Morphological evaluation of monocytes and their precursors

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ABSTRACT

The monocyte is still the most difficult cell to identify with confidence in the peripheral blood or in the bone marrow in healthy individuals as well as in patients with infections, and in those with leukemic proliferations. The goal of this study was to establish morphological definitions so that monocytes, including immature monocytes, could be separated from the spectrum of monocyte precursors. Cells from peripheral blood or bone marrow were selected to provide a large panel of normal and leukemic cells at different maturational stages and were submitted to 5 experts, who had previously reached a consensus, on the basis of microscopy, in defining 4 subtypes: *monoblast, promonocyte, immature monocyte, mature, monocyte*. They achieved a good concordance rate of 76.6% and a high κ rate confirming that the criteria for defining the 4 subtypes could be applied consistently. It has now to be established whether these monocyte subtypes correlate with immunological or molecular markers and are clinically relevant.

Key words: monocytes, monoblasts promonocytes immature monocytes, acute myelomonocytic leukemia, chronic myelomonocytic leukemia.

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Introduction

The major and dominant role of the cells of the monocytic-macrophage lineage in the defence of the human organism has never been questioned. However, the identification of cells of this lineage has raised problems as attested by the variety of names used to designate monocytes and their precursors. One of the questions which arises is whether the lineage can be defined by a unique and recognizable marker (on the membrane, the cytoplasm or the nucleus). At the present time, immunology is still imperfect in identifying cells of this lineage, and precise morphological definitions are therefore required to provide the basis for clinical studies.

The monocytic subpopulations were described by Bessis[1] using terms that are summarized in *Living Blood Cells* (1973). Small monocytes were distinguished from large (classical) monocytes. These *small* cells cover *leukocytoid lymphocyte* (Downey 1936), *lymphocytiform histiocyte* (Policard 1957) and also the *flag-like* cell described by Dantchev in 1950. Bessis also proposed the terms *monoblast* and *promonocyte* but recognized that they were better defined by electron microscopy.

The French–American–British (FAB) group[2] proposed in 1976 that a leukemic monocytic proliferation be classified as *poorly differentiated* with monoblasts predominating, and *differentiated* with more promonocytes and they outlined some morphological criteria for identification of these two cell types. Since this publication focused only on leukemic proliferations, nothing was said about the circulating blood monocytes and the distinction between leukemic and non-leukemic cells.

In 2001 the WHO[3] published an updated version of *Tumours of haematopoietic and lymphoid tissues*, with the relevant authors

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proposing new criteria for recognition of monocytes [(in chronic myelomonocytic leukemia (CMML)] and promonocytes and monoblasts [in acute monoblastic/monocytic leukemia (AMoL)]. Despite these efforts to clarify definitions it has sometimes proved difficult in practice to make a reliable distinction between neoplastic and reactive cells of this lineage.

Morphological differences between *classical monocytes* and *monocytes after stimulation* (infections, during recovery from bone marrow suppression and after administration of growth factors) have already been described[4] and allow the identification of four morphologically recognizable maturation stages.

Given this background, a group of 5 experts in morphology met on different occasions and drew up new proposals after review of slides from normal individuals as well as cells of monocyte lineage from patients with CMML or AMoL. The group defined criteria to identify 4 subtypes: monoblast, promonocyte, immature monocyte and mature monocyte. Ninety different cells illustrating the difficulties in identifying the 4 subtypes, were selected (from a large library of 400 cells) by using digital pictures (high definition) and using the same technique that has already been published[5] for blast identification in myelodysplastic syndromes and acute myeloid leukemia. The set of digital pictures was circulated between the experts with the recommendation to use the newly proposed definitions. The present report concerns the validation of the criteria for the definition of the 4 monocytic subtypes.

Design and Methods

Definitions

Bone marrow smears (12 cases) from CMML and AMoL were reviewed by experts to give a critical overview on monocyte identification. Blood smears (7 cases) from patients within non-leukemic states but demonstrating a monocytic proliferation (after infection, post-partum, or newborn) were examined to increase the range of cells considered. Table 1 summarizes the criteria for the categorization of the 4 subtypes that were agreed by the group.

Materials

Two experts (*JG and JMB*) met in Rennes to produce a selection of digital pictures illustrating the difficulties in identifying the 4 subtypes. The selection of 90 monocytic cells was circulated by internet among the 5 experts who were asked to apply the proposed recommendations. All evaluations were made at a distance, independently.

Methodology

A first reading was performed and results were sent to one center for analysis. A second reading of some images was requested by the center to ensure there had been no errors of interpretation or recording.

Statistical analysis

Statistical analysis including a κ test was performed using SAS version 9.1 program.

