Pediatric myelodysplastic syndromes

Inga Hofmann^{1,2}

Received: 22 June 2015 / Accepted: 7 July 2015 / Published online: 26 July 2015
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Abstract Pediatric myelodysplastic syndromes (MDS) are a group of rare clonal hematopoietic stem cell disorders characterized by varying degree of cytopenias, ineffective and dysplastic hematopoiesis, and the risk of leukemic transformation. The clinical, laboratory, and histologic presentation of pediatric MDS shares significant overlap with other inherited and acquired bone marrow failure (BMF) disorders. Given that the majority of pediatric patients with MDS present with a hypocellular bone marrow, the histopathologic diagnosis is challenging and usually requires ancillary molecular studies. While rapid advancements have been made in the field of adult MDS, the underlying genetics and pathophysiology of pediatric MDS are still poorly understood. The recent discovery of germline mutations in *GATA2* leading to MDS suggests that some pediatric and young adult patients with MDS have an underlying genetic predisposition. Nevertheless, the molecular underpinnings remain poorly understood in most cases. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment option. Optimal timing of HSCT is not often straightforward, as some pediatric patients with low-grade MDS have an indolent disease course while others show rapid progression to advanced MDS and leukemia. Lastly, the classification of pediatric MDS has evolved over the years and is different from the terminology currently used in adult MDS. This review will focus on the current classification schemes of

pediatric MDS and address clinical, laboratory, and pathologic features, as well as diagnostic criteria, genomic advances, and therapeutic options and prognosis.

Keywords Myelodysplastic syndromes · Refractory cytopenia of childhood (RCC) · Refractory anemia with excess blasts (RAEB) · Bone marrow failure · GATA2 haploinsufficiency · Histopathology

Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by varying degree of cytopenias, ineffective and dysplastic hematopoiesis, and the risk of leukemic transformation. Pediatric MDS accounts for ~4 % of all pediatric hematologic malignancies [32, 34] and has an estimated annual incidence of approximately 1.8–4 cases per million children. While the median age of diagnosis is 6.8 years, the disease can occur in any age group. The male to female distribution of these disorders appears to be equal [34, 50, 63, 67].

Pediatric MDS can occur de novo without an apparent underlying cause (primary MDS). Secondary MDS can develop after chemo or radiation therapy, in association with an inherited bone marrow failure syndrome (IBMFS) or following an acquired bone marrow failure disorder (BMF), such as idiopathic acquired aplastic anemia (AA) or as a result of a genetic predisposition. Pediatric MDS, IBMFS, and AA represent a spectrum of conditions that share significant overlap. The majority of cases present with hypocellular bone marrow (BM), making them difficult to distinguish from acquired and inherited BMF disorders [54]. Additionally, BMF disorders can progress to MDS, further complicating diagnosis. Recent advancements in genomics have improved our understanding

✉ Inga Hofmann
Inga.Hofmann@childrens.harvard.edu

¹ Pediatric Hematology/Oncology, Dana-Farber/Boston Children's Cancer and Blood Disorders Center Boston, Boston, MA, USA

² Department of Pathology, Boston Children's Hospital, Harvard Medical School, 300 Longwood Ave, Bader 124.1, Boston, MA 02115, USA

of adult MDS, but little is known about the genetic events that lead to pediatric MDS. As a result, no targeted therapies are available and hematopoietic stem cell transplantation (HSCT) remains the only curative therapy.

In this article, we review and critique the current classification system for pediatric MDS outlined in the 2008 World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues. In addition, we review the clinical presentation, diagnostic work-up, histopathologic features, and classification of pediatric MDS and highlight important differences between pediatric and adult MDS. We review recent advancements in the genetics and genomics of adult MDS and their applicability to pediatric cases. Finally, we review the current available therapies and prognosis of pediatric MDS.

A historical perspective

MDS was first described by Giovanni Antonio Di Guglielmo in the 1920s as “*il morbo Di Guglielmo*” or chronic erythremic myelosis [6]. In the 1960s and 1970s, the prevailing terms used were “preleukemia” or “smoldering leukemia.” In an attempt to better classify these disorders, the first formal classification scheme was introduced in 1976 by the French-American-British Cooperative Group (FAB) as “dysmyelopoietic syndromes” and later, in 1982, using the modern term “myelodysplastic syndromes” (MDS). The FAB classification included 5 categories of MDS: (1) refractory anemia (RA), (2) refractory anemia with ringed sideroblasts (RARS), (3) refractory anemia with excess blasts (RAEB), (4) RAEB in transformation (RAEB-T), and (5) chronic myelomonocytic leukemia (CMML). This classification system established the basis for the WHO classification introduced in 2000, which was further updated in 2008 to accommodate newer diagnostic findings (blast count, degree of dysplasia, and cytogenetic changes).

Development of a pediatric classification

In response to the limited literature on pediatric MDS, Hasle and colleagues advanced a pediatric classification in 2003 that built on the 2000 WHO classification [33]. These guidelines established the first diagnostic criteria for pediatric MDS, requiring the presence of at least two of the following: (1) sustained unexplained cytopenia, (2) at least bilineage morphologic myelodysplasia, (3) acquired clonal cytogenetic abnormality in hematopoietic cells, or (4) increased BM blasts (>5 %) [33]. In addition, juvenile myelomonocytic leukemia (JMML) and MDS associated with Down syndrome were recognized as distinct entities and are therefore discussed separately in this special issue (please refer to Cantor and Calvo et al. in this issue).

