



The European Clinical, Molecular, and Pathological (ECMP) Criteria and the 2007/2008 Revisions of the World Health Organization for the Diagnosis, Classification, and Staging of Prefibrotic Myeloproliferative Neoplasms Carrying the JAK2^{V617F} Mutation

JAK2^{V617F} Mutasyonu Bulunan Prefibrotik Myeloproliferatif Neoplazmların Tanı, Sınıflandırma ve Evrelendirmesinde Dünya Sağlık Örgütü Kriterlerinin 2007/2008 Gözden Geçirilmesi ve Avrupa Klinik, Moleküler ve Patolojik (ECMP) Kriterleri

Jan Jacques Michiels^{1,2}, Fibo Ten Kate³, King H. Lam³, Wilfried Schroyens¹, Zwi Berneman¹, Hendrik De Raeve⁴

¹Antwerp University Hospital, Department of Hematology, Antwerp, Belgium

²Goodheart Institute, European Working Group on Myeloproliferative Neoplasms (EWG-MPN), Rotterdam, Netherlands

³Erasmus University Medical Center, Department of Pathology, Rotterdam, Netherlands

⁴OLV Hospital Aalst and University Hospital, Departments of Pathology, Brussels, Belgium

Abstract:

Objective: The prefibrotic stages of JAK2^{V617F} essential thrombocythemia (ET) and JAK2^{V617F} polycythemia vera (PV) can easily be diagnosed clinically without use of bone marrow biopsy histology. We assessed the 2008 WHO and European Clinical, Molecular, and Pathological (ECMP) criteria for the diagnosis of myeloproliferative neoplasms (MPNs).

Materials and Methods: Studied patients included 6 JAK2^{V617F}-mutated ET and 4 PV patients during long-term follow-up in view of critical analysis of the literature. The bone marrow biopsy histology diagnosis without use of clinical data was PV in 7 (of which 3 were cases of ET with features of early prodromal PV) and classical PV in 4.

Results: The ECMP criteria distinguish 3 sequential phenotypes (1, 2, or 3) of JAK2^{V617F}-mutated ET: normocellular ET-1; ET-2, with clinical and bone marrow features of PV (prodromal PV), and ET-3, with hypercellular dysmorphic megakaryocytic and granulocytic myeloproliferation (ET.MGM). The 3 patients with ET-2 or prodromal PV developed slow-onset PV after

Address for Correspondence: Jan Jacques MICHIELS, M.D.,
Antwerp University Hospital, Department of Hematology, Antwerp, Belgium
E-mail: goodheartcenter@upcmail.nl

Received/Geliş tarihi : April 12, 2013

Accepted/Kabul tarihi : May 27, 2013

a follow-up of about 10 years. Bone marrow biopsy histology differentiates MPNs of various molecular etiologies from all variants of primary or secondary erythrocytoses and thrombocytoses with sensitivity and specificity of near 100%.

Conclusion: Normocellular ET (WHO-ET), prodromal PV, and classical PV show overlapping bone marrow biopsy histology features with similar pleomorphic clustered megakaryocytes in the prefibrotic stages of JAK2^{V617F} mutated MPN. Erythrocytes are below $6 \times 10^{12}/L$ in normocellular ET and prodromal PV, and are consistently above $6 \times 10^{12}/L$ in classical PV and at the time of transition from prodromal PV into classical PV. Red cell count at a cut-off level of $6 \times 10^{12}/L$ separates ET from PV and obviates the need for red cell mass measurement when bone marrow histology and JAK2^{V617F} mutation screening are included in the diagnostic work-up of MPNs.

Key Words: Myeloproliferative disorders, Myeloproliferative neoplasm, Essential thrombocythemia, Polycythemia vera, Primary myelofibrosis, JAK2^{V617F} mutation, Bone marrow histopathology, Red cell mass, Erythrocyte count

Özet:

Amaç: JAK2^{V617F} esansiyel trombositemi (ET) ve JAK2^{V617F} polisitemia veranın (PV) prefibrotik evrelerinin tanısı, kemik iliği biyopsi histolojisine gerek kalmadan kolaylıkla klinik olarak konulabilir. Biz, myeloproliferatif neoplazmlarının (MPNs) tanısı için 2008 Dünya Sağlık Örgütü (DSÖ) ve Avrupa Klinik, Moleküler ve Patolojik (ECMP) kriterlerini değerlendirdik.

Gereç ve Yöntemler: Çalışmaya, literatür değerlendirmesi gözönünde bulundurularak uzun süreli gözlemlenilen 6 JAK2^{V617F} mutasyon pozitif ET ve 4 PV hastası dahil edildi. Dört klasik PV ve 7 PV olgusuna klinik veriler kullanılmadan, kemik iliği biyopsi histolojisine dayanarak tanı konuldu (bunların 3'ü erken prodromal PV özellikleri taşıyan ET idi).

Bulgular: ECMP kriterleri JAK2^{V617F} mutasyonu olan ET'yi 3 ardışık fenotipe ayırmaktadır. Normosellüler ET-1; PV'nin klinik ve kemik iliği özelliklerini taşıyan ET-2 (prodromal PV) ve hipersellüler dismorfik megakaryositik ve granülositik myeloproliferasyon ile birlikte olan, ET-3 (ET.MGM). ET-2 ya da prodromal PV'li 3 hasta, yaklaşık 10 yıllık bir izlemin ardından yavaş başlangıçlı PV'ye dönüşmüşlerdir. Kemik iliği biyopsi histolojisi, çok çeşitli moleküler etiyolojik etkenlere sahip olan myeloproliferatif neoplazmları neredeyse %100'e varan bir duyarlılık ve özgüllükle, birincil ve ikincil trombositoz ve eritrositozun hemen her tipinden ayırt etmektedir.

Sonuç: Normosellüler ET (DSÖ-ET), prodromal PV ve klasik PV'nin üçü de prefibrotik evrede benzer pleomorfik megakaryosit kümeleşmesi özelliği barındıran birbirleriyle örtüşen kemik iliği biyopsi histolojisine sahiptirler. Eritrosit sayısı, normosellüler ET ve prodromal PV'de $6 \times 10^{12}/L$ 'nin altındayken, klasik PV'de ve prodromal PV'den klasik PV'ye dönüşümde ise kalıcı olarak $6 \times 10^{12}/L$ 'nin üstünde seyretmektedir. $6 \times 10^{12}/L$ düzeyindeki kırmızı hücre cut-off değeri ET'yi PV'den ayırmakta ve ek olarak kemik iliği histolojisi ve JAK2^{V617F} mutasyon taramasının uygulanan prosedürler içinde olmasıyla myeloproliferatif neoplazmların tanısında kırmızı hücre kitle tayini ihtiyacını da ortadan kaldırmaktadır.

Anahtar Sözcükler: Myeloproliferatif hastalıklar, Myeloproliferatif neoplasm, Esansiyel trombositemi, Polisitemia vera, Primer myelofibrozis, JAK2^{V617F} mutasyonu, Kemik iliği histolojisi, Kırmızı kan hücresi kitlesi, Kırmızı kan hücresi sayısı

Introduction

Focusing on the elucidation of platelet-mediated erythromelalgia in essential thrombocythemia (ET) and polycythemia vera (PV) [1,2,3] and on the association of migraine-like microvascular cerebral transient ischemic attacks (MIAs) as specific presenting symptoms of thrombocythemia in ET [4], we were able to document the prefibrotic stages of ET and PV by the combined use of clinical, laboratory, and bone marrow histopathology features for each of the primary myeloproliferative neoplasms (MPNs). Since 1975 we have diagnosed and classified ET and PV patients according to the Rotterdam Clinical and Pathological (RCP [5,6]), European Clinical and Pathological (ECP; http://www.mpn-stichting.nl/doctors_brochure_2004.pdf [7,8,9]), and European Clinical, Molecular, and Pathological (ECMP [10,11]) criteria for prefibrotic ET and PV and primary

