Myelodysplastic syndromes revisited

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Massachusetts General Hospital and Harvard Medical School
Outline of presentation

• Review criteria required to establish a diagnosis of MDS according to the 2016 WHO Classification

• Present the revised 2016 WHO MDS disease categories
  – Distinguishing features of each category
  – Changes from 2008 Classification based on new data
Myelodysplastic syndromes

• Clonal hematopoietic stem cell diseases
  – At diagnosis, the vast majority of hematopoietic cells are part of the neoplastic clone
  – Clone has recurring genetic abnormalities
• Ineffective hematopoiesis with one or more peripheral cytopenias
• Morphologic dysplasia of maturing hematopoietic elements
• Variable increase in myeloblasts (<20%)
  – May progress to AML with differing propensities depending on disease subtype
Challenges in MDS diagnosis

- Does the patient have a neoplasm?
- Should the patient be treated for MDS or should another diagnosis be sought?

Risk-adapted therapy according to prognosis

- Should the patient receive induction or other intensive chemotherapy with a goal of remission?
Components of MDS diagnosis and classification (2016 WHO)

Unexplained cytopenias are a sine qua non of MDS

Dysplasia is a defining feature of MDS

90% of MDS cases have a demonstrable clonal genetic abnormality

Dysplasia and blasts

Dysplasia is defining feature of MDS
Information needed by pathologist to diagnose MDS

- **Clinical history**
  - Full CBC and WBC differential results
  - Knowledge of duration of cytopenias and possible other causes of cytopenia

- **Morphology review**
  - Blood smear
  - Bone marrow aspirate or touch prep
    - Wright-Giemsa and iron stains
  - Bone marrow biopsy

- **Complete bone marrow karyotype**
Complications in defining cytopenia

ANC x $10^9$/L

- <1.0
- 1.1 to 1.4
- 1.5 to 1.7
- 1.8 to 1.9
- ≥2.0

HGB g/dL

- 8.0 to 10.0
- 11.0 to 12.0
- 13.0
- 14.0

Platelets x $10^9$/L

- 80 to 100
- 110 to 140
- 150 to 180

Dysplasia assessment

• Threshold of 10% of cells in any lineage
• No distinction between different types of dysplastic morphologies
• Dysplasia is not always reproducible, even among experienced hematopathologists
• Dysplasia is not specific for MDS
  – Significant dysplasia in bone marrow of normal volunteers
  – Dysplastic changes are even more frequent in patients with non-neoplastic cytopenias

Can we do better than ≥10%?

<table>
<thead>
<tr>
<th>Morphological abnormalities</th>
<th>Cutoff values</th>
<th>AUC</th>
<th>Cohen’s K-coefficient (inter-observer agreement)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Erythroid lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megaloblastoid changes</td>
<td>&gt; 5%</td>
<td>0.814, $P &lt; 0.001$</td>
<td>0.83</td>
</tr>
<tr>
<td>Bi- or multinuclearity</td>
<td>&gt; 3%</td>
<td>0.679, $P &lt; 0.001$</td>
<td>0.87</td>
</tr>
<tr>
<td>Nuclear lobulation or irregular contours</td>
<td>&gt; 5%</td>
<td>0.698, $P &lt; 0.001$</td>
<td>0.84</td>
</tr>
<tr>
<td>Pyknosis</td>
<td>&gt; 3%</td>
<td>0.674, $P &lt; 0.001$</td>
<td>0.84</td>
</tr>
<tr>
<td>Cytoplasmic fraying</td>
<td>&gt; 5%</td>
<td>0.677, $P &lt; 0.001$</td>
<td>0.81</td>
</tr>
<tr>
<td>Ring sideroblasts</td>
<td>≥ 15%</td>
<td>0.719, $P &lt; 0.001$</td>
<td>0.82</td>
</tr>
<tr>
<td>Ferritin sideroblasts</td>
<td>≥ 30%</td>
<td>0.670, $P &lt; 0.001$</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>Granulocytic lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>&gt; 3%</td>
<td>0.777, $P &lt; 0.001$</td>
<td>0.92</td>
</tr>
<tr>
<td>Auer rods</td>
<td>&gt; 5%</td>
<td>0.723, $P &lt; 0.001$</td>
<td>0.90</td>
</tr>
<tr>
<td>Pseudo Pelger–Hüet anomaly</td>
<td>≥ 1%</td>
<td>0.524, $P = 0.001$</td>
<td>0.87</td>
</tr>
<tr>
<td>Abnormal nuclear shape</td>
<td>&gt; 5%</td>
<td>0.814, $P &lt; 0.001$</td>
<td>0.86</td>
</tr>
<tr>
<td>Neutrophil hypogranulation</td>
<td>≥ 7%</td>
<td>0.700, $P &lt; 0.001$</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Megakaryocytic lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micromegakaryocytes</td>
<td>&gt; 5%</td>
<td>0.916, $P &lt; 0.001$</td>
<td>0.88</td>
</tr>
<tr>
<td>Small binucleated megakaryocytes</td>
<td>&gt; 5%</td>
<td>0.845, $P = 0.001$</td>
<td>0.81</td>
</tr>
<tr>
<td>Megakaryocytes with multiple separated nuclei</td>
<td>&gt; 5%</td>
<td>0.750, $P &lt; 0.001$</td>
<td>0.84</td>
</tr>
<tr>
<td>Hypolobated or monolobar megakaryocytes</td>
<td>&gt; 5%</td>
<td>0.646, $P &lt; 0.001$</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Della Porta MG Leukemia 2015;29:66
Flow cytometry assessment of MDS

