Myelodysplastic syndromes and related disorders, diagnosis and classification
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Bullet points

• Morphology is still the “gold standard” for diagnosis (requires high quality smears and biopsy sections).
• Do not forget the peripheral blood which is integral part to the WHO classification and particularly important in MDS, MDS/MPN and in MPN.
• To diagnose MDS: \( \geq 10\% \) of cells in a lineage should be dysplastic to consider the lineage as dysplastic. Be aware of conditions which can be responsible for non-clonal marrow dyspoiesis.
• Stain the bone marrow aspirate smear for iron.
• Perform a 500 cell differential count. Be aware of the definition of blast.
• Always correlate the aspirate smear findings with those seen in the bone marrow biopsy.
• Immunohistology with CD34 and other selected markers can be useful, particularly in cases with suboptimal aspirates and/or fibrotic and fatty marrows.
• Flow cytometry provides useful information but is not diagnostic on its own. Particularly useful in CMML. Also do not forget cytochemistry for nonspecific esterase to confirm marrow monocytosis.
• Cytogenetics is an integral part of the prognostic assessment by both IPSS and WPSS.
• In appropriate clinical setting, certain cytogenetic abnormalities are considered as presumptive of MDS (not yet evaluated for MDS/MPN).
• Remember to assess for PDGFR abnormalities (in the presence of eosinophilia).
• In the presence of myeloproliferation always assess for JAK2 mutation.
• The WHO classification is useful only if properly applied (e.g. do not forget to examine P.B. smear or to get adequate clinical information).

Key words: WHO classification, myelodysplastic syndromes, myelodysplastic/myeloproliferative neoplasms, flow cytometry, immunohistology, cytogenetics, JAK2, BCR-ABL

Text
Myelodysplastic syndromes (MDS) are hematopoietic stem-cell disorders characterized by ineffective hematopoiesis producing marrow failure and a propensity to progress into acute myeloid leukemia (1,2). The pathogenetic mechanisms causing primary MDS are still largely unknown. Development and progression of MDS suggests multistep alterations affecting the hematopoietic stem cell. Different molecular alterations have been described, affecting genes involved in cell-cycle control, mitotic checkpoints, and growth factor receptors. Secondary signal proteins and transcription factors which gives the cell a growth advantage over its normal counterpart, may be affected as well. The abnormal hematopoietic cell of MDS, although actively proliferating, is largely incapable of normal differentiation necessary for its release into the blood, and dies in the marrow through programmed cell death or apoptosis. The cause of the excess proliferation and apoptosis is unknown, although some have suggested that abnormal signaling from factors produced by marrow stroma, such as FAS ligand or TNF-alpha and TGF-beta may play a role. The event responsible for the initial genetic insult is generally unknown, but the predominance of MDS in the elderly suggests that exposure to a number of environmental or occupational toxins may play some role. Cytogenetic abnormalities are
frequent in MDS. Loss of cytogenetic material is the most common abnormality while balanced translocations are uncommon. This may suggest a tumor suppressor gene mechanism. However, loss of function of one allele followed by a “second hit” with loss of function of the alternate allele has not been detected in most MDS patients. Haploinsufficiency, i.e., the reduction in the level of one or more gene products critical for gene function, has been recently demonstrated in cases of MDS with isolated deletion of 5q (3). Anomalies in the epigenetic transcriptional control mechanisms have also been detected in a large proportion of patients with MDS. They include promoter hypermethylation that can cause transcriptional silencing of multiple genes. Of these, the most often associated with MDS has been \( P15 \). This may have important clinical implications because of the current interest in the development and clinical use of antimiethyting agents in MDS patients (4).

**The classification and prognostic scoring systems**

MDS display impressive clinical heterogeneity running the gamut from an indolent disease with a near normal life expectancy to an aggressive malignancy overlapping acute myeloid leukemia (AML). Following the original 1982 FAB classification scheme (5), various additional systems to improve prognostic predictive power in MDS have been proposed. In 1997, Greenberg et al developed the international prognostic scoring system (IPSS), based on bone marrow blast percentage, cytogenetic subgroups of outcome, and number of cytopenias (6). In 2001, the FAB guidelines were revised and their updated version was integrated into the World Health Organization (WHO) Classification System (7). More recently, transfusion dependency has been shown to have a significant effect on survival of MDS patients (8). The integration of transfusion dependency within the WHO system has produced the so-called WHO classification based prognostic scoring system (WPSS) (8). The WHO classification system has been recently updated and the revised WHO Classification (4th Edition) was published and made available in the fall of 2008 (1).

