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Proposal for refining the definition of dysgranulopoiesis in acute myeloid leukemia and myelodysplastic syndromes

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ABSTRACT

Studies of morphology of myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) refer to the definitions produced by the French-American-British (FAB) group and by the World Health Organization expert group. To clarify some points regarding the dysgranulopoiesis that are still unclear we analyzed a series of 98 neutrophils from MDS patients with regard to granularity, nuclear segmentation, the appearance of the chromatin, the presence of giant neutrophils, and the presence of nuclear chromatin extensions. We found that cells with at least 2/3 reduction of the content of granules, Pelger-like neutrophils, dysplastic non-Pelger cells, neutrophils with abnormal clumping of the chromatin, and macropolycytes could be recognized as dysplastic and included in the 10% count recommended by these two classifications. In addition, we suggest that neutrophils with more than 4 nuclear projections could be recognized as a relevant dysplastic feature.

1. Introduction

Most studies that consider the morphology of myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) refer to the definitions produced by the French–American–British (FAB) group in 1982 [1]. These definitions have been included in the classifications proposed by the World Health Organization (WHO) in 1999 [2] and 2008 [3] but with some modifications regarding the percentage of cells that define categories and the significance of promonocytes. Nevertheless some points are still unclear. For example, the FAB group mentioned that "*quantitative assessment of the qualitative features may be necessary in some cases*" but without giving the percentage of cells that might be considered relevant; in the WHO classification it was proposed that ≥10% of myeloid cells showing dysgranulopoiesis (DysG) be one of the criteria for the diagnosis of MDS and that ≥50% of cells of at least two lineages showing dysplasia be one of the criteria for AML with myelodysplasia-related changes. This was further defined in Table 5.03, page 92, where 6 features of dysgranulopoiesis are listed. However the quantitative features were not defined. This lack of strict definitions could have an impact on the evaluation of dysplastic features in MDS and AML and may explain the variability of some diagnoses in different studies [4,5].

The most frequent cytological criteria that are included in the usual definition of dysgranulopoiesis have been recognized for a long time. "Pelger" cells were observed a century ago [6], as having "*a short and compact nucleus with mosaic-like clumping of the chromatin that is especially eye catching*". Three years later, Huët [7] observed that "*the nucleus consisted of two very distinct segments connected by a very thin filament*". This definition (or the terms hypolobation or hypolobulation) is generally used by most morphologists for the diagnosis of dysplasia in MDS or AML [8,9], the cells also being called [pseudo]-Pelger-Huët (pPH) cells.

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Reduction of granules in neutrophils was also described by the FAB group [1] as: "*...the neutrophils may appear agranular or hypogranular*...", but the definition of hypogranular was not provided and was not clarified by the WHO classification, the term "*cytoplasmic hypogranularity*" being used without further explanation [2,3].

Most published papers have also included abnormal clumping of nuclear chromatin (large blocks of chromatin separated by clear zones) as a dysplastic feature, but this point is not included in the WHO description. This abnormality was first described as a feature of acquired dysplasia by Gutske in 1970 [10] and was confirmed by Felmann in 1988 [11], but the degree of nuclear clumping or condensation has been difficult to define.

Hypersegmentation or irregular segmentation of neutrophils was reported in both FAB and WHO publications but without any description, although in many cases it is associated with giant cells and then has a different significance. The FAB also described some neutrophils with nuclei of "*bizarre shapes*" and "*bizarrely segmented neutrophils*"; and the significance of macropolycytes showing dysplastic features has also been recently described [12] but is not specific.

As stated in the WHO classification [2]: "the characteristics of the dysplasia may be relevant in predicting biology of MDS and a relationship to specific cytogenetic abnormalities". Clearly there was a need to clarify all these definitions in order to have more consistency and to achieve a high concordance in differentiating Refractory Cytopenia with Unilineage Dysplasia (RCUD) from Refractory Cytopenia with Multilineage Dysplasia (RCMD) among cases of MDS and in recognizing AML with Myelodysplasia-Related Changes [3].

2. Materials and methods

The goal of the study was to establish standardized, reproducible criteria for defining granulocytic dysplasia. All observers reviewed the identical individual cells, each cell having a separate label, from a library of cells from previously identified MDS/AML patients.

Bone marrow films from six patients from a single institution with primary MDS or AML (3 RAEB-1, 2 RAEB-2 and one AML M2, as defined according to the WHO classification) were stained with a standardized Romanowsky stain. A cover slip was applied. Photographs of neutrophils were taken consecutively, without selection, for each case. A total of 98 digital images thus produced were viewed as JPEGs inserted into PowerPoint presentations and circulated by means of the internet to the co-authors. Each author independently completed a table describing the nuclear and cytoplasmic characteristics of each cell without knowing the gender of any case; this was then sent to the recording center (JG).

