

**Proposals and rationale for revision of the World Health
Organization diagnostic criteria for polycythemia vera, essential
thrombocythemia, and primary myelofibrosis: *Recommendations*
*from an ad hoc international expert panel***

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AT, JT, AO, and JWV prepared the initial draft of the proposals for revision of the WHO diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis. AT wrote the paper. JT, AO, and JWV participated in the writing of the paper. All the authors participated in the discussion and have reviewed and approved the current version of the manuscript.

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Abstract

The Janus kinase 2 mutation, *JAK2V617F*, is myeloid neoplasm-specific; its presence excludes secondary polycythemia, or thrombocytosis or bone marrow fibrosis from other causes.

Furthermore, *JAK2V617F* or a *JAK2* exon 12 mutation is present in virtually all patients with polycythemia vera (PV) whereas *JAK2V617F* also occurs in approximately half of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF). Therefore, *JAK2* mutation screening holds the promise of a decisive diagnostic test in PV while being complementary to histology for the diagnosis of ET and PMF; the combination of molecular testing and histological review should also facilitate diagnosis of ET associated with borderline thrombocytosis.

Accordingly, revision of the current World Health Organization (WHO) diagnostic criteria for PV, ET, and PMF is warranted; *JAK2* mutation analysis should be listed as a major criterion for PV diagnosis and the platelet count threshold for ET diagnosis can be lowered from 600 to 450 x 10⁹/L. The current document was prepared by an international expert panel of pathologists and clinical investigators in myeloproliferative disorders; it was subsequently presented to members of the Clinical Advisory Committee for the revision of the WHO Classification of Myeloid Neoplasms who endorsed the document and recommended its adoption by the WHO.

Introduction

Recent discoveries in the molecular pathogenesis of *BCR-ABL*-negative myeloproliferative disorders (MPDs) have raised the prospect of genetic classification as well as molecular diagnosis in these disorders.¹ The seminal reports in this regard were first published in early 2005 and described a somatic Janus kinase 2 (*JAK2*) mutation (*JAK2V617F*; an exon 14 somatic 1849G→T mutation) in polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF);²⁻⁵ mutational frequency is estimated at 95% for PV and 50% for both ET and PMF. Subsequently, *JAK2V617F* was demonstrated in other myeloid neoplasms, albeit at a much lower mutational frequency.^{6,7} Most recently, other MPD-associated *JAK2* (exon 12 mutations)⁸ and *MPL* (*MPLW515L/K*)⁹ mutations were described; the former in *JAK2V617F*-negative PV⁸ and the latter in < 5% of patients with PMF or ET.^{9,10} As such, a *JAK2* mutation is present in virtually all patients with PV and thus constitutes a sensitive diagnostic marker for the disease. In addition, the incidence of *JAK2V617F* in both ET and PMF is high enough to warrant diagnostic utility that is complementary to histological impression.

Prompted by these developments, members of the Steering Committee for the revision of the WHO Classification of Hematopoietic and Lymphoid Neoplasms approached key members of the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT), the Myeloproliferative Diseases-Research Consortium (MPD-RC) and the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) to enlist their cooperation in the development of revised diagnostic guidelines for the *BCR-ABL*-negative MPDs. E-conferences were initiated by two of the authors (AT and JWV). The key participants are listed as co-authors of the current document, and include clinical investigators, scientists and pathologists, two of whom are members of the WHO Steering Committee (JWV and JT). The resulting consensus

document was also presented to members of the Clinical Advisory Committee for the revision of the WHO Classification of Myeloid Neoplasms (meeting March 10, 2007, Chicago IL, Clara D. Bloomfield, Chair), who endorsed the document and recommended its adoption by the WHO.

Rationale for revising WHO criteria for the diagnosis of polycythemia vera

Table 1 outlines the current WHO criteria for the diagnosis of PV. As mentioned above, virtually all patients with overt (i.e. with increased hematocrit) or inapparent (i.e. with hematocrit that does not exceed the upper reference range) PV carry *JAK2V617F* or other functionally similar *JAK2* mutation (e.g. exon 12 *JAK2* mutations). Such mutations are absent in either secondary or spurious polycythemia. Therefore, most of the current WHO criteria, listed under either A-criteria or B-criteria (Table 1) are no longer necessary and can be replaced by adding “presence of *JAK2V617F* or other functionally similar *JAK2* mutation” as a major criterion (Table 2). Accordingly, we propose to replace the current list under the “A” criteria with two “major” criteria: i) laboratory evidence of increased hemoglobin, hematocrit, or red cell mass and ii) the presence of a *JAK2* mutation (Table 2). Similarly, we propose to replace the current list of B-criteria with three biologically relevant “minor” criteria: i) MPD-consistent bone marrow histology, ii) serum erythropoietin level below the reference range for normal, and iii) presence of endogenous erythroid colonies. Diagnosis of PV under the new proposed criteria will require either the presence of both major criteria and at least one minor criterion or the presence of the first major criterion and at least 2 minor criteria (Table 2).

