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REVIEW

PVSG and WHO *vs* European Clinical, Molecular and Pathological Criteria for prefibrotic myeloproliferative neoplasms

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Abstract

The Polycythemia Vera Study Group (PVSG), World Health Organization (WHO) and European Clinical, Molecular and Pathological (ECMP) classifications agree upon the diagnostic criteria for polycythemia vera (PV) and advanced primary myelofibrosis (MF). Essential thrombocythemia (ET) according to PVSG and 2007/2008 WHO criteria comprises three variants of JAK2^{V617F} mutated ET when the ECMP criteria are applied. These include normocellular ET, hypercellular ET with features of early PV (prodromal PV), and hypercellular ET due to megakaryocytic, granulocytic myeloproliferation (ET.MGM). Evolution of prodromal PV into overt PV is common. Development of MF is rare in normocellular ET (WHO-ET) but rather common in hypercellular ET.MGM. The JAK2^{V617F} mutation burden in heterozygous mutated normocellular ET and in heterozygous/homozygous or homozygous mutated PV and ET.MGM is of major prognostic significance. JAK2/MPL wild type ET associated with prefibrotic primary megakaryocytic and granulocytic myeloproliferation (PMGM) is characterized by densely clustered immature dysmorphic megakaryocytes with bulky (bulbous) hyperchromatic nuclei, which are never seen in JAK2^{V617F} mutated ET, and PV and also not in MPL^{515} mutated normocellular ET (WHO-ET). JAK2^{V617} mutation burden, spleen size, LDH, circulating CD34^{+} cells, and pre-treatment bone marrow histopathology are mandatory to stage the myeloproliferative neoplasms ET, PV, PMGM for proper prognosis assessment and therapeutic implications. MF itself is not a disease because reticulin fibrosis and reticulin/collagen fibrosis are secondary responses of activated polyclonal fibroblasts to cytokines released from the clonal myeloproliferative granulocytic and megakaryocytic progenitor cells in ET.MGM, PV and PMGM.

Key words: Myeloproliferative neoplasms; Essential thrombocythemia; Prodromal polycythemia vera; Polycythemia vera; Myelofibrosis; JAK2^{V617F} mutation; JAK2 wild type myeloproliferative neoplasm; Bone marrow pathology

Core tip: The integrated World Health Organization (WHO) and European Clinical, Molecular and Pathological classification of the myeloproliferative neoplasms include JAK2^{V617F} mutated normocellular essential thrombocythemia (WHO-ET), prodromal polycythemia vera (PV), classical PV, and hypercellular ET due to megakaryocytic, granulocytic myeloproliferation. Evolution of prodromal PV into overt PV is common. JAK2/MPL wild hypercellular ET associated with prefibrotic primary megakaryocytic

and granulocytic myeloproliferation is characterized by densely clustered immature dysmorphic megakaryocytes with bulky (bulbous) hyperchromatic nuclei, which are never seen in JAK2^{V617F} mutated ET and PV, and also not in JAK2 wild type normocellular ET (WHO-ET) carrying the MPL^{515} mutation.

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INTRODUCTION

Vaquez[1] and Osler[2] first described polycythemia vera (PV) as a distinct disease entity. In 1950, Dameshek[3] described PV as a trilinear myeloproliferation of the bone marrow with various degrees excessive production of red blood cells, granulocytes and platelets. Dameshek[3] proposed two highly speculative possibilities for the etiology of trilinear PV: first, the presence of excessive bone marrow stimulation by an unknown factor, second, a lack or a diminution in the normal inhibitory factor. This hypothesis is confirmed by the discovery of the JAK2^{V617F} mutation by James *et al*[4] in 2005 demonstrating that the JAK2^{V617F} mutation induces a loss of inhibitory activity of the JH2 pseudokinase part on the JH1 kinase part of JAK2, leading to enhanced activity of the normal JH1 kinase activity of JAK2. The JAK2^{V617F} mutation makes the mutated hematopoietic stem cells hypersensitive to hematopoietic growth factors TPO, EPO, IGF1, SCF and GCSF, resulting in trilinear myeloproliferation with clinical manifestations of essential thrombocythemia (ET), PV and myelofibrosis (MF) (Figure 1)[4,5].

The Polycythemia Vera Study Group (PVSG) followed the 1951 recommendations of Dameshek to define PV by increased red cell mass (RCM) as a major criterion and proposed criteria for the clinical diagnosis of Ph-negative ET, PV and agnogenic myeloid metaplasia (AMM) with MF[6-10]. The unifying concept of the lumping of the chronic myeloproliferative disorders (MPD) ET, PV, AMM, chronic myeloid leukemia (CML) by Dameshek in 1951 has been broken up by the PVSG in 1975 into Ph-positive (Ph$^+$) CML and Ph-negative ET, PV and AMM (Figure 1)[6-11]. The Ph-negative MPDs ET, PV and MF form a benign group of chronic MPD, whereas the Ph$^+$ chromosome is the result of the *BRC/ABL* fusion gene and protein. *BCR/ABL*-positive CML appears to be a real neoplasia (leukemia) with an inevitable transition into acute leukemia when strict morphological, biochemical, cytogenetic and molecular criteria are used in routine daily practice[12-14]. The Thrombocythemia Vera Study Group introduced bone marrow biopsy as a specific, pathognomonic clue for early stage ET and PV[15,16]. The PVSG[6-8] and the 2001 World Health Organization (WHO) criteria for the classification of the MPDs are not refined enough to also take the early prefibrotic stages of thrombocythemia in various MPDs into account[15-20]. The availability of the current clinical and molecular markers endogenous erythroid colony (EEC) formation, serum EPO levels, the JAK2^{V617F} mutation and bone marrow histology allow the detection of early stage ET and PV. Within the context of the European Working Group on MPD Michiels *et al*[15-17] contributed significantly to the European consensus criteria for ET, PV and chronic idiopathic MF (CIMF) by including bone marrow histology and subsequently defined between 2002 and 2005 the European Clinical and Pathologic (ECP) criteria (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf)[17-19]. In the present study, we extend the PVSG, the ECP[18-20], and the 2007/2008 WHO[21,22] MPD/myeloproliferative neoplasms (MPN) classifications into simpified and integrated WHO and European Clinical, Molecular and Pathological (WHO-ECMP) criteria by including bone marrow pathology together with a complete set of established laboratory and molecular markers for diagnostic differentiation of each of the latent (masked), early and overt MPNs[19,20].

DIAGNOSIS OF MPN

ET

In the 1980s, Georgii *et al*[23-25] and Thiele *et al*[26-29] defined the pathological features of ET, PV and chronic megakaryocytic granulocytic myeloproliferation (CMGM) or CIMF as derived from bone marrow histopathological morphology (Figure 1)[30]. ET was defined by Georgii *et al*[23-25] and Thiele *et al*[26-29] as persistent increase of platelets in excess of 400 × 10^9/L without the Ph$^+$ chromosome together with monolinear proliferation of mature enlarged megakaryocytes in the bone marrow with normal cellularity, normal erythropoiesis and normal granulopoiesis (Figure 1). This bone marrow definition for the diagnosis of normocellular ET has been used by Michiels *et al*[17,18] in the ECP classification of MPD (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) and by the 2007/2008 WHO classification (WHO-ET)[21,22]. Normocellular ET (WHO-ET) only comprises about one third of PVSG defined ET patients[21,22]. The 1997 PVSG and 2001 WHO classifications used a platelet count in excess of 600 × 10^9/L as the minimum criterion for the diagnosis of ET[7,14] and therefore did not include the early stages of ET, which consequently were diagnosed as masked or unclassifiable MPD[18]. This comprises about 30% of early stage or latent ET (Table 1) indicating the need to lower the platelet count cut-off to 400 × 10^9/L (upper limit of normal) for the diagnosis of thrombocythemia in various MPDs (Table 2)[12-20]. The relatively high incidence of early prefibrotic thrombocythemia with a platelet count between 400 and 600 × 10^9/L strengthen the need for use of specific laboratory and molecular markers to differentiate thrombocythemia from reactive thrombocytosis followed by bone marrow histopathological evaluation in order to correctly diagnose patients with suspected

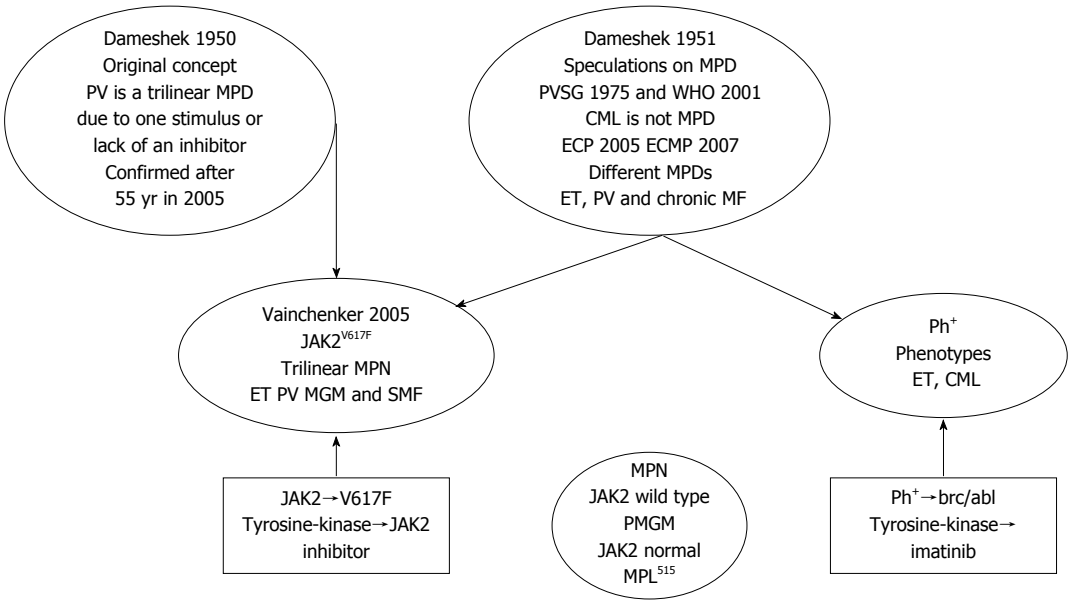

Figure 1 The concept of Dameshek in 1950 on polycythemia vera as a trilinear myeloproliferative disorder due to an unknown excessive bone marrow stimulating factor and/or a lack or a diminution in the normal inhibitory factor, which appeared to be caused by the acquired heterozygous and/or homozygous JAK2^{V617F} mutation discovered by James *et al*[5]. The unifying concept of Dameshek in 1951 on lumping the chronic disorders [myeloproliferative disorder (MPDs)] essential thrombocythemia (ET), polycythemia vera (PV), agnogenic myeloid metaplasia (AMM) has been broken up by the Polycythemia Vera Study Group (PVSG) in 1975 into Ph-positive (Ph$^+$) thrombocythemia and chronic myeloid leukemia (CML) and the Ph-negative MPDs ET, PV and myelofibrosis (MF). In 2005, PV indeed proved to be a JAK2^{V617F} mutated trilinear MPD, whereas ET and PMF are either positive or negative for the JAK2^{V617F} mutation. PMGM: Primary megakaryocytic and granulocytic myeloproliferation; MPN: Myeloproliferative neoplasm; WHO: World Health Organization; ECP: European Clinical and Pathologic; ECMP: European Clinical, Molecular and Pathological; MGM: Megakaryocytic, granulocytic myeloproliferation.

