European consensus on grading bone marrow fibrosis and assessment of cellularity

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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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Regarding assessment of bone marrow biopsy specimens, interest has been recently reawakened in the standardization of key histological parameters such as cellularity and fiber content. It is noteworthy that age-related quantitative changes in hematopoiesis must always be kept in mind because these have turned out to be predictive of adequate cell yield for transplantation.[1] Myelofibrosis is a concomitant cytokine-mediated process of the bone marrow stroma.[2] It has been associated with many different types of reactive conditions including, among others, autoimmune and granulomatous diseases[3,4] and a variety of neoplastic disorders.[5,6] The latter include myelodysplastic syndromes,[7,8] acute leukemic conditions[9,10] and chronic myeloproliferative diseases (CMPD).[11-15] In chronic myelogenous leukemia (CML) myelofibrosis was shown to be a significant predictor of therapeutic efficacy and outcome[16-20] including engraftment after transplantation.[21,22] In this context, therapy-related effects on the stabilization or regression of fibrosis in chronic idiopathic myelofibrosis (CIMF) or myelofibrosis with myeloid metaplasia have gained increasing attention,[14,15] particularly when considering novel therapeutic strategies.[23-26]

Scoring systems for normal values of bone marrow cellularity[27-31] and grading of myelofibrosis[32-35,31,36-40] are mainly based on subjective evaluations by individual pathologists using different grading systems (Table 1) and methods of processing the trephine biopsies, i.e. plastic[3,31,11-14] versus paraffin[32,33,16,17,35,37,15,40] embedding. The most frequently used grading systems applied to assess myelofibrosis are essentially based on the Baumeister scale[32,35] which was modified by Manoharan.[33] More recent scoring systems are focused on CMPD, and differentiate between reticulin and collagen, on the basis of which grading of myelofibrosis is assessed[11,12,36,13-15,40] (Table 1). The grading of myelofibrosis into four

Table 1. Survey of previous semiquantitative scoring systems on myelofibrosis in normal bone marrow and CMPD.

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

categories (0, 1, 2, 3) according to Georgii[12,13] and Thiele[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[27-31] and the grading of myelofibrosis,[32,33,17,35,11,15,40] 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 hemapathologists 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-

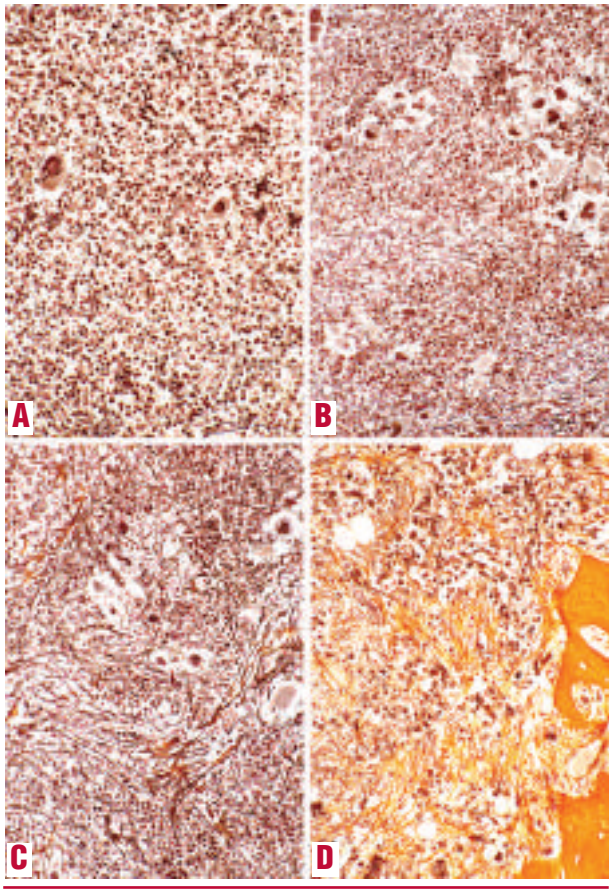

Figure 1. Grading of fiber density and quality, according to the proposed consensus, in bone marrow biopsy specimens of chronic idiopathic myelofibrosis (CIMF). (A) Grade 0 with single scattered reticulin fibers consistent with the appearance of the normal bone marrow. (B) Grade 1 showing a loose meshwork of thin reticulin fibers with many intersections. (C) Grade 2 with a dense and diffuse increase in reticulin forming extensive intersections and focal (yellowish) thick collagen fibers. (D) Grade 3 with dense reticulin fibers intermingled with bundles of (yellowish) collagen and associated with endophytic bone formation (osteosclerosis). A-D: silver impregnation after Gomori, A-D×180.