In practice, meeting both of the newly proposed major criteria should capture more than 97% of cases with PV. However, in order to minimize consequences of false positive molecular test results and further optimize diagnostic specificity, the additional presence of at least one of three minor criteria will be required in order to make a diagnosis of PV (Table 2). The alternative

diagnostic combination (i.e. first major criterion + 2 minor criteria) should capture occasional cases of true PV who might be either negative for a known *JAK2* mutation or associated with a mutation burden that is too low to be detected.¹¹ The requirement for two instead of one minor criteria in this instance reinforces the accuracy associated with each test and increases the comfort level to make the specific diagnosis in the context of a negative molecular test result.

The first major criterion, under the newly proposed criteria (Table 2), is notably different from that listed in the existing WHO criteria (Table 1). First, while retaining the hemoglobin and red cell mass threshold levels for PV diagnosis used in the current WHO criteria, the new first major criterion incorporates information on hematocrit in order to address potential discordance between hemoglobin level and hematocrit as well as accommodate the needs of medical centers which use hematocrit instead of hemoglobin level as their primary tool to evaluate polycythemia.¹² We are very much aware of the controversies regarding which one of the three red cell parameters (i.e. hemoglobin level, hematocrit value, measured red cell mass) is the most accurate in the determination of red cell volume. However, we do not believe that the issue will be resolved any time soon and thus we opted to accommodate individual choice on the matter. The second modification of the first major criterion for PV diagnosis (Table 2) aspires to capture biologically true PV cases with a documented, sustained increase in their baseline hemoglobin level in excess of 2 g/dL, without reaching the diagnostic threshold. The particular measure helps bypass the need for red cell mass measurement in such cases and complements bone marrow histology in identifying early PV cases.

We recognize the possibility that the newly revised criteria could still miss occult PV. However, the identification of such cases might not be therapeutically relevant because i) high-risk MPD patients receive similar drug therapy regardless of the specific MPD diagnosis and ii)

the value of aggressive phlebotomy in aspirin-treated low-risk disease, within hematocrit ranges between 40% and 55%, has been questioned by the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) Investigators.¹³ The newly proposed criteria will also allow identification of “atypical” or “early” PV that presents with the phenotype of “idiopathic erythrocytosis”¹⁴ since such cases have now been identified to display either exon 14 or exon 12 *JAK2* mutations.^{11,15} Some cases of idiopathic erythrocytosis remain molecularly undefined but we do not believe that the situation warrants establishment of a separate set of diagnostic criteria. We are also aware of the possibility that certain cases of *JAK2* mutation-positive “idiopathic” abdominal vein thrombosis could progress into overt PV but more information is needed in this regard before recognizing such cases in formal diagnostic criteria.¹⁶

Finally, hemoglobin level is expected to be lower in the presence of iron deficiency and this confounds the accurate interpretation of the hemoglobin/hematocrit level. However, for the purposes of research or clinical trials, a formal PV diagnosis requires demonstration of meeting the WHO criteria in terms of hemoglobin/hematocrit level after iron replacement. Whether or not such a measure is clinically prudent is left to the discretion of the physician. In other words, in routine clinical practice, one should not be prevented from making a working diagnosis of PV, in the presence of iron deficiency, just because the WHO criteria are not met.

