The 2016 revision of WHO classification of myeloproliferative neoplasms: Clinical and molecular advances

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A R T I C L E   I N F O

Keywords:
Polycthemia vera
Essential thrombocythemia
Prebrotic/early primary myelofibrosis
Overt primary myelofibrosis
Revision
Clinical aspects

A B S T R A C T

Clinical evidence supports the need of changing the diagnostic criteria of the 2008 updated WHO classification for polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). In JAK2-mutated patients who show characteristic bone marrow (BM) morphology, clinical studies demonstrated that a hemoglobin level of 16.5 g/dL in men and 16.0 g/dL for women or a hematocrit value of 49% in men and 48% in women are the optimal cut off levels for distinguishing JAK2-mutated ET from "masked/prodromal" PV. Therefore BM morphology was upgraded to a major diagnostic criterion. Regarding ET the key issue was to improve standardization of prominent BM features enhancing differentiation between "true" ET and prebrotic/early primary myelofibrosis (prePMF). These two entities have shown a different epidemiology and clinical outcomes. Concerning prePMF a more explicit clinical characterization of minor criteria is mandated for an improved distinction from ET and overt PMF and accurate diagnosis and outcome prediction.

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1. Introduction

The 2001 introduced and 2008 updated diagnostic criteria of the World Health Organization (WHO) classification for myeloproliferative neoplasms (MPNs) regarded molecular (JAK2, MPL), laboratory and morphological features [1,2]. By applying these diagnostic criteria for almost 8 years (Table 1), a new clinical epidemiology of MPN patients emerged. The reasons may be due to an earlier diagnosis, different clinical and hematologic features at presentation and, more importantly, different rates of thrombo-hemorrhagic event, progression to myelodysplasia syndrome (MDS) or transformation to blast phase (BP). Consequently, the relevant clinical outcomes registered in contemporary cohorts of patients with MPN enrolled in several observational studies, were not concordant with the findings obtained before the 2008 WHO update [3–6], and therefore generated new proposals for a new update of the 2008 diagnostic criteria (Table 2).

The aim of this review is to report the current scientific and clinical evidence supporting the need of changing the diagnostic criteria of the 2008 updated WHO classification concerning polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).

2. Polycythemia vera

2.1. Hematocrit and hemoglobin thresholds

In WHO-2008 classification, diagnostic criteria for PV were focused on the threshold value for hemoglobin (Hb) levels > 18.5 g/dL in men and >16.5 g/dL in women) and not so much on hematocrit (Hct) [2]. This has generated a conflict of opinion questioning which one of these parameters is the most reliable to characterize red cell mass (RCM) [7] or monitoring response to therapy [8–10]. Contrasting the WHO criteria, the British Committee for Standards in Haematology (BCSH) group considered the presence of JAK2V617F mutation and Hct >52% in men and >48% in women the main criteria to qualify for the diagnosis of PV [11]. These criteria used Hct rather than Hb as a surrogate marker of increased RCM and do not require a bone marrow (BM) examination in those patients for whom a clonal marker is shown [12]. According to the results gained from CYTO-PV randomized clinical trial showing that an Hct value of <45% is the optimal threshold to lower cardiovascular complications [8]. Therefore, our new proposal is to add to the diagnostic Hb also the Hct threshold value (Table 2).