MPN[18-20]. The 2007/2008 WHO classification reduced the platelet count from 600 × 10^9/L to 450 × 10^9/L and added bone marrow features as major criteria for normocellular ET (WHO-ET, but did not define criteria for hypercellular ET, Table 2)[21,22]. Recent studies clearly show that PVSG defined ET according to ECMP criteria include at least three phenotypes of ET at the bone marrow level (Tables 2 and 3, Figure 2)[19,20]. About 50% of PVSG defined ET patients show not only spontaneous EEC but also increased score for leukocyte alkaline phosphatase (LAP) together with low serum EPO levels (Table 2)[31-36] indicating that EEC-positive ET with low serum EPO comprises a biologically distinct subgroup of ET patients reflecting early PV ("forme fruste" PV, Table 2) that is at risk for progression to overt PV (Table 3). Spontaneous EEC formation is the hallmark of PV. In a study of 170 PVSG-defined ET patients, spontaneous EEC formation was seen in all 11 (6.5%), who later developed PV, but also in 60% of 159 patients with stable ET during a median follow-up of 29 mo (12-138 mo)[37]. This overlap of EEC in ET and PV points to the need for specific molecular and pathological markers to better differentiate between normocellular ET and hypercellular ET from prodromal PV and classical PV (Table 2, Figures 2-5)[38,39].

PV

In the 1980s bone marrow, Georgii *et al*[23-25] and Thiele *et al*[26-29] used a typical trilinear hypercellular bone marrow with increased megakaryopoiesis, erythropoiesis and granulopoiesis (panmyelosis) as mandatory criteria for the diagnosis for classic PV (Figure 2). This definition is used by the ECP (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) and the 2007/2008 WHO classification to confirm the diagnosis of PVSG defined PV in cases with increased RCM or increased hemoglobin and hematocrit above the upper level of normal and in cases with JAK2^{V617F} mutated erythrocythemia[15-22]. The PVSG and WHO criteria use increased RCM or persistent high levels for hemoglobin and hematocrit as a major crude inclusion criterion and a histological bone marrow picture characteristic for PV as a minor criterion for the diagnosis of PV, thereby excluding early thrombocythemic stage PV mimicking ET (Table 3, Figure 2). Spontaneous EEC formation and low serum EPO levels are used as specific criteria for the diagnosis of PV, but have insufficient diagnostic sensitivity as isolated parameters to differentiate between PV, congenital polycythemia (CP), secondary erythrocytosis (SE), ET and normal controls[31-34]. RCM measurement is still the WHO gold standard to distinguish ET from PV and to distinguish idiopathic from apparent erythrocytosis. In patients with so-called idiopathic erythrocytosis (increased RCM, hemoglobin and hematocrit but normal leukocyte and platelet counts and no splenomegaly), the histological evaluation of the bone marrow clearly differentiate between erythrocythemic early stage PV showing increased erythropoiesis and loosely clustered large pleomorphic megakaryocytes from CP and SE with increased erythropoiesis and megakaryocytes of normal size and morphology[15-19]. Increased RCM alone does not distinguish early erythrocythemic PV from CP or SE, indicating the need of specific clinical, molecular markers including JAK2^{V617F} and MPL^{515} mutations and bone marrow histology. In patients with JAK2^{V617F} mutated ET,

Table 1 Blood and bone marrow features in one prospective study of Thrombocythemia Vera Study Group-defined essential thrombocythemia and one retrospective study of Polycythemia Vera Study Group defined essential thrombocythemia at platelet counts above the upper limit of normal

Ref.	Michiels *et al*[11]	Lengfelder *et al*[30]	
Type of study	Prospective 1975-1985	Retrospective 1975-1995	
Diagnosis ET	TVSG criteria	PVSG criteria	
Inclusion criterion	ET	ET	Tentative diagnosis
Platelet count × 10^9/L	> 400	> 350	WHO-ECMP
Number of ET patients	30	143	
Platelets × 10^9/L range	420-1500	< 350-> 000	
Below 600	13%	29%	Early latent ET
Between 600-1000	54%	45%	Fits with ET
Above 1000	33%	26%	Fits with ET
Leukocytes			
Above 12 × 10^9/L	10%	51%	
Hemoglobin			
Below 16 g/dL	-	80%	
Below 17 g/dL	-	100%	
Above 16 g/dL	-	20%	Fits with PV
Splenomegaly			
No	63%	56%	
Yes	37%	44%	
Spleen size on echogram (cm)			
n < 12/12-15/> 15	2019/8/3	-	
Bone marrow biopsy			
Normal cellularity	17 (57%)	52%	Fits with true ET
Increased cellularity	13 (43%)	60%	
Increased erythropiesis	13 (43%)	17%	Fits with early PV
Increased granulopiesis	0	45%	Fits with CMGM
Myelofibrosis	No	No	

Essential thrombocythemia (ET) according to Polycythemia Vera Study Group (PVSG) criteria appears to be a spectrum of normocellular ET, prodromal polycythemia vera (PV) and ET due to megakaryocytic, granulocytic myeloproliferation or ET associated with chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM/PMGM) when diagnostic World Health Organization and European Clinical, Molecular and Pathological (WHO-ECMP) bone marrow features are applied. TVSG: Thrombocythemia Vera Study Group.

slight splenomegaly and borderline erythrocytosis RCM is the gold standard to make the distinction between hypercellular ET due to increased erythropoiesis with normal RCM (prodromal PV) from classical PV with increased RCM. In our experience, cases of prodromal PV, masked PV do show a typical PV picture in the bone marrow histopathology (Figure 3). This controversial topic has been addressed in a separate report (manuscript in press). PV patients do have erythrocytes above 6 × 10^{12}/L even when the hemoglobin and hematocrit are in the upper range of normal due to microcytosis of erythrocytes caused by iron deficiency and/or significant splenomegaly[3,39]. Consequently, RCM measurement is of debatable additional diagnostic value in classic PV carrying the JAK2^{V617F} or exon 12 mutation, since all patients with 2008 WHO/ECMP defined PV do have erythrocyte counts above 6 × 10^{12}/L and demonstrate a bone marrow histology that is pathognomonic for PV (Table 3).

Nomenclature, clinical and bone marrow diagnosis of primary MF

The terms AMM and IMF are applied to hypercellular advanced fibrotic stages of MPN[8,23-29]. MF is a reactive feature secondary to progressive disease seen in AMM, CIMF, PV and CML. In 1988, 1996 and 1999 Thiele *et al*[26-29] clearly defined the bone marrow features of normocellular true ET (WHO-ET, Table 2, Figure 2), of hypercellular trilinear PV (Table 3, Figure 2), and prefibrotic CIMF with associated thrombocytosis (Figure 2). According to Thiele *et al*[26-29], "true" ET clearly differs from ET associated with prefibrotic CIMF labeled as "false" ET. In true ET megakaryocytes display large to giant megakaryocytes showing hyperlobulated staghorn-like nuclei in a normocellular bone marrow (WHO-ET, Table 2, Figure 2). Interestingly, the megakaryocytes in true ET are larger than in PV[26]. PV is typically featured by small to large (pleomorphic) megakaryocytes with hyperploid nuclei in a hypercellular bone marrow due to increased erythropoiesis or increased erythro-granulocytic myeloproliferation (WHO-PV, Table 3, Figures 2, 3 and 5). In 1980, Georgii *et al*[23] described CMGM as a distinct MPD entity apart from ET. In 1990, Georgii *et al*[24,25] proposed the Hannover Bone Classification of the myeloproliferative disease and defined CMGM as hypercellular prefibrotic stages preceding AMM or IMF (Figure 2). As prefibrotic CIMF-0 is a contradiction of terms and MF is not idiopathic but secondary seen in various MPDs, Georgii *et al*[24] replaced the term CIMF by CMGM as the third entity of prefibrotic MPD different from ET and PV at the bone marrow pathology level (Hannover Bone Marrow Classification of MPD). The prefibrotic stage of CMGM or CIMF precedes the fibrotic stages of AMM (Figure 2) and initially present as primary megakaryocytic and granulocytic myeloproliferation (PMGM) defined by Michiels and Thiele in Table 4. PMGM is characterized by a specific disturbance of a hypercellular bone marrow with striking abnormalities of megakaryocyte maturation (dysmegakaryopoiesis) (Figures 6-10), which consist of variations in size including giant forms and deviations of the nuclear-cytoplasmic ratio accompanied by bulbous and hyperchromatic cloud-like nuclei, which are not seen in ET and PV. Thiele *et al*[29] in their 1999 Cologne Clinical and Bone Marrow Classification of the MPDs used the term prefibrotic chronic IMF (CIMF-0) for this third CMGM MPD entity. Prefibrotic CIMF/CMGM is typically featured by hypercellular ET associated with megakaryocytic, granulocytic myeloproliferation (MGM) at the bone marrow level with no or slight increase of reticulin fibrosis (RF) in the Gomorri's silver or Gordon Sweet stain of bone marrow biopsy specimen (Table 4, Figure 2). With the advent of the JAK2^{V617F} mutation, Michiels distinguish in this report two variants of MGM (Figure 2): JAK2^{V617F} mutated ET due to MGM (ET.MGM) (Table 2, Figure 4) and JAK2 wild type ET associated with primary MGM (PMGM, Table 4, Figures 6-8 and 10). ET associated with JAK2 wild type PMGM (Table 4, Figure 2) is not preceded by any variant of JAK2 or MPL mutated ET or PV.

