The New WHO Classification of MDS

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DISCLOSURE

I have no relevant financial relationships to disclose.
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
Why should we classify myeloid disorders?

• Serves as a ‘lingua franca’
  – Pathologists and clinicians should be sure that they are diagnosing, treating, and studying the same disease entities

• Opportunity to define diagnostic features of individual diseases
  – Reference point for diagnosticians

• Process of classifying diseases (and re-examining existing classification systems) highlights areas that warrant further study
The 2008 WHO Classification (4th Edition)

• Sponsored by American (SH) and European (EAHP) Hematopathology Societies
• 8 editors selected by the societies
• 75 authors: U.S., Canada, Europe, Asia, Australia
• WHO Clinical Advisory Committee Meetings in Chicago and Virginia in 2007
  – Over 100 international hematologists and oncologists participated
• Publication in Sept. 2008 as “Blue Book”
The revised 4th edition classification

• Clinical advisory committee of 33 clinicians and 18 pathologists met in Chicago in April 2014
  – Prior to the meeting, participants proposed updates to the disease categories, which were discussed at the meeting

• Chapter revisions began in fall 2014
• Final versions of chapters submitted April 2016
• Myeloid editor (Thiele) and senior advisors (Arber, Orazi, Hasserjian, Le Beau) currently reviewing galley proofs

• Revised classification due to be published as a new “Blue Book” in early 2017
### Organization of the 2016 WHO Classification

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPN</td>
<td>Myeloproliferative neoplasms</td>
</tr>
<tr>
<td>Mastocytosis</td>
<td></td>
</tr>
<tr>
<td>MDS/MPN</td>
<td>Myelodysplastic/myeloproliferative neoplasms</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic syndromes</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td><strong>Red</strong></td>
<td>Myeloid neoplasms with germline predisposition</td>
</tr>
<tr>
<td><strong>Red</strong></td>
<td>Myeloid/lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, *FGFR1*, or <strong>PCM1-JAK2</strong></td>
</tr>
</tbody>
</table>
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)

Dysplasia and blasts

Unexplained cytopenias are a sine qua non of MDS

90% of MDS cases have a demonstrable clonal genetic abnormality

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 1.9
  - ≥2.0

- **HGB g/dL**:
  - 8.0 to 10.0
  - > 10.0

- **Platelets x 10^9/L**:
  - 80 to 100
  - > 100

- **HGB g/dL**:
  - < 10 (WHO/IPSS)
  - < 11 (2007 MDS Consensus)
  - < 12/13 (WHO anemia definition)

- **Platelets x 10^9/L**:
  - < 100 (WHO/IPSS)
  - < 150 (normal reference)

References:
WHO 2016 cytopenic thresholds

• ‘Traditional’ original IPSS thresholds still apply
  – Absolute neutrophil count <1.8 x 10⁹/L
  – Hemoglobin <10 g/dL
  – Platelets <100 x 10⁹/L

• MDS may be diagnosed with milder cytopenias if definitive diagnostic criteria are present
  – Hemoglobin <12/13 g/dL for females/males
  – Platelets <150 x 10⁹/L
  – Should use individual laboratory reference ranges as applicable

