Hematopathology Evening Conference Case 4

Magdalena Czader, MD, PhD
Indiana University, Indianapolis, IN

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Clinical history

- 22 year-old male presented to hematology/oncology clinic for annual follow-up
- Originally diagnosed with anemia at 13 years of age
- Transfusion dependent normocytic anemia, intermittent neutropenia and thrombocytosis
- No reported family history of anemia
- Father with a mild bleeding tendency

Evaluation of cytopenias in children and young adults

Clinical history:
- Timing and severity of cytopenia(s)
- Transfusion dependency
- Current comorbidities including infections and bleeding
- Medication history
- Three generational family history

Laboratory tests:
- Current and previous CBC and differential counts
- Reticulocyte counts
- Serum vitamin B12
- RBC folate levels
- Serum iron studies (iron, TIBC and ferritin)
- Lactate dehydrogenase

Complete physical examination, if necessary imaging studies
Bone marrow exam

Current CBC and differential count

WBC 4.8 k/ul
RBC 2.34 million/ul
Hemoglobin 7.3 g/dL
MCV 96 fl
RDW 18.1%
Platelet count 384 k/ul

Bands 1%
Segmented neutrophils 54%
Lymphocytes 38%
Monocytes 7%

Peripheral blood smear
Evaluation of cytopenias in children and young adults

- Conventional karyotyping of bone marrow aspirate by G-banding
- Molecular testing to exclude inherited bone marrow disorders is crucial in children and has been recommended for young adults (up to 50 years of age)

Babushok D, Bessler M. Best Pract Res Clin Haematol. 2015;28:55

Why adult patients should be tested for inherited bone marrow disorders?

Up to 10% of hematologic malignancies are associated with genetic predisposition
- hereditary BM failure syndromes
- non-syndromic familial MDS/AML predisposition

Feurstein et al. Sem Oncol 2016;43:598
Why adult patients should be tested for inherited bone marrow disorders?

- Significant proportion of patients come to medical attention later in life and may lack congenital malformations and/or classic cytogenetic/genetic lesions classically associated with inherited bone marrow disorders
- Syndromes with presentation in adulthood:
  - Fanconi anemia
  - Dyskeratosis congenita
  - GATA2 haploinsufficiency
  - Myeloid neoplasms with germ line RUNX1 mutation
  - AML with germ line CEBPA mutation
  - Myeloid neoplasms with germ line SRP72 mutation
- Expanding NGS panels and broadening availability of WES calls for periodic re-testing of children and adults with cytopenias without previously identified etiology.

Feurstein et al, Sem Oncol 2016;43:588

Unique clinical management of patients with inherited bone marrow disorders

- Genetic counseling of patient and family, and extended molecular testing of family members
- Conventional chemotherapy and immunosuppressive regimens used in primary MDS and AML may not be recommended:
  - Patients with Diamond-Blackfan anemia frequently respond to corticosteroids
  - Patients with Fanconi anemia and dyskeratosis congenita with aplastic/hypoplastic marrows are less likely to respond to immunosuppression
  - Fanconi anemia requires reduced intensity conditioning prior to stem cell transplantation
  - Emerging evidence of response to lenalidomide in patients with DDX41 mutations/deletions

Feurstein et al, Sem Oncol 2016;43:588

Hematopoietic stem cell transplantation and follow-up in inherited bone marrow disorders

- Informed donor selection to avoid affected family members
- Higher incidence of post-transplant complications:
  - Higher frequency of graft failure and graft-versus-host disease in Fanconi anemia
  - Pulmonary and cardiac complications
- Life-long cancer surveillance may be required
- Bone marrow exam and cytogenetic surveillance in bone marrow failure syndromes scheduled every 6-12 months