Rationale for revising WHO criteria for the diagnosis of essential thrombocythemia

Table 3 outlines current WHO criteria for the diagnosis of ET. Many investigators have effectively argued that the use of the $600 \times 10^9/L$ platelet threshold level compromises the detection of early phase disease since the 95th percentile for normal platelet count, adjusted for gender and race, is below $400 \times 10^9/L$.¹⁷⁻²¹ Therefore, we start by proposing a change in the platelet threshold level required for ET diagnosis by lowering it to $450 \times 10^9/L$ from $600 \times 10^9/L$

(Table 4; criterion 1); a proposal supported by the current availability of a clonal marker (*JAK2V617F*) that is present in 50% of ET patients. However, unlike the case with PV, the utility of mutation screening for *JAK2V617F* for the diagnosis of ET or PMF is limited by suboptimal negative predictive value and lack of diagnostic specificity within the context of myeloid neoplasms.^{7,22} Therefore, a bone marrow biopsy is still required to help with the differential diagnosis between *JAK2V617F*-negative ET and reactive thrombocytosis as well as differentiate ET from other chronic myeloid neoplasms including cellular phase/pre-fibrotic PMF and myelodysplastic syndromes (MDS).²³ As such, we propose keeping bone marrow examination as one of the required criteria for ET diagnosis (i.e. criterion 2; Table 4).

In addition, we propose getting rid of separate categorization of negative exclusionary criteria and instead suggest the addition of two criteria (criteria 3 and 4), which serve in the same capacity, to the main list (Table 4). Criterion 3 requires the absence of WHO criteria that would otherwise classify a patient as having PV, PMF, chronic myelogenous leukemia (CML), or MDS. In this regard, it is important to exclude occult PV in the iron deficient patient by a trial of iron replacement therapy. Similarly, careful bone marrow histological assessment is necessary in order to exclude the possibility of cellular phase/pre-fibrotic PMF or MDS. Criterion 4 underlines the need to exclude reactive thrombocytosis either through the demonstration of *JAK2V617F* or, in mutation-negative patients, by clinical assessment.^{23,24} Accordingly, the diagnosis of ET would require fulfillment of all 4 main criteria listed in Table 4. However, it is possible that a *JAK2V617F*-negative ET patient might have a concomitant condition known to be associated with reactive thrombocytosis. In this instance, diagnosis of ET can be made provided the first three criteria are met.

In ET, bone marrow findings are remarkable for the presence of large but mature-appearing megakaryocytes with deeply lobulated and hyperlobulated nuclei that are most often dispersed throughout the biopsy sections, but sometimes also found in loose clusters.²⁵ Often the bone marrow is normally or only slightly hypercellular for the patient's age, and the increased trilineage proliferation (panmyelosis) that characterizes PV or the granulocytic proliferation and highly bizarre megakaryocytes that characterize the pre-fibrotic stage of PMF are not found in ET.²⁶ The presence of dyserythropoiesis, macrocytosis, monocytosis, pseudo Pelger-Huet anomaly or other dysgranulopoietic changes of neutrophils and/or predominance of small megakaryocytes with monolobated nuclei suggest MDS rather than ET.^{23,27} On the other hand, a spectrum of cytogenetic abnormalities, including del(20q), del(5q), and unbalanced whole-arm translocation between 1q and 7p have been reported in otherwise typical ET^{28,29} and their presence, unless accompanied by histological features of MDS, should not exclude the diagnosis of ET.³⁰ Nevertheless, we encourage obtaining cytogenetic studies at time of diagnostic bone marrow examination for ET and re-evaluation of the histological impression in case of unusual findings.

Rationale for revising WHO criteria for the diagnosis of primary myelofibrosis

The current WHO criteria for PMF³¹ have separate tables for “pre-fibrotic” and “fibrotic” PMF (Tables 5 and 6). Under the newly proposed revised criteria (table 7), 3 major and 4 minor criteria are enlisted. The first major criterion underscores histology as a critical diagnostic criterion for PMF. In this regard, there is little doubt about the existence of the histopathologic phenotype of PMF with minimal or no demonstrable excess in reticulin fibers, i.e. pre-fibrotic or “cellular phase”. Therefore, the demonstration of reticulin fibrosis, although characteristic, is not a required criterion for the diagnosis of PMF. Instead, the cardinal and therefore required

features for PMF include increase in megakaryocyte growth associated with conspicuous abnormalities as well as granulocyte proliferation.²⁶ However, in the absence of reticulin fibrosis, meeting the first major criterion for PMF diagnosis requires, in addition to the aforementioned megakaryocyte changes, presence of bone marrow hypercellularity, granulocyte proliferation, and decrease in erythroid precursors.

