially with equivocal bone marrow morphology. Certain syndromes of bone marrow failure (BMF) may mimic MDS and formulating a correct diagnosis is vital for adequate prognostication as well as therapeutic approaches. Metaphase karyotyping (MK) is a very important diagnostic tool and marker of prognosis and can be an indicator of response to certain therapies. Unfortunately chromosomal abnormalities may only be found in approximately 50% of patients with MDS. In this review, we discuss the diagnostic approaches to patients with pancytopenia with a particular focus on the growing number of somatic mutations through new molecular testing.

Keywords

Bone marrow failure; Myelodysplastic syndrome; Aplastic anemia; Large granular lymphocytosis; Paroxysmal nocturnal hemoglobinuria; Molecular markers; SNP-arrays; Somatic mutations

Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematopoietic stem cell disorders characterized by peripheral blood cytopenias and ineffective hematopoiesis [1]. MDS is part of a wider spectrum of bone marrow failure (BMF) conditions that clinically and pathologically may share features. Despite distinct underlying

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etiology of the specific BMF, similar phenotypic characteristics may hamper the correct diagnosis, especially at early stages. Familiarity with various MDS mimics, their etiology, natural history and prognosis is essential during diagnostic work-up, prognostication and subsequent therapeutic decision-making [1-4].

Disorders categorized as BMF include MDS (including hypocellular MDS [hMDS]), aplastic anemia (AA), inherited BMF syndromes (IBMFS), large granular lymphocytosis (LGL), pure red cell aplasia (PRCA), and paroxysmal nocturnal hemoglobinuria (PNH) (Figure 1). Some also consider myeloproliferative neoplasms (MPN) in this arena. BMF may be classified in multiple ways: by bone marrow cellularity (cellular or hypocellular), clonality (neoplastic or non-clonal), etiology (inherited or acquired) and manifestation (acute or chronic). Additionally, some degree of overlap among BMF exists, which can complicate making the diagnosis. AA frequently occurs with a detectable PNH clone, MDS may overlap with MPN or LGL and the correct diagnosis may remain unclear even after a thorough evaluation. Given that the management paradigm differs depending on the diagnosis, the evaluation should focus on distinguishing conditions such as MDS, AA or PNH, whose therapies are distinctly different. Currently, several therapeutic options are available for MDS patients including hypomethylating agents (HA) and immunomodulators (IMIDs); thus, identifying the correct diagnosis is crucial. More importantly, hematopoietic stem cell transplantation (HSCT), the only curative therapy for MDS, can be considered as a vital option for select patients. These aforementioned therapies have potential serious side effects and thus it is essential that patients without MDS avoid their toxicity, and those patients with lower-risk (and potentially self-limited conditions) are not unnecessarily directed to HSCT.

In this review we will focus on diagnostic strategies, which include standard hematopathological techniques, with a particular emphasis on the growing field of molecular diagnostics. While early diagnosis of BMF syndromes may be challenging, appropriate application of these newly available tests may facilitate timely diagnosis resulting in early introduction of therapies.

Diagnostic Approach to Marrow Failure

Virtually all patients with BMF present with peripheral cytopenias affecting at least one lineage. It can be challenging to distinguish relatively benign BMF from clonal malignant conditions[5]. After non-malignant causes of cytopenias such as nutritional deficiencies or bone marrow toxins are excluded, the diagnostic approach to the suspected BMF disorder begins with a detailed history and physical exam followed by review of peripheral blood and bone marrow mophology along with flow cytometry and MK (Table 1). More in-depth testing, including an assortment of molecular techniques, should be considered through referral to BMF centers. Repeat peripheral blood and bone marrow examinations are often helpful in making the correct diagnosis and in assessing the tempo of disease progression, and should be considered when the clinical picture changes.