The concept of the WHO system is that classification of hematopoietic neoplasms should be based not only on morphologic findings, but also clinical, genetic, immunophenotypic, and biologic information should be used to define separate disease entities (1). However because a single biologic or genetic marker that reliably identifies all or most of the cases of MDS has not yet been discovered, bone marrow morphology remains the most important tool for classifying the majority of patients with MDS. Although concordance of the diagnosis of MDS is generally reported to be ~ 80%, this only applies to cooperative clinical trials. Outside of this setting, concordance among different observers is often considerably less (2). The factors which are largely responsible for inconsistencies in diagnosis and classification include the variability in the quality of the specimens, inaccurate enumeration of blasts, and the erroneous inclusions in the MDS category of patients with non-clonal dysplastic hematopoiesis. Therefore, the importance of a critical evaluation of morphology in the light of other laboratory results and clinical information cannot be overemphasized (2,9).

**How to diagnose MDS**

Morphological examination requires peripheral blood smear, bone marrow aspirate and bone marrow trephine biopsy. Biopsies should be of an adequate size (at least 1.5 cm, excluding cortical bone) and bone marrow aspirates should be both particulate and cellular enough to
perform a 500 cells differential count (1). The blood and marrow aspirate smears should be examined for dysplasia (defined as \( \geq 10\% \) of the cells for each given lineage), the percentage of blasts, and the iron stained marrow aspirate for ring sideroblasts. Cells counted as “blasts” include myeloblasts, monoblasts, and megakaryoblasts. Micromegakaryocytes are not blasts, and erythropoietic precursors are also not counted as blasts, except in rare cases of “pure” erythroleukemia, in which abnormal proerythroblasts account for the majority of cells (1).

Flow cytometry diagnosis of MDS is still a “work in progress”. Substitution of the percent of blasts determined by flow cytometry (e.g. by counting CD34 positive cells) for a visual blast count is discouraged because not all blasts express CD34. Furthermore, dilution of the marrow sample by peripheral blood, as well as further cellular loss during processing of the sample may cause discrepancy between the visual estimate and the blast value obtained by flow cytometry. Besides the blast count, multiparameter flow cytometry can also provide evidence of abnormal maturation of the myeloid lineages. There is no single abnormality that is specific, but abnormal light scatter properties, abnormal antigen density, loss of antigens and/or anomalous expression of antigens have all been reported in MDS and may even correlate with the grade of disease. However, the specificity of aberrant antigen expression for MDS, as compared to diseases in which secondary dysplasia may be seen, has not been extensively studied, and aberrant antigen expression has been documented in non-hematologic disorders, such as autoimmune disease, that morphologically can mimic MDS (1,2). The WHO approach recommends that cases with inconclusive morphologic and cytogenetic findings and three or more aberrant features by flow cytometry should be re-evaluated over several months for definitive morphologic or cytogenetic evidence of MDS (1). The value of bone marrow biopsy in this group of disorders is generally well established (1,9,10). It can increase the diagnostic accuracy and helps in refining the risk evaluation system (11). The current 4th edition of the WHO classification provides more information in this regard. Besides a “quality check” of the adequacy of the marrow aspirate, the biopsy provides information on cellularity and stroma (e.g. presence of myelofibrosis) and yields tissue for a number of studies including immunohistochemistry, in-situ hybridization, or other molecular procedures that can provide additional diagnostic information (9,12). An important application of bone marrow biopsy immunohistochemistry is in evaluating MDS associated neoangiogenesis (13). Additionally, bone marrow biopsy may help in confirming a suspected diagnosis of MDS by excluding reactive conditions in which dysmyelopoietic changes may be at times prominent (e.g. HIV infection, autoimmune diseases). An underappreciated role of the biopsy is that it may provide evidence for another disease that can mimic MDS clinically, such as hairy cell leukemia, lymphoma, or metastatic non-hematopoietic malignancy (2). Among the alterations detected by bone marrow biopsy, a prognostically important finding is the presence of aggregates or clusters of blasts, a typical finding in aggressive subtypes of MDS (1,9). These can also be identified by immunohistochemistry with CD34 (1,9,14). The blast enumeration by immunohistologic analysis is especially helpful in cases of MDS with fibrosis (MDS-F) (15) and cases of MDS associated with hypocellular marrows (hypoplastic MDS) (16,17). In both these variants, the presence of reticulin fibrosis (in the former) or fatty changes (in the latter) can make accurate disease characterization very difficult or impossible using bone marrow aspirates. Moreover, the often low cellular yield of the bone marrow aspirate in these cases may also be insufficient to obtain adequate cytogenetic information. Specific diagnostic examples include the separation of MDS-F from several subtypes of acute myeloid leukemia characterized by bone marrow fibrosis (e.g. acute panmyelosis with myelofibrosis, acute megakaryoblastic leukemia, therapy related AML) and the identification of hypoplastic MDS and its separation from aplastic
Anemia (17-19). A description of these two morphologic variants of MDS is included in the current 4th WHO edition (“MDS Overview” section).

