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Abstract

The classifications of Myeloproliferative Disorders (MPD) by the Polycythemia Vera Study Group (PVSG) and World Health Organization (WHO) used crude criteria for the diagnoses of Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Primary Myelofibrosis (PMF). The PVSG and the 2007 WHO criteria for the diagnosis of ET and PV overlook the very early pre-blastic stages of MPN. The 2008 European Clinical, Molecular and Pathological (2008 ECMP) criteria are sensitive for the detection of early stages of JAK2V617F trilinear Myelo Prolyliferative Neoplasms (MPN) and could delineate three stages JAK2V617F mutated ET: normocellular ET; ET with features of early PV (prodomal PV); and ET with Hypercellular Megakaryocytic Granulocytic Myeloproliferation (EMGM). The 2008 ECMP classification distinguishes six clinical PV stages that have important prognostic and therapeutic implications. Spontaneous EEC, low serum erythropoetin (EPO) levels and JAK2 mutations are highly specific for ET with PV features (prodomal PV), masked PV and classical PV. JAK2 wild type ET and MF have no blood and bone marrow features of PV.

The detection and quantitation of JAK2V617F mutation allele burden play a key-role in the diagnostic work-up and staging of ET, PV and MF patients. The JAK2V617F mutation allele burden in heterozygous mutated ET and in combined heterozygous-homozygous or homozygous mutated PV and EMGM is of major clinical and prognostic significance. Pre-treatment bone marrow histopathology is of huge importance to document and stage the broad spectrum JAK2 mutated and JAK wild type MPN. JAK2 wild type ET carrying the MPLV515 mutation is a separate and distinct MPN entity of ET and MF without features of PV at diagnosis and during follow-up. JAK2 wild type hypercellular ET associated with Primary Megakaryocytic Granulocytic Myeloproliferation (PMGM) is the third MPN entity of elusive etiology. Myelofibrosis (MF) is not a primary MPN disease entity because Reticulin Fibrosis (RF) and Reticulin/Collagen Fibrosis (RCF) are a secondary response of polycylic fibroblasts to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells.

Keywords: Myeloproliferative neoplasm; Essential thrombocythemia; Polycythemia vera; Myelofibrosis; JAK2V617F mutation; MPLV515 mutation; Bone marrow pathology; World Health Organization

Introduction

In the 19th century Chronic Myeloid Leukemia (CML) and Polycythemia Vera (PV) have been described as primary distinct disease entities [1-3]. In 1951 Dameshek lumped dissimilar diseases of polycythemia vera, erythroleukemia, idiopathic and agnogenic myeloid metaplasia, 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) [3]. Lumping erythroleukemia with PV, and putting together Chronic granulocytic or Myeloid Leukemia (CML) with PV appeared to be without scientific foundation (Figure 1). In 1960 Nowell and Hungerford described the presence of a minute chromosome in leukemic cells called Philadelphia (Ph) chromosome after the city of discovery as a diagnostic clue CML [4]. Using banding techniques Janet Rowley (1973) discovered that the Ph chromosome originated from a translocation between the chromosomes 9 and 22, t(9;22)(q34;q11) [5]. Three Dutch investigators discovered that a hybrid gene is generated by the translocation consisting of the BCR gene on chromosome 22 and the ABL oncogene originating from chromosome 9 [6-8], which results in a BCR/ABL fusion gene with high tyrosine kinase activity and CML-transformation capacity [9,10]. Ninety-five percent of all CML patients are Ph"; 90% are Ph"/BCR/ABL", 5% are Ph"/BCR/ ABL", and 5% are Ph"/BCR/ABL", the latter group usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia [11].

The PVSG used in 1975 the Ph" negative ET, PV and AMM from the Ph" positive ET and Chronic Myeloid Leukemia (CML) with various degrees of thrombocythemia and myelofibrosis [12,13]. According to strict morphological, biochemical, cyto genetic and molecular criteria including the Ph" chromosome and bcr/abl fusion gene and protein, 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 BCR/ABL negative chronic Myeloid Pro liferative Disorder (MPD) featured by a benign proliferation of the three hematopoietic cell lines [14].

Regarding etiology of PV, Dameshek proposed two highly speculative possibilities: first, the presence of excessive bone marrow stimulation by an unknown factor or factors, and second, a lack or a diminution in the normal inhibitory factor or factors [15]. The discovery of the JAK2V617F mutation in 2005 by Vainchenker confirmed that the PVSG used in 1975 the Ph" chromosome to separate the Ph" negative ET, PV and AMM from the Ph" positive ET and Chronic Myeloid Leukemia (CML) with various degrees of thrombocythemia and myelofibrosis [12,13]. According to strict morphological, biochemical, cyto genetic and molecular criteria including the Ph" chromosome and bcr/abl fusion gene and protein, 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 BCR/ABL negative chronic Myeloid Proliferative Disorder (MPD) featured by a benign proliferation of the three hematopoietic cell lines [14].