This first pediatric MDS classification was crafted to address two challenges: (1) to distinguish MDS with a low blast count from acquired AA and other BMF conditions and (2) to differentiate MDS with excess blasts (RAEB) from de novo acute myeloid leukemia (AML). Based on this classification scheme, pediatric MDS was divided into three main categories: refractory cytopenia (RC), RAEB, and RAEB-T (Table 1).

In recent years, the community has come to recognize that there are major differences between pediatric and adult MDS. Pediatric patients commonly present with thrombocytopenia and neutropenia, as opposed to isolated anemia [45]. Therefore, the term refractory cytopenia (RC) was used in children rather than refractory anemia (RA), which is used in adults. In addition, RARS and deletions in 5q- are rare in children [5, 31–33, 50, 64, 67, 69, 79]. If ringed sideroblasts are observed in a pediatric BM, other etiologies particularly nutritional deficiencies, drug toxicity, and congenital sideroblastic anemias, including Pearson marrow-pancreas syndrome, should be considered [19, 48]. Building on the pediatric proposal by Hasle in 2003 [33], the 2008 WHO classification introduced a pediatric MDS classification for the first time [45].

Current classification of pediatric MDS

The current WHO classification updated the term RC to refractory cytopenia of childhood (RCC). The diagnostic categories for pediatric MDS are largely distinguished by the peripheral blood (PB) and bone marrow (BM) blast percentage (see Table 1) [13].

Refractory cytopenia of childhood

RCC is the most common subtype of pediatric MDS accounting for about 50 % of cases [54, 63]. The majority of patients present with hypocellular BM resembling acquired or inherited BMF conditions [53]. The presence of a clonal cytogenetic marker can confirm the diagnosis; however, about 61–67 % of patients with RCC have normal cytogenetics [53].

One of the drawbacks of the WHO classification is that the provisional entity of RCC includes a wide range of phenotypes, ranging from hypo- to hyper-cellular RCC with limited unilineage dysplasia to overt multilineage dysplasia. RCC with multilineage dysplasia (RCMD) is easily appreciated as unequivocal MDS. In contrast, hypocellular RCC with minimal or unilineage dysplasia is difficult to distinguish from acquired AA and IBMFS. As opposed to adults where it is prognostic [25, 52, 83], the implications of multilineage (compared to unilineage) dysplasia is uncertain in childhood [14, 33]. Therefore, the recommendation was made to group all pediatric MDS without excess blasts into the RCC category, regardless of the degree of dysplasia and cellularity.

Table 1 Classification of childhood MDS

Diagnostic category	PB blasts %	BM blasts %
Refractory cytopenia of childhood (RCC)	<2	<5
Refractory anemia with excess blasts (RAEB)	2–19	5–19
RAEB in transformation (RAEB-T)	20–29	20–29

Adapted from WHO classification of neoplastic diseases of the hematopoietic and lymphoid tissue (2008) [15]

Recent studies suggest that the category of RCMD in pediatric MDS patients might be of prognostic value since pediatric RCC patients with multilineage dysplasia have a significantly higher incidence of disease progression [29].

Differentiating severe aplastic anemia from hypocellular refractory cytopenia of childhood

Due to limitations in the reproducibility of the diagnostic criteria and limited data on the clinical implications [29, 90], the provisional diagnosis of RCC remains controversial. The WHO defines RCC as (1) dysplasia in one or more hematopoietic lineages or in at least 10 % of cells in one cell line and (2) less than 5 % blasts in the marrow [13]. Given the challenges in differentiating hypocellular RCC from acquired AA, the WHO outlined key histological differences present in the BM between the two disorders (Table 2; Fig. 1), which heavily relies on a topographic diagnosis with specific histologic patterns. It is important to note that patients with IBMFS such as Fanconi anemia (FA), dyskeratosis congenital (DC), or Shwachman-Diamond syndrome (SDS) resemble the histologic features of RCC [17, 33, 92]. Therefore, these conditions must be excluded by molecular testing.

Few studies have assessed the validity of the current classification and the provisional entity of RCC [7, 29, 90]. Forester et al. assessed the reproducibility and clinical implications of the current criteria for RCC and AA outlined in a retrospective single-center study of pediatric patients diagnosed with AA [22]. They found that concordance between pathologists in the diagnosis of AA or RCC was modest and the diagnosis of RCC was not prognostic of immunosuppressive therapy (IST) treatment failure. Interestingly, studies by Forester²³ and Yang²⁷ both suggest that there is a correlation between moderately severe AA (MAA) and RCC [22, 90]. In addition, Forester et al. found a low rate (<5 %) of clonal evolution, which was observed exclusively in patients classified as MAA. This evidence suggests that, if it were possible, precise separation of severe AA from hypocellular RCC might help predict development of clonal disease [7, 22].

While some studies suggest that the classification of RCC is valuable and reproducible [7], others suggest that the diagnosis is difficult to establish with certainty [22] and does not accurately predict response and outcomes after IST [22, 29]. Although the development of clonal hematopoiesis

exclusively observed in patients classified as MAA [22] and RCC [7] is intriguing, this needs to be confirmed in larger prospective studies.

Advanced MDS

The classification of advanced pediatric MDS is based on the morphology and blast count in the BM. The WHO classification divides advanced pediatric MDS into two categories: (1) RAEB-1 (5–10 % blasts) or RAEB-2 (11–19 % blasts) and (2) RAEB-T (20–29 % blasts).