**Refractory cytopenia with unilineage dysplasia**

This designation, used for the first time in the current 4th WHO edition, encompasses those MDS that present a refractory cytopenia (of at least 6 months duration, in the absence of a confirmatory cytogenetic abnormality) associated with dysplasia in ≥10% of cells in a single cell line. Subtypes include refractory anemia (RA) and the rare cases of isolated refractory neutropenia (RN) and refractory thrombocytopenia (RT). Refractory bicytopenia may be included in this category if accompanied by unilineage dysplasia, but refractory pancytopenia that is associated with unilineage dysplasia should be considered as MDS, unclassifiable. The clinical presentation, in most cases, is related to anemia (these cases are properly designated as RA). In RA, anemia may be normocytic and normochromic, but is often macrocytic. In the blood, blasts are absent or represent <1% of circulating leukocytes, and they account for fewer than 5% of the nucleated marrow cells. The marrow is usually hypercellular due to erythroid hyperplasia, and dyserythropoiesis is present, but ring sideroblasts account for fewer than 15% of the erythroid cells. In RA, fewer than 10% of the cells in the granulocytic or megakaryocytic lineages show dysplasia. Monocytes are <1 × 10^9/L in the blood, and there is no monocytosis (<5% monocytes; normal range 0–4%) in the bone marrow. No Auer rods are present. The diagnosis is one of exclusion—other causes of anemia with dyserythropoiesis, such as megaloblastic anemia and congenital dyserythropoiesis must be carefully excluded. In general, RA can be considered as a “low-grade” MDS. Median survival times with unilineage dysplasia are reported to be 6 to 7 years, and only ~10% progress to overt acute leukemia. RN and RT are very rare and likely account for less than 1% to 2% of all cases of MDS. Other causes of neutropenia and thrombocytopenia need to be excluded, and extreme caution should be used in making such diagnoses.

**Refractory anemia with ring sideroblasts (RARS)**

RARS is an MDS characterized by unexplained anemia, morphologic dysplasia in the erythroid lineage, and ring sideroblasts comprising 15% or more of the erythroid precursors. There is no significant (<10%) dysplasia in granulocytic or megakaryocytic lineages. Anemia is the principal finding. The RBCs often exhibit a “dimorphic” pattern of normochromic and hypochromic cells, but macrocytosis is frequently observed as well. The criteria are similar to those described for RA with unilineage dysplasia, except that in the bone marrow, ring sideroblasts account for ≥15% of the erythroid precursors. Like RA, RARS is a “low-grade” process. In most series, RARS is reported to have the best prognosis and lowest rate of conversion to AML of all of the subtypes of MDS. Median survival times of 7 to 9 years or longer are commonly reported, and the conversion rate to acute leukemia is <5%. Whether some patients diagnosed with RARS, who have no evidence of a cytogenetic abnormality, may have ring sideroblasts only due to mitochondrial DNA abnormalities without clonal abnormalities in nuclear DNA is not clear. If the platelet count is greater than 450 × 10^9/L, and the megakaryocytes have features of those described in the MPNs, an analysis for a JAK2 V617F mutation and assignment to the provisional entity of refractory anemia with ring sideroblasts and thrombocytosis (RARS-T, see MDS/MPN section) should be considered.

**Refractory cytopenia with multilineage dysplasia (RCMD)**
This MDS subtype was an addition which was first introduced in the classification of MDS by the WHO 2001. Its identification has improved the prognostic utility of the morphologic classification of MDS. RCMD is characterized by one or more cytopenias in the peripheral blood and dysplastic changes in ≥10% of cells in two or more of the myeloid lineages: erythroid, granulocytic, and/or megakaryocytic series. In RCMD, there are <1% blasts in the blood and <5% blasts in the bone marrow, and Auer rods are not found. If 15% or more of the erythroid precursors are ring sideroblasts, the designation of RCMD with ring sideroblasts (RCMD-RS) can be made; although in the setting of multilineage dysplasia, there is no clear prognostic impact if ring sideroblasts are found or not. Cases that meet the criteria for RCMD, but have persistently 1% blasts in the blood, should be considered as MDS, unclassifiable (see below), while those with multilineage dysplasia and <5% blasts in the bone marrow but 2% to 4% blasts in the blood should be classified as RAEB-1. Patients with RCMD have a worse outcome (reported median survival times are 17–33 months) than patients with RA.

