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REVIEW

Problems and pitfalls regarding WHO-defined diagnosis of early/prefibrotic primary myelofibrosis versus essential thrombocythemia

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Reproducibility and clinical usefulness of the WHO classification of chronic myeloproliferative neoplasm (MPN) persist to be a controversial issue. Major arguments are focused on the critical impact of histopathology, particularly concerning the distinction between essential thrombocythemia (ET) versus early/prefibrotic primary myelofibrosis (PMF). Regarding bone marrow morphology, WHO guidelines strictly require the recognition of characteristic histological patterns based on standardized features and a consensus of clinical and molecular-genetic data. Molecular-genetic findings as JAK2V617F, may aid to exclude reactive thrombocytosis, although in ET and PMF only 50–60% of the cases show these aberrations. Considerable doubts over the existence of early/prefibrotic PMF have been expressed with the consequence to include this entity in the ET category. On the other hand, it has to be argued that some of the critical studies failed to adhere very strictly to the WHO guidelines. Contrasting this situation, recently published retrospective and prospective clinico-pathological studies featuring the WHO criteria provided an important information on disease outcomes supporting the existence of early/prefibrotic PMF as a distinct clinico-pathologic entity in patients presenting clinically with ET. Therefore, this controversy suggests a scientific project, including the community of pathologists and hematologists, for providing sound, objective and reproducible criteria for diagnosing early/prefibrotic PMF.

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INTRODUCTION

At variance with previous classification systems of chronic myeloproliferative neoplasm (MPN), it has been generally recognized that the 2008 updated diagnostic criteria[1] proposed by a panel of expert hematopathologists, physician scientists, and clinicians to the WHO[2] strongly emphasize a synoptical approach. This key issue implicates a combination of laboratory data with morphological features and molecular-genetic findings.[3,4] The rationale underlying this classification scheme was to create a useful interface between all these different parameters aimed to increase diagnostic sensitivity and specificity as well as to obtain readily applicable algorithms for routine clinical practice.[5] However, serious concern has been repeatedly expressed arguing against this concept, but particularly the inclusion of bone marrow (BM) features as an integral part of the diagnosis.[6] It is evident that criticism is mainly directed toward the strong inclination to give such a high diagnostic relevance to BM morphology and not so much to clinical features, and it is particularly this issue that the reproducibility and clinical usefulness of the WHO classification[1] superscript is questioned.[6–8] This conflict of opinion is predominantly focused on the recognition of a unique entity referred as early/prefibrotic primary myelofibrosis (PMF) and its distinction from essential thrombocythemia (ET).

The aim of this review is to critically discuss the problems and pitfalls that may be responsible for the current uncertainties and debates.

MORPHOLOGICAL CRITERIA

In the WHO morphological criteria, patients presenting with ET may be separated into two groups: the first is called 'true ET' the second early/prefibrotic PMF. This distinction is based on clinically distinctive and specific histological BM patterns. In BM biopsy specimens derived from ET patients, usually neither a relevant increase in overall hematopoietic cellularity nor a significant proliferation or a left-shifting of the neutrophil granulo- or erythropoiesis are recognizable. On the other hand, randomly distributed or loosely clustered large to giant mature megakaryocytes with deeply folded (hyperlobulated) nuclei (so-called staghorn type) surrounded by correspondingly mature cytoplasm are the outstanding features. Only in a very small subfraction of patients a minor accumulation of reticulin fibers may be observed.[1,2] In early/prefibrotic PMF, histopathology of the BM is characterized by hematopoietic hypercellularity consisting of a prominent neutrophil granulocytic and megakaryocytic proliferation, which is often associated with a slight to moderate reduction of nucleated red cell precursors. Most important are conspicuous deviations of the megakaryocytic cell lineage, including abnormal arrangement and localization in the marrow space (histotopography with endosteal-paratrabecular dislocation, formation of dense or loose clusters) and a high variability in size (small and giant forms). Moreover, there are significant aberrations of nuclear organization (marked hypolobulation, irregular folding, condensed chromatin patterns) generating bulbous or so-called cloud-like/balloon-shaped nuclei,

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increased nuclear-cytoplasmic ratio (maturation defect) as well as increased numbers of bare (denuded) nuclei.[1,2] Some of these features are shown in Figure 1 to demonstrate the differences in BM appearance between ET (Figure 1, panels a and c) versus early/prefibrotic PMF (Figure 1, panels b and d).