http://dx.doi.org/10.1016/j.blre.2016.06.001
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Please cite this article as: Barbui T, et al, The 2016 revision of WHO classification of myeloproliferative neoplasms: Clinical and molecular advances, Blood Rev (2016), http://dx.doi.org/10.1016/j.blre.2016.06.001
Arguments for a lowering of the Hb/Hct threshold values in PV may not only be derived from patients presenting with an ET-like appearance [13–15] but also by regarding the addition of the WHO guidelines; (1) a Hb or Hct level that is > 99th percentile of reference range for age, sex, or altitude of residence; or red cell mass > 25% above mean normal predicted; or (2) RCM that is > 25% above mean normal predicted, or (3) Hb level > 17 g/dL (> 15 g/dL in women) associated with a sustained increase of 2 g/dL from baseline that cannot be attributed to correction of iron deficiency. The latter three components were designed to capture PV patients with borderline increased Hb that is ≤ 18.5 g/dL in men and ≤ 16.6 g/dL in women [2]. For this reason, we have re-introduced the term “masked PV” [16] for JAK2-mutated patients who reveal a PV-characteristic BM morphology but display Hb levels between 16.0 and 18.5 g/dL for men and 15.0 and 16.5 g/dL for women [17,18]. Subsequently, a Hb level of 16.5 g/dL in men and 16.0 g/dL for women or a Hct level of 49% in men and 48% in women has been determined to be the optimal cut off levels for distinguishing JAK2-mutated ET from “masked/prodromal” PV [19].

2.2. Bone marrow morphology

Lowering the diagnostic Hb/Hct levels to those thresholds might simplify the first major diagnostic criterion for PV, but would require morphological confirmation [15,20–23]. This postulate could avoid the possibility of underdiagnosis and has therapeutic relevance because of the associations between increased thrombotic complications and borderline increased Hct (45% to 50%) in PV and JAK2 mutation in ET [8,24,25]. Moreover, the possibility of missing PV is demonstrated by a recently conducted clinical trial on 538 WHO-defined patients younger than 40 years including 97 PV, 66 “masked” PV and 375 JAK2-mutated ET cases [26]. In this cohort, multivariate analysis showed that the only factor accounting for the risk of thrombosis was the less. Frequent application of phlebotomies or cytoreductive treatment in the young patients diagnosed after 2005 presented with significant increase of at least 2 g/dL from baseline not attributable to iron deficiency.

2.3. JAK2 mutation

According to the criteria of the Polycythemia Vera Study Group (PVSG) in PV an elevation of the RCM was regarded as the most important major diagnostic criterion, irrespective of Hct or Hb values, along with normal arterial O2 saturation and splenomegaly [23,28,29]. Although the PVSG has to be credited for conducting a number of important clinical trials [30], the discovery of JAK2V617F (~95% of patients) and the less common JAK2 exon 12 mutations (3%) has significantly reduced the use of these criteria [25] and greatly simplified the diagnostic approach. A large cohort of WHO-defined PV patients stratified by a calendar period before or after 2005, i.e. in the JAK2V617F era, revealed remarkable differences, since patients diagnosed after 2005 presented with significantly lower Hb values associated with older age. This finding is likely to be ascribed to the widespread use of assays addressing the JAK2V617F mutation even in the presence of modestly increased Hb levels that resulted in earlier diagnosis particularly in the older age groups, also as a result of the increased attention and health monitoring for the elderly [31]. Regardless of the contributing factors, these observations underscore the need to re-evaluate critically not only the diagnostic criteria applied but also the current demographics and the frequencies of thrombosis in contemporary series of patients when embarking in clinical trial design that includes thrombosis prevention in PV as main endpoint.

2.4. Serum erythropoietin level

The inclusion of BM morphology as a major criterion allows the diagnosis of PV based on major criteria alone without the need for additional minor criteria. In order to address the rare possibility of a JAK2-unmutated PV, a “subnormal level” of serum erythropoietin (EPO) was maintained as the only minor criterion [32]. On the other hand, several authors expressed their concern regarding an overreliance on serum EPO values as a diagnostic criterion in view of the relatively high frequency of normal EPO values in overt cases of PV. Whereas a low serum EPO is very specific for PV and therefore clinically useful, approximately 20% of PV patients present with normal and a few even with high EPO values [23,33,34].
2.5. Endogenous erythroid colony growth

The former minor criterion endogenous erythroid colony growth (EEC) is now removed from the list of diagnostic parameters because performance is limited to special institutions and lacks accurate standardization. Furthermore, this procedure is time consuming and very costly and therefore of limited practical use [32].

