European consensus on grading bone marrow fibrosis and assessment of cellularity

Quantification of characteristic bone marrow biopsy features includes basic parameters such as cellularity and fiber content. These are important to assess the dynamics of disease processes with a significant impact on risk stratification, survival patterns and, especially, therapy-related changes. A panel of experienced European pathologists and a foreign expert evaluated, at a multi-headed microscope, a large number of representative slides of trephine biopsies from patients with myelofibrosis in an attempt to reach a consensus on how to grade cellularity and fibrosis. This included a critical evaluation of previously described scoring systems. During the microscopic analysis and subsequent discussion and voting, the importance of age-dependent decrease in cellularity was recognized. Grading of myelofibrosis was simplified by using four easily reproducible categories including differentiation between reticulin and collagen. A consensus was reached that the density of fibers must be assessed in relation to the hematopoietic tissue. This feature is especially important in order to avoid a false impression of a reduced fiber content in fatty and/or edematous bone marrow samples after treatment. The consensus for measuring myelofibrosis by clear and reproducible guidelines achieved by our group should allow for precise grading during the disease process and after therapy.

Key words: bone marrow, cellularity, grading of myelofibrosis, standardization, trephine biopsies

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categories (0, 1, 2, 3) according to Georgii\cite{12,13} and Thiele\cite{36,15,40} is predominantly based on expert evaluation by pathologists and lacks strict hematologic criteria. Since controversy and discussion continues about the best practical means to determine cellularity and fiber content routinely and reproducibly in bone marrow trephine biopsy specimens, a number of pathologists interested in this issue convened with the explicit aim of recommending an appropriate grading system.

Methods

Following a general as well as critical discussion on the most widely applied previous scoring systems for normal values of bone marrow cellularity\cite{27-31} and the grading of myelofibrosis,\cite{32,33,15,11,12,15,40} our panel of hematopathologists reviewed more than 150 trephine biopsies from various medical institutions. Generally, specimens included different lesions, but were predominantly cases of CMPD, in particular CIMF and essential thrombocythemia, before and after therapy, derived from adult patients (ranged from 25 to 87 years) and obtained from the posterior iliac crest. Following fixation in buffered formalin, biopsies were decalcified in ethylene-diamine tetra-acetate acid and embedded in paraffin wax. The paraffin sections studied were usually stained with hematoxylin-eosin and a silver impregnation method (Gomori; Gordon-Sweet). Parameters were assessed through a multiple eye-piece microscope and grading was performed in an independent fashion by each of the 13 participants. The hemopathologists were unaware of the clinical characterization of the patients from whom the samples had been taken except for age and gender. To achieve a consensus, the six authors first tried to reach an agreement by one vote (a consensus requiring agreement of at least five of the six authors). This was followed by a subsequent validation of the corresponding results by the rest of the participating investigators by revoting to test the reproducibility. Overall consensus was assumed when at least 11 of the 13 pathologists assigned the same score. Particularly following Gomori’s silver impregnation technique, which turned out to be superior to the Gordon-Sweet reaction, thin (black) reticulin fibers were discriminated from the thick yellowish appearing collagen fibers (see also Figure 1D). In case of a focal increase or regression of fibers, i.e. a patchy distribution of myelofibrosis, this feature was explicitly recorded in the final report. Grading of cellularity (amount of hematopoiesis) was re-assessed in all specimens in accordance with the age of the patients.

Results

Unequivocal consensus was reached that a basic requirement for an assessment of cellularity is a representative, i.e. artefact-free, biopsy of a certain length taken at an orthograde direction (i.e. at right angles to the cortical bone) and that the sections are of constant thickness. Consequently, positive characteristics included: a non-tangential biopsy at least 1.5 cm in length (to enable the evaluation of ten, at least partially preserved, intertrabecular areas) and an optimal thickness of the paraffin sections ranging between 3 and 4 μm. Based on the experience gained from bone marrow specimens without hematologic disease, cellularity was documented in relation to age and with respect to normally occurring ranges (Table 2). Quantity and quality (reticulin/collagen) of the fiber content was determined only in areas of hematopoiesis by using a scoring system comprising four grades (Table 3). This semi-quantitative grading was easily reproducible in samples derived from patients presenting with different stages of CMPD (Figures 1 A-D). However, several points must be considered when applying our scoring system. The first of these is the quality of the reticulin stain, which should be assessed by detection of normal staining in vessel walls as internal controls. Furthermore, lymphoid nodules and vessels as well as fibers framing adipocytes must be disregarded. Finally, areas of prominent scleredema and/or scarring should be included in the overall grading of myelofibrosis. The 13 pathologists involved in this study reached a consensus of more than 95%, including a similar grade of reproducibility. It was conclud-