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thrombocythemia associated with Ph+ and negative ET, PV and PMGM

BCR/ABL positive ET and CML versus BCR/ABL- 

Factor (GCSF), resulting in trilinear MPN (ET, PV and MF [16,17]. Thrombopoietin stages of heterozygous and homozygous JAK2V617 mutations (Figures 1 and 2) [16,17] The sequential enhanced activity of the normal JH1 kinase activity of JAK2 as the cause of the JH2 pseudokinase part on the JH1 kinase part of JAK2, leading to MPD by the discovery and demonstration that loss of inhibitory activity appeared to be caused by the acquired somatic JAK2V617F mutation discovered by Vainchenker and his team in 2005 [16]. The unifying concept of Dameshek in 1951 on the chronic MPDs essential thrombocytosis (ET), polycythemia vera (PV), agnogenic myeloid metaplasia (ANM) and chronic myeloid leukemia (CML) has been separated by the PVSG into Ph-positive CML and Ph-negative ET, PV and AMM. The 2008 ECMP classification separated the JAK2 wild type MPN into MPL mutated ET/MF as the second and JAK2 wild type hypercellular ET associated with primary granulocytic megakaryocytic myeloproliferation (PMGM) as the third distinct MPN disease.

Figure 1: The 1950 concept of Dameshek on polycythemia vera (PV) as a trilineal myeloproliferative disorder (MPD) [15] due to one hypothetical cause appeared to be caused by the acquired somatic JAK2V617F mutation discovered by Vainchenker and his team in 2005 [16]. The unifying concept of Dameshek in 1951 on the chronic MPDs essential thrombocytosis (ET), polycythemia vera (PV), agnogenic myeloid metaplasia (ANM) and chronic myeloid leukemia (CML) has been separated by the PVSG into Ph-positive CML and Ph-negative ET, PV and AMM. The 2008 ECMP classification separated the JAK2 wild type MPN into MPL mutated ET/MF as the second and JAK2 wild type hypercellular ET associated with primary granulocytic megakaryocytic myeloproliferation (PMGM) as the third distinct MPN disease.

BCR/ABL positive ET and CML versus BCR/ABL- negative ET, PV and PMGM

The morphological distinction between Ph+ and BCR/ABL- ET and thrombocytosis associated with Ph+ and BCR/ABL+ CML versus the Ph-negative thrombocytosis in various MPDs is primarily based upon conspicuous differences in the form and size of megakaryocytes in bone marrow smears and sections of bone marrow biopsy [14]. This difference in bone marrow smears observed by Michiels et al in 1987 is reproducible in bone marrow biopsies by pathologists to distinguish between small mononucleated megakaryocytes in Ph+ diseases versus clustered large megakaryocytes with hyperlobulated nuclei in Ph- negative MPDs [14,18-26]. Georgii et al defined in 1990 [22] the Hannover Bone Marrow classification of the MPDs, which had the great advantage to pick up the early stages of prefibrotic MPD ET, PV and PMGM, which are overlooked by the PVSG criteria. Thiele introduced in 2001 bone marrow features on top of PVSG criteria and defined the World Health Organization (2001 WHO MPD) classification [25,26]. The PVSG [12,13], the 2001 WHO [26] and the 2007 WHO [27] criteria are rather crude to adequately classify the MPDs as ET, PV and CIMF and overlook the very early prefibrotic stages of MPD by definition [19-23]. In the present manuscript we could integrate the PVSG and the 2001 and 2007 WHO criteria into the 2008 ECMP classification (Tables 1-3) 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 or MPNs.

Hannover Bone Marrow Classification of the MPDs ET, PV and PMGM

With the improvement of bone marrow biopsy and tissue processing in the 1980s and 1990s, Georgii and Thiele defined the pathological features of ET, PV and CIMF or AMM on bone marrow histopathological morphology [18-24]. Georgii regarded Myelofibrosis (MF) as a reactive feature secondary to progressive disease [18] seen in AMM, PV and CML [22-24]. Georgii reasoned that the terms Agnogenic Myloid Metaplasia (AMM) or CIMF lack accuracy since they are applied to both the pre-fibrotic hyper cellular and advanced fibrotic stages [22]. As Myelofibrosis (MF) is not a disease but a secondary complication of MPD he used the term MF for grading of the MPD disease burden based on the degree of Reticulin Fibrosis (RF) and reticulin-collagen fibrosis (Tables 1-3). Accordingly, Georgii et al replaced the terms AMM and CIMF by chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM or PMGM). The Hannover Bone Marrow classification have defined ET by persistent increase of platelets in excess of 400 x10⁹/l without the Ph chromosome together with mononuclear proliferation of mature enlarged megakaryocytes in the bone marrow with normal cellularity, normal erythropoiesis and normal granulopoiesis [22-24]. The Hannover Bone Marrow classification have defined PV as a trilinear proliferation of megakaryopoiesis, erythropoiesis and granulopoiesis in which the erythropoiesis was most prominent together with variable degrees of increased platelets, erythrocytes and granulocytes in the peripheral blood in the absence of the Ph chromosome [22-24]. The diagnosis of prefibrotic CMGM/PMGM [18-20] has been labelled as chronic idiopathic myelofibrosis (CIMF) in the 2001 WHO classifications [26]. The diagnosis of CMGM/PMGM according to the 1996 Hannover...
but never disturbed in maturation and 3) no features of PV with cloud-like nuclei not seen in ET and PV, 2) increased granulopoiesis dysmorphic megakaryocytes with immature cytoplasm and immature