3. Essential thrombocythemia

3.1. Bone marrow morphology

Concerning ET the main issue for a prospective change in diagnostic criteria is to improve standardization of prominent features of BM morphology for a more easy recognition of histological patterns facilitating a better differentiation from the other MPN subtypes. This improvement is needed because criticisms regarding the WHO classification [2] are mainly focused on subjectivity and lack of inter-observer reproducibility of morphological criteria, but especially their reliability of distinguishing ET from early/prefibrotic PMF (prePMF) and “prodromal/masked” PV [35–38]. A careful evaluation of these arguments revealed among others failing standardization of prominent BM features of distinctive value precluding correct histological pattern recognition including fiber grading [21,22,39–42]. The wide ranges reported for fiber grading [35,37] are in striking contrast with previous results describing a substantially to almost perfect concordance between hematopathologists [22,43,44]. In initial WHO-diagnosed ET minor (grade 1) reticulin fibrosis [44,45] is a very rare finding [15,21,46] and in 891 patients was described to occur in only 3% [47], contrasting the results reported by another group [36]. In this context it is noteworthy that several studies on large cohorts of patients demonstrate overall consensus rates ranging between 76% and 88%, significantly depending on the study design (all subtypes of MPN or restriction to ET versus prePMF, inclusion of control cases with reactive changes, blinded evaluation or in combination with clinical data as required by the WHO diagnostic guidelines) [22,41,42,47,48]. In consideration of these data a revision is needed in enhancing efforts to differentiate “true” ET from prePMF and other MPN subtypes by careful examination of BM biopsy specimens emphasizing among other features the lack of reticulin fibrosis and a more scrutinized histological pattern recognition (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Polycythemia vera (PV)*</th>
<th>Essential thrombocythemia (ET)*</th>
<th>Primary myelofibrosis (PMF)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major criteria</strong></td>
<td></td>
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</tr>
<tr>
<td>1. Hemoglobin &gt; 16.5 g/dl (men) &gt; 16 g/dl (women) or hematocrit &gt; 49% (men) &gt; 48% (women) or increased red cell mass (RCM)</td>
<td>Platelet count ≥ 450 × 10^9/l</td>
<td>prePMF: Megakaryocytic proliferation and atypia≥, without reticulin fibrosis &gt; grade 1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation and often decreased erythropoiesis</td>
</tr>
<tr>
<td>BM with age-adjusted hypercellularity and trilineage myeloproliferation with pleomorphic mature megakaryocytes (differences in size)</td>
<td>BM with megakaryocyte proliferation with large and mature morphology. No significant left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers</td>
<td>Not meeting WHO criteria for BCR-ABL1 + CML, PV, ET, MDS or other myeloid neoplasms</td>
</tr>
<tr>
<td>Presence of JAK2 mutation</td>
<td>Not meeting WHO criteria for BCR-ABL1 + CML, PV, PMF, MDS or other myeloid neoplasms</td>
<td>Presence of JAK2, CALR or MPL mutation or in the absence of these mutations, presence of another clonal marker or absence of minor reactive bone marrow reticulin fibrosis†</td>
</tr>
<tr>
<td><strong>Minor criteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subnormal serum erythroproteint level</td>
<td>Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive thrombocytosis</td>
<td>Presence of one or more of the following‡: • Anemia not attributed to a comorbid condition • Palpable splenomegaly • Leukocytosis ≥ 11 × 10^9/L • Elevated LDH†</td>
</tr>
<tr>
<td>Presence of JAK2, CALR or MPL mutation</td>
<td>Presence of one or more of the following‡: • Anemia not attributed to a comorbid condition • Palpable splenomegaly • Leukocytosis ≥ 11 × 10^9/L • Elevated LDH† • Leukoerythroblastosis‡</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome.

