The myelodysplastic syndromes: Diagnosis, molecular biology and risk assessment

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Abstract
Myelodysplastic syndromes (MDS) are heterogeneous group of neoplastic clonal stem cell diseases characterized by dysplastic morphological features and clinical bone marrow failure. The FAB (French-American-British) system served as the gold standard for MDS classification for more than two decades. The WHO classification, built on the backbone of FAB classification, is an attempt to further improve the prognostic value of MDS classification as well as establish its clinical utility as a tool to select different treatments. In this article we review the epidemiology, pathogenesis, molecular biology, diagnosis and classification of MDS. We highlight the major differences between the FAB classification and the WHO MDS classification. We discuss in more detail the experience of using the new WHO classification since its publication and review the studies that tried to validate the prognostic value of the new classification or apply it to predict clinical responses to various treatments.

Introduction
Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of hematopoietic stem cells. The definition of MDS has two parts, as it is essentially a clinico-pathologic description. MDS can be defined as a clonal disease of the bone marrow with:

- The clinical manifestation of bone marrow failure as well as a tendency to transform into an acute leukemic phase (e.g. variable percentage of leukemic blast cells).
- The pathological manifestation of morphological abnormalities (termed “dysplasia”, although it is a clonal disorder, and hence, neoplastic) of the peripheral blood and bone marrow cells such as ringed sideroblasts, megaloblastic erythroid precursors, hypogranulation/hypossegmentation of the granulocytes, and micromegakaryocytes.

In the early 1970s, a number of investigators gathered to form the French-American-British (FAB) Working Group. The goal was to provide uniform terminology for the myriad of different definitions for the leukemias and related diseases. At the time, new therapies and supportive care measures for hematologic disorders were evolving rapidly. Exciting new drugs were active and more were in clinical development. The group believed strongly that for international groups to be able to exchange information about these different entities, it was critical to agree on common definitions. The FAB Working Group developed a series of proposals and published its first article on the acute leukemias in 1976, which discussed two of the components of what are now called myelodysplastic syndromes (MDS)- refractory anemia with excess blasts (RAEB) and chronic myelomonocytic leukemia (CMML).

It was recognized that some patients could present with a disease that bore some resemblance to acute myeloid leukemia (AML), but that this entity, unlike AML, did not have many leukemic blasts in the bone marrow. It was associated with some alteration in maturation of the three major cell lines (granulocytes,
erythroid precursors, and megakaryocytes), which resulted in pancytopenia and increased risk of infection and bleeding but did not necessarily progress to acute leukemia. Different terms were applied, including dysmyelopoietic anemias. The FAB Working Group applied the term MDS to these disorders to indicate that the common disease pathway began with a common neoplastic stem cell. The evolution from that stem cell could be highly variable: some patients never evolved to acute leukemia and others evolved quickly.

In 1980, a larger number of cases were reviewed with the intent to determine if specific morphologic abnormalities, singly or in groups, would predict for a different biologic outcome. This larger review of cases led to an expanded definition of the myelodysplastic syndromes into the well-known FAB five subgroups.

**Epidemiology**

*MDS is primarily a disease of the elderly. It is more common than AML and appears to be increasing in incidence.*

Most investigators believe MDS is at least twice as common as AML. Current projections are an annual incidence of approximately 12,000 cases in the United States, which makes it the most common leukemia observed, even more common than chronic lymphocytic leukemia (CLL).

One of the limitations in determining the true incidence and prevalence of MDS is the inability of tumor registries to record cases accurately. Most rely on tissue pathology, and many patients with MDS are diagnosed in a hematologist’s office where a bone marrow aspirate may be performed without a biopsy, or the diagnosis is made accurately by the process of elimination without ever performing a bone marrow aspirate. This is not the case with AML or any other malignancy.

Addressing the question of whether MDS is increasing in incidence is equally if not more difficult. Older literature is unreliable because different disease classifications existed: idiopathic sideroblastic anemias, refractory anemias, preleukemias, dysmyelopoietic anemias, smoldering acute leukemias, and subacute myeloid leukemias. All of these entities presumably described a similar disease.

We suspect that the incidence and prevalence of MDS are rising, but there are no data to prove this. It makes sense, however, because people are living longer, and MDS is a disease of the aging population. Increasing numbers of people are also developing MDS as a result of exposure to the drugs used to treat patients with solid tumors, the acute leukemias, and autoimmune disorders, as well as in patients receiving bone marrow, liver, and cardiac transplantation.

The acceptance of the FAB classification has facilitated the determination of true age-specific incidences in confined populations, and the best estimates come from selected institutions, cities, and countries that are able to define the entire population at risk. Reports from England, Germany, France, and Thailand have been similar, and there is no evidence to suggest that the incidence of MDS varies worldwide. The approximate incidence is 6 to 10 cases per 100,000 individuals, with an increasing incidence above the age of 60. This compares with an incidence of AML of approximately 3 cases per 100,000. By age 80, the incidence of MDS may approach 65 to 100 per 100,000.