Therefore, the crucial discrimination of ET from early/prefibrotic PMF or other entities that present with thrombocythemia needs an elaborate evaluation of these histological parameters, including a qualitative and quantitative assessment as well.[9,10] This implies that not any single BM feature is sufficient for a final diagnosis of any MPN.[1–3,5,9–11] Recognition of these histological patterns requires not only a standardization of prominent BM features,[10] but also a scrutinized analysis taking into account the variable incidences of characteristic parameters.[9,11] For a number of pathologists this postulate seems to be a weak point of the WHO classification,[1,2] as according to their opinion it may be impaired by subjectivity and can only insufficiently be validated.[12–14] In this regards, some relevant studies deserve to be considered.

In the first study by Wilkins *et al.*,[12] entering 370 specimens at diagnosis from the UK-PT1 trial[15] agreement was better for

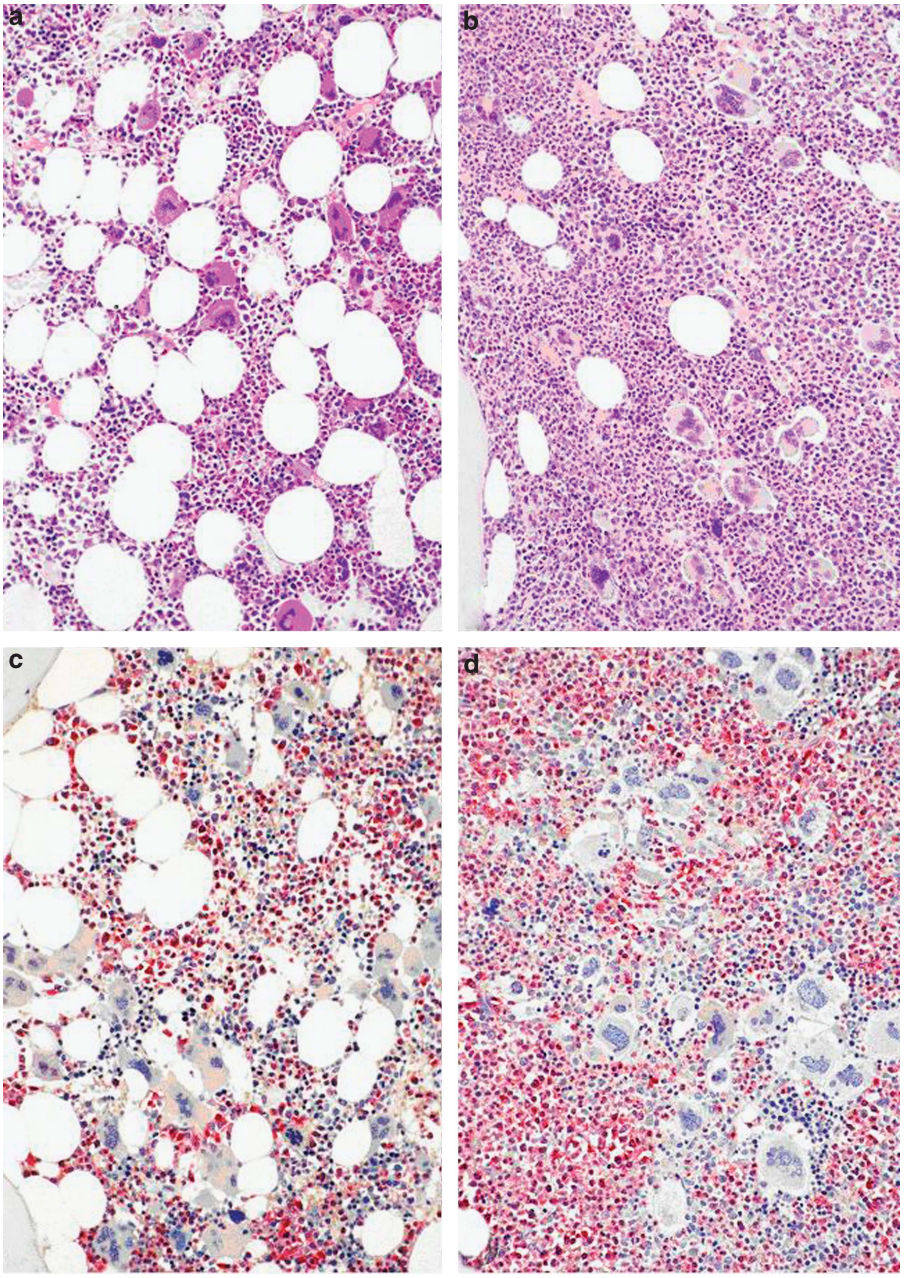

Figure 1. BM biopsy histology in ET and early/prefibrotic PMF in low-power (× 180) or medium-power (× 260) field. (**a**) In ET survey with periodic acid Schiff reaction-stained section shows an age-matched hematopoietic cellularity, but an increase in large megakaryocytes mostly with a random arrangement within the marrow space. (**b**) In early/prefibrotic PMF clinically mimicking ET an increase in normal cellularity is noticeable and accompanied by conspicuous megakaryopoiesis revealing a variety of size ranging from small to giant ones as well as different degrees of nuclear lobulations and dense and loose clustering. (**c**) Chloroacetate esterase reaction discloses that in ET the large megakaryocytes are hyperlobulated, well-differentiated and surrounded by an inconspicuous white and red cell lineage. (**d**) In early/prefibrotic PMF chloroacetate esterase reaction confirms a prevalent and left shifted neutrophil granulopoiesis surrounding clustered megakaryocytes with dense, hyperchromatic and clumsy nuclei or aberrations of nuclear lobulation (cloud-like features).

