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Quality control initiative on the evaluation of the dysmegakaryopoiesis in myeloid neoplasms: Difficulties in the assessment of dysplasia

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ABSTRACT

Evaluation of megakaryocyte morphology is difficult but can be essential for the diagnosis of myelodysplastic syndromes (MDS) and other myeloid neoplasms. We agreed upon descriptions and provided images of megakaryoblasts and of normal and dysplastic megakaryocytes, which were used as a basis for assessing the concordance of expert morphologists in their recognition. We showed a high rate of concordance for the recognition of micromegakaryocytes and confirmed their strong association with hematologic neoplasia, including MDS. Concordance was also found to be good for the recognition of multinucleated megakaryocytes, which showed a significant association with MDS. However cytoplasmic abnormalities were found not to be useful in MDS recognition. The occurrence of appreciable numbers of nonlobulated and hypolobulated megakaryocytes in individuals without a myeloid neoplasm was confirmed. We demonstrated that subjects without a myeloid neoplasm can have some megakaryocytes that are assessed as 'dysplastic' or 'possibly dysplastic' and that to avoid over diagnosis of dysplasia, 'possibly dysplastic' forms should be excluded from the count of dysplastic cells. Our results demonstrate that the nature as well as the presence of megakaryocyte dysplasia is important in the diagnosis of MDS; although evaluation of 30 megakaryocytes is strongly recommended, it may be possible to recognize diagnostically important dysplasia when fewer megakaryocytes are present but highly diagnostic forms are seen.

1. Introduction

The diagnosis of low grade myelodysplastic syndromes is greatly dependent on morphologic evaluation of blood and bone marrow films for the detection of dysplasia in myeloid cells. The appearance of micromegakaryocytes in the peripheral blood, associated with atypical forms of megakaryocytes in the bone marrow has been noted in the literature from more than hundred years [1], but it was only in 1982 that most of the abnormalities were described under the designation '*Dysplastic features*' by the French–American–British (FAB) group [2]. These observations were then complemented in 1985 by the recognition of the cytologic features of megakaryoblasts [3]. More recently in 2008 the World Health Organization (WHO) expert group reiterated the features of megakaryoblasts and dysplastic megakaryocytes, including micromegakaryocytes and megakaryocytes with hypolobulated or nonlobulated nuclei [4].

The 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues requires the recognition of dysplastic features in the diagnosis of the myelodysplastic syndromes (MDS) and acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) [4,5]. In the case of MDS, the presence of 10% or more

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Table 1
Summary of the 13 categories that were used for this evaluation.

1.	Megakaryoblast
2.	Immature megakaryocyte
3.	Normal mature megakaryocyte
4.	Normal late megakaryocyte—'bare nucleus'
5.	Megakaryocyte with nonlobulated nucleus, but of normal size
6.	Megakaryocyte with hypolobulated nucleus, but of normal size
7.	Small megakaryocyte, nonlobulated or hypolobulated
8.	Micromegakaryocyte (mononuclear or binucleated)
	a. Immature
	b. Mature
9.	Multinucleated megakaryocyte (two or more separate nuclei)
10.	Large megakaryocyte with hyperlobulated nucleus
11.	Megakaryocytes with a cytoplasmic abnormality
	a. Vacuolation
	b. Agranular or hypogranular cytoplasm
	c. Persisting basophilia (nucleocytoplasmic asynchrony)
12.	Damaged (crushed/squashed) megakaryocyte, not evaluable[a]
13.	Not megakaryocyte lineage

[a] Damaged megakaryocytes that were assessable were assigned to the relevant category.

dysplastic cells restricted to one myeloid lineage is required for a diagnosis of refractory cytopenia with unilineage dysplasia – MDS with single lineage dysplasia (MDS-SLD) [4–6] – while the diagnosis of refractory cytopenia with multilineage dysplasia – MDS with multilineage dysplasia – [6] requires the presence of dysplasia in at least 10% of cells in two or more myeloid lineages. Recognition of AML-MRC on the basis of morphologic abnormalities requires at least 50% of cells in at least two lineages to be dysplastic. In the case of the megakaryocyte lineage, examination of a minimum of 30 cells is required. In many cases both the degree and characteristics of the dysplasia readily lead to the diagnosis of MDS or AML-MRC. In other instances, both the percentages of dysplastic cells and the characteristics of the dysplasia are marginal, resulting in uncertainty with regard to both the diagnosis and the classification. This is particularly so for cells of the megakaryocytic lineage where less is known of the maturation sequence and morphologic features in normal marrow. Well recognized dysplastic forms include micromegakaryocytes and nonlobulated megakaryocytes of normal size. The former have long been recognized as almost pathognomonic of MDS [7] while the latter show an abnormality that is less specific for a hematologic neoplasm but is highly characteristic of MDS with an isolated deletion of 5q (the "5q-syndrome"). There are other forms, such as multinucleated megakaryocytes, that are less strongly linked to MDS since they are occasionally seen in non-neoplastic disorders and even in the bone marrow of healthy subjects [8].