Despite similar incidences worldwide there is a difference in the median age and subtype classification in Asian vs. American/European countries. Lee et al. have published data on a higher incidence of trisomy 1q in MDS patients in Korea (15.2%). In addition there is a lower incidence of refractory anemia with ringed sideroblasts (RARS) in Korea and Japan and a younger median age (45–50 years).

Like AML, MDS can occur as a primary or de novo disease, or as a treatment-related or secondary event.

A number of retrospective studies suggest a correlation between MDS and occupational exposure to agents such as benzene. Although cigarette smoking has a slight but significant association with the development of AML, data suggesting an effect on the incidence of MDS are sparse.

Two types of secondary leukemias/MDS can occur following treatment with antineoplastic agents (Table I). The first type, initially recognized in survivors of Hodgkin’s disease, generally presents 5 to 15 years after exposure to alkylating agents (e.g. mechlorethamine and procarbazine as part of the MOPP regimen). It shares many of the dysplastic features of MDS, and has a high incidence of chromosomal abnormalities, involving chromosomes 5 and 7 in particular. Patients have trilineage dysplasia and significant marrow fibrosis, and usually progress.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemogen</td>
<td>Alkylating</td>
<td>Topoisomerase II Inhibitor</td>
</tr>
<tr>
<td>Onset</td>
<td>5–15 yr</td>
<td>&lt;5 yr</td>
</tr>
<tr>
<td>Classification by FAB Group</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cytogenetic result</td>
<td>Unbalanced</td>
<td>Balanced</td>
</tr>
<tr>
<td>(chromosomes 5 and 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS phase</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Response to therapy</td>
<td>Variable</td>
<td>CR likely</td>
</tr>
</tbody>
</table>

CR = complete response; FAB = French-American-British; MDS = myelodysplastic syndromes.
rapidly to acute leukemia. These secondary leukemias are difficult to classify as one of the FAB subtypes.

The second, more recently recognized type of secondary leukemia is associated with administration of topoisomerase II inhibitors (e.g. etoposide, the anthracyclines, cisplatin). Interestingly, these leukemias are associated with the translocations present in de novo acute leukemia. For example, there are alterations involving chromosome 11 (11q23), translocations involving t(8;21), and translocations of t(15;17) and inv.16.

Survivors of testicular or lung cancer are now presenting with these type II secondary leukemias, and patients previously treated with alkylating agents and anthracyclines as adjuvant therapy for breast cancer are receiving diagnoses of a mix of type I and II secondary leukemias. Recent results of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-25, which evaluated high-dose cyclophosphamide combined with doxorubicin as adjuvant therapy in 2548 breast cancer patients, revealed 16 cases of AML (3 preceded by MDS) and 4 cases of MDS (4-year cumulative incidence of 0.87%), including a mix of both secondary leukemias associated with alkylating agents and topoisomerase II inhibitors (epipodophyllotoxins, anthracyclines). These results suggest a 60-times -higher incidence than would be expected in a control population.

**Classification**

*FAB classification divides MDS into five subgroups according to the percentage of blasts in the marrow, percentage of ringed sideroblasts, presence of monocytes, and severity of dyspoiesis (Table II)*

After publishing the FAB classification for MDS in 1982, investigators found that they could apply it reasonably well. Separations in survival curves, ranging from 5 to 6 years for the most favorable prognostic forms of MDS to less than 1 year for the least favorable forms, were demonstrated. However, the FAB classification has not been without its critics, and modifications have been suggested. For example, evidence suggests that patients with greater than 10% leukemic blasts in the bone marrow (11–19%) experience disease progression different from those with 5–9% blasts. Patients with 20–30% blasts had outcomes similar to AML. It was necessary to look at the natural survival of the FAB categories and to confirm that the percentage of blasts is an important factor for prognosis. It was also noted that the degree of dysplasia, whether uni-lineage or multilineage, played an important prognostic value not completely addressed by the FAB classification.

Because CMML contains "leukemia" in its name, critics often object to its inclusion with the preleukemic states and myelodysplasia. A similar objection was raised several years ago regarding atypical chronic myeloid leukemia (aCML). A group of patients with elevated white blood cell counts – usually greater than 12,000ul⁻¹ – have a disease resembling CML, but with many of the morphologic features of MDS. These patients have an outcome similar to that of RAEB patients. Investigators differ in referring to the diagnosis as proliferative, leukemic, or myelodysplastic; however, the important point is that patients with aCML whose WBC counts are only slightly elevated tend to resemble more closely patients with MDS.

Their disease is unlikely to proliferate, and they can be treated successfully in the same way MDS patients are treated. Another small group of patients have an elevated monocyte count (proliferative CMML), dysplastic changes in their peripheral blood and bone marrow, do not have the Philadelphia chromosome or BCR-ABL gene rearrangement, and resemble patients with MDS, but have a proliferative illness. These patients may require CML-type treatment with drugs such as hydroxyurea, interferon-alfa, or busulfan.

There are also patients who meet the diagnostic criteria for MDS, but have only granulocytopenia and thrombocytopenia and no anemia. Some authorities justifiably question the diagnosis of RA when the patient is not anemic. A better term for these patients is "uncategorized MDS". The original description of RA was intended to include patients with mild pancytopenias and dysplasia, but since there was no category in which to put other kinds of patients, it has become a catchall phrase.

