Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2$^{V617F}$ trilinear polycythemia vera (PV), JAK2$^{exon12}$ PV and JAK2$^{V617F}$, CALR and MPL$^{515}$ thrombocythemia: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels

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Submitted: 09 March 2020
Approved: 30 March 2020
Published: 03 April 2020

How to cite this article: Michiels JJ, Lam KH, Kate FT, Kim DW, Kim M, et al. Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2$^{V617F}$ trilinear polycythemia vera (PV), JAK2$^{exon12}$ PV and JAK2$^{V617F}$, CALR and MPL$^{515}$ thrombocythemia: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels. Int J Bone Marrow Res. 2020; 3: 001-020.

DOI: 10.29328/journal.ijbmr.1001011

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Keywords: Myeloproliferative neoplasms; Essential thrombocythemia; Polycythemia vera; Primary megakaryocytic granulocytic myeloproliferation; Myelofibrosis; JAK2$^{V617F}$ mutation; MPL$^{515}$ mutation; Calreticulin mutation; JAK2 wild type; Bone marrow histology
The Myeloproliferative Neoplasms (MPN) of trilinear polycythemia vera (PV) and megakaryocytic leukemia (ML = primary megakaryocytic granulocytic myeloproliferation: PMGM) and Essential Thrombocythemia (ET) in the studies of Dameshek and Michiels are caused by the MPN driver mutations JAK2V617F, JAK2exon12, CALR and MPL515 discovered by Constantinescu-Vainchenker, Green and Kralovics. The JAK2V617F mutated trilinear myeloproliferative neoplasms (MPN) include a broad spectrum of clinical laboratory and bone marrow features in essential thrombocythemia (ET), prodomal PV and erythrocytic PV, classical PV and advanced stages of masked PV and PV complicated by splenomegaly and secondary myelofibrosis (MF). Heterozygous JAK2V617F mutated ET is associated with low JAK2 allele and MPN disease burden and normal life expectancy. In combined heterozygous and homozygous or homozygous JAK2V617F mutated trilinear PV, the JAK2 mutation load increases from less than 50% in prodomal PV and classical PV to above 50% up to 100% in hypercellular PV, advanced PV and PV with MF. Bone marrow histology show diagnostic features of erythrocrypto, megakaryocytic and granulocytic (EMG) myeloproliferation in JAK2V617F mutated trilinear MPN, which clearly differs from monolinear megakaryocytic (M) myeloproliferation in MPL and CALR thrombocythemia and dual megakaryocytic granulocytic (MG) myeloproliferation in CALR mutated thrombocythemia. The morphology of clustered large pleomorphic megakaryocytes with hyperlobulated nuclei are similar in JAK2V617F thrombocythemia, prodomal PV and classical PV patients. Monolinear megakaryocytic (M) myeloproliferation of large to giant megakaryocytes with hyperlobulated staghorn-like nuclei is the hallmark of MPL515 mutated normocellular thrombocythemia. CALR mutated thrombocythemia usually presents with high platelet count around 1000x10/L and normocellular megakaryocytic (M) proliferation of immature megakaryocytes with cloud-like hyperchromatic nuclei followed by dual megakaryocytic granulocytic (MG) myeloproliferation followed by various degrees of bone marrow fibrosis. Natural history and life expectancy of MPN patients are related to the response to treatment and the degree of anemia, splenomegaly, myelofibrosis and constitutional symptoms. The acquisition of epigenetic mutations at increasing age on top of MPN disease burden independently predict unfavorable outcome in JAK2V617F, MPL515 and CALR mutated myeloproliferative neoplasms (MPNs, which mutually exclude each other).

**Introduction**

The combination of plethoric appearance, splenomegaly, erythrocyte count above 6x10^{12}/L, elevated platelet count and the presence of large megakaryocytes and pannymelosis in the bone marrow is diagnostic for trilinear polycythemia vera [1,2]. Venesection aiming at a haematocrit of 0.40 is the first choice life saving treatment option in newly diagnosed PV that prevents major thrombosis and controls hypervolemic symptoms during long-term follow-up in the majority of PV patients [2-7]. PV is a trilinear erythrocytic, thrombocythemic and granulocytic (EMG) myeloproliferation caused by either one unknown bone marrow stimulation factor or the lack of one inhibitory factor, [2,8,9]. Megakaryocyte leukemia (ML) is distinct from PV [10] and has been recognized by Georgii, et al. [11,12], as hypercellular thrombocythemia due to dual chronic or primary megakaryocytic granulocytic myeloproliferation (CMG/PMGM) without features of PV [13-15].

The Hannover Bone Marrow criteria proposed by Georgii, et al. [11], translated the PVSG criteria in the Hannover BM criteria for ET, PV and PMGM and stages of each by grading of myelofibrosis (MF) as a secondary event in advanced stages of MPDs complicated by anemia, splenomegaly and fibrosis in the bone marrow [11-14,16]. Michiels drew attention to the importance of bone marrow histology as a pathognomonic clue to each of the MPDs ET, PV and PMGM [14-17]. The number and size of mature megakaryocytes in bone marrow biopsies are typically increased in ET and PV. Large megakaryocytes with mature cytoplasm and multilobulated nuclei and the tendency to cluster in small groups close to the sinuses represent the hallmark feature of ET (Figure 2). The histologic background of hematopoiesis in ET at platelet counts above 400x10^{12}/L is one of normal cellularity in the early stage [14,17] (Table 3). A slight to moderate increased cellularity due to increased erythropoiesis may be seen in ET with increasing platelet counts between 400 to above 1000x10^{12}/L against a background of normally maturing granulopoiesis and erythropoiesis comparable with the early stage of PV [18-20]. Increase in number and size of clustered large megakaryocytes comparable to ET and a moderate to marked increased cellularity due to increased erythropoiesis/megakaryopoiesis (EM) and erythro-megakaryo-granulopoiesis (EMG) are the diagnostic features of untreated PV [14,17] (Figure 3, Table 4). Increase of large megakaryocytes with mature cytoplasmas and multilobulated nuclei in a hypercellular bone marrow is even more conspicuously altered in PV than in ET or early prodomal stage PV. The megakaryocytes in PV usually have a pleomorphic appearance with a wide range of megakaryocyte sizes including small, medium sized and large forms (Tables 3,4) as can be demonstrated in immune stained bone marrow biopsies using monoclonal antibodies against platelet glycoprotein. The characteristic increase and clustering of large megakaryocytes and proliferation of erythropoiesis with hyperplasia of dilatated sinuses are the diagnostic hallmark of untreated PV to distinguish it from secondary erythrocytosis [12,15,17,18], from Ph+ chronic granulocytic leukemia and Ph+ ET [13,15] and most importantly from PMGM [12,15,16]. Bone marrow histology in PMGM is dominated by atypical immature megakaryocytes, which are conspicuously large due to increase of nuclear as well as cellular size. The nuclei of megakaryocytes in PMGM are bulky with lobuli becoming clumsy. The lightly stained chromatin and irregular roundish nuclear forms give rise to the so-called cloud-like nuclei, which are almost never seen in ET and PV [11,12,14,16,21].

megakaryocytic granulocytic myeloproliferation PMGM as the third distinct MPD [11,12,14-17,22] (Table 2). The present appraisal of the myeloproliferative neoplasms (MPN) from Dameshek to Michiels review the clinical laboratory molecular and pathological (CLMP) characteristic of the MPNs caused by the driver mutations JAK2V617F, MPL515, JAK2exon12, CALR and MPL515 discovered by Constantinescu-Vainchenker, Pardani, Green and Kralovics respectively (Tables 1,2, Figure 1).

Diagnostic differentiation of ET and PV by erythrocyte count and BM histology

According to Dameshek, [2], Georgii, et al. [11,12], and Michiels, et al. [6,7,14-16,21,23-36], the diagnosis of ET according to PVSG and WHO criteria is one of exclusion. Bone...
marrow and erythrocyte count were not used in the PVSG/WHO classification as a specific clue to ET in various MPNs [6,7,15-17,31,32]. Wassermann, et al. [37], introduced crude inclusion criteria for the PVSG-01 randomized clinical trial to be sure that patients do have PV because they were subjected to potential leukemogenic agents P32 and chlorambucil as compared phlebotomy aiming at a hematocrit to below 0.50 [37]. These crude criteria are used by the PVSG since 1975 as diagnostic criteria for PV [38]. The PVSG [38] and 2008 and 2016 WHO criteria for PV [39-41] did not measure erythrocyte diagnostic criteria for PV [38]. The PVSG [38] and 2008 and [37]. These crude criteria are used by the PVSG since 1975 as compared phlebotomy aiming at a hematocrit to below 0.50

The spectrum of JAK2V617F mutated ET, PV and MF versus JAK2 wild type normocellular ET and hypercellular ET associated with prefibrotic PMGM [11,12,15] according to WHO criteria [8] by integrating the PVSG/WHO bone marrow features into the ECP and ECMP criteria of myeloproliferative Disorders [15,17], MPD Doctor’s Brochure 2004, Michiels, et al. [8].

