Changing concepts on the myeloproliferative disorders/neoplasms (MPD/MPNs), chronic myeloid leukemia and thrombocythemia in various MPDs: From Dameshek 1950 to Vainchenker 2005 and Michiels 2012 in view of the ECMP criteria for the diagnosis, classification and staging of MPNs

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Abstract

The PVSG classification (1975) distinguished the Philadelphia (Ph+) chromosome positive chronic myeloid leukemia (CML) from the Ph- negative myeloproliferative disorders (MPD) essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). Normocellular ET and intermediate hypercellular prefibrotic ET stages in between PV or ET and post-PV or post ET myelofibrosis (MF) are not considered by the PVSG and WHO classifications. Half of PVSG/WHO defined ET patients show low serum erythropoietin (EPO) levels, and carry the JAK2V617F mutation, indicating prodromal PV when the European Clinical, Molecular and Pathological (ECMP) criteria are applied. The positive predictive value of a JAK2V617F PCR test for the diagnosis of PV is 95%, and for ET about 50%. ET and PV show overlapping bone marrow histology features with similar pleomorphic clustered large megakaryocytes. Erythrocytes are below 6x10^12/L in norm cellular ET and prodromal PV, and consistently above 6x10^12/L in PV obviating the need to measure red cell mass. The WHO defined JAK2V617F positive ET comprises three ECMP defined phenotypes of ET at clinical and the bone marrow level: normocellular ET, early PV mimicking ET (prodromal PV) and ET with hypercellular megakaryocytic-granulocytic myeloproliferation (ET-MGM or PV). Bone marrow histology in JAK2V617F-positive ET, PV, and masked PV clearly differ from JAK2 wild type ET associated with primary megakaryocytic granulocytic myeloproliferation (PMGM). JAK2 wild type ET carrying one of the MPL515 mutations is featured by increase of clustered small and giant megakaryocytes with hyperlobulated stag-horn-like nuclei, in a normal cellular bone marrow, and has no laboratory and bone marrow features of prodromal PV, overt PV, or PMGM at diagnosis and during follow-up. The third MPN entity of JAK2/MPL wild type PMGM is characterized by a hypercellular dual megakaryocytic granulocytic myeloproliferation of dense clustered enlarged immature dysmorphic megakaryocytes with bulky (bulbous) hyper chromatic nuclei, which are not seen in JAK2V617F mutated ET, prodromal PV, masked PV (ET-MGM) and PV, and also not in JAK2 wild type normocellular ET carrying the MPL515 Mutation.

Keywords: Myeloproliferative Disorders; Essential Thrombocythemia; Polycythemia Vera; Essential Megakaryocytic Granulocytic Myeloproliferation; Primary Myelofibrosis; JAK2V617F Mutation; MPL515 Mutation; JAK2 Wild Type; Myeloproliferative Neoplasms; Bone Marrow Pathology

Myeloproliferative Disorders (MPD) and chronic myeloid leukemia (CML)

In his seminal article in 1950, Dameshek (1900-1969) described polycythemia vera (PV) as a trilineal myeloproliferative disorder (MPD) [1]. Individual cases of PV differ greatly in the relationship of the three different marrow elements to each other. Some cases show moderately elevated erythrocytes (erythrocythemia) with an extreme degree of thrombocytosis (thrombocythemia) and other cases present with slight increase in red cells and platelets but with leukocytosis of mature granulocytes close to leukemic levels (granulocythemia). Dameshek concluded that PV is a trilinear MPD (erythrocythemia, thrombocythemia, granulocythemia or panmyelosis) caused by one hypothetical factor and proposed two highly speculative possibilities: either excessive bone marrow stimulation by an unknown factor, or the lack or diminution of an inhibitory factor [1].