• Ethnic and conditional variation should be taken into account

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

## Specificity of dysplastic findings

<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, <em>P</em> &lt; 0.001</td>
<td>0.83</td>
</tr>
<tr>
<td>Bi- or multinuclearity</td>
<td>&gt; 3%</td>
<td>0.679, <em>P</em> &lt; 0.001</td>
<td>0.87</td>
</tr>
<tr>
<td>Nuclear lobulation or irregular contours</td>
<td>&gt; 5%</td>
<td>0.698, <em>P</em> &lt; 0.001</td>
<td>0.84</td>
</tr>
<tr>
<td>Pyknosis</td>
<td>&gt; 3%</td>
<td>0.674, <em>P</em> &lt; 0.001</td>
<td>0.81</td>
</tr>
<tr>
<td>Cytoplasmic fraying</td>
<td>≥ 7%</td>
<td>0.602, <em>P</em> &lt; 0.001</td>
<td>0.82</td>
</tr>
<tr>
<td>Ring sideroblasts</td>
<td>&gt; 5%</td>
<td>0.650, <em>P</em> &lt; 0.001</td>
<td>0.95</td>
</tr>
<tr>
<td>Ferritin sideroblasts</td>
<td>≥ 15%</td>
<td>0.719, <em>P</em> &lt; 0.001</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>≥ 30%</td>
<td>0.670, <em>P</em> &lt; 0.001</td>
<td></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, <em>P</em> &lt; 0.001</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>&gt; 5%</td>
<td>0.723, <em>P</em> &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Auer rods</td>
<td>≥ 1%</td>
<td>0.524, <em>P</em> = 0.001</td>
<td>0.90</td>
</tr>
<tr>
<td>Pseudo Pelger–Hüet anomaly</td>
<td>&gt; 3%</td>
<td>0.714, <em>P</em> &lt; 0.001</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>&gt; 5%</td>
<td>0.814, <em>P</em> &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Abnormal nuclear shape</td>
<td>≥ 7%</td>
<td>0.700, <em>P</em> &lt; 0.001</td>
<td>0.86</td>
</tr>
<tr>
<td>Neutrophil hypogranulation</td>
<td>&gt; 3%</td>
<td>0.791, <em>P</em> &lt; 0.001</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>&gt; 5%</td>
<td>0.821, <em>P</em> &lt; 0.001</td>
<td></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, <em>P</em> &lt; 0.001</td>
<td>0.88</td>
</tr>
<tr>
<td>Small binucleated megakaryocytes</td>
<td>&gt; 5%</td>
<td>0.845, <em>P</em> = 0.001</td>
<td>0.81</td>
</tr>
<tr>
<td>Megakaryocytes with multiple separated nuclei</td>
<td>&gt; 5%</td>
<td>0.750, <em>P</em> &lt; 0.001</td>
<td>0.84</td>
</tr>
<tr>
<td>Hypolobated or monolobar megakaryocytes</td>
<td>&gt; 5%</td>
<td>0.646, <em>P</em> &lt; 0.001</td>
<td>0.86</td>
</tr>
</tbody>
</table>

*9% false positive*

*5% false positive*

*11% false positive*  
*30% cutoff better than 10%*  

Della Porta MG et al. Leukemia 2015;29:66
Can we develop a more objective way to diagnose MDS?

• Flow cytometry abnormalities
  – Hematopoiesis in most MDS cases is phenotypically abnormal

• Genetic abnormalities
  – Karyotype abnormalities (only 50% of cases)
  – Sub-karyotypic acquired genetic alterations
    • Microdeletions (SNP array)
    • Mutations (next-generation sequencing)
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.

# MDS-defining cytogenetic abnormalities (WHO 2016)

<table>
<thead>
<tr>
<th>Unbalanced</th>
<th>Primary MDS</th>
<th>Therapy-related MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7 or del(7q)</td>
<td>10%</td>
<td>50%</td>
</tr>
<tr>
<td>-5 or del(5q)</td>
<td>10%</td>
<td>40%</td>
</tr>
<tr>
<td>i(17q) or t(17p)</td>
<td>3-5%</td>
<td></td>
</tr>
<tr>
<td>-13 or del(13q)</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>del(11q)</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>del(12p) or t(12p)</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>del(9q)</td>
<td>1-2%</td>
<td></td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td>1-2%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Balanced</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;16)(q23;p13.3)</td>
<td>3%</td>
</tr>
<tr>
<td>t(3;21)(q26.2;q22.1)</td>
<td>2%</td>
</tr>
<tr>
<td>t(1;3)(p36.3;q21.2)</td>
<td>1%</td>
</tr>
<tr>
<td>t(2;11)(p21;q23)</td>
<td>1%</td>
</tr>
<tr>
<td>inv(3)(q21q26.2)</td>
<td>1%</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>1%</td>
</tr>
</tbody>
</table>

~50% of MDS have a normal karyotype

+8, -Y, and del(20q) are common in MDS, but can occur in non-neoplastic conditions and are not MDS-defining
Some genetic abnormality is present in ~90% of MDS cases.
“Clonal Hematopoiesis of Indeterminate Potential” (CHIP)

• A proportion of apparently healthy older individuals harbor somatic MDS-type mutations in hematopoietic cells
  – *DNMT3A, TET2, ASXL1, TP53, JAK2, SF3B1*
  – 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

• CHIP phenomenon precludes the current use of mutations in isolation to diagnose MDS

Summary: what is sufficient to diagnose MDS in according to WHO 2016?