The PVSG-01 randomized clinical trial to be sure that patients do have PV because they were subjected to potential leukemogenic agents P32 and chlorambucil as compared phlebotomy aiming at a hematocrit to below 0.50 [37]. These crude criteria are used by the PVSG since 1975 as diagnostic criteria for PV [38]. The PVSG [38] and 2008 and 2016 WHO criteria for PV [39-41] did not measure erythrocyte counts and MCV and did not use bone marrow histology features and persisted to use only crude cut-off levels for hemoglobin and hematocrit (Hb> 18.5 g/dl and Ht> 0.60 in men and Hb> 16.5 and Ht> 0.56 in women) as surrogate measures of red cell mass (RCM) to separate ET from PV.

Michiels, Thiele & De Raeeve used bone marrow histology and erythrocyte and platelet counts as pathognomonic clue to distinguish all variants of MPN from reactive thrombocytosis, BCR/ABL positive thrombocytosis in chronic myeloid leukemia (CML), and thrombocytosis in myelodysplastic syndromes (MDS, 5q minus syndrome) by demonstrating that clustered mature large megakaryocytes occur in MPN, small monolobulated megakaryocytes in CML and dysmorphic megakaryocytes in MDS [6-8,14,15,23,31,32,34,42].

Megakaryocytes are identical large, mature and pleio-morphic in prefibrotic JAK2V617F positive ET and PV patients (Tables 2-5) and clearly different from the large giant mature megakaryocytes in MPL thrombocytosis (Table 6) and from the large immature megakaryocytes with ‘cloud-like’ nuclei in CALR positive thrombocytasia (Table 7).

Erythrocyte count above the upper limit of normal (> 5.8 x10^{12}/L in males and > 5.6 x10^{12}/L in females) on top of characteristic bone marrow histology obviates the need to measure RCM [5,16,27,31,32,43,44] (Figure 2, Table 3). Bone marrow histology of sequential stages in prodromal, overt and advanced PV is typically featured by increased cellularity due to increase erythrocytic megakaryocytic (EM), erythrocytic, megakaryocytic granulocytic (EMG), and predominant megakaryocytic granulocytic (MG) myeloproliferation (Figures 5,6 and Tables 4,5). PV is frequently preceded by ET or

Table 2: The spectrum of JAK2V617F mutated ET, PV and MF versus JAK2 wild type normocellular ET and hypercellular ET associated with prefibrotic PMGM [11,12,15] according to ECP criteria [8] by integrating the PVSG/WHO bone marrow features into the ECP and ECMP criteria of myeloproliferative Disorders [15,17], MPD Doctor’s Brochure 2004, Michiels, et al. [8].

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>Normocellular ET</th>
<th>Hypercellular ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ET MF: RF3/4 = MF-2/3 (WHO criteria)</td>
<td>Hypercellular due to increased erythrocytic, megakaryocytic and granulocytic myeloproliferation (EMG, masked PV, MG fibrotic stages)</td>
<td>Hypercellular due to increased erythrocytic, megakaryocytic and granulocytic myeloproliferation (EMG, masked PV, MG fibrotic stages)</td>
</tr>
</tbody>
</table>

Table 3: Clinical, Laboratory, Molecular and Pathobiology (2015-2020 CLMP) criteria for diagnosis of JAK2V617F mutated essential thrombocytopenia (ET).

<table>
<thead>
<tr>
<th>Clinical, laboratory, and molecular (CLM) criteria</th>
<th>Bone marrow Pathology (P) criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prefibrotic ET</strong></td>
<td>Normocellular bone marrow (&lt; 60%), Megakaryocytic (M) proliferation of clustered of medium sized to large (pleiomorphic) mature megakaryocytes in anormocellular bone marrow (&lt; 60%), no proliferation of erythropoiesis and granulopoiesis.</td>
</tr>
<tr>
<td>1. Platelet count of ≥ 350 x10^{11}/L</td>
<td>Reticuline fibrosis (RF) 0 or 1</td>
</tr>
<tr>
<td>2. Heterozygous JAK2V617F mutation, and low JAK2 allele mutation load</td>
<td></td>
</tr>
<tr>
<td>3. Normal erythrocytes &lt; 5.8x10^{12}/L males, &lt; 5.6 x10^{12}/L females</td>
<td></td>
</tr>
<tr>
<td>4. Hemoglobin (Hb) and hematocrit (Ht) normal or upper range of normal</td>
<td></td>
</tr>
<tr>
<td><strong>Prefibrotic prodomal PV</strong></td>
<td>Increased cellularity (60%-80%) due to increased erythrocytic, megakaryocytic (EM) proliferation or trilinear erythrocytic, megakaryocytic, granulocytic (EMG) proliferation.</td>
</tr>
<tr>
<td>1. Platelet count of ≥ 350 x10^{11}/L</td>
<td>Increase of clustered medium sized to large (pleiomorphic) mature megakaryocytes.</td>
</tr>
<tr>
<td>Hb and Ht in upper range of normal, but erythrocyte count &lt; 5.8x10^{12}/L males, &lt; 5.6x10^{12}/L females</td>
<td>Spontaneous EEC.</td>
</tr>
<tr>
<td>2. Presence of JAK2- V617F mutation and variable JAK mutation load</td>
<td>RF 0 or 1</td>
</tr>
<tr>
<td>3. Low serum EPO, increased LAP score</td>
<td></td>
</tr>
<tr>
<td><strong>Prefibrotic hypercellular ET</strong></td>
<td></td>
</tr>
<tr>
<td>1. Platelet count of ≥ 350 x10^{11}/L</td>
<td>Hypercellular due to increased erythrocytic, megakaryocytic and granulocytic myeloproliferation (EMG, masked PV, MG fibrotic) or increased megakaryocytic, granulocytic (MG, fibrotic) proliferation with relative reduced erythroid precursors.</td>
</tr>
<tr>
<td>2. Presence of JAK2- V617F mutation and high JAK mutation load</td>
<td>Loose to dense clustering of more pleiomorphic megakaryocytes with hyperplasia or clumpy nuclei</td>
</tr>
<tr>
<td>3. Moderate myeloid neoplasia of the spleen – splenomegaly</td>
<td>Grading of reticulin fibrosis (RF, [113]) and myelofibrosis (MF, [11,12,109]).</td>
</tr>
<tr>
<td>4. No preceding or allied CML, PMGM, RARS-T or MDS</td>
<td></td>
</tr>
</tbody>
</table>

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https://doi.org/10.29328/journal.ijbmr.1001011
https://www.heighpubs.org/hbmr
Table 4: Clinical, Laboratory, Molecular and Pathobiology (2015-2020 CLMP) criteria for the diagnosis of prodromal, masked and classical JAK2 mutated polycythemia vera (PV) versus primary or secondary erythrocytoses

<table>
<thead>
<tr>
<th>Clinical, laboratory, molecular (CLM) criteria</th>
<th>Bone marrowPathology (P) criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major criteria for PV</td>
<td></td>
</tr>
<tr>
<td>A 1. Erythrocytes &gt; 5.6x10^12/L males and &gt; 5.6x10^12/L females. Hemoglobin and hematocrit upper range of normal or increased.</td>
<td></td>
</tr>
<tr>
<td>A 2. Heterozygous and/or homozygous JAK2V617F or JAK2exon12 mutation.</td>
<td></td>
</tr>
<tr>
<td>A 3. Low serum Epo level.</td>
<td></td>
</tr>
<tr>
<td>Confirmative criteria</td>
<td></td>
</tr>
<tr>
<td>B 1. Persistent increase of platelet count x10^3/L: grade I: 400-1500, grade II: &gt; 1500.</td>
<td></td>
</tr>
<tr>
<td>B 2. Granulocytes &gt; 10 x10^3/L or Leukocytes &gt; 12 x10^3/L and raised LAP-score or increased CD11b expression in the absence of fever or infection.</td>
<td></td>
</tr>
<tr>
<td>B 3. Myeloid neoplasia of the spleen → splenomegaly on ultrasound echogram (&gt; 12 cm length in diameter) or on palpation.</td>
<td></td>
</tr>
<tr>
<td>B 4. Spontaneous endogenous erythroid colony (EEC) formation (optional).</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: 2015-2020 CLMP staging of JAK2V617F positive prodromal PV, erythrocytic PV, classical PV, masked PV and MF, inapparent PV, spent phase PV and post-PV myelofibrosis (MF).