Clinical and molecular criteria | Bone marrow pathology (P) criteria (WHO)
---|---
JAK2V617F ET | Normocellular ET
1. Platelet count of >350 × 10^9/L and the presence of large platelets in a blood smear | Predominant proliferation of enlarged mature megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities.
2. Presence of JAK2V617F mutation | No increase, proliferation or immaturity of granulopoiesis or erythropoiesis.
3. Normal erythropoiesis <5.8 × 10^11/L males, <8 × 10^11/L females | Reticuline fibrosis (RF) 0 or 1
4. Normal haemoglobin (Hb) and hematocrit (Ht) | Prefibrotic: RF- 0/1, MF-0, no/minor splenomegaly

JAK2V617F prodomal PV | ET with bone marrow features of PV
1. Platelet count of ≥350 × 10^9/L and normal h/t male <0.51, female <0.48, normal erythrocyte <5.8 × 10^11/L males, <8 × 10^11/L females is mandatory. | Increased cellularity with due to increased erythropoiesis or trilineage myeloproliferation (i.e. panmyelosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes.
2. Presence of JAK2V617F mutation | Absence bone marrow features consistent with congenital polycythaemia and secondary erythropoiesis.
3. Low serum EPO level and/or increased LAP score | RF 0 or 1
4. Spontaneous EEC. | No preceding or allied CML, PV, RARS-T or MDS.

JAK2V617F hypercellular ET | ET, MGM
1. Platelet count of ≥350 × 10^9/L, 2. No signs of leuko-erythroblastosis 3. Slight or moderate splenomegaly on ultrasound | Hypercellular ET due to chronic megakaryocytic and granulocytic myeloproliferation (EMGM) and normal or reduced erythroid precursors.
4. Presence of JAK2V617F mutation | Loose to dense clustering of more pleiomorphic megakaryocytes with hyperplor or clumpsy nuclei (not or some cloud-like).
5. No preceding or allied CML, PV, RARS-T or MDS. | Bone marrow stage:

ET, MGM clinical staging:

Early stage: No anemia with hb and h/t 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

A1 Platelet count grade I 400-1500, grade II >1500 × 10^9/L
A2 Splenomegaly on ultrasound or CT (>12 cm) or splenomegaly on palpation
A3 Granulocytes >10 × 10^9/L or leukocytes >12 × 10^9/L and raised LAP score >100 in the absence of fever and no increase of ESR
A4 Absence of any cause of primary or secondary erythropoiesis
A5 Low plasma or serum EPO level
A6 Clinical criteria MPL515 mutated ET
A1 Persistent increase of platelet count grade I 400-1500, grade II >1500 × 10^9/L
A2 Normal spleen or only minor splenomegaly on echogram
A3 Normal LAP score, normal ESR and increased MPV
A4 Absence of Philadelphia chromosome
A5 Staging according to no, mild or severe anemia
A1 No preceding or allied other subtype of MPN, PV, MDS or CML
A2 No or only borderline increase in reticulin fibers
A3 Slight or moderate splenomegaly of ≤12 cm on ultrasound or CT
A4 Absence of any cause of primary or secondary erythropoiesis
A5 Absence of Philadelphia chromosome

Pathological criteria PV
B1 Increased cellularity due to increased erythropoiesis or due to trilinear myeloproliferation of megakaryopoesis, erythropoiesis and granulopoiesis (e.g. panmyelosis). Proliferation of small medium sized and large (pleomorphic) megakaryocytes.Absence ofstainable iron, No or slight increase of reticulin fibers.
B2 Sponantaneous erythroid colony (ECF) formation
B3 No or only borderline increase in reticulin fibers
B4 Combination of A1 and B1 + B2 establish ‘true’ ET. Any other criterion confirms ET. LAP=leukocyte alkaline phosphatase; ESR=erythrocyte sedimentation rate; MPV=mean platelet volume; MPN=myeloproliferative neoplasms; PV=polycthyemia vera; MDS=myelodysplastic syndrome; CML=chronic myeloid leukemia

Pathological criteria MPL515 mutated ET
B1 Predominant proliferation of enlarged to giant megakaryocytes with hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicuous cytophological abnormalities
B2 No proliferation or immaturity of granulopoiesis or erythropoiesis
B3 No or only borderline increase in reticulin fibers

Pathological criteria MPL515 mutated ET
B1 Predominant proliferation of enlarged to giant megakaryocytes with hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicuous cytophological abnormalities
B2 No proliferation or immaturity of granulopoiesis or erythropoiesis
B3 No or only borderline increase in reticulin fibers

Staging of myelofibrosis (MF) according to MF grading
B1 Megakaryocytic and granulocytic myeloproliferation (MGM) and relative or absolute reduction of erythropoiesis (erythroid precursors). Abnormal clustering and increase of atypical immature medium-sized large to giant megakaryocyte containing (Cloud-like) hyperlobulated nuclei and definitive maturation defects
B2 No proliferation or immaturity of granulopoiesis or erythropoiesis
B3 No or only borderline increase in reticulin fibers