Even for experienced morphologists the decision as to whether a megakaryocyte is a normal variant or dysplastic can be difficult. For this reason, the International Working Group on Morphology of Myelodysplastic Syndromes (IWGM-MDS) set out to provide a comprehensive description of normal and dysplastic megakaryocytes and to assess the concordance between hematologists and hematopathologists in the recognition of these forms on the basis of the descriptions and illustrations provided. The correlation of specific individual features with diagnosis was also evaluated.

2. Materials and methods

A planning group of the IWGM-MDS (JEG, RDB, BJB, JMB) met in London in October 2014 to examine blood films and bone marrow aspirates using a multi-headed microscope. In addition, electronic images were analyzed collectively at the meeting and also individually before and after the meeting. A table of morphologically distinguishable subtypes of megakaryocyte was prepared (Table 1). Descriptions of normal and abnormal megakaryocytes were agreed on and appropriate illustrative images were selected to serve as an introductory tutorial.

2.1. Descriptions of the morphologic categories

1. A **megakaryoblast** is a normal cell but is not recognized in normal bone marrow aspirates because of its infrequency and the lack of distinctive distinguishing features. When megakaryoblasts are present in increased numbers their nature can be identified by immunophenotyping and their cytologic features can then be defined. Megakaryoblasts are similar in size to myeloblasts. They have a delicate or diffuse chromatin pattern and may have nucleoli. Sometimes they have no distinguishing features (Fig. 1: 1a) but in other patients they are observed to have cytoplasmic blebs (Fig. 1: 1b) and early platelet-type granulation of the cytoplasm [9,10]. They do not produce platelets. Megakaryoblasts are observed in significant numbers in acute megakaryoblastic leukemia (e.g. in acute megakaryoblastic leukemia associated with t(1;22)(p13.3;q13.1)) or in children with Down syndrome, in transient abnormal myelopoiesis (TAM) in neonates with Down syndrome, and in megakaryoblastic transformation of myeloproliferative neoplasms [11].
2. An **immature megakaryocyte** (Fig. 1: 2) is a normal cell with low ploidy. The nuclear cytoplasmic ratio is high. The nucleus shows some chromatin condensation and may show early lobulation. The cytoplasm is scanty and basophilic, often forming blebs but with few if any granules.
3. A **normal mature megakaryocyte** (Fig. 1: 3) is a large polyploid cell, ranging from 4 N (tetraploid) to 64N. Its nucleocytoplasmic ratio is lower than that of the immature megakaryocyte and its cytoplasm is less basophilic. Sometimes the cytoplasm is demarcated into a central basophilic zone and a peripheral more weakly basophilic zone. With increasing cytoplasmic maturation, there is granule formation and sometimes demarcation of proplatelets is apparent within the cytoplasm. Platelets may be apparent being detached from the surface. The nucleus shows chromatin condensation and is usually lobulated but it should be noted that some normal megakaryocytes are large but with nonlobulated nuclei and are separately identified (see below).
4. A **normal late megakaryocyte** (Fig. 1: 4) has shed almost all its cytoplasm as platelets. All that remains is a thin rim of weakly basophilic cytoplasm. These cells are often referred to as '*bare*' or '*naked*' nuclei but careful inspection shows a thin rim of residual cytoplasm. The nucleus shows pronounced chromatin condensation and may be hyperchromatic since it is near to apoptosis.
5. A **megakaryocyte with a nonlobulated nucleus and a normal size** (Fig. 1: 5) is a cell of normal size with a nucleus that is round or slightly oval. The cytoplasm is usually mature. Such cells can be seen in small numbers in the bone marrow of healthy individuals but they are increased in number in some patients with MDS and are particularly characteristic of the 5q-syndrome. It should be noted that the term '*mononuclear megakaryocyte*' should not be used when what is intended is a nonlobulated megakaryocyte. The majority of megakaryocytes are mononuclear so the term should only be used when it is necessary to make a distinction from a binucleated or other multinucleated megakaryocyte.
6. A **megakaryocyte with a hypolobulated nucleus and a normal size** (Fig. 1: 6) is a cell of normal size with a nucleus that is less lobulated than expected for the size of the nucleus. Such cells can be seen in normal bone marrow but numbers are increased in some patients with MDS, including the 5q-syndrome.