In 1997 the World Health Organization (WHO) appointed a committee to revise and update the diagnostic categories of the Lymphomas and the

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**Table II. FAB Working Group Classification of MDS**

<table>
<thead>
<tr>
<th>FAB Type</th>
<th>Cases</th>
<th>Bone Marrow Blasts</th>
<th>Dyspoiesis</th>
<th>Ringed Sideroblasts</th>
<th>Monocytes</th>
<th>Progression to AML, %</th>
<th>Survival Rangers Yrs.</th>
<th>Survival Median Yrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>35</td>
<td>&lt;5</td>
<td>+</td>
<td>&lt;15</td>
<td>Rare</td>
<td>10</td>
<td>2–5</td>
<td>4</td>
</tr>
<tr>
<td>RARS</td>
<td>15</td>
<td>&lt;5</td>
<td></td>
<td>&gt;15</td>
<td>Rare</td>
<td>5</td>
<td>3–10</td>
<td>4</td>
</tr>
<tr>
<td>RAEB</td>
<td>20</td>
<td>5–20</td>
<td>++</td>
<td></td>
<td>Rare</td>
<td>45</td>
<td>0.5–2</td>
<td>1.5</td>
</tr>
<tr>
<td>CMML*</td>
<td>15</td>
<td>&lt;20</td>
<td>++</td>
<td>Variable</td>
<td>Increased</td>
<td>20</td>
<td>1–5</td>
<td>2</td>
</tr>
<tr>
<td>RAEB-t</td>
<td>15</td>
<td>20–30</td>
<td>++</td>
<td>Variable</td>
<td>Variable</td>
<td>60</td>
<td>&lt;1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Blood monocyte counts must be >1×10⁻¹.
Leukemias. One of us (John M. Bennett) was privileged to be appointed to the subcommittee for acute leukemias and MDS. Changes have been suggested that include the following:

1. Eliminate RAEB-t and establish AML when the percentage of marrow blasts is 20% or greater.
2. List CMML in a separate chapter entitled: myelodysplastic/myeloproliferative disorders. Subclassify CMML into CMML-1 and 2, based on the percentage of blasts in the marrow (1–10 vs. 11–19%).
3. List 2 types of RAEB: RAEB-I (5–10% blasts) and RAEB-II (11–19% blasts).
4. Include under RARS and RA a subtype for dysplasia in granulocytic and (or) megakaryocytic lineage. Dysplasia is defined as 10% or greater dysplastic progeny (granulocytes in peripheral blood and (or) granulocytes, megakaryocytes in bone marrow).
5. (5q -) Syndrome was recognized as a separate entity acknowledging the importance of Cytogenetics in MDS, recognizing this subgroup as unique clinical entity that have good prognosis, and may have more effective therapies geared to.
6. Add a new category for cases that do not fit the other subtypes, namely MDS, unclassified. (Table III)

Pathogenesis model

Occurrence of MDS is best viewed in the frame of a multi-hit theory. Hereditary and multiple environmental factors result in a neoplastic stem cell clone. The MDS clone is characterized by altered gene functions, the gene alterations result either from single gene mutations, chromosomal abnormalities (mostly deletions), or gene silencing. Many of those altered genes are suppressor genes that function in a recessive manner. The various gene alterations of the MDS clone result in an intrinsic increase in the susceptibility of the clone to apoptosis. The MDS clone is also recognized by the immune system leading, in some cases, to clonal T cell proliferation that leads to release of various cytokines including TNF-α. The cytokines not only lead to the apoptosis of the MDS clone but more so the normal hematopoietic cells. This intrinsic and immune mediated susceptibility to apoptosis are the hallmark of early MDS pathogenesis explaining the clinical findings of peripheral cytopenia in spite of hypercellular bone marrow. The changes that occur as the MDS progresses and transform to AML are not well understood yet but decrease in the apoptosis, clonal evolution as well as angiogenesis are thought to be contributing factors.

Evidence of clonality

The neoplastic MDS clone arises from a pluripotent stem cell. The evidence of clonality was originally shown by evidence of G6PD mosaicism. Cytogenetic studies showed presence of two clones with and without trisomy eight in patients with sideroblastic anemia. The clonality is also supported by restriction fragment length polymorphisms (RFLPs) of the X-chromosome genes and by the use of fluorescence in situ hybridization (FISH). Several studies showed evidence of clonality in lymphoid lineage as well suggesting that the MDS clone arises from early pluripotent stem cell capable of myeloid and lymphoid differentiation. It is important to note that karyotypic evolution and complex karyotypic changes may occur with progression of MDS and transformation to AML.