Refractory anemia with excess of blasts (RAEB)

RAEB is used to describe MDS with 5% to 19% blasts in the bone marrow or blood. However, if there are <5% blasts in the bone marrow, the finding of 2% to 4% blasts in the peripheral blood is sufficient for the diagnosis. Two subcategories are recognized. RAEB-1 is defined as having 5% to 9% blasts in the bone marrow or 2% to 4% in the blood. If blasts are 10% or more in the marrow, or 5% or more in the blood, the designation should be RAEB-2. These patients have a worse survival and higher rate of transformation to AML than those with 5% to 9% blasts in bone marrow (RAEB-1). RAEB is a serious disorder, regardless of whether it transforms to overt acute leukemia. Although 30% to 40% of patients with RAEB develop AML, more will die from the complications of neutropenia, thrombocytopenia, or anemia. Median survival times for RAEB-1 are ~18 months versus 10 months for those with RAEB-2.

MDS associated with an isolated del(5q) chromosomal abnormality

This subtype of MDS is characterized by anemia, with or without other cytopenias, and interstitial deletion of the long arm of chromosome 5q as the sole cytogenetic abnormality. Myeloblasts comprise <5% of nucleated bone marrow cells and <1% of peripheral blood leukocytes; Auer rods are not seen. An interstitial deletion of the long arm of chromosome 5 is a common abnormality in MDS, and may be seen in isolation or as part of a more complex karyotype. Patients with isolated del(5q) have a high response rate to the drug lenalidomide.

Myelodysplastic syndrome, unclassifiable (MDS, U)

This subtype encompasses those cases that do not fit easily into the other categories of MDS. Three possible situations which qualify a patient for this category include (1) patients with RCUD or RCMD with 1% blasts in the blood, (2) MDS with morphologic unilineage dysplasia associated with pancytopenia, and (3) patients with persistent cytopenias lacking diagnostic morphologic features of MDS or of any specific subgroups of MDS (i.e., <10% dysplastic cells in any lineage) but with cytogenetic abnormalities considered as presumptive evidence of MDS.

MDS related disorders: myelodysplastic/myeloproliferative neoplasms (MDS/MPN)

The MDS/MPN are rare conditions which demonstrate, at disease presentation, hybrid characteristics with myelodysplasia plus atypical findings more suggestive of MPN, such as
leukocytosis, thrombocytosis, and/or significant organomegaly (1,20). They do not fit well into any given category and can only be accurately categorized by using a systematic approach similar to that previously illustrated for the MDS (9,20). The best characterized members of the MDS/MPN group include chronic myelomonocytic leukemia (1,20,21) and, in pediatric patients, juvenile myelomonocytic leukemia (1). Other subtypes because of their rarity are less well characterized. These include atypical myeloid leukemia (aCML), a BCR-ABL and JAK2 negative (1,22) leukemic disorder most often seen in older individuals which is characterized by a poor prognosis, as well as several other poorly understood syndromes. One such syndrome is termed refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T) (23,24). The hallmark of this condition is the presence of erythroid cells similar to those seen in patients with refractory anemia with ring sideroblasts (RARS), which are found in association with large megakaryocytes, more similar to those seen in cases of Ph’ chromosome negative MPN (e.g., essential thrombocythemia) (24). The demonstration in RARS-T of a high frequency of positivity for JAK2 V617F mutation, possibly acquired as a secondary genetic event, gives further support to a possible relationship with a classical myeloproliferative neoplasm (23). Less frequently MPL W515K/L mutation has also been described (25). An additional example of an MDS/MPN-like condition includes rare cases of MDS with isolated 5q- abnormality and myeloproliferative characteristics (26). This hybrid condition must be differentiated from the classical 5q- syndrome, a well defined subtype of MDS. Further examples of a MDS/MPN-like overlapping syndrome are rare cases of chronic myeloid neoplasms associated with isolated isochromosome 17q (27). This syndrome is characterized by neutrophilia with dysplasia (hyposegmentation of neutrophil nuclei), variable monocytosis, and a hypercellular marrow with pleomorphic megakaryocytes, variable fibrosis, and a high rate of transformation to AML.