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)

PVSG, 2007 WHO and 2008 ECMP criteria for the diagnoses of ET, PV and PMGM

The PVSG failed to use BMB and introduced 3 major and 4 minor clinical criteria as inclusion criteria the diagnosis of PV in the PVSG-01 study of which increased RCM was mandatory [12,13]. Increased
RCM in PV patients corresponded to hematocrit values between 0.48 and 0.76 in all, platelet count above 400 x10^9/L in two-third and palpable spleen in two-third of about 400 PV patients in the PVSG-01 study [13]. The PVSG and WHO classifications excluded ECP of Idiopathic Erythrocythemia (IE) by definition [31,32]. IE is featured by increased hemoglobin, haematocrit, erythrocytes and increased red cell mass but normal leukocytes, thrombocytes and spleen size on palpation [31,32]. Minor B criteria did appear in untreated IE patients during follow up and was associated with a high incidence of major or lethal cerebrovascular thrombotic disease 31. This category of IE or the PVSG-01 classification and the 2007 WHO Revised Criteria for Myeloproliferative Neoplasms. J Hematol Thromb Dis 3: 190.

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Table 3: Grading of reticulin fibrinosis (RF) according to Ellis [34], and European grading of myelofibrosis (MF) according to Thiele et al. [75] in bone marrow biopsies of patients with a chronic myeloproliferative neoplasms (MPN).

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

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

Between 2000 and 2007 it turned out that various degrees of characteristic PV bone marrow histology features (irrespective of RCM measurements) are seen in the four different stages of newly diagnosed patients with the JAK2 mutated MPN (Tables 1, 2 and 4) [44-47]. First, early thrombocytopenic PV mimicking ET with a hematocrit in the upper limit of normal \(< 0.50\) but increased platelet count \(> 400 \times 10^9/L\) without or with slight splenomegaly \(\text{stage 0 PV}\); Second, “idiopathic erythrocythysis” with increased RCM, high hematocrit, low serum EPO, but normal platelet count and spleen size \(\text{stage 1 PV, Table 4}\); Third, classic PV with increased RCM, high hematocrit and one or more B criteria \(\text{overt stage 2 and 3 PV, Table 4}\); Fourth, unclassifiable or masked MPD with splenomegaly, normal hemoglobin and hematocrit, normal or slightly elevated platelet count \(\text{apparent PV–IPV, Table 4}\). Thiele et al confirmed that characteristic PV bone marrow histopathological features are seen in classic PV and in latent PV or early thrombocytopenic PV mimicking ET [42].

The 2007 WHO revision of the PVSG and 2001 WHO classifications decided to lower the platelet counts from 600 to around 450x10^9/L for the diagnosis of ET [27]. The 2007 WHO changed the term MPD into myeloproliferative neoplasia (MPN) and only defined the minimal criteria for ET, PV and Primary Myelofibrosis (PMF). We could improve the 2007 WHO revision of the MPNs the introduction of the 2006 → 2008 ECMP criteria for diagnoses of three distinct MPNs: JAK2\(^{V617F}\) mutated ET and PV with various degrees of MF; JAK2 wild type ET and MF carrying the MPL\(^{517T}\) mutation; and JAK2/ MPL wild type PMGM with features of hypercellular ET and various degrees of MF and splenomegaly (Tables 1-3) [27-32]. As compared to the 2008 ECMP classification, the 2007 WHO criteria by Tseferi et al are crude and not specific enough for three reasons. First, for ET they only include normocellular ‘true’ ET, but the diagnoses of early thrombocytopenic PV (hemoglobin <18.5 for men and <16.5 for women) and ET associated with prefollicular PV or MGM bone marrow \(\text{MF-0}\) without leukoerythroblastosis, anemia or myelofibrosis \(\text{MF-0}\) remain unclassifiable. Second, the 2007 WHO criteria for PV arbitrarily exclude the early idiopathic stage of PV and overlooked masked PV just by the main crude inclusion criterion of a high hemoglobin level disregarding the importance increase of leukocytes, platelets and spleen size as typical features of masked trilinear PV. Simple tests like cell blood counts including platelets, leukocytes, hematocrit and erythrocytes above \(6x10^9/L\) and spleen size on echogram are not taken into account to distinguish the early thrombocytopenic and erythrocythemic stages of PV from the classical overt trilinear polycythemic stage of classic PV as documented by bone marrow biopsy showing typical erythroid, granulocytic and megakaryocytic myeloproliferation [46,47]. Third, the 2007 WHO criteria defined Primary Myelofibrosis (PMF) as an endstage MPN disease complicated by anemia and splenomegaly, but the third prefollicular entity of so-called prefollicular PMGM (MF-0) without leukoerythroblastosis or anemia remained unclassifiable. These shortcomings of the 2007 WHO diagnostic criteria for MPN by Tseferi et al [27] will hamper to prospectively evaluate the natural history, and therapeutic implications by objective staging of ET and PV MPN disease burden related to therapy. To overcome the shortcomings of the PVSG and WHO classifications of the MPD and MPNs respectively, we here update and extend the ECMP criteria for the diagnosis, classification and staging of true ET, PV and PMGM (Tables 1-4).