<table>
<thead>
<tr>
<th>PV: CLMP stage</th>
<th>0</th>
<th>Clinical Diagnosis Prodromal PV</th>
<th>Erythrocythemia (E)</th>
<th>Early PV</th>
<th>Classical PV</th>
<th>Masked PV</th>
<th>Advanced PV</th>
<th>Post-PV MF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAP-score, CD11B</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑/↑/↑↑↑/↑↑/↑↑</td>
<td>↑/↑↑↑</td>
<td>↑/↑↑↑/↑↑↑/↑↑</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>EEC</td>
<td>+</td>
<td>+</td>
<td>↑</td>
<td>↑/↑/↑↑↑/↑↑/↑↑</td>
<td>↑/↑↑↑</td>
<td>↑/↑↑↑/↑↑↑/↑↑</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>Red Cell Mass</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>↑/↑/↑↑↑/↑↑/↑↑</td>
<td>↑/↑↑↑</td>
<td>↑/↑↑↑/↑↑↑/↑↑</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>Erythrocytes x10^12/L</td>
<td>&lt; 5.8</td>
<td>&gt; 5.8</td>
<td>&gt; 5.8</td>
<td>&gt; 5.8</td>
<td>N</td>
<td>N</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Leukocytes x10^9/L</td>
<td>&lt; 10</td>
<td>&gt; 10-12</td>
<td>12-15</td>
<td>&gt;15</td>
<td>↑↑↑↑/↑↑↑↑/↑↑↑↑</td>
<td>↑↑↑↑/↑↑↑↑/↑↑↑↑</td>
<td>↓ or ↑↑↑↑/↑↑↑↑</td>
</tr>
<tr>
<td></td>
<td>Platelets x10^9/L</td>
<td>&gt; 400</td>
<td>&lt; 400</td>
<td>&lt; or &gt; 400</td>
<td>&gt; 400</td>
<td>+1000</td>
<td>↑↑↑↑/↑↑↑↑/↑↑↑↑</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>CLMP bone marrow histology</td>
<td>EM</td>
<td>EM</td>
<td>EM</td>
<td>EM</td>
<td>EMG</td>
<td>EMG-MF</td>
<td>MF</td>
</tr>
<tr>
<td></td>
<td>BM cellularity (%)</td>
<td>50-80</td>
<td>50-80</td>
<td>60-100</td>
<td>80-100</td>
<td>80-100</td>
<td>60-100</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Grading RF</td>
<td>MF 0</td>
<td>RF 0-1</td>
<td>RF 0-1</td>
<td>RF 1/2</td>
<td>RF 3/4</td>
<td>RF 3/4</td>
<td>MF 2/3</td>
</tr>
<tr>
<td></td>
<td>Grading MF52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BM cellularity (%)</td>
<td>50-80</td>
<td>50-80</td>
<td>60-100</td>
<td>80-100</td>
<td>80-100</td>
<td>60-100</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Grading RF</td>
<td>MF 0</td>
<td>RF 0-1</td>
<td>RF 0-1</td>
<td>RF 1/2</td>
<td>RF 3/4</td>
<td>RF 3/4</td>
<td>MF 2/3</td>
</tr>
<tr>
<td></td>
<td>Grading MF52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleen size:</td>
<td>&lt; 12-15</td>
<td>&lt;13</td>
<td>12-15</td>
<td>12-16</td>
<td>18&gt; 20</td>
<td>16&gt; 20</td>
<td>&gt; 20</td>
</tr>
<tr>
<td></td>
<td>On echogram Below MCL</td>
<td>&lt; 12-15</td>
<td>&lt;13</td>
<td>12-15</td>
<td>12-16</td>
<td>18&gt; 20</td>
<td>16&gt; 20</td>
<td>&gt; 20</td>
</tr>
<tr>
<td></td>
<td>JAK2V617F load</td>
<td>Low</td>
<td>Low</td>
<td>Moderate 50%+</td>
<td>Mod/High +/+</td>
<td>High &gt;50% ++</td>
<td>High &gt;50% ++</td>
<td>High &gt;50% ++</td>
</tr>
<tr>
<td></td>
<td>Granulocytes %</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>High IFN if non Responsive HU-JAK2 inh</td>
<td>JAK2 inhibitor</td>
</tr>
<tr>
<td>Risk stratification</td>
<td>Therapeutic implications</td>
<td>Low Aspirin</td>
<td>Low Aspirin</td>
<td>Low Aspirin</td>
<td>Low Aspirin</td>
<td>Intermediate IFN</td>
<td>High IFN if non Responsive HU-JAK2 inh</td>
<td>JAK2 inhibitor</td>
</tr>
</tbody>
</table>

Table 6: 2015-2020 Clinical Laboratory, Molecular and Pathobiology (CLMP) criteria for the diagnosis of normocellular ET carrying one of the MPL515 mutations.

<table>
<thead>
<tr>
<th>Clinical, laboratory, molecular (CLM) criteria</th>
<th>Bone marrow Pathology (P) criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet count &gt; 350x10^12/L and presence of large platelets in blood smear.</td>
<td></td>
</tr>
<tr>
<td>2. Normal Hemoglobin, haematocrit and erythrocyte count.</td>
<td></td>
</tr>
<tr>
<td>4. Normal serum EPO.</td>
<td></td>
</tr>
<tr>
<td>5. Normal LAP score (CD11b).</td>
<td></td>
</tr>
<tr>
<td>6. No or slight splenomegaly.</td>
<td></td>
</tr>
<tr>
<td>7. No preceding or allied CML, PV, PMGM, RAS-T or MDS.</td>
<td></td>
</tr>
<tr>
<td>Clinical staging similar as in CALR thrombocytocemia based on the degree of anemia, splenomegaly and myelofibrosis.</td>
<td></td>
</tr>
</tbody>
</table>
below 5.8 x10^{12}/L with hematocrit values ranging from 0.40 to 0.45 (Figure 1, Tables 2-4). At erythrocytes above 5.8 x10^{12}/L, tear drop erythrocytes, increased LDH, increased CD34+ cells, pronounced splenomegaly, normal or decreased platelet counts, leucocytosis or leukopenia.

prodromal PV for several up to more than 10 years. Bone marrow iron stain is usually positive in normocellular ET, but negative in PV. At red cell mass (RCM) values above 1000x10^9/L, which are clearly below the 2008/2016 WHO-defined criteria for PV, platelet count around or above 1000x10^9/L. The incidence of major arterial and venous episodes in PV correlated positively with increased haematocrit level [46-49]. A mean haematocrit of 0.60 and a mean platelet count of 512x10^9/L at time of diagnosis of PV were associated microvascular ischemic events and major thrombosis in 49%. The risk of major vascular episodes were the lowest at hematocrits below 0.44, higher at hematocrits above 0.45 and the highest at hematocrits above 0.50 as was the case in the PVSG 01 study when not on low dose aspirin for the prevention of platelet-mediated microvascular ischemic disturbances, TIAs and acute coronary syndromes [36,50,51]. Low dose aspirin at hematocrits of around 0.40 in JAK2^{V617F} mutated ET and PV significantly reduces the incidences of both microvascular as well as major vascular events as compared to not using aspirin in randomized clinical trials [30,36,52]. Phlebotomy on top of low dose aspirin (40 to 80 mg OD) is the cornerstone of treatment of newly diagnosed PV patients with low, intermediate and high MPN disease burden [34-36,53].

In the late 1970s, the London PV study Group of Pearson, Messinezy, Thomas and Weitherley-Mein demonstrated that on top of the microvascular disease of thrombocythemia, the incidence of major arterial and venous episodes in PV correlated positively with increased haematocrit level [46-49]. A mean haematocrit of 0.60 and a mean platelet count of 512x10^9/L at time of diagnosis of PV were associated microvascular ischemic events and major thrombosis in 49%. The risk of major vascular episodes were the lowest at hematocrits below 0.44, higher at hematocrits above 0.45 and the highest at hematocrits above 0.50 as was the case in the PVSG 01 study when not on low dose aspirin for the prevention of platelet-mediated microvascular ischemic disturbances, TIAs and acute coronary syndromes [36,50,51]. Low dose aspirin at hematocrits of around 0.40 in JAK2^{V617F} mutated ET and PV significantly reduces the incidences of both microvascular as well as major vascular events as compared to not using aspirin in randomized clinical trials [30,36,52]. Phlebotomy on top of low dose aspirin (40 to 80 mg OD) is the cornerstone of treatment of newly diagnosed PV patients with low, intermediate and high MPN disease burden [34-36,53].