* PV diagnosis requires meeting either all three major criteria or the first two major criteria and one minor criterion.
† ET diagnosis requires meeting all four major criteria or another clonal marker or absence of minor reactive bone marrow reticulin fibrosis.
‡ Minor (grade 1) reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.
§ Confirmed in two consecutive determinations.
• Degree of abnormality can be borderline or marked and institutional reference range should be used for lactate dehydrogenase (LDH).

http://dx.doi.org/10.1016/j.blre.2016.06.001
3.2. Clinical findings

Several observational studies following the WHO diagnostic guidelines for ET [2] have been conducted in the past years [47–50], showing different data at presentation, new risk stratifications, differences between true ET vs. prePMF and efficacy of treatment when comparing these with former clinical trials [6,51]. Following the diagnostic criteria of the PVSG [52], a multicenter, randomized comparison of hydroxyurea (HU) with anagrelide (ANA) in treatment was conducted in patients with high-risk ET (UK-PT1 study) [51]. As compared with HU plus aspirin, ANA plus aspirin was associated with increased rates of arterial thrombosis, major hemorrhage and progression to MF, but decreased rates of venous thrombosis. A comparison with other studies on ET using the WHO classification [2] revealed different results [48,49]. According to the UK-PT1 Study BM fiber grading showed a wide range of 37–76% in the patients and included higher levels of MF [35], implicating grades 3 and 4 on a four-graded scale [53], displaying even new bone formation (osteosclerosis). In another study of the same group [54] on 361 patients with ET mostly derived from the UK-PT1 trial, 60% of the cases presented already initially with increased BM fibrosis including 20% with moderate to overt MF (consistent with grades 3 and 4) [35], implicating grades 3 and 4 on a four-graded scale [53], displaying even new bone formation (osteosclerosis). In this context these data are hardly compatible with a strict application of the PVSG diagnostic guidelines for ET [52] and have a significant impact on clinical presentation and outcome [47]. In this regard a challenging finding is the correlation between the leukocyte count, in turn related to granulocytic proliferation in the BM and degree of fibrosis [54]. This result gives more credence to the assumption that patients diagnosed as ET with presenting fibrosis would more likely meet the criteria for WHO-defined MF, where granulocytic proliferation is considered a characteristic feature [2,55]. Moreover, the argument that there is no difference in presenting laboratory or clinical features between WHO-confirmed ET and prePMF [38] is erroneous and not supported by a number of clinicopathological studies from different groups [42,47,49,55]. Altogether data reported by the UK-PT1 study are in keeping with a heterogeneous population including PMF patients as may be derived from a scrutinized analysis of BM features in PVSG- diagnosed MPN with presenting thrombocytopenia [56].

Contrasting these results based on the PVSG criteria [53], another study focused on high-risk patients with strictly WHO-diagnosed ET [2] displayed noninferiority of ANA in comparison with HU and no evidence for progression into post-ET MF or transformation to BP [48]. In this regard this investigation confirms and extends the notion that ANA does neither induce disease progression to myelofibrosis [50] or BP in strictly WHO-defined ET patients nor provokes in the absence of aspirin medication hemorrhagic events [48]. Concerning vascular events, it has been reported that leukocytosis and a higher degree of BM reticulosis fibrosis add prognostic significance to existing risk factors for major arterial thrombosis in PVSG-defined ET and therefore, these patients had a major advantage from HU in comparison to ANA [48,51,54,57–59]. In contrast, the ANAHYDRET cohort was not presenting a higher degree of leukocytosis or BM fibrosis, consequently the platelet reducing effect could be sufficient to prevent adverse arterial events. For this reason, ANAHYDRET trial enrolling patients with WHO-defined ET, failed to demonstrate the superiority of a panmyeloid myelosuppressive treatment with HU [48]. These findings were confirmed by an investigation on prePMF patients where leukocytosis has been shown to be an important risk factor for arterial thrombosis [59]. Differences in bleeding complications in the ET patient of the UK-PT1 study [51] compared with those of the ANAHYDRET trial [48] may be again explained by the differences of diagnostic criteria (PVSG-ET vs. WHO-ET) and previous treatment status. In addition, the restrictive use of aspirin in the ANAHYDRET study may have been responsible for the decreased bleeding rate in ANA-treated patients because it is known that hemorrhagic complications associated with aspirin may be provoked in not — WHO-confirmed ET patients, especially when aspirin is combined with ANA [60,61]. Low-dose aspirin could exacerbate bleeding tendency as may be concluded from the finding that major bleeding events associated with thrombocytosis have been described in prePMF compared to WHO-defined ET [62].