The other 2 major criteria underline the need to exclude either myelofibrosis associated with another myeloid neoplasm (major criterion 2) or reactive bone marrow fibrosis (major criterion 3). The former requires the absence of meeting WHO criteria for another myeloid neoplasm and the latter the demonstration that either *JAK2V617F* (or other clonal markers such as *MPLW515L/K*) is present or bone marrow fibrosis is not attributed to a non-clonal process like (chronic) toxic myelopathies due to a variety of exogenous agents, lymphoid neoplasms such as hairy cell leukemia and lymphoma, or a metastatic malignancy.²⁴ The diagnosis of PMF according to the revised WHO criteria requires meeting all 3 major criteria and, in order to increase diagnostic accuracy, in the context of prefibrotic/cellular phase disease, two of four minor criteria (Table 7).

The distinction between PMF on one hand, and MDS with fibrosis, ET, or acute panmyelosis with myelofibrosis (in many cases a hyperfibrotic subtype of AML according to the WHO),^{32,33} on the other, requires careful morphological assessment.^{26,34} PMF is characterized by megakaryocytes that are often found in sizable loose to tight clusters, and range in size from small to large with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei. Bare megakaryocytic nuclei are common.³⁵ In contrast, MDS is characterized by its classic dysmyelopoietic features. The dysplastic megakaryocytes seen in MDS are usually small and often have monolobated, hypolobated or widely dispersed nuclei, and

are not usually found in large clusters (with the exception of some cases of fibrotic MDS) as are typical for MPD.³⁴ On the other hand, the presence of ringed sideroblasts, although seen frequently in MDS, may also be found in some cases of MPD, and therefore lacks diagnostic specificity.^{36,37} Such contention is consistent with the demonstration of *JAK2V617F* in a high proportion of cases with "refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T)".³⁸⁻⁴² A proportion of these cases might "represent" true MPD cases (i.e. ET or PMF) with ringed sideroblasts.⁴³ Other cases, with more pronounced MDS-like abnormalities such as dyserythropoiesis and anemia, might or might not display additional morphological abnormalities of megakaryocytes that resemble those seen in MPD.³⁸⁻⁴²

Careful bone marrow morphological examination is crucial in distinguishing ET from pre-fibrotic PMF, an important detail that was overlooked by the original Polycythemia Vera Study Group (PVSG) criteria,^{44,45} despite its potential confounding effect on both survival and rate of myelofibrotic transformation.⁴⁶ Such distinction takes into account the degree of bone marrow cellularity (marked hypercellularity in cellular phase PMF), presence of prominent granulocyte proliferation with left-shifted forms (typical in PMF but absent in ET), and in particular megakaryocyte morphology (with the aforementioned nuclear features in PMF and giant, mature-appearing megakaryocytes with deeply lobulated and hyperlobulated nuclei in ET).¹⁷ Nevertheless, in order to reinforce diagnostic accuracy, the revised WHO diagnostic criteria for PMF require the presence of at least two of four PMF-characteristic peripheral blood or clinical features: leukoerythroblastosis, increased serum lactate dehydrogenase level, anemia, and palpable splenomegaly.

Patients with acute (malignant) myelofibrosis or panmyelosis with myelofibrosis usually present with severe constitutional symptoms, pancytopenia, mild or no splenomegaly, and

feature an increase in blood and/or bone marrow blast count that will partially approach or fulfill the required threshold for AML diagnosis.³³ In this context, it has been shown that the latter is a heterogeneous entity that should be differentiated from acute megakaryoblastic leukemia³³ as well as from MDS with accompanying myelofibrosis and a low blast count.^{34,47} Nevertheless, in all cases with myelofibrosis, blasts may be difficult to accurately estimate and CD34 assessment by immunohistochemistry may be invaluable in such cases.⁴⁸ Finally, the presence of either del(13)(q11~13q14~22) or der(6)t(1;6)(q21~23;p21.3) is strongly suggestive but not diagnostic for PMF.^{49,50}

Concluding remarks

The proposed revisions to the diagnostic guidelines require the time-honored integration of clinical, laboratory and pathologic findings. However, now the laboratory data includes evaluation for a molecular defect, *JAK2V617F*, that when present clearly identifies the process as neoplastic and thus eliminates the need for additional tests previously performed to exclude reactive myeloproliferation due to other diseases. The revised criteria also take advantage of the recently appreciated contribution of specific morphologic abnormalities to the diagnosis of the MPDs. The histological differences among the entities outlined in the current manuscript are recognized by experienced hematopathologists and there is on-going investigation to establish even more precise quantitative definitions similar to those established for other myeloid neoplasms.⁵¹

