A European consensus report on blood cell identification: terminology utilized and morphological diagnosis concordance among 28 experts from 17 countries within the European LeukemiaNet network WP10, on behalf of the ELN Morphology Faculty

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Received 9 June 2010; accepted for publication 1 July 2010
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Summary
This paper describes the methodology used to develop a consensual glossary for haematopoietic cells within Diagnostics-WP10 of European-LeukemiaNet EU-project. This highly interactive work was made possible through the use of the net, requiring only a single two-day meeting of actual confrontation and debate. It resulted in the production of a freely accessible tool that could be useful for training as well as harmonization of morphological reports in onco-haematology especially, without geographic limitation, not limited to European countries. Moreover, this collective work resulted in the production of a consensus statement, taking into account individual practices, collegial agreement and literature data.

Keywords: blood cell morphology, myelodysplasia, haematological malignancies diagnosis.

Morphological evaluation of peripheral blood (PB) and bone marrow (BM) cells through microscopic examination of properly stained smears remains crucial in haematological diagnosis mostly for differences in BM processing procedures, staining, degree of skill in interpretation and terminology used, contributing to a lack of standardization of this diagnostic tool. The new World Health Organization (WHO) classification (Swerdlow et al 2008) highlights the importance of morphological aspects, quantitative as well as qualitative, for the diagnosis and follow-up particularly of myeloid neoplasms, and above all myelodysplastic syndromes: therefore the microscope still remains a very important tool in the integrated diagnostic process of haematological diseases.

Information and Communication Technology (ICT) provides the opportunity to exchange images and information without geographic limitation, saving time and resources and many studies highlight the robustness of ICT for diagnostic assessment of blood cell morphology. (Flandrin,1997; Riley et al, 2002; Luethi et al, 2004).

European LeukemiaNet (ELN) is an European Union project that includes 162 centres from 33 countries with the major goal of organizing a network for improving leukaemia diagnosis, care and research. The ELN Morphology Faculty (EMF), composed of 28 expert morphologists from 17 European countries (Appendix 1), was organized within the activities of the Diagnostic WP10 with the goal to increase quality of diagnostics based on cytomorphology, as the first technique worldwide, and to support this by a web-based consensus report, including a uniform nomenclature.

This study was carried out by using anonymous files in three consecutive steps, aimed to take advantage of individual competences, to train each other, and to reach a full consensus by the end of the study. Statistical analyses were performed with the MEDCALC software (Mariakerke, Belgium).

First phase

To test the methodology, 50 images with 139 consecutively numbered cells were uploaded onto a restricted web page together a database containing name proposals (cell lineage and maturation stage). The initial lineage/morphological categories were six: erythroid (Ery), granulocytic (Gra), lymphoid (Lym), megakaryocytic (Mgk), monocytic (Mon), blast (Bl) and ‘other’ (Oth). EMF members were asked to indicate whether they agreed with proposed terminology or give an alternative definition. After collecting data, a preliminary version of a consensual ELN Blood Cells Glossary (EBCG) of morphological terms was created.

Five meaningful cell images together with the proposed cell definition(s) (according to the EBCG nomenclature when available) were provided by each of the 21 members of the EMF and submitted to all for the cell definition. A cell definition was considered approved if agreement from at least 17 members was obtained (consensus >80%).

Results

One hundred and sixty-four images containing 438 labelled blood cells were initially collected from the EMF members with the submitter’s proposal(s) of term(s) for each labelled cell. A full consensus (≥17/21) was achieved for 250 cells (59-4%): major discrepancies concerned blasts and monocytic series and discrepancies in the nomenclature used to identify the differentiation stages of the erythroid series: the EMF decided to add the alternative denomination in brackets, i.e. ‘erythroblast basophilic (early erythroblast)’, and the EBCG was prepared. The first Delphi questionnaire was applied to a total of 216 cells. The EMF created a new category, ‘Cell to delete’, for a set of eight cells, since failure to reach a firm decision was mostly due to the poor quality of the images. Full agreement

Second phase

The EMF was extended to 28 morphologists to achieve a broader representation: each member submitted two new images without providing cell names. Participants were asked to name these cells using terminology from the EBCG, whenever possible. Data were collected, grouped and analysed with the same requirement of at least 80% agreement for definite term used for a given cell.

Third phase

During a 2-day consensus meeting, EMF members collectively reviewed (i) the set of 79 cells with a scoring difference <7 between two options after the first Delphi round and (ii) the set of 98 not fully agreed cells submitted in the second phase.