chronic megakaryocytic granulocytic myeloproliferation (CMGM). The ECMP criteria for ET, PV, and CMGM or chronic idiopathic myelofibrosis were published in June 2007 [9,10,11] and preceded the World Health Organization (WHO) revised diagnostic criteria for PV, ET, and primary fibrosis (PMF) in August 2007 [12]. The RCP, ECP, and ECMP criteria included a minimum platelet count of 350 to $400 \times 10^9/L$ for the diagnosis of ET and the presence of enlarged megakaryocytes in bone marrow biopsy as the pathognomonic clue to prefibrotic ET and PV. According to the ECP and ECMP criteria, the criteria for ET defined by the Polycythemia Vera Study Group (PVSG) overlooked about 30% of masked ET with thrombocythemia at platelet counts below $600 \times 10^9/L$ [13] until the official introduction of the 2008 WHO classification of MPN using a minimum count of $450 \times 10^9/L$ for the diagnosis of ET [14,15]. In this report, we present 10 cases of MPNs with the JAK2^{V617F} mutation

diagnosed without the use of bone marrow histopathology in 6 ET and 4 PV patients. Based on bone marrow biopsy histology alone by 3 expert pathologists, 3 ET patients showed a normocellular ET bone marrow picture and 3 ET and 4 PV patients showed a hypercellular PV picture. In the present study we applied the ECP and ECMP [9] criteria for diagnosis, classification, and staging of JAK2^{V617F}-mutated MPN (Tables 1, 2, and 3). This study reveals the existence of 3 sequential phenotypes of JAK2^{V617F}-mutated ET patients clearly in between normocellular ET (WHO-ET) and post-ET myelofibrosis. As compared to the 2008 WHO classification, the 2008 ECMP classification encompasses a wider scale of specific clinical, laboratory, and bone marrow biopsy histology features of JAK2^{V617F}-positive ET and PV, thereby making possible a proper diagnosis, staging, and timely initiation of appropriate first-, second-, or third-line treatment options.

Materials and Methods

Ten patients who presented with aspirin-responsive migraine-like transient ischemic attack as the clue to prefibrotic stage ET or PV according to WHO, ECP, and ECMP criteria were selected (Tables 1 and 2) from 22 MPN patients with various fibrotic degrees of MPN that were referred from European countries to JJM at the Antwerp University Hospital between January 2001 and August 2007 for expert evaluation and treatment recommendation. The ET and PV patients were subsequently followed up by their local hematologists until 2013.

Diagnostic evaluation included a complete clinical history, physical examination, retrospective clinical and laboratory data collection from hospital records, actual blood cell counts, white blood differential count, leukocyte alkaline phosphatase (LAP: normal value <100) score, peripheral blood morphology for the presence of large platelets, tear drop red cells, erythroblasts, screening for the JAK2^{V617F} mutation in peripheral blood using polymerase chain reaction testing according to Baxter et al. [16], bone marrow aspirate for morphology, serum erythropoietin (EPO) levels, spontaneous endogenous erythroid colony (EEC+) formation, red cell mass (RCM) measurement, and spleen size on echogram [6,7,8,9]. The presence of a Philadelphia chromosome or BCR-ABL fusion protein was excluded in all patients.

Bone marrow biopsies from the iliac crest were stained with hematoxylin and eosin (H&E) for histopathology evaluation. All bone marrow biopsies were evaluated for morphology, grading of cellularity, and scoring of reticulin fibers according to PVSG recommendations according to Ellis et al. [17] (Table 3). In addition to silver impregnation for the detection of reticulin fibers, the collagen staining of Mason was used for objective detection of collagenization of reticulin fibers and grading of myelofibrosis according to Thiele et al. [18] to clearly distinguish among prefibrotic, early fibrotic, and advanced fibrotic stages of ET and PV

[17,18] (Table 3). Peripheral blood and bone marrow biopsy histology data were evaluated according to the 2008 WHO classification and the ECMP criteria for ET and PV (Tables 1 and 2).

Results

Thrombocytopenia-Specific Symptoms in JAK2^{V617F}-Positive ET and PV

The age of patients at the time of the first MPN-specific symptoms and increased platelet count ranged from 23 to 56 years for patients with clinical presentation of ET (n=6) and from 37 to 49 years for PV (n=4). The MIAs in 6 ET patients ranged from attacks of transient blindness, diplopia, scotomas, and migraine-like attacks followed by throbbing headaches, nausea, vomiting, or even seizures. The time lapse between the first symptoms of MIAs and the delay in the diagnosis of ET in 5 patients ranged from 4 to 12 years (Table 4). Six ET patients had normal erythrocyte counts below $6 \times 10^{12}/L$ and normal values for red cell mass (Table 4). Four PV patients with MIAs (Table 4, cases 7, 8, 9, and 10) had a documented short history of MIAs of about 1 year and presented with increased red cell mass and increased values for red blood cell counts in excess of $6 \times 10^{12}/L$, consistent with acute-onset PV.

Diagnosis of JAK2^{V617F}-Positive ET and PV without Bone Marrow Histology

At the time of presentation, 1 PV (case 7) and all ET patients revealed a heterozygous JAK2^{V617F} mutation of less than 40%, but the percentage of mutated alleles increased from 25% to 65% after 5 years in PV case 8 and from 38% to 71% in PV case 9. PV case 10 presented with 75% JAK2^{V617F} allele mutation burden. The diagnoses based on the clinical, laboratory, and molecular criteria without the use of bone marrow histology were ET in 6 and PV in 4 patients (Table 4A). All patients except 1 (PV case 8, platelets $397 \times 10^9/L$) presented with elevated platelet counts at the time of initial MIAs ranging from 405 to $924 \times 10^9/L$. The erythrocyte counts at time of diagnosis were below $6.0 \times 10^{12}/L$ in 6 ET and above $6.0 \times 10^{12}/L$ in 4 PV patients. The erythrocyte counts rose from below to above $6 \times 10^9/L$ in 3 ET patients at the time of evolution into classical PV (Table 4A, Figure 1). The hemoglobin and hematocrit levels remain low in PV in remission by phlebotomy alone for periods of months and years and parallel each other (Figure 1). The red blood cell counts remained above 6.0 to $7.5 \times 10^{12}/L$ in treated PV patients by phlebotomy alone (Table 4A, Figure 1), indicating that correction of hemoglobin and hematocrit is related to reduction of mean corpuscular volume to less than 70 to $65 \mu m^3$ due to iron deficiency.

Diagnosis of ET and PV by Bone Marrow Histology Alone

The bone marrow histology findings are summarized in Table 4B. All MPN patients carrying the JAK2^{V617F} mutation showed an increase of pleomorphic enlarged

Table 1. Diagnosis and classification of prefibrotic JAK2^{V617F}-positive ET and prodromal PV according to the European Clinical, Molecular, and Pathological (ECMP) criteria to distinguish 3 phenotypes of ET and subsequent early prefibrotic and fibrotic stages between ET and post-ET myelofibrosis.

Clinical and molecular criteria	Pathological criteria
Platelet count of $\geq 350 \times 10^9/L$ Normal hematocrit (males < 0.51 , females < 0.48) and normal erythrocytes ($< 6 \times 10^{12}/L$) are mandatory Presence of JAK2 ^{V617F} mutation Increased LAP score Low serum EPO level Spontaneous EEC No or minor splenomegaly on echogram No preceding or allied CML, PV, RARS-T, or MDS ET: presence of JAK2^{V617F} mutation and one or more of the above Clinical ET stage: Borderline or mild anemia Borderline or increased LDH Slight to moderate splenomegaly Hetero-/homozygous JAK2 ^{V617F} mutation Post-ET myelofibrosis Splenomegaly Anemia, increased CD34+ cells	Normocellular ET (WHO-ET) Predominant proliferation of enlarged mature megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. No increase of cellularity or immaturity of erythropoiesis. ET with features of PV: Prodromal PV Increased cellularity (60%-80%) with increased erythropoiesis or trilineage myeloproliferation (75%-100%). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes with hyperlobulated nuclei. ET.MGM Increased cellularity due to chronic megakaryocytic and granulocytic myeloproliferation (MGM) and normal or reduced erythroid precursors. Loose to dense clustering of more pleomorphic megakaryocytes with hyperloid or clumpy nuclei (not or some cloud-like). Grading of reticulin fibrosis (RF), reticulin collagen fibrosis (RCF, PVSG [17]), and myelofibrosis (MF, Thiele et al. [18]): Prefibrotic: RF-0/1 = MF-0 Early fibrotic: RF-2 = MF-1 Fibrotic: RCF-3 = MF-2 Sclerotic: RF-4 = MF-3

Masked ET or PV: In the setting of splanchnic vein thrombosis (SVT, Budd-Chiari syndrome or portal vein thrombosis) in 241 patients, platelet counts were between 238 and $456 \times 10^9/L$ (mean: 333) in 74 patients carrying the JAK2^{V617F} mutation and between 104 and $258 \times 10^9/L$ (mean: 159) in 147 JAK2 wild-type SVT patients [42]. Applying WHO bone marrow features in 539 PVSG-defined ET patients, the relative frequency of ET stage 1 versus ET stages 3, 4, and 5 was 1.1:1.5:2.2:0.7 [33].

megakaryocytes. Bone marrow cellularity ranged from 60% to 90% with increased erythropoiesis in 9 patients and increased myelopoiesis in 1 ET and 3 PV patients. Two patients fulfilled the histological WHO bone marrow features for ET (cases 1 and 2, Table 4, Figure 2). Seven patients (3 ET and 4 PV, Table 4B) fulfilled the histological WHO bone marrow features for PV (Figures 3, 4, 6, 7, and 8). There was no (MF-0, n=5) or slight (MF-1, n=2) increase of reticulin fibers (Table 4B).