Table 2 2008 World Health Organization and European Clinical, Molecular and Pathological criteria for the diagnosis and classification of JAK2^{V617F} mutated essential thrombocythemia into 3 stags or phenotypes: important to differentiate because natural history differs

Clinical and molecular criteria	WHO bone marrow criteria
ET stage 1	Normocellular ET
Platelet count of > 350 × 10^9/L and the presence of large platelets in a blood smear in all stages of ET	Predominant proliferation of enlarged megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis
Presence of JAK2^{V617F} mutation	No progression to post-ET myelofibrosis
ET stage 2	Prodromal PV
Platelet count of ⩾ 350 × 10^9/L and normal hematocrit: male < 51%, female < 48% erythrocytes < 6 × 10^{12}/L	Increased cellularity with trilineage myeloproliferation (*i.e.*, panmyelosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes No pronounced inflammatory reaction (plasmacytosis, cellular debris). Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis
Presence of JAK2^{V617F} mutation	Progression to overt PV during follow-up
Low serum EPO level and/or increased score for leukocyte alkaline phosphatase	
Spontaneous EEC	
ET stage 3	ET.MGM
Platelet count of ⩾ 3500 × 10^9/L and no signs of leuko-erythroblastosis	Increased cellularity due to MGM and normal or relative reduction of erythroid precursors with various degrees pleiomorphic loosely clustered megakaryocytes containing dysmorphic (not cloud-like) nuclei and maturation defects
Erythrocytes < 6 × 10^{12}/L	No or slight RF (RF 0 or 1)
Presence of JAK2^{V617F} mutation	Progression to post ET myelofibrosis
Slight splenomegaly on ultrasound and no anemia Hb > 12 g/dL	
No preceding or allied of CML, PV, RARS-T or MDS	

Masked myeloproliferative neoplasms: normal platelets, leukocytes and hematocrit, but slight splenomegaly on echogram with the presence of JAK2^{V617F} mutation and/or a World Health Organization (WHO) bone marrow is rare (rather frequent in patients with splanchnic vein thrombosis and/or Budd Chiari syndrome). ET: Essential thrombocythemia; PV: Polycythemia vera; ET.MGM: ET due to megakaryocytic, granulocytic myeloproliferation; EEC: Endogenous erythroid colony; CML: Chronic myeloid leukemia; RARS-T: Thrombocythemia associated with refractory anemia with increased ringed sideroblasts; RF: Reticulin fibrosis.

Table 3 The 2008 World Health Organization and European Clinical, Molecular and Pathological criteria for the diagnosis of polycythemia vera and diagnostic differentiation between polycythemia vera and congenital or acquired erythrocytosis

Clinical and molecular criteria	Pathological criteria (WHO)
Major PV criteria	P1. Early PV
A0. Early PV. Hematocrit in the upper limit of normal: Ht: 0.45 to 0.51 in male and 0.43 to 0.48 in female, Erythrocytes < 6 × 10^{12}/L	Increased cellularity of bone marrow predominantly due to increased erythropoiesis and loose clusters of large megakaryocytes with hyperlobulated nuclei. No or slight increase of granulopoiesis and RF
A1. Classical WHO defined PV: Hematocrit > 0.51/> 0.48 in male/female, Erythrocytes > 6 × 10^{12}/L	P2. Overt PV
A2. Presence of JAK2^{V617F} mutation (sensitivity 95%) or exon 12 mutation	Hypercellular (75%-100%) bone marrow due to trilinear increase of erythropoiesis, megakaryopoiesis and granulopoiesis and clustering of small to giant (pleomorph) megakaryocytes with hyperlobulated nuclei. Absence of stainable iron
A3. Low serum EPO level and/or spontaneous endogenous erythroid colony formation	
Minor MPD criteria	P3. Erythrocytosis
B1. Persistent increase of platelet count: grade Ⅰ: 400-1500, grade Ⅱ: > 1500	Selective increase of erythropoiesis, normal granulopoiesis and megakaryocytes of normal size, morphology and no clustering of megakaryocytes in primary or secondary erythrocytosis
B2. Granulocytes > 10 × 10^9/L or Leukocytes > 12 × 10^9/L and/or raised LAP-score or increased PRV-1 expression in the absence of fever or infection	Grading of RF (RF 0, 1, 2, 3)
B3. Splenomegaly on palpation or on ultrasound echogram (> 12 cm length in diameter)	Grading of reticulin and collagen fibrosis; myelofibrosis MF grade 1, 2 and 3

World Health Organization (WHO) and European Clinical, Molecular and Pathological (ECMP) criteria criteria for early and overt polycythemia vera (PV). A0, A2, B1 and P1 establish prodromal PV (ET stage 2) PV ECMP stage 0, or masked PV; A1, A2, P1 and none of B establish so-called idiopathic erythrocytosis or polycythemic PV ECMP stage 1. A1, A2, P2 and one or more of B establish WHO defined classic and advanced PV ECMP stage 2 and 3. A1 and P3 with normal or increased values of serum EPO is consistent with congenital or secondary erythrocytosis. A3 confirms early and overt PV without the need of red cell mass measurement for clinicians who do not have access to a hematopathologist expert in myeloproliferative neoplasms. MPD: Myeloproliferative disorders; LAP: Leukocyte alkaline phosphatase; MF: Myelofibrosis; RF: Reticulin fibrosis.

The diagnosis of 2008 WHO fibrotic primary MF (PMF) (Figure 2) is identical to fibrotic stages of PMGM as based on the presence of at least 2 minor criteria and typical bone marrow features including: (1) dense clusters

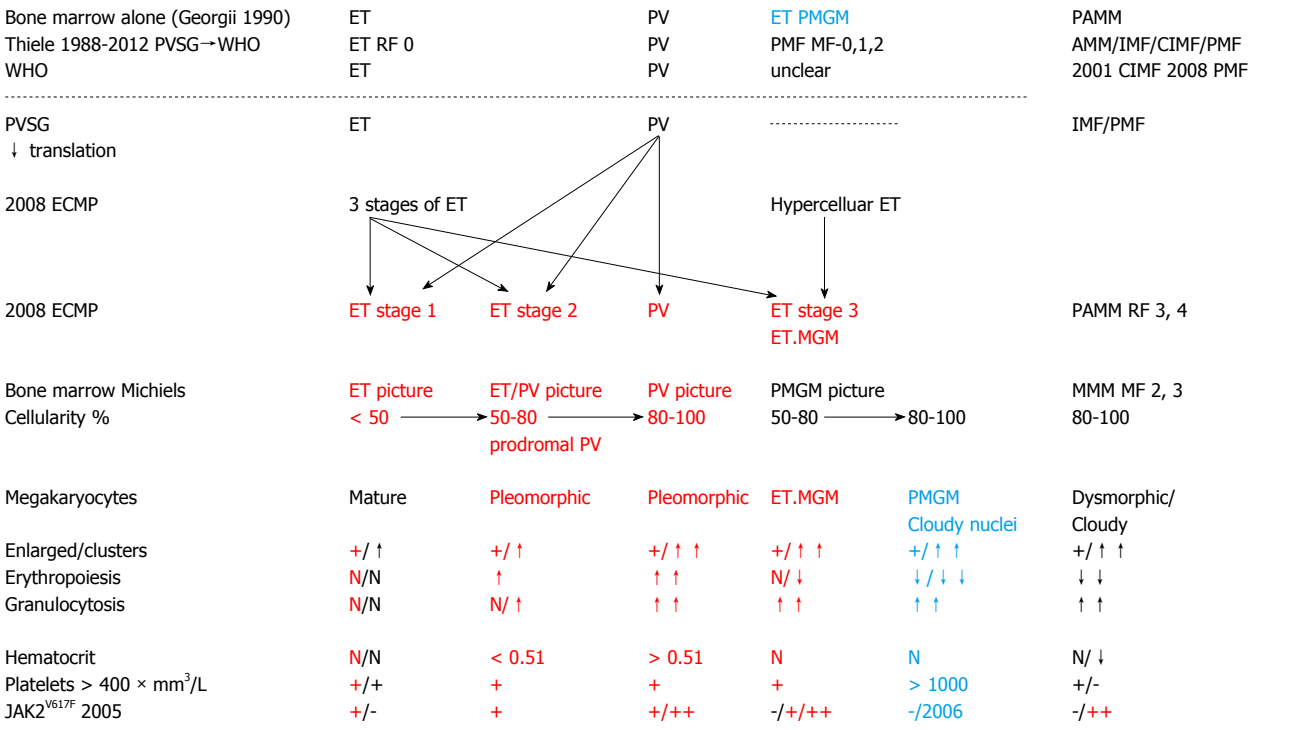

Figure 2 Bone Marrow diagnosis alone: chronic megakaryocytic granulocytic myeloproliferation by Georgii *et al*[24] *vs* chronic idiopathic myelofibrosis by Thiele *et al*[29,75], and comparative World Health Organization and European Clinical, Molecular and Pathological criteria for prefibrotic essential thrombocythemia, polycythemia vera and primary megakaryocytic and granulocytic myeloproliferation or chronic idiopathic myelofibrosis/primary myelofibrosis or agnogenic myeloid metaplasia. Translation of Polycythemia Vera Study Group (PVSG) and 2008 World Health Organization (WHO) defined essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF), chronic megakaryocytic granulocytic myeloproliferation (CMGM) or primary megakaryocytic and granulocytic myeloproliferation (PMGM) according to European Clinical, Molecular and Pathological (ECMP) criteria subdivided in $JAK2^{V617F}$ mutated ET, prodromal PV, overt PV and ET.MGM (red) *vs* prefibrotic PMGM (blue) and 2 types of normocellular ET ($JAK2^{V617F}$ + left red, MPL^{515} + black). PMF: Primary myelofibrosis; AMM: Agnogenic myeloid metaplasia; ET.MGM: ET due to megakaryocytic, granulocytic myeloproliferation; MF: Myelofibrosis.

of large dysmorphic megakaryocytes with immature cloud-like nuclei not seen in ET and PV; (2) increased and normal maturation of granulopoiesis; and (3) various degrees of MF, consistent with fibrotic stage of AMM (Figure 2, clinical stage C2 and C3 in Table 4). Fibrotic stage of PMF is also observed in post-ET MF and post-PV MF (Figure 2).