Noteworthy is that the first set of the 2014 updated BCSH criteria allows ET diagnosis without BM biopsy examination [63]. In this context it should be underscored that PV and especially PMF were diagnosed according to the updated BCSH criteria implying that prePMF was not taken into account [64]. A recently published multicenter study compared clinical and morphological features between ET patients diagnosed by applying either the first set of the updated BCSH criteria [63] or the 2008 WHO [1,2] diagnostic guidelines [49]. A re-evaluation of the 238 BCSH-confirmed ET patients by following the WHO criteria revealed a heterogeneous population (59.2% “true” ET, 32.4% PMF, 6.7% PV, and 1.7% PMF) and in contrast to the 232 patients with WHO-defined ET a significantly unfavorable fibrosis-free survival and prognosis [49].

Significant difficulties arise to provide accurate data about the other adverse events as progression to post-ET MF [65] because most studies do not follow the WHO classification [2]. In a study including 891 strictly WHO-defined ET patients after a median observation time of about 6 years a MF rate of 4% was found with a 10-year cumulative incidence of 0.8%, significantly contrasting prePMF with a total rate of 8% and a 10-year cumulative incidence of 12.3% [47]. As recently reviewed contrasting these favorable data other groups that were not or not strictly applying the WHO guidelines showed very different 10-year incidences for post-ET MF ranging from 5.0% to 9.7% [3,4,6]; A comparison with the study by Barbui and coworkers [47] suggests that the high frequencies are probably confounded by the inadvertent inclusion of prePMF patients and therefore are not consistent with the diagnostic guidelines of the WHO classification [2] In a similar way the frequency rates for BP (acute leukemia) transformation revealed wide ranges with 2.6% and up to 9.7% risk at 10 years [6]. According to the aforementioned study [47] the 10-year cumulative incidence was 0.7% with a total event rate of 1% significantly contrasting again prePMF with a 10-year cumulative incidence of 5.8% and a total event rate of 5%. These figures are consciously lower than those previously reported in large retrospective studies from independent groups of investigators [6], and in context with the discussed clinical studies provide persuasive evidence that a clear-cut discrimination between “true”, i.e. WHO-defined ET and prePMF is essential.

3.3. Molecular findings

The previous WHO criteria included the JAK2V617F mutation as major diagnostic criterion; it is found in 50% to 60% of the patients diagnosed as ET. An additional 3 to 5% of the patients may present with mutations of MPL, the gene encoding the thrombopoietin receptor, typically affecting the 515 codon with different aminoacid substitutions (W > L,K,A). The discovery of mutations in calreticulin gene (CALR) in 2013 added significantly to the molecular characterization of ET (and PMF) patients by reducing the molecular diagnostic gap in JAK2/MPL-unmutated ET [66,67], with reported frequencies ranging from 15% to 32% in the largest published series. By combining these mutational frequencies, a novel category of patients who are negative for the above mentioned 3 driver mutations, but are operationally called as “triple–negative” was highlighted, that account for 10 to 16% of ET cases [68–71]. Compared to JAK2-mutated cases, the association of CALR mutation with younger age, male gender, lower leukocyte count, lower hemoglobin level and higher platelet counts, and mostly important lower thrombotic risk, was reported in different cohorts of patients [68,70,71]. Additionally, there might be different phenotypic effects associated with distinct mutant CALR variants including the higher platelet count observed in type 2/type 2-like variants in ET [69,71,72]. Concerning the so-called “triple–negative” ET patients, it has been recently reported, by applying whole exome sequencing or other
sensitive molecular techniques that a proportion of them may actually present non-canonical gain of function mutations in MPL, such as MPLS204P and MPL591N, or less frequently in JAK2 [73,74]. However, interestingly enough, almost half of the patients in these series were lacking evidence of underlying clonal hematopoiesis. These findings are in keeping with the assumption that JAK2/CALR/MPL-unmutated ET does not represent a homogenous entity and that it includes cases with polyclonal hematopoiesis some of which might represent an otherwise unrecognized hereditary disorder [73].