The Delphi technique (Spivey, 1971; Keeney et al, 2006) is a structured process based on the collection of knowledge from a group of experts and rounds of examination of proposals/questionnaires until consensus is obtained: this methodology has been previously applied in several clinical studies, including attempts to obtain consensus in histopathology (Nagy et al, 1982). It was applied to obtain better consensus for cells without a full agreement during the first round. As a prerequisite, one alternative term had to have been proposed by at least three members to be included into the options of the Delphi questionnaire. Cells with a full agreement (≥17/21) but with a different classification provided by at least 3/2 members were also submitted to the Delphi questionnaire, in order to widen the discussion. A list of cells together with the proposed options for terminology was sent to the participants: for each option, the rate of initial agreement was indicated as the number of EMF members in agreement. Each option was scored between 3 and 1, to indicated full agreement, partial agreement and full disagreement, respectively. After collecting data, a new Delphi questionnaire was performed for those cells presenting with a low final score (<7) resulting from at least two full agreements.

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was reached on all of the 216 submitted cells and the EBCG was implemented. Seventy-nine cells showing a scoring difference $<$7 between two options were listed to be discussed during the consensus meeting together with several additional issues, such as the limitation of microscopic evaluation alone to define a lymphocyte as atypical or reactive, the term which had to be used to identify a ‘morphologically abnormal’ plasma cell (atypical vs. dysplastic), the question of whether the term ‘dysplastic’ should be used only for the three myeloid lineages or not. The majority of morphological discrepancies concerned: (i) 34 cells (2 Mgk, 8 Ery, 24 Gra) if they should be considered normal or dysplastic, (ii) seven cells (3 Gra, 3 Mon, 1 Ery) concerning the differentiation stage, (iii) 10 cells, whether they should be identified as blast versus monoblast (5), promonocyte (4) and promyelocyte (1).

In the second phase of the study, 64 new images with 162 labelled cells were collected and submitted to EMF members without any cell name proposal. According to the cell name provided by the submitters, the initial distribution of these 162 cells showed an increase in monocytes and an equivalent decrease in granulocytes compared to the proportions of the first set of 438 cells. Full agreement, including use of the same denomination, was reached immediately for 60 cells (36-14%).

### Table I. Cell lineage distribution and agreements in the different steps of the study.

#### A) First phase of the study: cell lineage distribution according to initial submitters’ proposals, EMF Full agreement and EMF Not-full agreement

<table>
<thead>
<tr>
<th>Cell series</th>
<th>Initial proposal of 438 submitted cells</th>
<th>Agreement $\geq$17/21 on 260 cells (59-4% of submitted cells)</th>
<th>Agreement $&lt;$17/21 on 178 cells (40-6% of submitted cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytic</td>
<td>126 (29%)</td>
<td>75</td>
<td>51</td>
</tr>
<tr>
<td>Erythroid</td>
<td>77 (17-5%)</td>
<td>53</td>
<td>24</td>
</tr>
<tr>
<td>Monocytic</td>
<td>35 (8%)</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>Lymphoid</td>
<td>107 (24-5%)</td>
<td>64</td>
<td>43</td>
</tr>
<tr>
<td>Blast</td>
<td>29 (6-5%)</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Megakaryocytic</td>
<td>23 (5%)</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>41 (9-5%)</td>
<td>34</td>
<td>7</td>
</tr>
</tbody>
</table>

#### Distribution of 216* cells Before and After Delphi round

<table>
<thead>
<tr>
<th>#216 cells (49-3% out of 438 submitted cells)</th>
<th>Cell distribution Before the Delphi round</th>
<th>Cell distribution After the Delphi round</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytic series</td>
<td>65 (30%)</td>
<td>61 (28%)</td>
</tr>
<tr>
<td>Erythroid series</td>
<td>28 (13%)</td>
<td>27 (12-5%)</td>
</tr>
<tr>
<td>Monocytic series</td>
<td>24 (11%)</td>
<td>24 (11%)</td>
</tr>
<tr>
<td>Lymphoid series</td>
<td>52 (24%)</td>
<td>48 (22%)</td>
</tr>
<tr>
<td>Blast</td>
<td>26 (12%)</td>
<td>30 (14%)</td>
</tr>
<tr>
<td>Megakaryocytic series</td>
<td>8 (4%)</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>Other</td>
<td>13 (6%)</td>
<td>12 (5-5%)</td>
</tr>
<tr>
<td>To delete</td>
<td>8 (3%)</td>
<td></td>
</tr>
</tbody>
</table>

