Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts

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ABSTRACT

The classification of myelodysplastic syndromes is based on the morphological criteria proposed by the French-American-British (FAB) and World Health Organization (WHO) groups. Accurate enumeration of blast cells, although essential for diagnosis of myelodysplastic syndrome and for assignment to prognostic groups, is often difficult, due to imprecise criteria for the morphological definition of blasts and promyelocytes. An International Working Group on Morphology of Myelodysplastic Syndrome (IWGM-MDS) of hematopathologists and hematologists expert in the field of myelodysplastic syndrome reviewed the morphological features of bone marrows from all subtypes of myelodysplastic syndrome and agreed on a set of recommendations, including recommendations for the definition and enumeration of blast cells and ring sideroblasts. It is recommended that (1) agranular or granular blast cells be defined (replacing the previous type I, II and III blasts), (2) dysplastic promyelocytes be distinguished from cytologically normal promyelocytes and from granular blast cells, (3) sufficient cells be counted to give a precise blast percentage, particularly at thresholds that are important for diagnosis or prognosis and (4) ring sideroblasts be defined as erythroblasts in which there are a minimum of 5 siderotic granules covering at least a third of the nuclear circumference. Clear definitions and a differential count of a sufficient number of cells is likely to improve precision in the diagnosis and classification of myelodysplastic syndrome. Recommendations should be applied in the context of the WHO classification.

Key words: myelodysplastic syndrome, myelodysplastic syndrome, myeloblast, ring sideroblast.

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Introduction

The term myelodysplastic syndrome (MDS) is used to describe a heterogeneous group of disorders that are characterized by clonal and ineffective hematopoiesis, morphological dysplasia, peripheral blood cytopenias and progressive bone marrow failure. MDS transforms to acute myeloid leukemia (AML) in approximately 30% of cases. Survival following a diagnosis of MDS varies from a few months to more than ten years (comparable to age/sex matched normal popu-

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lations).[1] This highly variable prognosis underscores the importance of a classification system, supplemented by a prognostic index, to predict the survival of patients with MDS and the likelihood of transformation to AML. With the recent development and introduction of several effective treatment options for MDS,[2,3] the need for a classification system to predict responsiveness to treatment and clinical outcomes for individual patients has become even more important.

During the past 20 years, several MDS classification and prognostic scoring systems have been proposed.[4-7] Several of these systems have gained acceptance with the French-American-British (FAB) classification as modified by the World Health Organization (WHO), the International Prognostic Scoring System (IPSS) being the most widely used. In addition, the recent identification of transfusion burden and a modification of the IPSS by Malcovati and co-workers,[8] the so-called WPSS (World Prognostic Scoring System), have surfaced as an important component of our understanding of the natural history of MDS. Refinements in classification are needed as research continues to advance our knowledge of the etiology and the pathogenesis of MDS.

To address these issues, a panel of experts in the classification of MDS, the International Working Group on Morphology of MDS (IWGM-MDS) convened on three occasions in 2005/06 to review and refine the morphological criteria for the classification of MDS. This group consisted of both clinical hematologists and hematopathologists. The latter attended all three meetings and participated actively in the review and characterization of many individual cases (slide review). The former provided clinical input as to the relevance of the precise determination of morphological cell types in the assessment of patients with MDS. This model has been utilized with success in the development of the 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.

The goals of this Working Group were to (i) define minimal diagnostic criteria for MDS; (ii) develop standardized definitions for myeloblasts and promyelocytes; and (iii) propose a classification for sideroblasts. This article details the proposals of the IWGM-MDS for the definition of myeloblasts, promyelocytes and ring sideroblasts. Proposals for minimal diagnostic criteria and for dealing with cases of possible MDS that do not meet these criteria are dealt with in an accompanying paper.[9]

Background: current myelodysplastic syndrome classification systems

Several classification systems have been developed to predict survival or transition to AML following MDS diagnosis. The first of these, the FAB system, was introduced in 1982 and is based on the percentage of blasts and morphological dysplastic features of blood and bone marrow.[4] According to this system, patients are diagnosed with MDS when dysplastic bone marrow hematopoiesis is present and/or myeloblasts are between 5 and 30% of all bone marrow cells. The FAB system served as the standard for MDS classification for two decades and provided considerable prognostic information. Nonetheless, the clinical outcomes of patients assigned to the same MDS subgroup remain too variable to accurately predict survival or transformation to AML in individual patients.