<table>
<thead>
<tr>
<th>Observation</th>
<th>Sufficient to diagnose MDS in a cytopenic patient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplastic morphology (≥10%)</td>
<td><strong>Yes</strong>, provided possible secondary causes of cytopenia and dysplasia are excluded clinically</td>
</tr>
<tr>
<td>Excess marrow blasts (≥5%)</td>
<td><strong>Yes</strong>, provided marrow recovery or growth factor effect are excluded</td>
</tr>
<tr>
<td>Cytogenetic abnormality</td>
<td><strong>Yes</strong>, provided it is on the WHO list of ‘approved’ abnormalities (excluding +8, -Y, del20q)</td>
</tr>
<tr>
<td>Flow cytometry abnormality</td>
<td><strong>No</strong>, but can support an MDS diagnosis suspected by other observations</td>
</tr>
<tr>
<td>MDS-type mutation</td>
<td><strong>No</strong>, 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
# Prognostic schemes in MDS

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dysplasia</strong></td>
<td>Yes: single versus multilineage and ring sideroblasts</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cytopenias</strong></td>
<td>Yes: Pancytopenia is only defining feature</td>
<td>Yes: both number and depth of cytopenias</td>
</tr>
<tr>
<td><strong>Blast % in blood</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Blast % in bone marrow</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Karyotype</strong></td>
<td>Yes: isolated del(5q) is the only defining feature</td>
<td>Yes, 5 prognostic groups</td>
</tr>
<tr>
<td><strong>Molecular genetic abnormalities</strong></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Flow cytometry abnormalities</strong></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 is not part of WHO 2016
- Precise blast count should be specified in report so that IPSS-R can be applied

Greenberg PL et al. Blood 2012;120:2454
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 the existing primarily morphologic classification

• New data help refine definitions 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
<table>
<thead>
<tr>
<th>WHO 2016</th>
<th>WHO 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS with single lineage dysplasia (MDS-SLD)</td>
<td>Refractory cytopenia with unilineage dysplasia (RCUD)</td>
</tr>
<tr>
<td>MDS with multilineage dysplasia (MDS-MLD)</td>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
</tr>
<tr>
<td>MDS with ring sideroblasts</td>
<td>Refractory anemia with ring sideroblasts (RARS)</td>
</tr>
<tr>
<td>- MDS-RS with single lineage dysplasia (MDS-RS-SLD)</td>
<td>Refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS)</td>
</tr>
<tr>
<td>- MDS-RS with multilineage dysplasia (MDS-RS-MLD)</td>
<td>MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>MDS, unclassifiable (MDS,U)</td>
</tr>
<tr>
<td>MDS, unclassifiable (MDS,U)</td>
<td>MDS, unclassifiable (MDS,U)</td>
</tr>
<tr>
<td>MDS with excess blasts (MDS-EB)</td>
<td>Refractory anemia excess blasts (RAEB)</td>
</tr>
<tr>
<td><em>Refractory cytopenia of childhood (RCC)</em> (provisional)</td>
<td><em>Refractory cytopenia of childhood (RCC)</em> (provisional)</td>
</tr>
</tbody>
</table>
MDS with isolated del(5q)
MDS with isolated del(5q): new data

No adverse effect with one additional cytogenetic abnormality

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

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

• Broaden definition to allow one additional cytogenetic abnormality (except -7 or del7q)
• Recommend 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 with ring sideroblasts: strong association with SF3B1 mutation