TruSight Myeloid Sequencing Panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>TRK</th>
<th>IRF8</th>
<th>MYD88</th>
<th>SF3B1</th>
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<tr>
<td>ABL1</td>
<td>CEBPA</td>
<td>IRAK</td>
<td>MYD88</td>
<td>SF3B1</td>
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<tr>
<td>ASXL1</td>
<td>CSF3R</td>
<td>IDH</td>
<td>NOTCH1</td>
<td>SM3B</td>
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<td>ATRX</td>
<td>GATA1</td>
<td>IDH2</td>
<td>NPM1</td>
<td>SMAD1A</td>
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<tr>
<td>BCROR</td>
<td>DNM1</td>
<td>KIT</td>
<td>NRAS</td>
<td>SRSF2</td>
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<tr>
<td>BCROR1</td>
<td>ETFB</td>
<td>JAK2</td>
<td>PDGFR</td>
<td>STAG2</td>
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<tr>
<td>BRAF</td>
<td>EZH2</td>
<td>JAK3</td>
<td>PHF6</td>
<td>TET2</td>
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<tr>
<td>CALR</td>
<td>FABX</td>
<td>KDM6A</td>
<td>PTPN1</td>
<td>TP53</td>
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<tr>
<td>CBL</td>
<td>FLT3</td>
<td>KIT</td>
<td>PTPN11</td>
<td>U2AF1</td>
</tr>
<tr>
<td>CBLB</td>
<td>GATA1</td>
<td>KRA</td>
<td>RAD1</td>
<td>ZRSR2</td>
</tr>
<tr>
<td>CBLC</td>
<td>GATA2</td>
<td>MLL</td>
<td>RUNX1</td>
<td>WT1</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>GRAS</td>
<td>MPL</td>
<td>SETBP1</td>
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</table>

Germline mutation testing in hematopoietic disorders

- Blood and bone marrow are not suitable to confirm germline abnormalities
- Buccal swab samples may be contaminated by peripheral blood cells
- Preferred sample: cultured skin fibroblasts
  - Small punch biopsy (3 mm) or skin ellipse, transported fresh
  - Grown for 3 to 6 weeks
  - Extracted DNA used for germline mutation testing
- Useful source after HSCT

Cytogenetic and molecular genetic studies

- Cytogenetic study: 46,XY[20]
- Next generation sequencing (Trusight Myeloid Panel, Illumina; 54 genes including all exons of 15 genes, and 39 genes analyzed for hot spot mutations common in myeloid malignancies, Miseq platform) GATA1 NM_002049.3 c.220 (+1)G>A, allelic frequency 98% Predicted to likely affect splicing
- Confirmed by MarrowSeq™ NGS panel including genes associated with familial MDS/acute leukemia predisposition syndromes and inherited marrow failure syndromes (peripheral blood and fibroblasts sent, University of Washington)
- No other mutations associated with inherited bone marrow disorders
GATA1 (globin transcription factor 1)

- Member of GATA family
- Involved in development of erythroid and megakaryocytic lineages, mast cells, eosinophils and basophils
- In animal models loss of GATA1 function lead to decreased erythropoiesis due to an increased apoptosis, and increased megakaryopoiesis with disrupted differentiation
- Decreased GATA1 function results in impaired mast cell differentiation and marrow fibrosis

GATA1 mutations associated with human disease

**Deletions**

**Somatic**
- Acute megakaryoblastic leukemia associated with Down syndrome
- Transient abnormal myelopoiesis of Down syndrome

**Germline**
- Diamond-Blackfan anemia
- Dyserthropoietic anemia

**Missense mutations**

**Germline**
- Variant congenital dyserthropoietic anemia
- X-linked familial dyserthropoietic anemia and thrombocytopenia
- X-linked macrothrombocytopenia
- Erythropoietic porphyria
- X-linked thrombocytopenia with β-thalassemia
- Gray platelet syndrome

GATA1 mutations affecting DNA and cofactor binding

**Clinical features**

- Spectrum of thrombocytopenia
- Bleeding diathesis
- Anemia
- Variable severity

**BM morphology**

- Hypercellular BM
- Small hypolobated megakaryocytes
- Increased megakaryopoiesis
- Increased erythropoiesis
- Dyserthropoiesis