Diagnostic Evaluation of MDS

MDS frequently presents as unilineage or multilinage cytopenias due to a hematopoietic stem cell (HSC) defect, leading to ineffective hematopoiesis and the propensity to malignant transformation in approximately 30% of patients. A peripheral blood morphology is often helpful in assessing dysplasia in myeloid cells. Hypogranulation, hypo- or hypersegmentation and the presence of pseudo Pelger-Huet cells are frequently observed in MDS and usually not appreciated in other disorders such as AA. A bone marrow biopsy with aspirate to assess marrow cellularity, dysplasia, number of blasts and underlying fibrosis is requisite. Additional flow cytometry and MK are also vital [6]. In approximately 90% of patients with MDS, the bone marrow examination is overtly hypercellular and dysplasia affecting erythroid or megakaryocytic lineages can be observed [1]. A finding of excess blasts (≥5%) is supportive of MDS or of an evolving acute leukemia. Bone marrow fibrosis may be an indicator of underlying MPN or of an overlap MDS/MPN. The confirmation of clonal hematopoiesis by MK is invaluable in cases with mild or very subtle dysplasia. Additionally, MK is the single most important predictor of survival. Some non-random chromosomal abnormalities are virtually diagnostic of a myeloid neoplasm [del(7/7q), del(5q), inv(3)] [7-9]. Others, like del(Y) may be seen in healthy elderly males with a significance that is not entirely clear [10, 11]. Given the fact that MDS is a very heterogeneous group of diseases, even in cases with established diagnosis, morphologic discrepancies still exist and correct classification of MDS subtypes may be problematic [12].

The diagnosis of MDS may be difficult in patients with a normal or non-informative karyotype, especially when frank dysplasia or characteristic morphologic markers like ring sideroblasts or increased blasts are not appreciated. As immediate therapy is not indicated at early stages of the disease, such patients can be closely monitored and repeat evaluation of the bone marrow can be performed at the time of worsening cytopenias, or at approximately 6 months from initial examination.[6] Increased numbers of CD34+ myeloid blasts may aid the diagnosis of MDS, especially in cases with a hypocellular marrow [13]. Blasts can be assessed either by multicolor flow cytometry or immunohistochemistry [14]. The latter test would allow for more precise quantification of CD34+ cells as hemodilution artifact is not a major problem in core biopsy evaluation, but may result in spuriously lower CD34+ cells during aspirate analysis.

Finally, novel diagnostic tools including array-based karyotyping and massive parallel sequencing may prove useful in equivocal cases and are discussed in detail below [15-17]. In most cases, these techniques can provide objective evidence of clonal hematopoiesis, prognostic stratification and serve as markers of disease monitoring.

Distinguishing MDS from other BMF syndromes (MDS mimics)

Evaluation of cytopenias in younger patients (acquired vs. inherited BMFs)

When evaluating the etiology of cytopenias in younger patients (usually <30 years old), one should strongly consider the possibility of an inherited condition. Often, acquired hypocellular conditions like AA may be challenging to distinguish from IBMFS [2, 3]. A detailed medical history, including prior complete blood counts and family history, can be

particularly illustrative. Thorough physical examination may identify skeletal, skin or nail anomalies indicating the presence of an inherited syndrome. In some instances however, when phenotypic distinction is problematic, genotyping may be the only available alternative. Most IBMFS are caused by well-defined germline mutations: DNA repair (Fanconi anemia [FA]), mutations involving telomeres maintenance (dyskeratosis congenita [DKC]), ribosomopathies (Schwachman-Bodian-Diamond syndrome [SDBS], Diamond-Blackfan anemia [DBA]), mutations in thrombopoietin receptor (congenita amegakaryocytic thrombocytopenia), or other rare causes [18, 19]. The confirmation of underlying mutations is required to make a diagnosis of an IBMFS and referral to specialized center is usually recommended.

DKC is another inherited BMF characterized by germline defect in telomere maintenance leading to premature telomere shortening [20]. DKC, however is not the only condition associated with short telomeres [21]. The definition of clinically significant short telomere length varies in the literature due to methodology, but it is accepted that length below the first percentile is significant and can confirm the inherited form of marrow aplasia like DKC [22]. Given the significant variation of critical telomere length in BMF literature, its use as a precise diagnostic test remains a challenge. Widely accepted methods to determine telomere length (TL) in peripheral blood leukocytes include flow cytometry based fluorescence in situ hybridization (Flow-FISH) and polymerase chain reaction assay (PCR), with the former being the only test certified for clinical use [23-26]. Confirmation of short telomeres, although helpful, cannot definitively distinguish inherited from acquired forms of BMF [21]. Decreased TL has been described in a subset of patients with acquired AA [27, 21, 24, 28]. The exact role and consequences of short telomeres in acquired AA, though, are not entirely clear. The prognostic value of TL and its use to guide therapeutic options is limited at present though active investigations are ongoing [23]. Telomeres have also been studied in MDS, with TL found to be only mildly reduced as compared to age-matched controls [29, 30]. In summary, TL is a useful test in patients in whom the diagnosis of DKC is suspected. In such cases the TL is dramatically reduced reaching the level below 1st percentile and confirmation of presence of telomerase mutations is imperative.

