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Review Article

Incorporation of the 2008 WHO into 2008/2020 ECMP/CLMP Classification for Staging and Prognosis Assessment of JAK2, MPL, TPO, MPL and CALR Mutated Myeloproliferative Neoplasms: From Dameshek to Vainchenker and Michiels 1940 - 2020: A Historical Appraisal and Novel Therapeutic Implications

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Abstract

In this critical appraisal of the literature Michiels and De Raeve incorporated the 2007/2008 WHO classification of myeloproliferative neoplasms into the European Clinical Molecular and pathological (2008 ECMP 2020 CLMP) criteria by including bone marrow biopsy as a pathognomonic clue with a 100% sensitivity and specificity. The 2008 ECMP classification distinguish the *BCR/ABL*-negative Myeloproliferative Disorders/Neoplasms (MPD/MPN) essential thrombocythemia (ET) and polycythemia Vera (PV) and primary megakaryocytic granulocytic myeloproliferation (PMGM) from *BCR/ABL* positive chronic myeloid leukemia (CML) and ET, and thrombocythemia associated with myelodysplastic syndromes in RARS-T and 5q-minus syndrome. The megakaryocytes in Ph-positive in *BCR/ABL*-positive ET and CML are small with mono- or bi-lobulated nuclei whereas the megakaryocytes in *BCR/ABL*-negative MPD/MPN are increased, clustered and enlarged as compared to controls showing variable degrees hyperlobulated nuclei in JAK2, TPO, MPL mutated and JAK2 wild type MPN. The 2007/2008 WHO criteria overlook the early latent stages of ET and PV and primary myelofibrosis (PMF) is not a disease but a secondary event in all variants of MPD/MPN.

The 2008 ECMP 2020 CLMP criteria distiguish at least six $JAK2^{V617F}$ mutated MPN stages that have important prognostic and therapeutic implications: normocellular ET, prodromal PV, classical PV, hypercellular PV, EMGM = masked PV, advanced PV with various degrees of myelofibrosis. The combination of spontaneous EEC, increased leukocyte alkaline phospatase score, decreased serum erythropoietin (EPO) and $JAK2^{V617F}$ mutation is specific for $JAK2^{V617F}$ mutated ET, prodromal PV, masked PV and classical PV but are normal in JAK2 wild type MPL and CALR mutated ET and MF. MPL^{515} mutated thrombocythemia is a separate and distinct MPN entity without features of PV. JAK2/MPL wild type ET associated with PMGM is the third distinct CALR mutated MPN entity. Acetylsalicylic acid (Aspirin) cures platelet mediated erythromelalgia and microvascular disturbances by inhibiting platelet cyclooxygenase. Platelet ADP-receptor inhibitors (ticlopedin, clopidrogrel, ticagrelor) and anticoagulation with vitamine K antagonist (VKA) and direct oral anticoagulants (DOAC) do not relief the aspirin responsive microcirculatory disturbances in $JAK2^{V617F}$ and MPL^{515} mutated ET and PV patients. The 'Early Interferon Intervention Strategy has become the first line myeloreductive treatment option in early stage ET and PV to postpone or even eliminate hydroxyurea and ruxolitininb during long-term or even lifelong follow-up.

Keywords: Myeloproliferative Neoplasm; Essential Thrombocythemia; Polycythemia Vera; Myelofibrosis; $JAK2^{V617F}$ Mutation; MPL^{515} Mutation; Bone Marrow Pathology; World Health Organisation; European; Classification

Introduction

In the 19th century chronic myeloid leukemia (CML) and polycythemia vera (PV) have been described as primary distinct disease entities [1-3]. The minute chromosome discovered by Nowell and Hungerford (1960) called Philadelphia (Ph) chromosome after the city of discovery is linked to CML and absent in all variants of the MPDs ET PV and AMM [4]. The Ph-chromosome originates from a translocation between chromosomes 9 and 22, t(9;22) (q34;q11) [5]. According to Dutch clinical and basic research investigators of the Erasmus University Medical Center (EUMC) Rotterdam this hybrid gene is generated by the translocation consisting of the *BCR* gene on chromosome 22 and the *ABL* oncogene originating from chromosome 9 [6]. The *BCR/ABL* fusion gene has high tyrosine kinase activity and is the driver cause of CML [7,8]. Patients clinically diagnosed as CML patients are $Ph^+/BCR/ABL^+$ in 90%, $Ph^-/BCR/ABL^+$ in 5% and $Ph^-/BCR/ABL^-$ in 5% (atypical CML) at the genetic level [7-10].

The combination of plethoric appearance, splenomegaly, erythrocyte counts above 6×10^{12}/L (Tables 1 and 2), elevated platelet counts above 400×10^9/L and the presence of large megakaryocytes and trilinear panmyelosis of erythrocythemic megakaryocythemic and granulocythemic (EMG) myeloproliferation in the bone marrow was pathognomonic diagnostic for polycythemia vera (PV) in the study of Dameshek and Hentel in 1940. Michiels confirmed that the combined use of clinical presentation, increased erythrocytes above 6×10^{12}/L, platelet count above 400×10^9/L and a typical ET/PV bone marrow histology showing clusters of large megakaryocytes indeed proved to be diagnostic for Ph-negative ET and PV by the use of the 1980 RCP 200 ECP and 2006 ECMP criteria for ET and PV (Tables 1 and 2) obviating the need to measure red cell mass (RCM).

Trilinear PV: From Dameshek 1950 to Vainchenker 2005 and Michiels 2008

In 1950, Dameshek proposed the one cause hypothesis of PV as a trilinear myeloproliferative disorder (MPD) of the bone marrow with excessive erythrocytosis, leukocytosis and thrombocytosis (Figure 1) [11]. The one cause concept of trilinear PV of Dameshek (1950) is preceded by ET and followed by myelofibrotic spent phase PV has been completely overlooked by the PVSG and WHO investigators Wasserman, Berlin, Spivak, Silver, Tefferi and Thiele. The discovery of the $JAK2^{V617F}$ mutation in 2005 by Vainchenker confirmed the one cause hypothesis of Dameshek (1950) that PV indeed appeared to be a trilinear MPD by the discovery and demonstration that 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 is the driver cause of trilinear MPNs ET, PV and MF (Figures 1 and 2) [12,13]. The sequential stages of heterozygous and homozygous $JAK2^{V617}$ mutations (Figures 2 and 3) render the TPO and EPO receptors constitutively activated and hypersensitive to hematopoietic growth factors thrombopoietin (TPO), erythropoietin (EPO) and granulocyte colony stimulating factor (GCSF), resulting in trilinear MPN (ET, PV and MF [12,13].

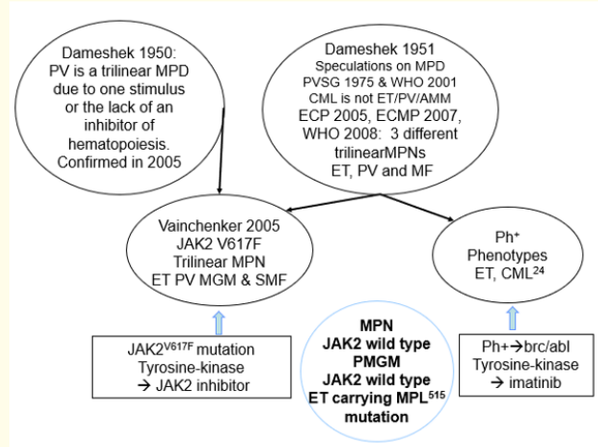

Figure 1: In 1950 Dameshek proposed the one cause hypothesis of trilinear polycythemia vera (PV) either the presence of a excessive bone marrow stimulation by an unknown factor or the lack of an inhibiting factor. This one cause hypothesis of trilinear PV has been confirmed by Vainchenker in 2005 by his discovery of the $JAK2^{V617F}$ mutation as the driver cause of trilinear PV, preceded by essential thrombocythemia (ET) and followed by myelofibrosis (MF) in the studies of Michiels., *et al*. 1985 - 2006.

In 1951 Dameshek speculated that ET, PV agnogenic myeoid metaplasia (AMM) and chronic myeloid leukemia (CML) form the myeloproliferative disorders (MPDs). Subsequent genetic and bone marrow histology studies in the 1980s in The Netherlands revealed that Ph-positive ET and CML are caused by the *BCR/ABL* fusion gene and protein that induces constitutively activated tyrokinase activity (TK) as the driver cause of Ph-positive ET and CML. The 1990 Hannover Bone marrow classification defined the three distinct MPDs ET, PV and primary megakaryocytic granulocytic myeloproliferation (PMGM), whereas myelofibrosis is a secondary event in Ph-negative ET, PV and PMGM and in Ph-positive ET and CML after long-term or lifelong follow-up as documented by Michiels,, De Raeve, Hebeda, Lam, Bot, Berneman, Schroyens. Biology, Diagnosis and Classification of MPD. 1st International Lymphoma-Leukemia-Myeloma (LLM) Congress. Turk J Hematol Proceeding May 2007; 24(Supp 1):37-53. The 1950 concept of PV as a trilinear MPD has been overlooked by the 1975 PVSG and the 2008 - 2016 WHO investigators Tefferi, Thiele and Barbui and Arbor., *et al.* as well.

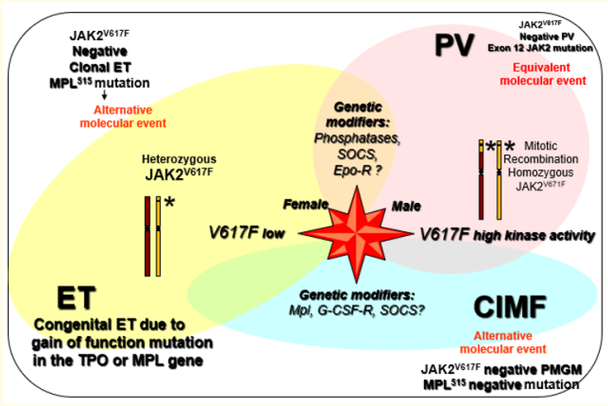

Figure 2: The JAK2^{V617F} "dosage" and "additional events" hypothesis as the underlying cause of essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF) (Modified according to Delhommmeau., *et al.*) [82]. Heterozygosity is the first acquired genetic step in which one allele of hematopoietic cells (HSCs) acquires the Jak2^{V617F} mutation. Homozygosity is the second genetic step in which both alleles of HSCs carry the JAK2^{V617F} mutation due the mitotic recombination occurring at times that HSCs are dividing [82,83]. For explanation see text. (Modified by Michiels and reproduced with the courtesy of Francois Delhommeau and William Vainchenker, and by permission of Birkhäuser Verlag, Basel: Oncogenic mechanisms in myeloproliferative disorders. Cell Mol Life Sci 2006;63:2939-2953).