The objective of establishing diagnostic criteria is neither to capture all biologically true cases of disease nor guarantee 100% diagnostic specificity. For example, recent communications have disclosed the presence of *JAK2V617F* in “idiopathic” abdominal vein thrombosis in patients who otherwise do not fulfill conventional diagnostic criteria for either

PV or ET.¹⁶ Some of these patients subsequently developed erythrocytosis and/or thrombocytosis while others appear to have stable counts for many years. Regardless, such cases represent unclassified MPDs and should not be forced into a specific diagnosis, for research purposes. We provide this example to emphasize the point that not fulfilling a given set of published criteria does not prevent one from making a specific working diagnosis that is based on sound clinical judgment.

Table 1. 2001 World Health Organization criteria for polycythemia vera. Diagnosis requires the presence of the first two ‘A’ criteria together with either any one other ‘A’ criterion or two ‘B’ criteria.

A-criteria

1. Elevated red cell mass > 25% above mean normal predicted value, or hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women, or > 99th percentile of method-specific reference range for age, sex, altitude of residence.
2. No cause of secondary erythrocytosis, including:
 - a. Absence of familial erythrocytosis
 - b. No elevation of erythropoietin due to;
 - i. Hypoxia (arterial pO₂ ≤ 92%)
 - ii. High oxygen affinity haemoglobin
 - iii. Truncated erythropoietin receptor
 - iv. Inappropriate erythropoietin production by tumor
3. Splenomegaly
4. Clonal genetic abnormality other than Philadelphia chromosome or *BCR-ABL* fusion gene in marrow cells
5. Endogenous erythroid colony formation *in vitro*

B-criteria

1. Thrombocytosis > 400 x 10⁹/L
2. Leukocytosis > 12 x 10⁹/L
3. Bone marrow biopsy showing panmyelosis with prominent erythroid and megakaryocytic proliferation
4. Low serum erythropoietin levels

Table 2. Proposed revised World Health Organization criteria for polycythemia vera. Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria.**

Major criteria

1. Hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women **or** other evidence of increased red cell volume*
2. Presence of *JAK2*V617F or other functionally similar mutation such as *JAK2* exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation *in vitro*

**Hemoglobin or hematocrit > 99th percentile of method-specific reference range for age, sex, altitude of residence*

***or** Hemoglobin > 17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from an individual's baseline value that can not be attributed to correction of iron deficiency,*

***or** Elevated red cell mass > 25% above mean normal predicted value*

Table 3. 2001 World Health Organization criteria for essential thrombocythemia.

Positive Criteria

1. Sustained platelet count $\geq 600 \times 10^9/L$
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes

Criteria of exclusion

1. No evidence of polycythemia vera
 - a. Normal red cell mass or hemoglobin < 18.5 g/dL in men, 16.5 g/dL in women
 - b. Stainable iron in marrow, normal serum ferritin or normal MCV
 - c. If the former condition is not met, failure of iron trial to increase red cell mass or hemoglobin levels to the PV range
2. No evidence of chronic myeloid leukemia
 - a. No Philadelphia chromosome and no *BCR-ABL* fusion gene
3. No evidence of chronic idiopathic myelofibrosis
 - a. Collagen fibrosis absent
 - b. Reticulin fibrosis minimal or absent
4. No evidence of myelodysplastic syndrome
 - a. No del(5q), t(3;3)(q21;q26), inv(3)(q21q26)
 - b. No significant granulocytic dysplasia, few if any micromegakaryocytes
5. No evidence that thrombocytosis is reactive due to:
 - a. Underlying inflammation or infection
 - b. Underlying neoplasm
 - c. Prior splenectomy

Table 4. Proposed revised World Health Organization (WHO) criteria for essential thrombocythemia (ET). Diagnosis requires meeting all four criteria.