Based solely on the bone marrow histology as judged by 3 expert hematopathologists, diagnosis was consistent with ET in 2 cases, PV in 7/10 cases, and ET with hypercellular dysmorphic megakaryocytic and granulocytic myeloproliferation (ET.MGM [5]) or prefibrotic PMF-0 [7,8] in 1 case (Table 4B). The morphology of clustered large pleomorphic megakaryocytes was diagnostic for ET and PV in all 10 MPN patients as

compared to controls, reactive thrombocytosis, or reactive or congenital erythrocytosis (Figure 7C). The bone marrow was normocellular or slightly hypercellular ($\leq 65\%$) in 2 ET cases, hypercellular ($\geq 75\%$) due to increased erythropoiesis in 2 ET cases (Figure 3), hypercellular due to increased erythropoiesis and granulopoiesis (trilinear) in 1 ET and 2 PV cases (Figure 4), and with increased granulopoiesis with relatively reduced erythropoiesis in 1 ET case (Figure 5). The bone marrow was hypercellular ($\geq 75\%$) in 4 PV patients due to increased erythropoiesis in 2 (Figures 6 and 7) and increased erythropoiesis and granulopoiesis (trilinear PV) in 2 (Figure 8).

2008 WHO and ECMP Diagnoses of JAK2^{V617F}-Mutated MPN

The initial diagnoses were ET in 5, MPN unclassifiable in 1, and PV in 4 patients according to 2008 WHO criteria [14,15] (Table 4B). Diagnoses in the 6 JAK2^{V617F}-mutated

Table 2. European Clinical, Molecular, and Pathological (ECMP) criteria for the diagnosis of PV and diagnostic differentiation between PV and congenital or acquired erythrocytosis.

Clinical and molecular criteria	Pathological criteria (WHO)
<p>Major</p> <p>A0. Prodromal PV: Hematocrit upper limit normal, 0.45 to 0.51 in males and 0.43 to 0.48 in females; erythrocytes <6x10¹²/L.</p> <p>A1. Classical PV: Hematocrit >0.51/>0.48 in male/females, erythrocytes >6x10¹²/L.</p> <p>A2. Presence of JAK2^{V617F} or JAK2 exon 12 mutation, mandatory.</p> <p>A3. Low serum EPO level.</p> <p>Minor</p> <p>B1. Persistent increase of platelet count: grade I, 400-1500; grade II, >1500.</p> <p>B2. Granulocytes >10 x10⁹/L or leukocytes >12 x10⁹/L and/or raised LAP score in the absence of fever or infection.</p> <p>B3. Splenomegaly on palpation or on ultrasound echogram (>12 cm length in diameter).</p> <p>B4. Spontaneous EEC formation (optional).</p>	<p>P1. Bone marrow pathology: increased cellularity (60%-100%) due to trilinear increase of erythropoiesis, megakaryopoiesis and granulopoiesis, and clustering of small to giant (pleomorphic) megakaryocytes with hyperlobulated nuclei.</p> <p>Absence of stainable iron. No pronounced inflammatory reaction (plasmacytosis, cellular debris).</p> <p>P2. Selective increase of normoblastic erythropoiesis, normal granulopoiesis and megakaryocytes of normal size and morphology, and no clustering in primary/secondary erythrocytosis.</p> <p>P3. Grading of reticulin fibrosis (RF), reticulin collagen fibrosis (RCF [17]) and myelofibrosis (MF [18]):</p> <p>Prefibrotic: RF-0/1 = MF-0</p> <p>Early fibrotic: RF-2 = MF-1</p> <p>Fibrotic: RCF-3 = MF-2</p> <p>Post-PV MF: RF-4 = MF-3</p>

2008 WHO bone marrow and ECMP criteria for early and overt PV: A0, A2, B1, and P1 establish early PV (mimicking ET), PV ECP stage 0, or masked PV. A1, A2, P1, and none of B establish polycythemic PV ECP stage 1. A1, A2, P1, and one or more of B establish classic and advanced PV ECP stages 2 and 3. A1 and P2 with normal or increased values of serum EPO is consistent with erythrocytosis. B3 and P1 is consistent with latent (masked) primary myeloproliferative disease (PMD). A3 confirms PV. B4 is an important research option.

Table 3. Grading of bone marrow cellularity according to Ellis et al., PVSG, 1975 [17].

Grading reticulin fibrosis (RF=+) [17]	Grading myelofibrosis (MF), Thiele et al. [18]	Description of reticulin fibers (RFs) [17] and reticulin/collagen fibers (RCFs) [7] in MF [18] as a secondary event in myeloproliferative neoplasms (MPNs)
Normal RF-0	N MF-0	No RFs, occasional individual fibers or focal areas with tiny amount of reticulin fiber network
Slight increase RF-1	+ MF-0	Fine RF network throughout much of the section and no course RFs
Moderate increase RF-2	+ + MF-1	Diffuse fine reticulin network with focal collections of thick coarse RFs and no collagenization
Marked increase, BM dry tap RF-3 = RCF	+++ MF-2	Diffuse and dense increase in reticulin with extensive intersections and presence of collagen fibers and no or minor osteosclerosis
OS dry tap RF-4 = RCF&O	Sclerotic MF-3	Diffuse and dense reticulin with coarse bundles of collagen associated with significant osteosclerosis (O)

N: normal cellularity (<50%-60%).

+: increased cellularity as judged by decrease in fat cells (cellularity: 60%-80%).

++: hypercellular with absence of fat cells (80%-100%).

Grading of reticulin and collagen fibrosis by Ellis et al. (PVSG, 1975) [17] and Thiele et al., 2005 [18].

Table 4A. Clinical and laboratory data, bone pathohistology, and diagnosis of ET or PV.

4A: Case	1	2	3	4	5	6	7	8	9	10
A. Clinical data										
Age (years), F/M	56/M	60/M	66/F	37/F	31/F	40/F	43/F	50/M	47/F	38/M
Platelets, x10 ⁹ /L	575	814	544	553	576	425	405	397	924	384
Duration of MIAs	2 years	11 years	8 years	<1 year	8 years	14 years	<1 year	1 year	<1 year	1 year
JAK2 ^{V617F} *	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/+	+/+
Serum EPO	Normal	Zero	Decreased	Decreased	Decreased	nt.	Decreased	Zero	Zero	Zero
Leukocytes, x10 ⁹ /L	6.7	5.3	12.9	8.2	6.2	6.1	14.3	7.3	13.1	8.0
LAP score	-	160	197	N	186	N	263	163	232	284
Hemoglobin, g/dL	13.6	15.5	14.2	14.4	140	13.4	18.31	18.6	16.3	12.8
Hematocrit	0.40	0.45	0.44	0.44	0.41	0.40	0.52	0.63	0.53	0.60
Erythrocytes, x10 ¹² /L	4.5	5.3	4.7	4.8	4.9	4.6	6.1.	6.3	7.4	6.7
EEC	+	+	+	+	+	nt.	+	+	+	+
Red cell mass	N	N	N	N	nt.	N	↑	↑	↑	↑
Spleen, cm	-	13	16	13	11.8	16.5	13	13.7	14.3	16
Clinical Diagnosis	ET	ET	ET	ET	ET	ET	PV	PV	PV	PV

JAK2^{V617F} * : +/- is heterozygous, +/+ is homozygous.

PV patients with documented increased RCM had erythrocytes above 6.0x10¹²/L at diagnosis and at time of transition of prodromal PV into classic PV.