An evaluation was made of granularity, pPH neutrophils, increased chromatin clumping, nuclear projections, and the presence of macropolycytes or of other dysplastic forms, not already listed. Pseudo-Chédiak-Higashi and irregular hypersegmentation (mentioned in the WHO classification) and neutrophils with ring-shaped nuclei were not investigated because they are uncommon features of MDS.

To assess the degree of cytoplasmic granularity each observer was asked to rank cells according to the content of granules. From the initial ranking (by each participant), the mean and standard error for each cell were determined. pPH neutrophils were defined as by Huët [7], with mature neutrophils with a total lack of nuclear segmentation also being included. Neutrophils that were considered to be clearly abnormal but that did not meet defined criteria for specific abnormalities were recognized as 'other dysplastic forms' (or dysplastic [non]-pPH).

Abnormal clumping of chromatin was identified as condensation of the chromatin into large blocks separated by clear zones, mimicking nuclear fragmentation [10,11]. Macropolycytes were defined as cells at least twice normal size with an appropriately large nucleus [12].

An attempt was made to evaluate the number of nuclear projections (NP), to verify their potential to be included as a criterion of dysplasia, taking into account the expected nuclear drumsticks present in a proportion of neutrophils in normal females. These projections have previously been designated by different names: Karyoschize, drumstick, nuclear extension, sex chromatin, appendages, etc. We noted these chromatin extensions and classified them according to the proposal by Kosonow in 1956 [13]: Type A: drop-like projection, symmetrical with a very thin connection, type B: like a drop, but with a large connection to the nuclear mass, type C: linear projection symmetric or not, type D: very rare, linear extension ending with a pocket (rarely seen in our experience). In the statistical study we excluded type A projections, since they were seen largely in women, and are known to be sex chromosome-related [15]; we included both B and C types.

Finally a total of 98 cells (MDS/AML) were evaluated independently by seven morphologists (one performed only the evaluation of the nuclear projections) with regard to 6 different factors: hypogranularity, Pelger-like, other abnormal nuclear shape, abnormally clumped chromatin, macropolycytes and nuclear projections. In addition, we investigated the peripheral blood from 4 healthy controls and 6 further MDS patients for the evaluation of nuclear projections. A total of 711 cells were investigated in this supplementary series (366 cells of healthy controls, and 345 cells in MDS); evaluation was done without knowledge of whether the images were from a healthy control or an MDS patient. Genders for all were hidden for the duration of the morphology evaluation.

3. Statistical considerations

The statistical analysis was performed using a Chi-squared test and a Kappa test [14] to evaluate any possible association between different features. Cells were classified for their content of granules according to their rank (means of rank and standard deviation). Concordance between observers was determined to be present when at least 4/6 observers were in agreement. A Somers test (Test of tendency) was applied to compare both controls populations for NP evaluation.

4. Results

The classification of the degree of granularity is shown in Fig. 1 and Table 1. Cells are represented according to their rank (mean of rank) for the content of granules. We found (Table 2) that at least 2/3 reduction of granules was required for hypogranularity to be recognized consistently by different observers and therefore to be accepted as a criterion for granulocytic dysplasia (DysG).

The segmentation of the nucleus was evaluated as pPH, dysplastic non-pPH (Fig. 2), or normal. The evaluation of pPH cells achieved 99% concordance between observers, and dysplastic non-pPH: 99% concordance. Abnormal clumping of the chromatin (Fig. 3) was assessed with 93% concordance.

All observers agreed on the identification of the 9 macropolycytes (Fig. 2).

The NP evaluation was performed by 7 morphologists (Table 3). In the original series (A: MDS/AML with a total of 98 cells) we found 53% of cells without NP, 21% with only 1 NP, 12% with 2 NP and 13% with more than 2 projections. The results of the supplementary series from the blood of 6 new MDS cases and four specimens from healthy biotechnologists with normal blood counts are displayed in Table 3. We found that 51% of cells in the healthy controls had no nuclear projections, compared to 36% of cells in the MDS cases. Cells with more than 2 NP were 9% in the healthy controls and 13% in MDS patients. Cells with more than 4 NP were not observed in healthy controls whereas they were seen in 6% in the original MDS series and 3% in the second MDS series. These results are significantly different since the p value is <0.0001 or =0.0001 when B and C, or A, B, and C are compared respectively (see results in Table 3).

Relationship between dysplastic criteria

In the original series (98 cells all from MDS/AML cases) we analyzed 6 criteria: one cell met 4 criteria; 7 cells met 3 criteria; 28 cells met 2 criteria; 39 cells met 1 criterion and 23 cells met none. Using the Kappa test (Table 4) we observed that there was no relationship between the presence of pPH neutrophils and the reduction of granularity, but that pPH was often associated with abnormal chromatin clumping in the same patient ($p = 0.002$) despite the fact that 12 and 7 cells were discordant.

The presence of macropolycytes was significantly associated with a non-pPH dysplastic nucleus ($p < 0.0001$), this providing the