1. Sustained^a platelet count $\geq 450 \times 10^9/L$
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes. No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
3. Not meeting WHO criteria for polycythemia vera,^b primary myelofibrosis,^c chronic myelogenous leukemia,^d myelodysplastic syndrome,^e or other myeloid neoplasm
4. Demonstration of *JAK2V617F* or other clonal marker,

or in the absence of a clonal marker, no evidence for reactive thrombocytosis^f

a. During the work-up period.

b. Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels and red cell mass measurement is not required.

c. Requires the absence of relevant reticulin fibrosis, collagen fibrosis, peripheral blood leukoerythroblastosis, or markedly hypercellular marrow for age accompanied by megakaryocyte morphology that is typical for primary myelofibrosis – small to large with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei and dense clustering

d. Requires the absence of BCR-ABL

e. Requires absence of dyserythropoiesis and dysgranulopoiesis

f. Causes of reactive thrombocytosis include iron deficiency, splenectomy, surgery, infection, inflammation, connective tissue disease, metastatic cancer, and lymphoproliferative disorders. However, the presence of a condition associated with reactive thrombocytosis does not exclude the possibility of ET if the first three criteria are met.

Table 5. 2001 World Health Organization criteria for pre-fibrotic stage primary myelofibrosis.

Clinical findings		Morphological findings	
Spleen and liver	No or mild splenomegaly or hepatomegaly	Blood	No or mild leukoerythroblastosis No or mild RBC poikilocytosis Few if any dacryocytes
Hematology	Variable but often; Mild anemia Mild to moderate leukocytosis Mild to marked thrombocytosis	Bone marrow	Hypercellularity Neutrophilic proliferation Megakaryocytic proliferation Megakaryocytic atypia* Minimal or absent reticulin fibrosis

*clustering of megakaryocytes, abnormally lobulated megakaryocytic nuclei, naked

megakaryocytic nuclei. RBC, red blood cells.

Table 6. 2001 World Health Organization criteria for fibrotic stage primary myelofibrosis.

Clinical findings		Morphological findings	
Spleen and liver	Moderate to marked splenomegaly or hepatomegaly	Blood	Leukoerythroblastosis Prominent RBC poikilocytosis Prominent dacryocytosis
Hematology	Moderate to marked anemia WBC decreased to elevated Platelet count decreased to elevated	Bone marrow	Reticulin and/or collagen fibrosis Decreased cellularity Dilated marrow sinuses Intraluminal hematopoiesis Neutrophilic proliferation Prominent megakaryocytic proliferation Megakaryocytic atypia* New bone formation (osteosclerosis)

Key: WBC, white blood cells; RBC, red blood cells. *clustering of megakaryocytes, abnormally lobulated megakaryocytic nuclei, naked megakaryocytic nuclei

Table 7. Proposed revised World Health Organization criteria for primary myelofibrosis. Diagnosis requires meeting all three major criteria and two minor criteria.

Major criteria

1. Presence of megakaryocyte proliferation and atypia,^a usually accompanied by either reticulin and/or collagen fibrosis,
or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (i.e. pre-fibrotic cellular-phase disease).
2. Not meeting WHO criteria for polycythemia vera,^b chronic myelogenous leukemia,^c myelodysplastic syndrome,^d or other myeloid neoplasm
3. Demonstration of *JAK2V617F* or other clonal marker (e.g. *MPLW515L/K*),
or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases^e

Minor criteria

1. Leukoerythroblastosis^f
2. Increase in serum lactate dehydrogenase level^f
3. Anemia^f
4. Palpable splenomegaly^f
 - a. *Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering*
 - b. *Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels. Red cell mass measurement is not required*
 - c. *Requires the absence of BCR-ABL*
 - d. *Requires absence of dyserythropoiesis and dysgranulopoiesis*
 - e. *Secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies. It should be noted that patients with conditions associated with reactive myelofibrosis are not immune to primary myelofibrosis and the diagnosis should be considered in such cases if other criteria are met*
 - f. *Degree of abnormality could be borderline or marked*