In 1951 Dameshek lumped such apparently dissimilar diseases as polycythemia vera, erythroleukemia, idiopathic or agnogenic myeloid metaplasia, megakaryocytic leukemia and proposed a unifying theory that all these variable manifestations represent one myeloproliferative activity of bone marrow cells due to one hypothetical stimulus [2]. Such an illuminative concept proposed by Dameshek as Editor in Chief of Blood might be conceivable until it’s proving or disapprove. Lumping erythroleukemia with PV, and putting together chronic granulocytic or myeloid leukemia (CML) with PV was without scientific foundation. Dameshek corrected himself in 1969 by describing that all variations of the chronic and acute erythroleukemias form a distinct entity, the Di Guglielmo syndrome [3, 4]. The Di Guglielmo syndrome, when running its full course, appeared to pass through three stages:
Stage 1: Refractory anemia (RA) with predominant erythroid hyperplasia and maturation arrest of the entire bone marrow

Stage 2: Progression to trilineal dysplastic features and gradual transition into a mixed erythroid/myeloblastic proliferation

Stage 3: Transformation into acute myeloblastic leukemia

According to Michiels the early and intermediate stage of the Di Guglielmo Syndrome proved to be consistent with a trilineal myelodysplastic syndrome (MDS) followed by transformation into acute leukemia mainly M2 or M4,5.6. Consequently, the Di Guglielmo Syndrome and erythroleukemia disappeared as nosologic disease entities by the introduction of the FAB classification for MDS. Each of the 3 variants of common, sideroblast and trilineal MDS run through the stages of RA, RA with excess (RAEB) RAEB in blastic transformation (RAEB-T) followed by acute blastic leukemia [5, 6].

In 1969, Glaser and Walker found no evidence that PV and CML represent parts of a spectrum of one single disease [7]. Based on careful clinical and basic research studies, Ward and Block (1971) splitted the 1951 Dameshek concept on the myeloproliferative syndrome into two distinct disease entities [8]. First, chronic myeloid leukemia (CML) is a distinct neoplasia that destroys normal hematopoiesis in the bone marrow. Second, the myeloproliferative disorders (MPD) idiopathic (essential) thrombocythemia (ET), polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) are characterized by a benign myeloproliferation of trilineal hematopoietic cells in the bone marrow and spleen [8].

In 1973, Gilbert of the Polycythemia Vera Study Group (PVSG) reviewed the PVSG concept on the spectrum and typical patterns of cellular involvement seen in various myeloproliferative syndromes with particular emphasis on the main characteristic features that occur in leukocyte alkaline phosphatase activity (decreased in CML, increased in PV), bone marrow morphology and histology and the Ph-positive chromosome [9]. First, chronic myeloid leukemia (CML) is a distinct neoplasia that destroys normal hematopoiesis in the bone marrow. Second, the myeloproliferative disorders (MPD) idiopathic (essential) thrombocythemia (ET), polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) [9]. The PVSG used in 1975 the Ph-positive chromosome to distinguish the Ph-negative MPDs from the Ph-positive ET and chronic myeloid leukemia (CML) with various degrees of thrombocytopenia and myelofibrosis [9-11].

Discovery of BCR/ABL in Ph-positive chronic myeloid leukemia (CML)

Chronic myeloid leukemia (CML) has been described in the nineteen century as a distinct disease entity [12, 13]. Nowell and Hungerford discovered a disease specific minute cytogenetic marker in patients with CML, labelled after the city of discovery the Philadelphia (Ph) [14]. Using improved banding techniques, Janet Rowley (1973) [15] showed that the Ph chromosome in CML represents a deletion of the long arm of chromosome 22 (22q-) resulting in the minute Ph. Additional studies showed that a large part of 22q was translocated to 9q, and that a small part of 9q was translocated to 22q resulting in the translocation (t) t (9; 22) (q34; q11) (Rowley1980) [16].

The discovery of the BCR/ABL translocation in the 1980s resulted from original research by three Dutch investigators Nora Heisterkamp, John Groffen and Gerard Grosveld [17-20]. They worked together at the Erasmus Medical University Rotterdam (EUR), and at the National Health Institute (NIH) in Frederick, MD USA (personal communications Gerard and Frank Grosveld 2008-2012). John Groffen and Nora Heisterkamp obtained their Drs Degree in Groningen and moved to the USA in 1981 to work in John Stephenson’s lab in Frederick to study viral oncogenes. Gerard Grosveld was working at the Erasmus University in Rotterdam on a project to identify the Ph-positive chromosome breakpoint. The BCR/ABL was discovered in a three step process.

1. John Groffen learned to make cosmid libraries in Dick Flavell's lab in the MRC in London and took the technique along to the USA. There John Groffen and Nora Heisterkamp cloned parts of the human ABL gene and in collaboration with Walter Bodmer’s group in the UK localized ABL to chromosome 9. Using a v-abl probe Heisterkamp and Groffen had localized ABL on human chromosome 9 [17].