Acquired JAK2\(^{V617F}\) mutation as the driver cause of trilinear MPN: Dameshek-Vainchenker’s disease 1950-2005 [15,16]

The one cause hypothesis of trilinear PV proposed by Dameshek in 1950 [15] has been confirmed by Vainchenker in 2005 by the discovery of the JAK2\(^{V617F}\) mutation as the driver cause of ET, PV, masked PV and MF (Figures 1and 2) [16,17]. Detection of JAK2\(^{V617F}\) has become the first intention diagnostic test to differentiate between PV and myeloproliferative Idiopathic Erythrocythemia (IE) from...
of a few TPO receptors by low levels of JAK2V617F (heterozygous) where it controls physiological TPO levels. It is possible that activation [48, 49]. TPOR/MPL is expressed at high levels in megakaryocytic cells and duration of JAK2V617F directly contribute to the phenotypic Chain Reaction (PCR) analysis in PVSG-defined MPD patients, a high platelets and granulocytes (Table 5). Applying allele-specific Polymerase bone marrow cells, erythroblasts, in cells of spontaneous EEC, blood patients are homozygous for the JAK2V617F mutation [50, 51]. Only 3 to 4% of ET, 24 to 27% of PV and 6 to 18% of MF erythrocytosis with a sensitivity of 95% and specificity of 100%. The discovery of the JAK2V617F mutation by James et al [16] was immediately appreciated as an evolutionary event, and rapidly confirmed by several investigators (reviewed by Michiels et al 2006) [45]. JAK2 plays an essential role in cytokine-induced signalling from receptors to the nucleus by several hematopoietic cytokines including Erythropoietin (EPO), Thrombopoietin (TPO), and granulocyte colony stimulating factor (G-CSF) [48-50]. The JAK2V617F mutation renders the receptors (EPO), Thrombopoietin (TPO), and granulocyte colony stimulating factor (G-CSFR) leading to extramedullary hematopoiesis (splenomegaly) and cytokine mediated secondary myelofibrosis (Figure 3 and Table 5). The percentage of JAK2V617F positivity and progression from heterozygous to homozygous is strongly correlated with the ability to form spontaneous EEC formation and with progressive post-PV myelofibrosis (Figure 3 and Table 5). The percentage of JAK2V617F positivity and progression from heterozygous to homozygous is strongly correlated with the ability to form spontaneous EEC formation and with progressive post-PV myelofibrosis (Figure 3 and Table 5).