measures of general morphologic patterns such as cellularity, number of clusters and reticulin grade, and weaker for measures of individual cellular features such as megakaryocyte morphology and whether clustering is tight or loose. In addition, the three hematopathologists showed poor agreement in synthesizing the various parameters when assigning cases to individual diagnostic categories using WHO criteria. The conclusion was that histologic criteria described in the WHO classification are difficult to reproducibly apply and questions the validity of distinguishing true ET from early/prefibrotic PMF. However, some weak points of this study should be discussed. First, the reported wide range from 37 to 76% of BM fibrosis (grades 3 and 4 on a four-graded scale) and new bone formation, that is, osteosclerosis, does not meet the diagnostic criteria of true ET at onset and is more consistent with PMF. Second, the small size of the evaluated BM trephines ($\geqslant$0.5 cm instead of 1.5 cm as required by the WHO)[1] may have precluded an accurate recognition of localized features like clustering of megakaryocytes, exact grading of fibrosis or assessment of age-matched hematopoietic cellularity. Third, no information concerning a self-assessment exercise (intra-observer evaluation) was provided as is usually required in testing the quality of histologic reproducibility. In addition, the very poor scoring results for the basic hematopoietic feature erythroid cellularity that may have served as control for reliability contrasting the relatively high scores for reticulin grade or new bone formation, raises serious concerns.[9,16]