2. Groffen and Heisterkamp contacted Gerard Grosveld mediated by Frank Grosveld and collaborated. Using somatic cell hybrids made by Anne Hagemeijer, (chief of Medical Cytogenetics EMC), they found c-ABL moved to the Ph-positive chromosome. Using hybrid cell lines containing the segregated Philadelphia translocation products (generated by Dr. A. Geurts van Kessel, EUR). Groffen, Heisterkamp and Gerard Grosveld investigated whether c-ABL moved from the long arm of chromosome 9 to the long arm of the Ph' chromosome. A Southern blot confirmed this possibility [18]. Indeed c-ABL was found to translocate to the Ph' chromosome even in patients with complex chromosomal translocations but not in Ph'-negative CML patients with apparently normal karyotypes [19].

3. John Groffen and Nora Heisterkamp cloned more to the 5' of ABL and discovered and cloned a breakpoint fragment from a CML patient DNA. Subsequent chromosome walking upstream from ABL identified a probe that recognized the chromosome 9 breakpoint in the DNA of a CML patient. Cloning of this fusion fragment provided probes of the breakpoint cluster region on chromosome 22, which detected the Philadelphia breakpoints in almost all CML patient samples in judging those with complex cytogenetic translocations. In CML patients provided by Dr Abels & Michiels from the Department of Hematology Erasmus University Medical Center, Rotterdam, the chromosomal breakpoints were clustered within a limited region on chromosome 22, for which they propose the term “breakpoint cluster region”: BCR. The specific molecular BCR/ABL translocation on chromosome 22 in the t (9; 22) of Ph'-positive CML patients was predicted to have functional significance for the disease [20]. There was no serendipity in the discovery of the BCR/ABL translocation t (9; 22) and "we all were very lucky to have had this collaboration" (personal communication Nora Heisterkamp 2012).

The sequential discoveries of the Ph'-chromosome in the t (9; 22) (q34; q11), and the BCR/ABL fusion gene on chromosome
22 appeared to become the cause of a clearly defined human myeloproliferative neoplasia, including \( BCR/ABL \)-positive CML, \( BRC/ABL \) positive ET and \( BCR/ABL \) positive thrombocytopenia associated CML. The \( BCR/ABL \) fusion gene is detectable in hematopoietic bone marrow cells but not in fibroblasts of CML patients. The \( BCR/ABL \) fusion gene produces a \( BCR/ABL \) protein, which has a high tyrosine kinase activity and CML-transformation capacity in animal models [21-23]. Ninety percent of all CML patients are \( Ph^+/-BCR/ABL^+ \), 5% are \( Ph^+/BCR/ABL^+ \), and 5% are \( Ph^+/ BRC/ABL^+ \), the latter group usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia[24].

In the Rotterdam cohort of CML 50 MPD patients seen between 1975 and 1987, Michiels and Hagemeijer could demonstrate that all MPD patients diagnosed as ET, PV and AMM were negative for the \( Ph^+ \)-chromosome and \( BRC/ABL \) translocation, and could detect (using the method of Groffen et al) the \( BCR/ABL \) transcript in a case of \( Ph^+ \)-positive essential thrombocytopenia [25, 26]. According to existing strict morphological, biochemical, cytogenetic and molecular criteria, in duding the \( Ph^+ \)-chromosome and \( BCR/ABL \) fusion gene and protein, CML is a malignant disease with an obligate transition into acute lymphoid, lymphatic or megakaryoblast leukemia, whereas ET, PV and agnogenic myeloid metaplasia (AMM) or chronic idiopathic myelofibrosis (CIMF) form the \( Ph^+ \)-chromosome and \( BRC/ABL \) negative MPDs featured by a benign proliferation of the three hematopoietic cell lines with a low incidence of leukemic transformation in PV and AMM [25].