On the other hand, one should be aware that determination of JAK2/CALR mutational status alone without BM morphological examination is not sufficient to differentiate PV from JAK2-mutant ET and that distinction of WHO-defined ET [2] into JAK2 and CALR subtypes has limited prognostic relevance in terms of overall survival or leukemia trasformation, unlike for PMF [71]. In a multicenter study Tefferi and coworkers [48] described 84 WHO-defined “triple-negative” ET patients presenting with a sustained median platelet count of $854 \times 10^9/L$ (range 500–3300 $\times 10^9/L$) and no significant differences in survival or leukemia-free survival when compared to the JAK2- or CALR-mutated cases [71].

4. Primary myelofibrosis

4.1. Clinical features

Although in PMF there is consensus on the diagnostic and clinical implications concerning the overt manifestations of this MPN subtype, the concept of prodromal presentations (prePMF) implicating a stepwise evolution of disease has to be re-enforced by defining more clearly the clinical criteria supporting diagnosis [15,73,74]. Preblastic PMF was not included in the diagnostic scheme of the 2014 updated BCSH criteria [64] since only advanced stages consistent with myelofibrosis with myeloid metaphasia (MMM) were included [77]. However, different groups based on clinical and morphological findings including follow-up studies have confirmed that prePMF exists [46,78–81]. In particular, controversy was focused on the clinical criteria with borderline expression as defined by the 2008 updated WHO classification [2]. The diagnostic relevant, so-called minor clinical parameters, consisted of at least two including leukoerythroblastosis, increase in serum lactate dehydrogenase, anemia and splenomegaly (Table 1). On the other hand, it has to be conceded that the so-called borderline expression of leukoerythroblastosis has caused a persistent debate because only a very few peripheral blasts (erythroblasts and myeloblasts) were described in single patients [42,55,82]. Contrasting this rarity of blasts in prePMF the leukocyte count ($\geq 11 \times 10^9/L$) seemed to be a more suitable candidate (Table 2) for entering this parameter into the set of clinical criteria [15,42,55,75,76,82], especially when referring to vascular events [59,62]. A study on 180 patients with prePMF and thrombocytosis revealed that major bleeding may be a relatively specific event as opposed to WHO-confirmed ET and that low-dose aspirin exerted a synergistic hemorrhagic effect [62]. Regarding major thrombotic complications no difference were encountered between prePMF and WHO-defined ET [47].

There are only a few data available referring to the progression of prePMF to advanced PMF, formerly termed MMM [77]. It is well-known that this evolution is unpredictable and in a series of 180 patients based on the clinical diagnosis after a follow-up of 6.2 years showed a total frequency of 8% with a 15-year cumulative incidence of approximately 17% [47]. Data derived from sequential BM biopsy evaluations are usually higher since they are biased by the tendency of the clinicians to perform such an examination if there is any change in the course of disease. In a cohort of 196 prePMF patients with repeated biopsies the relative risk to progress to MF at the same time point of grade 0 needs a significantly longer interval to develop MF compared to prePMF-fiber grade 1 [55]. Moreover, several groups have provided convincing data that compared to WHO-defined ET initial BM fiber grading in PMF reveals not only a correlation with hematological data but also with survival [42,47,49,55,83–85]. The combination between the parameters of the International Prognostic Scoring System (IPSS) [86] and fiber grades [45] suggests that a better prognostication can be achieved by considering this morphological parameter in addition to the clinical and mutation data [87].