Evaluation of cytopenias in in the context of hypocellular marrow (AA and hMDS)

Most patients with MDS have normal or hypercellular marrow at the time of diagnosis. However, 5-20% of patients may initially present with decreased marrow cellularity [31, 32]. The diagnosis in such patients may be challenging, as the presentation closely resembles AA. The diagnosis is even more difficult in cases without obvious bone marrow dysplasia, especially since mild erythroid dysplasia can be also seen in some cases of AA [31]. The evidence of clonal hematopoiesis as determined by cytogenetic studies is considered diagnostic of MDS. Unfortunately, 40-60% of MDS patients have normal or non-informative MK. Non-informative results may be particularly problematic in this group of patients given their deeply aplastic marrow and frequently limited amount of cells available for analysis. The limited amount of diagnostic material may also hamper the morphological evaluation of the bone marrow. Appropriate therapy is guided by morphological and cytogenetic evidence of clonal hematopoiesis and differs between AA

and MDS patients. Although immunosuppressive therapy is effective in some patients with hMDS, an accurate diagnosis has major prognostic implications [33, 34].

Newer molecular techniques, discussed later in this review, may be an important adjunct in diagnostically challenging cases. In one study, single nucleotide polymorphism array (SNP-A) karyotyping applied along with MK in patient with AA and hMDS resulted in improved detection of chromosomal lesions allowing for better distinction between AA and hMDS. The fact that some clonal abnormalities including copy-neutral loss of heterozygosity (CN-LOH) were found in AA patients may raise the question whether the correct diagnosis was made. It is however possible that some degree of clonality exists in AA due to heavily depleted HSC pool, especially that some of the lesions may subside after immunosuppressive therapy [35]. Whether the detected lesions are associated with an increased risk of malignant transformation is yet to be determined.

It is well accepted that an MDS clone originates from an immature HSC and the level of $CD34^+$ progenitors correlates with clonal expansion and disease progression [36, 37]. Conversely, in AA hematopoietic stem and progenitor cells are profoundly depleted due to immune attack [38, 39]. Thus, quantitative analyses of $CD34^+$ hematopoietic progenitors may be helpful in distinguishing AA from hMDS. In one single center study, patients with a normal or increased $CD34^+$ cells were found to have clonal cytogenetic abnormalities, suggesting the diagnosis of hMDS rather than AA. The opposite was true for patients with a low percentage of $CD34^+$ cells who were ultimately diagnosed with AA and responded to immunosuppression [13].

Evaluation of cytopenias in the context of normo- and hypercellular marrow (LGL, PRCA and inflammatory diseases)

The majority of MDS cases are characterized by normo- or hypercellular bone marrow. Unlike hMDS, the differential diagnosis of a hypercellular marrow with no clonal abnormalities is relatively broad and includes PRCA, LGL, marrow suppression associated with systemic inflammation, and other immune-mediated cytopenias. Patients with clonal hematopoiesis due to, for instance, PNH may also present with cytopenias and a hypercellular marrow, but PNH is relative easy to rule-out using flow cytometric analysis (absence of membrane CD55 and CD59 in PNH cells). Distinguishing MDS from PRCA, LGL and inflammatory diseases is more challenging [40].

Unlike MDS, in which a block in differentiation and ineffective hematopoiesis is largely attributed to cell-intrinsic defects, cytopenias associated with PRCA, LGL and inflammatory diseases are usually driven by cell extrinsic immune attack. Functional *in vitro* colony forming assays are helpful in establishing the correct diagnosis. As expected, colony growth *in vitro* is preserved in cases of immune mediated attack (PRCA, inflammatory diseases). Additionally, robust colony formation is a predictor of response to immunosuppressive therapy [41-44]. Burst forming units-erythroid (BFU-E) assays can also be used to exclude MDS in cases where dysplasia or markers of clonal hematopoiesis are equivocal. BFU-E growth above 40 colonies per 10^5 marrow mononuclear cells virtually eliminates MDS as an underlying etiology [42].