**Follow-up**

- No related malignancies
Germline GATA1 mutations associated with loss of full length protein

<table>
<thead>
<tr>
<th>Ref 1</th>
<th>Mutation</th>
<th>Clinical features</th>
<th>BM morphology</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.2T&gt;G</td>
<td>Severe macrocytic anemia, reticulocytopenia, occasional neutropenia, thrombocytosis, elevated hepatic transaminases, splenomegaly and frequent acquired point mutations</td>
<td>Hypocellular BM, pancytopenia with dysmegakaryopoiesis, 1 patient refractory to steroids</td>
<td>At least partial response to steroids</td>
<td>1 patient developed MDS</td>
</tr>
<tr>
<td>c.229G&gt;C</td>
<td>Macrocytic anemia, reticulocytopenia, mild neutropenia, 1 patient developed mild thrombocytopenia, no fetal hemoglobin</td>
<td>Reticulocytopenia, no significant abnormalities in other lineages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.225G&gt;A</td>
<td>Anemia, WBC and platelets within normal limits</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.229G&gt;C</td>
<td>Moderate to severe normocytic to macrocytic anemia, neutropenia, and/or thrombocytopenia</td>
<td>Predominantly hypocellular BM, pancytopenia and granulopoiesis, common dysmegakaryopoiesis with absent megakaryocytes, common trilineage deficiencies</td>
<td>At least partial response to steroids</td>
<td>1 patient developed MDS</td>
</tr>
<tr>
<td>c.215G&gt;C</td>
<td>Normocytic anemia, reticulocytopenia, WBC and platelets initially within normal limits, slightly elevated erythrocyte adenosine deaminase, no congenital anomalies</td>
<td>Reticulocytopenia, no significant abnormalities in other lineages; at age 4 developed progressive complex karyotype, -7, +3, +8, +21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>T&gt;C</td>
<td>Normocytic to macrocytic anemia, reticulocytopenia</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>
**Bone marrow 2006**

**Bone marrow 2010**

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**Diagnosing Diamond-Blackfan anemia**

**Diagnostic criteria:**
- Age <1 year
- Macrocytic anemia with no other significant cytopenias
- Reticulocytopenia
- Normocellular marrow with paucity of erythroid precursors
- **Supporting criteria**
  - Major:
    - Gene mutations described in classical DBA
    - Positive family history
  - Minor:
    - Elevated RBC adenosine deaminase activity
    - Congenital abnormalities associated with classical DBA
    - Elevated HbF
    - No evidence of another inherited BM failure syndrome

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**Take home**

- Critical role of clinicopathological correlation including longitudinal reviews of bone marrow morphology and ancillary studies
- Concerning dysplastic morphology associated with certain genetic lesions may persist throughout long periods of time without signs of disease progression/transformation
- Dysmegakaryopoiesis is associated with germline GATA1 mutations
- Phenotypes associated with specific germline mutations vary considerably among patients

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**Patient management**

- Previously transfusion dependent
- Considered for hematopoietic stem cell transplant
- Currently on steroids and showed initial improvement of anemia (hemoglobin 7 → 8.7 g/dL)
- Unknown long term risk of developing malignancy

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Take home
• Genetic testing for inherited bone marrow disorders is crucial in children and young adults (<50 years) with cytopenias of unclear etiology, including those with clinical suspicion of MDS
• Incidental finding of mutations at allelic frequency suggestive of germline abnormality requires confirmatory testing
• Periodic re-testing of individuals without clearly established etiology may be helpful (expanding gene panels, whole exome sequencing)

Case 4 - Panelists Diagnosis
• Inherited BM failure/germline disorder (DDx: RCC/MDS, toxins, nutritional). More molecular studies/clinical information needed.
• Likely familial MDS predisposition entity
• MDS with multilineage dysplasia (rule out MDS with germline predisposition)

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