Table 2. Normal ranges of bone marrow cellularity for selected age groups, as adapted from the literature.[27-29,3,31]

Age (years)	*% Hematopoietic area**
20-30	60-70
40-60	40-50
≥70	30-40

Table 3. Consensus on the grading of myelofibrosis (MF) as adapted from the literature.[32,33,36]

Grading	*Description**
MF - 0	Scattered linear reticulin with no intersections (cross-overs) corresponding to normal bone marrow
MF - 1	Loose network of reticulin with many intersections, especially in perivascular areas
MF - 2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis
MF - 3	Diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis

**Fiber density should be assessed in hematopoietic (cellular) areas.*

ed 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.[27,3,31] 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[30] which are also dependent on the method of evaluation.[29] 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.[28,3,34,31] Determining alterations in cellularity is not only important in patients following cytoreductive treatment in order to assess therapeutic efficacy,[39,40] but also in CMPD for diagnosis and staging.[13] 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.[38,25] 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

(grades 0 and 1) in the early stages of CIMF.[37,14,15]

The present study simplifies all previous descriptions of fiber scorings (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,[32,33,35] 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 consensus study. In addition, all authors reviewed the histological slide material under the supervision of the first author. Table 1 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

1. Rosenthal NS, Farhi DC, Fox RM, Lazarus HM. Marrow cellularity as a predictor of adequate cell yield for transplantation. Am J Clin Pathol 1994; 101:81-4.
2. Le Bousse-Kerdiles MC, Martyre MC. Dual implication of fibrogenic cytokines in the pathogenesis of fibrosis and myeloproliferation in myeloid metaplasia with myelofibrosis. Ann Hematol 1999;78:437-44.
3. Frisch B, Bartl R, Burkhardt R. Bone marrow biopsy in clinical medicine: an overview. Haematologia (Budap) 1982; 15:245-85.
4. Aharon A, Levy Y, Bar-Dayan Y, Afek A, Zandman-Goddard G, Skurnik Y, et al. Successful treatment of early secondary myelofibrosis in SLE with IVIG. Lupus 1997;6:408-11.
5. Thiele J, Langohr J, Skorupka M, Fischer R. Reticulin fibre content of bone marrow infiltrates of malignant non-Hodgkin's lymphomas (B-cell type, low malignancy)-a morphometric evaluation before and after therapy. Virchows Arch A Pathol Anat Histopathol 1990;417:485-92.
6. Schmidt U, Ruwe M, Leder LD. Multiple myeloma with bone marrow biopsy features simulating concomitant chronic idiopathic myelofibrosis. Nouv Rev Fr Hematol 1995; 37:159-63.
7. Lambertenghi-Deliliers G, Orazi A, Luksch R, Annaloro C, Soligo D. Myelodysplastic syndrome with increased marrow fibrosis: a distinct clinicopathological entity. Br J Haematol 1991; 78:161-6.
8. Maschek H, Georgii A, Kaloutsi V, Werner M, Bandecar K, Kressel MG, et al. Myelofibrosis in primary myelodysplastic syndromes: a retrospective study of 352 patients. Eur J Haematol 1992;48:208-14.
9. Thiele J, Grashof K, Fisher R. Follow-up study on bone marrow reticulin fibrosis in AML. Anal Cell Pathol 1991; 3:225-31.
10. Orazi A, O'Malley D P, Jiang J, Vance GH, Thomas J, Czader M, et al. Acute panmyelosis with myelofibrosis: an entity distinct from acute megakaryoblastic leukemia. Mod Pathol 2004:1-12.
11. Buhr T, Georgii A, Choritz H. Myelofibrosis in chronic myeloproliferative disorders. Incidence among subtypes according to the Hannover Classification. Pathol Res Pract 1993; 189:121-32.
12. Georgii A, Buhr T, Buesche G, Kreft A, Choritz H. Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. Leuk Lymphoma 1996; 22 Suppl 1:15-29.
13. Georgii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. Baillieres Clin Haematol 1998;11:721-49.
14. Buhr T, Busche G, Choritz H, Langer F, Kreipe H. Evolution of myelofibrosis in chronic idiopathic myelofibrosis as evidenced in sequential bone marrow biopsy specimens. Am J Clin Pathol 2003; 119:152-8.
15. Thiele J, Kvasnicka HM, Schmitt-Graeff A, Diehl V. Dynamics of fibrosis in chronic idiopathic (primary) myelofibrosis during therapy: a follow-up study on 309 patients. Leuk Lymphoma 2003;44:949-53.
16. Lazzarino M, Morra E, Castello A, Inverardi D, Coci A, Pagnucco G, et al. Myelofibrosis in chronic granulocytic leukaemia: clinicopathologic correlations and prognostic significance. Br J Haematol 1986;64:227-40.
17. Dekmezian R, Kantarjian HM, Keating MJ, Talpaz M, McCredie KB, Freireich EJ. The relevance of reticulin stain-measured fibrosis at diagnosis in chronic myelogenous leukemia. Cancer 1987;59:1739-43.
18. Thiele J, Kvasnicka HM, Titius BR, Parpert U, Nebel R, Zankovich R, et al. Histological features of prognostic significance in CML-an immunohistochemical and morphometric study (multivariate regression analysis) on trephine biopsies of the bone marrow. Ann Hematol 1993;66:291-302.
19. Kvasnicka HM, Thiele J, Schmitt-Graeff A, Diehl V, Zankovich R, Niederle N, et al. Prognostic impact of bone marrow erythropoietic precursor cells and myelofibrosis at diagnosis of Ph1+ chronic myelogenous leukaemia-a multicentre study on 495 patients. Br J Haematol 2001;112:727-39.
20. Buesche G, Hehlmann R, Hecker H, Heimpel H, Heinze B, Schmeil A, et al. Marrow fibrosis, indicator of therapy failure in chronic myeloid leukemia - prospective long-term results from a randomized-controlled trial. Leukemia 2003;17:2444-53.
21. Rajantie J, Sale GE, Deeg HJ, Amos D, Appelbaum F, Storb R, et al. Adverse effect of severe marrow fibrosis on hematologic recovery after chemoradiotherapy and allogeneic bone marrow transplantation. Blood 1986; 67:1693-7.
22. Thiele J, Kvasnicka HM, Beelen DW, Zirbes TK, Jung F, Reske D, et al. Relevance and dynamics of myelofibrosis regarding hematopoietic reconstitution after allogeneic bone marrow transplantation in chronic myelogenous leukemia-a single center experience on 160 patients. Bone Marrow Transplant 2000;26:275-81.
23. Barosi G, Elliott MA, Canepa L, Ballerini F, Piccaluga PP, Visani G, et al. Thalidomide in myelofibrosis with myeloid metaplasia: a pooled-analysis of individual patient data from five studies. Leuk Lymphoma 2002; 43: 2301-7.
24. Tefferi A. Treatment approaches in myelofibrosis with myeloid metaplasia: the old and the new. Semin Hematol 2003;40:18-21.
25. Dingli D, Mesa RA, Tefferi A. Myelofibrosis with myeloid metaplasia: new developments in pathogenesis and treatment. Intern Med 2004; 43: 540-7.
26. Mesa RA, Elliott MA, Schroeder G, Tefferi A. Durable responses to thalidomide-based drug therapy for myelofibrosis with myeloid metaplasia. Mayo Clin Proc 2004;79:883-9.
27. Hartsock RJ, Smith EB, Petty CS. Normal variations with aging of the amount of hematopoietic tissue in bone marrow from the anterior iliac crest. A study made from 177 cases of sudden death examined by necropsy. Am J Clin Pathol 1965;43:326-31.