**JAK2^{V617F} mutated trilinear MPN**

EPO-independent progenitor colony-forming unit-erythroid (CFU-E) and burst forming unit-erythroid (BFU-E) labelled as spontaneous endogenous erythroid colony formation (EEC) became the hallmark of PV [54]. Analysis of about 500 PV patients from 26 studies indicated that EEC in expert hematological laboratories has a near 100% diagnostic specificity for overt and masked PV and ET mimicking PV or latent PV [54,55].

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**Table 7:** Clinical Laboratory, Molecular and Pathobiology (2015-2020 CLMP) criteria for hypercellular thrombocythemia associated with primary megakaryocytic, granulocytic myeloproliferation (PMGM) caused by calreticulin (CALR) mutations.

<table>
<thead>
<tr>
<th>CLM criteria</th>
<th>Bone marrow Pathology (P) criteria</th>
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<tbody>
<tr>
<td>A1</td>
<td>Megakaryocytic (M) myeloproliferation of dense clustered atypical large immature megakaryocytes with hypolobulated nuclei in a normocellular bone marrow.</td>
</tr>
<tr>
<td>A2</td>
<td>Prefibrotic dual megakaryocytic granulocytic (MG) myeloproliferation and relative or absolute reduction of erythropoiesis and erythroid precursors. Abnormal dense clustering and increase in atypical medium sized, large to giant immature megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects.</td>
</tr>
<tr>
<td>C</td>
<td>No features of PV in blood and bone marrow.</td>
</tr>
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</table>

Grading reticulin fibrosis (RF), Wilkins, et al. [113], and myelofibrosis (MF) Georgil, et al. [11,12], Thiele, et al. [109].

MF 0 Prefibrotic CALR MG, no reticulin fibrosis RF 0/1.

MF 1 Early fibrotic CALR MG slight reticulin fibrosis RF 2.

MF 2 Fibrotic CALR MG increase RF grade 3 and slight to moderate collagen fibrosis.

MF 3 Advanced fibrotic CALR MG with collagen fibrosis-osteosclerosis.

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**Figure 4:** Megakaryocytic (M) proliferation in a normocellular bone marrow (60%) with hematocrit values ranging from 0.40 to 0.45 (Figure 1, Tables 2-4). At erythrocytes above 5.8 x10^{12}/L, diagnostic for PV, (Table 4), the hematocrit values ranged from 0.46 to 0.72 and Hb values ranged from 15.0 to 20.9 g/dL, which are clearly below the 2008/2016 WHO-defined criteria for PV [39,41,45]. Seven ET patients had normal RCM at erythrocyte counts between 4.4 to 5.3 x10^{12}/L of whom 4 had normocellular (< 60%) ET and 3 had hypercellular (60%-80%) prodromal PV bone marrow histology. Erythrocyte counts remain above 5.8x10^{12}/L in phlebotomy induced PV in hematological remission due to microcytic erthrocytosis (MCV below 70 fl) caused by iron deficiency [2,10] (Figure 4). In newly diagnosed PV patients the state of PV in remission by phlebotomy due to iron deficiency induced microcytosis of erythrocytes is reached after about 1 to 2 years of repeated venesections (Figures 3,4). This is associated with the relief of hypervolemic symptoms in PV patients during long-term or even life-long follow-up [2,4,31,32].
The 9p loss of heterogeneity (9pLOH) due to mitotic recombination of chromosome 9p is the most frequent chromosomal lesion described in PV (∼33%) not detectable by cytogenetic analysis [56]. Kralovics, et al. sequenced 19 candidate genes mutations within the 9pLOH region and no mutations were found in the Janus kinase (JAK) gene, but Kralovics did not screen the JH2 pseudokinase gene thereby overlooking the JAK2V617F in the JH2 pseudokinase gene (Figure 1). Constantinescu & Vainchenker searched for a mutation in the 9pLOH region of the complete JAK2 gene and did found the JAK2V617F in the JAK2 pseudogene of 3 PV and 2 controls by detection of a G-to-T mutation at nucleotide 1849 in exon 12 leading a substitution of valine to phenylalanine at position 617 (V617F) in the JAK2 pseudo-gene. This V617F substitution of the JAK2V617F mutation was present in 40 of 45 PV patients, in 9 of 21 ET patients and in 3 of 7 MF patients [57], (Figure 1). The JAK2V617F substitution was absent in patients with secondary erythrocytosis (N = 35) and 15 controls [57]. Thirty percent of PV patients are homozygous for JAK2V617F mutation without so called 9pLOH due to mitotic recombination, whereas heterozygous JAK2V617F mutated ET and PV patients showed the presence of 9pLOH [57,58] (Table 1, Figure 1).
Constantinescu & Vainchenker, [59] demonstrated that the acquisitions of heterozygous, hetero-homozygous and homozygous due to mitotic recombination JAK2V617F mutation on chromosome 9p are the driver causes of sequential megakaryocytic (M), erythrocytic megakaryocytic (EM) and erythro-megakaryo-granulocytic (EMG) myeloproliferations seen in normocellular ET, prodromal PV and classical and advanced PV in trilinear MPN (Figures 5,6), [21,57,60], (Table 1, Figure 1). The JAK2V617F mutation as the driver cause of trilinear MPN was immediately confirmed in three large groups of ET, PV and MF patients due to inside information during the peer review process in 2004 [58,59,61]. JAK2V617F mutation induces a loss of inhibitory activity of the JAK2 pseudokinase part on the JAK2 JH kinase part leading to enhanced activated of the normal JAK2 JH kinase activity, which makes the TPO, EPO and granulocyte growth factor receptors on the hematopoietic progenitor cells hypersensitive to their growth factors TPO, EPO and granulocyte growth factor (Figures 5,6). The JAK2V617F mutated hematopoietic cells produce Contitutively activated platelets and leukocytes (increased leukocyte alkaline phosphatase: LAP) and quantitative increase of platelets erythrocytes and granulocytes (Table 1). The sequential occurrence of low heterozygous, combined heterozygous and homozygous normal homozygous JAK2V617F allele load can readily explain the sequential occurrence of ET, prodromal PV, classical PV and advanced PV followed by secondary MF in trilinear MPN during lifelong follow-up. JAK2V617F trilinear MPN is clearly different from JAK2 wild type hypercellular ET associated with PMGM [11,12,14,16] as the third distinct entity of MPD without features of PV (Figure 2, Tables 12-15 in Michiels, et al. [8]). According to 2006 ECMP criteria the sequential transitional states of JAK2V617F disease entity ranged from heterozygous normocellular ET and latent PV mimicking ET labelled as prodromal PV or forme fruste PV followed by heterozygous/homozygous mutated erythrophagocytic and early PV, and homozygous mutated advanced PV and post-PV myelofibrosis (Figures 1,2, Table 1), [8,21,62]. The JAK2V617F mutated trilinear MPN phenotypic expression includes normocellular ET, prodromal PV, erythrocytic PV with normal platelet and leukocyte count, classical PV, masked PV, and various degrees of splenomegaly and myelofibrosis (MF) [57,59] (Figure 1, Table 1). The quantitative JAK2V617F allele burden in neutrophils and in CD34+ cells from the same blood sample in 96 JAK2-positive MPN patients (17 ET, 64 PV and 15 MF mean follow-up 7 years) were below 50% in the majority of ET and about half of the PV patients [63]. The JAK2V617F CD34+ allele burden were above 50% in about half of the PV patients and the majority of MF indicating advanced trilinear MPN disease burden. The neutrophil JAK2V617F allele burden is usually below 50% in ET and above 50% in PV and MF. The CD34+ allele burden is much lower than the neutrophil JAK2V617F allele burden in ET and early stage PV with no splenomegaly [64]. This is completely in line with the concept that some maturation of JAK2V617F mutated hematopoietic stem cells is needed to neoproliferate because of hypersensitivity megakaryopoiesis to TPO and erythropoiesis to EPO [64,65] (Figures 5,6, Table 1). The neutrophil JAK2V617F allele burden alone can overestimate the MPN disease burden at the bone marrow progenitor cell level in early stage ET and PV neoproliferative disease [63,64].