LGL associated with other BMFs

LGL can overlap with other acquired BMF such as PRCA, MDS and AA. The pathophysiology of LGL cells in this setting is not entirely clear, but may signal an immune system response to clonal cells. It is important to determine whether the LGL clone is an underlying cause of pancytopenia or rather a co-existing condition. In some instances, even though LGLs are seen on peripheral blood smear or flow cytometric analysis they remain polyclonal and are likely reactive. Given its overlap with other BMF disorders, when LGLs are present, looking for other underlying bone marrow disorder like MDS is important. Using *STAT3* mutational status to distinguish LGL is not helpful because these mutations are also detected in AA and MDS when associated with LGL, as well as in classical LGL phenotype (Felty's syndrome) of isolated neutropenia and rheumatoid arthritis.[45, 46] *STAT3* mutations have also been reported in PRCA [47]. The presence of LGL cells concurrently with MDS correlated with poor response to IST as compared to “pure” LGL [48]. Better response to IST was, however, observed in PRCA associated with an LGL clone [41, 42]. The identification of concurrent LGL cells, although important, may not warrant LGL-directed therapies.

Markers of clonal hematopoiesis in the diagnosis and prognosis of MDS

Chromosomal abnormalities

MK remains the diagnostic gold standard and is invaluable in cases with peripheral cytopenias, absence of increased bone marrow blasts and discrete or no dysplasia. Unfortunately, the test is informative in only 40-60% MDS patients. In addition to its diagnostic value, MK has been the single most important predictor of survival and, in some cases, response to targeted therapies [49]. For example, lenalidomide has been shown to be effective in patients with 5q deletion [50, 51]. Methodology of MK requires bone marrow aspirates in order to obtain adequate bone marrow material containing viable cells. Additionally, the test is non-informative in approximately 10% of patients due to difficulties in cell culture. The detection rate of relatively small copy number changes is limited by the resolution of the microscopy and the lesions <1 million base pairs are frequently missed. Finally CN-LOH which is frequently seen in MDS, cannot be detected by MK.

Given these limitations, there has been a search for more sensitive and precise cytogenetic methods. Microarray-based assays have proved to be a useful diagnostic and prognostic tool. SNP-A are used concurrently with MK in a variety of hematological malignancies. The high resolution allows for detection of small, submicroscopic copy number changes in patients with unremarkable MK. Moreover, SNP-A allow for detection of CN-LOH, which is a frequent genetic aberration in cancer and remains undetectable by traditional MK. SNP-A is particularly useful in instances of inaspirable bone marrow. In such patients, diagnosis can be facilitated using SNP-A performed on DNA isolated from peripheral blood. Analogically, useful results can be obtained in patients with non-informative MK due to poor cell growth. In a study of 174 patients with MDS, MDS/MPN and secondary leukemias, using SNP-A, clonal chromosomal aberrations were detected in 75% cases and CN-LOH was seen in 20-30% of patients [52]. SNP-A also allowed for detection of additional copy number changed and CN-LOH in 119 patients with low-risk MDS [53]. In addition to being a

complementary diagnostic test, chromosomal abnormalities detected by SNP-A are an important prognostic marker [52, 17, 54].

Somatic mutations

Since the introduction of powerful genetic tools like next-generation sequencing, many new somatic mutations have been identified in MDS. Genetic defects tend to affect genes responsible for epigenetic modifications, RNA splicing machinery and tyrosine kinase signaling. These are helpful molecular markers of clonal hematopoiesis that are also important prognostically (Table 2).