The International Prognostic Scoring System[5,6] provided a prospective risk assessment from the initial diagnosis but was dependent on having both an accurate bone marrow blast assessment and cytogenetic analysis. Increasing blast percentages (5-10%, 11-20%, 20-30%) indicated an increase in the risk of leukemic transformation and of death from all causes. Therefore, an accurate definition of the blast percentage and separation of blast cells from promyelocytes is critical.

In 2001, the WHO[7] proposed a revision of the FAB morphological approach. The revisions included lowering the threshold for the percentage of blasts required to make the diagnosis of AML from 30% to 20%, thus elimination of the MDS subcategory of refractory anemia with excess blasts in transformation (RAEB-T). In addition, chronic myelomonocytic leukemia (CMML) was reclassified from a subcategory of MDS to a subcategory of myelodysplastic/myeloproliferative disorder.

Of considerable importance was the introduction of a new subtype: refractory anemia with multilineage dysplasia without ring sideroblasts (RCMD) or with ring sideroblasts (RCMD-RS) with ≥10% of dysplasia in at least two cell lines, and refractory anemia (RA) or refractory anemia with ring sideroblasts (RARS) with dysplasia restricted to the erythroid lineage. The precise qualitative features of the dysplastic cells were described with several illustrations, particularly of the erythroid dysplasia.

Developing a classification system

All of the classification systems described above depend on an assessment of dysplastic changes in the marrow. In addition, recognition and enumeration of blast cells is of critical importance both in the diagnosis of AML and MDS, and for stratifying MDS patients into prognostic groups.[10,11] According to the IPSS, for example, patients with -10% bone marrow blast cells would be assigned to the intermediate 1 or 2 risk groups, and would have a worse prognosis than low-risk patients.[5]

It is often assumed that definitions of blast cells are applied uniformly by hematologists/pathologists worldwide, and that blast cells could be identified and counted very easily. Unfortunately this is not so. The FAB group defined type I and type II blast cells, both having a high nucleocytoplasmic ratio, a diffuse chromatin pattern and usually visible nucleoli; type I blast cells are agranular and type II have scanty granules.[4] Subsequently Goasguen and colleagues analyzed bone marrow smears obtained from 18 patients with MDS classified according to the FAB classification and defined a type III blast, with more than 20 fine azurophilic granules but otherwise with the characteristics expected of a blast cell.[12] In the FAB classification such cells were categorized as promyelocytes. The inclusion of type III blast cells in the blast cell count led to 7 patients (39%) being reclassified from refractory anemia with excess of blasts (RAEB) to RAEB-T and was found to give a better separation of survival curves of different FAB categories

of MDS. Despite the ability of this classification system to refine survival estimates for patients with MDS it was unclear how often type III blasts have been utilized in myelograms in typical clinical practice. Subsequent to this publication protocols for some clinical trials have included type III blast cells in the blast cell count but this has not been universal practice. The WHO classification does not give any specific recommendations for the definition of blast cells.[7]

In practice, although FAB type I and type II blasts can generally be readily distinguished from each other it has proved difficult to distinguish FAB type II blasts from type III blasts. In addition, the enumeration of promyelocytes, which are often abnormal in MDS, remains problematic and their separation from type II and type III blasts has remained imprecise.

Statistical analysis

Concordance was determined using the κ statistics.[13]

Results

The Working Group participants reviewed previous attempts to define blasts (agranular *vs.* granular) and promyelocytes. Each member of the group was asked to bring blood and bone marrow slides obtained from patients with various subtypes of MDS and/or AML that would serve as the basis for discussion of the identification of different types of blasts and promyelocytes. Myelograms were determined from these slides, and the data were captured and recorded electronically for subsequent statistical analysis. The starting point for developing definitions was the 1991 paper by Goasguen and colleagues.[12] In addition, criteria for separating granular blast cells from promyelocytes were developed.

