Diagnosis of Myelodysplastic Syndromes (MDS) and WHO Classification

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Normal Hematopoiesis is an orderly regulated process by morphology and immunophenotype.

**Erythroid**
- Pronormoblast
- Basophilic
- Polychrom. Orthochrom.
- Retic-
- Erythrocyte
- Normoblast
- Normoblast
- Normoblast
- Ulocyte

**Myeloid**
- Myeloblast
- Promyelocyte
- Myelocyte
- Meta-
- Myelocyte
- Band form
- Segmented Neutrophil

**Bone marrow**

**PB**

**Megakaryocytes**
- Megakaryoblast
- Promegakaryocyte
- Megakaryocyte

**Platelets**
What is Myelodysplastic Syndrome?

A heterogeneous group of clonal hematopoietic stem cell diseases resulting in INEFFECTIVE HEMATOPOIESIS:

One or more persistent peripheral cytopenias (REQUIRED):

- Anemia (IPSS <10 g/dL; MDS-IWG <13 g/dL)
- Neutropenia (IPSS and MDS-IWG ANC < 1.8K/uL)
- Thrombocytopenia (IPSS <100 K/uL; MDS-IWG <150 K/uL) (K/uL = x 10^9/L)

• Typically a disease of the elderly .......
• Dysplasia in one or more lineages (≥10% of cells in lineage)
• Hypercellular bone marrow (5-10% hypocellular)
• Less than 20% blasts (if 20% or greater the Dx is acute leukemia)
• Characteristic cytogenetic abnormalities seen in 50%
• Increased risk of developing AML (“pre-Leukemia”)
Diagnosis of MDS: Three Pilars

Peripheral Cytopenias

Morphologic Dysplasia

Cytogenetic Analysis

Courtesy of Dr. Adam Bagg
Morphologic features of dysplasia in ERYTHROID Lineage

**Nuclear:**
- Binucleation
- Nuclear budding
- Megaloblastic changes
- Karrhorhexis
- Chromatin bridging

**Cytoplasmic:**
- Ringed Sideroblasts
- Vacuolization

**Iron stain:**
- Ringed sideroblast

**Criteria for dysplasia:**
- Ringed Sideroblasts ≥ 15% of erythroid precursors
- If *SF3B1* mutation ≥ 5% of erythroid precursors
Morphologic features of dysplasia in MYELOID Lineage

- Hyposegmentation, “Pseudo-Pelger Huet”
- Hypogranularity
- Binucleation
- Left-shift with increased blasts
- Auer rods
- Pseudo-Chidiak-Higashi granules
- Abnormal maturation
Morphologic features of dysplasia in MEGAKARYOCYTIC Lineage

Dysplastic Megas:
- Separation of nuclear lobes
- Mononuclear Megakaryocytes
- Micromegakaryocytes
- Clustering

Normal Megakaryocyte

CD61 IHC

micromegakaryocytes

CD61 IHC
Left shift with increased myeloblasts = MDS with excess blasts
MDS EB 1 (5-9%), MDS EB 2 (10-19%)

Blast enumeration on Aspirate smear

Blast enumeration on core biopsy: CD34 IHC

10-15% CD34 positive cells c/w blasts
### Flow cytometric analysis of marrow in MDS

<table>
<thead>
<tr>
<th>Myeloid maturation</th>
<th>Myeloblast immunophenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD13 vs. CD16</strong></td>
<td><strong>CD38 vs. CD34</strong></td>
</tr>
<tr>
<td>Normal</td>
<td>CD7 vs. CD2</td>
</tr>
</tbody>
</table>

#### Normal

<table>
<thead>
<tr>
<th>CD13 vs. CD16</th>
<th>CD13 vs. CD11b</th>
<th>CD38 vs. CD34</th>
<th>CD7 vs. CD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.01%</td>
<td>36.62%</td>
<td>14.01%</td>
<td>1.73%</td>
</tr>
</tbody>
</table>

#### MDS

<table>
<thead>
<tr>
<th>CD13 vs. CD16</th>
<th>CD13 vs. CD11b</th>
<th>CD38 vs. CD34</th>
<th>CD7 vs. CD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD16 FITC</td>
<td>CD11b APC</td>
<td>CD38 APC-Cy7-A</td>
<td>CD7 APC-A</td>
</tr>
<tr>
<td>0.71%</td>
<td>45.66%</td>
<td>36.62%</td>
<td>17.01%</td>
</tr>
</tbody>
</table>