The blast cutoff for the diagnosis of AML was historically set at 30 % and had been lowered to 20 % for pediatric and adult de novo AML. Therefore, the category of RAEB-T,

Table 2 Histopathologic criteria of hypocellular RCC and SAA as outlined in the WHO classification

Lineage characteristics	RCC	SAA
Erythroid	Patchy, left-shifted erythropoiesis with increased mitoses	Lacking foci or left-shifted erythroid cells or only showing single small focus of <10 cells of erythroid cells with maturation
Myeloid	Markedly decreased, left-shifted myelopoiesis	Lacking or markedly decreased myelopoiesis with very few small foci of granulopoiesis with maturation
Megakaryocytes	Markedly decreased megakaryopoiesis	Lacking or only very few megakaryocytes present
	Dysplastic changes (micromegakaryocytes) ^a	No dysplastic changes or micromegakaryocytes*
Lymphoid	Lymphocytes, PC, MC may be focally increased or dispersed	Lymphocytes, PC, MC may be focally increased or dispersed
CD34+ cells	Not increased	Not increased

Adapted from the 2008 WHO classification [15]

PC plasma cells, MC mast cells

^a Immunohistochemistry with CD61 staining is required for the detection of micromegakaryocytes

Fig. 1 Histology of hypocellular refractory anemia of childhood (RCC) compared to severe aplastic anemia (SAA). **a** The image shows an example of a child's marrow with hypocellular RCC (*left*) and illustrates a hypocellular marrow with patches of erythroid predominant hematopoiesis (BM). Erythroid islands show increased early forms, mitosis, and occasional nuclear contour abnormalities. Scattered mildly left-shifted myeloid elements are noted in the background.

Megakaryocytes are not present. **b** In contrast, an image of a marrow from a child with severe aplastic anemia (SAA) (*right*) shows a severely hypocellular, adipose-rich marrow with scattered lymphocytes and fully maturing myeloid cells including segmented neutrophils. Clusters of immature erythroid cells are not seen. Megakaryocytes are absent. Magnification $\times 400$

which is based on an old FAB category, was omitted in the recent WHO classification of adult MDS and these cases are now regarded as AML with myelodysplastic-related changes (AML-MRC). In contrast, RAEB-T continues to be recommended in the current pediatric classification [30, 33, 55] given that there is little evidence that a blast cutoff of 20 % is of clinical significance.

In the absence of correlative data, the blast cutoff for the definition of AML is arbitrary and, in reality, the transition of MDS to AML is likely a continuum. Nevertheless, the different terminologies in the pediatric and adult classification have introduced confusion. These classification schemes are not universally applied in the literature of pediatric MDS further complicating the interpretation of clinical outcomes.

Challenges of the current WHO classification

The WHO classification is the current standard for diagnosis of pediatric MDS. While these guidelines allow for accurate classification of most pediatric MDS [17], there are several limitations that need to be addressed in future editions.

First, classification based on blast count alone is a crude method of distinguishing clinical phenotypes. With the advent of next generation sequencing (NGS), additional molecular markers may emerge that can be incorporated into future classifications. Second, the provisional entity of RCC includes a wide range of phenotypes that might be due to different underlying molecular genetics. For example, the lack of differentiation of cellularity and degree of dysplasia, in particular the exclusion of RCMD from the classification, makes it difficult to interpret clinical studies. Finally, the different terminologies and definitions used for advanced MDS in pediatric and adult patients cause confusion. For this reason, it is important to use consistent contemporary terms and definitions.

Secondary MDS

Secondary pediatric MDS can result from a number of inherited predispositions or acquired conditions (Table 3). The underlying molecular mechanisms for these two groups are distinct, and therefore, it is important to segregate them in clinical studies. In cases of constitutional abnormalities associated with MDS, most often an underlying IBMFS with

Table 3 Inherited and acquired conditions associated with pediatric myelodysplastic syndrome (MDS) leading to secondary MDS

Conditions associated with MDS
Inherited conditions
Inherited bone marrow failure syndromes (IBMFS)
Fanconi anemia
Shwachman-Diamond syndrome
Severe congenital neutropenia
Dyskeratosis congenita
Diamond-Blackfan anemia
<i>GATA2</i> haploinsufficiency (MonoMac syndrome, Emberger syndrome, Familial MDS/AML)
Other familial non-syndromic MDS (<i>ETV6</i> , <i>RUNX1/AML1</i> , <i>CEBPA</i>)
Familial MDS (at least one first degree relative with MDS/AML) without identified genetic cause ^a
Trisomy 8 mosaicism
Acquired conditions
Prior chemotherapy
Prior radiation therapy
Acquired aplastic anemia ^b

^a Familial cases of MDS not due to *GATA2*, *ETV6*, *RUNX1/AML1* and *CEBPA*

^b A subset of cases currently classified as acquired aplastic anemia might be due to underlying genetic predisposition and may in the future be considered as an inherited condition (see main text)

defects in DNA repair, telomere biology or ribosomal protein synthesis is present. Other genetic predisposition syndromes such as trisomy 8 mosaicism and *GATA2* haploinsufficiency have been described (Table 3).

Therapy-related MDS after chemo or radiation therapy causes DNA damage and most frequently involves changes in chromosomes 7, 5, and 11q23, which are observed in more than 90 % of the cases [1, 78]. Therapy-related MDS more often presents as advanced MDS.

Secondary MDS following IST for AA has traditionally been regarded as an acquired cause of secondary MDS [17, 33] although it is possible that some pediatric patients regarded as acquired AA in fact have an underlying inherited genetic predisposition, possibly explaining the poor outcomes of some AA patients with clonal progression to MDS.