Definition of myeloblasts

After a review of the literature, assessment of blood and bone marrow films individually and collectively, and much discussion, the participants arrived at a consensus regarding the definition of a myeloblast. Myeloblasts were defined in terms of several nuclear characteristics, including a high nuclear/cytoplasmic ratio, easily visible nucleoli and usually, but not invariably, fine nuclear chromatin. Nuclear shape is variable. Cytoplasmic characteristics include variable cytoplasmic basophilia; there may or may not be granules or Auer rods but no Golgi zone is detected (Figure 1). The exception to this last observation may be seen in cases of AML with t(8;21) where there may be blast cells with a small distinct Golgi, with or without an Auer rod, but with no other features of a promyelocyte. After reviewing all the available bone marrow smears, the IWGM group recommended that myeloblasts in MDS should be classified as agranular or granular. The agranular blasts correspond to the type I blasts of the FAB classification. Granular blasts are cells that have the nuclear features of blast cells but also have cytoplasmic granules. These cells will thus include type II blasts as defined by FAB, as well as type III blasts as defined by Goasguen *et al.*[12]

Granular blasts must be distinguished from promyelocytes (*see below*).

Promyelocytes

The group discussed the morphological features that define normal promyelocytes. Nuclear characteristics of normal promyelocytes included a central or eccentric nucleus and chromatin, which may still be fine or may be intermediate. The nucleolus is usually easily visible and prominent (Figure 1). The group determined that the principal distinguishing characteristic of the normal promyelocyte was the presence of a visible Golgi zone. Other cytoplasmic characteristics include uniformly dispersed azurophilic granules, and in most instances basophilic cytoplasm. Dysplastic promyelocytes have the recognizable features of a promyelocyte including a round, oval, or indented nucleus that is often eccentric, a Golgi zone (at least faintly visible) and a nucleus with fine or coarse chromatin and an easily visible nucleolus. Abnormal features that lead to recognition of promyelocytes as being dysplastic include reduced or irregular cytoplasmic basophilia, a poorly developed Golgi zone,

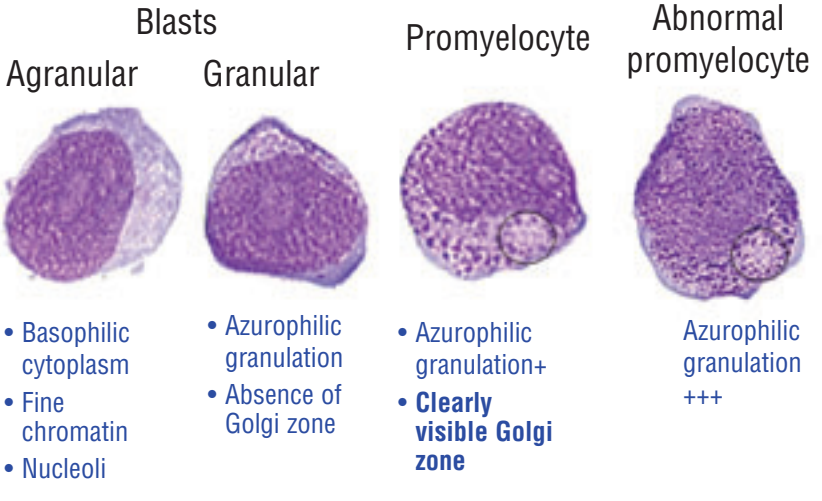

Figure 1. Blasts, promyelocytes, abnormal promyelocytes.

hypergranularity, hypogranularity and irregular distribution (clumps) of granules.

The group agreed, therefore, on the following morphological categories: normal promyelocytes, blasts (which are differentiated as simply agranular or granular, irrespective of the number of granules) and dysplastic promyelocytes. To verify the reproducibility of these propositions five experts were asked to review 264 consecutive cells from one case of AML (FAB-M2). The pictures were captured utilizing a unique digital image, capable of merging multiple consecutive fields (600x800 pixels). Each observer performed the task on his/her own computer by downloading the file from a dedicated website of the MDS Foundation. A drop-down menu was provided with the following choices: blasts (agranular and granular), promyelocytes (normal or abnormal), mature granulocytes, others (Figure 2). Results were sent electronically to the MDS Foundation headquarters and analyzed by JG.[14]

Individual assessments can be seen in Table 1. If we consider that a very good concordance would be agreement of 5/5 or 4/5 experts, then an 89.4% concordance was achieved in separating blasts from promyelocytes. Examining the data with kappa statistics demonstrated a high concordance when viewing one observer versus another (Table 2).