**Caution:** Bone marrow blast enumeration by flow cytometry can be skewed if blood contamination is present => blast count may be underestimated
Before a diagnosis of MDS is rendered all other causes of cytopenias or dysplastic features must be excluded

- Vitamin/mineral deficiency: iron, B12, Folate, Copper
- Infections: Parvovirus, CMV, HSV
- Immune Thrombocytopenic Purpura (ITP) or platelet disorder
- Drug effect, chemotherapy, growth factors, toxic exposure
- Autoimmune cytopenias
- Hemolytic anemia, anemia of chronic disease
- Rheumatologic disease, SLE
- Bone marrow failure: inherited or acquired aplastic anemia
WHO 2016: Diagnostic criteria for MDS entities

<table>
<thead>
<tr>
<th>Entity name</th>
<th>Dysplastic Lineages</th>
<th>Cytopenias</th>
<th>Ringed Sideroblasts</th>
<th>Blasts</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS-SLD</td>
<td>1</td>
<td>1-2</td>
<td>$&lt; 15% / &lt; 5%$</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-MLD</td>
<td>2-3</td>
<td>1-3</td>
<td>$&lt; 15% / &lt; 5%$</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-RS</td>
<td>1</td>
<td>1-2</td>
<td>$\geq 15% / \geq 5%$</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-RS-SLD</td>
<td>2-3</td>
<td>1-3</td>
<td>$\geq 15% / \geq 5%$</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-EB</td>
<td>1-3</td>
<td>1-2</td>
<td>None or any</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>del(5q) alone or with 1 additional abnormality, except loss of chromosome 7 or del(7q)</td>
</tr>
<tr>
<td>MDS-EB-1</td>
<td>1-3</td>
<td>1-2</td>
<td>None or any</td>
<td>BM 5–3% or PB 2–4%, BM &lt;10% and PB &lt;5%, no Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-EB-2</td>
<td>1-3</td>
<td>1-2</td>
<td>None or any</td>
<td>BM 10–19% or PB 5–19% or Auer rods, BM and PB &lt;20%</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-U</td>
<td>1-3</td>
<td>1-3</td>
<td>None or any</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-U with 1% blood blasts</td>
<td>1-3</td>
<td>1-3</td>
<td>None or any</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-U with SLD and pancytopenia</td>
<td>1</td>
<td>3</td>
<td>None or any</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-U based on defining cytogenetic abnormality</td>
<td>0</td>
<td>1-3</td>
<td>$&lt; 15%$</td>
<td>BM $&lt; 5%$, PB $&lt; 1%$, no Auer rods</td>
<td>MDS-defining abnormality$^6$</td>
</tr>
</tbody>
</table>

- **MDS w/ single lineage dysplasia**
- **MDS w/ multi-lineage dysplasia**
- **MDS with Ringed Sideroblasts**
- **MDS with Isolated del(5q)**
- **MDS with excess blasts – MDS-EB-1**
- **MDS-EB-2**
- **“High grade” 5-19% blasts => Increased risk of evolution to AML**
- **MDS Unclassifiable – MDS-U**
<table>
<thead>
<tr>
<th>Prognostic Group (%)</th>
<th>Cytogenetic abnormalities</th>
<th>Median Survival (yrs)</th>
<th>AML evolution (25%, yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Good</strong> (4%)</td>
<td>-Y, del(11q)</td>
<td><strong>5.4</strong></td>
<td>NR</td>
</tr>
<tr>
<td><strong>Good</strong> (69%)</td>
<td>Normal, del(5q), del (12p), del(20q) Double including del(5q)</td>
<td><strong>4.8</strong></td>
<td><strong>9.4</strong></td>
</tr>
<tr>
<td><strong>Intermediate</strong> (16%)</td>
<td>Del(7q), +8, +19, l(17q), any other single or double independent clones</td>
<td><strong>2.7</strong></td>
<td><strong>2.5</strong></td>
</tr>
<tr>
<td><strong>Poor</strong> (4%)</td>
<td>-7, inv(3), double including -7/del(7q) Complex: 3 abN</td>
<td><strong>1.5</strong></td>
<td><strong>1.7</strong></td>
</tr>
<tr>
<td><strong>Very Poor</strong> (7%)</td>
<td>Complex: &gt; 3abN</td>
<td><strong>0.7</strong></td>
<td><strong>0.7</strong></td>
</tr>
</tbody>
</table>