The diagnosis of secondary MDS following IBMFS or more recently identified cases of *GATA2* haploinsufficiency is challenging as most bone marrows from patients with IBMFS show some background dysplasia. For example, dysmyelopoiesis (in particular in the neutrophil series) is often seen in SDS [88]. Patients with *GATA2* haploinsufficiency show unique megakaryocytic dysplasia with large separated, “pulled apart” appearing nuclei, that often precedes the development of MDS [12, 74] [internal observation from the pediatric MDS and BMF Registry]. Therefore, minimal diagnostic criteria for secondary MDS following an underlying IBMFS have been developed [33] (increased BM blast count, development of a hypercellular marrow in the presence of persistent PB cytopenias and acquired clonal chromosomal abnormalities). However, studies in FA and SDS indicate that clonal cytogenetic abnormalities are not universally associated with disease progression and poor outcomes since cytogenetic changes may persist for years without evolution to MDS/AML [3, 4, 51].

Patients with IBMFS, such as FA, frequently present with a morphological picture of RCC [92]. Therefore, it is also difficult to distinguish primary pediatric MDS from IBMFS, further highlighting the importance of molecular testing.

Clinical presentation

The clinical presentation in pediatric MDS patients is variable. Some patients, especially those with RCC, come to medical attention because of an incidental finding of cytopenia [45]. In other cases, patients present with symptoms related to cytopenias. In contrast to adult MDS, most pediatric MDS patients present with bleeding due to thrombocytopenia or infection secondary to neutropenia. Isolated anemia is less frequently seen in children. If anemia is present, it is usually macrocytic and accompanied by thrombocytopenia or neutropenia.

Diagnostic work up

Laboratory evaluation

Given that MDS is often a diagnosis of exclusion, the workup of patients with suspected MDS requires a comprehensive laboratory evaluation to rule out secondary causes (Table 4).

A number of non-hematologic disorders can cause cytopenias and a hypoplastic marrow in children and must be ruled out, including vitamin and mineral deficiencies and zinc toxicity [23], viral infections and toxin or drug exposure [10], rheumatologic disorders (juvenile rheumatoid arthritis), mitochondrial disorders (Pearson syndrome), metabolic disorders (mevalonate kinase deficiency) and inherited anemias (e.g., CDA), and IBMFS [53].

Histopathology

First, careful evaluation of the PB smear to assess morphological abnormalities and the presence of circulating blasts should be performed. Both a high-quality BM aspirate and biopsy are required for an accurate assessment. The BM aspirate allows for assessment of cytologic dysplasia and blast count, while the biopsy is essential to assess cellularity, the presence of reticulin fibrosis, and the marrow architecture. It may also facilitate the identification of dysplastic megakaryocytes. A biopsy is particularly important for the assessment of pediatric MDS as the majority of patients present with a hypocellular marrow that has patches of cellularity that may be missed on a sub-optimal, hypospicular aspirate.

In addition to routine May-Grunwald-Giemsa-stained BM aspirate and H&E-stained biopsy slides, a number of ancillary immunohistochemistry (IHC) and special stains are often needed. The WHO classification for MDS in childhood mandates the addition of a CD61 or CD41 stain to identify micromegakaryocytes, which are supportive of the diagnosis of RCC and important in the differentiation from acquired AA. Additional lineage- or stage-specific IHC stains (CD34, CD117 (C-KIT), CD71, glycophorin A, MPO, and lysozyme) can also be helpful. Other IHC studies such as CD3, CD20, CD79a, and TdT, to assess lymphoid populations, are indicated when an increase in lymphocytes is observed, which can often be noted in younger children or in a reactive process. A reticulin stain is helpful in the assessment of background fibrosis (associated with monosomy 7 and germline *GATA2* mutations) [12, 24, 74].

Cytogenetics and molecular studies

Routine karyotype and fluorescence in situ hybridization (FISH) for 7-, 7q-, 5q, +8, 20q- and *MLL* (11q23) rearrangements should be performed in all patients with suspected MDS.

Table 4 Suggested diagnostic workup for pediatric patients with suspected MDS

Diagnostic checklist	
Basic laboratory	<ul style="list-style-type: none"> ✓ CBC with differential (ANC, AMC) ✓ Reticulocyte count ✓ Basic chemistry studies (electrolytes, LDH, uric acid) ✓ Renal and liver function tests ✓ Hemoglobin F (hemoglobin electrophoresis)
Immunology	<ul style="list-style-type: none"> ✓ Lymphocyte subsets (B and T cell subsets)
Other laboratory	<ul style="list-style-type: none"> ✓ High-resolution HLA typing^a ✓ Nutritional deficiencies (folate, B12, copper) ✓ Infectious studies (CMV, EBV, HSV, parvovirus B19, VZV, HIV, leishmaniasis, etc.)
Bone marrow failure workup	<ul style="list-style-type: none"> ✓ Fanconi anemia (chromosomal breakage with DEB from PB) ✓ Dyskeratosis congenita (telomere length in lymphocyte subsets, gene sequencing as indicated^b) ✓ Shwachman-Diamond syndrome (<i>SBD</i> gene sequencing, isoamylase, stool elastase) ✓ Other IBMFS based on clinical index of suspicion ✓ PNH clone on PB by flow cytometry
Molecular diagnostics	<ul style="list-style-type: none"> ✓ <i>GATA2</i> (on all cases) ✓ <i>CBBPA</i>, <i>RUNX1/AML1</i>, <i>ETV6</i> based on clinical suspicion in cases of familial MDS
Histopathology	<ul style="list-style-type: none"> ✓ Peripheral blood smear review ✓ Bone marrow aspirate for morphology ✓ Bone marrow biopsy for histology ✓ IHC staining (at minimum CD34 and CD61 suggested) ✓ Reticulin stain ✓ Iron stain on aspirate
Additional studies	<ul style="list-style-type: none"> ✓ Flow cytometry ✓ Karyotype form BM ✓ FISH on BM (−7, 7q−, +8, −5, 5q−, 20q−)