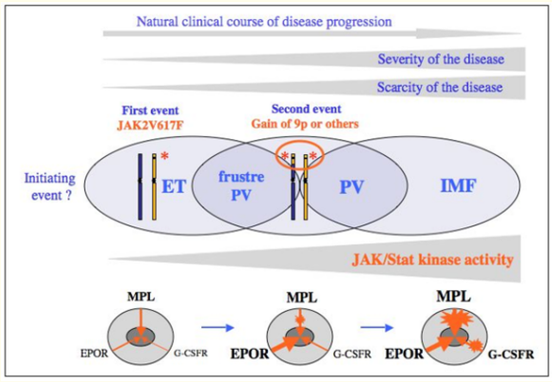

Figure 3: Molecular pathogenesis on the natural history of heterozygous into homozygous JAK2^{V617F} mutated trilinear myeloproliferative neoplasms (MPN) and associated disease progression of the sequential occurrence of the clinical phenotypes essential thrombocythemia (ET), polycythemia vera (PV), and idiopathic myelofibrosis (IMF) related to increased JAK2/Stat kinase activity (Courtesy of Dr Villeval., *et al.* 2006) [12,13,82,83].

In 1951 Dameshek lumped such apparently dissimilar diseases as polycythemia vera (PV), erythroleukemia, CML, agnogenic myeloid metaplasia (AMM), megakaryocytic leukemia and proposed an unifying theory that all these variable manifestations represent one myeloproliferative activity of bone marrow cells due to one hypothetical stimulus (Figure 1 right) [3]. Such speculations in the mind of Dameshek as Editor in Chief of Blood was conceivable but lacked scientific evidence. The PVSG used between 1973 and 1975 the Ph^1-chromosome to separate the Ph^1- negative ET, PV and AMM from the Ph^1-positive ET and chronic myeloid leukemia (CML) with various degrees of thrombocythemia and myelofibrosis [9-11]. According to strict morphological, biochemical, cytogenetic and molecular criteria including the Ph$^+$ chromosome and *bcr/abl* fusion gene and protein, Michiels (1987) concluded that CML is a malignant disease with an obligate transition into acute leukemia, whereas essential thrombocythemia (ET), polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) or primary megakaryocytic granulocytic myeloproliferation (PMGM) form the Ph-chromosome and BRC/ABL negative chronic myeloproliferative disorder (MPD) featured by a benign proliferation of the three hematopoietic cell lines (Figure 1) [10].

Hannover bone marrow classification of the MPDs ET, PV and PMGM: from Georgii 1980 - 1990 to Michiels 1987 - 1997

The morphological distinction between Ph$^+$ and *BCR/ABL*$^+$ ET and thrombocythemia associated with and *BCR/ABL*$^+$ CML versus the Ph-negative thrombocythemia in various MPDs is based upon conspicuous differences in megakaryocyte size and morphology in bone marrow smears and [10]. This enabled Bone marrow pathologist Georgii and Thiele to distinguish between small megakaryocytes in Ph-positive diseases versus enlarged megakaryocytes with hyperlobulated nuclei in Ph-negative MPDs [10,14-17]. The distinction of small mono- or binucleated small megakaryocytes in Ph-positive ET and CML (Figure 4) versus large megakaryocytes in the chronic MPD ET and PV (Figure 5) could reproduced by Georgii between 1980 and 1990 [18-20], by Michiels between 1987 and 1997 [10,14-17].

Georgii., *et al*. (1990) [18] described the Hannover Bone Marrow classification of the MPDs to pick up the early stages of prefibrotic MPD ET and PV at the marrow and laboratory level. The early latent stages of prefibrotic MPD has been overlooked by the crude crude PVSG criteria for PV and ET. The 1975 PVSG and its extension into the 2001/2008 WHO clinical criteria of ET, PV and PMF are suboptimal as compared to the Hannover BM criteria [19-23]. Michiels incorporated the Hannover BM features of ET, PV and PMGM into the ECP and ECMP to pick up all early and latent prefibrotic stages of ET, PV and PMGM. In the present manuscript we could integrate the PVSG and the 2001 and 2007 WHO criteria of Tefferi and Thiele into the 2008 ECMP classification of Michiels and De Raeve by including bone marrow pathology and the use of specific laboratory features and molecular markers for diagnostic differentiation of each of the latent (masked), early and overt MPDs.

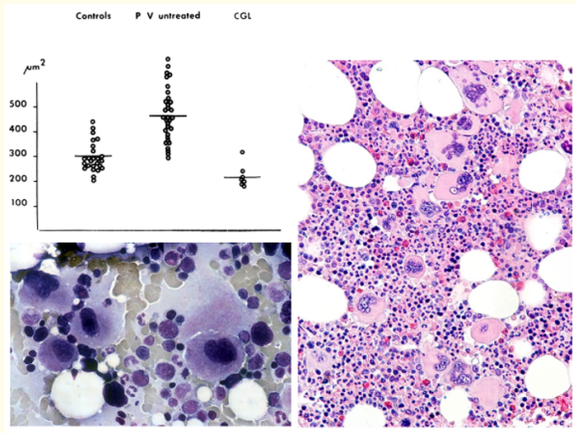

Figure 4:

Upper left: Microplanic studies on megakaryocytes in chronic granulocytic leukemia (CGL = CML) and polycythemia vera (PV untreated) showed that the average size of megakaryocytes in CGL is less than normal.

Lower left: Small sized megakaryocytes with monolobulated and bilobulated nuclei in a bone marrow smear from the patients with *BCR/ABL*+ ET, who developed *BCR/ABL*+ megakakaryoblast leukemia (Michiels., *et al.* 1987) [23].

Right: Bone marrow histology in PV showing increased cellularity due to increased erythropiesis and increase and clustered large mature megakaryocytes with hyperlobulated nuclei.

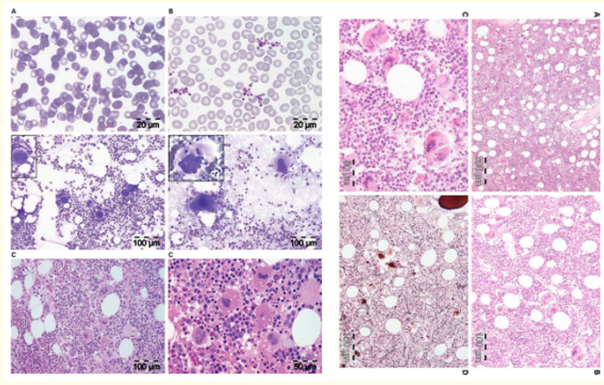

Figure 5: JAK2^{V617F} positive ET in a case of portal vein thrombosis and platelets 453 x 10^9/L. Large platelets in peripheral blood smear B as compared to control A left panels. Bone marrow smear: large megakaryocytes with multilobulated nuclei B as compared to control A middle panels. Bone marrow biopsy hypercellular due to increased erythropoiesis and clustered large pleomorphic megakaryocytes. Bonemarrow features of homozygous JAK2^{V617F} acute onset PV (right).

With the improvement of bone marrow biopsy and tissue processing in the 1980s and 1990s, Georgii., *et al*. defined the pathological features of ET, PV and PMGM [14-21]. The terms agnogenic myloid metaplasia (AMM) and CIMF used in the PVSG/WHO classification lack accuracy and represent the sequential prefibrotic hypercellular and fibrotic MPD. Myelofibrosis is a secondary response of polyclonal fibroblast activation underlying myeloproliferation (Table 1, 2, 3) [18-20]. Georgii and Michiels replaced the terms AMM and CIMF by primary megakaryocytic granulocytic myeloproliferation (PMGM). The 1980 Rotterdam Clinical and Pathological (RCP) and the 1990 Hannover Bone Marrow classification define Ph-negative ET by persistent increase of platelets in excess of 400 x 10^9/l myeloproliferation of mature enlarged megakaryocytes in a normocellular bone marrow [18-20]. The diagnosis of PV is based on increased bone marrow cellularity (60 to 80%) due to increased erythrocythemic and thrombocythemic (EM) myeloprolifeation (earl stage PV), and trilineage hypercellularity (80 to 100%) in classical PV (Figure 5) or trilinear "panmyelosis" exactly as described by Dameshek in 1950, The diagnosis of PMGM is based on three specific bone marrow histology criteria: 1) the presence of large dysmorphic megakaryocytes with immature cytoplasm and immature cloud-like nuclei not seen in ET and PV, 2) increased granulopoiesis but never disturbed in maturation and 3) no features of PV with relatively decreased erythropoiesis (Figures 6 and 7) [18-24].

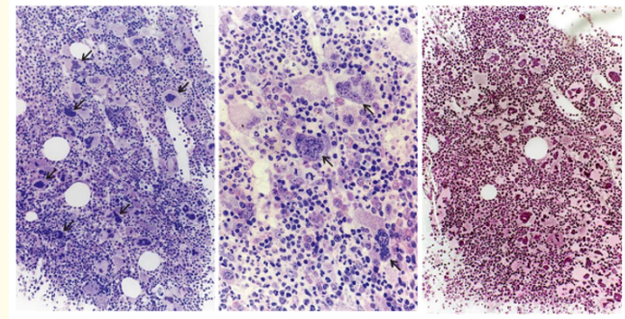

Figure 6: Hypercellular ET entity presenting with JAK2 wild type prefibrotic (right panel) primary megakaryocytic and granulocytic myeloproliferation (PMGM, left and middle), which is characterized by a hypercellular bone marrow due to dual myeloproliferation of granulopoiesis and dense clustered enlarged immature dysmorphic megakaryocytes (left and middle panels) with bulky (bulbous) hyperchromatic nuclei (arrows) (personal observation), which are never seen inMPL^{515} mutated PT and also not in the prefibrotic JAK2^{V617F} mutated ET, prodromal PV, EMGM and trilinear PV entities.

Clinical and molecular criteria	Bone marrow pathology (P) criteria
JAK2^{V617F} ET	**Normocellular ET**
Platelet count of >350 x 10^9/l and presence of large platelets in a blood smear Presence of JAK2-^{V617F} mutation Normal erythrocytes <5.8 x 10^{12}/L males, <5.6 x 10^{12}/L females Normal haemoglobin (Hb) and hematocrit (ht)	Predominant proliferation of enlarged mature megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. Reticuline fibrosis (RF) 0 or 1
JAK2^{V617F} prodromal PV	**ET with bone marrow features of prodromal PV**
Platelet count of >350 x 10^9/l and normal ht male <0.51, female <0.48, Normal erythrocyte <5.8 x 10^{12}/L males, <5.6 x 10^{12}/L females Presence of JAK2^{V617F} mutation Low serum EPO level and/or increased LAP score Spontaneous EEC.	Increased cellularity with due to increased erythropoiesis or trilineage myeloproliferation (masked PV). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes. Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis. RF 0 or 1
JAK2^{V617F} hypercellular ET	**EMGM (masked PV)**
Platelet count of >350 x 10^9/l, No signs of leuko-erythroblastosis Slight or moderate splenomegaly on ultrasound Presence of JAK2^{V617F} mutation No preceding or allied CML, PV, RARS-T or MDS. **EMGM clinical staging** Early stage: No anemia with hb and ht in the normal low normal range: hb >13 g/dl: early clinical stage Intermediate: Hb < 13 to >12 g/dL, LDH N or ↑, no leukoerythroblastosis Advanced: Hb <10 g/dL, LDH↑↑, CD34+, leukoerythroblastosis, tear drop	Hypercellular ET due to JAK2 mutated essential megakaryocytic and granulocytic myeloproliferation (EMGM) and normal or reduced erythroid precursors. Loose to dense clustering of more pleiomorphic megakaryocytes with hyperploid or clumsy nuclei (not or some cloud-like). RF grading PVSG, MF Georgii and Thiele (Table 3) Prefibrotic: RF-0/1, MF-0, no/minor splenomegaly Bone marrow staging: Early fibrotic ET: RF 2, MF 1, splenomegaly no/minor Fibrotic ET: RF3, RCF or MF2, overt splenomegaly Post-ET MF: RF3/4, or MF-2/3, huge splenomegaly

Table 1: The 2008 European Clinical Molecular and Pathobiological (ECMP) criteria for the diagnosis JAK2^{V617F} mutated essential thrombocythemia (ET) [68].