Gene alterations

Loss or gain of gene function can result from single gene mutations, chromosomal translocations (unbalanced or balanced), and by epigenetic alterations such as silencing of the gene expression by hyper-

Table III. WHO Classification

<table>
<thead>
<tr>
<th>Category</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a. RA without dysplasia</td>
<td>Blasts &lt;1%; monos &lt;1000/cmm</td>
<td>Blasts &lt;5%; ringed sideroblasts &lt;15%</td>
</tr>
<tr>
<td>1b. RA with dysplasia</td>
<td>Same + dysgranulo and or giant platelets</td>
<td>Same + dysgranulo and or dysmegao.</td>
</tr>
<tr>
<td>2a. RARS without dysplasia</td>
<td>Blasts &lt;1%; monos &lt;1000/cmm</td>
<td>Blasts &lt;5%; = or &gt;15%; ringed sideroblasts</td>
</tr>
<tr>
<td>2b. RARS with dysplasia</td>
<td>Same + dysgranulo and or giant platelets</td>
<td>Same + dysgranulo. and or dysmegao.</td>
</tr>
<tr>
<td>3a. RAEB-1</td>
<td>Blasts 1–4; monos &lt;1000/cmm</td>
<td>Blasts: 5–9%</td>
</tr>
<tr>
<td>3b. RAEB-2</td>
<td>Blasts 5–19%; monos &lt;1000/cmm</td>
<td>Blasts: 10–19%</td>
</tr>
<tr>
<td>4. CMML*</td>
<td>Blasts &lt;1–19%; &gt;1000 monos/cmm</td>
<td>Blasts: 0–19%</td>
</tr>
</tbody>
</table>

*list under myelodysplastic/myeloproliferative category.
methylation. The net result is either gain of an oncogene function or loss of tumor suppressor gene function. Tumor suppressor genes function in a recessive fashion that requires loss of both alleles function. Haploinsufficiency (loss of a single gene copy) can result in reduction of the gene products and predisposition to malignancies.

The RAS gene family is the most studied in MDS. Ten to forty percent of patients with MDS have RAS mutation. The most common mutation is single base change at codon 12 of the N-RAS family. The resultant mutated N-RAS protein retains active GTP form promoting continuous signaling to the nucleus. N-RAS mutation carries higher risk of AML transformation and worse prognosis. The farnesyl transferase inhibitors are a group of pharmaceutical agents that specifically targets RAS but have broader actions as well.

Other gene mutations described in MDS include P53 tumor suppression gene (5–10% of cases), FLT3 oncogene receptor kinase (5% of cases), P15 ink4b a tumor suppressor gene that is transcriptionally repressed through promoter silencing by hypermethylation (can be present in up to 50% of high risk cases-high grade MDS). The abnormality is seen in association with 7 q- syndrome and is associated with shorter survival.

Certain co-existing gene mutations may increase the individual susceptibility to develop MDS: for example NQO1 gene mutation increases the risk for t-MDS in both the homozygotic and heterozygotic states. NQO1 is a quinone oxidoreductase required for detoxifying benzene derivatives.

**Microarray analysis in MDS**

The introduction of microarray analysis revolutionized the analysis of gene profiles. Not only can thousands of genes be analyzed together, but the technique is also promising to identify gene profiles “molecular signatures” that can help identify the disease, categorize its subtypes, better predict the outcomes, and hopefully in the future deliver tailored treatments.

Microarray analysis studies in MDS identified new important genes, profiles that may help distinguish MDS from AML as well as low risk from high risk MDS. In one study investigators were able to discriminate between healthy control bone marrow samples and MDS patients bone marrow samples using the expression profile of 11 selected genes representing different gene classes. The gene expression profile was also able to discriminate between low risk and high risk MDS. The retinoic acid induced gene (RAI3), radiation-inducible immediate early response gene (IEX1) and the stress induced phosphoprotein 1 (STIP1) gene were among the genes down-regulated in low risk MDS reflecting that the CD34 MDS stem cells may lack the defensive proteins and thus is more susceptible to damage. In another study authors were able to distinguish between AML blasts and MDS blasts by certain gene profiles. Delta like gene (Dlk), Tec gene, and inositol 1,4,5-triphosphate receptor type 1 gene were among the genes highly specific for MDS. The Dlk 1 gene for example may be an important gene in cell proliferation and may allow stromal cells to support stem cells. Certain gene sets were identified for early stage MDS including the PIASy gene (PIAS family are group of signaling proteins) that function as tumor suppressor gene. As MDS progresses and transforms to AML those gene expressions are decreased.

**Cytogenetics**

Chromosomal abnormalities are described in 40–70% of all MDS cases. Chromosomal abnormalities are usually unbalanced loss, deletion or translocation. It may be surprising to find a normal karyotype in 30–60% of a clonal disease; however, this could be explained by technical failures as well as karyotypic evolution overtime. In spite of that, the normal karyotype carries a better prognosis similar to 5q-syndrome, 20q- or loss of chromosome Y. Complex karyotype is defined as presence of 3 or more different cytogenetic abnormalities. It is present in 10–20% of primary MDS and up to 90% of therapy-related MDS. More cytogenetic abnormalities occur in high risk MDS and therapy related MDS. The reported frequency of cytogenetic abnormalities under the new WHO classification are: refractory anemia 25%, refractory anemia with ring sideroblasts 10%, refractory cytopenia with multi-lineage dysplasia 50%, refractory anemia with excess blasts type I & II 30–50%. Cytogenetic abnormalities do not correlate with WHO subtypes except the 5-q syndrome that represents a separate entity.