Results and Discussion

The 90 monocytic cells were identified by all five experts (*JG, JMB, BB, RB, TV*) strictly applying the recommendations as described in Table 1. Results and concordances between observers are given in Table 2. All 5 experts made the same identification (concordance between experts 5/5) on 25 cells (3 monocytes, 5 immature monocytes, 8 promonocytes, 9 monoblasts) and 4 of 5 experts gave an identical interpretation for 9 monocytes, 21 immature monocytes, 7 promonocytes and 7 monoblasts (44 cells). The percentage of good concordance (at least 4 of 5 experts) was 76.6% (69 of 90 evaluated cells).

The 21 remaining cells were more difficult to identify since the concordance rate was 3/5 or 2/5. For 3/5 concordances (15 cells) the subtype could be validated since the majority of experts concurred and in that way 4 monocytes, 8 immature monocytes, 2 promonocytes and one monoblast were classified. When the concordance rate was 2/5 (6 cells) it could mean (for example cell #37), that 2 experts identified a cell as *monocyte* and 2 others as *an immature monocyte*, but the fifth observer identified it as a promonocyte. Following this analysis,

Table 1. Recommendations for monocyte evaluation in the blood or bone marrow smears. Four subtypes may be identified with high concordance between experts.

	Nuclear shape	Chromatin	Cytoplasm	Comments
Monoblast	Round/oval	Delicate / lace-like Nucleolus prominent	Basophilic Rare azurophilic, Granules	Large: 20-30 µm
Promonocyte	Convoluted / indented	Delicate / lace-like Nucleolus prominent	Variably basophilic Variable azurophilic Granules	Except for nuclear shape, very similar to monoblast
Immature monocyte	Convoluted / indented	More condensed Rare nucleolus	Less basophilic than promonocyte or blast, but more basophilic than mature monocyte	Resemble monocytes but less mature and smaller
Monocyte	Lobulated/ indented	Condensed No visible nucleolus	Gray Occasional azurophilic granules. Occasional vacuole	Large : 20-25 µm

cell#37 was interpreted as an *immature monocyte* to have a final identity for statistical analysis. By this procedure, all 6 cells with only 2/5 concordances had a second reading and were confirmed as immature monocytes.

Finally, a consensus was obtained on 17 blasts, 17 promonocytes, 40 immature monocytes and 16 monocytes (90 cells). The highest percentage of good concordance (at least 4/5) was clearly obtained for the monoblast subtype (16/17, 94%) followed by the promonocytes (15/17, 88%), then the monocytes (12/16, 75%) and the immature monocytes (26/40, 65%). The degree of consistency among experts and the consistency of each with the final consensus may be evaluated by the percentage of agreement for all pairs of readers and extended to the final consensus (κ test). The percentage of agreement varies from 0.6244 to 0.8634 demonstrating a high concordance rate for the large majority of comparisons but with the limitation that the test has been applied on a small set of data.

The monocyte is still the most difficult cell to identify with confidence in healthy individuals as well as in patients with infections, and in those with leukemic proliferations. This term (monocyte) refers to a lineage that has always presented difficulties with regard to description and delineation. In the past, monocytes and macrophages have been included in the *reticulo-endothelial* system and today most of the research in this field is directed at identifying the place of the *macrophage and dendritic cell progenitor* in the lineage and to understanding monocytic and macrophage trafficking in the blood and tissues. Immunophenotyping, cytogenetic and genetic technologies are deployed to investigate the different subsets of this lineage. Our goal was to establish morphological definitions so that monocyte subtypes could be separated from the spectrum of normal and reactive promonocytes and leukemic monoblastic cells.

Recent reviews[6-11] have reported immunological studies to try to identify monocytic subpopulations. They demonstrate that the monocytic lineage may be identified by expression of CD14 or CD16 or CD62. Some correlations were found between a small-cell subset and a reactive monocytosis.

A comparative analysis, based on morphology, cytochemistry, immunophenotype and functional characteristics of human monocytes was published in 2001 by Almeida *et al.*[12] Despite the quality of their investigations, the morphological assessment was performed after purification and centrifugation and, for this reason, cannot be applied to defining morphological criteria on unmanipulated blood or bone marrow smears.

Based on all the data above, we propose newly defined criteria for 4 subtypes of cells of monocyte lineage, in order to be able to correctly assess various cell types during the follow-up of patients. These new crite-

Table 2. Concordances between experts according to the final diagnosis: 16 mature monocytes (M), 40 immature monocytes (I), 17 promonocytes (P) and 17 monoblasts (B) have been identified.

Concordances	M	I	P	B	Total
2/5	M/I=2	I/P=4			6
3/5	4	8	2	1	15
4/5	9	21	7	7	44
5/5	3	5	8	9	25
Total	16	40	17	17	90

All cells with only 2/5 concordances were classified as immature monocytes. Concordance rate for at least 4/5 concordances is 76.6% (69/90).