#### B) Second phase of study: cell lineage distribution of the submitted cell Before and After the discussion at the 2-day meeting

<table>
<thead>
<tr>
<th>Cell distribution</th>
<th>Cell distribution Before the meeting (162 cells)</th>
<th>Cell distribution After the meeting (162 plus 4 added cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytic series</td>
<td>38 (23-5%)</td>
<td>39 (23-5%)</td>
</tr>
<tr>
<td>Erythroid series</td>
<td>30 (18-5%)</td>
<td>31 (19%)</td>
</tr>
<tr>
<td>Monocytic series</td>
<td>20 (12%)</td>
<td>17 (10%)</td>
</tr>
<tr>
<td>Lymphoid series</td>
<td>42 (26%)</td>
<td>35 (21%)</td>
</tr>
<tr>
<td>Blast</td>
<td>8 (5%)</td>
<td>14 (8-5%)</td>
</tr>
<tr>
<td>Blast NOC</td>
<td>11 (7%)</td>
<td>15 (9%)</td>
</tr>
<tr>
<td>Megakaryocytic series</td>
<td>13 (8%)</td>
<td>7 (4%)</td>
</tr>
<tr>
<td>Other</td>
<td>13 (8%)</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>To delete</td>
<td></td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Cytologically unclassifiable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOC, not otherwise categorized.

*This number includes 178 cells with an agreement $<$17/21 plus n = 37 cells with an agreement $\geq$17/21 associated with the same alternative proposal by three or four faculty members.
In general, bone marrow aspirates are not appropriate for the diagnosis of cancer. However, collections of immature cells in a syncytial cluster should be reported as 'Cytologically unclassifiable' and should be confirmed with a bone marrow biopsy and appropriate immunostains.

When significant extra information that makes the photograph more informative is available, this should be added. When brief information is available, it should be provided in brackets, e.g. erythroblast, vacuolated (alcohol excess).

Dysplasia is a description of morphologically abnormal development and is not synonymous with myelodysplastic syndromes.

The term 'dysplastic' should only be used for the three myeloid lineages. For cells of other lineages showing similar morphologically abnormal development, e.g. lymphocytes and plasma cells, the convention of using the term 'atypical' should be followed. Despite their myeloid nature, the convention of applying 'atypical' also to mast cells is supported.

Dysplastic haemopoiesis can lead to the production of cytologically abnormal erythrocytes (e.g. poikilocytes or a dimorphic population) or platelets (e.g. giant, hypogranular or with abnormal granules); however the term 'dysplasia' should be confined to nucleated cells.

It was emphasized that dysplasia should not be assessed in patients receiving growth factors and, furthermore, that, as heavy neutrophil granulation is often the result of sepsis, its presence should not be included in the quantification of dysplastic features for the diagnosis of myelodysplastic syndromes or for the recognition of multilineage dysplasia in acute myeloid leukaemia.

In scoring the percentage of dysplastic cells for the purpose of diagnosis and classification, subtle abnormalities should not be considered sufficient to categorize a cell as dysplastic, as in the WHO classification.

The abnormal cells of acute promyelocytic leukaemia should be designated as in the WHO classification as hypergranular and microgranular (hypogranular) promyelocytes respectively. Although both forms are dysplastic, the word 'dysplastic' is not needed in the designation.

Hypogranular promyelocytes of other types should be included in the general category of 'dysplastic promyelocyte'.

The blast definition proposed by the International Working Group on Morphology of MDS (Mufti et al, 2008) should be used. Briefly, this designation recognizes agranular and granular blast cells with the latter differing from type II blast cells as defined by the French–American–British (FAB) group in that they may have more than 'scanty' granules but have all the other characteristics of blast cells. Blast cells that cannot be recognized as belonging to a specific lineage should be designated 'blast, not otherwise categorized'. Assigning lineage to blast cells is facilitated by assessing cells in relation to each other rather than in isolation.

Monoblasts and promonocytes should be defined as in the WHO classification (Swerdlow et al, 2008). A monoblast has a round or oval nucleus. A promonocyte has a convoluted, folded or grooved nucleus. In the WHO classification, a promonocyte is a blast equivalent and the term should therefore be applied only to a cell that has a delicate or dispersed chromatin pattern, equivalent to that of a monoblast. Distinction between a monoblast and a promonocyte is not of practical importance since they are regarded as having the same significance.

