Myelodysplastic Syndrome: a pathologist’s perspective

Kajal Sitwala, MD, PhD

Case 1:
- 64 y.o. man with longstanding low back pain (unrelated)
- MRI showed abnormal bone marrow signal → referred to Hematology
- Had normocytic anemia dating back 15 years
- Bone marrow biopsy showed fairly normal overall cellularity, but relative expansion in erythropoiesis. Iron stain showed many ring sideroblasts
- Diagnosis of RARS (low-grade MDS)
  - Refractory Anemia with Ring Sideroblasts
- Interval treatment supportive (erythropoietin to boost RBC production)
- Follow-up bone marrow

MYELODYSPLASTIC SYNDROMES:

Part 1 – Example MDS cases coming into our practice over the last several weeks

Part 2 – Review of MDS definition, features, morphology, and biology

Dyserythropoiesis in aspirate smear

Ring sideroblasts (special stain of aspirate smear)

Hypercellularity; odd megakaryocytes, but not typical for MDS
Diagnosis:

**Persistent RARS**

- No increase in blasts
- Frank dysplasia still limited to the erythroid lineage
- Cytogenetic analysis showed same abnormality as before (+8)
- Trisomy 8 can be seen in myeloid disorders including MDS and AML
- For RARS, it’s worse prognosis to see it (versus normal karyotype)
- Flow cytometry: main contribution is confirming no increase in blasts
- Mild aberrancy of CD56 co-expression on maturing granulocytes and monocytes
  (supportive finding, not enough to definitively diagnose MDS)
- Recently, MDS-RARS shown to have strong association with gene mutation:
  - Haploinsufficiency of SF3B1

Case 2:

- 62 y.o. man with anemia and thrombocytopenia
- Bone marrow showed RAEB-1 (high-grade MDS)
- Refractory Anemia with Excess Blasts
- Treatment/management course complicated by cold agglutinin disease and transfusion refractoriness
- Recently, transfusion requirements became too severe to manage supportively, hospitalized for aggressive chemotherapy
- Bone marrow performed to assess response

Peripheral blood:
Granulocyte with mature, clumped chromatin but lack of granulation or nuclear lobation
(Pseudo-Pelger-Huet)

Increased blasts in aspirate smear

Diagnosis:

**Persistent MDS, now best classified as RAEB-2**

- In subsequent bone marrows, I only reclassify MDS if worse improvements are described, but disease isn’t “downgraded” as a new MDS subtype
- 14% blasts in differential count of aspirate smear
- Reticulin stain confirmed the presence of fibrosis
- Historically normal karyotype in leukemia cells; not repeated with this specimen
- Flow cytometry with 15% blasts, similar phenotype as prior cells
- Patient given even more aggressive treatment (AML induction), however, disease is persisting
Case 3:
- 83 y.o. woman with history of MDS dating back to 2008
  - When seen here in 2010, classified as RCMD
  - Refractory Cytopenia with Multilineage Dysplasia
- 2010 bone marrow with similar cytogenetic abnormalities, still low-grade (no increase in blasts)
- Since then, CBC counts have held pretty steady with lenolidamide treatment
  - uniquely efficacious in cases involving chromosomal deletions on 5q
- Some concern with low hemoglobin values, so bone marrow reassessed

![Basophilic stippling in peripheral blood](image)

![Dyserythropoiesis in aspirate smear](image)

![Small and hypolobated megakaryocytes in aspirate smear](image)

![Ring sideroblasts in aspirate smear](image)

**Diagnosis:**
**Persistent RCMD**

- Cytogenetics unchanged over past 5 years
  - 46,XX,del(5)(q13q33),add(11)(q23)[19]/46,XX[1]
  - doesn’t fit for isolated 5q deletion ("5q-minus syndrome") but some features overlap
  - often, dramatic worsening (e.g. increase in blasts) corresponds with clonal evolution
- Flow cytometry: increased basophils, light scatter changes (but no aberrant phenotype)
  - No increase in blasts
- Later: discuss role of lenolidamide in 5q deleted cases
**MDS**

A group of clonal hematopoietic stem cell diseases characterized by cytopenias, dysplasia, ineffective hematopoiesis, and increased risk of developing acute myeloid leukemia.