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 x 10^9/L (mean 333) in 74 patients carrying the JAK2V617F mutation and between 104 and 258 x 10^9/L (mean 159) in 147 JAK2 wild type SVT patients [41].

Clinical criteria JAK2^{V617F} mutated PV	**Pathological criteria PV**
A1: Erythrocyte count above 5.8 x 10^{12}/L, hemoglobin >18.5 g/dL male and >16.5 g/dL females. Raised red cell mass (RCM optional) male >36 ml/kg, female >32 ml/kg (PVSG, WHO) **A2**: Persistent increase of platelet count grade I 400-1500, grade II >1500 x 10^9/L **A3**: Splenomegaly on ultrasound or CT (>12 cm) or splenomegaly on palpation **A4**: Granulocytes >10x10^9/L or leukocytes >12 x 10^9/L and raised LAP score >100 in the absence of fever and no increase of ESR **A5**: Absence of any cause of primary or secondary erythrocytosis **A6**: Low plasma or serum EPO level **Clinical criteria MPL^{515} mutated ET** **A1**: Persistent increase of platelet count grade 1400 - 1500, grade II > 1500 x 10^9/L **A2**: Normal spleen or only minor splenomegaly on echogram **A3**: Normal LAP score, normal ESR and increased MPV **A4**: Spontaneous megakaryocyte colony formation (CFU-Meg) **A5**: No signs or cause of reactive thrombocytosis **A6**: No preceding or allied other subtype of MPN, PV, MDS or CML **A7**: Absence of Philadelphia chromosome. **Clinical criteria JAK2 wild type ET and PMGM** **A1**: No preceding or allied other subtype of MPN, PV, CML or MDS, JAK2 and MPL wildtype Early clinical stage: No anemia Normal hemoglobin, or anemia grade I: hemoglobin >12 g/dL, slight or moderate splenomegaly on palpation or >11 cm on ultrasound or CT. Thrombocythemia > 400 x 10/L Intermediate clinical stage: Mild anemia Anemia grade II, hemoglobin > 10 g/dL, definitive leuko-erythroblastic blood picture and/or tear-drop erythrocytes. Splenomegaly on palpation, no adverse signs Advance clinical stage: severe anemia Anemia grade III, hemoglobin < 10 g/dL, significant splenomegaly and one or more adverse signs	**B1**: Increased cellularity due to increased erythropoiesis or due to trilinear myeloproliferation of megakaryopoiesis, erythropoiesis and granulopoiesis (e.g. panmyelosis). Proliferation of small medium sized and large (pleomorphic) megakaryocytes. Absence of stainable iron, No or slight increase of reticulin fibers. **B2**: Spontaneous erythroid colony (EEC) formation. A1 + B1 and none of the others is idiopathic erythrocythemia: IE A2 + B1 and none of the others is ET with features of PV (prodromal PV) A3 and B1 and none of the other is primary MPD or latent PV A1 + B1 plus one of A2 to A6 or B2 is overt classical PV. **Pathological criteria MPL^{515} mutated ET** **B1**: Predominant proliferation of enlarged to giant megakaryocytes wit hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicious cytological abnormalities **B2**: Normocellular and no proliferation or immaturity of granulopoisis or erythropoiesis **B3**: No or only borderline increase in reticulin fibers. **Abbreviations** LAP: Leukocyte Alkaline Phosphatase; ESR: Erythrocyte Sedimentation Rate; MPV: Mean Platelet Volume; MPN: Myeloproliferative Neoplasm; PV: Polycythemia Vera; MDS: Myelodysplastic Syndrome; CML: Chronic Myeloid Leukemia. **Staging myelofibrosis (MF) according to MF grading** **Pathological criteria JAK2 wild type PMGM** **B1**: Megakaryocytic and granulocytic myeloproliferation (MGM) and relative or absolute reduction of erythropoiesis. Abnormal clustering and increase of atypical immature medium-sized large to giant megakaryocyte containing (Cloud-like) hypolobulated nucle and definitive maturation defects. **Staging of myelofibrosis: MF in PV and PMGM** MF 0: No reticulin fibrosis RF 0/1 MF 1: Slight reticulin fibrosis RF 2 MF 2: Marked increase RF grade 3 and slight to moderate collagen fibrosis MF 3: Advanced collagen fibrosis-osteosclerosis (endophytic bone formation)

Table 2: The 2008 European Clinical and Pathological (2008 ECMP) criteria [3] for the diagnosis of JAK2 mutated polycythemia vera (PV) [1,2], MPL^{515} mutated 'true 'ET [3-5] and JAK2 wild type hypercellular ET associated with primary megakaryocytic granulocytic myeloproliferation (PMGM) [5-8].

USA Subjective	UK Subjective	Grading of myelofibrosis (MF) Descriptive: silver impregnation, Masson stain	MF
RF 1	RF 1+	Scattered linear fine fibers with no intersections (cross-overs) and rare course reticulin fibers	MF 0 Prefibrotic
RF 2 No Masson stain	RF 2+ RF 3+ No Masson stain	Loose network of reticulin with intersections around megakaryocytes and in perivascular areas: silver impregnation No collagenisation: Masson stain	MF 1 Early reticulin fibrosis: RF
RF 3 No Masson stain	RF 4+ Dry tap	Diffuse and dense increase in reticulin with extensive intersections, occasionally only focal bundles of collagen and/or focal osteosclerosis	MF 2 Fibrotic
RF 4 No Massaon Stain	Dry tap	Diffuse and dense increased in reticulin with extensive interactions with coarse bundles of collagen, often associated with significant osteosclerosis	MF 3 Sclerotic

Table 3: Grading of reticulin fibrosis (RF) according to Ellis, Baumeister, USA, Manoharan, UK, and European grading of myelofibrosis (MF) according to the Hannover Bone Marrow Classification according to Georgii., *et al*. 1990 - 1996 and Thiele., *et al.* 2005 in bone marrow biopsies of patients with a chronic myeloproliferative disorder (MPD, 2001 WHO) or myeloprolifrative Neoplasms (MPN, 2008 WHO).

Reticulin fiber (RF) density should be assessed in cellular hematopoietic areas.

Source: Michiels JJ, Kvasnicka HM, Thiele J. MPD Doctors Brochure. http://www.mpn-stichting.nl/doctors_brochure_2004.pdf

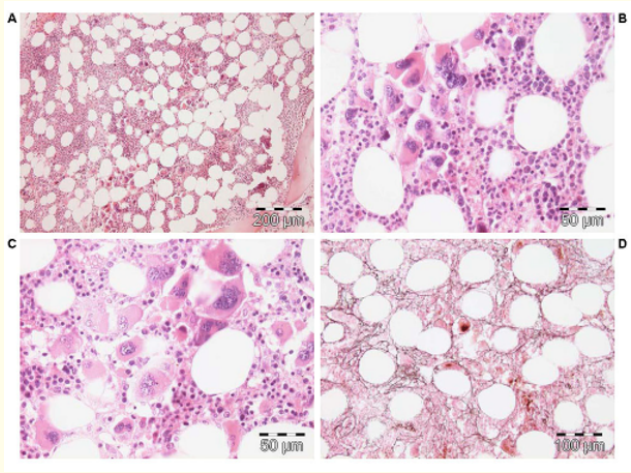

Figure 7: Clinical MF, JAK2 wild type ET: platelets 1430 x 109/L, anemia hemoglobin 11.3 g/dL, splenomgaly and high LDH 2730 → JAK2 wild type hypercellular (65 - 70%) bone marrow associated with early fibrotic PMGM, reticulin fibrosis grade. (Dr De Raeve)

PVSG versus ECMP and WHO criteria for ET, PV and PMF or PMGM: from Wasserman and Tefferi to Georgii and Michiels

Wasserman (1954) distinguished five stages in the natural history of PV [33,34] and proposed in 1971 a new set of crude major (A) and minor (B) inclusion criteria for PV in order to be sure that patients included in the PVSG 01 study indeed suffered from PV and not from secondary erythrocytosis. These crude PVSG criteria proposed by Wasserman (1971) were used by Berlin as the 1975 PVSG criteria, which has never been validated in prospective cohort studies [35]. The 1975 PVSG classification used increased red cell mass (RCM) but not bone marrow histology [36] and therefore needed 3 major and 4 minor clinical criteria as inclusion criteria the diagnosis of PV [35]. Analyses by Michiels revealed that PV patients with increased RCM are featured by hematocrit values between 0.48 and 0.76 in all, platelet count above 400 x 10^9/L in two-third and palpable spleen in two-third of about 400 PV patients in the PVSG-01 study [35]. Idiopathic Erythrocythemia (IE) is featured by increased hemoglobin, haematocrit, erythrocytes above 6 x 10^{12}/L and increased red cell mass but normal leukocytes, thrombocytes

and spleen size on palpation not meeting the official 1975 PVSG criteria for PV [37,38]. The category of IE comprises about 10 to 20% of the PV cases at time of presentation [37,38]. The 1980 RCP criteria proposed by Michiels used bone marrow histology plus platelet and erythrocyte counts to pick up myeloproliferative PV and IE and separate both IE and PV from primary, idiopathic and secondary erythrocytoses [39-45].

The PVSG reduced in 1986 the platelet count from 1000 to 600 x 10^9/l as the arbitrary minimum for the diagnosis of ET [25,25,26]. Michiels and Georgii used since 1980 a minimum platelet count of 400 x 10^9/L for thrombocythemia in ET, PV and PMGM. Michiels., *et al.* defined between 1999 and 2006 the ECP characteristics of the three Ph-negative MP.Ds ET, PV and PMGM [27] by including the bone marrow histopathology according to Georgii., *et al.* 1990 - 1998 to replace PVSG defined ET, PV and AMM [27,28]. The combination of a typical ET histological bone marrow picture with platelet counts in excess of 400 x 10^9/l is diagnostic for ET and latent PV when the ECP and ECMP criteria are applied (Table 4) [28-30].