WHO-ECMP CRITERIA TO DIAGNOSE AND CLASSIFY MPD

The 2008 WHO[21,22] classification of the MPNs ET, PV and PMF is a very important step forward as compared to the PVSG diagnostic MPD criteria for ET, PV and AMM[7-10], but do not meet the needs in daily practice for four reasons[18-20]. First, 2008 WHO criteria for ET only define normocellular ET (WHO-ET), and the diagnosis of ET type 2 with features of early PV (prodromal PV) in blood and bone marrow but normal RCM and erythrocytes ($< 6 \times 10^{12}$/L) is suggested by the 2008 WHO but not clearly defined. Second, the 2008 WHO defined ET include ECMP defined $JAK2^{V617F}$ positive normocellular ET (WHO-ET), prodromal PV and ET.MGM, MPL^{515} positive ET (Figure 2 middle part in red), as well as JAK2 wild type PMGM (Figure 2 right in blue). There is good evidence that $JAK2^{V617F}$ positive hypercellular ET with no leukoerythroblastosis but with increased megakaryocytic granulocytic myeloproliferation (ET.MGM) is featured by reduced erythropoiesis and loose clusters of slight to moderate dysmegakaryopoiesis is rather frequent (ET.MGM, Table 2)[40]. Third, the diagnostic differentiation between $JAK2^{V616F}$ positive ET.MGM without leukoerythroblastosis in Table 2 and JAK2 wild type PMGM without leukoerythroblastosis is clinically relevant and not addressed by the 2008 WHO classification. Fourth, the 2008 WHO classification disregard the importance of increased *vs* normal or decreased erythrocytes, leukocytes, LAP score, platelets and spleen size for diagnosis, classification and staging of thrombocythemia in MPNs of various molecular etiology. Simple tests like blood cell counts including platelets, leukocytes, hematocrit and erythrocytes, spleen size on echogram, EEC, and LAP score are even not taken into account to distinguish the latent (masked), early and overt thrombocythemic and erythrocythemic stages of PV from the overt trilinear polycythemic stage of classic PV. These shortcomings of the 2008 WHO MPN criteria prompted us to propose integrated WHO-ECMP criteria for the diagnosis of ET (Table 2), PV (Table 3) and PMF or PMGM (Table 4).

THREE STAGES OF ET

Sustained increase of platelet counts ($> 350 \times 10^9$/L) associated with slight splenomegaly on echogram (> 12 cm),

Table 4 World Health Organization and European Clinical, Molecular and Pathological criteria for diagnosis and staging of primary megakaryocytic granulocytic myeloproliferation, or primary myelofibrosis

Michiels JJ	Clinical criteria (2005)	Thiele J	pathological criteria (2005/2008)	
A1	Hypercellular JAK2/MPL wild type ET and no preceding or allied other subtype of myeloproliferative neoplasm: JAK2^{V617F} or MPL^{515} normocellular ET, prodromal or classical PV, Ph1+ CML or MDS	B1	PMGM and relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant to medium sized dysmorphic megakaryocytes containing bulky/clumsy (cloud-like) hypolobulated nuclei and definitive maturation defects	
C	Clinical stages	MF	Staging of myelofibrosis	
C1	Early clinical stages			
	Normal hemoglobin or slight anemia, grade Ⅰ: hemoglobin > 12 g/dL	MF 0	Prefibrotic stage PMGM/PMF	RF 0/1
	No, slight or moderate splenomegaly on ultrasound scan or CT	MF 1	Early fibrotic PMGM/PMF	RF 2
	Hypercellular ET, platelets in excess of 400, 600 or even > 1000 × 10^9/L			
	No leuko-erythroblastic blood picture and/or tear drop erythrocytes			
C2	Intermediate clinical stage			
	Anemia grade II: hemoglobin > 10 g/dL	MF 2	Manifest fibrotic PMGM/PMF	RF 3 = RCF
	Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes	MF 3	Advanced fibrotic PMGM/PMF	RF 4 = RCF
	Splenomegaly, increased LDH			
C3	Advanced clinical stage			
	Anemia grade Ⅲ: hemoglobin < 10 g/L	MF 3	Osteosclerosis	
	Splenomegaly and increased, normal or decreased platelet count			
	Thrombocytopenia, leukocytosis,leukopenia, increased circulating CD34+ cells			

ET: Essential thrombocythemia; PMGM: Primary megakaryocytic and granulocytic myeloproliferation; CT: Computed tomography; LDH: Lactodehydrogenase; PV: Polycythemia vera; CML: Chronic myeloid leukemia; PMF: Primary myelofibrosis; MF: Myelofibrosis; RF: Reticulin fibrosis; RCF: Reticulin/collagen fibrosis.

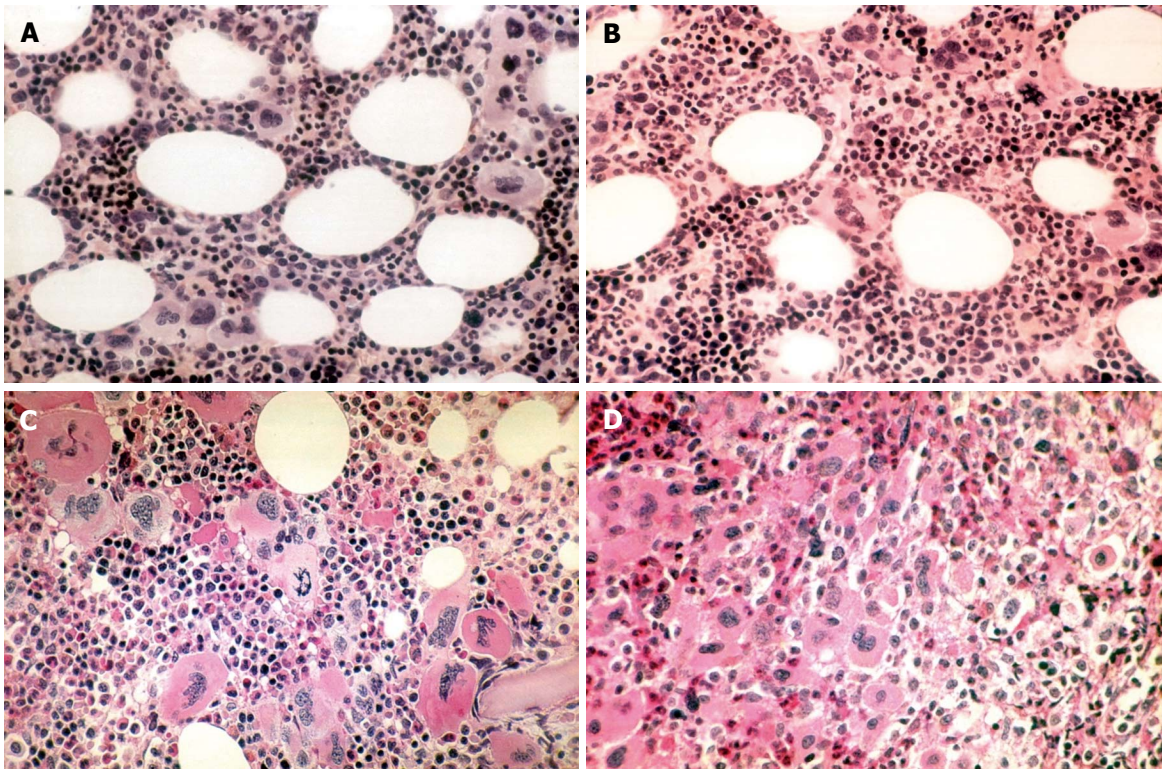

Figure 3 Bone marrow histology features in essential thrombocythemia and polycythemia vera patients. A: Normocellular essential thrombocythemia (ET) bone marrow histology [World Health Organization (WHO)-ET] with increase of clustered pleomorphic megakaryocytes similar as in prodromal and overt polycythemia vera (PV); B: ET/PV bone marrow histology with pleomorphic megakaryocytes and increased cellularity due to increased erythropoiesis as can be seen in WHO and European Clinical, Molecular and Pathological defined prodromal PV and overt PV patients; C: PV bone marrow histology with increased cellularity due to increased erythropoiesis/granulopoiesis and increase of clustered pleomorphic megakaryocytes and no increase of reticulin fibrosis; D: Advanced PV bone marrow histology with dense clustered pleomorphic/dysmorphic megakaryocytes (not cloud-like) and increase in reticulin fibrosis grade 2.