**Conclusion**

In conclusion, while eagerly awaiting a “genetically based” classification scheme free of morphologic subjectivity, the WHO 2008 classification offers a valuable tool in the diagnosis and classification of MDS. The overall goal remains the same: keeping the classification flexible and usable worldwide while, at the same time, making it open to the inclusion of new information.

**References**

22. Fend F, Horn T, Koch I, Vela T, Orazi A. Atypical Chronic Myeloid Leukemia as defined in the WHO classification is a JAK2 V617F negative neoplasm. Leuk Res. 2008;32:1931-5.


MDS are hematopoietic stem-cell disorders characterized by:

- Ineffective hematopoiesis
- Marrow failure
- Propensity to progress into AML

[Diagram showing the relationship between MDS and AML with the process of escape from apoptotic control]
Molecular pathogenesis of MDS

- **Cytogenetics** -- Loss of cytogenetic material is most common (balanced translocations are rare)
- **Tumor suppressor gene mechanism** -- Loss of function of one allele via chromosomal deletion, point mutation or transcriptional silencing. None found in the majority of MDS
- **Haploinsufficiency** -- Reduction in the level of one or more gene products critical for gene function. Haploinsufficiency for \( RPS14 \) been described in cases of 5q- syndrome
- **Epigenetic transcriptional control mechanism alterations** -- Promoter hypermethylation can cause transcriptional silencing of multiple genes, e.g., \( P15 \) (>50% of MDS). Predicts poor prognosis in early-stage MDS
MDS are clinically heterogeneous: Classification/Scoring Systems

- Based on morphologic differences including % blasts
- FAB plus dysplasia (10% rule; uni vs. multi), and cytogenetics (5q-)

FAB
1976, 1982

IPSS
1997

WHO
2001

WHO
2008

WPSS
2007

Based on % blasts, karyotype, # of cytopenias

Based on WHO classification, karyotype, and transfusion

Modified from Dr. John Bennett
## WHO classification-based Prognostic Scoring System (WPSS)


<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td><strong>WHO category</strong></td>
<td>RA, RARS, 5q-</td>
<td>RCMD, RCMD-RS</td>
<td>RAEB-1</td>
<td>RAEB-2</td>
</tr>
<tr>
<td><strong>Karyotype</strong>*</td>
<td>Good</td>
<td>Intermediate</td>
<td>Poor</td>
<td>-</td>
</tr>
<tr>
<td><strong>Transfusion Requirement</strong></td>
<td>No</td>
<td>Regular°</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Karyotype: Good = normal, -Y, del(5q), del(20q); Poor = complex, -7 or 7q-; Intermediate = other abnormalities

°RBC transfusion dependency: Defined as having at least one RBC transfusion every 8 weeks over a period of 4 months

**Risk groups:** very low (score 0), low (1), intermediate (2), high (3-4), very high (5-6)
Survival & Risk Based on WPSS

Diagnostic Guidelines: MDS

Adapted from: Greenberg, et al: NCCN Practice Guidelines in Oncology v.4.2006

Cytopenia(s) and its duration
- Hemoglobin <10 g/dL
- Neutrophil count <1,800/μL
- Platelet count <100,000/μL

History of blood diseases /relevant conditions, e.g., autoimmune disorders, malignancies, therapy

Physical [e.g. splenomegaly]

Labs: Vit B12, Folate, Iron/ferritin, EPO, LDH
Morphologic Guidelines for the Diagnosis and Classification of MDS:

Well-prepared blood and bone marrow aspirate smears should be examined for:

- Dysplasia (10% or more rule)
- Ring sideroblasts (iron stain on aspirate)
- Blast percentage (500 cell differential)

[Exclude monocytosis]

Correlate the findings with marrow biopsy
Erythroid precursors - Highly suggestive features include: multinucleation/asymmetrical nuclei, nuclear bridging
Erythroblasts with 1/3 or more of the nucleus encircled by ≥5 siderotic granules
Granulocytic cells - Highly suggestive features include: Pelger-Huet like nuclei, hypogranular cytoplasms

Megakaryocytes - Highly suggestive features include: Dwarf forms non/hypo-lobated including micro megakaryocytes
Non-clonal dysplasia

**Dyserythropoiesis**
- B12/folate/copper deficiencies
- Methotrexate and other chemotherapy
- Alcohol, toxic compounds (e.g. arsenic, zinc)
- Autoimmune conditions
- Malignancy associated (paraneoplastic)
- Aplastic anemia/PNH
- Infections (parvo, HIV)

**Dysgranulopoiesis**
- Cytokines (G-CSF)
- Bone marrow regeneration
- Status post BMT

**Dysmegakaryopoiesis**
- Infections (HIV)
- Chemotherapy
- Status post transplant
- TMPD (Down syndrome)
Blast Count

• 500 cell differential
• Blasts:
  <5 %; 5-10 %; 11-19%
• Cells counted as “blasts” include:
  - Myeloblasts
  - Monoblasts
  - Mk-blasts
  But not erythroblasts!
How to count the blasts?