ANC absolute neutrophil count, AMC absolute monocyte count, DEB diepoxybutane, PB peripheral blood

^a High-resolution HLA should be send early on the patient and any full siblings once diagnosis is confirmed

^b Gene sequencing should be performed if telomere length shows very short telomeres diagnostic for DC

An underlying IBMFS, particularly FA, DC, or SDS, should be ruled out by molecular testing, as these diagnoses can have cryptic presentations and their presence will impact clinical management. Heterozygous germline mutations in *GATA2* can occur in patients with sporadic and familial MDS and therefore should be assessed in MDS patients as well as any potential related HSCT donors [28], [11, 39, 40, 46, 75] [60].

Pediatric MDS and other BMF disorders, in particular those due *GATA2* mutations, can present with immunodeficiency. Therefore, an immunology workup with analysis of lymphocyte subsets (B- and T-cell subsets) should be considered.

Flow cytometry

Flow cytometry to assess blast count and the presence of a PNH clone should also be obtained. While small subclinical PNH clones can be found in patients with RCC [2], overt PNH

with thrombosis and hemolysis is a rare finding in children [81].

In adults, flow cytometry analysis with the assessment of immunophenotypic abnormalities in myelomonocytic, erythroid, and myeloid blast cells can provide complementary information aiding in the diagnosis and distinguishing MDS from other non-clonal cytopenias [87]. However, studies assessing the value of flow cytometry in pediatric patients are limited. Veltroni and colleagues showed that immunophenotypic characterization of advanced MDS in pediatric patients might be a useful diagnostic and prognostic tool in differentiating from de novo AML [82]. Similar to adults, co-expression of CD7 was associated with poor outcomes [20, 21, 57, 58, 80, 82].

Simple flow cytometric scoring systems first developed by Ogata and others have also been shown to be useful in adult low-grade MDS [15, 56]. However, the diagnostic utility of flow cytometry scoring in low-grade pediatric MDS, in particular in RCC, remains uncertain. Furthermore, flow

cytometric analysis for the assessment of abnormal hematopoietic maturation (as a surrogate for dysplasia) requires a highly standardized approach and specialized expertise that is not uniformly available.

Genetics and genomic advances

Cytogenetics

The cytogenetic profile of pediatric and adult MDS patients varies significantly. The majority of pediatric MDS cases have a normal karyotype. In pediatric MDS, monosomy 7 is the most commonly observed change, occurring in 30 % of cases [26], followed by trisomy 8. A complex karyotype involving 3 or more cytogenetic changes occurs only in a small subset of patients and is associated with a poor prognosis [26]. Abnormalities in chromosome 5, in particular the 5- syndrome commonly seen in adults, rarely occur in children. Other changes such as 20q- and loss of chromosome X are infrequently seen in children.

Recent genetic advances in MDS

The advent of novel sequencing technologies has accelerated the pace of discovery in adult MDS. Since the early genetic studies that lead to discovery of somatic mutations in oncogenes (*NRAS/KRAS*) and tumor-suppressor genes (*TP53*, *RUNX1*) [44, 65, 66], over 40 MDS-associated gene mutations have been discovered in the last 7 years, including identification of epigenetic regulators (*TET2*, *ASXL1*, *EZH2*, *DNMT3A*, *IDH1/IDH2*), and most recently, genes in the splicing machinery (*SFRB1*, *U2AF1*, *ZRSR2*) and cohesion complex genes (*STAG2*, *SMC3*, *RAD21*). In adult MDS, somatic mutations in splicing factors and epigenetic regulators are present in about 75 % of cases, followed by isolated *TP53* mutations and mutations in a variety of other genes including transcription factors (*RUNX1*, *ETV6*, *GATA2*, *PHF6*), kinase signaling (*NRAS*, *KRAS*, *JAK2*, *CBL*), and cohesion complex genes [61, 85, 91]. Interestingly, the majority of these changes—in particular those of splicing factors and epigenetic regulation—do not appear to be present in children [35], suggesting that pediatric MDS is indeed a distinct disorder. A recent large scale whole-exome sequencing study on DNA samples from over 17,000 individuals showed that patients under the age of 40 years rarely had somatic mutations in adult MDS-associated genes. The most common mutations identified were *DNMT3A*, *TET2*, and *ASXL1* disease-associated variants, suggesting that mutations in epigenetic pathways accumulate with age and likely play a less prominent role in children [42].

Based on these data, it has been speculated that pediatric MDS is more frequently due to an inherited genetic

predisposition. The identification of *RUNX1/AML1* in familial platelet disorder with predisposition to AML, *CEBPA*, and more recently the discovery of germline mutations in *GATA2* leading to haploinsufficiency and development of familial MDS/MonoMac syndrome and germline mutations in *ETV6* [94] leading to familial MDS, support this. Nevertheless, in contrast to adults, the underlying genetic mechanism remains uncertain for most pediatric MDS patients.