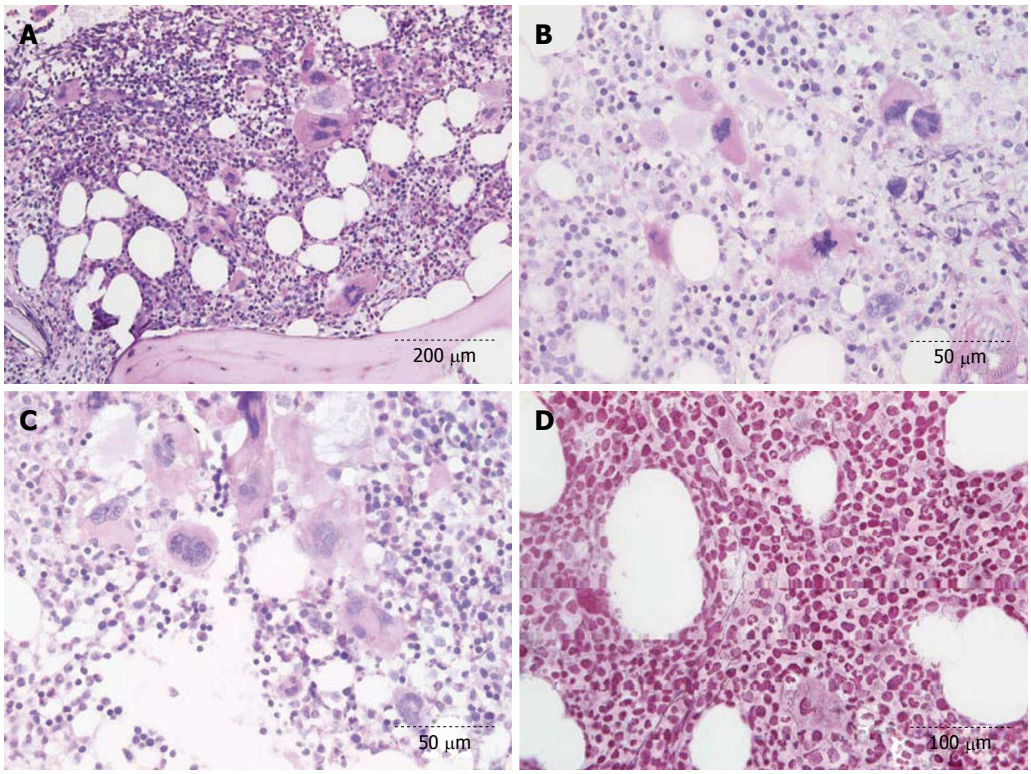

Figure 4 JAK2^{V617F} mutated essential thrombocythemia due to megakaryocytic, granulocytic myeloproliferation with slight splenomegaly (spleen 16 cm on echogram) and a hypercellular megakaryocytic granulocytic bone marrow and clusteried pleomorphic clumpsy megakaryocytes with dysmorphic (not cloud-like) nuclei: prefibrotic essential thrombocythemia due to megakaryocytic, granulocytic myeloproliferation. A-C: JAK2^{V617}-positive essential thrombocythemia due to megakaryocytic, granulocytic myeloproliferation (ET.MGM) featured by hypercellular ET due to increased megakaryocytic granulocytic myeloproliferation and the presence of pleomorphic/dysmorphic megakaryocytes (not cloud-like); D: Reticulin fibrosis grade 1, myelofibrosis grade 0.

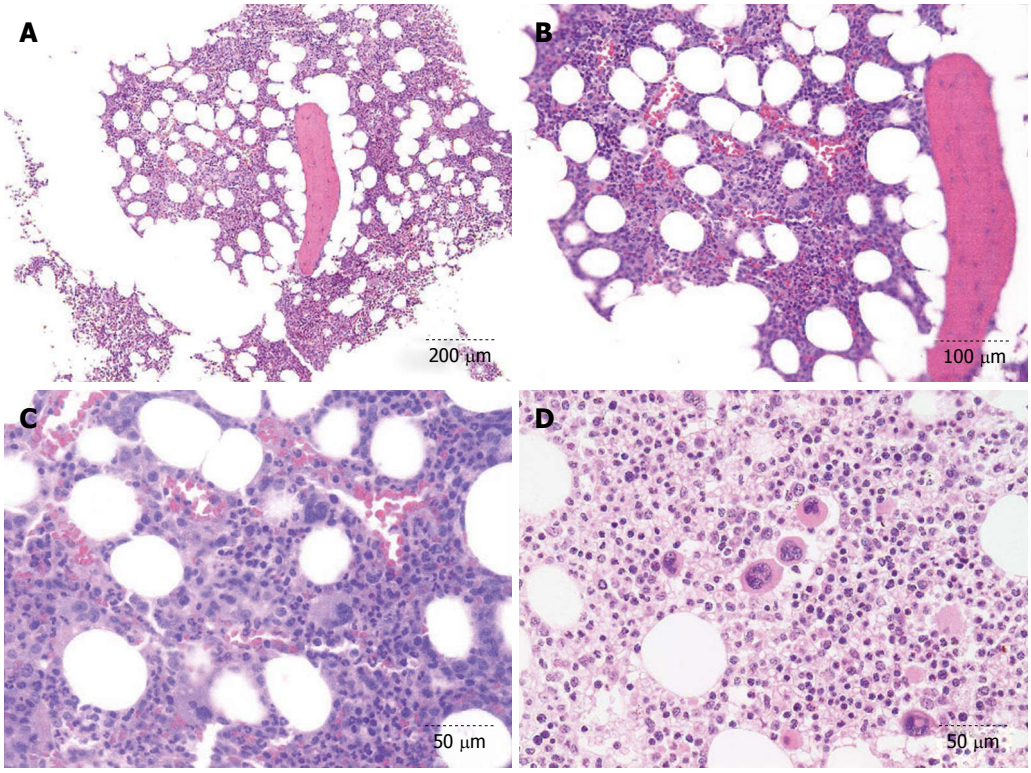

Figure 5 Forty-three-year-old female with positive polycythemia vera (platelets 405 × 10^{9}/L, low serum erythropoietin, leukocyte alkaline phosphatase score 283, hematocrit 0.52, erythrocytes 6.1 × 10^{12}/L, increased red cell mass) with a diagnostic essential thrombocythemia/polycythemia vera bone marrow picture. Such essential thrombocythemia (ET)/polycythemia vera (PV) pictures are regularly seen in prodromal PV and overt PV. A-D: JAK2^{V617F} positive early stage PV with a ET/PV bone marrow histology with loose clusters of pleomorphic megakaryocytes.

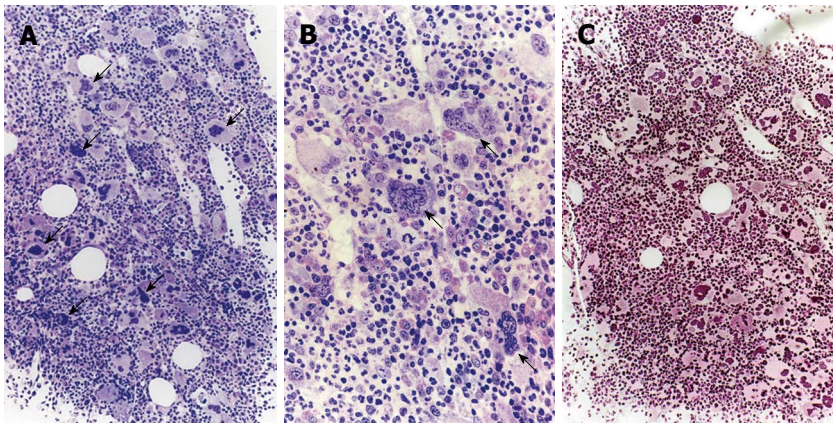

Figure 6 JAK2 wild type hypercellular essential thrombocythemia with platelet counts of 2180 × 10^9/L, no splenomegaly, normal lactodehydrogenase and normal white blood cell differential counts with a characteristics picture of prefibrotic primary dysmegakaryocytic granulocytic myeloproliferation. A, B: JAK2 wild type hypercellular essential thrombocythemia with a typical primary megakaryocytic and granulocytic myeloproliferation bone marrow histology with the presence of abnormal clustering and increase in atypical giant to medium sized dysmorphic megakaryocytes containing bulky, clumsy (cloud-like) hypolobulated nuclei and definitive maturation defects; C: Reticulin fibrosis grade 0. Arrows indicate: Immature dysmorphic megakaryocytes with cloud-like nuclei.

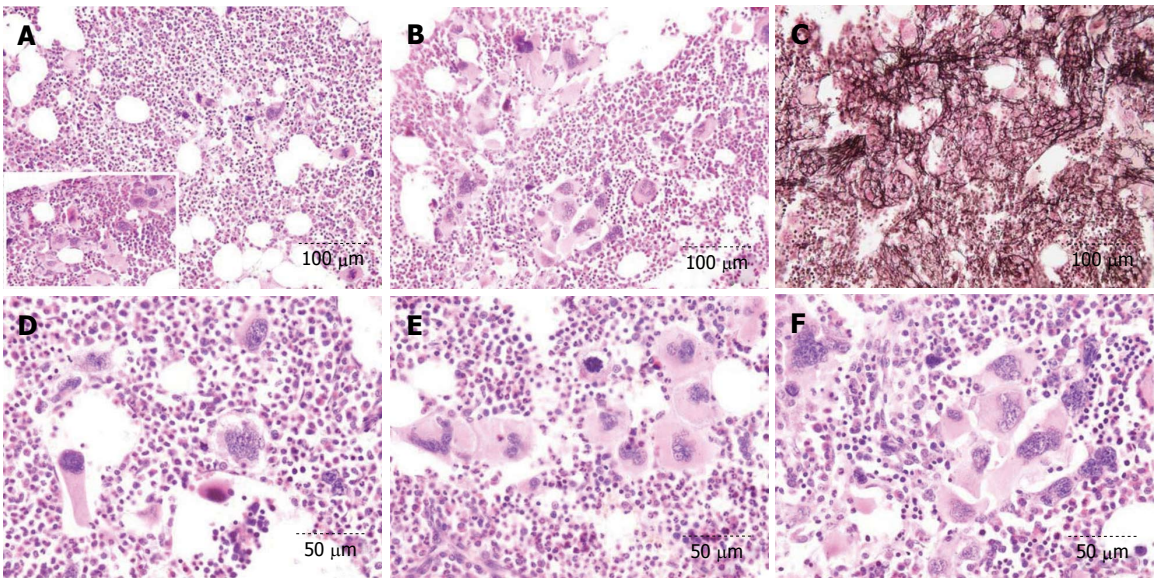

Figure 7 Thirty-seven-years old woman (asymptomatic except fatigue) with JAK2 wild type hypercellular essential thrombocythemia: platelets 1205 × 10^9/L, Hb 12.5 g/dL, erythrocytes 4.9 × 10^{12}/L, leukocytes 18 × 10^9/L, slightly increased lactodehydrogenase, no splenomegaly on palpation as the presenting features of primary megakaryocytic and granulocytic myeloproliferation (Table 5). A, B, D-F: JAK2 wild type hypercellular bone marrow histology due to primary megakaryocytic and granulocytic myeloproliferation with the presence of clustered atypical giant to medium sized dysmorphic megakaryocytes containing bulky (cloud-like) hypolobulated nuclei and definitive maturation defects; C: Reticulin fibrosis grade 2.