<table>
<thead>
<tr>
<th>PV: WHO-ECMP stage</th>
<th>Clinical Diagnosis</th>
<th>WHO-ECMP stage</th>
<th>Prodomal PV</th>
<th>Erythrythemic PV</th>
<th>Early PV</th>
<th>Manifest PV</th>
<th>Combined PV</th>
<th>Inapparent PV</th>
<th>Spent PV</th>
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</thead>
<tbody>
<tr>
<td>LAP-score</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>EEC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serum EPO</td>
<td>N/↓</td>
<td>N/↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Erythrocytes x10^12/l</td>
<td>&gt;5.8</td>
<td>&lt;5.8</td>
<td>&gt;5.8</td>
<td>&gt;5.8</td>
<td>&gt;5.8</td>
<td>Normal</td>
<td>&lt;5.5 Decreased</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leukocytes x10^9/l</td>
<td>&lt;12</td>
<td>&lt;12</td>
<td>&lt;12</td>
<td>&lt;12</td>
<td>&lt;12</td>
<td>&gt;15</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Platelets x10^9/l</td>
<td>&gt;400</td>
<td>&gt;400</td>
<td>&lt;400</td>
<td>&gt;400</td>
<td>&gt;400</td>
<td>&lt;400</td>
<td>&gt;1000</td>
<td>N low or ↑</td>
<td>+</td>
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<td>WHO-ECMP bone marrow</td>
<td>Early PV</td>
<td>Early PV</td>
<td>Early PV</td>
<td>Triline PV</td>
<td>Triline PV</td>
<td>Triline PV</td>
<td>Triline PV</td>
<td>Myelofibrosis</td>
<td>+</td>
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<td>Bone marrow cellularity (%)</td>
<td>50-80</td>
<td>50-80</td>
<td>60-100</td>
<td>50-80</td>
<td>60-100</td>
<td>50-80</td>
<td>80-100</td>
<td>60-100 Decreased</td>
<td>+</td>
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<td>Grading reticulin fibrosis: RF</td>
<td>RF 0-1</td>
<td>RF 0-1</td>
<td>RF 0-1</td>
<td>RF 0-1</td>
<td>RCF 1/2/3</td>
<td>RCF 2/3</td>
<td>RCF 3/4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Grading myelofibrosis: MF</td>
<td>MF 0</td>
<td>MF 0</td>
<td>MF 0</td>
<td>MF 0</td>
<td>MF 0/2</td>
<td>MF 2/3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Splenomegaly on palpation: No/±</td>
<td>No</td>
<td>No</td>
<td>No/±</td>
<td>+</td>
<td>+/+++</td>
<td>+/+++</td>
<td>+/+++</td>
<td>/large</td>
<td>+</td>
</tr>
<tr>
<td>Spleen size on palpation: NP</td>
<td>0-3</td>
<td>0-3</td>
<td>0-3</td>
<td>0-3</td>
<td>4-6</td>
<td>6</td>
<td>&gt;6</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>JAK2V617F in Granulocytes%</td>
<td>Low</td>
<td>Low</td>
<td>Moderate &lt;50</td>
<td>High &gt;50</td>
<td>High &gt;50</td>
<td>High &gt;50</td>
<td>High &gt;50</td>
<td>High &gt;50</td>
<td>+</td>
</tr>
<tr>
<td>JAK2V617F in BFU-e (exon 12)</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Intermediate risk PV</td>
<td>High risk PVeryar MF</td>
<td>WaitSee IFN JAK2 Inhibitor</td>
<td>IFN Resistant JAK2 inhibitor</td>
<td>IFN Resistant JAK2 inhibitor</td>
<td>JAK2 Inhibitor Bone marrow transplant</td>
</tr>
<tr>
<td>First line Aspirin/Phlebotomy</td>
<td>Aspirin Phlebotomy</td>
<td>Aspirin Phlebotomy</td>
<td>Phlebotomy Aspirin Low dose IFN → responsive</td>
<td>JAK2 inhibitor</td>
<td>IFN resistant Hu or JAK2 inhibitor</td>
<td>JAK2 inhibitor</td>
<td>JAK2 inhibitor</td>
<td>Bone marrow transplant</td>
<td></td>
</tr>
<tr>
<td>Second line IFN versus Hydroxyurea (HJ)</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Intermediate risk PV</td>
<td>High risk PVeryar MF</td>
<td>WaitSee IFN JAK2 Inhibitor</td>
<td>IFN Resistant JAK2 inhibitor</td>
<td>IFN Resistant JAK2 inhibitor</td>
<td>JAK2 Inhibitor Bone marrow transplant</td>
</tr>
<tr>
<td>Third line JAK2 inhibitor</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Intermediate risk PV</td>
<td>High risk PVeryar MF</td>
<td>WaitSee IFN JAK2 Inhibitor</td>
<td>IFN Resistant JAK2 inhibitor</td>
<td>IFN Resistant JAK2 inhibitor</td>
<td>JAK2 Inhibitor Bone marrow transplant</td>
</tr>
</tbody>
</table>

**↑**=increased, **↓**=decreased, **N**=normal, +=present or heterozygous; +++=homozygous

**Table 4. Staging of JAK2V617F positive prodomal PV, erythrythemic 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 (Black) and 2014 (Red) [78-80].**
counts in ET [53]. Tefferi et al detected JAK2 V617F in 75% of ET (n=60) and in 97% of PV patients (n=62), whereas allelic ratios exceeding 50% JAK2 V617F indicating homozygosity were found in 70% of PV at diagnosis but never in ET [54]. 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 and post-ET myelofibrosis (Table 5). JAK2 V617F heterozygous and homozygous PV patients have increased haemoglobin, hematocrit and erythrocyt counts (> 6x10^{12}/L) at time of diagnosis, experience increased incidence of pruritus, and a higher rate of fibrotic transformation as compared to ET. Sex appears to be a powerful genetic background modifier in JAK2 V617F-positive MPDs as ET is more common in females and PV in males.

Mechanisms other than mitotic recombination such as duplication of the mutated allele is observed in a proportion of PV and MF patients displaying a gain of 9p, mostly due to trisomy 9 [55,56]. Campbell et al reported that the JAK2 V617F mutation was associated with trisomy 9 with all 10 MPN patients investigated and was found in 28 of 29 MPN patients (PV, ET or MF) with a 29q deletion [57]. Scott et al identified JAK2 exon 12 mutations in 10 erythrocytosis patients with increased red cell mass but no JAK2 V617F, of which according to PVSG criteria 6 could be diagnosed as PV and 4 as idiopathic erythrocytosis [58].

Pre-treatment bone marrow biopsies in 5 JAK2 exon 12 mutated PV patients showed a characteristic pattern of erythroid hyperplasia without morphological abnormalities of the megakaryocyte or granulocyte lineages [58]. Therefore, an overlap between “dosage” and “additional molecular events” hypotheses is very likely in patients with trilinear PV (Figure 3).