GATA2 diagnostics and unique pathology

In 2011, heterozygous germline mutations in *GATA2* was independently observed in four seemingly distinct disease entities: (1) autosomal dominant familial MDS/AML [27]; (2) Emberger syndrome, characterized by lymphedema, warts, and predisposition to MDS/AML [59]; (3) MonoMac syndrome, comprised of monocytopenia and nontuberculous mycobacterial infection [41, 84]; and (4) dendritic cell, monocyte, and B and natural killer cell lymphoid deficiency (DCML) [8, 9, 16]. It is now recognized that these conditions are a spectrum of hematopoietic, lymphatic, and immune system disorders due to *GATA2* haploinsufficiency [74]. Mutations in *GATA2* can arise sporadically or be inherited in an autosomal dominant pattern [41, 84]. To date, several different *GATA2* mutations have been identified: (1) missense or frame-shift mutations often involving the C-terminal zinc finger, which is important for DNA binding, (2) null mutations, and (3) mutations in the regulatory enhancer region of intron 5 [16, 27, 38, 41, 43, 47, 59, 62].

While the clinical hallmarks of *GATA2* deficiency are immunodeficiency, predisposition to MDS/AML, pulmonary alveolar proteinosis (PAP), and congenital lymphedema, other clinical phenotypes have been observed, including sensorineural hearing loss, thrombotic events, panniculitis/erythema nodosum, increased incidence of miscarriages, other infections (severe *Clostridium difficile*, fungal, and EBV), and solid tumors (often related to viral infections including HPV- and EBV-driven leiomyosarcoma). Furthermore, there is significant heterogeneity in the clinical presentation, onset, and severity of the disease with some patients being asymptomatic and other presenting with life-threatening infections or MDS/AML. Intriguingly, there is also pronounced variation in the expression and severity of phenotypes and onset within a single family. Genotype-phenotype associations have been attempted, but only limited correlations have been found [74].

Patients with *GATA2*-related BMF disorders and acquired AA show significant overlap in presentation. Ganapathi et al. demonstrated that routine laboratory values, flow cytometry on the PB and BM, BM morphology, and cytogenetic studies can aid in the differential diagnosis and identify those patients for whom *GATA2* sequencing is indicated [24]. They found that hemoglobin levels, platelet count, and absolute neutrophil count (ANC) were not as severely decreased in *GATA2*

patients compared to AA patients. In contrast, the monocyte and lymphocyte counts were severely decreased in *GATA2* patients but usually only mildly reduced in patients with AA [24].

Flow cytometry of the BM has now been shown to be capable of identifying those that are at high risk of carrying a *GATA2* mutation. BM of *GATA2*-mutated patients often show a reduction in monocytes, B cells and NK cells, and a complete loss of B-cell precursors (CD19+, CD10+, CD20-). The loss of hematogones is intriguing and suggests disordered B-cell development. While hematogones are decreased in AA patients, they are usually detectable. Interestingly, the BM of patients with *GATA2* mutations showed an increase in overall T cells, an inverted CD4:CD8 ratio, and an expansion of large granular lymphocyte (CD3+, CD16+, CD56+) cells. An increase in myeloid blasts is often noted [12, 24, 84]. CD34+ cells were overall reduced in AA and *GATA2* patients compared to controls but similar to each other.

The morphology of BM specimens from *GATA2* patients has unique characteristics (Fig. 2). The majority of patients (84 %) meet histomorphologic criteria for MDS often with multilineage dysplasia and less than 5 % blasts [74]. In keeping with most pediatric MDS, patients with *GATA2* haploinsufficiency usually show a hypocellular marrow. Using the WHO classification scheme, *GATA2* patients would typically be best classified as RCC. A subset of patients present with a hypercellular marrow, often with evidence of advanced MDS (RAEB) or progression to leukemia. This suggests that *GATA2* haploinsufficiency can be regarded as an inherited BMF disorder with risk to progression to MDS and AML likely mediated through secondary clonal somatic events.

Based on recent studies [12, 24, 74] and our own observations [36, 37], a unique megakaryocytic dysplasia is the most common finding in 92 % of the patients and can even be seen prior to or without overt progression to MDS [12, 24, 74]. Megakaryocytes are often large and show separated and peripherally placed nuclear lobes, although occasional micromegakaryocytes have been observed in the adult population as well [24]. Other dysplastic features, including erythroid dysplasia with megaloblastoid changes, nuclear contour irregularities and bi-nucleation and myeloid dysplasia characterized by maturation dyssynchrony and hypogranulation, tend to be less frequent, occurring in 50 % of the cases. The myeloid to erythroid ratio is frequently reversed. A varying degree of reticulin fibrosis is also seen in about 50 % of the cases [24].

Clonal cytogenetic abnormalities have been identified in large subset of *GATA2* patients. Emerging evidence supports a strong association between *GATA2* germline mutations and development of monosomy 7 and trisomy 8. Other karyotype

abnormalities have been seen (gain of 1q and deletions of parts or entire chromosome 6) [24, 74].

Historic reports have described a pediatric entity termed “monosomy 7 syndrome” characterized by MDS, monosomy 7 and a histopathology with leukoerythroblastic anemia with leukocytosis, thrombocytopenia and slight increase in blasts, and increased reticulin in some cases [70]. Most of the early reports on monosomy 7 syndrome ultimately fit the diagnosis of JMML. However, a study by Hasle et al. [31] analyzed myeloid disorders in children, including pediatric MDS, and AML with monosomy 7 some of which were familial in origin. Given the overlap of the clinical features and histologic description with *GATA2* patients and the association with monosomy 7, we can now surmise that a subset of the patients that were historically described as monosomy 7 syndrome or MDS/AML with monosomy 7 and did not fulfill the criteria for JMML are likely cases of *GATA2* haploinsufficiency (Fig. 3). These patients may resemble chronic myelomonocytic leukemia (CMML). In fact, CMML can be seen in a small subset of patients with *GATA2* haploinsufficiency and might indicate disease progression to a proliferative state with increased myelomonocytic cells [74].