PV: WHO-ECMP stage	**0**	**1**	**2**	**3**	**4**	**5**	**6**
WHO-ECMP Clinical Diagnosis	Prodromal PV	Erythrocythemic PV	Early PV	Manifest PV Classical PV	PV early MF Masked PV	Inapparent PV	Spent PV Post-PV MF
LAP-score	↑	↑	↑	↑	↑/↑↑	↑	Variable
EEC	+	+	+	+	+	+	+
Serum EPO	N/↓	N/↓	↓	↓	↓	↓	Variable
Erythrocytes x 10^{12}/l	<5.8	>5.8	>5.8	>5.8	>5.8	Normal <5.5	Decreased
Leukocytes x 10^9/l	<12	<12	<or >12	< or->15	>15	N or ↑	>20
Platelets x 10^9/l	>400	<400	< or >400	>400	< or >1000	N low or ↑	variable
WHO-ECMP bone marrow	Early PV	Early PV	Early PV	Trilinear PV	Trilinear PV	Prilinear PV	Myelofibrosis
Bone marrow cellularity (%) Grading reticulin fibrosis: RF Grading myelofibrosis: MF[57]	50 - 80 RF 0-1 MF 0	50 - 80 RF 0-1 MF 0	60 - 100 RF 0-1 MF 0	80 - 100 RF 0/1, MF 0	80 - 100 RCF1/2/3 MF 0/1	60 - 100 RCF 1/2/3 MF 0/2	Decreased RCF 3/ 4 MF 2/3
Splenomegaly on palpation	No/+	No	No/+	+	++/+++	++/+++	/Large
Spleen size, echogram cm Spleen size on palpation cm	<12 - 15 0 - 3	<13 NP	12 - 15 0 - 3	12 - 16 4 - 6	18->20 >6	16 >20 >6	>20 >8
JAK2^{V617F} in Granulocytes % JAK2^{V617F} in BFU-e (exon 12)	low +(++)	low +(++)	Moderate <50 +(++)	High >50 ++	High >50 ++	Mod/High +	High >50 ++
Risk stratification → Therapeutic implications Anno 2014	Low risk	Low risk	Low risk	Intermediate risk PV	High risk PV early MF	Wait/See IFN JAK2	Post-PV MF Spent phase PV
First line Aspirin/Phlebotomy Second line IFN versus Hydroxyurea (HU) Third line JAK2 inhibitor	Aspirin Phlebotomy	Aspirin Phlebotomy	Phlebotomy Aspirin Low dose IFN → resonsive	Phlebotomy* Aspirin IFN → resistant → HU	If IFN resistant → HU or JAK2 inhibitor	IF IFN Resistant JAK2 inhibitor	JAK2 Inhibitor → Bone marrow transplant

Table 4: Staging of JAK2^{V617F} positive prodromal PV, erythrocythemic PV, classical PV, early MF, inapparent PV, spent phase PV and post-PV myelofibrosis (MF) according to WHO-ECMP criteria related to therapy anno 2008 and beyond.

*↑: Increased; ↓: Decreased; N: Normal; +: Present or Heterozygous; ++: Homozygous.

Treatment recommendation of Polycythemia Vera related to MPN disease burden.
Designed by Michiels 2008 - 2020.

Using the minimum platelet count of 600 x 10^9/l for ET according to the 1986 PVSG, the Lengfelder ET study did excluded early (masked) ET at platelet count below 600 x 10^9/l in 29% of 143 ET cases whereas 97% of all 143 ET patients showed a typical MPD bone marrow histology [31]. Normal cellularity in 52% was consistent with ET, increased erythropoiesis in 17% was consistent with early PV, and increased cellularity due to pronounced granulopoiesis in 45% was consistent with prefibrotic PMGM [31]. From this it is concluded that both the 1975 PVSG and 2001 WHO classifications did overlook latent and early stages of MPD in patients with thrombocythemia: 1) initial ET with a typical ET bone marrow but platelet count below 600 x 10^9/l; 2) initial PV with a typical PV bone marrow, platelet count less than 600 x 10^9/l, low serum erythropoietin (EPO), normal red cell mass and hematocrit less than 0.51; 3) initial masked MPD with splenomegaly and normal or slightly increased platelet count and hematocrit. The translation of the 1975 PVSG into the 2007 WHO criteria by Tefferi and Thiele lowered the platelet counts from 600 to around 450 x 10^9/l for the diagnosis of ET and changed the term MPD into three variants of myeloproliferative neoplasia (MPN) [32]. ET, PV and primary myelofibrosis (PMF). Michiels and De Raeve improved the 2007 WHO revision of the MPNs by the introduction of bone marrow histology and erythrocyte count by the introduction of the European Clinical, Molecular and Pathological (2006 - 2008 ECMP) criteria for three distinct MPNs: $JAK2^{V617F}$ mutated ET PV and advanced PV with MF versus JAK2 wild type ET and MF carrying the MPL^{515} mutation; and JAK2/MPL wild type PMGM with features of PV (Tables 1, 2, and 3) [27-32].

Including bone marrow histology as a pathognomonic clue to each of the MPDs has been implemented in Europe since 1980 by Georgii, Vykoupil and Thiele [18], by Georgii in the Hannover bone Marrow classification (1990) [19,20], by Michiels (1987 - 1997) [10,24] and by Michiels and Thiele [45,46] but not in the UK and USA [46-55]. In retrospect Michiels demonstrated that the pretreatment bone marrow histology findings in 191 PV patients with increased RCM in the PVSG-01 study [40,41] did show up with a normal bone marrow cellularity with no increase of clustered large megakaryocytes (idiopathic erythrocytosis) in 7.5%, increased bone marrow cellularity (60 to 80%) in two-thirds (erythrocythemic, thrombocythemic early stage PV Figure 5), and trilineage hypercellularity (80 to 100%, classical PV, Figure 5) in one-third classic similar to trilinear "panmyelosis" as described by Dameshek (1950), Georgii., *et al.* (1990) [18-20], Michiels and Thiele (2002 - 2005) [45-50], and Michiels and De Raeve (2006 - 2020) as shown in Tables 1, 2 and 4 [28,45-55].

The 2007 WHO criteria only recommended bone marrow histology as a minor for ET and PV and introduced the $JAK2^{V617F}$ mutation as a major inclusion criterion for PV [32]. The translation of 1975 PVSG into 2007 WHO criteria by Tefferi and Thiele remained crude and not specific enough for three reasons [32]. First, for ET they only include normocellular ET, but the diagnoses of early $JAK2^{V617F}$ mutated PV (hemoglobin <18.5 for men and <16.5 for women) and ET associated with prefibrotic $JAK2^{V617F}$ mutated PV or EMGM bone marrow (MF-0) with no leukoerythroblastosis, anemia (hemoglobin below 12 g/dl) or myelofibrosis (MF-0) remain unclassifiable**.** Second, the 2007 WHO criteria for PV arbitrarily exclude the early idiopathic stage of $JAK2^{V617F}$ mutated ET mimicking PV and overlooked masked PV. Simple tests like platelet, leukocyte and erythrocyte counts above 6 x 10/L [11,50], and spleen size on echogram are not taken into account to distinguish the early thrombocythemic and erythrocythemic stages of PV from the classical trilinear PV showing erythroid, granulocytic and megakaryocytic (EMG) myeloproliferation in the bone marrow. Third, in the application of the 2007 WHO criteria proposed by Tefferi and Thiele for primary myelofibrosis (PMF) without leukoerythroblastosis or anemia proved to become the third prefibrotic entity of so-called prefibrotic PMGM (MF-0) when the ECMPN criteria are applied [32]. These three shortcomings of the 2007 WHO diagnostic criteria for MPN by Tefferi., *et al.* [32] will hamper to prospectively evaluate the natural history, and therapeutic implications of objective staging of ET and PV MPN disease burden related to therapy. To overcome the shortcomings we here update and extend the 2008 ECMP criteria for the diagnosis, classification and staging of true ET, PV and PMGM (Table 1, 2 and 3) [28,30,52-54].

Limitations of 1975 PVSG and 2007/2008 WHO criteria for ET and PV

Red cell mass (RCM) is increased in Inapparent PV (IPV) due to pronounced splenomegaly with hypersplenism as the cause of normal values for haemoglobin, haematocrit and erythrocytes [55]. In 105 patients with WHO-defined PV, RCM had a sensitivity of 76% in the diagnosis of PV and a specificity of 79% in distinguishing PV and non-clonal polycythemia [56]. Early and over PV patients with no or minor splenomegaly and increased RCM usually have increased erythrocyte values above 6 x 10^{12}/L [50,57]. In 77 WHO defined PV patients (31 males and 46 females) only 35% of male and 63% of female PV patients had WHO defined Hb values above 18.5 and 16.5 g/dL respectively [58]. Initial latent PV as documented by typical PV bone marrow histology increased platelet counts (600 - 1260 x 10^9/L) could be diagnosed as masked PV by the ECP and ECMP criteria [48].

Spontaneous EEC formation and decreased serum EPO levels are specific confirmative criteria for the diagnosis of $JAK2^{V617F}$ mutated ET and PV [58-71]. De Stefano and Michiels (1997) reviewed 120 cases with splanchnic vein thrombosis (Budd-Chiari syndrome 51, and portal/splenic and/or mesenteric vein thrombosis in 69) and found that MPN was diagnosed in 80 MPD patient using spontaneous EEC and bone marrow data were overt PV in 37 (31%), ET in 2, MF in 2, and latent (masked) MPD in 39 (32.5%) [72]. Accounting clustered enlarged or giant megakaryocytes as diagnostic for MPN enabled Dr Briere of Paris MPD study group to subsequently diag-

nose MPD in 46 out of 128 patients with splanchnic vein thrombosis (SVT) either hepatic vein or portal vein thrombosis [73,74]. The sensitivity as compared to bone marrow as the gold standard for the diagnosis MPN was 63% for increased RCM, 52% for low serum EPO level, 72% for EEC formation, and 74% for splenomegaly [73,74]. JAK2^{V617F} mutation screening of 274 patients with splanchnic vein thrombosis in four studies appeared to be a specific clue to MPN in 86 cases (31%) [71-74]. Additional studies in patients with SVT including Budd-Chiari syndrome (BCS) showed that the combination of JAK2^{V617F} mutation screening and bone marrow histology assessment became has a near to 100% sensitivity and specificity for the diagnose of trilinear JAK2^{V617F} mutated MPNs ET, PV, EMGM and MF [75-80].

Signs and symptoms in ECP/ECMP defined Dutch ET, PV and MF patients

The 2008 MPN Questionaires of the Dutch MPN Patient Foundation used the ECMP guidelines for diagnosis, staging and treatment recommendations of ET and PV patients in The Netherlands between 2000 and 2008 [24,27,28,30,46,81]. Using ECMP criteria, 497 MPDs patients were diagnosed as ET in 181 (36%), PV in 244 (50%), MF in 67 (13%), and MPN unclassifiable in 5 (1%). The JAK2V617F mutation was detected in 60% of ET, 91% of PV and 52% of MF patients, which is in line with literature as reviewed by Michiels., *et al.* 2006 [30,52]. Based on the Dutch MPN questionaire including 36 questions the top 20 complaints at time of diagnosis in 399 out of 497 (81%) MPN patients is shown in table 6 [81]. The most frequent complaint is fatigue (81%) equally high in ET, PV and MF patients. The microvascular erythromelalgic signs and symptoms in ET are characterized by tingling and prickling sensations in footsoles, handpalms, toes and fingers, cognitive concentration and visual disturbances (Table 5) [81]. Itching was recorded in 58% of PV vs 30% of ET patients. Fatigue was much more prominent in PV. Night sweats related to splenomegaly was noted in about half of the MPN patients and bone pain inone third of MPN patients (Table 5). MF patients suffered from combined constitutional symptoms, fatigue, night sweats and symptomatic splenomegaly [81].

