EUROPEAN HEMATOLOGY ASSOCIATION

Diagnosis of inherited bone marrow failure and myelodysplastic syndromes

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Take-home messages

- Diagnosis of inherited bone marrow failure (BMF) and inherited myelodysplastic syndromes (MDS) informs surveillance strategies and treatment decisions
- Classical clinical stigmata of these inherited syndromes may be absent
- Understand the indications and caveats of genetic screening strategies for the diagnosis of patients with bone marrow failure.

Introduction

Accurate and timely diagnosis of inherited bone marrow failure syndromes (BMF) and inherited myelodysplastic syndromes (MDS) is essential to guide clinical management. The inherited BMF/MDS syndromes are characterized by an increased risk of progression to leukemia, typically acute myelogenous leukemia.Early diagnosis and surveillance allows initiation of a hematopoietic stem cell transplant (HSCT) prior to the onset of leukemia thus avoiding the need for intensive leukemia-directed therapies for remission induction prior to transplant and reduces the risk of subsequent leukemia relapse or refractory disease. Many of the BMF/MDS syndromes are associated with an increased risk of treatment-related toxicities which may arise from impaired DNA repair, hyperactive stress responses, or organ co-morbidities.Early diagnosis therefore allows tailoring of transplant with reduced intensity conditioning regimens to avoid excessive toxicity. Diagnosis of an inherited BMF/MDS disorder also informs treatment of marrow failure since these syndromes respond poorly or transiently to immunosuppressive therapies used for acquired aplastic anemia.Diagnosis of a genetic BMF/MDS disorder also allows testing of family members to avoid inadvertently choosing a related stem cell donor afflicted with the same disorder.The identification of increasing numbers of genetic BMF/MDS disorders together with the availability of multiplexed genetic testing has expanded our diagnostic approach for BMF and MDS.Recent advances in the diagnostic evaluation for inherited BMF/MDS will be discussed.The benefits and caveats of genetic testing will be explored.

Current state of the art

Distinguishing inherited from acquired bone marrow failure/myelodysplastic syndrome is often challenging. Clinical history and the physical exam provide important clues to the diagnosis of an underlying genetic BMF/MDS disorder. Many of the BMF/MDS disorders initially come to medical attention with characteristic stigmata such as congenital anomalies, dysmorphic features, short stature, poor growth or additional suggestive clinical features.Unexplained red cell macrocytosis or elevated fetal hemoglobin may hint at a genetic BMF disorder. Family history provides another important clue to an underlying genetic BMF/MDS disorder. Suspicion is aroused by a family history of unexplained cytopenias, red cell macrocytosis, cancers presenting in multiple family members, cancers presenting at an unusually young age, excessive toxicity with chemotherapy/radiation, poor stem cell yield after marrow harvest or stem cell mobilization[1], or other characteristic stigmata of inherited BMF/MDS syndromes.See presentation by Dr. Kratz for additional discussion of the clinical features of inherited BMF/MDS syndromes which should arouse clinical suspicion and prompt diagnostic testing.

In the past, only those patients suspected to have a genetic disorder based on clinical stigmata or family history were referred for directed testing of the suspected gene. However, the clinical phenotypes of these disorders are now recognized to vary widely and a significant subset of patients may lack the clinical findings classically associated with these disorders. Indeed, BMF or MDS might be the sole presenting finding for these genetic disorders. Many of these syndromes share overlapping features leading to erroneous diagnosis. Accurate diagnosis is critical because many syndromes are associated with specific treatment considerations, additional organ sys-

tem co-morbidities, or solid tumor risks that affect medical management. The family history may fail to flag an inherited disorder due to a *de novo* constitutional mutation arising in the proband, parental gonadal mosaicism, incomplete penetrance or variable expressivity, or latency to cancer development which has yet to be manifested in family members. A comprehensive and current history of all family members is often unavailable.