<table>
<thead>
<tr>
<th>Authors</th>
<th>Reference#</th>
<th>Number of grades</th>
</tr>
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<tbody>
<tr>
<td>Bauermeister 1971</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Manoharan 1979</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>Lazzarino et al. 1986</td>
<td>16</td>
<td>4</td>
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<tr>
<td>Dekmezian et al. 1986</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Beckman and Brown 1990</td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td>Buhr et al. 1993, 2003</td>
<td>11, 14</td>
<td>4</td>
</tr>
<tr>
<td>Georgii et al. 1996, 1998</td>
<td>12, 13</td>
<td>4</td>
</tr>
<tr>
<td>Thiele et al. 1996, 2003</td>
<td>36, 15, 40</td>
<td>4</td>
</tr>
<tr>
<td>Kvasnicka et al. 1997</td>
<td>37</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1. Survey of previous semiquantitative scoring systems on myelofibrosis in normal bone marrow and CMPD.
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that simplifying former classification systems (Table 1), in particular regarding myelofibrosis in relation to bone marrow cellularity, may help to stage the dynamics of hematologic disorders not only more accurately, but also in a more easily reproducible way.

Discussion

It has long been recognized that an age-related quantitative change must be considered when evaluating any given bone marrow biopsy specimen for hematopoiesis or cellularity. In this context the question arises whether and to what extent trephine biopsy material may be compared to aspirates. Reports in the literature offer rather contradictory observations which are also dependent on the method of evaluation. On the other hand, most investigators concur that in comparison with smears and imprints, biopsy examination has proven to be an important and reliable tool to validate bone marrow cellularity. Determining alterations in cellularity is not only important in patients following cytoreductive treatment in order to assess therapeutic efficacy, but also in CMPD for diagnosis and staging. Moreover, the progression of the disease process can be documented and consequently different risk groups with variable survival patterns may be defined.

By definition, myelofibrosis is consistent with an increase in the bone marrow fiber content beyond the normal range and therefore, this term does not denote quality (reticulin versus collagen) nor quantity (borderline to marked). However, in relation to CMPD, myelofibrosis is frequently used by the clinicians to describe a situation characterized by the laboratory findings of anemia, splenomegaly and a leuko-erythroblastic blood picture with appearance of tear drop erythrocytes. It should be emphasized that these changes indicate an advanced stages of (collagen) fibrosis associated with myeloid metaplasia, but usually these peripheral findings are not encountered in minimal to mild increase in reticulin

Table 2. Normal ranges of bone marrow cellularity for selected age groups, as adapted from the literature.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>% Hematopoietic area*</th>
</tr>
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<tbody>
<tr>
<td>20-30</td>
<td>60-70</td>
</tr>
<tr>
<td>40-60</td>
<td>40-50</td>
</tr>
<tr>
<td>≥70</td>
<td>30-40</td>
</tr>
</tbody>
</table>

Table 3. Consensus on the grading of myelofibrosis (MF) as adapted from the literature.

<table>
<thead>
<tr>
<th>Grading</th>
<th>Description*</th>
</tr>
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<tbody>
<tr>
<td>MF - 0</td>
<td>Scattered linear reticulin with no intersections (cross-overs) corresponding to normal bone marrow</td>
</tr>
<tr>
<td>MF - 1</td>
<td>Loose network of reticulin with many intersections, especially in perivascular areas</td>
</tr>
<tr>
<td>MF - 2</td>
<td>Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis</td>
</tr>
<tr>
<td>MF - 3</td>
<td>Diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis</td>
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*Fiber density should be assessed in hematopoietic (cellular) areas.
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The present study simplifies all previous descriptions of fiber scarring (Table 1) by reducing them to four grades, including normal reticulin density, in order to avoid excessive overlapping and to achieve a higher degree of reproducibility in routine diagnosis. Confusion created in former systems, in which normal reticulin is classified as grade 1, was reduced by classifying normal as N - normal or grade 0.

In conclusion, the consensus reached by our group of experienced hematopathologists, including both European pathologists and a foreign expert, on the guidelines to be used for measuring cellularity and bone marrow fiber content may provide a useful tool for assessing both important dynamic aspects of the disease process, and therapy-related changes.

 JT drafted the manuscript, all six authors of the current communication fully and directly participated in the concept, design, data analysis and critical revision of the results of this study. In addition, all authors reviewed the histological slide material under the supervision of the first author. Table 4 was composed by the first two authors who also provided Figure 1a-d. Tables 2 and 3 were provided by all six authors of this paper.

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References