Table 4B. Bone marrow (BM) histology features and diagnosis according to 2008 WHO and ECMP criteria in 10 patients with JAK2^{V617F}-positive MPN at diagnosis and during long-term follow-up.

4B: Case	1	2	3	4	5	6	7	8	9	10
B. BM Histology	-	Figure 2	Figure 4	-	Figure 3	Figure 5	Figure 6	Figure 7	Figure 8	-
Cellularity	65%	60%	90%	75%	75%	80%	75%	80%	80%	80%
M:E ratio	1	1	1	0.5	0.7	4	1	0.7	1.5	-
Megakaryocytes	MPN	MPN	MPN	MPN	MPN	MPN	MPN	MPN	MPN	MPN
Myeloid lineage	N	N	Increased	N	N	Increased	N	N	Increased	Increased
Erythroid lineage	Increased	Increased	Increased	Increased	Increased	N	Increased	Increased	Increased	Increased
Iron	Stainable	Stainable	-	US	US	US	US	US	US	US
Fibrosis	MF-0	MF-1	MF-0	MF-0	MF-0	MF-0	MF-0	MF-0	MF-1	MF-0
C. MPN Diagnosis										
WHO 2008	ET	ET	ET	ET	ET	MPNuc	PV	PV	PV	PV
ECMP 2008	ET	ET	Pro-PV→	Pro-PV→	Pro-PV→	ET.MGM	PV	PV	PV	PV
Follow-up years →PV	-	-	10 years PV	7 years PV	11 years PV	pPMF	-	-	-	-

M:E = myeloid erythroid ratio, US = unstainable, uc = unclassifiable.

ET cases according to ECMP [10,11] criteria (Tables 1 and 2) included normocellular ET (WHO-ET) in 2 (Figure 2) and prodromal PV (Figure 3) in 3. Prodromal PV is characterized by PV bone marrow picture, low serum EPO, presence of EEC, normal values for hemoglobin and hematocrit, erythrocyte count of <6.0x10¹²/L, and normal RCM (Table 4A). The diagnosis in 1 ET patient was prefibrotic ET (case 6) with hypercellular megakaryocytic-

granulocytic (MGM [5]) bone marrow with normal (relatively reduced) erythropoiesis, no reticulins (Figure 5), and moderate splenomegaly (spleen size on echogram of 16 cm). ET.MGM [5] is consistent with prefibrotic primary myelofibrosis pPMF-0 according to the 2004 ECP criteria (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) [7,8,9].

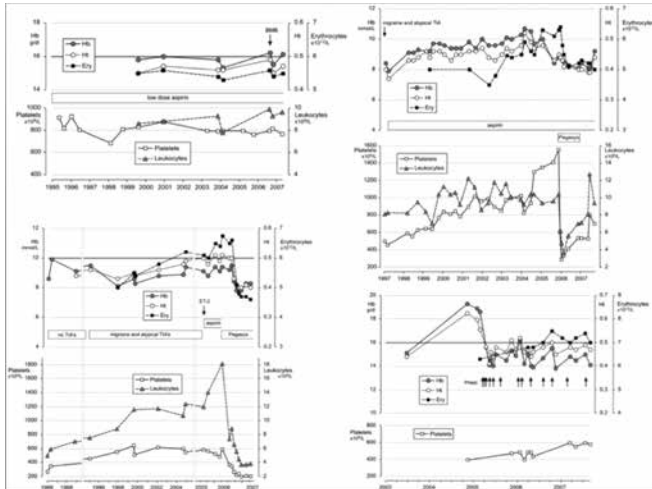


Figure 1. Upper left: ET case 2 (Table 4) with a typical ET bone marrow picture (Figure 2); the patient remained asymptomatic for a documented period of 8 years at increased platelet counts (730 to 915x10⁹/L) while treated with low-dose aspirin (80 mg).

Lower left: ET case 3 (Table 4); the patient had increased platelet counts between 500 and 600x10⁹/L and atypical migraine-like cerebral ischemic manifestations that were overlooked until 2005. In 2005, ET with a 5-year history of fatigue and aquagenic pruritus was diagnosed and PV with erythrocyte count above 6x10¹²/L developed in 2006. Low-dose PEGASYS (45 µg/week for 6 months followed by 30 µg/week) induced a maintained complete hematologic remission of the PV with relief of pruritus, obviating the need for aspirin and phlebotomy during 6 years of follow-up (2006-2012).

Upper right: ET case 3 was diagnosed in 1997, but bone marrow biopsy was hypercellular, consistent with PV (Figure 4). In 2004, PV with erythrocyte counts above 6x10¹²/L developed. Low-dose PEGASYS (45 µg/week for 6 months followed by 45 µg once per 3 to 4 weeks) induced a maintained complete hematologic remission, obviating the need for phlebotomy (2006-2013).

Lower right: Case 8 (Table 4); the patient presented with rapid-onset JAK2^{V617F}-positive erythrocythemic PV with a typical ET/PV bone marrow picture (Figure 7) in 2005 carrying the homozygous JAK2^{V617F} mutation on repeated testing. The erythrocyte counts were far above 6x10¹²/L and remained above 6x10¹²/L at the time of hematological remission of PV by phlebotomy alone on top of low-dose aspirin.

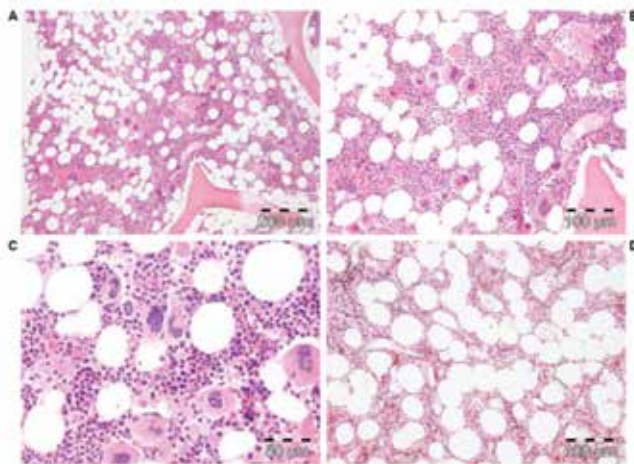


Figure 2. ET of stage 1 showing a typical ET bone marrow picture with increased cellularity (60%) due to slight increase of erythropoiesis in case 2 with a 10- to 15-year history of stable ET disease up to 2013 (case 2, Table 4, Figure 1) (A, B, and C: H&E stain; D: silver stain for reticulin).

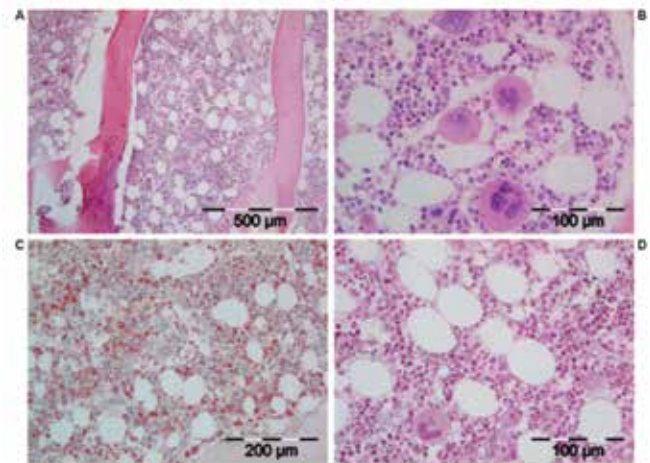


Figure 3. ET stage 2, case 4 (Table 4), with prodromal PV with increased cellularity of the bone marrow due to increased erythropoiesis (A and B: H&E stain; C: Lederle stain for granulo- and reticulin; D: silver stain for reticulin).