Taken together, the unique clinical and histologic features that are present in patients with *GATA2* haploinsufficiency should be carefully evaluated in any pediatric patient with suspected MDS or BMF. The histopathology of *GATA2* patients is unique, and in some patients, the megakaryocytic dysplasia is striking enough to provide instant pattern recognition. Because of the clinical and genetic implications for patients with *GATA2* haploinsufficiency, genetic testing should be performed on all patients presenting with classical phenotypes of familial MDS, Emberger syndrome, and MonoMac syndrome and be considered in all pediatric patients with MDS or BMF. Given the high association between monosomy 7 and trisomy 8, *GATA2* testing should also be considered in patients with hematologic malignancies especially AML. Testing of family members and genetic counseling should be offered to all patients with documented *GATA2* mutation but is essential for any related HSCT donors.

Treatment options in pediatric MDS

HSCT remains the only curative therapy for pediatric MDS. High-resolution HLA typing should be performed early to expedite the search for a matched related donor (MRD) or unrelated donor (URD). Evaluation of any MRD should include a CBC with differential, BM aspirate and biopsy, and cytogenetic and FISH studies to rule out familial MDS. For patients with *GATA2* mutation or patients with MDS

Fig. 2 Unique histopathology in pediatric MDS due to *GATA2* mutation. a–g Representative images from the marrow of a 17-year-old male with fevers, lymphadenopathy, and splenomegaly secondary to primary EBV infection. He eventually developed persistent pancytopenia (absolute neutrophil count (ANC) 400 cells/uL, absolute monocyte count (AMC) 0 cells/uL, platelet count 44×10^3 cells/uL) with morphological dysplasia, despite normalizing EBV titers and negative laboratory and molecular work up for HLH. The BM aspirate showed hypocellular spicules (a) ($\times 200$) with a relative erythroid hyperplasia with megaloblastoid changes and dyserythropoiesis (b and g) ($\times 400$). Megakaryocytes displayed marked dysplasia with hyperlobated pulled apart nuclei (c–f) ($\times 1000$). The biopsy showed a hypocellular marrow with erythroid hyperplasia

and dysplastic megakaryocytes appearing in loose clusters (h) ($\times 400$). Images i–n are from an 18-year-old young male with a hydrocele and an incidental finding of pancytopenia (WBC 1.9×10^3 cells/uL, Hgb 10.2 g/dL, MCV 106 fL, platelet count 134×10^3 cells/uL). The subsequent BM aspirate shows characteristic dysplastic megakaryocytes (i), myeloid dysplasia with pseudo-pelger huet forms, nuclear satellite (j), and hypogranularity (k). Erythroid dysplasia with nuclear contour abnormalities (k) (i–m $\times 1000$). The biopsy showed a hypocellular marrow with erythroid hyperplasia and dysplastic megakaryocytes (n) ($\times 400$). Both patients were ultimately diagnosed with MDS due to mutations in *GATA2*.

secondary to an IBMFS, *GATA2* mutations and IBMFS should be ruled out by molecular testing in any possible MRD.

The literature on HSCT outcomes for pediatric MDS is sparse and includes small retrospective studies with a wide range of MDS-related diagnoses, varying pre-transplant therapies and conditioning regimens, stem cell sources, and graft versus host disease (GVHD) prophylaxis. Most studies used a myeloablative-conditioning regimen, although more recently a reduced intensity-conditioning (RIC) approach has successfully been employed in a selected group of patients with low-grade MDS [76], in an attempt to reduce treatment-related toxicity and mortality.

Several studies indicate that transplanting pediatric MDS patients with RCC prior to disease progression is associated

with improved outcomes [73, 89], supporting a referral to HSCT immediately after diagnosis.

The use of pre-HSCT chemotherapy in MDS remains controversial. Historically, pediatric MDS patients with advanced disease (RAEB, RAEB-T) have been treated with AML induction chemotherapy [16]. However, recent data suggest that intensive chemotherapy before HSCT does not improve outcomes or relapse incidence (RI) in most patients with advanced MDS with the exception of MDS with progression to AML ($>30\%$ blasts) [72, 77]. Therefore, expedited HSCT should be considered in most patients with RAEB, particularly if a donor can be available promptly. Induction chemotherapy would uniformly be recommended for patients with MDS with progression to AML and perhaps RAEB-T.

Fig. 3 Monosomy 7 syndrome due to *GATA2* mutation. Representative bone marrow aspirate and biopsy images of the marrow from a 19-year-old male with “monosomy 7 syndrome.” The patient presented with easy bruising and a leukocytosis of 39.0×10^3 cells/uL with 2 % circulating blasts and the presence of metamyelocytes and myelocytes on the peripheral blood smear. The absolute monocyte count was 2.15×10^3 cells/uL. The Hgb was 10.0 g/dL, and the platelet count was 76×10^3 cells/uL. Cytogenetic studies showed monosomy 7 in 20 out of 20 metaphases. Molecular analysis confirmed a pathogenic *GATA2* mutation in the critical zinc-finger region. **a, b** Bone marrow aspirate ($\times 200$ and $\times 400$) showed a hypercellular myeloid predominant marrow. The myeloid elements were left shifted and dysplastic with hypolobated forms. The

erythroid series showed megaloblastoid maturation. Megakaryocytes showed frequent dysplastic forms. **c** High-power view ($\times 1000$) of two dysplastic megakaryocytes with separated nuclear lobes. **d** Illustration of dysplastic early promonocyte to monocyte with two nuclei and nuclear satellite. **e** Bone marrow biopsy ($\times 100$) showed a hypercellular marrow with **f** myeloid predominance and clusters of early myeloid forms ($\times 200$). **g** Immunohistochemistry (IHC) for CD34 showed scattered blasts comprising less than 5 % of the overall cellularity (200x). **h** ICH for CD61 highlighted increased often smaller sized megakaryocytes, including micromegakaryocytes, small hypolobated forms, and dysplastic forms with separated nuclear lobes ($\times 100$)