References

1. Tefferi A, Gilliland DG. Oncogenes in Myeloproliferative Disorders. *Cell Cycle*. 2007;6.
2. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*. 2005;365:1054-1061.
3. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005;7:387-397.
4. Kralovics R, Passamonti F, Buser AS, et al. A gain of function mutation in Jak2 is frequently found in patients with myeloproliferative disorders. *New England Journal of Medicine*. 2005;352:1779-1790.
5. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005;434:1144-1148.
6. Steensma DP, Dewald GW, Lasho TL, et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. *Blood*. 2005;106:1207-1209.
7. Jones AV, Kreil S, Zoi K, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood*. 2005;106:2162-2168.
8. Scott LM, Tong W, Levine R, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007;356:459-468.
9. Pikman Y, Lee BH, Mercher T, et al. MPLW515L Is a Novel Somatic Activating Mutation in Myelofibrosis with Myeloid Metaplasia. *PLoS Med*. 2006;3:e270.
10. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*. 2006;108:3472-3476.
11. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007;356:459-468.
12. Johansson PL, Safai-Kutti S, Kutti J. An elevated venous haemoglobin concentration cannot be used as a surrogate marker for absolute erythrocytosis: a study of patients with polycythaemia vera and apparent polycythaemia. *Br J Haematol*. 2005;129:701-705.
13. Di Nisio M, Barbui T, Di Gennaro L, et al. The haematocrit and platelet target in polycythemia vera. *Br J Haematol*. 2006;Published article online: 8-Dec-2006 doi: 10.1111/j.1365-2141.2006.06430.x.
14. Finazzi G, Gregg XT, Barbui T, Prchal JT. Idiopathic erythrocytosis and other non-clonal polycythemia. *Best Pract Res Clin Haematol*. 2006;19:471-482.
15. James C, Delhommeau F, Marzac C, et al. Detection of JAK2 V617F as a first intention diagnostic test for erythrocytosis. *Leukemia*. 2006;20:350-353.
16. Patel RK, Lea NC, Heneghan MA, et al. Prevalence of the Activating JAK2 Tyrosine Kinase Mutation V617F in the Budd-Chiari Syndrome. *Gastroenterology*. 2006;130:2031-2038.
17. Thiele J, Kvasnicka HM. A critical reappraisal of the WHO classification of the chronic myeloproliferative disorders. *Leuk Lymphoma*. 2006;47:381-396.
18. Tefferi A, Hanson CA, Inwards DJ. How to interpret and pursue an abnormal complete blood cell count in adults. *Mayo Clin Proc*. 2005;80:923-936.

19. Ruggeri M, Tosetto A, Frezzato M, Rodeghiero F. The rate of progression to polycythemia vera or essential thrombocythemia in patients with erythrocytosis or thrombocytosis. *Ann Intern Med.* 2003;139:470-475.
20. Lengfelder E, Hochhaus A, Kronawitter U, et al. Should a platelet limit of $600 \times 10^9/l$ be used as a diagnostic criterion in essential thrombocythaemia? An analysis of the natural course including early stages. *Br J Haematol.* 1998;100:15-23.
21. Sacchi S, Vinci G, Gugliotta L, et al. Diagnosis of essential thrombocythemia at platelet counts between 400 and $600 \times 10^9/L$. Gruppo Italiano Malattie Mieloproliferative Croniche(GIMMC). *Haematologica.* 2000;85:492-495.
22. Tefferi A, Gilliland DG. The JAK2V617F tyrosine kinase mutation in myeloproliferative disorders: status report and immediate implications for disease classification and diagnosis. *Mayo Clin Proc.* 2005;80:947-958.
23. Thiele J, Kvasnicka HM. Clinicopathological criteria for differential diagnosis of thrombocythemias in various myeloproliferative disorders. *Semin Thromb Hemost.* 2006;32:219-230.
24. Tefferi A, Pardanani A. Mutation screening for JAK2V617F: when to order the test and how to interpret the results. *Leuk Res.* 2006;30:739-744.
25. Thiele J, Kvasnicka HM, Diehl V, Fischer R, Michiels JJ. Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disorders by histopathology, histochemistry and immunostaining from bone marrow biopsies. *Leukemia & Lymphoma.* 1999;33:207-218.
26. Thiele J, Kvasnicka HM. Hematopathologic findings in chronic idiopathic myelofibrosis. *Semin Oncol.* 2005;32:380-394.
27. Bennett JM. The myelodysplastic/myeloproliferative disorders: the interface. *Hematol Oncol Clin North Am.* 2003;17:1095-1100, v.
28. Panani AD. Cytogenetic findings in untreated patients with essential thrombocythemia. *In Vivo.* 2006;20:381-384.
29. Mitelman F, Johansson B, Mertens F, eds. Mitelman database of chromosome aberrations in cancer, 2007, 2/2007 update. <http://cgap.nci.nih.gov/Chromosomes/Mitelman>. 2007; Accessed March 14, 2007.
30. Steensma DP, Tefferi A. Cytogenetic and molecular genetic aspects of essential thrombocythemia. *Acta Haematol.* 2002;108:55-65.
31. Mesa R, Verstovsek S, Cervantes F, et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): Consensus on terminology by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT). *Leukemia Research.* 2007;in press.
32. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood.* 2002;100:2292-2302.
33. Orazi A, O'Malley DP, Jiang J, et al. Acute panmyelosis with myelofibrosis: an entity distinct from acute megakaryoblastic leukemia. *Mod Pathol.* 2005;18:603-614.
34. Lambertenghi-Delilieri G, Orazi A, Luksch R, Annaloro C, Soligo D. Myelodysplastic syndrome with increased marrow fibrosis: a distinct clinico-pathological entity. *Br J Haematol.* 1991;78:161-166.