Another study not in favor of the WHO classification was performed by two pathologists who tried to reproduce the corresponding diagnostic criteria on BM biopsy specimens in patients originally diagnosed with ET according to Polycythemia Vera Study Group (PVSG) criteria.[17] This evaluation resulted in a significantly low concordance of only about 35% regarding so-called WHO-confirmed ET versus early/prefibrotic PMF diagnosis.[13] Unfortunately, the panelists collected their BM samples up to 3 years after clinical diagnosis, provided no data on previous drug treatments nor reported on biopsy size. The most conspicuous finding, however, was that 54% of their cases, explicitly claimed to represent WHO-defined ET, disclosed a minor to moderate reticulin fibrosis. It may be speculated that the authors have probably overlooked that at onset only very rarely reticulin fibers are minimally increased in strictly WHO-diagnosed ET.[1,2] This specific feature was confirmed by several independent groups describing a frequency of minor reticulin fibrosis (grade 1 of a three-graded scoring system)[18] in WHO-defined patients ranging between 3 to 5%.[9,19–23]

A third study was performed by six hematopathologists from five European countries. They focused on 102 patients with ET and early/prefibrotic PMF presenting without or with fibrosis grade 1 (ref. 18) and compared clinical criteria with BM features.[14] Blinded evaluation disclosed that at least four out of six panelists concurred in 63% of cases consistent with a low to moderate agreement according to kappa values (0.28–0.57, average 0.41). In this context, it has to be emphasized that the panelists were not provided with any adequate clinical input. Moreover, this level of consensus is comparable with that derived from international studies aimed at assessing the diagnostic reproducibility of malignant B- and T-cell lymphoma subtypes.[24,25]

The study by Koopmans *et al.*[26] reported the results of a blinded evaluation of 56 prefibrotic MPN cases by four hematopathologists. A significantly high degree of a consensus (83%) was obtained for the WHO-defined individual major histological features, particularly megakaryocytes. Of note is that these results are in contrast with the findings of Wilkins *et al.*[12] Agreement on the final histological classification was 70%, thus slightly exceeding the study by Buhr *et al.*[14] The conclusion of the authors that the translation to a final diagnosis is problematic because, in addition to the identification of specific morphological parameters, their frequency and ranking has a crucial role[26] seems to be reasonable, and can only be ameliorated by strictly regarding standardized BM features.[9–11]

A major criticism of WHO morphologic classification is that it is mainly based on investigations generated from the single Cologne group and therefore may not be extended in clinical practice.[12] This impression is denied by a number of clinico-pathological studies performed without interference by any of the authors from Cologne. These investigations are in concordance with the existence of early/prefibrotic PMF to be distinct by morphological and clinical features from ET.[22,27–31] This notion was recently confirmed by Barosi *et al.*[32] who investigated whether prefibrotic PMF may be aligned along a clinical and biological continuum in 683 consecutive patients who received a WHO diagnosis of PMF. They distinguished prefibrotic myelofibrosis (fiber grade 0) from PMF with early/prefibrotic myelofibrosis (fiber grade 1),[18] while the WHO regarded both manifestations together as early/prefibrotic PMF.[32]