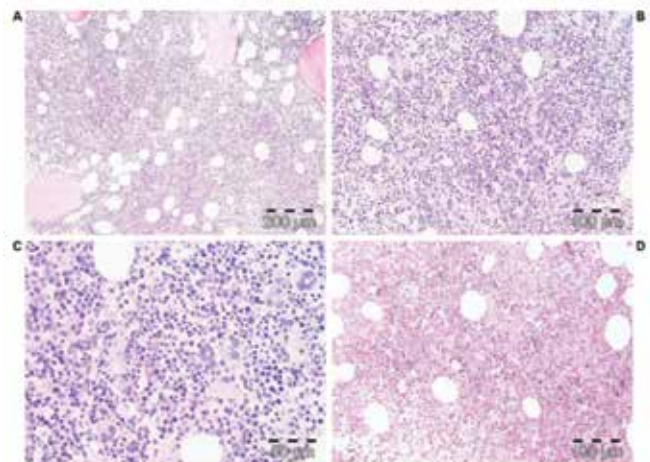


Figure 4. ET, case 3 (Table 4), showing a typical PV bone marrow picture at time of diagnosis of ET according to ECP criteria not meeting the PVSG criteria (Table 2); after 7 years of follow-up, the ET evolved into PV featuring increased red cell counts above 6x10¹²/L at the time of PV diagnosis in 2004 and a complete hematological response to low interferon alpha-2a in 2005 (Figure 1) for 8 years up to 2013.

Bone Marrow Biopsy Histology Features in PVSG-Defined PV and ET

Characteristic histology findings in bone marrow biopsies of 155 evaluable PV patients with documented increased RCM in the PVSG 01 study revealed a broad spectrum of no, slight, and moderate to marked (>80%) increase of bone marrow cellularity from 50% to 60% in 10 cases, from 60% to 80% in 45 cases, and from 80% to 100% in 100 cases (compare Figure

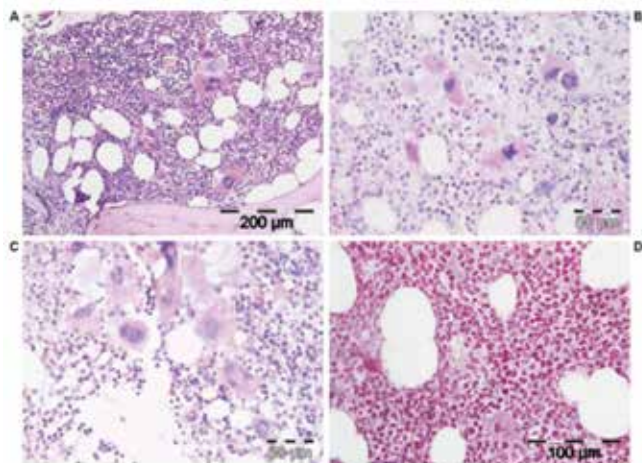


Figure 5. ET stage 3, case 6 (Table 4), showing an ET.MGM or “pPMF” bone marrow picture with increased granulopoiesis and pleomorphic megakaryopoiesis with dysmorphic nuclei (not cloud-like) but no increase of reticulin fibrosis (RF-0/1), with a long history of MPD disease, normal LAP score, and moderate splenomegaly at time of bone marrow diagnosis (A, B, and C: PAS stain; D: silver stain for reticulin).

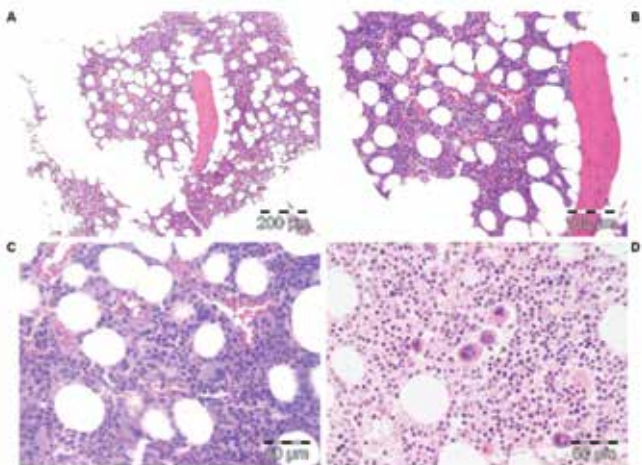


Figure 6. Bone marrow picture with loose clusters of pleomorphic megakaryocytes and slight increase of cellularity (ET/PV bone marrow picture) due to increased erythropoiesis in a case of rapid-onset PV (case 7, Table 4).

9 and Table 5) [17]. Reticulin fiber (RF) content was normal (RF-0 and -1: prefibrotic) in 94 cases, slightly increased (RF-2: early fibrotic) in 40 cases, and moderately to markedly increased (RF-3 and -4) in 21 cases (Table 3). Reticulin fibrosis was absent (RF-0 and -1) in 9 of 10 PV patients with a normocellular ET bone marrow picture, in 45 PV patients with a mixed ET/PV bone marrow picture, and in 40 of 90 PV patients with a hypercellular PV bone marrow picture. Out of 155 evaluable PV patients with increased RCM in the PVSG 01 study, 94 PV cases (61%) had no increase of reticulin

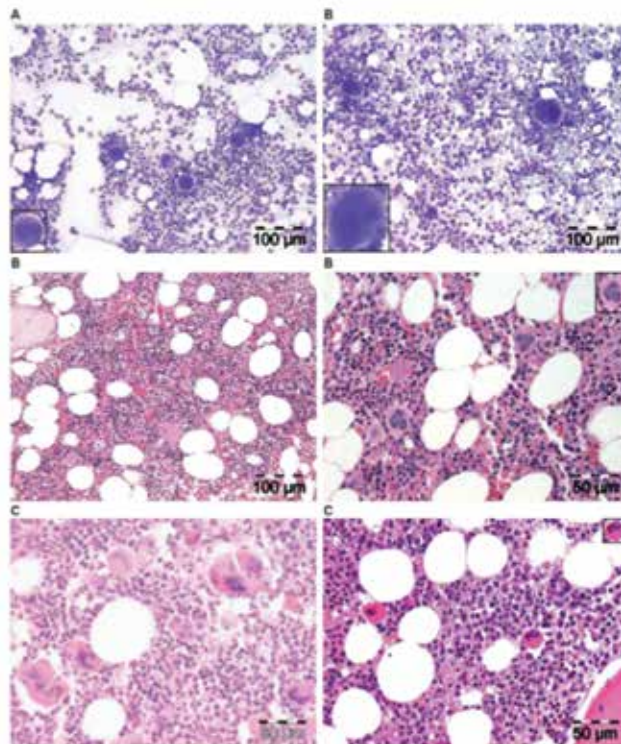


Figure 7. Upper A and B: Bone marrow smears of case 8 (Table 4) with rapid-onset erythrocythemic PV show large megakaryocytes and hypercellularity (B) as compared to normal-sized megakaryocytes and normal cellularity in a control bone marrow smear (A).

Middle panels, B: Typical ET/PV picture in bone marrow biopsy specimen of case 8 with rapid-onset erythrocythemic PV and increased RCM, homozygous for the JAK2^{V617F} mutation in 2005 (Table 4). Please note that loosely clustered pleomorphic enlarged megakaryocytes in this PV case are somewhat less enlarged and have hyperlobulated nuclei (B, right) as compared to larger megakaryocytes in classical PV with a hypercellular trilinear bone marrow proliferation (C, left bottom).

Bottom panels, C: Direct comparison in case 10 with trilinear PV showing pleomorphic large megakaryocytes (C, left) as compared to small and isolated normal-sized megakaryocytes in the bone marrow biopsy of a patient with idiopathic erythrocytosis (C, right).

fibrosis (very early stage PV upfront treated with leukemogenic agents), 40 PV cases (26%) had increased RF of grade 2 (= early MF 1 with no collagenization), and 21 PV patients (13.5%) had advanced reticulin/collagen fibrosis (RF/RCF) (grade 3 and 4 fibrosis in the bone marrow = overt MF grade 2 and 3 with collagenization) (Table 3) [7,17,18]. We conclude from the PVSG 01 and the present study that bone marrow histology alone cannot clearly distinguish among WHO- and ECMP-defined ET, prodromal PV, and PV (prefibrotic and fibrotic stages) in JAK2^{V617F}-positive groups of MPN

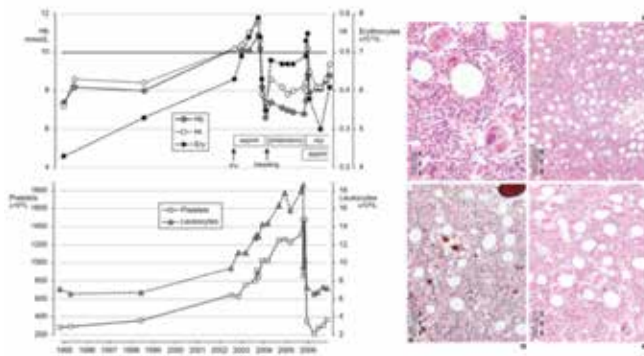


Figure 8. Rapid onset trilinear PV, case 9 (Table 4), with a typical trilinear PV bone marrow picture complicated by bleeding and poor response to pegylated interferon, indicating the need to treat with hydroxyurea on top of low-dose aspirin, which resulted in eliminating the thrombohemorrhagic diathesis and significant improvement of quality of life during long-term follow-up (2006-2013).