Acquired MPL515 gain of function mutations as the driver cause of normocellular ET:

The JAK2 kinase activity in MPNs is dependent on the amount of heterozygous and homozygous JAK2 V617F mutant protein, but also influenced by the various steps upstream or downstream the signalling pathways including MPL, JAK2, STAT-3 (Figure 2). This has been demonstrated in animal models overexpressing c-MPL [59]. MPL transgenic mice manifest with typical features of ET with a fourfold increase of platelet count, increased colony formation of megakaryocytes, and increase of clustered enlarged megakaryocytes in the bone marrow. The ET animals appeared healthy, had a very slight decrease of hematocrit (0.39 versus 0.42 in controls) despite an increase of bone marrow EEC, and survived normally with no evidence of pruritus, and a very slight decrease of hematocrit (0.39 versus 0.42 in controls) despite an increase of bone marrow EEC, and survived normally with no evidence of pruritus, and a very slight decrease of hematocrit (0.39 versus 0.42 in controls). The general features of bone marrow reports cellularity in MPL515/L/K positive MPDs as ET is more common in females and PV in males.

The 2008 ECMP criteria 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 (Figure 6) and ET with MGM (MF-0) bone marrow (EMGM) without features of leuko-erythroblastosis in the peripheral blood (Table 1). These ECMP defined JAK2 V617F mutated ET phenotypes do not differ significantly with regard to peripheral blood features, thrombocytopenia related clinical presentation or laboratory findings and are to be treated equally based on clinical risk stratification for thrombotic and bleeding complications, irrespective of bone marrow features [51,63,64]. Pretreatment bone marrow biopsy will allow clinicians and pathologists to diagnose the early stages of thrombocytopenia in various MPNs irrespective of JAK2 V617F mutation status (Figure 6). The 2008 ECMP criteria classify JAK2 mutated ET (Table 1) as: normocellular ET (Table 2); early PV mimicking ET (Table 3); prefibrotic ET associated with MGM (EMGM) with RF-0 or RF-1 and without features of leukaemophyocytes and no significant increase in reticulin fibrosis in a normocellular bone marrow (Figure 6) without features of PV or PMGM [63-65]. In 2008 we discovered that JAK2/MPL wild type hypercellular ET associated with a PMGM bone marrow appeared to be the third distinct MPN entity (Figures 7 and 8).

### 2008 ECMP criteria for the diagnosis and classification of MPNs

- **The 2008 ECMP criteria** 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 (Figure 6) and ET with MGM (MF-0) bone marrow (EMGM) without features of leuko-erythroblastosis in the peripheral blood (Table 1). These ECMP defined JAK2 V617F mutated ET phenotypes do not differ significantly with regard to peripheral blood features, thrombocytopenia related clinical presentation or laboratory findings and are to be treated equally based on clinical risk stratification for thrombotic and bleeding complications, irrespective of bone marrow features [51,63,64]. Pretreatment bone marrow biopsy will allow clinicians and pathologists to diagnose the early stages of thrombocytopenia in various MPNs irrespective of JAK2 V617F mutation status (Figure 6). The 2008 ECMP criteria classify JAK2 mutated ET (Table 1) as: normocellular ET (Table 2); early PV mimicking ET (Table 3); prefibrotic ET associated with MGM (EMGM) with RF-0 or RF-1 and without features of leukaemophyocytes and no significant increase in reticulin fibrosis in a normocellular bone marrow (Table 1). Post-ET MGM with MF-1, 2 and 3 features of leucoerythroblastosis (Table 1). The 2008 ECMP criteria distinguish thrombocytopenia in various MPNs from thrombocytopenia associated with Ph-1 chromosomes and bcr-abl positive chronic myeloid leukemia (CML) [65] or Myelodysplastic Syndromes (MDS) including the so-called 5q-syndrome, which clearly differs from refractory anemia with ringed sideroblasts and significant thrombocytopenia (RARS-T) [66-69]. Among 9 RARS-T patients, 6 showed the presence of JAK2 V617F mutation [68,69]. As compared to JAK2 wild type ET, JAK2 V617F positive ET is characterized by higher values for hemoglobin, hematocrit, neutrophil counts, LAP score, by lower values for serum EPO levels, serum ferritin and MCV, and by increased cellularity of the bone marrow in biopsy material [65,66], indicating early thrombocytopenic PV mimicking ET (“forme fruste” PV; stage 1 PV, Figure 5, Tables 2 and 4). JAK2 wild type ET patients represent a distinct category who had significantly higher platelet counts, normal serum EPO levels, a typical bone marrow picture of ET, no features of early PV, and are at lower risk for the development of thrombotic complications [70,71].
Grading of myelofibrosis in myeloproliferative disorders 1980-2008

2007 and 2008 WHO defined Primary Myelofibrosis (PMF) itself is not a disease because reticulin and collagen fibrosis are produced by polyclonal fibroblasts in response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in both PV and MF (Table 3) [34,74]. Transformation to myelofibrosis is rare in ET and does occur in about one third of PV and in the majority of patients with ET associated with PMGM (MF-0) during long-term follow-up [22-24]. The grading of Reticulin Fibrosis (RF) was developed grading of myelofibrosis in bone marrow biopsies by pathologist (Table 3) [75]. A scoring system based on morphometric analysis (point intersection with an ocular grid) and quality of fibers (reticulin and collagen fibers) and the bone marrow fiber density (fine or coarse reticulin and some or course bundles of collagen) has been proposed by European consensus for grading of MF (Table 3) [34,75]. According to defined standardized semiquantitative grading of reticulin and collagen fibrosis in the bone marrow, MF can reliably be graded at the pathological bone marrow level as 0 in prefibrotic, as 1 in early fibrotic, as 2 in classical fibrotic and as 3 in classical sclerotic MF (Table 3) [34,75].