Laboratory screening is also helpful in the assessment of BMF/MDS. Increased chromosomal breakage with mitomycin C or diepoxybutane is the diagnostic hallmark of Fanconi anemia.[2] Shortened telomere lengths in multiple lymphocyte subsets raises suspicion for a telomere biology disorder.[3] Low serum trypsinogen or pancreatic isoamylase for age is characteristic of Shwachman Diamond syndrome.[4] Immunologic abnormalities may be seen with *GATA2* disorders, dyskeratosis congenita, or Shwachman Diamond syndrome.[5] Monocytopenia may be seen with GATA2 disorders.[5] Bone marrow clonal cytogenetic abnormalities involving deletion of 20q or isochromosome 7 commonly arise in Shwachman Diamond syndrome.[6,7]

To assess for cryptic presentations of genetic BMF/MDS disorders, a cohort of 71 pediatric and young adult patients with BMF or MDS who remained diagnostically undefined after an initial medical and laboratory evaluation were screened for mutations in 85 BMF/MDS genes.[8] Thirty-two patients, including 6 of the 13 adults, had a positive family history.Causative germline mutations were identified in eight out of these 71 patients (11%) with idiopathic BMF/MDS.[8] All eight of these patients lacked classical clinical stigmata or laboratory findings of these syndromes and only four had a family history suggestive of inherited disease. A subsequent retrospective targeted BMF/MDS genetic screen of samples banked from 98 children and young adults transplanted for aplastic anemia or MDS identified causative mutations in 5.1% (5/98) of aplastic anemia patients and 13.6% (15/110) of MDS patients.[9] Family history or physical examination failed to reliably predict the presence of germline BMF/MDS mutations. Similar findings were reported by Ghemlas *et al*., who identified causative genes in 15 of 83 patients (18%) with idiopathic unclassifiable bone marrow failure screened with targeted sequencing.[10] A study of patients with inherited bone marrow failure from Japan identified causative mutations in 53 out of 121 (44%) patients screened by targeted sequencing and 68 out of 250 patients (27%) screened by whole exome sequencing.[11] The initial clinical diagnosis for a subset of patients was re-classified to a different disorder after genetic testing.[8,10,11] These studies demonstrated that although any single specific BMF/MDS genetic disorder is rare, genetic BMF/MDS disorders in aggregate affect a significant subset of patients presenting with BMF/MDS.

Referral to a center with expertise in the diagnosis of inherited BMF/MDS syndromes is recommended. Navigation of the rapidly growing number of available genetic testing options requires detailed knowledge of the limitations of the specific test ordered.[12,13] Gene panels vary widely with respect to genes included, coverage of a specific gene, detection of copy number

Table 1. Diagnostic evaluation for inherited bone marrow failure (BMF)/MDS.*

Personal history
Cytopenias
Short stature
Congenital anomalies
Other features of inherited BMF/MDS syndromes[12,17]
Excessive treatment-related morbidity (TRM) with cancer treatment/hematopoietic stem cell transplant (HSCT)
Family history
Cytopenias
Congenital anomalies
Other features of inherited BMF/MDS syndromes[12, 17]
Cancers at atypically young age
Multiple first or second-degree relatives with malignancy
Excessive TRM with cancer treatment/HSCT
Physical exam/imaging studies
Short stature
Failure to thrive
Dysmorphologies
Congenital anomalies
Other features of inherited BMF/MDS syndromes[12, 17]
Laboratory testing
Cytopenias
Elevated red cell MCV (mean cell volume)
Elevated fetal hemoglobin
Low Trypsinogen, Pancreatic isoamylase
Elevated erythrocyte adenosine deaminase
Low immunoglobulin levels
Abnormal lymphocyte subsets (often B cell lymphopenia)
Other clinically directed testing
Functional testing
Telomere length
Chromosomal breakage for Fanconi anemia
Germline genetic testing
Single gene Sanger sequencing
Targeted gene panels
Whole exome sequencing

**This is a general schematic outline that may be tailored as clinically indicated.*

variants, coverage of regulatory non-coding regions. Whole exome sequencing may fail to cover specific regions of interest. Importantly, the inability to identify a causative gene mutation does not rule out an inherited marrow failure disorder. Patients may be diagnosed with an inherited marrow failure disorder based on clinical diagnostic criteria for the known syndromes or based on a family history of a familial BMF/MDS disorder. Therefore, a diagnostic approach considering both clinical and genetic diagnostic criteria is essential.