normal erythrocytes (< 6 × 10^{12}/L) obviating the need of RCM, normal or increased leukocytes (> 12 × 10^9/L) with normal erythrocyte sedimentation rate (ESR) is suspicious of myeloproliferative ET in the absence of any cause for reactive thrombocytosis. Pre-treatment bone marrow biopsy is needed as the final step in the diagnostic workup to correctly classify the $JAK2^{V617F}$ positive and JAK2 wild type thrombocythemias in various prefibrotic MPDs. The presence of giant platelets in a peripheral blood smear and clustered large or giant mature megakaryocytes in bone marrow smear and biopsy are the pathognomonic clues to the clinical diagnosis of ET. PVSG defined ET without leukoerythroblastosis includes three phenotypes of ET when the WHO-ECMP criteria are applied (Table 2)[18-22]. The WHO-ECMP criteria classify clinical ET as normocellular ET (WHO-ET), prodromal PV mimicking ET, and ET associated with a prefibrotic MGM bone marrow picture without features of leukoerythrocytosis and extramedullary hematopoiesis (ET. MGM) and ET associated with PMGM. The screening for $JAK2^{V617F}$ mutation is very helpful in the diagnostic workup of MPN patients[41-63]. Prodromal PV patients carry the $JAK2^{V617F}$ mutation (Figures 3 and 4). Only half of the patients with PVSG or WHO defined ET carry the $JAK2^{V617F}$ mutation (sensitivity 50% to 60%) and only a very few carry the MPL^{515} mutation[59,60]. A typical MPN bone marrow histology (either ET, PV or PMGM) excludes reactive thrombocytosis, congenital or secondary eryth-

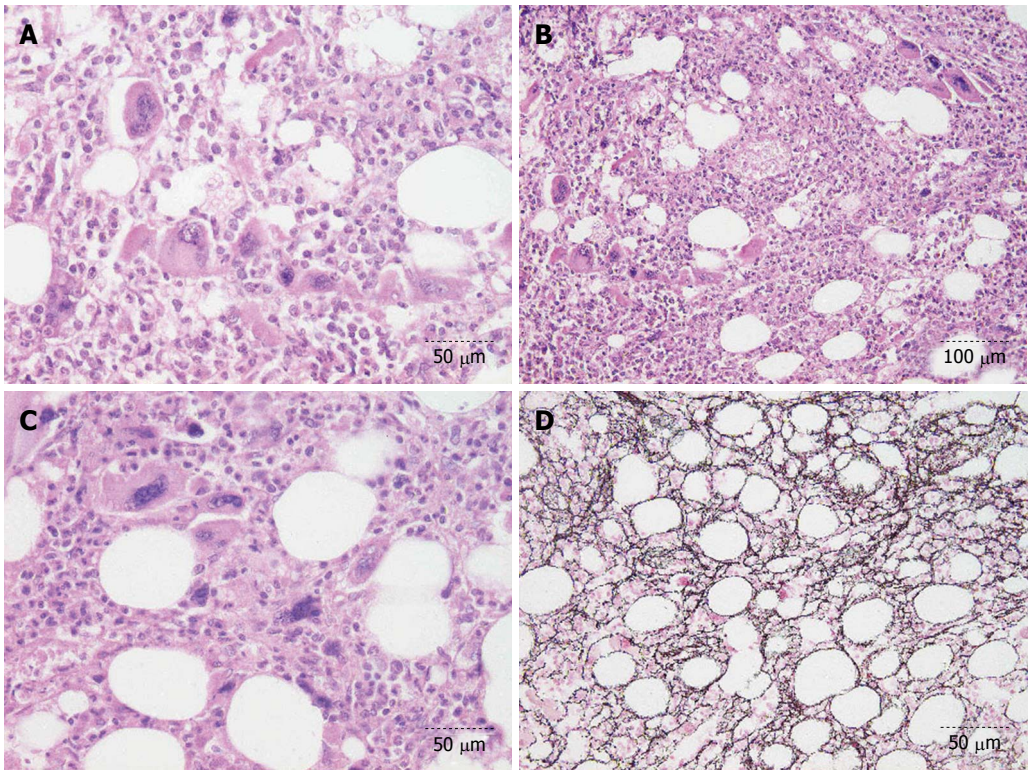

Figure 8 Chronic megakaryocytic granulocytic myelosis according to the Hannover Bone Marrow Classification at time of diagnosis in 1995, and JAK2 wild type primary megakaryocytic granulocytic myeloproliferation according to World Health Organization and European Clinical, Molecular and Pathological criteria in 2006. A-C: Hypercellular bone marrow histology with the presence of Abnormal clustering and increase in atypical giant to medium sized dysmorphic megakaryocytes containing bulky/clumsy (cloud-like) hypolobulated nuclei and definitive maturation defects; D: Reticulin fibrosis grade 2, myelofibrosis grade 1.

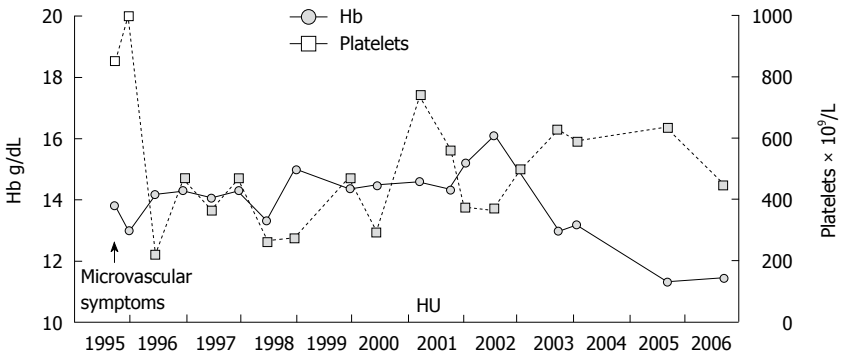

Figure 9 The case of primary megakaryocytic and granulocytic myeloproliferation in Figure 7, who presented in 1995 with microvascular circulation disturbances treated with hydroxyurea for 11 years complicated by mild anemia at platelet counts of 600 × mm^3/L after 10 years of hydroxyurea (HU) for 10 years (1996-2006).

rocytoses, CML, and thrombocythemia associated with refractory anemia with increased ringed sideroblasts[61-63]. WHO-ECMP defined prefibrotic JAK2 wild type PMGM is featured by a hypercellular bone marrow due to pronounced granulopoiesis and dominated by dense clusters of dysmorphic megakaryopoiesis with atypical immature megakaryocytes which are conspicuously enlarged due to increase of nuclear and cellular size with bulky and irregular, round-shaped (cloud-like) nuclei (Table 4, Figures 6-8).

PV *VS* PRIMARY OR SECUNDARY

Characteristic features suspicious for PV include increased hematocrit (> 0.51), increased erythrocytes (> 6×10^{12}/L), slight splenomegaly, increased leukocytes (> 12×10^9/L) or LAP score with normal ESR, an increased platelets (> 400×10^9/L) (Table 3). The detection of JAK2^{V617F} in granulocytes with sensitive polymerase chain reaction (PCR) techniques plays a key-role in the diagnostic work-up of patients with suspected PV (Table 3)[64,65]. In the context of erythrocytosis the presence of the JAK2^{V617F} mutation has a sensitivity of 95% and a positive predictive value of 100% for the diagnosis of PV, and excludes CP and SE without the need of RCM measurement (Figure 2)[18]. In the context of a JAK2^{V617F} positive erythrocythemia (hematocrit > 0.51 in males and > 0.48 in females) the presence of

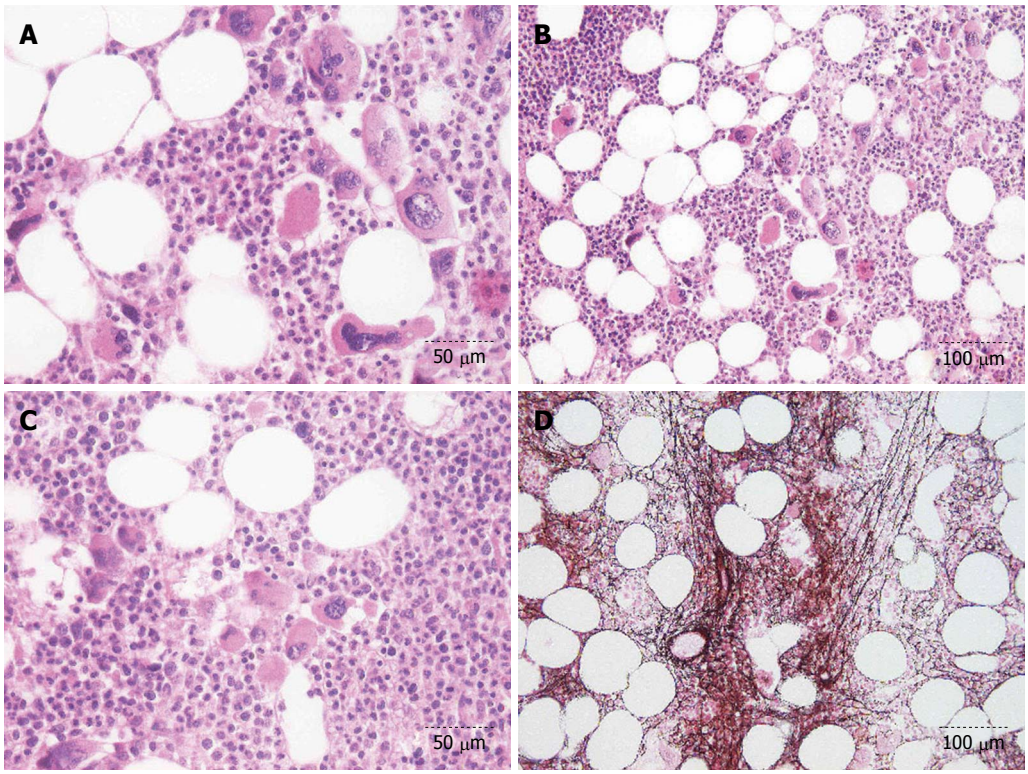

Figure 10 Essential thrombocythemia case diagnosed in 1995 as chronic megakaryocytic granulocytic myelosis, and as JAK2 wild type primary megakaryocytic granulocytic myeloproliferation in 2006 (Figure 8) was complicated by slight anemia and increased bundles of reticulin fibrosis grade 2 after 10 years of hydroxurea treatment (Figure 9). A-C: Bone marrow histology findings in 2006 show thightly clustered immature megakaryocytes with low degree of dysmegakaryopoiesis and cloud-like nuclei. Sometimes the nuclei have an irregular contour and no real hyperchromasia; D: Increase in reticulin fibrosis with many cross-sections grade 2/myelofibrosis grade 1 (Table 5).