While the use of hypomethylating agents has been shown to improve overall survival (OS) in high-risk adult MDS patients [18], these agents have some limitations since the response can take several months [68] and are associated with a risk of febrile neutropenia [18, 49, 71]. While isolated cases, particularly in the palliative care setting, have shown a possible response, their use has not been systematically evaluated in children and should be limited to clinical trials and compassionate use.

Prognosis

Some patients with low-grade pediatric MDS, in particular RCC without high-risk cytogenetic abnormalities, need for transfusions or risk of neutropenia have relatively stable

disease for weeks to years. In this setting, a “watch and wait” strategy may be indicated. Nevertheless, pediatric MDS patients remain at risk for clonal transformation and progression to leukemia that is estimated at about 30 % for patients with RCC over 5 years. While monosomy 7 and complex karyotype (≥ 3 abnormalities) are known to be associated with increased risk for disease progression to leukemia, it is difficult to predict when this will occur [26, 33, 45]. Given the clinical heterogeneity and lack of precise prognostic factors, guidelines on the optimal timing for HSCT and the need for upfront chemotherapy in advanced MDS do not currently exist.

Reported outcomes of pediatric MDS after HSCT vary. Earlier studies showed a 3-year OS between 18–74 % depending on stage, with RAEB-T having the worst outcomes [93]. A recent large study from the Center of International Blood and

Fig. 4 Relationship between marrow failure conditions and myelodysplastic syndromes (MDS) and disease progression to myeloid leukemia. This diagram illustrates the clinical and possible genetic overlaps and relationships between bone marrow failure (BMF) conditions, low-grade MDS, and high-grade MDS with progression to acute myeloid leukemia (AML). The *left panel* shows the overlap between acquired BMF conditions such as severe aplastic anemia (SAA), IBMFS, refractory cytopenia of childhood (RCC), and familial MDS such as patients with *GATA2* haploinsufficiency. These conditions present with cytopenias and a hypocellular marrow in most cases and share significant clinical and histopathologic overlap suggesting a possible underlying inherited genetic defect in patients with pediatric MDS. Most SAA is thought to be an immunologic process. Immune dysregulation may even play a role in RCC, since some of these patients respond to

immunosuppressive therapy; a significant subset of patients with SAA and RCC do not respond to IST suggesting an underlying stem cell defect or exhaustion possible due to an inherited genetic defect in some of those patients. *PNH clones can develop secondary to SAA but are also seen in RCC. The *right panel* shows the other end of the disease spectrum with high-grade pediatric MDS such as a RAEB and RAEB-T with progression to AML. It has been hypothesized that an underlying genetic defect on a germline level is the underlying basis of many cases of pediatric MDS, which predisposes them to marrow failure, dysplastic hematopoiesis, and risk for clonal evolution. Secondary-acquired mutations are likely involved in clonal evolution and disease progression to advanced MDS and AML. Identifying such somatic clonal markers might help predict which patients are at high risk for rapid disease progression and inform therapeutic interventions and timing to transplant

Marrow Transplant Research showed [89] an 8-year disease free survival of 40–65 % for RCC and 28–48 % for RAEB/RAEB-T and a 1-year transplant-related mortality of 13–42 %. The European Working Group of Myelodysplastic Syndromes in Childhood reported more favorable outcomes for children with advanced MDS with a 5-year OS of 63 % with a TRM and RI of 21 %, each [77].

Recent evidence supports the hypothesis that somatic clonal evolution in the context of an underlying inherited predisposition to MDS and BMF may indicate clinical disease progression (Fig. 4). For example, acquired somatic *ASXL1* mutations have been identified in patients with *GATA2* haploinsufficiency and were associated with progression from MDS to leukemia [86]. With the rapid progression of NGS technologies, we may be equipped to identify more patients at risk for leukemic transformation prior to overt clinical leukemia.

Conclusions

Pediatric MDS is a rare and heterogeneous group of clonal hematopoietic stem cell disorders that remain difficult to diagnosis. While emerging evidence suggests that an underlying genetic predisposition might be the cause for pediatric MDS, the molecular underpinnings remain unclear. Pediatric MDS is diagnosed using the 2008 WHO classification system. Within this classification scheme, RCC remains a provisional entity and its clinical implications are

uncertain. Diagnosis of pediatric MDS often requires analysis by a team of experienced pediatric hematologists and hematopathologists, in conjunction with multiple molecular studies. With the rapid advancement of NGS technologies, we hope to diagnose and characterize these disorders more effectively in the future. Comprehensive multidisciplinary analysis of these rare patients in large collaborative studies will be valuable for improving in our understanding of these disorders.

Conflict of interest The author declares that she has no competing interests.

Funding This study was funded by the National Institutes of Health career development award from the National Cancer Institute (1K08CA140723).

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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