**Loss of chromosome 5/del (5q)**

Loss of chromosome 5 or interstitial deletion of its long arm is one of the most common chromosomal abnormalities described in primary and therapy related MDS. This abnormality is associated with previous exposure to carcinogens including benzene, alkylating agents, and radiation. It is very important to distinguish this −5/(del 5q) abnormality from the 5q-syndrome that for the first time is recognized as a separate entity in the WHO classification. The 5-q syndrome occurs in the form of refractory macrocytic and often thrombocytosis; more commonly in upper middle age females. It carries the best prognosis of MDS subtypes. It seems that the deletion in 5-q syndrome breakpoint, which involves band 5q33, contains a different myeloid tumor suppressor gene
from 5q31 band that is commonly involved in –5/del (5q).

**Loss of chromosome 7/ del (7q)**

Monosomy 7 or deletion of the long arm of chromosome 7 is well described in therapy related MDS and primary MDS. The breakpoint 7q22 seems to be involved in MDS cases. A monosomy 7 syndrome entity is described in the pediatric literature and common in juvenile myelomonocytic leukemia (JMML). Interestingly, -7/del (7q) is the most common abnormality described in patients with hereditary predispositions to MDS like Fanconi anemia (FA).

**Loss of 7 q is associated with AML1 gene mutations.**

Monosomy 7 or deletion of the long arm of chromosome 7 is associated poor outcome in children and adults. Trisomy 8 is described in different hematological malignancies including MDS. Its significance is not well understood.

**Loss of Y chromosome**

Loss of chromosome Y is described in patients with hematological and non-hematological diseases so by itself does not represent a diagnostic evidence of a hematological process. Once present, however, in MDS it may carry a favorable prognosis.

**Loss of chromosome 17 Short Arm**

17p- syndrome is associated morphologically with the classical pseudo-Pelger-Huet hypolobulation. P53 gene is located on 17p 13.1 and is often involved in this syndrome.

**Deletion of Long Arm of chromosome 20**

Del (20q) carries a favorable prognosis by itself. It is more often seen in early MDS. Prominent erythrophagocytosis and megakaryocytic dysplasia is often seen. Mature granulocytes from peripheral bone marrow may lack the abnormality suggesting increased propensity of apoptosis for the clone carrying this abnormality. Isochromosome 20q with loss of interstitial material i (20q-) was described recently in six MDS patients out of 998 in a registry. This was seen in older patients and behaved different clinically from 20q- syndrome with rapid progression and shorter survival. The i (20q-) could represent a further evolution of the 20q karyotype, thus disease progression.

**11q23 syndrome**

Translocations involving 11q23 are classically described in therapy-related MDS secondary to topoisomerase II class drugs. The mixed lineage leukemia (MLL) gene is located on 11q23. The translocations involving 11q23 are described in acute leukemia with a biphenotypic phenotype that usually carries poor prognosis. The exact involvement of MLL gene in translocation in primary MDS is not well defined.

**Apoptosis in MDS**

A major advance toward understanding the pathogenesis of MDS has been the observation of apoptosis, programmed cell death, in MDS. The group of Raza/Preisler et al. have carried out cell kinetic studies from MDS bone marrow biopsies using intravenous infusions of either iododeoxyuridine or bromodeoxyuridine, or both, and estimated the degree of apoptosis by in situ end-labeling of DNA. Virtually all marrows studied demonstrated increased rates of apoptosis as well as rapid cell proliferation.

Apoptosis may carry the explanation for the paradoxical observation of peripheral cytopenia and a normo- or hypercellular bone marrow in MDS. Evidence of apoptosis in MDS is supported by various techniques. Earlier on apoptosis was observed by electron microscopic examination of the bone marrow in MDS patients. Evidence of apoptosis is also shown by biochemical techniques, in situ methods and flowcytometric studies.

Apoptosis seems to be higher in early stages of MDS and decreases as MDS progresses and transforms to AML. Flowcytometric studies revealed that the proportion of CD 34+ cells in G1-DNA phase are more in early MDS. Also, the ratio of C-Myc (pro-apoptotic gene) to BCL 2 (anti-apoptotic gene) is decreased as MDS progresses to AML. It is controversial whether apoptosis is restricted to CD34+ progenitors or it also includes mature cells.

Several mechanisms can explain the observation of excessive apoptosis in MDS. The MDS clone itself may carry an intrinsic liability for apoptosis due to altered gene functions and expression, however, there is lack of correlation between cytogenetics abnormalities and apoptosis suggesting that the phenomenon is not only restricted to MDS clone. Increased apoptosis in MDS could also be secondary to inhibitory cytokines mainly TNF-α that can induce apoptosis not only affecting the MDS clone but also the normal cells. Increased expression of FAS ligand (CD 95 cell surface protein) in MDS bone marrow cells could also be one of the mechanisms contributing to apoptosis. Other possible involved mechanisms include cell cycle abnormalities and mitochondrial abnormalities leading to increased apoptosis. Mutations of mitochondrial DNA may also impair iron metabolism contributing to sideroblastic anemia.