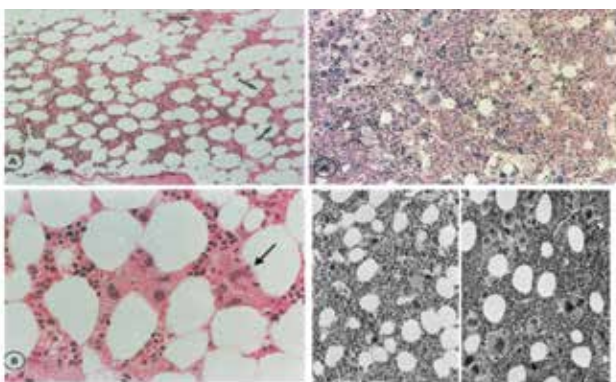


Figure 9. The spectrum of ET bone marrow picture (left panels, A and B) and PV bone marrow picture (right panel, A) in PVSG-defined PV in 155 bone marrow biopsies of the PVSG 01 study [17]. Please note the presence of clustered pleomorphic enlarged megakaryocytes in the ET and PV bone marrow pictures of 155 PVSG-defined PV cases with documented increased RCM. ET/PV bone marrow pictures (black and white) have increased cellularity (range: 60%-80%) with loose clusters of pleomorphic enlarged megakaryocytes according to Ellis et al. in the PVSG 01 study of 1975 [17].

patients. A typical ET/PV bone marrow picture is frequently seen in ET and PV in the PVSG 01 study (Figure 9) and in the present study (Table 4, Figures 2,3,4,5,6,7,8). The finding of a mixed ET/PV bone marrow histopathology picture in PV was previously observed by Thiele et al. in cases of so-called initial (latent) PV with thrombocytopenia at platelet counts between 600 and 1260x10⁹/L, mimicking ET (Table 6) [27]. The mixed ET/PV bone marrow histopathology picture in the latter study showed a clustered distribution of pleomorphic large

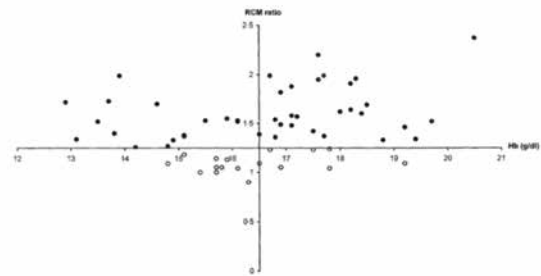


Figure 10. An elevated venous hemoglobin concentration could not be used as a surrogate marker for absolute erythrocytosis in a series of 77 consecutive Swedish PV patients (31 males and 46 females) with increased RCM (black dots): only 35% of male and 63% of female PV patients had hemoglobin values above the 2008 WHO defined criterion of 18.5 and 16.5 g/dL, respectively [30].

megakaryocytes with slight to moderate increase of trilinear hematopoietic cellularity (60%-80%) [27]. The laboratory data of these 23 cases diagnosed as initial (latent) PV did not meet the PVSG- and WHO-defined levels of hemoglobin and hematocrit required for diagnosis of PV [27], but did meet the criterion of increased erythrocyte counts above 6x10¹²/L in men and above 5.5x10¹²/L in women (Table 6) when the ECMP criteria for PV in Table 2 are applied.

In 37 evaluable 1975 PVSG-defined ET patients with platelet counts above 1000x10⁹/L, only 11% had normocellular bone marrow consistent with ET while about 80% had pretreatment ET/PV or PV bone marrow with increased cellularity between 50% and 90%, but only 11% had typical PV hypercellular bone marrow greater than 90% (Table 5) [28,29]. The majority of bone marrow biopsies showed marked megakaryocyte hyperplasia with atypical large megakaryocytes. Bone marrow reticulin content was essentially normal in 90% of 1975 PVSG-defined ET patients (prefibrotic MPN) (Table 5). The PVSG concluded that megakaryocyte morphology in PV and ET in the PVSG 01 and ET studies were identical in appearance, and ET and PV cannot be distinguished from one another based on bone marrow histology alone. Leukocytosis is common in the 1975 PVSG-defined ET and PV cases. LAP scores of over 100 were seen in 42% of ET cases, whereas 70% of PV cases had values over 100. Pruritus was observed in 14% of ET cases, which is clearly less than the 43% incidence in PV patients. The spleen was palpable in 38% of ET and 70% of PV patients, and when enlarged in ET cases the spleen was only 2 to 4 cm below the costal margin.

Discussion

Dameshek believed in 1940 that the minimal criteria for a definite diagnosis of PV were definitively elevated erythrocyte count (>6x10¹²/L), and elevated hematocrit

Table 5. Bone marrow biopsy data of 155 cases in 1975 PVSG-defined PV patients [17].

PVSG 01 Study						Increase of silver stained reticulin in bone marrow biopsy:	
Grading:	RF-0/1	RF-2	RF-3	RF-4	Bone marrow (BM) picture, Figure 9		
Cellularity	-----	-----	-----		Number		
Normal, <50%	5	1	-	=	6 ET BM		
Slight increase							
50%-60%		4	-	-	4 ET BM		
Moderate increase							
60%-80%	45	-	-		45 ET/PV BM		
80%-90%	25	25	5		55 PV BM		
Marked increase							
90%-100%	15	14	8	8	45 PV BM		
Total RF	RF0/1	RF-2	RF-3	RF-4			
	94	40	13	8	155	13+8 PV-RF-3/4	

Conclusion: The spectrum of bone marrow pictures of 155 bone marrow biopsies in 1975 PVSG-defined PV patients of the PVSG-01 study showed an ET picture in 10, an ET/PV picture in 45, and a typical PV picture in 90 PVSG-defined PV cases of the PVSG 01 study [17].

Bone marrow biopsy data in 37 PVSG-defined ET patients with platelet counts of >1000x10⁹/L [28,29].

Cellularity	Normal increased	Slightly increased	Moderately increased	Markedly increased
Overall cellularity	11%	19%	60%	10%
Megakaryocyte number	0%	8%	27%	65%
Erythroid elements	16%	32%	41%	11%
Myeloid elements	19%	30%	46%	5%
Reticulin fibers	RF-0/1: 78%	RF-2: 19%	RF-3: 3%	RF-4: 0%

Conclusion: The 1975 PVSG-defined ET patients have platelet counts in excess of 1000x10⁹/L and normocellular bone marrow in 11% consistent with ET, slightly increased cellularity in 19% consistent with ET, and moderate or marked increased cellularity due to increased erythropoiesis (PV-like) or granulopoiesis in 70% consistent with prefibrotic or early fibrotic hypercellular ET with a PV or ET.MGM (pPMF) bone marrow picture.

and platelet count together with erythrocytic and megakaryocytic hyperplasia in bone marrow aspirate [19]. In a doubtful case, blood volume estimation may be helpful. According to Dameshek in 1950, PV is a trilinear chronic myeloproliferative disorder (MPD) characterized by excessive production of nucleated red cells, granulocytes and megakaryocytes, peripheral blood erythrocytosis, leukocytosis, and thrombocytosis [20]. Some PV cases show only a moderate elevation of erythrocytes clearly above 6x10¹²/L with an extreme degree of thrombocytosis, while other PV patients present with increased leukocyte counts close to leukemic levels, with only a slight increase in platelets [19]. As to the etiology of hypercellular trilinear hematopoiesis in PV, Dameshek proposed 2 highly speculative possibilities in 1950 [20]: first, the presence of excessive bone marrow stimulation by an unknown factor or factors, and, second, a lack of or a diminution in the normal inhibitory factor or factors. Dameshek's hypothesis of PV as a trilinear MPD in 1950 could be confirmed

by Vainchenker's discovery in 2005 of the JAK2^{V617F} mutation as the cause of 3 phenotypes of MPDs: ET, PV, and secondary myelofibrosis [21,22]. The JAK2^{V617F} mutation became a pathognomonic clue to MPN because it makes the mutated hematopoietic stem and progenitor cells hypersensitive to TPO, EPO, and G-SCF, thereby leading to a growth advantage of the mutated cells in comparison to the normal trilinear hematopoietic progenitor cells in the bone marrow with the consequence of increased peripheral blood platelet, leukocyte (granulocyte), and/or erythrocyte counts. About half of the PVSG-defined ET patients carry the JAK2^{V617F} mutation, have low serum EPO levels, and show spontaneous erythroid colony formation (EEC+) [9,10,15]. PVSG-defined PV patients carry the JAK2^{V617F} mutation in 95% of cases and are characterized by high LAP scores, are EEC+, and have decreased serum EPO levels; the few JAK2^{V617F}-negative PV patients (5%) carry the exon 12 mutation in about 3% of cases [9,10,11,15].