However, distinction between a promonocyte and an atypical/abnormal/immature monocyte can be very difficult. A lack of rigour in applying the defining criteria of promonocyte definition may lead to monocytes being misclassified as promonocytes, which could in turn lead to an erroneous assessment of the number of blasts plus blast equivalents and thus to a misdiagnosis of chronic myelomonocytic leukaemia as acute monocytic leukaemia. Appreciation of the immature chromatin pattern is crucial in recognition of a cell of monocytic lineage as a blast equivalent.

Based on morphology alone, atypical lymphocytes should be further divided into (i) Atypical lymphocyte, suspect reactive (ii) Atypical lymphocyte, suspect neoplastic (iii) Atypical lymphocyte, uncertain nature.

In general it is preferable to avoid the use of eponymous names to identify cells or peculiar morphological patterns. However some names are well established and have a clear meaning (e.g. Auer rods) and their use should continue.

'Mott cell' and 'Russell bodies' are used differently in different European countries. The original papers were therefore reviewed after the meeting. Dutcher bodies, single or multiple Russell bodies and the inclusions of Mott cells (Russell,1890; Mott,1905; Dutcher & Fahey,1959) are the morphological evidence of the same cytoplasmic inclusion(s), in the case of Dutcher bodies being invaginated into the nucleus. Mott cells contain Russell bodies. The proposed term to identify plasma cells with this morphological pattern is: plasma cell, atypical, with nuclear inclusions or plasma cell, atypical, with cytoplasmic inclusions/vacuoles.

The term 'macrophage' should be used for a potentially phagocytic cell derived from a monocyte. The term 'histiocyte' has a broader use, including macrophages and dendritic cells, such as Langerhans cells, not normally seen on peripheral blood or bone marrow smears.

When examining a single cell, cytological criteria do not permit a distinction between Gaucher cells and pseudo-Gaucher cells and both should therefore be categorized as 'Gaucher cells' with an explanation between quotes, e.g. 'Gaucher disease' or 'pseudo-Gaucher cell' in chronic myeloid leukaemia'.

In general, bone marrow aspirates are not appropriate for the diagnosis of cancer. However, collections of immature cells in a syncytial cluster is suggestive of metastatic cancer and should be reported as 'Cytologically unclassifiable' and should be confirmed with a bone marrow biopsy and appropriate immunostains.
This was considered an important achievement, especially in the view of the heterogeneity of the glossaries used in practice in haematology laboratories all over Europe. The remaining 102 cells were collectively discussed, agreed upon and named during the 2-day meeting. Three additional cells were deleted, due to the poor quality of the images. During the interactive discussion on the images, the EMF decided to label four more cells according to relevance in the milieu (1 blast not otherwise categorized, 1 promyelocyte, 1 promonocyte and 1 megakaryoblast, respectively) and to add the category ‘Cytopologically unclassifiable’ for five images displaying metastatic cells, previously included in the category ‘other’. The ECBG was updated.

Major discrepancies on this set of cells concerned the appropriate use of terms, such as dysplasia, atypical or Mott cell (Russell, 1890; Mott, 1905; Dutcher & Fahey, 1959).

Cell distribution and agreements in the different steps of this study are summarized in Table I.

All the problems faced and all the points discussed were listed and a Consensus statement document was approved (Table II).

At the end of the meeting 228 images with 604 labelled blood cells were uploaded onto the ELN website together with an Excel file (ECBG) where each cell is identified by its code, the type of stain used, the lineage and the consensus name agreed by the EMF. This image library is currently freely available on the ELN web site (http://www.leukemia-net.org) and is also linked to the European Haematology Association web site (http://ehaweb.org).

Finally, a set of 239 cells was submitted via internet to a recognized expert morphologist (JMB) external to the EFM: after the first round, 34 cells were rejected as not well focused and 205 were identified, with a full agreement on 96 cells (46%) with the EFM identification. After a meeting focused on re-examining disagreements, only seven cells were confirmed as disagreed and a final agreement was reached on 205 evaluable cells (96.6%). Main additional suggestions concern the nomenclature of ‘immature monocyte’ instead of ‘dysplastic monocyte’ (Swerdlow et al., 2008; Goasguen et al., 2009) and a better definition of the term ‘dysplastic promyelocyte’ (Mufﬁ, 2008). The quite full concordance achieved after the second interactive reviewing process support the realistic need in the field of cytomorphology to share consensus, including nomenclature, to increase quality of diagnostics according to WHO 2008 goals and guidelines (Swerdlow et al., 2008).

Significant omissions in the archive should be identified in the future and contributions should be sought for inclusion after validation.

References


Appendix 1

ELN Morphology Faculty

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