Bone marrow morphology of clustered medium to large mature megakaryocytes is similar in heterozygous-mutated JAK2V617F ET and homozygous-mutated JAK2V617F PV patients at time of diagnosis (Figures 2,3, Tables 3-5), [8,9,13,24,25,66]. The bone marrow in heterozygous JAK2V617F ET is normocellular with increased clustered large megakaryocytes (M) proliferation and no or slight increase of erythropoiesis [8,9,24,61,66-68]. The bone marrow in JAK2V617F PV patients with less than 50% mutation load is hypercellular (60-80%) due to increased erythropoiesis and megakaryopoiesis in prodromal PV. Classical, masked and advanced stages of PV typically show a trilinear 90-100% hypercellular bone marrow [2] due to increased erythro-megakaryo-granulocytic (EMG, [15,17,18]). The JAK2V617F allele burden in WHO-defined advanced PV and post-PV myelofibrosis(MF) patients ranged from 50% to 100% [63,67,69-71]. According to the Vainchenker’s “dosage” concept, heterozygosity for the JAK2V617F mutation in acquired ET and autosomal dominant JAK2 or TPO mutated hereditary ET (HET) is enough to activate megakaryocytes to induce the ET clinical phenotype [8,21,27,28,60,72] (Figure 5, Table 1). Patients with dominant hereditary ET (HET) heterozygous for the JAK2V617F and JAK2R564I germ line mutations have a clinical ET phenotype with normal values for Hb, Ht, erythrocytes, thrombopoietin (TPO), and erythropoietin (EPO) levels. The response to EPO in the EEC assay was normal in congenital JAK2V617I and JAK2R564I [73-75], but increased in acquired JAK2V617F mutated ET and PV (Figure 5, Table 1) [8]. According to the JAK2V617F dosage hypothesis the JAK2V617F mutation load is low in heterozygous mutated ET and increases from below to above 50% inpatients with homozygous JAK2V617F mutated PV, advanced PV and post-PV myelofibrosis [8,58,64,76] (Figure 6). According to Vainchenker’s “dosage” concept the higher intracellular levels of JAK2V617F kinase activity in homozygous mutated progenitor stem cells preferentially activate the erythropoietin receptor (EPOR) and generate a PV-like phenotype with erythrocytes above 5.8x10¹²/L and increased activated platelet and leukocyte counts (Figures 5, Table 1) [8,9,24,35,63,66,70,72].

The JAK2V617F allele burden was directly correlated with increased levels of hematocrit, neutrophil count, LDH and leukocyte alkaline phosphatase (LAP) score, spleen size on echogram [8] (Table 1) and with decreased values for platelets, serum ferritin, and erythropoietin, with higher relative risks for aquagenic pruritus, spleen size on echogram, total thrombosis and the need for myelosuppressive treatment [69]. The JAK2V617F allele burden in granulocytes in a prospective study of 175 PV patients could be quantified as 1%-25%, 25% to 50%, 50%-75% and 75%-100% in 57, 50, 34
and 32 PV patients respectively [69]. Prefibrotic heterozygous JAK2V617F mutated ET usually runs a benign course with low JAK2 and MPN burden and a normal to near normal life expectancy (Table 1) [8,9,21] Figure 7, [76]. Prefibrotic heterozygous JAK2V617F mutated prodromal and classical PV usually have a low mutation burden associated with microvascular and major thrombosis at time of presentation (Table 1). Homozygous JAK2V617F mutated trilinear PV result in high JAK2V617F and hypercellular MPN burden associated with progressive extramedullary myeloid neoplasia of the spleen (MNS), splenomegaly and cytokine mediated MF during long-term follow-up [70,76] (Table 1, Figure 6). Transition of heterozygous into homozygous JAK2V617F mutation due to mitotic recombination of chromosome 9p (9pLOH) is strongly correlated with progression into advanced PV and masked PV with splenomegaly in figures 1 and 2 [77], Table 4, [31,32] followed by post-PV myelofibrosis and associated with high JAK2 allele burden [6,7,24,34,63,70,76].

The UK MPN Study Group [71,78] elegantly confirmed the Vainchenker’s ‘dosage’ concept at the biological EEC level by studying the genotype of individual BFU-E in a crosssectional cohort of 29 JAK2V617F mutated ET and 30 JAK2V617F mutated PV patients (Figure 8). The JAK2 mutation load was expressed as a percentage (%) of EEC colonies genotyped as homozygous (red), heterozygous (purple) or wild type [78] (Figure 8). All 29 JAK2V617F positive ET patients have heterozygous JAK2 mutated EEC colonies: 9 of them have a low percentage (< 10%) of homozygous JAK2 mutated colonies. Out of 30 JAK2V617F positive PV patients, 8 have heterozygous JAK2 mutated EEC, 13 have homozygous EEC colonies of more than 50% and 7 of less than 50%. Homozygous EEC colonies were absent or rare in heterozygous ET, but prevalent in JAK2V617F-positive PV [78] (Figure 6). These observations are completely in line with Vainchenker’s “dosage” concept (Figures 5,6, Table 1) [8,9,21,60,72]. Additional cytogenetic [79], genetic or epigenetic alterations in PV and MF patients are of huge prognostic significance [80-84]. The presence of epigenetic factors like TET2 or ASXL1 etc on top of the JAK2, MPL and CALR driver mutations of MPN is associated with impaired prognosis in MPN, MDS and other myeloid malignancies as well. The targeted search for epigenetic factors will become hugely important to the understanding of differences in biology, prognosis and outcome of MPN patients [81-84]. Using next generation sequencing (NGS) on top of the JAK2 or CALR mutation, the Swiss MPN investigators in Basel found one, two or more epigenetic somatic mutations in 65 (33%) of 197 WHO defined MPN patients (94 PV, 69 ET, 34 MF) [82]. Seventeen of 69 (25%) ET patients, 11 of 34 (32%) MF and none (0%) of 94 PV patients carried mutations in CALR. In addition to JAK2V617F and CALR, the most frequently observed epigenetic somatic mutations affecting the biology and natural history of MPN disease included TET2, ASXL1, DNMT3A, EZH2, and IDH1 [82-84]. Rare epigenetic mutations were NF1, NFE2, and RUNX1. The presence of one, two or more somatic mutations appeared to impair prognosis in JAK2 and CALR mutated MPN [82]. Tefferi, et al. [83,84], confirmed the Lundberg observations in large scale retrospective studies in WHO defined ET, PV and MF patients demonstrating that epigenetic somatic mutation detection on top of the JAK2, CALR and MPL mutational load and subtype MPN characterization is far superior to classify the distinct MPN diseases as compared to the crude WHO classification, that cannot clearly distinguish between ET, prodromal overt and masked PV and PV with MF. Dr. Green, addressed the key question whether the sequence of acquisition of somatic mutations can be inferred from the genotypes of detectable subclones [85]. For instance, if some tumor cells have JAK2V617F, and others from the same patient bear JAK2V617F with an additional somatic mutation, then this indicates that JAK2V617F came first. Genotyping individual hematopoietic colonies has shown that the order of acquisition of JAK2V617F, relative to mutations in TET2 or DNMT3A, influences subclonal composition within HSPCs and mature cell compartments, disease presentation, and clinical outcome. In JAK2-first patients, the HSC compartment is dominated by double-mutant cells, and such patients present at a younger age, often with PV. Conversely, in TET2-first patients, the HSC compartment is dominated by single mutant cells, and such patients present at an older age, usually with ET. JAK2-first patients had a greater likelihood of presenting with PV than with ET, had an increased risk of thrombosis, and an increased sensitivity of JAK2 mutant progenitors to ruxolitinib in vitro.

Erythromelalgic microvascular circulation disturbances or platelet thrombophilia in PV and ET: From Dameshek to Michiels & Van Vliet

Dameshek & Henthel, [1] described the presenting clinical manifestations in 20 newly diagnosed PV patients including quite severe headaches in 17, attacks of migraine in 14, visual disturbances, particularly spots before the eyes and coloured scotomas in 6, paresthesias numbing and tingling

![Figure 7: Granulocyte JAK2V617F mutated allele burden in WHO defined 250 ET, 212 PV, 18 post-ET and 35 post-PV patients from the study of Rumi et al. [76]. JAK2V617F allele burden was recorded in granulocytes positively above 52% (change from heterozygous-homozygous) in 2%, 41%, 72%, and 93% of ET, PV post-ET and post-PV myelofibrosis patients. Granulocyte CALR mutation allele load in granulocytes of selected 38 CALR ET patients at time of diagnosis was below 50% (median 32%) and around 50% in 10 CALR post-ET MF patients (median 50%) in the study of Rumi et al. [76].](https://doi.org/10.29328/journal.ijbmr.1001011)
Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2V617F trilinear polycythemia vera (PV), JAK2exon12 PV and JAK2V617F, CALR and MPL515 thrombocythemias: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels

Figure 8: Proportions of JAK2 genotypes in BFU-Es from 59 patients with JAK2V617F-mutated essential thrombocythemia (ET) and polycythemia vera (PV) [78]. Each vertical bar represents 1 patient, divided according to the proportion of wild-type, heterozygous, and homozygous-mutant colonies obtained, with the absolute colony numbers shown above: (wild type white), heterozygous (purple) homozygous (red). Results of EEC colony genotypes are presented for 29 JAK2V617F-positive ET (B) patients (total 2277 colonies; mean 79 per patient) and for 30 JAK2V617F-positive PV (A) patients (total 2287 colonies; mean 76 colonies per patient). All 29 JAK2V617F-positive ET patients have heterozygous JAK2 mutated EEC colonies and less than 10% homozygous colonies in 9 and 20% in 1 of them. Out of 30 JAK2V617F positive PV patients 8 have heterozygous JAK2 mutated EEC, 13 have homozygous EEC colonies of more than 50% and 7 of less than 50%.