CLINICAL DATA

Generally, any study on MPN without adequate clinical and morphological input does not comply with the concept of the WHO classification and is definitely prone to generate diagnostic uncertainty and controversy.[2,3,5] Concerning early/prefibrotic PMF, previous studies including clinical parameters were in keeping with the finding of minor/borderline age- and gender-matched anemia, slight increase in the serum lactate dehydrogenase level and the white blood cell count, minor to slight splenomegaly and occurrence of a very few myelo- and erythroblasts in the majority of patients.[16,23,27,31–34] An accurate differentiation of both MPN entities was shown to exert a significant difference in terms of hemostatic complications, overall and relative survival, hematologic transformation in overt PMF and acute leukemia.[23,30,31,35–37] These initial data were significantly extended and validated by an international study, including 891 ET and 180 early/prefibrotic PMF patients strictly diagnosed according to WHO criteria.[23] Compared with the large cohort of WHO-diagnosed ET patients a significantly expressed worsening of overall- and event-free survival, increased progression into overt myelofibrosis and transformation to acute leukemia were found.[23] Concerning the overall incidence of major thrombosis, no differences were revealed in both WHO-defined entities.[38] Fatal and non-fatal arterial and venous thrombotic events in this large cohort of WHO-confirmed ET patients revealed a rate of 1.9% patient-years and therefore grossly corresponds with frequencies found in studies including PVSG-diagnosed patients.[23,39–41] In these patients, the parameters age $>$60 years, previous thrombosis, JAK2V617F mutation and cardiovascular events identified three different risk categories to develop thrombosis[42] suggesting a new stratification of vascular risk for future clinical trials. Concerning early/prefibrotic PMF a significant impact of an increased white blood cell count on the overall thrombotic incidence both in arterial and venous districts has been recently reported.[43] This finding is in keeping with the UK-PT1 study[15] that is assumed to contain early/prefibrotic and fibrotic PMF patients according to relevant BM findings.[12,16,44] On the contrary, a clear and significant difference of the hemorrhagic complications was shown in early/prefibrotic PMF as compared with ET. In a large international study, major bleeding during follow-up occurred in WHO-diagnosed ET and early/prefibrotic PMF patients with a rate of 0.79 and 1.39% patient-years, respectively ($P=0.039$). In multivariable analysis, predictors of bleeding included diagnosis of PMF, leukocytosis ($P=0.04$; hazards ratio, 1.74), previous hemorrhage and aspirin therapy ($P=0.001$).[45] The analysis restricted to patients with WHO-defined ET, confirmed previous hemorrhage as independent risk factors. These results demonstrate a higher risk of major bleeding in patients with early/prefibrotic PMF versus WHO-confirmed ET, particularly after aspirin medication, and thus establishes incidence

and risk factors for this complication in strictly defined WHO-diagnosed ET patients. It is interesting to compare this finding with the results of the UK-PT1 study.[15] In a *post-hoc* analysis of 311 patients diagnosed with ET, according to the PVSG criteria[17] mostly derived from this prospective trial that received aspirin with either hydroxyurea or anagrelide, increased BM reticulin at presentation predicted higher rates of major bleeding during follow-up.[44] In this context it has been argued whether those patients were more likely compatible with thrombocythemic manifestations of PMF.[16] Altogether these results question the rationale of using aspirin as primary prophylaxis in ET patients without accurate morphological diagnosis.[46]

Finally, it has been argued that no prospective, clinically controlled study validating the WHO criteria,[1,2] particularly concerning early/prefibrotic PMF versus ET was conducted.[6] In the meantime there is one prospective, long-term follow-up study confirming the results of the retrospective evaluations[16,23] that a blinded re-evaluation of BM biopsy samples according to the WHO guidelines was not only able to discriminate ET from early/prefibrotic PMF, but also showed a marked difference in the outcome.[36] A very recently published randomized clinical trial on strictly WHO-defined ET, revealed that there were no significant differences between anagrelide versus hydroxyurea therapy regarding incidences of minor and major arterial and venous thrombosis or severe bleedings and transformation into myelofibrosis or secondary leukemia during an observation time of 12 and 36 months.[37] These findings are in sharp contrast with PT1 trial that enrolled PVSG patients.

MOLECULAR-GENETIC FINDINGS

The discovery of the JAK2V617F mutation, followed by the description of mutations at codon 515 (W > L, K, A) of MPL and in exon 12 of JAK2, has represented the strongest impetus for promoting a far-reaching revision of the diagnostic criteria of MPN. As a matter of fact, the novelty of the revised criteria concerned the fact that, for the first time, molecular information were used as affirmative variables in the diagnostic make-up, as they easily allow to differentiate MPN from reactive erythrocytosis and thrombocytosis. This axiom is best exemplified by the case of patients with just slightly increased platelet count who can be found to harbor JAK2V617F; it was on the basis of such findings that the diagnostic level of platelet count was decreased from 600 to 450×10^9/l in the current WHO criteria.