Future perspective

While genomic testing is a powerful addition to the diagnostic armamentarium for inherited BMF/MDS, this remarkable advance also brings additional challenges.[12-16] Pathologic damaging mutations must be distinguished from benign variants or polymorphisms.Variants reported in the literature may lack rigorous assessment of pathogenicity. Development of functional assays to test variants and access to such functional analysis as part of clinical testing would complement the rapid advances in genetic testing.There is an urgent need for centralized database(s) of all identified BMF/MDS genes and their variants with expert annotation regarding the evidence for disease causation. Research is needed to investigate the significant population of patients with familial BMF/MDS who remain genetically undefined. Referral to a center with expertise in the diagnostic evaluation and medical management of these complex patients is recommended.

References

1. Rojek K, Nickels E, Neistadt B, et al. Identifying inherited and acquired genetic factors involved in poor stem cell mobilization and donor-derived malignancy. Biol Blood Marrow Transplant 2016;22:2100-2103.
2. Auerbach AD. Fanconi anemia diagnosis and the diepoxybutane (DEB) test. Exp Hematol 1993;21:731-733.
3. Alter BP, Baerlocher GM, Savage SA, et al.Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita. Blood 2007;110:1439-47.
4. Ip WF, Dupuis A, Ellis L, et al Serum pancreatic enzymes define the pancreatic phenotype in patients with Shwachman-Diamond syndrome. J Pediatr 2002;141:259-65.
5. Hsu AP, McReynolds LJ, Holland SM. GATA2 deficiency. Curr Opin Allergy Clin Immunol 2015;15:104-9.
6. Dror Y, Durie P, Ginzberg H, et al. Clonal evolution in marrows of patients with Shwachman-Diamond syndrome: a prospective 5-year follow-up study. Exp Hematol 2002;30:659-69.
7. Pressato B, Valli R, Marletta C, et al. Cytogenetic monitoring in Shwachman-Diamond syndrome: a note on clonal progression and a practical warning. J Pediatr Hematol Oncol 2015;37:307-10.
*8. Zhang MY, Keel SB, Walsh T, et al. Genomic analysis of bone marrow failure and myelodysplastic syndromes reveals phenotypic and diagnostic complexity. Haematologica 2015;100:42-8.

A genomic screen demonstrating a genetic cause in patients lacking clinical features or history suggestive of an inherited BMF disorder.

*9. Keel SB, Scott A, Sanchez-Bonilla M, et al. Genetic features of myelodysplastic syndrome and aplastic anemia in pediatric and young adult patients. Haematologica 2016;101:1343-50.

A retrospective study demonstrating that a significant subset of pediatric and young adult patients with BMF or MDS have a genetic etiology.

*10. Ghemlas I, Li H, Zlateska B, et al. Improving diagnostic precision, care and syndrome definitions using comprehensive next-generation sequencing for the inherited bone marrow failure syndromes. J Med Genet 2015;52:575-84.

This study demonstrates the utility of a broad genetic screening approach to the diagnosis of inherited BMF.

*11. Muramatsu H, Okuno Y, Yoshida K, et al. Clinical utility of next-generation sequencing for inherited bone marrow failure syndromes. Genet Med 2017 doi: 10.1038/gim.2016.197. [Epub ahead of print].

A broad genomic screen demonstrating genetic causes of BMF in a significant subset of patients.

12. Shimamura A. Aplastic anemia and clonal evolution: germ line and somatic genetics. Hematology Am Soc Hematol Educ Program 2016;2016:74-82.
13. Furutani E, Shimamura A. Germline genetic predisposition to hematologic malignancy. J Clin Oncol 2017;35:1018-28.
*14. Babushok DV, Bessler M, Olson TS. Genetic predisposition to myelodysplastic syndrome and acute myeloid leukemia in children and young adults. Leuk Lymphoma 2016;57:520-36.

Excellent summary of genetic predisposition to MDS and AML.

*15. DiNardo CD, Bannon SA, Routbort M, et al. Evaluation of patients and families with concern for predispositions to hematologic malignancies within the hereditary hematologic malignancy clinic (HHMC). Clin Lymphoma Myeloma Leuk 2016;16:417-428 e2.

Nice discussion of diagnostic evaluation for genetic MDS/AML predisposition.

16. Feurstein S, Drazer MW, Godley LA. Genetic predisposition to leukemia and other hematologic malignancies. Semin Oncol 2016;43:598-608.
*17. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev 2010;24:101-22.

These papers provide evidence to support broad genomic testing to identify cryptic presentations of inherited BMF/MDS and show that constitutional genetic causes are found in a significant subset of patients with BMF/MDS.