Epigenetic modifiers

TET2: *TET2* (Ten-Eleven translocation 2) gene is a putative tumor suppressor gene localized on chromosome 4q24. Loss of function mutations have been described in both myeloid and lymphoid malignancies as well as in healthy elderly individuals with clonal hematopoiesis. *TET2* mutations are present in approximately 20-30% of patients with MDS, 20-43% MDS/MPN (including CMML) and 25%-40% of patients with secondary leukemia. Moreover, *TET2* mutations are frequent in patients with normal MK, making them convenient indicators of clonal hematopoiesis in equivocal cases [16]. Data regarding the prognostic significance of *TET2* mutations remain divisive. In one study by Kosmider et al. mutated *TET2* was a favorable prognostic marker with 5-year survival of 76.9% in *TET2* mutated group vs. 18.3% in the *TET2* wild-type group [55]. Several studies however, showed no difference in survival based on *TET2* mutation status [56, 57]. Although *TET2* may seem to be a promising molecular marker facilitating diagnosis, mutations in this gene can be observed in up to 5% of elderly individuals with clonal hematopoiesis as determined by X chromosome inactivation studies and no apparent hematological abnormalities [58]. The exact risk of transformation in such patients is unknown and it is unclear whether *TET2* alone or additional genetic events are necessary for development of overt hematologic malignancy. Consequently, mutations in *TET2* appear to be an early genetic event likely predisposing to clonal evolution and eventually malignant transformation. As such, it may persist in antecedent clone even after successful therapy, which precludes its use as a marker of response or residual disease.

DNMT3A: Another example of a gene frequently mutated in hematologic malignancies and involved in epigenetic regulation via DNA methylation is DNA methyltransferase 3A (*DNMT3A*). Although more frequent in *de novo* AML, mutations in this gene have been identified in about 3-8% of MDS patients [59, 60]. Single institution experience suggests that mutated *DNMT3A* is a prognostic factor for both worse overall survival and higher rates of progression to leukemia [60]. Recent data suggest the *DNMT3A* is an initial genetic event occurring in pre-leukemic stem cells and may be present in <0.1% of otherwise healthy population. HSC clones harboring *DNMT3A* mutations can be also detected in post-remission samples [61]. Whether the presence of *DNMT3A* mutations in complete remission samples is a predictor of disease relapse remains to be seen.

IDH1 and IDH2: Two genes encoding isocitrate dehydrogenase (*IDH1* and *IDH2*) were found to be frequently mutated in AML and to a lesser degree in MDS and MDS/MPN with

a frequency of <5% [62-64]. *IDH1/2* mutations are mutually exclusive with *TET2* mutations indicating their functional redundancy and involvement in the same pathologic pathway. Also, similarl to *TET2*, *IDH1* mutations were more frequently encountered in patients with normal karyotype. Unlike in AML, where IDH mutations are favorable prognostic markers, *IDH1* but not *IDH2* mutations seem to be an indicator of inferior outcome in MDS [65, 66].

Genes involved in chromatin modification

ASXL1: Loss of function mutations in additional sex comb-like 1 (*ASXL1*) gene involved in polycomb-group repressive complex 1 (PRC1) have been described in a variety of human malignancies including MDS. Approximately 17% of MDS and up to 49% of MDS/MPN patients harbor *ASXL1* mutations [16, 67-69]. Frameshift and nonsense mutations are also an independent marker of adverse overall survival and shorter time to AML progression both in MDS and MDS/MPN [16, 70, 68]. Although *ASXL1* seems to be a marker or inferior outcome, it does not contribute to leukemic transformation and progression [70]. Also, no association has been found between *ASXL1* mutational status and response to lenalidomide or hypomethylating agents [71, 72]. Mutations in *ASXL1* may also serve as an individualized marker of minimal residual disease (MRD) in patient undergoing allogeneic HSCT, although the presence of mutation did not predict survival in the setting of BMT [73].

EZH2: Enhancer of Zest homolog 2 (*EZH2*) located on the long arm of chromosome 7 encodes a histone methyltransferase subunit of PRC2 complex. Loss-of-function mutations have been observed in approximately 6% or MDS patients [74, 75]. The presence of *EZH2* mutations was mostly seen in lower-risk patients and was strongly associated with decreased overall survival in multivariate model [16, 76].

Spliceosome machinery—Genes encoding proteins involved in mRNA splicing (*SF3B1*, *ZRSR2*, *SRSF2* and *U2AF1*) were found to be the most frequently mutated group of genes in MDS [77-80]. Mutations in *SF3B1* affected approximately 30% of patients and were nearly pathognomonic for dysplasia associated with the presence of ringed sideroblasts [77, 78, 81]. Interestingly, mutations in *U2AF1*, *ZRSR2* and *SRSF2* were seen in MDS cases without ring sideroblasts. *SF3B1* mutations were found to be an independent predictor of better overall survival and lower risk of transformation to AML [82]. Although affecting the same pathway, mutations in *U2AF1* and *SRSF2* were associated with worse overall survival and shorter progression to AML [79, 80]. It is now well accepted that mutations affecting mRNA spicing machinery are likely to be early/initiating events in disease evolution and are present in virtually all malignant cells. The presence of point mutations as detected by PCR may also serve as a molecular marker of MRD after chemotherapy or hematopoietic stem cell transplant [79, 80, 83].