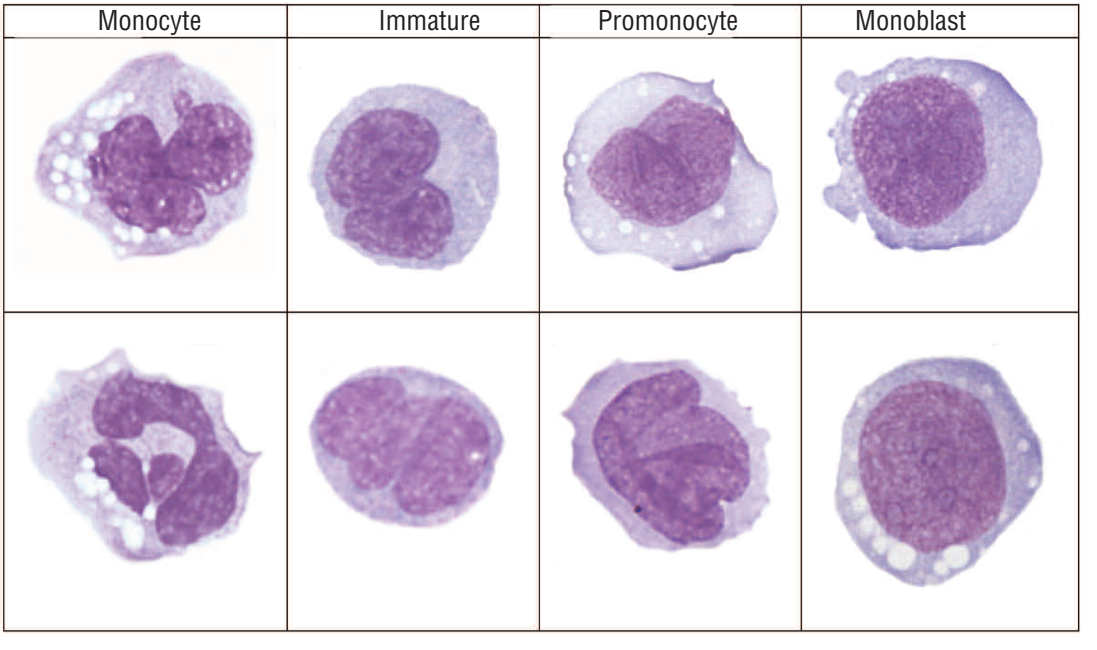

Figure 1. Example of monocyte subtypes as circulated for evaluation.

ria have been established after long and detailed observation (of smears) by the group of experts. To be sure that all were discussing the same cells, it was decided to supplement the initial microscopy with digital images of a selected set of cells. The pictures came from different patients (healthy, infected, with inflammatory disease or with leukemic proliferation).

The result of 76.6% good concordance in this study is considered very encouraging for cells that have always proved problematic. The *immature subtype* is over-represented (40/90) in this series since it was the goal of the study to distinguish this cell type from other more mature or less mature cells. A high concordance was achieved despite the deliberate inclusion of this difficult subtype. We have demonstrated that this subtype can be identified on blood or bone marrow smears and can be used for classification and follow-up of patients. On the basis of this experience and the available literature, we propose the recognition of 4 morphological subtypes for application in future studies.

The interest of this new morphological sub-classification will be to work on its correspondence with clinical data and immunological or molecular markers. Almeida *et al.* in 2001 were nearing this goal but it is necessary to work on native blood or bone marrow smears (MGG stain) and not on centrifuged cells.[12]

It has now to be established whether the 2 most mature proposed subtypes (monocytes and immature monocytes) have any correspondence with the immunological subtypes as proposed in different published papers. The identification of the 2 subtypes most characteristic of acute leukemia (promonocytes and monoblasts) may help distinguish better between FAB AML M5 (a or b), FAB AML M4, and CMML. The identification of immature monocytes and their distinction from promonocytes is of critical importance in making the distinction between AML and CMML with increased blast cells. Morphologically similar cells can be seen in reactive conditions and in leukemias; the term *immature monocyte* has been chosen since it is a purely morphological term and does not imply either a leukemic state or a reactive condition; this is consistent with the WHO report from 2008[13] where these cells are called *abnormal monocytes* but without being considered as monoblast equivalents.

Conclusion

Since the definition of mature monocytes, immature monocytes, promonocytes and monoblasts has not been absolutely clear in the literature, improved criteria for identifying these cells have been established by reviewing blood films and by circulating a series of digital images among experts. The high rate of concordance and the results of the κ test lead us to hope that these proposals will help in the diagnosis and follow-up of patients with monocytic proliferation.

Authorship and Disclosures

Morphological evaluation was performed by JG, JMB, BB, RB, and TV. Definitions and the manuscript were elaborated by all authors. All pictures were selected by JG and JMB and produced by JG.

The authors reported no potential conflicts of interest.

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