large platelets in peripheral blood smear, large megakaryocytes in smears from aspirated bone marrow, low serum EPO, ferritin and slight splenomegaly on echogram are diagnostic for prodromal or overt PV showing a typical ET/PV or PV bone marrow histology picture (Figure 2). As compared to bone marrow histopathology, EEC and serum EPO levels are specific but not sensitive enough to differentiate between myeloproliferative PV, primary erythrocytosis and SE[66]. EEC and serum EPO levels do not differentiate between prodromal PV (normal RCM and normal erythrocyte count) *vs* classic PV (increased erythrocyte count > 6×10^{12}/L and RCM). EEC in the clinical research setting surely will contribute to a better understanding of the role of $JAK2^{V617F}$ in the etiology of heterozygous *vs* homozygous mutated MPN. Pre-treatment bone marrow histology is very insightful and the most powerful tool to stage PV, and to differentiate trilinear hypercellularity in PV from an isolated increase of erythropoiesis in CP and SE with a specificity and sensitivity approaching 100% (Table 3)[67-70]. In SE[69,71] and in CP due to a gain of function mutation in the Epo-receptor, the megakaryocytes are of normal size and morphology and there is no tendency to cluster[72,73]. Differential diagnosis of JAK2 wild type PV, early erythrocythemic PV and idiopathic erythrocytosis (increased RCM and erythrocytes) is problematic and can best be solved by the combined use of bone marrow histology and molecular screening including $JAK2^{V617F}$, JAK2 exon and MPL^{515} mutations obviating the need to measure RCM. About 5% of WHO defined PV patients are $JAK2^{V617F}$ negative and half of them may carry a JAK2 exon 12 mutation[57,58]. Scott *et al*[57] identified JAK2 exon 12 mutations in 10 erythrocytosis patients with increased RCM but negative for the $JAK2^{V617F}$ mutation, which according to PVSG criteria could be diagnosed as PV in 6 and idiopathic erythrocytosis in 4. Pre-treatment bone marrow biopsies in 5 patients carrying one of the JAK2 exon 12 mutations showed characteristic erythroid hyperplasia with slight morphological abnormalities of the megakaryocyte in the study of Scott *et al*[57]. In another report, 5 cases of $JAK2^{V617F}$ negative PV carrying exon 12 mutation (F537-K539delinsl or N542-E534del) were diagnosed as idiopathic erythrocytosis with increased hemoglobin and hematocrit, low serum EPO, normal platelet and leukocyte counts, no or palpable spleen and a typical hypercellular bone histopathology predominantly due to erythroid hyperplasia and clusters enlarged megakaryocytes with hyperploid nuclei was observed in 2 cases[58].

ROLE OF JAK2 AND MPL MUTATIONS IN THE ETIOLOGY AND PROGNOSIS OF MPN

Applying allele-specific PCR analysis in PVSG-defined MPD patients, a high frequency of the $JAK2^{V617F}$ muta-

tion of 95% (92%-97%) is described in PV, and a lower frequency of 53% (49%-57%) in ET and 52% (44%-55%) in MF (post PV, post ET and PMF)[18,74]. Only 3%-4% of ET, 24%-27% of PV and 6%-18% of MF patients are homozygous for the JAK2^{V617F} mutation[18,72]. Within the JAK2^{V617F}-positive MPNs, good evidences accumulate that the majority of PVSG defined ET patients are heterozygous for the JAK2^{V617F} mutation[41,42] and behaves as an indolent slow onset myeloproliferation of mature enlarged megakaryocytes with no progression to homozygosity and MF during long-term follow-up[43-46]. As compared to JAK2^{V617F}-negative ET, the presence of JAK2^{V617F} mutation has been significantly associated with higher hemoglobin level, higher leukocyte counts and less pronounced thrombocytosis[43-48]. Two studies showed that erythroid burst forming unit colonies are already homozygous for the JAK2^{V617F} mutation in PV patients with a heterozygous pattern of JAK2^{V617F} in their peripheral blood granulocytes[41,42]. Homozygous JAK2^{V617F} PV and PMF refers to a more rapid onset and slowly progressive disease in about one third during long-term follow-up[49-52]. The percentage of JAK2^{V617F} mutation and progression from heterozygous to homozygous due to mitotic recombination of chromosome 9p (loss of heterogeneity of chromosome 9p: LOH 9p) is strongly correlated with increased LAP score, with the ability to form spontaneous EEC and with progressive post-PV MF[50,53]. Homozygous JAK2^{V617F} PV and PMF patients belong to an advanced stage of MPN and displayed significantly higher hemoglobin at time of diagnosis, increased incidence of aquagenic pruritus, higher LAP scores in granulocytes, and higher rate of fibrotic transformation[49-52]. Homozygous MPN patients are older, had larger spleen, more frequent leukocytosis, and displayed evolution to secondary MF and a significantly higher risk of cardiovascular events as compared to heterozygous and wild type MPN patients. Vannucchi *et al*[53] employed quantitative assays for JAK2^{V617F} allele levels in granulocytes in a prospective study of 175 PV patients at time of diagnosis. The JAK2 mutant allele burden could be quantified as 1%-25%, 25%-50%, 50%-75% and 75%-100% in 57, 50, 34 and 32 PV patients respectively at time of investigation. The burden of JAK2^{V617F} allele was directly correlated with abnormally increased levels of hematocrit, white cell and neutrophil count, LDH and LAP score, spleen size on echogram and with decreased values for serum ferritin, and erythropoietin[53]. The JAK2^{V617F} allele burden nicely correlated with a progressively higher relative risk for aquagenic pruritus, spleen size on echogram, total thrombosis and the need for receiving myelosuppressive therapy[53]. Mechanisms other than the mutated JAK2^{V617F} in exon 14 is observed in a proportion of PV and MF patients displaying a gain of chromosome 9p, mostly due to trisomy 9[54-56]. Campbell *et al*[56] reported that the JAK2^{V617F} mutation was found in all 10 MPN patients with trisomy 9, and in 28 of 29 MPN patients (PV, ET or CMF) with a 20q deletion. The finding of the JAK2 exon 12 mutations in patients with PV or idiopathic erythrocytosis, but not in ET further confirms the strong association between the JAK2 mutations and MPN and clearly demonstrates the pivotal role of JAK2 mutations as pathogenic events in variable phenotypes of MPN[57,58]. MPL^{W515L} and MPL^{W515K} mutations has been found in some ET and MF patients indicating the importance of the MPL signalling pathway in the etiology of clonal MPN[59,60].

DIAGNOSTIC DIFFERENTIATION AND NATURAL HISTORY OF JAK2V617F MUTATED ET, PV AND PMF

We propose to extend the PVSG and WHO criteria into a broader set of integrated WHO-ECMP criteria not only for the diagnosis and classification but also for staging of MPN burden at the peripheral blood, spleen and blood and bone marrow level. Upon application of the integrated set of WHO-ECMP criteria, the JAK2^{V617F} mutated ET comprises three phenotypic manifestations of ET including normocellular ET (WHO-ET) (Figure 3), ET 2 with early features of PV (prodromal PV, Figure 3) and ET.MGM with loose or dense clusters of pleomorphic to dysmorphic megakaryopoiesis in a hypercellular bone marrow due to increased granulopoiesis (Figure 4).

The bone marrow histology of JAK2^{V617F} mutated ET.MGM show different grades of granulocytic hypercellularity, which can appear to overlap with PV (hematocrit < 0.51) presenting with a hypercellular bone marrow with more or less pronounced increase of granulopoiesis. The histology of ET.MGM bone marrow showing slightly dysmorphic megakaryopoiesis and may overlap with that of ET cases with mild hyperplasia of granulopoiesis and/or a mixture of mainly mild dysmorphic megakaryocytes (Table 3). Therefore, we may predict a significant overlap (grey zones of about 20%-30%) between ET.MGM and PV with increased granulopoiesis due to an inter-observer disagreement among hematopathologists.

The prognostic importance of the WHO bone marrow features and grading of MF (Table 5) has demonstrated by Kvasnicka and Thiele in a large retrospective study of 865 PVSG defined normocellular ET patients with a platelet count in excess of 600×10^9 mm^3/L[75,76]. In this study, Kvasnicka and Thiele reclassified PVSG defined ET as normocellular true ET (WHO-ET) in 167, and prefibrotic CIMF in 174 and early fibrotic CIMF-1 in 135 according to WHO bone marrow criteria[75]. WHO-ET patients showed no significant loss of life expectancy compared to significant loss of life expectancy in CIMF 0 and CIMF 1 ($P = 0.0001$). The 15 years relative survival was 84% for WHO-ET compared to 68% for CIMF 0 and 55% for CIMF 1. Interestingly, WHO-ET patients were 10 to 12 year younger compared to CIMF 0 and CIMF 1. A similar, large retrospective study has been performed by Barbui *et al*[77]. A total of 1104 PVSG defined ET patients (platelet count > 600×10^9/L) from seven centers in Italy and the United States diagnosed between 1975 and 2008 were analyzed retrospectively using the 2008 WHO clinical and

Table 5 Grading of reticulin fibrosis and myelofibrosis

Grading[78,79]	Grading of MF[80]	Description of RF and RCF in MF as a secondary event in MPN
Normal RF-0	MF 0	No reticulin fibers, occasional individual fibers or focal areas with tiny amount of reticulin fiber network
RF 1 Slight increase	MF 0	Fine reticulin fiber network throughout much of section and no course reticukin fibers
RF 2 Moderate increase	MF 1	Diffuse fine reticuline network with focal collections of thick course reticulin fibers and no collagenisation
RF 3 = RCF Marked increase	MF 2	Diffuse and dense increase in reticulin with extensive intersections, and presence of collagen fibers and no or minor osteosclerosis
RF 4 = RCF and O OS Dry tap	MF 3 Sclerotic	Diffuse and dense reticulin with with coarse bundles of collagen associated with significant O

MF: Myelofibrosis; RF: Reticulin fibrosis; RCF: Reticulin/collagen fibers; MPN: Myeloproliferative neoplasms; O: Osteosclerosis.

