Hematopathology Specialty Conference
Case #4
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Dr. Sherrie L. Perkins declares affiliation with ARUP Laboratories

History
• The patient is an 80-year-old man with previous history of iron deficiency anemia (treated) who is now noted to have macrocytic anemia on a recent physician's office visit. He is healthy except for seasonal allergies and increasing fatigue, and is currently on no medications. He drinks approximately 2-3 cocktails per night. His current CBC shows the following:
  • WBC: 4.37 x 10^3/mL
  • RBC: 2.21 x 10^6/mL
  • HGB: 9.1 g/dL
  • HCT: 27.8%
  • MCV: 126.1 fL
  • RDW: 11.2%
  • PLT: 377 x 10^3/mL
• Differential: 35% PMNs, 10% bands, 44% lymphocytes, 4% monocytes, 7% eosinophils
Cytopenia?

- Hemoglobin: <10 g/dL
- ANC: <1.8 x10^9/L
- Platelets: <100 x10^9/L

- Our patient meets criteria for macrocytic anemia and is on the lower end of normal for ANC
- Caveat: some patients with MDS may not have significant cytopenias will lead to revision of diagnostic terminology in upcoming WHO

Macrocytosis

- Most cases of MDS are macrocytic, however must exclude wide variety of other causes of macrocytosis
  - Vitamin deficiency- B12/folate- should see megaloblastic changes in other cell lines (PMNs)
  - Drugs, toxins
  - Reticulocytosis
Bone Marrow Aspirate

Moderate erythroid dysplasia, mild myeloid dysplasia, no increase in blasts. Iron stain showed NO ringed sideroblasts

Bone Marrow Biopsy

Hypercellular for age 40-50% (patchy), mild megakaryocytic dysplasia
Limits of Morphology

- Dysplasia may not necessarily = MDS
- Patients with MDS may not show definitive (>10%) morphologic evidence of dysplasia
- Dysplasia is not entirely reproducible among pathologists
- Sample quality

MDS-related cytogenetic abnormalities

- Complex karyotype*
- Cytogenetic abnormalities: 
  - +8
  - +18(2+)
  - 5q(4+)
  - 7q(4+)
  - 20q
  - +21
  - +11
  - -7, -7q
  - t(1;19)(q41;p13)
  - t(5;12)(q35;p13)
  - t(10;17)(q26.1;q25.3)

*2 aberrations
** rare occurrence of MDS in drugs this abnormality

Allows for presumptive diagnosis of MDS in absence of definitive morphology
Cytogenetic Anomalies Associated with MDS

Limits of Cytogenetics

- May not be available and requires special handling, good specimen
- Up to 50% of patients with MDS have a normal karyotype
- Non-specific abnormalities (e.g. del 20q, trisomy 8)

Cytogenetic Results for our Patient

- Cytogenetics- normal 46 (XY)
- MDS FISH Panel- no abnormalities detected

Utility of SNP Array in Cases of Suspected Myeloid Malignancy

- Paper examined the utility of array in 430 patients with myeloid malignancies
  - MDS: 250
  - MDS/MPN: 95
  - AML from MDS/MPN: 85
- In each of these patient subgroups, array was able to increase the diagnostic yield
  - MDS: 46% CHR to 73% CHR + Array
  - MDS/MPN: 38% CHR to 74% CHR + Array
  - AML from MDS/MPN: 45% CHR to 74% CHR + Array
Types of Genomic Changes Identified by SNP Arrays in MDS/MPD/AML

- Deletions on 5q and 7q
- Duplications on 5p, 1, 20p
- Triomies 8, 21
- UCN 3q, 4q, 7q, 11q, 21

Patients with SNP-array Findings or Chromosome Findings had Poorer Survival

OS: 16 vs. 43 months; P=.0001
EFS: 12 vs. 20 months; P=.0006
PFS: 11 vs 17 months; P=.002

Array Results for our Patient

- Single abnormality
- Loss of heterozygosity on chromosome 7q
  - This would not be picked up by cytogenetics but is in an area that is often associated with MDS and may be associated with poorer outcome
  - Indicates a loss of genetic material in this chromosome
Explosive Advances in Molecular Genetic Characterization of MDS

- Can mutations be used to diagnose MDS?
- Should MDS entities be defined by common molecular lesions or by common morphological / clinical features?
- Major caveats
  - Molecular genetic testing availability is not keeping up with its increasing relevance
  - Data is actively accumulating (moving target) as to utility in defining disease, prognosis and/or therapy

Somatic Mutation in MDS

Relation Between Number of Mutations and Outcome in MDS
Mutation Happens

Figure 1. Presence of Somatic Mutations, According to Age:
Colored bands, in increasing lighter shades, represent the 5th, 75th,
and 95th percentiles.