A. In total 29 PV patients: 5 were heterozygous, 13 heterozygous/homozygous and 11 predominant homozygous (high allele burden) for the JAK2V617F mutation.

B. In total 18 JAK2exon12 mutated PV: all are predominant heterozygous (low allele burden) for the JAK2exon12 mutation, but 7 of them had a minor clone of homozygous mutated BFU-Es.

C and D. EEC colony genotypes for 18 patients with JAK2exon12 mutated PV (total 1931 colonies; mean 107 per patient). D show example sequence traces for patients with patients with homozygous JAK2exon12 mutations in colonies. In total, 16 patients (5 “heterozygous-only” JAK2V617F-positive PV patients, 4 JAK2V617F positive PV patients with homozygous and heterozygous clones, 3 JAK2V617F positive ET patients with small homozygous clones, and 4 JAK2exon12 mutated PV patients with homozygous clones) were assessed in this way (mean time between experiments, 13 months; range, 2-32 months) and showed reproducibility of proportions of heterozygous and homozygous-mutant colonies.

Interpretation. The JAK2V617F dosage concept of Constantinescu & Vainchenker is in line with the EEC bone marrow findings in the UK study in Figures A and B [78]. A low level of JAK2V617F kinase activity only activate the MPL (TPO) receptor and favors the ET phenotype in acquired heterozygous (Figure 2, Table 1), [8,27,60,76] and in dominant heterozygous JAK2 or TPO mutated ET (Figure 2, HET), [28]. A high level of JAK2V617F activity in heterozygous/homozygous or homoygous mutated trilinear MPN is needed to activate the erythropoietin receptor (EPOR) and generate a PV-like phenotype (Figure 3), [8,21,31,32,57,59]. Similarly, high levels of JAK2exon12 activity of long duration in homozygous mutated trilinear MPN is needed to activate the granulocyte colony-stimulating factor receptor (C-GCSF, Figure 5) leading to EM or MG bone marrow phenotype and progressive secondary myelofibrosis (MF) [31,32,69,70]. Other mechanisms do occur in the pathobiologies of myeloid metaplasia and myelofibrosis in advanced stage of trilinear MPNs.
in toes and fingers in 12 and various types of transient major thrombosis (cerebral, coronary, venous) in 9 cases. Dameshek & Henthel, et al. [1] noted that the lack of large vessel involvement in PV and the associated high platelet counts suggested the possibility of ‘platelet thrombophilia’ as the cause of multiple small peripheral vascular thromboses in the peripheral cerebral and coronary circulation similar to aspirin-responsive platelet-dependent thrombophilia in JAK2exon12 mutated thrombocytopenia in ET and PV patients first discovered and described by Michiels Ten Kate & Van Vliet in [86] 1984-1985 and subsequently confirmed between 1985 and 2018 [20-22,35,36,86]. The broad spectrum of acrocyanosis, erythromelalgic and acrocyanotic ischemia or gangrene together with the episodic and transient neurologic symptoms of migraine accompaniments, attacks of amnesia, dysbasia, dysphasias, TIA, hemiparesis and acute coronary ischemic syndromes all are the consequence of one underlying disorder: arterial thrombophilia caused by JAK2 exon12 platelet-mediated and MPL515 platelet-mediated arteriolar inflammation and thrombosis in acquired thrombocytopenias [20-22,27,28,35,36,86]. Platelet-mediated erythromelalgic microvascular disturbances also occur in dominant hereditary ET (HET) caused by hyperactive germ line gain of function mutation in the TPO, JAK2 and MPL genes [34,73-75] (Figure 5). Erythromelalgia is rare CALR mutated thrombocytopenia and has never been observed in reactive thrombocytosis [35,36]. Platelet-mediated inflammatory and thrombotic processes in the end-arterial microcirculation typically respond to aspirin, but not to platelet ADP inhibitors and anticoagulation with vitamin K antagonists [20-22,35,36]. JAK2 V617F mutated platelets are constitutively activated, hypersensitive (sticky) and cause aspirin responsive platelet-mediated microvascular circulation disturbances, (Table 1). [20,86-94] has recently been discovered by Michiels as the novel Aspirin-responsive Sticky Platelet Syndrome in JAK2 V617F, MPL and TPO mutated thrombocytopenias [35,36].

JAK2exon12 mutations as cause of Isolated Erythrocythemia and PV

The finding of the JAK2exon12 mutations in the 5% PV patients negative for JAK2 V617F usually present with early stage PV or isolated erythrocythemia (IE, Figure 8) with increased red cell mass but normal leukocytes and platelets and no palpable spleen [95-98]. The frequency of JAK2exon12 mutations among all PV patients is estimated around 3% [95,98]. JAK2 N542-E543del is the most frequent among the different reported exon 12 mutations. JAK2exon12 mutated MPN patients with increased erythrocytes above 6.0x10^{12}/L and a typical PV bone marrow histology are diagnosed as benign IE or PV with a favourable outcome and normal life expectancy [95,96,98,99]. Pre-treatment bone marrow histology in JAK2exon12 mutated PV or IE showed characteristic erythroid hyperplasia with minor and distinct histology changes of the megakaryocytic lineage, which are not seen in primary or secondary erythrocytoses (PE and SE) [95]. Cases of JAK2exon12 mutated IE or PV have erythrocytes above 6x10^{12}/L [100], normal platelet and leukocyte counts, no or palpable spleen and a typical hypercellular bone histopathology predominantly due to erythroid hyperplasia and clusters of large megakaryocytes with hyperploid nuclei [95,98] (Figure 8). Bone marrow histology in 7 cases (4 IE, 3 PV) of JAK2exon12 mutated MPN in the pathology study of Lakey, et al. [97], showed prominent hyperplasia of erythropoiesis and atypical small to medium-sized large megakaryocytes (Figure 8). A low percentage of homozygosity was found for the JAK2 K539L-type and E543del-type exon 12 mutations (Figure 8) [78]. Godfrey, et al. [78], assessed the colony genotypes for 18 patients with JAK2exon12 mutated PV in a total of 1931 colonies; mean 107 per patient (Figure 8C).

Example sequence traces for patients with homozygous JAK2exon12 mutations in colonies are shown in figure 8D. In total, 16 patients (5 “homozygous-only” JAK2 V617F-positive PV patients, 4 JAK2 V617F-positive PV patients with homozygous and heterozygous clones, 3 JAK2exon12-positive ET patients with small homozygous clones, and 4) JAK2exon12 mutated PV patients with homozygous clones showed reproducibility of proportions of heterozygous and homozygous-mutant colonies (Figure 8D).

Acquired MPL515 mutated normocellular ET

The prevalence of the MPL515 mutated ET range from 3% of MPN to 8.5% of JAK2 wild type ET and MF [101-103]. The clinical presentation in 30 MPL515 mutated ET patients (9 males and 21 females, age 22-84, mean 56 years) was featured major arterial thrombosis in 23%, venous thrombosis in 10%, aspirin responsive microvessel disturbances in 60%, and major hemorrhage in 7% [101]. The clinical, laboratory, molecular and pathological (CLMP) findings in MPL515 mutated ET were increased platelet count, 956±331 x 10^{9}/L in all, slight splenomegaly in 5 (17%), and no PV features in blood and bone marrow in all table 6, [31,32,34]. Pretreatment bone marrow histology at the time of diagnosis in MPL515 mutated ET features large and giant megakaryocytes with hyperlobulated staghorn-like nuclei (Figure 10, Table 6), clearly different from JAK2exon12 PV (Figure 9), and distinct from JAK2exon12 ET and prodromal PV (Figures 2,3,9) and distinct from CALR thrombocytopenia (Figures 11,12).

Megakaryocyte Leukemia (ML) and CALR mutated Thrombocytopenia: From Dameshek 1951 to Kralovics 2013 and Michiels 2015

According to Dameshek,[10] megakaryocyte leukemia (ML) is defined by platelet counts around and above 1000x10^{9}/L without features of PV in blood and bone marrow smear and biopsy. The traditional classification of the myeloproliferative disorders (MPD) by the PVSG and used in textbooks was revised in the Hannover Bone Marrow classification to include PV, primary thrombocytopenia (PTH), and hypercellular thrombocytopenia related to primary megakaryocytic
myeloproliferation (PMGM, Table 7) without features of PV [11,12,14,16,104]. The discovery of the calreticulin (CALR) as the main cause of JAK2/MPL515 wild type thrombocytemia and PMF by Kralovics and his team [105] was identified by Michielis & De Raeve [31,32] as the driver cause of prefibrotic and fibrotic stages of PMGM without features of PV. This led to the second ground breaking event in the molecular landscape of the MPNs that induced a complete revision of all MPN classifications of the PVSG, WHO into the current Clinical Laboratory, Genetic and Pathobiological (2018 CLMP) criteria for JAK2V617F trilinear MPN (Tables 3 and 4), and JAK2exon12 PV as compared to two distinct MPL515 (Table 6) and CALR thrombocythemia and myelofibrosis (Table 7) without features of PV.