BM biopsy in MDS

• “Quality check” of the adequacy of marrow aspirate (e.g. hemodilution)
• Provides information on cellularity and stroma changes (e.g. myelofibrosis)
• Help to exclude unsuspected pathology
• Yields tissue for a number of studies:
  - immunohistochemistry
  - in-situ hybridization
  - molecular procedures, (e.g. microdissection)

Immunohistology

- **Blasts**: CD34, CD117
- **Erythroblasts**: HB, glycophorin A
- **Promyelocytes***: MPO+ (CD34-/CD117-)
- **Megakaryocytes**: CD42b, CD61
- **Monocytes**: CD68R, CD163
- **Lymphoid cells**: TdT, lymphoid markers

*Post G-CSF
Blast count is poorly reproducible particularly in thick paraffin sections. Clusters of blasts more specific.
CD34 pos. cell clusters: 3-5 cells

The identification of blast clusters made easier by immunohistology (e.g. CD34, CD117)
Clusters $\rightarrow$ Aggregates $\rightarrow$ Sheets in AML

Disease progression by CD34
Dwarf MKs: their identification is facilitated by immunohistology (e.g. CD42b, CD61)
Flow cytometry can provide evidence of abnormal myeloid maturation
(abnormalities in light scatter and antigenic expression)

Q: “Can flow cytometry parameters be added to the diagnostic criteria for MDS?”

A: “FC results are highly suggestive of MDS only if there are three or more aberrant features in erythropoietic, granulocytic or monocytic maturation; single aberrant features are not significant.

Cases with inconclusive morphologic and cytogenetic findings and three or more aberrant features by flow cytometry should be re-evaluated over several months for definitive morphologic or cytogenetic evidence of MDS.” Substitution of blast % determined by flow cytometry for a visual blast count is discouraged

WHO-CAC: discussion on MDS, Chicago, IL Feb. 2007
# Prognostic significance of cytogenetic abnormalities in MDS

<table>
<thead>
<tr>
<th>Risk</th>
<th>Abnormality</th>
<th>Median Survival</th>
<th>Time to AML*</th>
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<tbody>
<tr>
<td>Favorable</td>
<td>Normal</td>
<td>3.8 yrs</td>
<td>5.6 yrs</td>
</tr>
<tr>
<td></td>
<td>Isolated del(5q)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Isolated del(20q)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Isolated –Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>Other</td>
<td>2.4 yrs</td>
<td>1.6 yrs</td>
</tr>
<tr>
<td>Poor</td>
<td>-7, del(7q)</td>
<td>0.8 yrs</td>
<td>0.9 yrs</td>
</tr>
<tr>
<td></td>
<td>Complex</td>
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</table>

*25% of patients
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)
<table>
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<tr>
<th>Refractory Cytopenia</th>
<th>PB</th>
<th>BM</th>
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<tbody>
<tr>
<td>Refractory cytopenia with unilineage dysplasia (RA, RN, RT)</td>
<td>Mono-/bi-cytopenia, No or &lt;1% blasts</td>
<td>Dysplasia (≥10%) unilineage, &lt;5% blasts, &lt;15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with ring sideroblasts</td>
<td>Anemia, No blasts</td>
<td>Erythroid dysplasia only, &lt;5% blasts, ≥15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia</td>
<td>Cytopenia(s), No or &lt;1% blasts</td>
<td>Dysplasia in ≥10% of the cells of two or more myeloid lineages, &lt;5% blasts, ±15% ring sideroblasts</td>
</tr>
</tbody>
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Note: Monocytes <1 × 10⁹/L; No Auer rods
<table>
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<tr>
<th>Condition</th>
<th>PB</th>
<th>BM</th>
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<tr>
<td>Refractory anemia with excess blasts-1</td>
<td>Cytopenias</td>
<td>Uni- or multi-lineage dysplasia</td>
</tr>
<tr>
<td></td>
<td>&lt;5% blasts*</td>
<td>5–9% blasts*</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>No Auer rods</td>
</tr>
<tr>
<td></td>
<td>*Also if &lt; 5% blasts in BM but 2-4% in PB</td>
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<tr>
<td>Refractory anemia with excess blasts-2</td>
<td>Cytopenias</td>
<td>Uni- or multi-lineage dysplasia</td>
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<tr>
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<td>5–19% blasts</td>
<td>10–19% blasts</td>
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<tr>
<td></td>
<td>Auer rods +/-</td>
<td>Auer rods +/-</td>
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<tr>
<td>MDS with isolated del(5q)</td>
<td>Anemia, usually normal or mildly increased platelets</td>
<td>Normal to increased megakaryocytes with hypolobated nuclei</td>
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<tr>
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<td>&lt;5% blasts</td>
<td>&lt;5% blasts</td>
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<tr>
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<td>No Auer rods</td>
<td>No Auer rods</td>
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[Note: Monocytes <1 × 10⁹/L]
MDS: WHO 2008