Symptom	Top 20 MPN complaints	All MPN	MPN	ET	PV	MF
		N = 497	%	%	%	%
1	Fatigue, listless	399	81	80	81	85
2	Microvascular acra [97]	278	57	61	56	46
3	Cognitive disturbances [98,99]	262	53	52	56	45
4	Visual disturbances [98,99]	249	51	50	52	46
5	Night sweats	236	48	44	50	52
6	Itching	220	45	30	58	36
7	Dizziness	218	44	44	46	39
8	Bruises, bleedings	211	43	40	45	43
9	Splenomegaly constitutional symptoms	198	40	22	43	78
10	Tinnitus	188	38	38	39	37
11	Migraine headache without visual symptoms	184	37	46	35	22
12	Bone pain	172	35	33	36	34
13	Heart arrythmias	154	31	34	31	24
14	Dysarthria, dyslexia,	151	31	31	31	30
15	Hypersensitive to sounds and noices	149	30	29	32	28
16	Paleness	145	29	30	26	40
17	Claudicatio intermittens	140	28	28	30	24
18	Hypersensitive to lights	136	28	25	32	16
19	Visual disturbances without headache	18	33	54	3	90
20	Headache without visual symptoms	24	43	43	4	90

Table 5: Top 20 clinical manifestations in Dutch patients with ECMP defined myeloproliferative neoplasm (MPN) essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF) based on the Dutch MPN Questionaire 2009 - 2011 [81].

Microvascular acra: Tingling,prickeling sensations, redness,swelling and/or bluish discolouration of footsoles, handpalms, toes and/or fingers [96,97].

Cognitive disturbances of concentration and memory and sudden attacks of unconscienceness.

Visual disturbances of scintillating scotomas, light flashes, blurred vision, transient monocular blindness, rapid spreading of visual figure disturbances [96-99].

Attacks of migraine-like headaches followed by nausea or vomiting or loss of consciencenous or transient paresis of one extermity [96-99].

Molecular Step 1 V617F+	Molecular Step 1→ 2 V617F++ 9p LOH	Molecular Step 1→ 2 V617F++ 9p LOH
Spontaneous **CFU-MK/EEC** **BM: ET picture**	**Spontaneous** **EEC/CFU-MK** **ET-PV picture**	**PVR-1 =↑ LAP score↑** **Granulocytes growth/activation** **Myeloid metaplasia: PMGM**
Increase of enlarged hypersensitive platelets Platelet > 400 x 10^9/L	Increase of hematocrit to above 0.45–0.50: PV Platelets > 400 x 10^9/L	**Leukocythemia**/cytokines↑↑ Fatigue, splenomegaly, masked PV, MPD, MF
Clinical step 1 → Microvascular → Thrombosis Treatment Aspirin sensitive	Clinical step 2 → Macrovascular → Thrombosis Aspirin/phlebotomy	Clinical step 3 Secondary MF: 30% Constitutional symptoms IFN/hydroxyurea

Table 6: 2005 Molecular Etiology of Platelet-Mediated Microvascular Thrombosis, Increased Red Cell Mass, and Secondary Myelofibrosis in JAK2^{V617F}-Positive MPDs (ET, PV, and PMGM: JAK2^{V617F} Gain of Function Mutation in Trilinear Hematopoietic Cells of MPD Patients is Detectable in Platelets, Erythroblasts, and Granulocytes.

MPD: Myeloproliferative Disorder; ET: Essential Thrombocythemia; PV: Polycythemia Vera; PMGM: Chronic Secondary Myelofibrosis; LOH: Loss of Heterogeneity; CFU-MK: Colony-Forming Units Mega-Karyocytes; EEC: Endogenous Erythroid Colony; LAP: Leukocyte Alkaline Phosphatase; BM: Bone Marrow; IFN: Interferon.

Designed by Michiels 2006 [30,52].

The cohort of 497 MPN patients were treated low dose aspirin or calcium carbasalate aetylsalicylic acid (Ascal) in 70%, phlebotomy in 42% (mainly PV 91%), hydroxyurea in 29%, and pegylated interferon-alpha2a in 7% [81]. Additional treatment with myeloreductive agents on top of low dose aspirin during follow-up was instituted in 294 (60%) of MPN patients: ET in 64% (n = 115), PV in 59% (n = 143) and MF in 49% (n = 33). Out of 459 evaluable MPN patient adverse drug reactions or side effects were recorded in one third (N = 168 = 35%) of MPN patients, which could be related to HU in 41% (n = 69) and to IFN in 28% (n = 47). Side effects of HU included dry skin, skin lesions, skin ulcers, skin carcinoma, brittle nails, aphtous ulcers and hair loss [81]. Most frequent side effects of IFN were flue-like symptoms, fatigue and mood disturbances [81]. Low dose aspirin or Ascal induced gastritic complants in 11% for which treatment with metronazol was usually indicated [81].

JAK2^{V617F} mutated trilinear MPN: Dameshek - Vainchenker's disease 1940 - 2005 [12,13]

Trilinear PV [11] orignates from the JAK2^{V617F} mutation as the driver cause of ET, PV, masked PV and MF (Figures 1, 2 and 3, Table 6) [12,82-84]. The JAK2^{V617F} tyrosine kinase "dosage" hypothesis in heterozygous versus homozygous JAK2^{V617F}mutated MPN is based on different densities of TPO receptors (TPOR or MPL)) and EPO receptors (EPOR) on hematopoietic progenitor cells and on differences of response of TPOR and EPOR to various levels of JAK2^{V617F} activity Figures 2 and 3, Table 6) [83,84]. The TPO/MPL receptor TPOR-MPLR is the ligand expressed on megakaryocytic cells that binds and controls physiological TPO levels. Activation of a few TPO receptors by low levels of JAK2^{V617F} (heterozygous ET) is sufficient to send a signal to megakaryocytic cells [12,30]. A slight increase in numbers of mutated large (giant) megakaryocytes and platelets (about 50 to 100 x 10^9/l mutated platelets) will produce platelet-mediated microvascular circulation disturbances (Table 6) [30]. High levels of JAK2^{V617F} tyrosine kinase activity in homozygous mutated hematopiotic progenitor cells is required to activate EPOR and generate a PV-like phenotype [30,83,84]. Such high levels of JAK2^{V617F} activating both EPOR and GCSFR are associated with progressive MPN disease complicated by extramedullary hematopoiesis (splenomegaly) and cytokine mediated secondary myelofibrosis (Figure 3, Table 6). The percentage of JAK2^{V617F} positivity and progression from heterozygous to homozygous is strongly correlated with the ability to form spontaneous EEC formation. In the UK basic research study, BFU-e colonies are already homozygous for the JAK2^{V617F} mutation in PVSG defined PV patients in early, classical and advanced stages of PV (Figure 8) [85]. The BFU-E colonies from heterozygous in PVSG defined ET patients and did not contain a subpopulation of JAK2^{V617F} homozygous cells [85]. The result of a large French study of JAK2^{V617F} positive PV (N = 159, 36% homozygous) and ET (N = 147, 4% homozygous) and genotyped BFU-E colonies in in 20 PV and 6 ET patients were similar and showed that heterozygous mutated JAK2^{V617F} ET patients harbour heterozygous BFU-E clones, some PV patients have a purely heterozygous profile, and most PV patients harbour a mixture of heterozygous and homozygous BFU-E clones (Figure 8) [86].

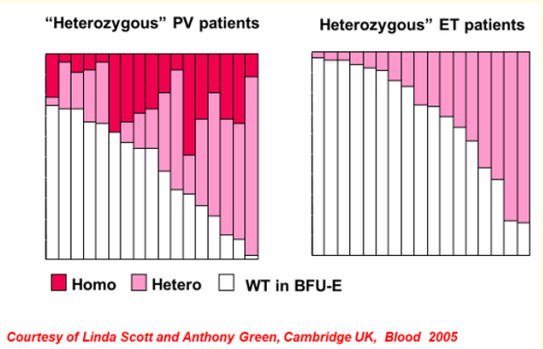

Figure 8: Genotype of individual BFU-E in PVSG defined 17 ET and 17 PV patients. ET patients are featured by a mixture of normal BFU-e and heterozygous JAK2^{V617F} mutated BFU-e clones. PV patients are featured by a mixture of normal and combined heterozygous/homozygous JAK2^{V617F} mutated BFUe clones in the study of Scott., *et al*. [85].

The JAK2^{V617F} allele load in heterozygous ET ranged from below 10% to 40% and in JAK2^{V617f} heterozygous/homozygous mutated OV from about 30% to 85%. Reproduced by the Courtesy of Dr Green, Cambridge.

Mutated erythroid progenitors are more sensitive to EPO than normal progenitors, and most homozygous progenitors are EPO independent. In this cohort of 306 JAK2^{V617F} positive MPD patients, homozygous JAK2^{V617F} mutated PV was associated with significantly lower platelet counts and higher hematocrit and granulocyte values than in heterozygous JAK2^{V617F} mutated ET patients. The highest platelet count was associated with low JAK2^{V617F} allele burden in PV, whereas high JAK^{V617F} allele burden correlated with increased haemoglobin, hematocrit and erythrocytes above 6 x 10^{12}/L [86]. Transition from heterozygosity to homozygosity for the JAK2^{V617F} mutation represents a very important step in the progression from early to classic PV and subsequent post-PV myelofibrosis (Figures 2 and 3, Table 6) [30,85,87]. Comparing JAK2^{V617F} heterozygous and homozygous PV patients showed that homozygous mutated JAK2^{V617F} PV patients have high JAK2^{V617F} mutation load and displayed significantly higher hemoglobin at time of diagnosis and have a higher rate of fibrotic transformation. Some PV and MF patients displaying a gain of 9p are due to trisomy 9 as the cause of advanced PV [88,89]. Campell., *et al.* reported JAK2^{V617F} mutation associated with trisomy 9 in nine MPN patients [90]. JAK2$^{exon\ 12}$ mutations in 10 erythrocytosis patients with increased red cell mass but no JAK2^{V617F} in the study of Scott el could be diagnosed as PVSG defined PV in 6 and idiopathic erythrocytosis (IE) in 4 [91].