Kralovics performed targeted whole-exome sequencing in 6 cases of WHO defined JAK2/MPL wild type PMF patients and found somatic calreticulin (CALR) mutations of 52-bp deletion in 1, of 1bp deletion in 1 and recurrent 5-bp insertion in 4
MF patients. The CALR somatic mutation was subsequently discovered as the driver cause of thrombocythemia in 78 of 311 (25%) ET patients and in 72 of 203 (35%) MF patients [105]. The CALR mutation was detected in none of 382 PV, 45 CML, 73 MDS, and 64 chronic myelomonocytic leukemia (CMML) patients. Three (12%) of 24 RARS-T cases were positive for both the SF3B1 and CALR mutation. A subsequent Italian-Austrian study of 1235 WHO-defined ET and MF patients detected the JAK2V617F, MPL 515 and CALR mutation in 63.3%, 23.5% and 4.4% respectively with 8.8% being negative for all three mutations [76] (Figure 6). Evolution into MF during follow up was as high in CALR mutated ET as in JAK2V617F mutated PV (about 20% after 20 years). CALR mutated MPN patients lacked features of PV (normal erythrocytes and hematocrit), had higher platelet counts and a lower incidence of major thrombosis compared to JAK2V617F positive ET [76,105]. The large UK study confirmed the presence of the somatic CALR driver mutations in 80 of 112 (70%) JAK2/MPL wild type ET patients, and in 18 of 32 (56%) JAK2/MPL wild type MF patients and in none of 120 (70%) JAK2/MPL wild type ET patients [106]. The distribution of the JAK2V617F, CALR and MPL 515 mutations or triple negative cases in 254 CAPM and 311 (25%) ET patients and in 72 of 203 (35%) MF patients. The CALR somatic mutation was subsequently discovered as the driver cause of thrombocythemia in 78 of 311 (25%) ET patients and in 72 of 203 (35%) MF patients [105]. The CALR mutation was detected in none of 382 PV, 45 CML, 73 MDS, and 64 chronic myelomonocytic leukemia (CMML) patients. Three (12%) of 24 RARS-T cases were positive for both the SF3B1 and CALR mutation. A subsequent Italian-Austrian study of 1235 WHO-defined ET and MF patients detected the JAK2V617F, MPL 515 and CALR mutation in 63.3%, 23.5% and 4.4% respectively with 8.8% being negative for all three mutations [76] (Figure 6). Evolution into MF during follow up was as high in CALR mutated ET as in JAK2V617F mutated PV (about 20% after 20 years). CALR mutated MPN patients lacked features of PV (normal erythrocytes and hematocrit), had higher platelet counts and a lower incidence of major thrombosis compared to JAK2V617F positive ET [76,105]. The large UK study confirmed the presence of the somatic CALR driver mutations in 80 of 112 (70%) JAK2/MPL wild type ET patients, and in 18 of 32 (56%) JAK2/MPL wild type MF patients and in none of 120 (70%) JAK2/MPL wild type ET patients [106]. The distribution of the JAK2V617F, CALR and MPL 515 mutations or triple negative cases in 254 WHO-defined ET patients was 58%, 25%, 8.3% and 8.7% with median overall survival of 8.2, 4.1, 4.3 and 2.5 years respectively reflecting advanced or end stage MPN disease [39].

The biological and clinical features of WHO-defined ET carrying the JAK2V617F and CALR mutation ET clearly differ [76]. The mutant allele burden was lower in JAK2V617F mutated than in CALR mutated ET (Figure 7). JAK2V617F ET patients were older, had higher hemoglobin and white blood cell counts but lower platelet counts. Serum erythropoietin levels are lower and frequently decreased in JAK2V617F ET but normal in CALR thrombocytemia. The cumulative risk of WHO-defined ET carrying the JAK2V617F mutation to transform into WHO-defined PV was 29% after 15 years but transformation into PV was never observed in CALR thrombocytemia. With the advent of the CALR mutation as the main driver cause of JAK2/MPL wild type ET, hypercellular ET associated with PMGM [8,11,12,14,15] and CALR thrombocytemia and myelofibrosis appeared to be the same distinct MPN entity as in JAK2V617F mutated PV (about 20% after 20 years). CALR mutated MPN patients lacked features of PV (normal erythrocytes and hematocrit), had higher platelet counts and a lower incidence of major thrombosis compared to JAK2V617F positive ET [76,105]. The large UK study confirmed the presence of the somatic CALR driver mutations in 80 of 112 (70%) JAK2/MPL wild type ET patients, and in 18 of 32 (56%) JAK2/MPL wild type MF patients and in none of 120 (70%) JAK2/MPL wild type ET patients [106]. The distribution of the JAK2V617F, CALR and MPL 515 mutations or triple negative cases in 254 WHO-defined ET patients was 58%, 25%, 8.3% and 8.7% with median overall survival of 8.2, 4.1, 4.3 and 2.5 years respectively reflecting advanced or end stage MPN disease [39].

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Bone marrow histology findings in 59 WHO-defined JAK2V617F positive ET and 44 JAK2 wild ET cases in the study of Pich, et al. [66], (Figure 11) revealed PV-like hypercellular morphological bone marrow changes of pleiomorphic enlarged megakaryocytes in JAK2V617F mutated ET similar as described previously (Figure 9), [8,15]. Various stages erythropoiesis and or myelopoesis with megakaryocyte proliferation as well as LDH and spleen size are more pronounced in PV-like phenotype in JAK2V617F mutated ET in particular at higher JAK2 mutation load (Figure 9), [66]. WHO defined JAK2V617F positive ET showing increased cellularity due to increased erythropoiesis is consistent with prodromal PV [62]. The prognosis of JAK2V617F mutated ET and prodromal PV is favorable and to be treated with low aspirin and additional phlebotomy in early PV to maintain ht below 0.45 in man and below 0.42 in women. This concept based on prospective clinical observations are completely in line with the present study of patients with JAK2V617F mutated ET, prodromal PV and PV.

Bone marrow histology analysis of bone marrow biopsies by Michiels & De Raeve from the Vannucchi’s study on WHO defined MPL515 mutated ET revealed that clustered large to giant maure megakaryocyte with staghorn nuclei and platelet count increase in a normocelluar bone marrow are characteristic for JAK2 wild type ET carrying the MPL515 mutation [31-33]. JAK2/CALR wild type ET carrying the MPL515 mutation indeed displayed clustered large and giant mature megakaryocytes with a greater number of large deeply lobulated ‘staghorn’ nuclei in a normocellular bone marrow as the hallmark of MPL515 thrombocytemia (Figure 10), [6,7,31,32].

Between 2015 and 2018 Michiels & De Raeve found typical PMGM pictures in 15 CLMP defined consecutive newly diagnosed CALR mutated ET (Figures 11,12) and MF patients [6,7,31-33]. CALR thrombocytemia patients appeared to be phenotypically identical to JAK2 wild type PMGM defined by the Hannover Bone Marrow Classification and in retrospect surely belong to the original description by Dameshek of megakaryocyte leukemia (ML) without features of PV [10]. CALR mutated thrombocytemia and MF are clearly distinct from MPL515 normocellular thrombocytemia (Figure 10), JAK2V617F ET, prodromal PV and PV cases with regard to clinical, hematological and bone marrow features at presentation and during follow-up (Figure 9).

The European Asiatic collaboration between Michiels & De Raeve (Rotterdam-Brussels) and Yongoo and Myungshin Kim (Seoul, Korea) translated the laboratory, molecular and pathological characteristics in a large cross sectional study of 407 WHO defined MPN patients into the 2015-2020 CLMP classification (Tables 2-7). The Large cohort of 407 MPN
patients included PV in 111 (29%), ET in 179 (44%) and PMF in 117 (29%). The three driver mutations were detected in 82.6% of 407 MPN patients with a mutation distribution of JAK2 in 275 (67.5%), CALR in 55 (13.7%), MPL in 6 (1.5%) [100]. In this report we analyzed the CLMP characteristics of 337 Korean evaluable WHO defined MPN patients subdivided into JAK2V617F in 268 (80%), JAK2exon12 in 7 (2.1%, CALC in 56 (17%) and MPL in 6 (1.8%) [6,100]. The values of hemoglobin (Hb), hematocrit (Ht) and erythrocytes in JAK2V617F mutated trilinear MPN ranged from anemic to polycythemic values with mean values of Hb 14.7 g/dL, Ht 0.44 and erythrocytes 5.0x10^{12}/L (Table 8). The bone marrow (BM) lineage proliferation class in MPN including 101 PV, 95 ET and 78 PMF WHO defined patients MPN consisted of M (WHO-ET) in 80; EM and EMG in 116 consistent with prodromal and classical PV; and GM myelofibrosis in 72. The mean JAK2V617F mutation load was high 69 to 80% in EM, EMG and 69% in MG bone marrow class, but low (37%) in M class ET patients (Table 8).