- Principally a disease of older adults
- Disorder of HSCs and their microenvironment

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**General (simplified) model of myeloid malignancies**

- **Class I**
  - Proliferation advantage
  - Survival advantage
  - Myeloproliferative disorders

- **Class II**
  - Differentiation arrest
  - Clonogenicity

**AML**

**So MDS is a stem cell neoplasm: but what does "immortalization" look like?**

- Clonogenic, but not rapid, proliferation of stem cells
- (There is also increased apoptosis)
- Inability to complete differentiation and leave marrow

- Hallmark of MDS – peripheral blood cytopenias PLUS bone marrow hypercellularity
- In contrast to MPDs...
  - Rapid proliferation but complete differentiation – cells accumulate in both places (clinical presentation from tumor burden rather than loss of function)
- Or acute leukemia...
  - Proliferation and lack of differentiation: blasts in bone marrow and blood

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**Clonogenicity:** (Can’t see whether a cell will keep dividing or not)

- Hypercellularity

**Maturation arrest:** (Marrow is full of precursors anyhow)

- Skew toward immaturity

As Hematopathologists, what we SEE is morphologic abnormalities that result from these molecular processes
Predictions about dysplasia, based on concept of “maturation arrest” –

1. immaturity of any kind
   a. cells that are more like a precursor form
   b. failure to develop characteristics of final state

2. dyssynchrony between different elements/split personality as far as further cell division

3. just plain weird

1a. Immaturity
   Excess blasts
   Erythroid immaturity (increase in early forms)
   Small megakaryocytes that have not divided the nucleus yet
1b. Failure to finalize
- Lack of granulation in circulating neutrophils
- Lack of nuclear lobation in circulating neutrophils
  *Mimic of harmless genetic state  
  (Pelger-Huet anomaly)

2. Dyssynchrony, or confusion about further division
- Hemoglobinized cytoplasm with still immature nucleus
- Nuclear budding in erythroid precursors
- Megakaryocytes with completely separated nuclear lobes
Patient with RAEB-1

Dysplastic erythroid precursor with hemoglobinization and large nucleus (ASH image Bank)

Patient with RCMD (core biopsy touch imprints)

Dysplastic megakaryocytes with separation of nuclear lobes (ASH image Bank)

Nuclear budding in erythroid precursor (ASH image Bank)

3. Just plain weird

- Ring sideroblasts
- Megaloblastoid chromatin in erythroid precursors
- Basophilic stippling in red blood cells
- Dimorphic circulating red cell population
- Vacuoles (especially erythroid precursors)
  * beware of MDS mimic – copper deficiency (sometimes caused by zinc toxicity)
That "classification" was purely speculative, a tool to mentally account for dysplasia.

Are there any cases where we have pinpointed the connection between genes and dysplasia?

**5q minus syndrome**
Anemia – can be quite severe
Normal to elevated platelet count; characteristic megakaryocytes
Hypercellular marrow, variable erythroid dysplasia
Female predominance – middle to older age

1974 – Nature – clinical syndrome with chr 5 long arm deletion reported
2001 – WHO classification recognizes 5q- as distinct subtype of MDS
2002 – Blood – commonly deleted region narrowed to 40 genes by FISH/Southern
2007 – ASH plenary abstract – identification of candidate gene

no biallelic deletions or point mutations in 40 genes – likely haploinsufficiency
therefore reduction in gene expression could be exploited: RNA interference
Megakaryocyte:erythroid ratio using markers CD41, glycophorin A

RPS14 participates in ribosomal synthesis
RPS19 mutations found in 25% Diamond-Blackfan Anemia cases

Haploinsufficiency of ribosomal proteins:
Phenotype of macrocytic anemia shows p53 dependence
-Cell cycle arrest, failure to complete erythroid differentiation
-Interestingly, 5q minus syndrome differs from other MDS by exhibiting erythroid hypoplasia

Studies in mice prove the genotype:phenotype correlation for anemia
Further research showed increased RPS14 levels after lenolidamide therapy
But, doesn't answer the whole question...megs are normal in those mice
Also, DBA patients (RPS19) don't have platelet or megakaryocyte problems

Targets of these microRNAs are transcription factors (TIARAP, TRAF6), and these in turn Regulate level of interferulin-6
Increased IL-6 can recapitulate megakaryocyte phenotype
Lenolidamide* reduces IL-6 levels
But wait...didn't they think lenolidamide worked by increasing RPS14 expression?
But wait...there's also the SPARC gene on 5q, necessary for mouse hematopoiesis, lenolidamide increases its expression as well!
*Thalidomide analog with many anti-cancer mechanisms in vitro and in vivo
Genotype:phenotype correlations are real, but messy and redundant
Hard to untangle, because of complexity of hematologic pathway, evolved in parallel to general embryogenic cascade
The genes used to regulate hematopoiesis are the same set of genes that determine body patterning during embryogenesis!

**Human genes:**

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</tr>
</tbody>
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-HOXA9 is most upregulated gene in AML
-Potent mediator of leukemia

**Summary – A pathologist’s perspective on MDS**

- WHO classification is our guide to approaching the diagnosis
  - Threshold is key
- CBC information is the predictor
  - This is how patients come to attention of hematology, then to us
- Morphology and cytogenetics make up our toolkit
  - Flow cytometry can sometimes contribute
  - FISH plays a more minor role than conventional karyotype
  - New studies support possible role for point mutations
- The biology guides our understanding, and contextualizes morphology
  - And genetics holds (some of) the answers