MPL^{515} mutated normocellular ET: From Vainchenker to Tefferi and Vannucchi

The JAK2 kinase activity in MPNs is not only dependent on the amount of heterozygous and homozygous JAK2^{V617F} mutant protein but may also be influenced by the various steps upstream or downstream the signalling pathways including MPL, JAK2, STAT-3 (Figure 2). MPL transgenic mice manifested with typical features of ET with a four-fold increase of platelet count, increased colony formation of megakaryocytes, and increase of clustered enlarged megakaryocytes in the bone marrow (Figure 9) [92]. Acquired MPL^{W515L} and MPL^{W515K} gain of function mutations has been discovered as the underlying molecular driver cause in MPN patients with normocellular ET [93,94] by screening of 1182 PVSG-defined MPD patients (318 ET, 242 PV, and 290 IMF) and 64 controls for MPL^{515} mutations. This basic research study discovered MPL mutations either MPL^{W515L} (n = 17) or MPL^{W515K} (n = 5) in 20 MPN patients (MF in 4%, ET in 4 = 1%, and post-ET myelofibrosis in 1), but not in the 242 PV patients and controls [93]. MPL^{515} mutated ET and MF is a distinct entity of JAK2 wild type MPN without features of JAK2^{V617F} mutated PV. The clinical presentation of 30 ET patients carrying the MPL^{515} mutation (18 MPL^{W515L} and 12 MPL^{W515K}) at diagnosis and follow-up in the Italian study (Vannucchi., *et al.*) revealed a high incidence of major arterial event in 23%, venous thrombosis in 10%, microcirculatory disturbances in 60%, and major hemorrhage in 7% [95]. Bone marrow histology in MPL$^{W515L/K}$ mutated patients is featured by increased number of clustered large to giant megakaryocytes and no significant increase in reticulin fibrosis in a normocellular bone marrow (Figure 9) without features of prodromal or classical PV (Figure 5) or PMGM (Figure 6 and 7) [85].

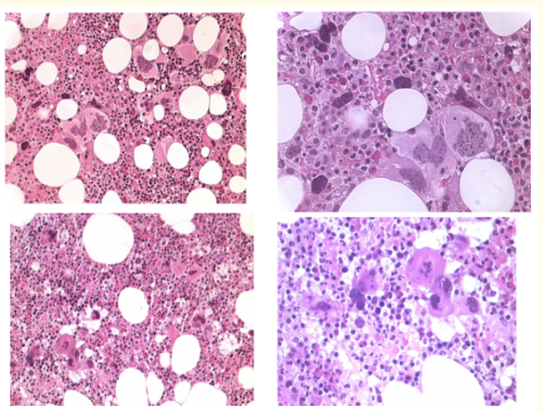

Figure 9: Bone marrow histology findings in JAK2 wild type, MPL^{515} mutated ET showing large and giant mature megakaryocytes with loose clusters of hyperlobulated, "stag-horn" hyperlobulated nuclei. Case 1, upper panel Case 2 lower panel, Bone marrow slides were kindly provided in 2008 by Dr. Vannucchi, Italy [95].

Acquired JAK2^{V617F} mutated ET and TPO mutated hereditary ET (HET)

Michiels (1985 = 2020) defined JAK2^{V617F} mutated ET preceding PV by platelet count in excess of 400 x 10^9/L and increase of clustered enlarged megakaryocytes in bone marrow smears and biopsies as pathognomonic clues to the diagnosis of ET, and increased platelets above 400 x 10^9/l and erythrocytes above 6 x 10^{12}/L for the diagnosis of JAK2^{V617F} mutated PV [97-100]. The Dutch family with autosomal dominant hereditary essential thrombocythemia (HET) due to a gain of function mutation in the TPO gene was diagnosed at the clinical bone marrow level (Figure 10) by Michiels as ET caused by marked increased TPO levels and associated with

microvascular circulation disturbances including erythromelalgia and atypical transient ischemic attacks similar to acquired ET [101,102]. Bone marrow morphology of affected member of the Dutch HET family showed large mature megakaryocytes with hyperlobulated nuclei in bone marrow smear and biopsy (Figure 10, personal observations Dr Michiels) similar to and indistinguishable from acquired heterozygous JAK2^{V617F} mutated ET. TPO mutated HET affected patients have no features of PV, normal EEC and no splenomegaly [103]. Autosomal dominant hereditary ET (HET) due to a gain of function mutation of the c-MPL gene (MPL^{S505N}) anno 2008 has been described in one family [104].

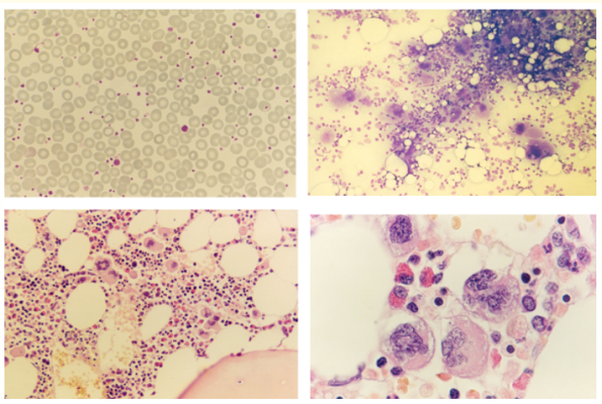

Figure 10: Peripheral blood and bone marrow findings from the proband of the Dutch Family with hereditary essential thrombocythemia (HET) caused by to a gain of function mutation in the TPO gene [96,97].

A: Increased platelet with the presence of a few large platelets in a peripheral blood smears from EDTA blood.

B: Smears from aspirated bone marrow showed a pronounced increase and clustering enlarged mature megakaryocytes, slightly increased cellularity, and normal maturation of erythropoiesis and granulopoiesis (erythroid, myeloid, lymphoid ratio = 43 : 47: 10).

C: Bone marrow biopsy specimen revealed a pronounced increase and ring of enlarged mature megakaryocytes with hyperlobulated nuclei, a slight increase of cellularity and normal maturation of erythropoiesis and granulopoiesis (hematoxylin and eosin stain: H&E).

D: Slightly scattered linear reticulin with no intersections (crossover) corresponding to normal bone marrow, myelofibrosis (MF) grade 0 (Gomorri stain). Source bone marrow biopsy 1991: Dr. R.W. Veldhuizen, Department of Pathology, Westeinde Hospital, The Hague, Netherlands.

Diagnostic work-up of ET patients 1980 - 2008 and beyond

The 2008 ECMP criteria proposed by Michiels and De Raeve separate JAK2^{V617F} mutated ET patients into three phenotypes of prefibrotic MPNs at the bone marrow level: normocellular ET, early PV mimicking ET (prodromal PV and ET with MGM (MF-0) bone marrow without features of leuko-erythroblastosis in the peripheral blood (Table 2) [103-112]. Suspicion of ET patients starts with the recognition of microvascular circulation disturbances including atypical and typical TIAs, ocular ischemic attacks, erythromelalgia (Table 6), and splanchnic vein thrombosis (Figure 11) [92-95]. Persistent platelet counts (>400 x 10^9/l) associated with slight splenomegaly on echogram (>12cm), increased leukocytes (>12 x 10^9/l), or LAP score with normal ESR (erythrocyte sedimentation rate) is highly suspicious of ET or thrombocythemia in various MPN (Figure 11). Presence of giant platelets in a peripheral blood smear and normal ESR is indicative for ET and excludes reactive thrombocytosis. JAK2^{V617F} mutation detection is diagnostic for ET and thrombocythemia in PV. Only half of ET and MF patients carry the JAK2^{V617F} mutation (sensitivity 50-60%). Bone marrow biopsy detects all variant of thrombocythemia in JAK2^{V617F} or MPL^{515} mutated MPNs and in JAK2/MPL wild type ET and MF (Figure 9). The 2008 ECMP criteria classify JAK2^{V617F} mutated ET (Table 1) as: normocellular ET (Table 2); early PV mimicking ET (Table 1); ET associated with MGM (RF-0 or RF-1) without features of leuko-erythrocytosis and extramedullary hematopoiesis (Table 1); and post-ET MGM with MF-1, 2 and 3 and features of leuco-erythroblastosis (Table 1). The 2008 ECMP criteria distinguish thrombocythemia in various MPNs from thrombocythemia associated with Ph^1-chromosome and *bcr/abl* positive CML [113] or myelodysplastic syndromes (MDS) including the so-called 5q-syndrome, which clearly differs from refractory anemia with ringed sideroblasts (RARS) and significant thrombocytosis (RARS-T) (Figure 11) [114-117]. Among nine reported cases of RARS-T patients, six showed the presence of JAK2^{V617F} mutation [116,117]. JAK2^{V617F} positive ET is characterized by higher values for hemoglobin, hematocrit, neutrophil counts, increased LAP score, decreased serum EPO levels, serum ferritin and MCV, and by increased bone marrow cellularity [113,114], which is consistent with thrombocythemic latent PV mimicking ET and has been described as "forme fruste" PV, stage 1 PV (Tables 2 and 4). JAK2 wild type ET patients represent a distinct category featured by significantly higher platelet counts, normal LAP score, normal

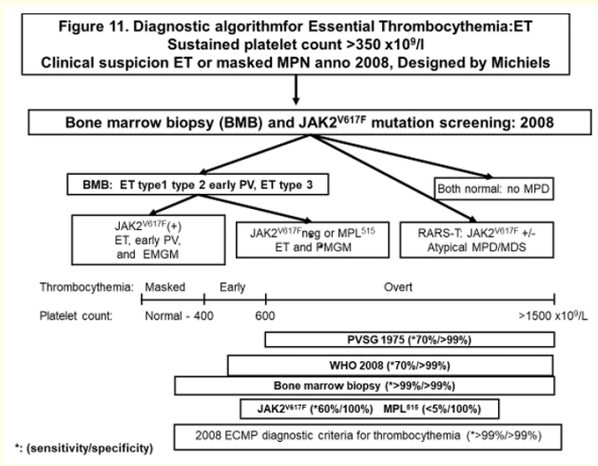

Figure 11: Algorithm for diagnostic work-up for patients with suspected thrombocythemia as the presenting feature of ET, early PV, prefibrotic PMGM (EMGM), early fibrotic PMGM or refractory anemia with increased ringed side oblasts (RARS-T) [112]. Designed by Michiels 2008. For explanation see text.

Diagnostic work-up of PV patients 1980 - 2008 and beyond

PV patients usually present with plethora, headache, TIAs, erythromelalgia (Figure 12) [97-100], splanchnic vein thrombosis [72-80], and microcytosis of erythrocytes due to iron deficiency [118,119]. ECMP features of PV include increased hematocrit (>0.51), increased erythrocytes (>6 x 10^{12}/l), slight splenomegaly, increased leukocytes (>12 x 10^9/l) or LAP score with normal ESR, increased platelets (>400 x 10^9/l). Bone marrow histology is normal in patients with congenital erythrocytosis due to gain of function mutation in the EPOR gene or acquired erythrocytosis. The detection of $JAK2^{V617F}$ in the diagnostic for PV In the context of erythrocytosis (hematocrit > 0.51 in males and >0.48 in females) the presence of a sensitivity of 95% and positive predictive value of 100% and excludes congenital and secondary erythrocytosis (Figure 12) [120,121]. EEC and low serum EPO significantly contribute but are not sensitive enough for the differential diagnosis of PV versus primary and secondary erythrocytosis, whereas JAK2exon 12 mutated PV is featured by EEC, decreased serum EPO and a typical PV bone marrow (Table 5) [68-70,88,127].