Other somatic mutations

RUNX1: Mutations affecting transcription factors have been reported in both *de novo* AML and in nearly 10% of MDS cases.[84] *RUNX1* mutations were also described as a germline variant in familial platelets disorder and myelodysplasia with a propensity to leukemic transformation [85].

c-CBL: Mutations in Casitas b-cell lymphoma (*c-CBL*) were found by candidate screening in area of CN-LOH spanning long arm or chromosome 7 [86]. *CBL* mutations were most prevalent in CMML (5% of cases), and sAML (nearly 10% of cases) but were relatively infrequent in MDS [87]. Mutations in *c-CBL* tend to arise in a subclone of malignant cells during disease progression. As such, they are an indicator of poor outcome.

Mutations in Ras superfamily (*NRAS*, *KRAS*, *NF1*, *PTPN11*), DNA repair (*TP53*), cohesion complex (*STAG2*, *SMC3*) and *SETBP1* have been observed in subsets of MDS patients and their frequencies and prognostic impact have been mentioned in Table 2 [88-95].

As above, 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, even independent of currently used clinical prognostic information [96, 16]. We have reviewed the most commonly mutated genes, some associated with worse outcome and decreased leukemia-free survival [76]. Certain mutational combinations are also likely critical in MDS pathogenesis. Specifically, several groups have now 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 [96]. Studies have found that the detection of these mutations had a significant impact on outcome even when not present in the dominant clone. From a diagnostic standpoint, this suggests that molecular tests might simultaneously provide both mutational data and copy number changes capable of supplanting recurrent cytogenetic testing in MDS. However, there are always limitations and caveats to this type of testing. Mutations in isolation without appropriate clinical context must be interpreted with some caution. As an MDS community, we have yet to realize the full clinical utility of these innovative markers. 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 their high risk disease. SNP-A or genomic analyses are also useful in detecting genetic alterations that contribute to disease phenotype, but they are not in and of themselves disease-defining. Therefore, these should again be used with caution in isolation in diagnosing a bone marrow failure condition as we gain additional knowledge and experience in the field.

Conclusions

Establishing the correct diagnosis in patients with cytopenias can be challenging. Clinicians may encounter a diagnostic dilemma, particularly in patients with only mild cytopenias, no obvious dysplasia and lack of cytogenetic abnormalities as determined by MK. A detailed work-up (Table 1) can direct the clinician towards the best diagnostic path and help distinguish acquired from inherited bone marrow failure. New molecular tools like SNP-A and somatic mutation analysis prove to be a helpful adjunct tool especially in equivocal cases. Early studies also suggest that certain somatic mutations and chromosomal changes detected by SNP-A may have an important prognostic value but prospective clinical studies will be required to prove their role as a risk stratification marker.

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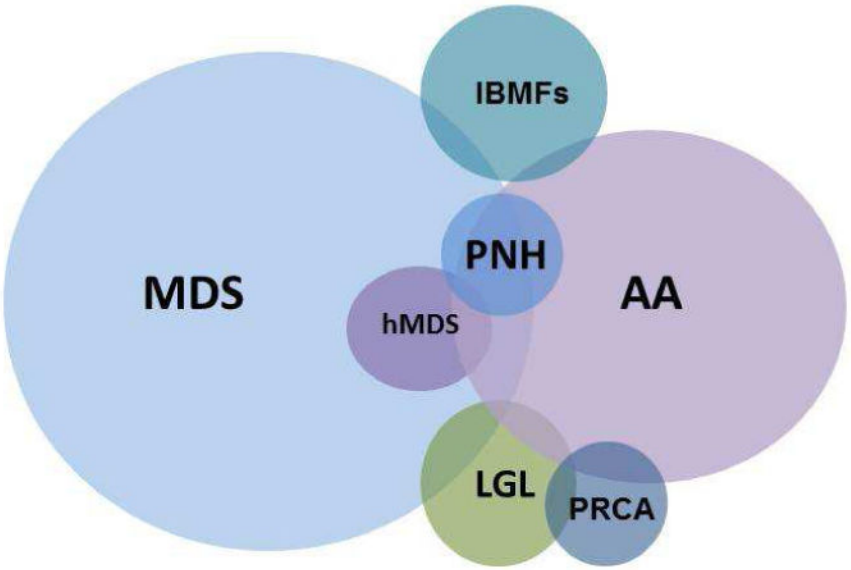