Targeting apoptosis could serve as a therapeutic strategy in MDS. In fact, treatment with erythropoie-
Diagnostic evaluation:

*The diagnosis of MDS is based on routine laboratory and peripheral blood evaluation. Bone marrow aspiration and biopsy along with cytogenetic analyses should be performed.*

The laboratory diagnosis of MDS is prompted by detection of cytopenia or clinical symptoms, such as fatigue, bleeding or infection that indicate the presence of anemia, thrombocytopenia, or severe granulocytopenia. There are no clinical phenomena associated specifically with MDS versus other pancytopenic states, including mild to moderate forms of aplastic anemia, which are occasionally difficult to differentiate from MDS. Sometimes a patient presents with an acellular bone marrow, thereby fulfilling a criterion for aplastic anemia, but the patient also has significant dysplasia, slight macrocytosis, and an abnormal karyotype, such as monosomy 7 or trisomy 8. This patient will eventually develop MDS or acute leukemia if not treated with allogeneic bone marrow transplantation (BMT).

The diagnosis of MDS depends on the process of elimination for half of the patients we observe. For the other half, the diagnosis is not difficult if patients have more than 5% blasts. Having less than 5% blasts and normal cytogenetic and fluorescence in situ hybridization (FISH) results, even in the presence of mild to moderate dysplasia, makes most clinicians reluctant to assign a diagnosis of MDS until a few months elapse. This allows time to rule out a correctable hematologic process, such as pyridoxine-responsive anemia. If the pancytopenic state is not readily reversible by normal interventions within 3–4 months, the chances are overwhelming that the patient has MDS.

Classifying MDS continues to be valuable. We recommend performing a 500 cell differential, eliminating lymphocytes and plasma cells for the count. It involves performing a bone marrow biopsy and aspirate utilizing hematoxylin & eosin; reticulin; Romanowsky and iron stains and counting the number of blasts. The percentage of blasts is calculated, and patients are categorized according to this percentage (e.g. <5%, 5–10%, 11–19%, >20–30%) or if they have CMML, which can be any percentage of blasts with a monocytosis of greater than 1,000ul⁻¹. (Figure 1)

If the absolute percentage of erythroid precursors is 50% or greater, the percentage blasts is based on the non-erythroid precursors (essentially granulocytes and monocytes). For example, a differential count of 6% blasts, 4% promyelocytes, 15% granulocytes, and 75% erythroid precursors would convert the percentage of blasts to "24% " blasts (6/25) changing the subgroup from RAEB to RAEB-t (in the FAB system).

Cytogenetic testing should be performed on every patient with MDS and cytogenetic and bone marrow evaluations repeated whenever there is a significant alteration in the peripheral blood parameters. Because chromosomal evolution frequently occurs in patients who become more pancytopenic, different treatment categories may be required. However, we are not suggesting a monthly bone marrow biopsy be performed in patients with MDS. Once a diagnosis is established, routine, repeated bone marrow testing is unnecessary, unless there is a valid indication or the patient is on a clinical trial that requires such studies.

Readable cytogenetic spreads can be obtained in approximately 75% of patients with MDS. Of these, 60% to 65% will be abnormal. The most common cytogenetic abnormalities occur with chromosomes 5, 7, and 8. There are abnormalities specific to MDS [e.g. 20q- or t(del(12p))] that are rare events in AML, specific translocations unique to AML not observed in primary MDS [e.g. t(15; 17)], and the same abnormalities seen in both. (Table IV)

**Prognostic factors**

* A number of indexes have been proposed to aid in predicting clinical outcome for patients with MDS

Every city or country with adequate numbers of MDS patients has developed its own prognostic scoring system. Most of these systems separate patients into three groups, and they all have the same outcome: median survival times of 60, 30, and 15 months for the good-, intermediate-, and poor-prognosis groups, respectively.

Recently an improvement in the existing systems was proposed, referred to as the International Prognostic Scoring System (IPSS), which includes a fourth group of patients. Greenberg et al. colleagues performed an analysis of 816 patients with de novo MDS to determine the critical prognostic variables. Patient subgroups were classified according to cytogenetics, percentage of blasts in the bone marrow, and number of cytopenias.

In this system cytogenetic availability is important, as it enables one to predict survival and evolution to AML in the low-risk group. This will help to individualize strategies for treating the patients in whom karyotyping is available. (Table V)

**Validation and application of WHO classification in MDS**

Several studies evaluated the validity and clinical utility of the WHO classification since its publication. The majority of those studies confirm that the WHO classification was a refinement of the predictive
prognostic ability of the FAB classification where it clearly identifies more homogenous subgroups with similar outcomes. Also recent studies suggest that the WHO classification can be used as a tool to tailor treatment to its subgroups and that response rates to different treatments differ among those subgroups. Some studies, however, still raise the question whether the WHO classification is a better classification system than FAB or they call for a further refinement of WHO classification. In this section we review in more details some of the recently published above-mentioned studies.