Reticulin and collagen fibrosis of the bone marrow (myelofibrosis MF) is a secondary event produced by polyclonal fibroblasts in response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in both PV and ET (Table 3) [18-20,24,128]. Transformation to myelofibrosis is rare in ET, occurs in about one third of PV and ET associated with PMGM (MF-0) during long-term follow-up [103-109]. The grading of reticulin fibrosis (RF) according to Georgii [19,20] and Thiele (Table 3) [129-131] can be quantified by using the European scoring system based on morphometric analysis (point intersection with an ocular grid) and quality of fibers (reticulin and collagen fibers) [19,20] and the bone marrow fiber density (fine or course reticulin and some or course bundles of collagen) (Table 3) [24,131].

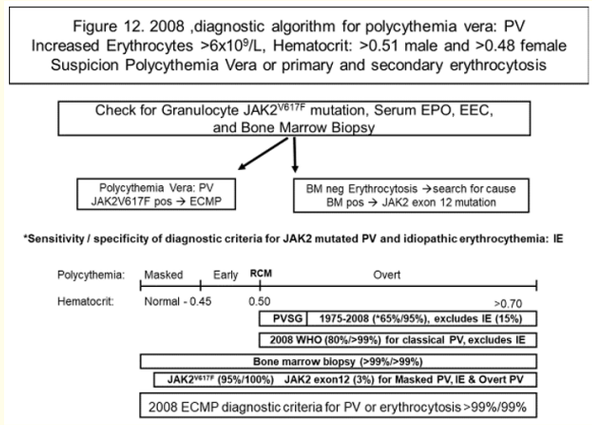

Figure 12: Algorithm for diagnostic work-up of patients with suspected polycythemia vera versus primary or secondary erythrocytosis. Designed by Michiels 2008. For explanation see text.

$JAK2^{V617F}$ allele burden and MPN disease progression in ET, PV and MF 2005 - 2008

In PVSG-defined USA cohort of 84 ET, 92 PV, and 19 fibrotic MF patients the $JAK2^{V617F}$ mutation was detected in 92% of PV, in 45% of ET, and in 42% of fibrotic MF [132]. Median $JAK2^{V617F}$ allele burden was significantly lower in ET (47%) than in PV (67%) in PV patients (p = <0.001) (Figure 13). When stratified for disease duration, a $JAK2^{V617F}$ burden of 100% (homozygosity) was present in only 15% of PV less than three years from diagnosis compared to 40% of PV three to 10 years and 40% of PV between 10 and 26 years since diagnosis, but in none of ET patients during very long-term follow-up (Figure 13) [132]. In the Italian study the $JAK2^{V617F}$ mutation was detected in 92% of 25 PV, in 53% of 19 ET, in 58% of 12 MF-0 (ET associated with MGM) in 56% of 18 fibrotic MF, and in 100% of 16 post-PV myelofibrosis patients (Figure 14) [84]. ET and p-MF patients had significantly lower percentage of mutated alleles than patients with PV (p = 0.01), whereas patients with f- MF had much higher values than p-MF or ET (p = 0.0008) (Figure 8). Circulating CD34 positive circulating cells were normal all patients with PV (N = 25), ET (N = 19) and p-CIMF (N = 12) and 6 out of 18 f-MF patients had normal (<10 x 10^6/L) circulating CD34 cells (figure 12). Conversely, 12 out of 18 f-MF and all post-PV MF (16) had increased CD34 circulating cells (Figure 14). Post-PV myelofibrosis had the highest percentages of mutant alleles approaching 100% homozygosity (Figure 8). PV and MF patients with a high mutation burden (granulocytes mutant alleles in excess of 50%) have leukocytosis, splenomegaly, increased LDH levels, increased circulating CD34-positive cells, a worse event free survival and a compromised overall survival as compared with those with lower mutation burden (granulocyte mutant alleles of 1 - 50%) mainly seen in ET and early stage PV [84].

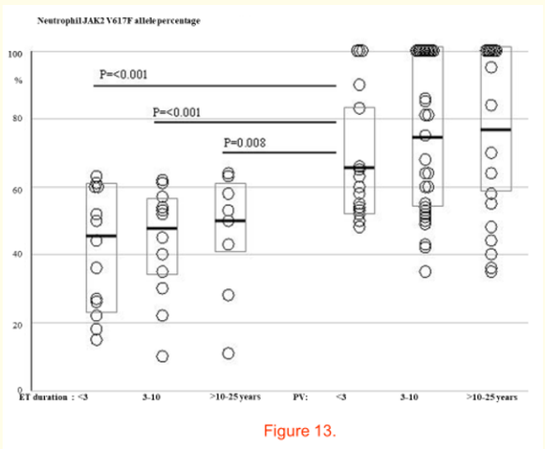

Figure 13.

Figure 13: Results of the USA study on neutrophil JAK2^{V617F} allele percentages (%) related to disease in 36 PVSG-defined ET and 77 PV patients (upper left) [132]. Median neutrophil JAK2^{V617F} allele % were significantly higher in PV than those for ET, regardless of disease duration. Within PV the differences in neutrophil JAK2^{V617F} allele % as a function of disease duration were not statistically significant. This may be indicative for good risk PV and poor risk PV with neutrophil JAK2^{V617F} allele burden between 30 to 80% and between 80 and 100% respectively.

Neutrophil genomic DNA and platelet cDNA from the same blood samples in 13 ET and 23 PV patients (upper right) [140]. First, median neutrophil JAK2^{V6217F} allele % in PV were greater than in ET (P = <0.001). Second, median platelet JAK2^{V617F} allele % in ET were lower than in PV (P = <0.002). Third, median neutrophil JAK2^{V6217F} allele % in ET were lower than platelet JAK2^{V617F} allele % in ET (P = <0.001). Reproduced with the courtesy of Dr Jerry Spivak, Baltimore, USA.

Bock., *et al.* evaluated the JAK2^{V617F} mutation using PCR techniques in bone marrow cells derived from bone marrow trephine biopsies from 79 MPN patients classified according to WHO bone marrow criteria [133,134]. The JAK2^{V617F} mutation was found in 90% of PV (n = 29), 22% in EMGM (MF-0, n = 18), 60% in advanced MF (n = 20), and 27% in ET (n = 15), but not in CML (n = 5), acute leukemia (n = 20) secondary erythrocytosis (n = 10, or normal bone marrow (n = 10). The JAK2^{V617F} mutation occurred at a lower frequency in ET never exceeding 50% of alleles indicating heterozygosity and exceeding 50% of alleles indicating homozygosity in PV and fibrotic MF. Horn., *et al.* studied 152 paraffin-embedded trephine bone marrow biopsies from patients with MPN diagnosed according to WHO bone marrow criteria for the presence of the JAK2^{V617F} mutation using PCR techniques [135]. The JAK2^{V617F} mutation was detected in 27 of 28 (96%) cases of PV, 17 of 23 (74%) cases of ET, 28 of 45 (75%) of MGM with MF-0 to 3, in 8 of 12 (75%) cases of MPN unclassifiable or with MDS/MPD syndrome, but not in Ph-chromosome positive CML (n = 4), secondary erythrocytosis or reactive thrombocytosis (n = 15) and controls (n = 19) [135]. 2008 ECMP defined JAK2 wild type ET and MF lack specific PV laboratory and pathological features at diagnosis and during follow-up. This has been demonstrated for MPL^{515} mutated (ET/MF) and for JAK2/MPL wild type hypercellular ET in PMGM in the MPN studies of Michiels and De Raeve.

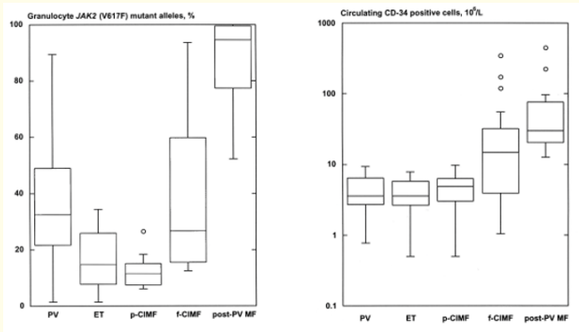

Figure 14: Results of the Italian study on granulocyte JAK2^{V617F} mutation burden (%) in WHO-defined MPN patients 23 PV, 10 ET, 7 prefibrotic CIMF (p-CIMF) 10 fibrotic CIMF (f-CIMF) and 16 post-PV MF patients (lower left) [81]. First, patients with PV had higher JAK2^{V617F} % than ET (p=0.01) and p-CIMF. Second, patients with p-CIMF had much lower JAK2^{V617F} allele % than f-CIMF. Third, patients with post-PV myelofibrosis (MF) had the highest JAK2^{V6217F} allele %. Please note that the term JAK2^{V617F} mutated CIMF has been replaced by EMGM = prodromal or masked PV when the ECMP classification is applied.

Circulating CD34 positive cells (lower right) [81]: all patients with PV (N = 25), ET (N = 19) and p-CIMF, (N = 12) and 6 out of 18 f-CIMF patients had normal (<10 x 10^6/L) circulating CD34 cells. Conversely, 12 out f 18 f-CIMF and all 16 post-PV MF patients had increased CD34 circulating cells. These data indicate that ET and p-CIMF are not different at the molecular (JAK2^{V617F}) and biological (CD34 cells) level. This arises the question whether WHO defined JAK2^{V6217F} p-CIMF and ET are the same (PVSG-defined ET) or distinct in their natural history during long-term follow-up. Reproduced by the courtesy of Dr Francesco Passamonti, Pavia, Italy [84].

Historic appraisal and therapeutic options of MPNs 1980 - 2020

Michiels and De Raeve changed and integrated the 1975 PVSG as well as the 2008/2016 WHO criteria into the ECMP and Clinical Laboratory Molecular and Bone Marrow Pathology (CLMP) classification for staging of the JAK2, MPL and CALR mutated MPNs (Figure 15) [136-156]. The incorporation of PVSG/WHO into the ECMP/CLMP classifications able clinicians and scientist to much better prognosis assessment and to targeted treatment options including aspirin/phlebotomy followed by interferon as the first option to postpone or even eliminate hydroxyurea in prefibrotic early stage ET and PV patients [136-156]. This incorporation proces of PVSG/WHO into ECP/ECMP classification started in 1998 with the Rotterdam Workshop on Myeloproliferative Disorders (MPD) organized by Jan Jacques Michiels [136]. Dr. Nathaniel Berlin was invited as honorary guest on behave of the PVSG and to introduce

the PVSG concept and classification of PV for the education of EWG. MPD investigators. PVSG defined MPD include ET, PV, and PMF as three distinct disease entities. Primary Myelofibrosis (PMF) of the bone marrow is not a disease but is a reactive feature consecutive to neoplastic myeloproliferation and of hematopoiesis when the Hannover Bone Marrow and ECMP criteria for MPD are applied. Advanced disorders of MPD of various molecular etiology comprise early myelofibrosis (MF) stage MF 0/1, myelofibrosis stage MF 2 and MF 3, advanced myelofibrosis with osteosclerosis, and excess of blasts and blasts crisis. Michiels and De Raeve extended the 2008 ECMP classification into the 2018-2020 CLMP based on bone marrow histology studies in well defined JAK2^{V617F}, JAK2^{exon12}, MPL^{515} and CALR mutated patients (Figure 15) with the help and scientifcic collaboratiion between the Rotterdam, Antwerp, Brussels and Seoul MPN investigators in Europe and Korea [139-156]. The definitive incorporation the 2008/2016 WHO classifications into the 2008-2020 ECMP/CLMP classification has been documented in a series of manuscript on the sequential updates of 2014/015 ECMP, and the 2018/2020 CLMP classification for at least five distinct clonal MPNs caused by the JAK2^{V617F}, JAK2^{exon12}, TPO, MPL^{515} and CALR driver mutation leaving a small group of quintuple negative group of MPN (Figure 15) [136-156].