Subtypes and changes
RCUD: Refractory Anemia WHO

- Mono- or bi-cytopenia
- No or <1% blasts in blood
- Blasts in marrow <5%
- Dyserythropoiesis only
- <15% of erythroblasts are ring sideroblasts
- If no cytogenetic evidence of clonality is present, observation for 6 months is recommended

Courtesy of J.W. Vardiman, 2008
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
RAEB: Differences Between 2001 and 2008 WHO: RAEB 1 and RAEB 2

RAEB-1:
5-9% blasts in bone marrow and <5% in blood, or
If blasts in bone marrow are <5%, the finding of 2-4% blasts in the P.B. makes it RAEB-1
• No Auer rods

RAEB-2:
• 5-19% blasts in blood
• 10-19% blasts in bone marrow
• Auer rods (when blasts in blood or bone marrow <20%)
MDS with isolated 5q-

- Haploinsufficiency of the ribosomal gene RPS14
- Lenalidomide: thalidomide like, immunomodulatory with anti-angiogenic and anti-neoplastic properties
- Problems with the proliferative variant
MDS, unclassifiable (3 settings)

1. Patients with refractory cytopenia with unilineage dysplasia (RCUD) or refractory cytopenia with multilineage dysplasia (RCMD) but with 1% blasts in the peripheral blood

2. Cases of MDS with unilineage dysplasia which are associated with pancytopenia. RCUD (in contrast with RCMD) only allows for a single cytopenia or bicytopenia
3. Persistent (6 mo.) cytopenia(s) lacking morphologic features of MDS but with cytogenetic abnormalities presumptive for MDS*

- +8*
- -7 or del(7q)
- -5 or del(5q)
- del(20q)*
- -Y*
- i(17q) or t(17p)
- -13 or del(13q)
- del(11q)
- del(12p) or t(12p)
- del(9q)
- idic(X)(q13)

* t(11;16)(q23;p13.3)
* t(3;21)(q26.2;q22.1)
* t(1;3)(p36.3;q21.2)
* t(2;11)(p21;q23)
* inv(3)(q21q26.2)
* t(6;9)(p23;q34)

* Their presence as sole cytogenetic abnormality, in the absence of morphologic criteria, is insufficient evidence for MDS
Subtypes of MDS difficult to distinguish from other myeloid neoplasms (discussed in the 4th edition)

- Hypoplastic MDS from acquired aplastic anemia
- MDS with fibrosis from other fibrotic myeloid neoplasms (e.g. PMF)
- MDS with $\geq 50\%$ erythroblasts from acute erythroid leukemia
MDS, hypoplastic
(<30% in patients ≤70 years old, and <20% in >70 years old)

MDS with fibrosis (≥2+ fibrosis)
Myeloid neoplasms with $\geq$ 50% BM erythroid cells

- MDS has $< 20\%$ bone marrow blasts -- calculated as a percentage of the bone marrow non-erythroid cells
- Acute erythroid/myeloid leukemia requires $\geq 20\%$ bone marrow blasts -- calculated as a percentage of all the bone marrow non-erythroid cells
- In cases of MDS with erythroid precursors accounting for $\geq 50\%$ of the bone marrow nucleated cells, the MDS subtyping is done according to the number of blasts in blood and blasts in bone marrow, the latter calculated as percentage of all the bone marrow nucleated cells

Note: lymphoid cells and plasma cells are excluded from the count of the non-erythroid cells
Myelodysplastic/Myeloproliferative Neoplasms
(Hematopoietic malignancies with hybrid features both of MPN and MDS)

Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia* (aCML)
MDS/MPN, unclassified (MDS/MPN,u)

[Juvenile myelomonocytic leukemia (JMML)]

*BCR-ABL negative
Diagnosis of MDS/MPN: WHO requires to exclude

In all cases the presence of
• Ph’ chromosome / $BCR/ABL1$ fusion gene

plus, in cases with eosinophilia
• PDGFR Alpha abnormalities
• PDGFR Beta abnormalities*

*May resemble CMML or aCML. Include cases formerly termed CMML with t(5;12)
CMML: WHO definition

• Monocytosis (PB) as major defining feature:
  > 1 x10⁹/L  [percentage of monocytes greater than 10% of WBC]

• Myelodysplasia (mono or multi-lineage). Not required if all other criteria are met and
  -- there is a cytogenetic abnormality
  -- or reactive causes are excluded and
  monocytosis has lasted for at least 3 months

• <20% blasts in BM or PB
**Monoblasts**
Large cells with abundant cytoplasm minimally vacuolated round nuclei with fine chromatin and nucleoli

**Promonocytes**
Irregular or folded nuclei, small indistinct nucleoli, fine chromatin and a more vacuolated and/or granular cytoplasm

**Abnormal monocytes**
More condensed chromatin, convoluted nuclei and granulated

Adapted from: Vardiman et al. Introduction and Overview of the Classification of the Myeloid Neoplasms. In *WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues*. IARC: Lyon 2008. Fig.1-04 (p. 21).
Immunophenotyping

• CD14 shows decreased expression
• Aberrant phenotypes, e.g. overexpression of CD56, aberrant expression of CD2 or decreased HLA-DR, CD13, CD15, CD64, CD36
• Immunohistology including the reactivity of plasmacytoid dendritic cells has been included in the 4th WHO edition

Atypical CML

- Atypical CML incidence is <2 cases for 100 cases of t(9;22) BCR/ABL positive CML
- Patients are older than in CML; poor prognosis
- Has some features of CML e.g. splenomegaly, neutrophilia (WBC: ≥13 x10^9/L) and anemia. No basophilia and thrombocytopenia.
- Difference with CML: dysgranulopoiesis (in aCML)
- Cytogenetics: +8, +13, del(20q), del (12p)
- JAK2 negative

aCML (*BCR-ABL1* neg.)
The MDS/MPN, unclassifiable Category: provisional entities and “overlap” syndromes

- Refractory anemia with ring sideroblasts and thrombocytosis (RARS-T)
- Isolated 5q- with myeloproliferative features
- Isolated isochromosome 17q syndrome
- Truly unclassifiable

(MDS features plus Plts ≥ 450x10⁹/L or ≥ 13x10⁹/L + splenomegaly)
MDS/MPD, u with ring sideroblasts associated with thrombocytosis (RARS-T)

Megaloblastoid changes, ring sideroblasts, thrombocytosis and MPN-like megakaryocytes
RARS-T

- Its incidence is rare (underdiagnosed?)
- JAK2 positive in up to 60% of cases
- MPL W515K/L (rare)
- RARS-T has a worse outcome than essential thrombocythemia (particularly in those pts. without the JAK2 mutation)
Isolated 5q- with myeloproliferative features

Uncertain categorization: include cases of 5q- associated with JAK2 mutation. These are described both in the section on MDS with isolated 5q- and also in the one on MDS/MPN.

May be a “biclonal disease”
Isolated 5q- with myeloproliferative features

Thrombocytosis plus granulocytic proliferation in the bone marrow and/or PB

The frequency of JAK2-V617F mutation is much higher in the myeloproliferative variant than that seen in the 5q- syndrome

Ingram et al: *Leukemia* 2006;20:1319-1321
MDS/MPN associated with isolated isochromosome 17q

- Neutrophilia with dysplasia and variable monocytosis
- Hypercellular marrow with pleomorphic megakaryocytes
- Poor prognosis, very aggressive
- Fibrotic marrow
MDS/MPN associated with isolated i(17q)

Uncertain categorization: is included in the CMML section in WHO 4th edition
Summary

• Morphology is still the “gold standard” for diagnosis (but only if used with high quality smears and biopsy sections)
• >10% of cells in a lineage should be dysplastic to consider the lineage as dysplastic
• Flow cytometry provides useful information but is not diagnostic on its own. Particularly useful in CMML
• In the appropriate clinical setting, certain cytogenetic abnormalities are considered as presumptive of MDS
• The WHO classification is useful if properly applied (remember PB smear and adequate clinical information)