The prevalence of JAK2V617F mutation in patients with an overt polycythemia vera phenotype is assumed to be >95%, and an additional 2–3% of polycythemia vera patients harbor mutations in JAK2 exon 12; accordingly, when an absolute erythrocytosis is defined, the absence of a JAK2 mutation makes a diagnosis of polycythemia vera very unlikely, although it cannot be currently ruled out provided the other criteria enlisted in the 2008 classification are satisfied.[1,2] The situation is less straightforward in case of thrombocytosis as only 50–60% of the subjects who are finally diagnosed as having ET using the combination of the four major criteria of the WHO classification are JAK2V617F positive and no more than 5–8% harbor MPL mutations; thus, at least 30% of patients with 2008 WHO-defined ET remain molecularly not characterized. Analogous considerations apply to subjects with a diagnosis of PMF where the prevalence of JAK2V617F mutation is 60% and that of MPL mutation is 8–10%. It is well documented that the JAK2V617F allelic burden (that is, the proportion of mutated versus wild-type alleles) varies in the MPN clinical entities, with ET and myelofibrosis lying at the opposite sides (the lowest for ET, the highest for myelofibrosis of a continuum of values). Therefore, even if population distribution is statistically different, the measured allelic burden lacks any practical diagnostic relevance at the individual level.

At present, no specific molecular asset helps in the differentiation of WHO-defined ET from early/prefibrotic PMF, although no extensive study has yet been performed to this end. In a study involving 230 patients with early/prefibrotic PMF and 90 ET subjects, diagnosed based on histology, Hussein *et al*.[47] observed comparable frequencies of JAK2V617F mutation (47 of 90 were mutated in ET (52%) versus 55% in early/prefibrotic PMF (52 of 95)); 50 similar data were reported by the large IWG-MRT study on 891 ET (61% were JAK2 mutated) and 180 early/prefibrotic PMF patients (58%).[23] Hussein *et al*.[47] also reported that the V617F allelic burden was lower in ET (median 24%, range 5–40%) than in early/prefibrotic PMF (38%, range, 7–92%).[54] Although statistically significant, this observation has modest clinical relevance due to the very close median levels and the large overlapping of the two patient populations. However, according to this report, no ET patients presented an allele burden >50% as compared with about one quarter of those having early/prefibrotic PMF; the difference appeared even more compelling when the V617F allele burden was measured at the mRNA level in a small subset of patients, consistent with previous observations of higher allelic ratio when measured as mRNA copies.[48] If confirmed on statistically meaningful number of patients, implication of these finding would be that finding a JAK2V617F allele burden >50% in a patient with thrombocytosis and no additional criterion for PMF or polycythemia vera suggests a diagnosis of early/prefibrotic PMF rather than ET. In ET patients, the presence of a JAK2V617F mutation, that is known to point to a greater risk of thrombosis,[38,49] was associated with a lower risk of overt fibrotic progression and had no impact on overall survival or leukemia as it did in patients with early/prefibrotic PMF; the prognostic relevance of the mutation in early/prefibrotic PMF, if any, is unknown.

CONCLUSIONS

Concerning MPN classification in comparison with the PVSG criteria, the WHO is taking over a provocative role when attempting to create an interface between clinical, morphological and molecular-genetic data trying to achieve a consensus-based working diagnosis. A number of clinico-pathological studies by independently working groups have demonstrated that an accurate morphological differentiation is a key issue. We are aware of the limited reproducibility of the currently applied WHO histological classification by a number of authors. Nevertheless, we emphasize that histopathological diagnosis and particularly discrimination between ET and early/prefibrotic PMF should be based on standardized morphological features, and recognition of distinctive BM patterns generating concordant results among pathologists as essential features for clinical studies. By regarding these postulates early stages of PMF presenting with thrombocythemia may be clearly separated from ET. On the other hand, it cannot be overlooked that the WHO classification is not aimed to capture all biological true cases of MPNs or guarantee a complete diagnostic specificity and should not be in need of minor improvement following several years of clinical experience. A good solution of the still ongoing controversy regarding these issues could be to launch a scientific project, including the community of pathologists and hematologists for providing scientifically sound, objective, repeatable quantitative criteria for the prefibrotic variant of PMF followed by a corresponding prospective clinico-pathological study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS CONTRIBUTION

TB, JT, AMV and AT contributed equally to this work and finally approved the text of the manuscript.

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