To underscore the clinical impact for a more easy identification of prePMF it was suggested to create distinct diagnostic guidelines for this entity (fiber grades, clinical features) separate from overt PMF [76], in order to improve differentiation from overt PMF (Table 2) that may have major influence not only on accurate diagnosis but also on outcome and prognosis.

4.2. Molecular findings

Similar to findings in ET, also in PMF patients CALR mutation was associated with younger age, lower Hb level, lower risk disease and lower incidence of spleenomegaly mutations [71,88]. The reported incidences of CALR mutants ranged from about 20% to more than 30% in PMF patients [71,88–91], leaving approximately 9% to 12% so-called “triple-negative” cases [71,89–91]. In contrast to ET, the JAK2/CALR/MPL mutational status provided significant prognostic information in PMF [71]. The favorable impact of CALR mutations on survival was confirmed by several groups, highlighting also the prognostically worse effect of a “triple-negative” mutational status [71,89–94]. Noteworthy is that the prognostic advancement of calreticulin mutations in PMF might be confined to type 1 or type 1-like CALR variants [94,95]. Multivariable analysis distinguished CALR (−) and ASXL1 (+) mutational status as the most significant risk factors for shortened survival [92,94] independent of the dynamic IPSS/DIPSS–plus model that employs clinical and cytogenetic variables [86]. For this reason, further studies are necessary to better evaluate the impact of molecular findings on prognosis in the settings of clinical scores, and particularly in patients with prePMF to test the frequency and clinical impact of their CALR/ASXL1 status on prognosis.

5. Conclusion

In conclusion, although the 2008 updated WHO classification has significantly improved the diagnostic criteria for existing MPN entities, as has been highlighted by experiences gained in the past 8 years, a number of modifications and amendments are needed regarding clinical, molecular and morphological features. In this context it is noteworthy that the diagnostic issues and proposals as previously suggested for MPNs [76] were fully adopted by the authors of the just published WHO 2016 revision regarding myeloid neoplasms [96].

Practice points

• Description of genetic markers other than JAK2V617F and MPL, in particular CALR mutation, supports the need for changing the diagnostic criteria of the WHO-2008, particularly regarding essential thrombocythemia (ET) and primary myelofibrosis (PMF).

• In JAK2-mutated patients with characteristic bone marrow morphology, a hemoglobin — hematocrit level of 16.5 g/dL — 49% in men and 16.0 g/dL — 48% for women are the optimal cut off levels for distinguishing JAK2-mutated ET from “masked/prodromal” polycythemia vera (PV).

• The existence of two distinct entities, “true ET” versus prefibrotic/early PMF (prePMF), with unique clinical and morphological patterns, is now reinforced by several observational studies.

• In overt/advanced stages of PMF, minor criteria for diagnosis have been more explicitly defined by clinical studies; these minor criteria are not fully or only borderline expressed in prePMF.
Research agenda

- The updated WHO classification is focused on bone marrow morphology and controversy persists on the subjectivity and lack of interobserver reproducibility. Therefore, future research should try to improve standardization of prominent BM features.
- The novel diagnostic criteria will lead to an increase in patients in earlier stages of the disease, thereby new clinical research is required to prospectively confirm the clinical epidemiology and outcomes described in retrospective cohorts.
- The clinical epidemiology of “true ET” and prePMF needs to be prospectively evaluated in well-designed clinical trials testing separately patients belonging to the two distinct entities.

Conflict of interest statement

None of the authors have any conflict of interest to disclose in regards to the current manuscript.

Acknowledgments

T.B., G.F. and A.M.V. were supported by a grant from Associazione Italiana per la Ricerca sul Cancro (AIRC, Milano) “Special Program Molecular Clinical Oncology 5 × 1000” to AGIMM (AIRC-Gruppo Italiano Malattie Mieloproliferative).

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