- Abnormal flow cytometry patterns predict MDS with good sensitivity and specificity
- WHO 2016 and ELN guidelines do not permit a diagnosis of MDS solely based on flow cytometry
  - Considered ‘supportive’ of a diagnosis
  - More data needed on reactive conditions

Abnormalities in blasts

Abnormalities in maturing elements


Courtesy Of Dr S Wang, MD Anderson Cancer Center
Somatic mutations in MDS

- Ribosomal proteins: \textit{RPS14}
- Epigenetic regulators: \textit{TET2}, \textit{ASXL1}
- RNA splicing: \textit{SF3B1}, \textit{SRSF2}, \textit{U2AF1}
- Transcription factors: \textit{RUNX1}, \textit{ETV6}
- Tyrosine kinase signaling: \textit{RAS}
- Tumor suppressor genes: \textit{TP53}

Some genetic abnormality is present in \textasciitilde 90\% of MDS cases
Impact of the explosive advance of molecular genetics on MDS

• Can mutations be used to diagnose MDS?
• Should MDS entities be defined by common molecular lesions or by common morphologic/clinical features?
• Major caveats
  – Molecular genetic testing availability is not keeping up with its increasing relevance
  – Data is actively accumulating ("moving target")
“Clonal Hematopoiesis of Indeterminate Potential” (CHIP)

- A proportion of apparently healthy aging individuals harbor somatic MDS-type mutations in hematopoietic cells
  - DNMT3A, TET2, ASXL1, TP53, JAK2, SF3B1
  - Allele burden typically 10-20% in blood, can be higher
  - Associated with increased risk of subsequent hematologic malignancy and death from other causes
  - Many patients never develop cytopenias or MDS even after many years of followup

Both CHIP and MDS affect older individuals, but CHIP is far more frequent

INCIDENCE OF MDS PER 100,000

<1% incidence

10-15% incidence

CHIP: “Clonal Hematopoiesis of Indeterminate Potential”

Risk stratify
Treat as appropriate

Seek other causes of cytopenias

Kwok B et al. Blood 2015;126:2355
Summary: what is sufficient to diagnose MDS in according to WHO 2016?

<table>
<thead>
<tr>
<th>Observation</th>
<th>Sufficient to diagnose MDS in isolation?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplastic morphology (≥10%)</td>
<td>Yes, provided possible secondary causes of cytopenia and dysplasia are excluded clinically</td>
</tr>
<tr>
<td>Excess marrow blasts (≥5%)</td>
<td>Yes, provided marrow recovery or growth factor effect are excluded</td>
</tr>
<tr>
<td>Cytogenetic abnormality</td>
<td>Yes, provided it is on the WHO list of ‘approved’ abnormalities (excluding +8, -7, del20q)</td>
</tr>
<tr>
<td>Flow cytometry abnormality</td>
<td>No, but can support an MDS diagnosis suspected by other observations</td>
</tr>
<tr>
<td>MDS-type mutation</td>
<td>No, these can be found in normal individuals (“clonal hematopoiesis of indeterminate potential”); may support an MDS diagnosis suspected by other observations</td>
</tr>
</tbody>
</table>
Morphologic diagnosis of MDS remains subjective