- RNA splicing factor
  - Mutated in 70-80% of MDS with >15% ring sideroblasts
  - Very rare in MDS lacking ring sideroblasts
- Appears to be an early founding mutation
- Associated with longer survival in MDS patients

SF3B1 mutation is associated with highly differential gene expression

Significant mutation-expression coefficients ($q<0.05$)

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 broadened to include:
  – “Traditional” RARS (single erythroid lineage dysplasia)
  – Cases with multilineage dysplasia
  – Cases with $SF3B1$ mutation and $\geq 5\%$ RS
    • If $SF3B1$ mutation status is negative or unknown, $\geq 15\%$ RS is required

• Presence of $SF3B1$ mutation or RS does not affect MDS with excess blasts or 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
MDS, unclassifiable (MDS-U): a heterogeneous group

- MDS with single lineage dysplasia, but with pancytopenia

  *All cytopenias must be below IPSS levels:*
  - ANC<1.8 x 10^9/L, HGB<10 g/dL, PLT<100 x 10^9/L

- Low-grade MDS 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
MDS with excess blasts (MDS-EB)

• ≥5% blasts in marrow or ≥2% blasts in blood
  – Subdivided into MDS-EB-1 and MDS-EB-2 based on marrow and blood blast levels
• Increased blasts are a very strong indicator of aggressive behavior in MDS, independent of cytogenetics, cytopenias, and mutations
• CD34 immunostaining useful in cases with fibrosis or poor aspirate

• Aspirate blast count is ‘gold standard’
• CD34 immunostaining of biopsy is important if aspirate is compromised
Controversies in blast counting...

Considering Bone Marrow Blasts From Nonerythroid Cellularity Improves the Prognostic Evaluation of Myelodysplastic Syndromes

Dysplastic erythroid precursors in the myelodysplastic syndromes and the acute myeloid leukemias: Is their biologic significance? (How should blasts be counted?)

Acute erythroid leukemia with <20% bone marrow blasts is clinically and biologically similar to myelodysplastic syndrome with excess blasts

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 and erythroids are ≥50% of marrow cells
- Small changes in blast or erythroid percentages can change diagnosis, with major clinical impact
Most AEL patients may not benefit from intensive chemotherapy

- AEL: ≥50% erythroids and blasts ≥20% of non-erythroids
- MDS-erythroid: ≥50% erythroids and 5-9% blasts
- MDS-typical: <50% erythroids and 5-19% blasts

Wang SA et al. Mod Pathol 2016;29:1221
New WHO 2016 recommendations for blast counting

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

• Myeloid neoplasms with ≥50% erythroids and blasts <20% all cells are now classified as MDS-EB, even if blasts are ≥20% of the non-erythroid cells
  – Merges most cases previously diagnosed as acute erythroleukemia into MDS-EB
  – Pure erythroid leukemia remains as an AML subtype
    • Malignant proliferation of *immature erythroblasts*

MDS in children

- ‘Conventional’ types of MDS (~50%)
  - MDS-EB, classified the same as for adults
  - Therapy-related MDS
  - MDS-RS and isolated del(5q) are very rare in children

- Refractory cytopenia of childhood (~50%)
  - Usually hypocellular, important differential diagnosis with aplastic anemia
  - Mutations are less common than in adult MDS (22% of cases) and have a different profile
    - SETBP1, ASXL1, NRAS/KRAS, RUNX1, BCOR/BCORL

References:
Myeloid neoplasms with germline predisposition: new WHO category

• Without a pre-existing platelet disorder or organ dysfunction
  – Germline CEBPA and DDX41 mutation

• With pre-existing platelet disorders
  – Germline RUNX1, ANKRD26, and ETV6 mutation

• Associated with organ dysfunction
  – Germline GATA2 mutation
  – Bone marrow failure syndromes, telomere biology disorders, neurofibromatosis, Noonan syndrome

Thrombocytopenia with germline *ANKRD26* mutation

AML with germline *GATA2* mutation

Courtesy of L Peterson and J Vardiman
Challenges in genetic predisposition myeloid neoplasms

• 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 and family history and use of experienced genetic counselors
  – Often newly arising mutations where family history is 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)

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