35. Thiele J, Kvasnicka HM, Vardiman J. Bone marrow histopathology in the diagnosis of chronic myeloproliferative disorders: a forgotten pearl. *Best Pract Res Clin Haematol.* 2006;19:413-437.
36. Steensma DP, Hanson CA, Letendre L, Tefferi A. Myelodysplasia with fibrosis: a distinct entity? *Leuk Res.* 2001;25:829-838.
37. Schmitt-Graeff A, Thiele J, Zuk I, Kvasnicka HM. Essential thrombocythemia with ringed sideroblasts: a heterogeneous spectrum of diseases, but not a distinct entity. *Haematologica.* 2002;87:392-399.
38. Renneville A, Quesnel B, Charpentier A, et al. High occurrence of JAK2 V617 mutation in refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Leukemia.* 2006;20:2067-2070.
39. Wang SA, Hasserjian RP, Loew JM, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis harbors JAK2 mutation and shows overlapping myeloproliferative and myelodysplastic features. *Leukemia.* 2006;20:1641-1644.
40. Szpurka H, Tiu R, Murugesan G, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. *Blood First Edition Paper*, prepublished online June 1, 2006; DOI 10.1182/blood-2006-02-005751. 2006.
41. Remacha AF, Nomdedeu JF, Puget G, et al. Occurrence of the JAK2 V617F mutation in the WHO provisional entity: myelodysplastic/myeloproliferative disease, unclassifiable-refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Haematologica.* 2006;91:719-720.
42. Ceesay MM, Lea NC, Ingram W, et al. The JAK2 V617F mutation is rare in RARS but common in RARS-T. *Leukemia.* 2006;20:2060-2061.
43. Gattermann N, Billiet J, Kronenwett R, et al. High frequency of the JAK2 V617F mutation in patients with thrombocytosis (platelet count >600x10⁹/L) and ringed sideroblasts more than 15% considered as MDS/MPD, unclassifiable. *Blood.* 2007;109:1334-1335.
44. Murphy S, Peterson P, Iland H, Laszlo J. Experience of the Polycythemia Vera Study Group with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment. *Semin Hematol.* 1997;34:29-39.
45. Thiele J, Kvasnicka HM. Chronic myeloproliferative disorders with thrombocythemia: a comparative study of two classification systems (PVSG, WHO) on 839 patients. *Annals of Hematology.* 2003;82:148-152.
46. Kvasnicka HM, Thiele J. The impact of clinicopathological studies on staging and survival in essential thrombocythemia, chronic idiopathic myelofibrosis, and polycythemia rubra vera. *Semin Thromb Hemost.* 2006;32:362-371.
47. Thiele J, Kvasnicka HM, Zerhusen G, et al. Acute panmyelosis with myelofibrosis: a clinicopathological study on 46 patients including histochemistry of bone marrow biopsies and follow-up. *Ann Hematol.* 2004;83:513-521.
48. Soligo D, Delia D, Oriani A, et al. Identification of CD34+ cells in normal and pathological bone marrow biopsies by QBEND10 monoclonal antibody. *Leukemia.* 1991;5:1026-1030.
49. Dingli D, Grand FH, Mahaffey V, et al. Der(6)t(1;6)(q21-23;p21.3): a specific cytogenetic abnormality in myelofibrosis with myeloid metaplasia. *Br J Haematol.* 2005;130:229-232.

50. Tefferi A, Mesa RA, Schroeder G, Hanson CA, Li CY, Dewald GW. Cytogenetic findings and their clinical relevance in myelofibrosis with myeloid metaplasia. *Br J Haematol.* 2001;113:763-771.

51. Thiele J, Kvasnicka HM, Diehl V. Standardization of bone marrow features--does it work in hematopathology for histological discrimination of different disease patterns? *Histol Histopathol.* 2005;20:633-644.