JAK2exon12 patients in the study of Kim, et al. [100], are featured by idiopathic erythrocythemia (IE) not meeting WHO-defined PV with normal platelet and leucocyte counts, no or palpable spleen and a hypercellular bone marrow predominantly due to erythroid hyperplasia (EM, Table 8) [71,95-98]. JAK2exon12 mutated MPN in the study of Kim et al presented with erythrocyte counts above 5.8x10^{12}/L, normal platelet counts of less than 350x10^{9}/L and no anaemia consistent with the diagnosis of erythrocythemic PV (Table 8). Increased erythropoiesis in bone marrow was absent in all cases of CALR and MPL mutated MPN (Table 8). Bone marrow histology in 56 cases of CALR mutated MPN typically featured predominant increased monolinear megakaryopoiesis M in two thirds and increased granulopoiesis and megakaryopoiesis (GM) in one third (Table 8).

The grade of bone marrow (BM) fibrosis in the study of

### Table 8: Change of 2008/16 WHO into European Asiatic 2015-2020 CLMP characteristics in 337 patients with Myeloproliferative Neoplasms (MPN) caused by the somatic driver mutations in the JAK2V617F, JAK2exon12, and CALR [100].

<table>
<thead>
<tr>
<th>337 MPN patients</th>
<th>JAK2V617F</th>
<th>JAK2exon12</th>
<th>CALR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients N</td>
<td>268</td>
<td>7</td>
<td>56</td>
</tr>
<tr>
<td>% of 337</td>
<td>80%</td>
<td>2.1%</td>
<td>16.6%</td>
</tr>
<tr>
<td>Age yrs</td>
<td>66</td>
<td>66</td>
<td>56</td>
</tr>
<tr>
<td>Range</td>
<td>22-89</td>
<td>46-76</td>
<td>20-89</td>
</tr>
<tr>
<td>Males (%)</td>
<td>45.5%</td>
<td>28.6%</td>
<td>41.1%</td>
</tr>
<tr>
<td>Hemoglobin g/dL</td>
<td>14.7</td>
<td>18.3</td>
<td>12.6</td>
</tr>
<tr>
<td>Range</td>
<td>6.2-22.6</td>
<td>13.7-21.1</td>
<td>7.5-16.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.9</td>
<td>49.9</td>
<td>38.4</td>
</tr>
<tr>
<td>Range</td>
<td>19.7-69.1</td>
<td>46.2-59.3</td>
<td>22.9-47.0</td>
</tr>
<tr>
<td>Red Blood cells</td>
<td>5.010^{12}/L</td>
<td>6.910^{12}/L</td>
<td>4.210^{12}/L</td>
</tr>
<tr>
<td>Range</td>
<td>1.89-9.72</td>
<td>5.83-8.50</td>
<td>2.25-5.32</td>
</tr>
<tr>
<td>Platelets 10^{9}/L</td>
<td>650</td>
<td>281</td>
<td>898</td>
</tr>
<tr>
<td>Range</td>
<td>13-3268</td>
<td>58-310</td>
<td>49-1795</td>
</tr>
<tr>
<td>Leukocytes 10^{9}/L</td>
<td>12.0</td>
<td>8.2</td>
<td>8.6</td>
</tr>
<tr>
<td>Range</td>
<td>2.2-177</td>
<td>6.2-22</td>
<td>4.8-31</td>
</tr>
<tr>
<td>2008/16 WHO Class</td>
<td>JAK2V617F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PV N</td>
<td>101</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>ET N</td>
<td>95</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>PMF N</td>
<td>78</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>BM CLMP Class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM N</td>
<td>32 PV</td>
<td>5 IE PV</td>
<td>0</td>
</tr>
<tr>
<td>EGM N</td>
<td>84 PV</td>
<td>2 PV</td>
<td>0</td>
</tr>
<tr>
<td>MET N</td>
<td>80</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>GMMF</td>
<td>72</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Mutation burden</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total-Range</td>
<td>67% (1.8-99)</td>
<td>43.5% (34.5-49.4)</td>
<td>48% (17.8-93)</td>
</tr>
<tr>
<td>EM + EGM</td>
<td>85% (13-99)</td>
<td>43.5% (34.5-49.4)</td>
<td>0</td>
</tr>
<tr>
<td>M (ET)</td>
<td>37 ET</td>
<td>No data</td>
<td>Type 1 53%</td>
</tr>
<tr>
<td>GM (MF)</td>
<td>69 ET PV</td>
<td>No data</td>
<td>Type 2 38%</td>
</tr>
</tbody>
</table>

**Abbreviations:** PV: Polycythemia Vera; ET: Essential Thrombocythemia; PMF: Primary Myelofibrosis; E: Erythroid; M: Megakaryocytic, G: Granulocytic myeloproliferation of increased bone marrow proliferation lineage. N: Number; Red = JAK2V617F mutated PV, ET or MF. JAK2 wild type either CALR (blue) or MPL (black) mutated.

*CALR type 1 revealed a higher mutant allele burden (53%) compared to CALR type 2 (38%) MPN. JAK2V617F mutated ‘forme fruste’ (prodromal PV) and early PV, and exon 12 PV patients presented with E(M) bone marrow proliferation without fibrosis.
Kim, et al. was divided into minimal fibrosis MF 0/1 and overt fibrosis MF 2/3 [7,11,12,21,109]. The frequency of overt fibrosis in JAK2 V617F- and CALR-mutated and triple-negative MPN patients was 22.2%, 27.1% and 29.3%, respectively. JAK2-GM and CALR-GM showed a high rate of overt fibrosis (46.0 and 42.1%), followed by JAK2-M (17.5%), CALR-M (17.2%) and JAK2-EMG) (10.4%; p < 0.001). None of the JAK2-EM (‘forme fruste’, early and overt PV and exon 12 PV) patients presented overt fibrosis.

The overall bone marrow histology findings of erythroid, granulocytic and/or megakaryocytic hyperplasia in JAK2 V617F mutated MPN, and of granulocytic and/or megakaryocytic hyperplasia in CALR mutated MPN patients in the Seoul study are completely in line with the 2015-2020 CLMP classification of six distinct MPN disease entities and transitional MPN states. Comparing the survival curves of 2008/2016 WHO defined PV, ET and PMF versus the 2015-2020 CLMP defined JAK2 V617F, JAK2 exon12, CALR and MPL 515 defined MPN without defined PV, ET and PMF versus the 2015-2020 CLMP defined states. Comparing the survival curves of 2008/2016 WHO of six distinct MPN disease entities and transitional MPN are completely in line with the 2015-2020 CLMP classification.

Conclusion

The present insight review is a strenuous joint effort by a multicentre MPN European Asiatic collaborative study group to demonstrate that scrutinized and integral clinical, laboratory, genetic and pathological (2015-2020 CLMP) approaches and intense communications amongst clinicians, scientist, molecular biologists, and pathologists are warranted to more precisely diagnose and treat each MPN patient before avoidable major complications had occurred. The change of 2008/2016 WHO into the 2015-2020 CLMP criteria for at least five distinct clonal MPNs are in urgent need of validation in well designed large clinical prospective unmet need (PUN) studies within the context of the International Collaborations and Academic Research on MPN (ICAR.MPN 2015 founded and chaired by Dr. Michiels Europe and Dr. Shuvaev, Russia) to even better define improved standards for diagnosis, classification, natural history and novel treatment options of JAK2 V617F, JAK2 exon12, CALR and MPL 515 mutated myeloproliferative neoplasms [7,35,36,110,111-122].

Acknowledgement

The authors are grateful to Alexander Georgii, Juergen Thiele, Ayalew Tiferry, Alessandro Vanucchi, Tiziano, Barbui, Stefan Constantinescu, William Vainchenker, Anthony Green, Heinz Gisslinger, Hans Hasselbalch, Jiri Schwarz and Jean Jacques Kiladjian for their original and expert contributions to our current understanding of the JAK2 V617F trilinear MPN and JAK2 V617F E or PV.
References