**MDS-U:**
MDS can be diagnosed in the absence of morphologic dysplasia if MDS associated cytogenetic abnormalities are detected, with the exception of:
- Trisomy 8
- Del(20q)
- Loss of Y

Recurrently mutated genes in MDS

- Splicing factors: SF3B1, SRSF2, U2AF1, ZRSR2
- DNA methylation: TET2, DNMT3A, IDH1/2

Why can’t we use mutations to diagnose MDS in the current WHO?

(with the exception of SF3B1 and ringed sideroblasts)
The mutations found in MDS are also found in Healthy Controls. “Clonal Hematopoiesis of Indeterminate Potential” (CHIP) and Aplastic Anemia.

Prevalence of Somatic Mutations in PBMCs, According to Age.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>No. with Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>20-29</td>
<td>240</td>
</tr>
<tr>
<td>30-39</td>
<td>855</td>
</tr>
<tr>
<td>40-49</td>
<td>2894</td>
</tr>
<tr>
<td>50-59</td>
<td>5441</td>
</tr>
<tr>
<td>60-69</td>
<td>5002</td>
</tr>
<tr>
<td>70-79</td>
<td>2300</td>
</tr>
<tr>
<td>80-89</td>
<td>317</td>
</tr>
<tr>
<td>90-99</td>
<td>86</td>
</tr>
<tr>
<td>100-108</td>
<td>17</td>
</tr>
</tbody>
</table>

Ogawa, S. Blood 2016
MDS in Pediatric Population

- RARE - Often presents with neutropenia and thrombocytopenia with RBC macrocytosis
- Hypocellular marrow more common (~80%) may overlap with AA or IBMFS
- Mutation landscape profile differs from adult MDS (RAS pathway mutations more common)
- Associated with germline mutations:
  - GATA2 (7%), RUNX1, SAMD9/SAMD9L, etc
  - Inherited bone marrow failure syndromes (Fanconi anemia, SDS, DBA, etc.)

WHO: Refractory Cytopenia of Childhood (RCC):
- Dysplasia in ≥ 10% of cells in ≥ 1 lineages
- < 5% blasts in marrow and < 2% blasts in PB

Increased blasts => same as MDS in adults:
MDS EB1: 5-9% blasts in marrow or 2-4% blasts in PB
MDS EB2: 10-19% blasts in marrow or PB 5-19% blasts or Auer Rods
New WHO 2016 Chapter: Myeloid Neoplasms with Germline Predisposition

Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction

- Acute myeloid leukemia with **CEBPA** mutation
- Myeloid neoplasms with germline **DDX41** mutation
- Myeloid neoplasms with germline predisposition and pre-existing platelet disorders
- Myeloid neoplasms with germline **RUNX1** mutation
- Myeloid neoplasms with germline **ANKRD26** mutation
- Myeloid neoplasms with germline **ETV6** mutation

Recognition of germline predisposition is important for:

- **Donor selection for HSCT**
  - avoid healthy matched related donors who may harbor same mutation to prevent donor-derived MDS/AML or failed engraftment
- **Treatment/conditioning regimens**
- **Genetic counseling**

Myeloid neoplasms with germline predisposition associated with other organ dysfunction

- Myeloid neoplasms with germline predisposition and **GATA2** mutation
- Inherited bone marrow failure syndromes
- Telomere biology disorders
- JMML associated with **Neurofibromatosis**, Noonan syndrome or Noonan-like disorders
- Myeloid neoplasms associated with **Down syndrome**
Therapy-Related Myeloid Neoplasm: t-MDS, t-AML and t-MDS/MPN

- 70-80% occur 5-10 yrs after exposure to alkylation agents or ionizing radiation => t-MDS

- 20-30% occur 1-5 yrs after topoisomerase II inhibitors => t-AML

- Prior cancer: 70% solid tumors, 30% hematolymphoid

- Subset of patients have heritable predisposition with germline mutations in TP53, BRCA1/2 or other genes.