Germing et al. conducted the largest retrospective validation study for the WHO classification so far. Their results verified the difference in outcome between RA/RARS and RCMD as well as between RAEB I and II. The WHO classification was retrospectively applied to about 1600 patients in the Dusseldorf MDS registry diagnosed between 1970 and 1999. In their original registry the following FAB subtypes were recorded: RA 26%, RARS 20%, RAEB 22%, RAEB-t 17% and CMML 15%. After re-classification according to WHO classification myeloproliferative CMML patients were excluded, dysplas-
tic CMML cases were reclassified. RAEB-t cases were reviewed and only included if auer rods were present but bone marrow blasts were less than 20%. The following subtype frequencies were observed: RA 8.5%, RARS 11%, RCMD 24%, RCMD-RS 15%, RAEB-I 21%, RAEB-II 18.5%, and 5q syndrome 2.2%. The median survival in months for WHO subtypes were RA 69 months, RARS 69 months, RCMD 33 months, RCMD-RS 32 months, RAEB-I 18 months, RAEB-II 10 months, and 5q- 116 months. The frequency of AML progression was: RA 7.5%, RARS 1.4%, RCMD 10%, RCMD-RS 13%, RAEB-I 21%, RAEB-II 34.5%, and 5q- 8%. Various scoring systems were applied to the reclassified group (IPSS, Dusseldorf, Bournemouth, and Spanish). All scoring systems were applicable and valid. RAEB-II subgroup had more patients in the high risk-IPSS group compared to RAEB-I, probably due to higher score denoted for blast counts.

A similar study was conducted in Brazilian population. The study again verified the difference in outcome between RA/RARS and RCMD and also suggested that the WHO classification correlated better with survival compared to FAB. One hundred fifty patients diagnosed between 1996 and 2002 were retrospectively re-classified according the WHO classification. In the original FAB classification they had the following subtypes: RA 90 patients, RARS 18 patients, RAEB 34 patients, CMML 5 patients, and RAEB-t 3 patients. Upon re-classification by the standard WHO criteria 47 patients were RA, 12 patients were RARS, 25 patients were RCMD, 34 patients were RAEB, 23 patients were unclassified MDS and one patient was 5q syndrome. The median survival for RCMD was in between RA/RARS and RAEB. No break between RAEB I and II was presented. In a regression model WHO classification better correlated with survival compared to FAB with and without IPSS in the model.

Some studies, however, did not confirm the superiority of WHO classification. Nosslinger et al. applied the new WHO classification again in a retrospective fashion. Four hundred and thirty one patients diagnosed with primary MDS between 1976 and 1999 at Hanusch Hospital in Vienna were included. In the original FAB classification 33% were RA, 11% RARS, 21% RAEB, 12% RAEB-t and 23% CMML. Only 281 patients were reclassified, 150 patients with RAEB-t and CMML were excluded. 43 patients were classified as RA, 4 as RARS, one patient as 5q syndrome, 91 were RCMD, 50 were RAEB-I, 42 were RAEB II, and 50 were unclassified. RCMD had better median survival than RA (RARS patients were excluded). No significant difference between RAEB-I and RAEB-II was seen. This study was criticized for a major limitation. The authors used 50% as cutoff to define presence of dysplasia in a cell line. This is clearly not what the WHO set as cut off where 10% or more dysplasia in two or more cell line defined RCMD. The whole reclassification would have been different if the WHO cutoff was used. The degree of dysplasia in a cell line is not a yes or no question but obviously a continuum and a certain threshold should be uniformly adapted to unify the classification.

### Table IV. Karyotypic Changes Associated with Different Disease Subgroups

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Most commonly associated Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>5q-</td>
</tr>
<tr>
<td>RARS</td>
<td>+8, 5q-, 7, t(del(11), 20q-</td>
</tr>
<tr>
<td>RAEB and</td>
<td>5q-, 7, +8, +5, 7q-, 21, -Y</td>
</tr>
<tr>
<td>RAEB-t</td>
<td></td>
</tr>
<tr>
<td>CMML</td>
<td>−7, +8, t(del(12p), 21, −Y, 7q-</td>
</tr>
<tr>
<td>AML (de novo)</td>
<td>t(8;21), t(15;17), t(9;11), inv(16), −7, +8</td>
</tr>
</tbody>
</table>

AML—acute myeloid leukemia; CMML—chronic myelomonocytic leukemia; RA—refractory anemia; RAEB—refractory anemia with excessive blasts; RAEB-t—RAEB in transformation; RARS—refractory anemia with ringed sideroblasts.