Table 6. Laboratory features of initial (latent) PV with clustered distribution of pleomorphic enlarged megakaryocytes in bone marrow with increased cellularity (60%-70%) diagnosed by Thiele et al. [27] as ET mimicking PV in 23 patients that did have erythrocytes above $6 \times 10^{12}/L$ in males and above 5.5 in the majority of females, consistent with the diagnosis of PV (Table 2).

		Normal values	
Erythrocytes, $\times 10^{12}/L$	Males	7.2 (6.1-8.6)	4.5-5.9
	Females	6.5 (5.2-7.6)	4.0-5.5
Hemoglobin, g/dL	Males	17.8 (17.0-18.3)	13.2-16.4
	Females	15.6 (15.0-16.4)	11.6-15.0
Hematocrit, %	Males	53.4 (50.3-60.4)	0.40-0.50
	Females	50.0 (43.0-58.0)	0.35-0.45
Leukocytes, $\times 10^9/L$		14.2 (6.0-17.3)	4.0-10.0
Thrombocytes, $\times 10^9/L$		780 (608-1260)	140-360
LAP		193 (85-391)	10-80
LDH, U/L		268 (183-536)	
Spleen size:			
on echogram/scan	Not tested		<12 cm
on palpation, cm below costal margin	2.0 (0-3.9)		Not palpable

Bone marrow histopathology may significantly contribute to diagnostic differentiation and staging of the primary JAK2^{V617F}-mutated MPNs of ET and PV, with the MPL515-positive ET and JAK2-MPL wild-type ET as the presenting feature of primary dysmegakaryocytic myeloproliferation (PMGM) [23,24]. The natural history of each MPN of various molecular etiology may pass through early, overt, and advanced stages of MPN by grading cellularity and bone marrow reticulin (MF-0/1) or reticulin-collagen fibrosis (MF-2/3) [23,24]. Megakaryocytes are pronouncedly pleomorphic and dysmorphic in the prefibrotic and fibrotic hypercellular (80%-100%) bone marrow stages in JAK2^{V617F}-positive PV and ET.MGM, and are very likely different from PMGM in prefibrotic and JAK2 wild-type PMF patients [7,8,9,10,23,24]. Bone marrow histopathology has the power to distinguish PV (either JAK2^{V617F}- or exon 12-mutated) from congenital and secondary erythrocytosis and to distinguish ET of various underlying molecular etiologies from reactive thrombocytosis with a sensitivity and specificity of very near 100% [7,8,9,10,25,26,27]. The present study shows that histology of pleomorphic megakaryocytes and bone marrow cellularity was not different in JAK2^{V617F}-positive normocellular ET, prodromal PV, and classical PV patients. The prefibrotic stage of prodromal PV may precede PV for about 10 years. This type of slow-onset PV (ET type 2, Table 4B) differs from acute-onset JAK2^{V617F} homozygous PV with a short previous MPN history (Figure 1, case 8; Figures 7 and 8; Table 4, cases 8 and 9). Three of the 4 patients with

acute onset PV shown in (Table 4) were homozygous for JAK2^{V617F}. Whether or not these 2 types of slow-onset PV versus rapid-onset PV differ at the biological level for the JAK2^{V617F} mutation load, MPN disease burden, and natural history with regard to progression to post-PV myelofibrosis remains to be evaluated in long-term prospective clinical and basic research studies of newly diagnosed MPN patients.

The JAK2^{V617F} mutation is causative for a broad spectrum of trilinear MPNs; its presence rules in prefibrotic and fibrotic stages of ET and PV and excludes erythrocytoses, thrombocytoses, and thrombocythemia and myelofibrosis of other clonal origins. With the advent of JAK2^{V617F} mutation and bone marrow histopathology as a pathognomonic clue to MPN, RCM measurement no longer has additional diagnostic value. It is erythrocytosis that distinguishes PV from all other variants of MPNs, but PV is usually hetero-/homozygous for the JAK2^{V617F} mutation and associated with thrombocythemia, granulocytopenia, and splenic hematopoietic neoplasia (SHN). For the diagnosis of PV, the minimal criteria of elevated erythrocyte count ($>6 \times 10^{12}/L$) and elevated hematocrit must be present, but bone marrow aspirate is diagnostic, showing a panmyelosis (increased trilinear hematopoiesis) and pleomorphic large megakaryocytes in diagnostic bone marrow biopsies. Both hemoglobin and hematocrit levels are clearly dependent on the iron status, related to low or decreased ferritin levels, and on increased spleen size in untreated PV patients who in fact do have or have had

Table 7. ECMP staging of PV patients related to currently available therapeutic options anno 2014.

PV, ECMP stage	0	0	1	2	3	4	5
Michiels 2008-2013 clinical diagnosis	Erythrocythemic PV	Prodrormal PV	Polycythemic PV prefibrotic	Classic PV prefibrotic	Masked or Inapparent PV (IPV) MF stage	Post-PV myelofibrosis, neoplastic features	Spent-phase 'anemic' PV MDS AL
LAP score	N/↑	↑	↑	↑	↑/↑↑	Variable	Variable
RCM	↑	N	↑	↑	↑	Variable	N/↓
Serum EPO	N/↓	N/↓	↓	↓	↓	Variable	N/↓
Leukocytes, x10 ⁹ /L	Ni: <12	<12	<12	Ni: >12	>15	>20	>20
Platelets, x10 ⁹ /L	Ni: <400	>400	N or >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, x10 ¹² /L	>6	<6	>6	>6	Normal	Normal, low/↓	N/↓
WHO bone marrow	Early PV	Prodrormal PV	Early PV	Trilinear PV	Trilinear PV	PV/MF	PV/MF
Bone marrow cellularity (%)	50-80	50-80	60-100	80-100	80-100	Decreased	Decreased
Grading myelofibrosis [17]	RF-0/1	RF-0/1	RF-0/1	RF-0/1-2	RCF-1/2	RCF-2/3	RCF-3
Splnomegaly on palpation	No	No/+	No/+	+	++/+++	Large	Large
Spleen size, echogram cm	Ni: <12	<12-15	12-15	12-18	18->20	>20	>20
EEC+	+	+	+	+	+	+	+
Low serum EPO	+	+	+	+	+	+	?
JAK2V617F in granulocytes and BFU-e	+	+	+	+/+++	+/+++	++	++
Therapeutic implications, Barbui et al. [48]	Low risk since 1985 [3]	Low risk since 1985 [3]	Low risk since 2000 [43]	Intermediate since 2000 [43,49]	High risk, 2013	Post-PV MF, 2013	Since 1950
First-line treatment option, Klidjian et al. [49]	Aspirin since 1985 [3,4]	Aspirin	Phlebotomy Aspirin	Phlebotomy Aspirin IFN →	Up front JAK2 inhibitor	JAK2 inhibitor	Supportive
Second-line IFN	Phlebotomy since 1950, Dameshek [20]	Low-dose IFN for Intermediate Stage PV [23,45]	Low-dose IFN → Response = MPN disease reduction [45]	if resistant → HU PVSG 2000 [23,44,45]	Supportive BMT to be defined	supportive palliative BMT	

increased RCM, thereby allowing the overlooking of initial or latent-stage PV. The authors of the 2008 WHO criteria for PV stated that the measured RCM could be preplaced by a surrogate marker of a hemoglobin value of above 18.5 g/dL in men and above 16.5 g/dL in women [14,15]. Johansson et al. showed that WHO-defined elevated hemoglobin concentration cannot be used as a surrogate marker for absolute erythrocytosis in PV patients (Figure 10) [30]. In a series of 77 consecutive patients (31 males and 46 females) with PV in the study of Johansson et al., only 35% of male and 63% of female PV patients had hemoglobin values above 18.5 and 16.5 g/dL, respectively (Figure 10). In our experience, treatment of PV by phlebotomy alone corrects the hemoglobin and hematocrit values but, due to microcytic erythrocytes caused by iron deficiency, the number of erythrocytes remains above $6.0 \times 10^{12}/L$ in PV and below $6.0 \times 10^{12}/L$ in JAK2^{V617F}-mutated ET patients (Figure 1, case 8). The erythrocyte count at a cut-off of $6.0 \times 10^{12}/L$ according to ECMP criteria (Tables 1 and 2) within JAK2^{V617F}-positive MPN entities appears to be a sensitive and specific criterion to distinguish ET and prodromal PV from overt PV. In a recent study of 26 PV patients with increased RCM, erythrocytes counts were above $6.5 \times 10^{12}/L$ in 100%, hemoglobin was above 18 g/dL in 50%, hematocrit above 0.55 in 46%, and decreased serum EPO was $<3.3 \mu\text{g/mL}$ in 94% [31].