Blood. 2015 Jul 2;126(1):9-16.

Clinical hematopoiesis of indeterminate potential (CHIP)

- Absence of definitive morphological evidence of a
  neoplastic process
- Does not meet diagnostic criteria for PMF, MCL, or MIB
- Presence of a somatic mutation associated with
  hematological neoplasia at a variant allele frequency of
  at least 2% (e.g., CMMTA, TdT, IGH, SORL, AML, TRIST,
  TYK2, JAK2, JUB1, BCL11A, BCR, DEK, EGR2, ERAS,
  PRKDC, CDKN2A, CDKN2B, MLL2, SETD2, SPTD2, IDH1, IDH2, FGFR1, BCL2L12)
- 50% of progression to overt neoplasia are approximately
  G6; G12; G16; MCL; MIB; MIB1

Blood. 2015 Jul 2;126(1):9-16.
Negative Predictive Value of Mutations in MDS

• Greater than 85% of patients with MDS have one or more somatic mutations (Papaemmanuil et al, Blood 2013)

• If diagnosing MDS, a negative NGS result should prompt re-evaluation other causes of cytopenia(s)

Prognostic Value of Specific Mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation Description</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>c1900_1922del</td>
<td>11%</td>
</tr>
<tr>
<td>ETV6</td>
<td>c167dup</td>
<td>31%</td>
</tr>
<tr>
<td>EZH2</td>
<td>c.1505+1G&gt;A</td>
<td>66%</td>
</tr>
</tbody>
</table>

Molecular Results for our Patient

• 3 Tier 1 (clinically significant) variants present
  – ASXL1 c1900_1922del 11%
  – ETV6 c167dup 31%
  – EZH2 c.1505+1G>A 66%

  – EZH2 frequency suggest homozygosity and is consistent with LOH at chromosome 7q where EZH2 gene is located
  – ASXL1 mutations are associated with adverse outcomes as are multiple mutations but at a level that may indicate a subclone

Genotype-Phenotype Correlation
Role of Mutation in Diagnosis of MDS

• Beware -10% of healthy individuals >65 years harbor MDS-type mutations in hematopoietic cells.
  – Most DNMT3A, TET2, ASXL1, TPS3, JAK2, SF3B1
  – Allelic burden typically 10-20% in blood
  – Associated with increased risk of subsequent hematologic malignancies
• Currently mutations identification should not be used to diagnose MDS (further studies are needed)
  – Multiple mutations, particular combinations of mutations and/or high allele burden may provide more specificity for diagnosing MDS

2008 WHO Classification of MDS

• Refractory Cytopenia with Unilineage Dysplasia (RCUD)
  – Refractory Anemia (RA) Refractory Neutropenia (RN) Refractory Thrombocytopenia (RT)
• Refractory Anemia with Ring Sideroblasts (RARS)
• Refractory Cytopenia with Multilineage Dysplasia (RCMD)
• Refractory Anemia with Excess of Blasts (RAEB) Subtypes: RAEB - 1, RAEB – 2
• Myelodysplastic Syndrome with isolated del(5q) chrom. Abnormality
• Myelodysplastic syndrome, Unclassifiable (MDS,U)

Refractory Cytopenia with Multilineage Dysplasia (RCMD)

• One or more cytopenia or pancytopenia and dysplastic changes in two or more of the myeloid lineages
• There are <1% blasts in the blood and <5% in the marrow
• Ring sideroblasts can be present
• Auer rods are not present
  ➢ MDS with multilineage dysplasia

Revised International Prognostic Scoring System

Prognostic factors

<table>
<thead>
<tr>
<th>Component</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 points</td>
<td>3 points</td>
</tr>
</tbody>
</table>
| Cytopenia | + | - | - | +
| Recurrent failure (R) | + | + | - | - |
| Prior treatment (P) | + | - | - | - |
| Patient age (A) | + | - | + | - |
| Leukocyte count (L) | + | + | + | - |

Treatment

- **Very low risk**
  - Monitor

- **Low risk**
  - Monitor
  - EPO if transfusion dependent
  - Lenalidomide if transfusion dependent ≤ 5
  - In AML of hypoplastic and < 10

- **Intermediate, high, and very high** (treat the disease course, avoiding progression to AML)
  - Allogeneic BMT if BMT eligible
  - Asparaginase/decitabine if BMT ineligible
  - Clinical trials

Take Home

- For patients with possible MDS, integration of clinical history, CBC, morphology, conventional cytogenetics, new cytogenetic techniques (SNP arrays) and NGS data is **essential**
- Sequencing capabilities have advanced much faster than our understanding of genomics
- Detection of somatic variant(s) alone is **insufficient** to diagnose MDS