- Morphologic dysplasia
  - ↑ Lineages involved
  - ↑ Number of dysplastic forms
  - ↑ Severity of dysplasia
- Severity and persistence of cytopenia(s)
- Unexplained ↑ MCV
- Flow cytometry abnormalities
- MDS-type mutations

- Younger patients
- Co-morbid conditions
- Paucity of clinical history
MDS classification: new terminology

**WHO 2016**
- MDS with single lineage dysplasia (MDS-SLD)
- MDS with multilineage dysplasia (MDS-MLD)
- MDS with ring sideroblasts
  - MDS-RS with single lineage dysplasia (MDS-RS-SLD)
  - MDS-RS with multilineage dysplasia (MDS-RS-MLD)
- MDS with isolated del(5q)
- MDS, unclassifiable (MDS,U)
- MDS with excess blasts (MDS-EB)
- *Refractory cytopenia of childhood (RCC)(provisional)*

**WHO 2008**
- Refractory cytopenia with unilineage dysplasia (RCUD)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory anemia with ring sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS)
- MDS with isolated del(5q)
- MDS, unclassifiable (MDS,U)
- Refractory anemia w/excess blasts (RAEB)
- *Refractory cytopenia of childhood (RCC)(provisional)*
## Prognostic schemes in MDS

<table>
<thead>
<tr>
<th></th>
<th>WHO Classification (2016)</th>
<th>IPSS-R* (2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplasia</td>
<td>Yes: single versus multilineage and ring sideroblasts</td>
<td>No</td>
</tr>
<tr>
<td>Cytopenias</td>
<td>Yes: Pancytopenia is only defining feature</td>
<td>Yes: both number and depth of cytopenias</td>
</tr>
<tr>
<td>Blast % in blood</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Blast % in bone marrow</td>
<td>Yes</td>
<td>Yes (include ≤2% group and up to 29%)</td>
</tr>
<tr>
<td>Karyotype</td>
<td>Yes: isolated del(5q) is the only defining feature</td>
<td>Yes, 5 prognostic groups</td>
</tr>
<tr>
<td>Molecular genetic abnormalities</td>
<td>Yes (SF3B1 mutation)</td>
<td>No</td>
</tr>
<tr>
<td>Flow cytometry abnormalities</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Revised International Prognostic Scoring System of MDS

Greenberg PL et al. Blood 2012;120:2454
Bone marrow blast percentage strongly influences overall survival in MDS

- 2% blast threshold will not be adopted by WHO
- Precise blast count should be specified in report so that IPSS-R can be applied

Greenberg PL et al. Blood 2012;120:2454
- Aspirate blast count is ‘gold standard’
- CD34 immunostaining of biopsy is important if aspirate is compromised
Blast counting in myeloid neoplasms with erythroid predominance (≥50% erythroids)

- 2008 WHO classification rule allows acute erythroid leukemia (AEL) diagnosis if blasts comprise ≥20% of non-erythroid cells if erythroids are ≥50% of marrow cells.

MDS-EB or AEL?

>20% of non-erythroid
Controversies in blast counting...


Dysplastic erythroid precursors in the myelodysplastic syndromes: Is there biologic significance? (How similar to myelodysplastic syndrome with excess blasts should blasts be counted? Acute erythroid leukemia with <20% bone marrow blasts is clinically and biologically

Erythroid predominant myelodysplastic syndromes: engraftment myelodysplastic risk stratification than total marrow blast counts provide...
New WHO 2016 recommendations for blast counting

• Blasts in BM always counted as % of total cells, never as % of non-erythroid cells

• Myeloid neoplasms with ≥50% erythroids and with blasts <20% all cells will now be classified as MDS-EB, even if blasts are ≥20% of non-erythroid cells
  – MDS-EB now includes most cases previously diagnosed as acute erythroleukemia (i.e. with ≥20% non-erythroid blasts, but 5-19% total blasts)
  – Pure erythroid leukemia will remain in AML