Figure 1. Bone Marrow Failure Syndromes

Table 1
Diagnostic Workup for Bone Marrow Failure

	Diagnostic test/tool	Application
History	Onset of cytopenias	Inherited vs. acquired BMF
	Medications/supplements Exposure	Reversible etiology
	Transfusion requirements	Disease severity
Family history	Cytopenias	IBMFS
	Hematologic malignancies	Inherited predisposition (e.g. *GATA2* mutations)
	Interstitial lung disease, idiopathic liver failure	Telomeropathies
Physical exam	Height	IBMFS
	Skeletal abnormalities (limbs)	FA
	Skin and nails abnormalities Premature greying	DKC
Peripheral blood	Complete blood count with differential	Diagnosis, severity
	Reticulocytes count	BMF vs. RBC destruction
	Metabolic panel Vitamin levels, copper	Metabolic derangement Nutritional deficiencies
	Hepatitis panel	Acquired AA
	Flow cytometry	FLAER assay for PNH LGL clone
	Telomere lengths	DKC
	Chromosomal breakage test	FA
	FISH SNP array Somatic mutations	Clonal abnormalities/prognosis
Bone marrow	Aspirate and biopsy	Diagnostic
	Iron stain/ringed sideroblasts	Sideroblastic anemia, nutritional deficiencies, RARS
	Flow cytometry	Phenotypic abnormalities, CD34 count
	Metaphase karyotyping FISH SNP-array Somatic mutations	Clonal abnormalities, prognosis
	Colony forming assay	Hematopoietic cells defect vs.extrinsic etiology

BMF Bone marrow failure

AA Aplastic anemia

RARS Refractory anemia with ringed sideroblasts

IBMFS Inherited bone marrow failure syndromes

RBC Red blood cells

FISH Fluorescence *in situ* hybridization

SNP Single nucleotide polymorphism

FA Fanconi anemia

DKC Dyskeratosis congenita

PNH Paroxysmal nocturnal hemoglobinuria

FLAER Fluorescent aerolysin

Table 2

Somatic Mutations in MDS

Mutation	Frequency	Prognostic impact	Comments
DNA methylation			
TET2	20-30% MDS 20-43% MDS/MPN	Unfavorable in CMML	More favorable in AML
DNMT3A	3-8%	Unfavorable	
IDH1/2	<5%	Unfavorable	*IDH1* associated with worse prognosis
Chromatin modification			
ASXL1	17% MDS 50% MDS/MPN	Unfavorable	
EZH2	6%	Unfavorable	
Spliceosome machinery			
SF3B1	30%	Favorable	Frequent in RARS and RCMD-RS
U2AF1	10%	Unfavorable	
SRSF2	15%	Unfavorable	
ZRSR2	10%	Unknown	
RAS signaling			
KRAS	<2%	Unknown	
PTPN11	<1%	Unknown	
NF1	<1%	Unknown	
Cohesin			
STAG2	5%	Unknown	
RAD21	<1%	Unknown	
SMC3	<1%	Unknown	
Others			
BCOR/BCROL1	4%	Unfavorable	
ETV6	3%	Unfavorable	
RUNX1	10-15%	Unfavorable	
p53	10-15%	Unfavorable	
JAK2	3%	Unknown	Frequent in RARS-t
c-CBL	1%	Unfavorable	Frequent in CMML
MPL	1%	Unknown	Associated with ET and MF
SETBP1	4-25%	Unfavorable	Frequent in aCML and CMML

RARS Refractory anemia with ringed sideroblasts

RCMD-RS Refractory cytopenias with multilineage dysplasia with ringed sideroblasts

RARS-T Refractory anemia with ringed sideroblasts associated with thrombocytosis

CMML Chronic myelomonocytic leukemia

aCML Atypical chronic myeloid leukemia

ET Essential thrombocythemia

PV Polycythemia vera