The 2008 WHO laboratory, molecular, and pathological criteria for the diagnosis of Philadelphia-chromosome (Ph1-) MPNs [14,15] failed to stage each of the MPNs, ET, PV, and ET.MGM, and failed to define transitional stages between ET and PV and between ET and post-ET myelofibrosis [32,33,34]. The 2008 WHO criteria for PMF only detect the advanced spent-phase state of fibrotic MPN complicated by pronounced myeloid metaplasia of the spleen. A clear-cut diagnosis of either JAK2^{V617F}-positive ET.MGM or JAK2 wild-type pPMF and prefibrotic, early fibrotic, or fibrotic ET/PMF cannot be made by the 2008 WHO classification [14,15]. Consequently, the various fibrotic stages of ET in various MPDs/MPNs with hypercellular megakaryo-granulocytic myeloproliferation (ET.MGM or prefibrotic PMF) in the bone marrow are to be diagnosed by expert hematopathologists as MPN unclassifiable (MPNuc) [34]. In future prospective studies, the first step in classification of MPNs should be the molecular classification of MPNs as JAK2^{V617F}-mutated, MPL515-mutated, and JAK2-MPL wild-type [23,24]. A set of relevant WHO bone marrow criteria combined with MPN-specific clinical and laboratory features including EEC, EPO levels, JAK2 mutation for ET, prodromal PV and PV, and ET.MGM according to ECMP has the potential to define main phenotypes or stages of JAK2^{V617F}-positive MPN. JAK2 wild-type ET carrying the MPL515 mutation has no clinical, laboratory, or bone marrow features of prodromal PV or PV at diagnosis and during follow-up [35,36,37,38]. JAK2 wild-type but MPL515-mutated ET cases have normal serum EPO and ferritin levels and no spontaneous EECs [23,24].

Proper WHO diagnosis of MPNs is difficult in patients with splanchnic vein thrombosis (SVT) as the Budd–Chiari syndrome (BCS) and/or portal vein thrombosis (PVT), since the typical peripheral blood changes are usually less pronounced [39,40,41,42,43]. In 241 SVT patients (104 with BCS, 137 with PVT; Kiladjian et al. [42]), JAK2^{V617F} was found in 94 (38%) and MPL515 mutations were not detected. The platelet counts were between 238 and $456 \times 10^9/L$ (median: 333) in 74 patients carrying the JAK2^{V617F} mutation and between 104 and $258 \times 10^9/L$ (median: 159) in 147 JAK2 wild-type SVT patients [42]. In this cohort of 241 SVT patients, the values for hemoglobin were 13.0 (range: 11.1-14.4) and 13.4 (range: 12.3-15.4) in the JAK2 wild-type and JAK2^{V617F}-mutated cases, respectively. In 62 evaluable JAK2^{V617F}-positive cases of SVT, RCM was increased ($>125\%$) in 38 cases despite normal values for hemoglobin, consistent with PV complicated by splenomegaly and hypersplenism (inapparent PV or masked PV, Table 7), and RCM was normal in 24 cases, consistent with prodromal PV, ET, or ET.MGM. In 147 JAK2 wild-type SVT patients, 144 bone marrow biopsies were evaluable and 10 were positive for the diagnosis ET or MF [42]. In a recent metaanalysis of 1062 BCS and 855 PVT patients, mean prevalence of MPN and JAK2^{V617F} was 41% and 41%, respectively, and JAK2^{V617F} screening in SVT patients without typical hematologic features identified MPN in 17% and 15% of BCS and PVT patients [43]. We conclude that the JAK2^{V617F} mutation and extensive thrombophilia screening and bone marrow biopsy should be included in the diagnostic work-up of SVT patients [40,41,42,43].

In view of new molecular biological insights and current novel treatment options, proper staging of ET, ET.MGM, prodromal PV, and overt and advanced PV patients according to ECMP criteria has a significant and determinative impact on first-, second-, and third-line treatment options in MPN patients carrying the JAK2^{V617F} mutation (Table 7) [44,45]. The broad spectrum of JAK2^{V617F} mutation load, heterozygous in ET, hetero-/homozygous in PV, and homozygous in advanced PV and post-MPN myelofibrosis [46,47] and its related pathobiological evolution into symptomatic and advanced stages including thrombohemorrhagic manifestations, the degree of splenomegaly and hypersplenism, the degree of bone marrow cellularity, and fibrosis [7,9,15,18,32,33] (Table 7) indeed characterize and classify the JAK2^{V617F}-mutated trilinear MPNs. The JAK2^{V617F} mutated trilinear MPN is a distinct clonal MPN with a very broad spectrum of clinical manifestations of ET and PV, and various stages in between prefibrotic ET and PV versus post-ET and post-PV myelofibrosis in terms of JAK2 mutation load, MPN disease burden, and sequential prefibrotic, early fibrotic, and fibrotic stages at the bone marrow level paralleling the degree of extramedullary hematopoietic neoplasm in the spleen, which can objectively be measured by the

degree of splenomegaly on echogram (Table 7). The ECMP criteria for the classification of JAK2^{V617F}-positive ET, prodromal PV, ET.MGM, and classic PV in terms of JAK2^{V617F} mutation load and MPN disease burden (Table 7) will allow much better staging of PV patients related to targeted treatment recommendations according to current guidelines for low-risk PV (aspirin-phlebotomy), for intermediate-risk PV (pegylated interferon), for high-risk PV (hydroxyurea versus JAK2 inhibitors), and for post-PV myelofibrosis (JAK2 inhibitors; Table 7) [23,24,45]. A similar risk stratification has been reached for primary advanced myelofibrosis, post-ET myelofibrosis, and post-PV myelofibrosis to clarify the targeted indication of JAK2 inhibitors [50,51,52]. In very early stage PV with absence of constitutional symptoms and itching, phlebotomy on top of low-dose aspirin is enough without the need of myeloreductive treatment with pegylated interferon (IFN). Evidence is accumulating that pegylated IFN is the first-line treatment option in symptomatic intermediate stage PV to postpone the use of hydroxyurea as long as possible or even life-long [23,44,45,49]. Patients with advanced PV, masked PV, or inapparent PV who have large spleens (Table 7) usually complicated by pronounced constitutional symptoms during their course of PV disease are candidates for targeted JAK2 inhibitor treatment since hydroxyurea is predicted to induce anemia and acceleration of myelofibrosis [23,24,44,45]. The JAK2 inhibitors exert a salutary effect on constitutional symptoms and splenomegaly, do not induce histologic or cytogenetic remission, and are predicted to improve the quality of life significantly and do improve survival [50,51,52; personal communications with Srdan Verstovsek, 2014].

Authors' Contributions

JJM and FTK designed the study. HDR coordinated the bone marrow pathology data. JJM, ZB, and WS collected and JJM analyzed the clinical, molecular, and pathological data. JJM, FTK, KL, and HDR interpreted the clinical and bone marrow pathology studies. JJM and HDR wrote the manuscript.

Acknowledgment

We gratefully thank Dr. Jürgen Thiele, who was a most respected active member of the EWG.MPD (1998-2005) and significantly contributed to the formulation of the ECP, WHO, and ECMP criteria for the diagnosis of the MPNs ET, PV, and MF and the various clinicopathological fibrotic stages of all variants of clonal MPNs.

Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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