It should also be noted that when performing a marrow differential count, the myeloblast percentage should be determined by counting at least 500 nucleated cells, with the total including at least 100 nonerythroid cells to improve precision. The Working Group emphasized the use of this number to be extremely important for correct classification of patients with MDS, especially when cells of the erythroid lineage exceed 50%. Other methods of determining the myeloblast percentage may result in some patients being classified incorrectly. Counting an adequate number of cells is of critical importance for the classification of patients whose blast counts fall near the boundary between MDS categories of different prognostic significance. It is similarly essential to perform a 500-differential count on the blood film of patients with circulating blast cells since relatively small differences in the percentage of blast cells are of prognostic significance; the 2008 WHO classification assigns patients to different MDS categories with a blast count of less than 1%, 1%, 2-4% or 5%.[15]

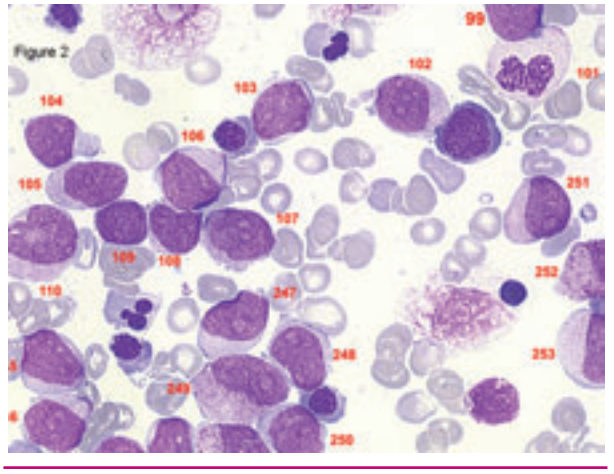

Figure 2. Example of individual cells counted by the experts.

Ring sideroblasts

The prognosis of patients with pure sideroblastic anemia may differ from that of patients with non-sideroblastic anemia; therefore, clear, standardized definitions of sideroblast types are necessary. Varying definitions of ring sideroblasts have led to confusion and controversy among clinicians. Early investigators defined ring sideroblasts as having iron granules in a perinuclear distribution surrounding the entire nucleus. Other investigators have required that perinuclear granules encircle at least one third of the perinuclear area, but not necessarily the entire nucleus.[16] Ringed sideroblasts were some-

Table 1. Agreement of the expert panel. (A) Light grey bar: Maturing granulocytes. (B) Medium grey bar: Promyelocytes. (C) Black bar: blast Cells.

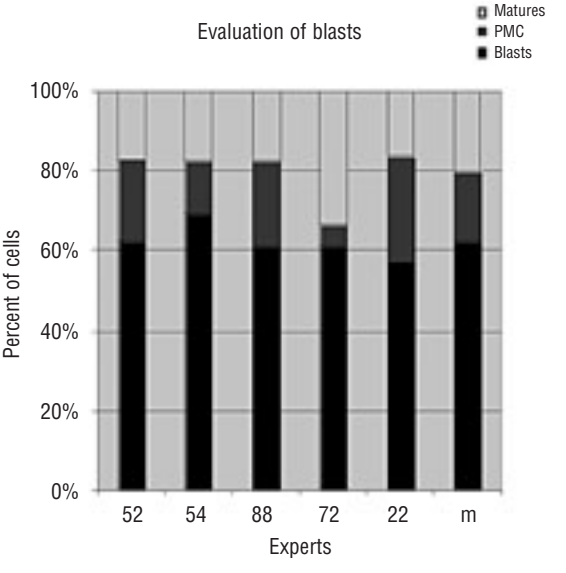

Table 2. Degree of consistency among experts: percent agreement/unweighted κ coefficients for pairs of 5 readers based on 264 cells divided into 4 categories (blasts, promyelocytes, matures, others).

Expert ref #	52	54	88	72	22
52	1	0.77/0.57	0.80/0.63	0.72/0.51	0.82/0.68
54	–	1	0.78/0.61	0.76/0.56	0.75/0.56
88	–		1	0.76/0.59	0.85/0.74
72	–	–	–	1	0.72/0.55
22	–	–	–	–	1

Consistency among readers is evaluated by percentage of agreement (first value) and the unweighted kappa coefficient (second value) for all pairs of readers. Conclusion: Percent agreement varies from 0.72 (pair (72×52) to 0.85 (pair 22×88) demonstrating a high concordance rate between experts. When adjusted for chance agreement, however, the κ values were somewhat lower indicating less than optimal agreement in some cases. The adjustment for chance agreement is influenced by small numbers of cells in two categories.

Figure 3. Perinuclear siderotic granules (cartoon of potential examples).