The detection of JAK2V617F in granulocytes with sensitive PCR techniques plays a key-role as a first intention diagnostic test for erythrocytosis, because it simplifies the diagnostic work-up of PV [72,73]. In the context of erythrocytosis (hematocrit >0.51 in males and >0.48 in females) the presence of the JAK2V617F mutation has a sensitivity of 95% and positive predictive value of 100% for the diagnosis of PV, and excludes congenital and secondary erythrocytosis. EEC and low serum EPO significantly contribute but are not sensitive enough to diagnose the broad spectrum of PV phenotypes [51]. Bone marrow histology assessment is a gold standard for the diagnosis of masked, overt and advanced JAK2V617F mutated and exon 12 mutated PV.

Table 5: 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 trilineal hematopoietic cells of MPD patients is detectable in platelets, erythroblasts, and granulocytes). Designed by Michiels et al. [51].

Molecular etiology of platelet-mediated microvascular thrombosis

Positive MPDs (ET, PV and PMGM: JAK2 V617F) gain of function mutation in trilineal hematopoietic cells of MPD patients is detectable in platelets, erythroblasts, and granulocytes).

Designed by Michiels et al. [51].

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JAK2 allele burden related to MPN disease progression in ET, PV and MF 2005-2008

Spivak and his co-workers assessed the burden of JAK2V617F mutation and PVSG-defined MPD in 84 ET, 92 PV, and 19 fibrotic MF patients [76]. The JAK2V617F mutation was detected in 92% of PV, in 45% of ET, and in 42% of fibrotic MF patients. The median burden of JAK2V617F alleles was significantly lower ET (47%) than in PV (67%) when stratified for disease duration, a JAK2V617F 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 none in ET patients during very long-term follow-up (Figure 9) [76]. Passamonti et al studied the burden of JAK2V617F in WHO-defined MPD ET, PV, prefibrotic MF (p-MF), fibrotic MF (f-MF) and 16 post-PV myelofibrosis (Figure 10), [50]. The JAK2V617F 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 [50]. Interestingly, 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) (Figures 10). 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 (<10x10⁶/L) circulating CD34 cells. Conversely, 12 out of 18 f-MF and all post-PV MF [16] had increased CD34 circulating cells (Figure 10). These data indicate that ET and p-MF are not different at the molecular (JAK2V617F) and biological (CD34 cells) level. 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 [50].

As compared to the 2007 → 2008 WHO classification [27,77], the use of the 2008 ECMP and the updated WHO-CMP [77-80] criteria clearly show that JAK2 wild type ET and MF lack specific PV laboratory and pathological features at diagnosis and during follow-up. This has been demonstrated for MPL515 mutated (ET/MF) (Figure 6) and for JAK2/MPL wild type hypercellular ET in PMGM (Figures 7 and 8). The flexible use of the 2008 ECMP → 2015 WHO-ECMP criteria should serve as pathognomonic diagnostic clues to each of the prefibrotic MPNs to distinguish early and overt PV MPN disease from primary or secondary erythrocytosis, and can be applied to document the natural history of myeloproliferative and fibrotic disease in JAK2V617F, MPLS15 and JAK2 wild type ET and MF patients. The 2008 ECMP were the critical responses on the shortcomings of the 2007 WHO criteria and were conceptualised before the publication of the final 2008 WHO classification [36,37]. The 2008 ECMP and the updated 2014/2015 WHO-CMP detect and distinguish five distinct clonal MPNs caused by the JAK2V617F, exon 12 JAK2, MPL515 and CALR driver mutation leaving a small group of triple negative group of MPN [78-80].
Figure 9: Neutrophil JAK2V617F allele percentages (%) related to disease in 36 PV-SG-defined ET and 77 PV patients (upper left) [76]. Median neutrophil JAK2V617F allele% were significantly higher in PV than those for ET, regardless of disease duration. Within PV the differences in neutrophil JAK2V617F 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 JAK2V617F 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). First, median neutrophil JAK2V617F allele% in PV were greater than in ET (P=0.001). Second, median platelet JAK2V617F allele% in ET were lower than in PV (P=0.002). Third, median neutrophil JAK2V617F allele% in ET were lower than platelet JAK2V617F allele% in ET (P=0.001). Reproduced with the courtesy of Dr Jerry Spivak, Baltimore, USA.

Figure 10: Granulocyte JAK2V617F mutation burden (%) in 23 PV, 10 ET, 7 prefibrotic CCMF (p-CCMF) 10 fibrotic CCMF (f-CCMF) and 16 post-PV MF patients (lower left) [50]. First, patients with PV had higher JAK2V617F% than ET (P=0.01) and p-CCMF. Second, patients with p-CCMF had much lower JAK2V617F allele% than f-CCMF. Third, patients with post-PV myelofibrosis (MF) had the highest JAK2V617F allele%.

References


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