28. Gruppo RA, Lampkin BC, Granger S. Bone marrow cellularity determination: comparison of the biopsy, aspirate, and buffy coat. Blood 1977; 49: 29-31.
29. Fong TP, Okafor LA, Schmitz TH, Thomas W, Westerman MP. An evaluation of cellularity in various types of bone marrow specimens. Am J Clin Pathol 1979;72:812-6.
30. Paul M, Chandy M, Pulimood R, Krishnaswami H. Cellularity of bone marrow: a comparison of trephine biopsies and aspirate smears. Indian J Pathol Microbiol 1989;32:186-9.
31. Frisch B, Bartl R. Atlas of bone marrow pathology. London: Kluwer. 1990:10-1.
32. Bauermeister DE. Quantitation of bone marrow reticulin - a normal range. Am J Clin Pathol 1971;56:24-31.
33. Manoharan A, Horsley R, Pitney WR. The reticulin content of bone marrow in acute leukaemia in adults. Br J Haematol 1979; 43:185-90.
34. Pasquale D, Chikkappa G. Comparative evaluation of bone marrow aspirate particle smears, biopsy imprints, and biopsy sections. Am J Hematol 1986;22:381-9.
35. Beckman EN, Brown AW, Jr. Normal reticulin level in iliac bone marrow. Arch Pathol Lab Med 1990;114:1241-3.
36. Thiele J, Kvasnicka HM, Werden C, Zankovich R, Diehl V, Fischer R. Idiopathic primary osteo-myelofibrosis: a clinico-pathological study on 208 patients with special emphasis on evolution of disease features, differentiation from essential thrombocythemia and variables of prognostic impact. Leuk Lymphoma 1996;22:303-17.
37. Kvasnicka HM, Thiele J, Werden C, Zankovich R, Diehl V, Fischer R. Prognostic factors in idiopathic (primary) osteomyelofibrosis. Cancer 1997;80:708-19.
38. Barosi G. Myelofibrosis with myeloid metaplasia: diagnostic definition and prognostic classification for clinical studies and treatment guidelines. J Clin Oncol 1999;17:2954-70.
39. Thiele J, Kvasnicka HM, Schmitt-Graeff A, Bundschuh S, Biermann T, Roessler G, et al. Effects of chemotherapy (busulfan-hydroxyurea) and interferon-α on bone marrow morphologic features in chronic myelogenous leukemia. Histochemical and morphometric study on sequential trephine biopsy specimens with special emphasis on dynamic features. Am J Clin Pathol 2000;114:57-65.
40. Thiele J, Kvasnicka HM, Schmitt-Graeff A, Diehl V. Bone marrow histopathology following cytoreductive therapy in chronic idiopathic myelofibrosis. Histopathology 2003; 43:470-9.