### Table V. Risk Analysis (IPSS)

<table>
<thead>
<tr>
<th>Risk Subgroup</th>
<th>Score</th>
<th>Median survival (years)</th>
<th>Percent AML risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>5.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>0.5–1.0</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Intermediate-2</td>
<td>1.5–2.0</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>High</td>
<td>&gt;2.5</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The score is based on the following parameters: Score

<table>
<thead>
<tr>
<th>Prognostic Variable</th>
<th>Score</th>
<th>Median survival (years)</th>
<th>Percent AML risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM blasts (%)</td>
<td>&lt;5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Karyotype</td>
<td>Good</td>
<td>5–10</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>(3 abnormalities or monosomy 7)</td>
<td>2.0</td>
</tr>
<tr>
<td>Cytopenias</td>
<td>0/1</td>
<td>2/3</td>
<td></td>
</tr>
</tbody>
</table>

(Hemoglobin <10 g%, absolute neutrophil count (ANC <1,800ul⁻¹, platelet count <100,000ul⁻¹).
Two recent studies addressed specifically the difference between uni-lineage and multi-lineage dysplasia. In the first study, 103 patients with low risk primary MDS RA subtype by FAB were reclassified according to WHO classification. 56 were labeled RCMD, 43 were labeled RA and 4 as 5q-. Using FISH for cytogenetic determination the authors reclassified the patients into 37 with pure RA, 37 with RCMD and 29 with 5q deletion. Patients with RCMD had shorter median survival compared to RA (47 month versus 85.2 month). The outcome of 5q patients was the best if it was the sole cytogenetic abnormality and worse especially if associated with other chromosome 5 cytogenetic abnormalities. In another very interesting study Cermak et al. showed the presence of clonal cell subpopulations in the peripheral blood and bone marrow of RCMD but not in RA/RARS or 5q- patients reflecting multistep pathogenesis of MDS. They studied clonality in 36 females with MDS using XCIP (X chromosome inactivation patterns) as well as FISH. The patients were classified according to FAB and WHO classifications. Patients with advanced MDS had clonal granulocyte subpopulation and simultaneously clonal CD14+ cells in both peripheral blood and bone marrow. When comparing RCMD versus RA, 12 out of 14 RCMD patients had clonal peripheral granulocyte population with 8 of them exhibiting CD 14+ clonal cells. In the bone marrow 10 and 8 of RCMD patients had clonal granulocytes and CD14+ cells respectively. Only 2 out of 11 patients with RA/RARS or 5q-syndrome had clonal granulocytes or CD14+ cells in the peripheral blood. For all patients, median survival for patients with clonal subpopulation in peripheral blood was shorter although not statistically significant.

Focusing more on RAEB-t subgroup in the FAB classification and its elimination from the WHO classification Strupp et al. examined this subset of patients in the Dusseldorf registry. They analyzed 310 patients with RAEB-t. They divided the patients into 3 subgroups: (1) patients diagnosed as RAEB-t with medullary blasts >20% (2) patients diagnosed as RAEB-t with medullary blasts <20% but peripheral blasts ≥ 5% and (3) patient diagnosed with RAEB-t based on presence of Auer rods with <20% medullary blasts. The median survival was 5.3, and 11 month respectively. In group 3 the survival and AML progression was similar to RAEB II but not in group 2. The authors concluded that their results support the WHO classification in eliminating RAEB-t and that presence of Auer rods by itself doesn’t carry a prognostic value, however, they cautioned that patients with peripheral blasts ≥ 5% carry a worse prognosis than what is classified as RAEB in WHO and may be should be addressed separately. Similar results were also presented in a Japanese study re-evaluating 113 patients with RAEB-t where they were subdivided into 2 groups (1) RAEB-t with medullary blasts >20% and (2) RAEB-t with medullary blasts ≦20% but peripheral blasts ≥ 5%. Interestingly, the median survival was shorter for the second group and when compared to RAEB II this group had more complex cytogenetics and poor prognosis.

The Nordic MDS group is the first to address the clinical utility of WHO classification in predicting clinical response. They re-classified 103 low risk MDS patients from FAB to WHO. Among 64 patients treated with G-CSF and EPO (erythropoietin) on previous Nordic group trials the WHO reliably predicted response rates. The response rate was 67% in RA versus 50% in RCMD. The difference was more pronounced between RARS (75%) and RCMD-RS (9%) (p value 0.003). All over response rate was 73% among patients with uni-lineage dysplasia and 35% among patients with multi-lineage dysplasia. More over, patients with uni-lineage dysplasia had better survival: 51% were alive at 62 month versus 50% at 28.5 month in the multi-lineage dysplasia group (P value =0.03). This study is a testimony that the WHO classification does make a difference from a therapeutic point of view. This study also addressed the interobserver variation in MDS classification. Three blinded and separate reviewers classified the MDS. There was 92% agreement on the WHO subtype.

Most discrepancies were on dysplastic features of neutrophils and megakaryocytes, the 10% cutoff of dysplasia in a cell line was sometimes difficult to apply, and the RAEB I and II borderline blast percentage was an issue in few cases.

Conclusion

The experience using the new WHO classification for MDS after its publication confirms its utility as a better prognostic classification system and as a tool able to predict clinical responses. The WHO should be used in adjunct with the IPSS. Future application will hopefully further validate its prognostic value and potentially better identify homogenous subsets of patients who will respond to different therapeutic options. The classification of MDS should be viewed as a continuous process that is refined as we learn more about the disease and its biology. A particular area that may undergo further evolution in the MDS classification includes the cutoff of dysplasia required in each cell line and the impact of peripheral blood blast counts.

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References