bone marrow criteria. Bone marrow biopsies were evaluated by one pathologist (Dr. Thiele). In this cohort of 1104 PVSG defined ET patients, 891 (81%) were diagnosed as normocellular WHO-ET (JAK2^{V617F} positive 61%); and 180 (16%) as hypercellular ET with prefibrotic PMF (pPMF) bone marrow histology (JAK2^{V617F} positive 58%). The overall survival curves show the expected differences in overall survival between WHO-ET and pPMF similar as shown by Kvasnicka *et al*[76]. When compared to the 2008 Eurostat age- and sex-standardized incidences for all causes of death, there was no or minimal loss of life expectancy in WHO-ET MPN patients. The difference of 15 years overall survival in WHO-ET *vs* pPMF (80% and 59%, respectively) was mainly due to the 15 years leukemia-free survival incidence in WHO-ET and pPMF (0.8% *vs* 12.3%, respectively). There were significant differences in leukocyte counts (8.6×10^9/L *vs* 9.7×10^9/L), LDH (298 mU/mL *vs* 429 mU/mL) and reticulin grade grade 1 (3% *vs* 24%) at time of diagnosis of WHO-ET and pPMF 0/1 respectively. Evolution of pPMF 0/1 into fibrotic PMF 2/3 (PAMM) after 10 and 15 years increased from 0.8% to 9.3% in WHO-ET and from 9.3% to 16.9% in pPMF 0/1. Unfavorable prognostic factors in pPMF include LDH above the upper limit of normal, increase of CD34+ circulating cells, spleen size growth of 0.5 to 1 cm/year, slight anemia hemoglobin < 13 g/dL, and constitutional symptoms. In this study 60% of WHO-ET and pPMF patients carried the JAK2^{V617F} mutation. Evolution of WHO-defined ET into myeloid metaplasia of the spleen with MF and leukoerythroblastosis is very rare and predicted to be rather frequent in JAK2^{V617F}-positive hypercellular ET.MGM or JAK2 wild type PMGM. Large scale collaborative prospective management study of newly diagnosed MPN patients comparing the various degrees of JAK2^{V617F} and MPL^{515} mutation load and JAK2 wild type PMGM are needed.

GRADING OF SECONDARY MF IN MPN

The terms RF and reticulin/collagen fibrosis (RCF) are well established in the literature[24,78-81]. Sequential biopsies indicate that initially there is a diffuse or patchy increase in fine RF fibers admixed with abundant hematopoietic elements in PV and PMGM during long-term follow-up. In sequential bone marrow biopsies, the marrow in fibrotic PV and PMF is replaced by course collagen fibers with a decreasing number of hematopoietic cells. This progression from RF to RCF in the bone marrow biopsy during long-term follow-up may be analogous to the similar wound healing, in which collagen composition changes as time passes (fine reticulin type Ⅲ collagen is replaced by course collagen type I collagen). The precise mechanisms by which cytokines from abnormal neoproliferative hematopoietic clone in the various MPNs do stimulate the host's polyclonal fibroblasts to produce excessive amounts of fine RF fibers and course RCF bundles are out of scope in this review.

Two kinds of fiber qualities can easily be distinguishes by common staining in light microscopy: RF[78,79] and RCF[24,80]. Gomorri's silver staining detects early and course RF and do not stain collagen fibers thereby underestimating advanced RCF MF grade 2 and 3. Collagen fibers stain with Mason's trichrome stains, and are negative in the Gomorri's silver stain. Consequently both Gomorri's stain for RF and trichrome stain for CF are to be used for optimal MF-grading of RF and RCF[78,79] and for grading of MF[80] (Table 5). The evolution of RF into RCF as documented by the combined use of silver and trichrome stains simple means a determinative change from reversible normal reticulin (= RF) into progressed pathological collagen scarring (RCF without or with osteosclerois). Clinically, RCF often results in cytopenia and dry tap, when aspiration is attempted. RF with very early RCF usually do occur without real scarring. Bone marrow aspiration in RF without collagen fibrosis (MF-1) usually does not cause the symptom of dry tap. Advanced MF (RCF = MF 2 and 3[80]) designates pronounced increased collagen fibrosis visible scarring spotted areas and sometimes with foci or larger areas of atrophic hematopiesis in the bone marrow in light microscopy.

MF itself is not a disease because RF and RCF is a secondary event induced by polyclonal fibroblasts in response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in both PV and PMF. The presence of RF is well documented in ET, PV, PT, PMGM, CML and in many other conditions. Various degrees of RF is rather rare in normocellular ET (WHO-ET) and does occur in about one third of PV and in

Table 6 World Health Organization and European Clinical, Molecular and Pathological staging of prodromal, classical and advanced polycythemia vera related to therapy

PV, ECMP stage	0		1	2	3	4	5
Michiels ECMP Clinical diagnosis	Erythrocythemic PV	Prodromal PV mimicking ET	Polycythemic PV prefibrotic	Classic PV prefibrotic	Advanced PV PMF stage	Post-PV MF Spent phase PV	Leukemic evolution MDS AL
LAP-score	N/↑	↑	↑	↑	↑/↑↑	Variable	Variable
Red cell mass	↑	N	↑	↑	↑	Variable	N/↓
Serum EPO	N/↓	N/↓	↓	↓	↓	Variable	N/↓
Leukocytes × 10^9/L	< 12	< 12	< 12	N-> 12	> 15	> 20	> 20
Platelets × 10^9/L	< 400	> 400	< 400	> 400	< or > 1000	Variable	Variable
Hemoglobin g/dL (mmol/L)	> 16 (10)	< 16 (10)	> 16 (10)	> 16 (10)	> 16 (10)	N/> 12	< 12
Hematocrit	> 0.51	< 0.51	> 0.51	> 0.51	> 0.51	Variable	N↓
Erythrocytes × 10^{12}/L	> 6	< 6	> 6	> 6	> 6	Variable	N/↓
ECMP bone marrow	Early PV	Pro-PV	Early PV	Trilinear PV	Trilinear PV	Myelofibrosis	AML
Bone marrow cellularity (%)	50-80	50-80	60-90	80-100	80-100	Decreased	Increased
Grading myelofibrosis[57]	RF 0-1	RF 0-1	RF 0-1	RF 0/1, MF 0	RCF 2/3 MF 1/2	RCF 3/4 MF 2/3	No MF
Splenomegaly on palpation	No	No/+	No/+	+	++/+++	/Large	Large
Spleen size, echogram cm	< 12	< 12-15	12-15	12-18	18-> 20	> 20	> 20
Spontaneous EEC+	+	+	+	+	+	+	No
JAK2^{V617F} in granulocytes	+	+	+	+/++	+/++	++	No or +
BFU-e (exon 12)	+(++)	+(++)	+(++)	++	++	++	No
Therapeutic implications	Low risk	Low risk	Low risk	Intermediate risk PV	High risk PV	Post-PV MF Spent phase	Acute leukemia
First line treatment option[82,83]	Aspirin phlebotomy	Aspirin phlebotomy low dose IFN?	Phlebotomy Aspirin Low dose IFN → Complete response	Phlebotomy[1] Aspirin IFN→ if resistant →HU	If IFN resistant→ HU or HU first line	JAK2 inhibitor →Bone marrow transplantation Aspirin?	Chemotherapy Bone marrow transplantation? Supportive
Asp/Phleb[82,83]							
IFN[84-86]							
MPN reductive treatment							
Hydroxyurea[83]							
JAK2 inhibitor[87-90]							

[1] ↑: Increased; ↓: Decreased; N: Normal; +: Present or heterozygous; ++: Homozygous. ET: Essential thrombocythemia; PV: Polycythemia vera; MPN: Myeloproliferative neoplasms; MF: Myelofibrosis; AMM: Agnogenic myeloid metaplasia; IFN: Interferon; EEC: Endogenous erythroid colony; ECMP: European Clinical, Molecular and Pathological; LAP: Leukocyte alkaline phosphatase; RCF: Reticulin/collagen fibrosis; RF: Reticulin fibrosis.

the majority of patients with PMGM during long-term follow-up[24,25]. The easiest way in grading of RF using the reticulin silver stain has been performed by the PVSG and in the recent UK study[78,79]. A scoring system of MF based on morphometric analysis (point intersection with an ocular grid) and quality of fibers (reticulin and collagen fibers) and the bone marrow fiber density (fine or course reticulin and some or course bundles of collagen) has been proposed by Thiele *et al*[80] 2005 (Table 5).

CONCLUSION

The underreported early stages of MPN are currently detected by the combined use of clinical, molecular and pathological markers as recommended by integrated WHO-ECMP MPN criteria for the classification and staging of ET, PV and PMGM (Tables 3-6). A wide diffusion and implementation of WHO-ECMP criteria are awaited to clarify their value in recognizing the prefibrotic stages of MPN and in predicting significant differences in long-term prognosis between JAK2^{V617F} normocellular WHO-ET and ET.MGM *vs* JAK2 wild type hypercellular ET associated with PMGM. The urgent need of prospective evaluation of integrated WHO-ECMP criteria include complete blood cell counts (erythrocytes, leukocytes, platelets, LAP score), spleen size on echogram, JAK2 mutation screening, JAK2 mutation load, serum EPO followed by bone marrow biopsy (Tables 2-4 and 6, Figure 2). This integrated approach by clinicians, scientists, molecular biologists and pathologists thereby creates the great advantage to detect all early thrombocythemic and erythrocythemic stages of ET and PV several to more than 10 years earlier. The proposed WHO-ECMP classification and staging of patients with MPN will be very helpful in predicting the natural history of JAK2^{V617F} mutated ET, PV and ET.MGM patients (Table 6), *vs* MPL^{515} mutated ET, *vs* JAK2 wild type PMGM. The WHO-ECMP criteria surely will have important implications in choosing proper treatment options for the management and prevention of thrombotic and bleeding complications and serious complications of progressive MPN disease burden in prodromal PV and classical PV (Table 6)[81-90]. A primary rigid venesection regimen according to Dameshek[3] aiming at a hematocrit below 0.45 in males and below 0.42 in females according on top of low dose aspirin will reduce the cumulative incidence of minor and major thrombosis from above 50% to less than 2% per patient/year during long-term follow-up[16,81,82]. According to current insights, interferon is the treatment of choice in intermediate stage PV patients[82-86]. High risk PV in terms of high JAK2^{V617F} allele burden, progressive MPN disease, splenomegaly and constitutional symptoms are candidates for myelosuppressive (hydroxyurea) or myeloreductive (JAK2 inhibitor) treatment[87-90].

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