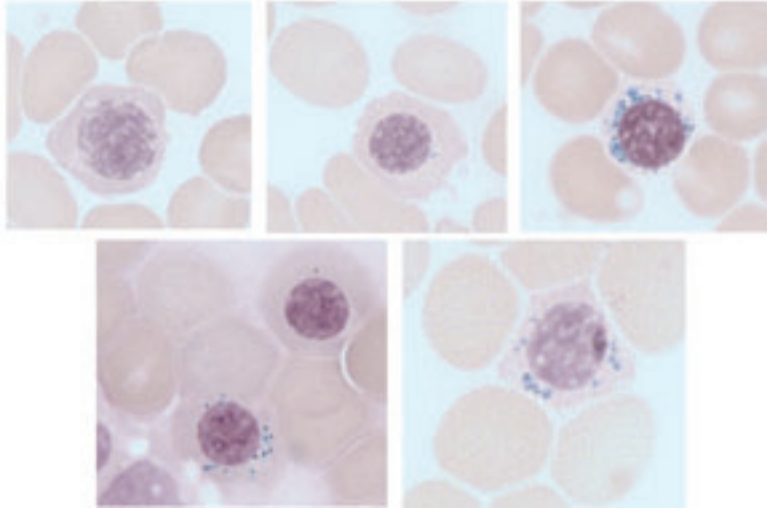

Figure 4. Prussian blue reaction of erythroid precursors. a. Upper panel (left to right): no siderotic granules; type 1 sideroblast (1 granule); type 3 sideroblast (numerous granules). b. Lower panel (left to right): type 1 sideroblast (upper cell), type 2 sideroblast (lower cell); type 3 sideroblasts (lower right); hematoxylin counter stain.

times required to have a minimum of 5 granules and sometimes a minimum of 10 granules.

After a review of many cases of sideroblastic anemia, the group determined that ring sideroblasts should have at least 5 granules in a perinuclear distribution; that these granules could either surround the entire nucleus, be localized to portions of the perinuclear area or cover at least one third of the nucleus (Figures 3 and 4).

The Working Group defined three types of sideroblast: Type 1 sideroblasts: fewer than 5 siderotic granules in the cytoplasm; Type 2 sideroblasts: 5 or more siderotic granules, but not in a perinuclear distribution; Type 3 or ring sideroblasts: 5 or more granules in a perinuclear position, surrounding the nucleus or encompassing at least one third of the nuclear circumference.

The group recommends that when counting ring sideroblasts all stages of erythroid precursors be counted and should include at least 100 nucleated erythroid precursors and for the definition of RARS the required number of ring sideroblasts remains at 15% as defined previously in the FAB and WHO classifications. The definition of a ring sideroblast proposed by the IWGMDS (an erythroblast with at least 5 siderotic granules covering at least a third of the circumference of the nucleus) has been incorporated into the 2008 WHO classification of Tumours of Haematopoietic and Lymphoid Tissues.[15]

The group also addressed the nuclear counterstain used to optimize the distinction of erythroid cells. The Working Group discussed the value of a number of stains such as neutral red, basic fuchsin, saffronin, hematoxylin, and light Giemsa, as well as staining for H-type ferritin and polyclonal antibody staining for siderotic granules. The group considered that further studies were needed to assess the value of these counterstains and methods but agreed that all had merits.

Only type 3 sideroblasts would qualify as *ring* sideroblasts to separate sideroblastic from non-sideroblastic anemia. This proposal will be tested in a similar manner to the blast definition by developing a web based digital image of multiple types of sideroblasts.

Discussion

In the absence of biological markers to stratify patients with MDS morphological assessment is essential for defining risk, regardless of which risk system is utilized. Because of the importance of determining the percentage of blasts as well as of ring sideroblasts, the IWGM-MDS focused on careful definitions that are illustrated and confirmed to be reproducible. The proposed definitions are intended to be used in conjunction with the WHO classification in order to make the categorization of patients with MDS more precise.

Authorship and Disclosures

GJM, JMB, JG, BJB, RB and TV reviewed the bone marrow preparations in the workshops and carried out the review of the digital images; GJM, JMB and BJB prepared the final manuscript; IB, MC, PF, UG, EH-L, IJ, AM, AkM, CMN, GS, MT and AY reviewed some of the material and contributed to the general discussions and all gave approval to the manuscript. The authors reported no potential conflicts of interest.

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