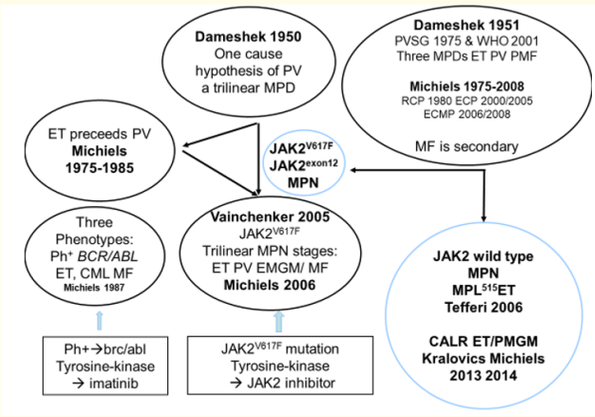

Figure 15

Left: Historical appraisal of myeloproliferative disorder/neoplasms: MPD/MPN classifications 1950-2020. The one cause hypothesis of PV (Dameshek 1950) has been confirmed by the discovery in 2005 by Constantinescu and Vainchenker of the JAK2^{V617F} mutation as the driver cause of ET, PV and MF.

Left below: *BCR/ABL*-positive JAK2 wild malignancy runs through sequential stages of ET, CML, MF and obligate blastic transformation within 10 to 15 years.

Right: The PVSG/WHO MPD/MPN classifications of ET, PV and PMF has been separated by the ECP and ECMP classifications into JAK2^{V617F} mutated ET and PV and in JAK2 wild type thrombocythemia without features of PV. The majority of JAK2 wild type ET/thrombocythemia are caused by the MPL^{515} and CALR mutation. Designed by Michiels.

The 'Early Interferon Intervention Strategy' in ET and PV 2000-2020

Silver of the PVSG and Michiels of the EWG>MPD proposed in 1998 to treat PV patients according to the least toxic approach with low dose aspirin and phlebotomy alone in early stage PV and to postpone potential leukemogenic agent hydroxyurea in the intermediate stage of as long as possible by using recombinant interferon (IFN) in the prefibrotic stages of PV [27,28,136,156]. If non responsive to IFN or serious side effects there is a clear indication to suppress advanced MPD disease in PV by hydroxyurea. Silver and Michiels stated in 1998 that IFN is a good candidate in the therapeutic armentarium for the best first line treatment option of newly diagnosed PV patients [27,136,156]. The reasons for using pegylated IFN-alpha in early stage prefibrotic ET and PV are manifold (Table 7). If non-responsive to IFN clear indications for hydroxyurea in symptomatic PV include uncontrolled platelet count around or above 1000 × 10^9/l; leukocytosis; symptomatic large spleen (splenomegaly); PV-related constitutional symptoms including pruritis; increased leukocyte count (hypercellular PV); leukoerythroblastic blood picture and signs of myeloid metaplasia and major arterial or venous thromboembolic complications despite eradication of vascular risk [27,136-156]. The main therapeutic benefits of recombinant interferon-alpha (rIFN-alpha) therapy for PV include induction of hematological remission with elimination or reduction in the need of phlebotomy, control of thrombocytosis and leukocytosis and resolution of classical PV disease associated symptoms in particular refractory pruritis [27,156].

Four recent studies demonstrate that Pegylated IFN-2a is now becoming the first line non-leukemogenic treatment option in prodromal PV and early or classical stages of PV to postpone or eliminate the use of the leukemogenic agent hydroxyurea as long as possible [157-160]. The Blood paper by Yacoud (2019) demonstrated that pegylated IFN-2a is effective in previously hydrocyurea treated PV patients who switched to IFN-2a [157]. The overall response rate (complete response CR plus partial response PR) at 12 months was 69.2% (43.1% CR/26.2% PR) in 65 ET, and 60% (22% CR/38% PR) in 50 PV patients. Complete hematological response rates were significantly higher in CALR mutated ET patients (56.5% vs. 28.0%) as compared to subjects lacking a CALR mutation. The median absolute reduction in JAK2^{V617F} variant allele fraction was -60% (range - 84%/-47%) in patients achieving a CR versus +40% (range 18%/56%) in patients with PR or non-responsive. Therapy was associated with a significant rate of adverse events, most were manageable, and IFN-2a discontinuation due to adverse events occurred only in 13.9% of subjects. In this study, IFN-2a is an effective therapy for patients with ET/PV who were previously refractory and/or intolerant to HU. In a post-hoc study of 83 ET/PV patients, Masarova and Verstovsek (2017) showed in a post-hoc study of 83 ET/PV patients, that IFN-alpha2a (Pegasys) induced durable hematological remission rates in 80% and molecular response rates in 63% of treated ET/PV patients

during long-term periods lasting from 5.7 to 12 years [158]. The Danish retrospective study (Czech., *et al.* 2018) treated 38 WHO-defined normocellular and hypercellular ET patients with IFN-2a Pegasys of whom 18 JAK2^{V617F} mutated ET and 20 CLAR mutated ET [159]. Starting IFN-2a dose was 45 ug/week. The calculated mean dose was 49 ug/week for JAK2V617F mutated ET and 45 ug/weel for CALR mutated ET. Baseline mutant allele burden was 35% (range 13%-62%) for JAK2^{V617F} mutated ET and 36% (range 21% - 63%) for CALR mutated ET patients. The JAK2^{V617F} mutation allele load increased in 3 cases of JAK2^{V617F} mutated ET. Fifteen of the 18 JAK2^{V617F} mutated ET patients (83%) and 10 or 20 CALR mutated ET patients (50%) demonstrated a decrease in mutation allele burden. The precentage reduction in mutation burden was minus 69% (range 3% - 98%) for JAK2^{V617F} mutated ET and minus 37% (range 4% - 73%) in CALR mutated ET.

The elegant 2020 Danish IFN/MPN basic research study presented a thorough analysis of serial JAK2^{V617F} measurements in 66 IFN-treated patients and in 6 untreated JAK2^{V617F} mutated MPN patients [160]. Without IFN treatment, the JAK2^{V617F} allele burden increased exponentially with a period of doubling of 1.4 year. During monotherapy with IFN, the JAK2^{V617F} allele burden decreased mono- or bi-exponentially for 33 responders of which 28 patients satisfied both descriptions. Bi-exponential description improved quality of health fitness in 19 cases being associated with late JAK2^{V617F} responses. The decay of the JAK2^{V617F} allele burden during IFN treatment was estimated to have half-lives of 1.6 year for the mono-exponential response and 1.0 year in the long term for the bi-exponential response. The authors concluded that through data-driven analysis of the JAK2^{V617F} allele burden, did provide novel information regarding the JAK2^{V617F} kinetics during IFN-treatment, arguing for early IFN intervention strategy in previously untreated and newly diagnosed JAK2^{V617F} mutated ET and PV patients**.** If non responsive to IFN-2a the cornerstone of today's treatment of ET and PV with progessed MPN disease like hypercellular PV are candidates for hydroxyurea and or the JAK2-inhibitor ruxolitinib or other targeting agents displaying a synergism with IFN at time points before intermediate and advanced myelofibrotic transformations do occur [27,136-156].

Declaration of Interest

This State-of-the-Art manuscript on the 2008 ECMP interim report was conceptualized in 2007, finalized in May 2008 and remained unpublished because it did not meet the PVSG/WHO criteria of the MPD/MPN and would have undermined the publication of the 2008 WHO classification of the (MPNs) proposed by Tefferi and Thiele. The original 2008 ECMP classification of the MPNs conceptualized and designed by Michiels has been validated as the 2015 ECMP 2018 CLMP classifications of the MPNs [140-156]. The content of the present manuscript is the extension of the 2006 ECP criteria of the MPDs (reference 30) and based on the plenary lecture entitled: Biology, Diagnosis and Classification of MPD. 1st International Lymphoma-Leukemia-Myeloma (LLM) Congress by Michiels JJ, De Raeve H, Hebeda K, Lam KH, Bot F, Berneman Z, Schroyens W. Turk J Hematol Proceeding May 2007; 24(Supp 1):37-53. The list of references 1 to 136 of the original 2008 ECMP manuscript is updated until May 2008 as the response to the 2007 WHO MPN classification before the publication of the 2008 WHO classification of the MPNs.

The critical appraisal and therapeutic implications 1998 - 2020 are added in February 2020 based on references 136-156. Dr Jan Jacques Michiels is Multidisciplinary Internist, Hematologist and Bloodcoagulation and Vascular Medicine Specialist, and founded the Goodheart Institute (GHI) in Nature Medicine and Health in 1998. Dr JJ Michiels served as staff member of the Department of Hematology (Chief Prof Dr Johan Abels) and as Director of Hemostasis and Thrombosis Research, Erasmus University Medical Center Rotterdam 1973 - 2000. Dr JJ Michiels wrote his Thesis in 1981 on Erythromelalgia and Thrombocythemia caused by Von Willebrand factor-platelet-mediated arteriolar inflammation and thrombosis) as the origin of a series of publications between 1985 - 2017, which could be labeled as a novel Sticky Platelet Thrombophilia in TPO, JAK2 and MPL mutated Thrombocythemia of myeloproliferative Neoplasms (MPN). Dr JJ Michiels is Founder of the European Working Group on Myeloproliferative Disorders EWG.MPD (1994 - 2006) and Myeloproliferative Neoplasms EWG.MPN (2006 - 2020) as a Scientific Working Group within the European Hematology Association: EHA and the European LeukemiaNET (ELN). Dr. JJ Michiels and Conny Luteijn founded in 2003 the Dutch MPD/MPN Patients Foundation to learn from the interaction of MPD doctors experiences and the ET, PV and MPN patient voices heir sorrows and sufferings from MPN disease. Dr JJ Michiels served since 2000 as consultant professor in Hematology and Bloodcoagulation at the University Hospital Antwerp with special attention to MPN, von Willebrand Disease and Bloodcoagulation Disorders. Dr Michiels recently created the International Collaborations and Research on Myeloprolifrative Neoplasms: ICAR.MPN.

Author Contribution

All authors met the conditions of substantial contributions to conception and design of the study, and the acquisition, analysis, and interpretation of data; drafting the article or revising for important intellectual content; and final approval of the version to be published.

Conflict of Interests

The author(s) declared no potential conflicts of interest with respect tot he research, authorship, and/or publication of this article.

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