Main new data incorporated into 2016 WHO Classification of MDS

• Significance of point mutations
  – Large body of information confirm significant impact of mutations on prognosis
  – Most data is still too immature to determine how to incorporate mutations into existing primarily morphologic classification

• New data help refine definition of MDS with isolated del(5q) and MDS with ring sideroblasts

• Elimination of acute erythroid leukemia, with inclusion of most cases in MDS with excess blasts
SF3B1: a spliceosome gene where mutation conveys a favorable prognosis

- Strongly correlates with the presence of ring sideroblasts (RS)
  - Mutated in 70-80% of MDS cases with >15% RS, very rare in cases lacking RS
- Appears to be an early founding mutation in MDS
- Associated with longer survival in MDS patients

SF3B1 mutation is associated with highly differential gene expression

Includes downregulation of ABCB7 gene due to altered exon usage

New handling of MDS with ring sideroblasts in WHO 2016

• MDS with ring sideroblasts (MDS-RS) is now broadened to include:
  – Traditional RARS (single lineage erythroid dysplasia)
  – Cases with multilineage dysplasia (“RCMD-RS”)
    • Less frequent $SF3B1$ mutation and poorer prognosis than MDS-RS with single lineage dysplasia
  – Cases with $SF3B1$ mutation and $\geq 5\%$ RS
    • If $SF3B1$ mutation status is negative or unknown, $\geq 15\%$ RS are required

• Presence of $SF3B1$ mutation or RS does not affect MDS with excess blasts or isolated del(5q)

Malcovati L et al. Blood 2015;126:233
No adverse effect with one additional cytogenetic abnormality

MDS with isolated del(5q): new data

TP53 mutation confers poor prognosis to del(5q) patients treated with lenalidomide

Changes to MDS del(5q) in the 2016 update

• Broaden definition to allow one additional cytogenetic abnormality (except -7 or del7q)
• Suggest $TP53$ mutation test or p53 immunostain for prognostic information
• Any cases with increased blasts in blood or bone marrow are still excluded from the MDS del(5q) category

MDS, unclassifiable (MDS-U): a heterogeneous group

- MDS with SLD but with pancytopenia

  *All cytopenias must be below IPSS levels: ANC<1.8 \times 10^9/L, HGB<10 \text{ g/dL}, PLT<100 \times 10^9/L*

- MDS-RS,-SLD,-MLD, del(5q) with exactly 1% PB blasts

  *1% PB blasts must be measured on at least two separate occasions*

- MDS without excess blasts or dysplasia, but with an MDS-defining cytogenetic abnormality
### 2016 WHO Classification of MDS-1

<table>
<thead>
<tr>
<th></th>
<th>Dysplastic lineages</th>
<th>Cytopenias</th>
<th>RS as % of erythroids</th>
<th>BM and PB blasts</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDS-SLD</strong></td>
<td>1</td>
<td>1 or 2&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;15%/&lt;5%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt;5% BM, &lt;1% PB&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Any&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>MDS-MLD</strong></td>
<td>2 or 3</td>
<td>1-3</td>
<td>&lt;15%/&lt;5%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt;5% BM, &lt;1% PB&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Any&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>MDS-RS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with SLD</td>
<td>1</td>
<td>1 or 2</td>
<td>≥15%/≥5%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt;5% BM, &lt;1% PB&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Any&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>with MLD</td>
<td>2 or 3</td>
<td>1-3</td>
<td>≥15%/≥5%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt;5% BM, &lt;1% PB&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Any&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>1-3</td>
<td>1 or 2</td>
<td>None or any</td>
<td>&lt;5% BM, &lt;1% PB&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Del(5q) alone or with 1 other abnormality (except -7/del7q)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Note that cytopenic lineage frequently does not correlate with dysplastic lineage in MDS-SLD

<sup>2</sup> If *SF3B1* mutation is present

<sup>3</sup> Also no Auer rods

<sup>4</sup> Unless fulfills all criteria for MDS with isolated del(5q)

Reorganization of low-grade MDS

2008 WHO

RCUD

RARS

RCMD (+/- RS)

MDS isolated del(5q)

2016 WHO

MDS-SLD

MDS-RS (+/- MLD)

MDS-MLD

MDS isolated del(5q)

≥5% RS + SF3B1 mutation

Multilineage dysplasia + ≥15% RS or ≥5% RS/SF3B1 mutation

del(5q) + one additional abnormality
## 2016 WHO Classification of MDS-2

<table>
<thead>
<tr>
<th></th>
<th>Dysplastic lineages</th>
<th>Cytopenias</th>
<th>RS as % of erythroids</th>
<th>BM and PB blasts</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS-EB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS-EB1</td>
<td>0-3</td>
<td>1-3</td>
<td>None or any</td>
<td>5-9% BM or 2-4% PB&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-EB2</td>
<td>0-3</td>
<td>1-3</td>
<td>None or any</td>
<td>10-19% BM or 5-19% PB or Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>MDS, unclassifiable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with 1% PB blasts</td>
<td>1-3</td>
<td>1-3</td>
<td>None or any</td>
<td>&lt;5% BM, =1% PB&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>Any</td>
</tr>
<tr>
<td>with SLD and pancytopenia</td>
<td>1</td>
<td>3</td>
<td>None or any</td>
<td>&lt;5% BM, &lt;1% PB&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Any</td>
</tr>
<tr>
<td>based on karyotype</td>
<td>0</td>
<td>1-3</td>
<td>&lt;15%</td>
<td>&lt;5% BM, &lt;1% PB&lt;sup&gt;2&lt;/sup&gt;</td>
<td>MDS-defining</td>
</tr>
</tbody>
</table>

<sup>1</sup> BM blast percentage always derived from all nucleated cells, even if erythroids are ≥50%

<sup>2</sup> Also no Auer rods

<sup>3</sup> 1% PB blasts must be documented on at least 2 separate occasions
Special situations in MDS

• Hypoplastic MDS
  – About 10% of cases
  – Differential diagnosis with aplastic anemia
  – CD34 and CD61 immunostains of biopsy

• MDS with fibrosis
  – 10-15% of cases
  – Differential diagnosis with MPN and MDS/MPN
  – CD34 and CD61 immunostains of biopsy
  – Adverse prognosis

• MDS in children
  – Refractory cytopenia of childhood still a provisional entity in 2016 WHO
    – Usually hypoplastic, differential diagnosis with aplastic anemia
    – Different mutational profile from adult MDS
  – MDS-EB and therapy-related MDS also occur in children

Myeloid neoplasms with germline predisposition: new WHO category

• Encompasses myeloid neoplasms (MDS, AML, MDS/MPN) arising in the background of a predisposing mutation or congenital syndrome

• Can include cases with or without a known syndrome, platelet disorder, or family history
  – Examples: CEBPA, RUNX1, ETV6, ANKRD26, DDX41, GATA2

• Germline predisposition should be appended to diagnosis, e.g.
  – Refractory cytopenia of childhood with germline GATA mutation
  – MDS with excess blasts associated with Fanconi anemia

Challenges in genetic predisposition syndromes

• Identifying the germline mutation
  – Need to sequence non-hematopoietic tissue to know for certain that the mutation is germline
  – Need to be alert to clues: detailed personal & family history and use of experienced genetic counselors
  – Often are newly arising mutations (family history unhelpful)

• Some entities present in adulthood
  – MDS/AML with \textit{DDX41} mutation

• Distinguishing platelet disorders and bone marrow failure conditions from MDS

• Implications for family members (including potential bone marrow donors)

Thrombocytopenia with germline ANKRD26 mutation

AML with germline GATA2 mutation

Courtesy of L Peterson and J Vardiman
Conclusions: MDS diagnosis and classification should optimally incorporate multiple modalities

- Impact of various factors on outcome in 124 MDS patients
- Optimal model is achieved by combining all information
- Future models must also take into account response to various therapies

SH/EAHP 2017 Workshop
September 7-9, 2017
Chicago, Illinois USA
Molecular Genetics of Hematolymphoid Neoplasms

Organizers
Robert Hasserjian, Frank Kuo, Olga Weinberg (Harvard)

Case submission opens on October 16!