The distinct entities of \( Ph^+ \) and \( BCR/ABL^+ \) ET and thrombocytopenia associated CML versus the \( Ph^+ \)- and \( BCR/-ABL-negative-thrombocytopenias-in-varying-MPDs-seen-between-1975-and-1987-at-the-Departments-Hematology-(Dr-Van-Lom) 
and Pathology (Drs Noorduin and Ten Kate, Erasmus University Medical Center, Rotterdam), showed conspicuous differences in the form and size of megakaryocytes in bone marrow smears and sections of bone marrow biopsy [25-27]. This difference of megakaryocyte histology appeared to be reproducible in bone marrow biopsies by the German pathologists Georgii and Thiele to distinguish between small megakaryocytes with hypolobulated nuclei in \( Ph^+ \)-CML diseases versus enlarged pleomorphic megakaryocytes with hyperlobulated nuclei in \( Ph^- \)-negative MPDs (Hannover Bone Marrow Classification of \( Ph^- \)-positive CML and the postfibrotic \( Ph^- \)-negative MPDs ET, PV and chronic granulocytic myeloproliferation, CMGM, Georgii et al 1990) [28-32].

**The PVSG criteria for Polycythemia Vera**

Wasserman extended the original concept of Dameshek (1950) on PV as a trilinear MPD and distinguished in 1954 five subsequent stages in the natural history of PV [33, 34].

**Stage 1:** Pure erythrocytosis is featured by increased hemoglobin, haematocrit, erythrocytes above 6x10^{12}/L and increased red cell mass with normal leukocytes, thrombocytes and spleen size, which is labelled by Pearson & Wetherley-Mein (1979) as idiopathic erythrocytosis [35].

**Stage 2:** The polycythemic stage of PV is featured by erythrocytosis, thrombocytopenia, granulocytopenia and no or early reticulin fibrosis in the bone marrow, with various degrees of thrombocytosis, leukocytosis and/or slight to moderate splenomegaly.

**Stage 3:** PV patients present with different grades of reticulin fibrosis (RF, tables 1 and 2) in the bone marrow and slowly progressive splenomegaly does occur in about one third of the cases during long-term follow-up.

**Stage 4:** Post-PV myeloid metaplasia of the spleen (splenomegaly) and various degrees of reticulin myelofibrosis (table 1C) following PV may elapse 5 to 25 years.

**Stage 5:** Spent phase PV may last several years. At this point the spleen is frequently large and very firm on palpation, the liver is enlarged to a moderately degree in most patients, thrombocytopenia is frequent and may be pronounced with bizarre and giant platelets, and granulocytic leukocytosis (granulocytopenia) [32]. Finally leukemic transformation may occur in only a few cases when treated by phlebotomy alone [33-36].

**The Dameshek Wasserman controversy in the treatment of PV**

Dameshek interpreted PV as a benign myeloproliferative disorder (MPD) [37, 38]. Wasserman disagreed, favouring the concept that PV is a myeloproliferative neoplasia (MPN) of the whole bone marrow [33, 34]. According to Dameshek in 1950 [1-38], it is best to consider the PV patients as fundamentally normal. As such, the PV patient may have a long life span and every attempt should be made to keep the treatment as physiologic as possible. According to Dameshek, venesection aiming at haematocrit of 0.40 proved a satisfactory method resulting in a state of iron deficiency [1-38]. Red cell formation under these circumstances is only partially reduced, but due to microcytosis of red cells hemoglobin and hematocrit levels remain low for periods of months to years during which time the patient may be completely asymptomatic. Red cell levels during this induced remission of PV by phlebotomy alone gradually rise and remained at erythrocytic levels above 6x10^{12}/L so that the red cell count as an index of therapy is of little value. The best index of therapy is the hematocrit value, although the haemoglobin concentration alone may be used since this correlates fairly closely with the hematocrit level [1]. During the state of chronic iron deficiency, the patient himself presents a normal appearance. On this program it is possible to control PV patients for several up to ten to fifteen years and is in as good health now as comparable persons of the same age group. Dameshek hesitated to use a potentially dangerous radioactive material in an individual with a relatively long life span and questioned whether the acute leukemic states which have occurred in some cases are due to the potentially leukemogenic drug P32 or are associated with the natural history of polycythemia. Whether or not the amounts of radioactivity as administered in the ordinary dose of P32 used in the treatment of PV were harmful or productive of leukemia was not known at that time [36-39]. In the experience of Dameshek in about 50 reasonably well followed cases of polycythemia, acute leukemia developed in only 1 (2%) instance without previous roentgen ray or radioactive phosphor therapy [36-39].

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As compared to phlebotomy, radioactive phosphor (P32) significantly reduced the incidence of major thrombosis in PV from about 30% to less than 5% in the studies of Lawrence (1949) [40], Stroebel et al 1951 [41], and Wasserman and Bassen 1959 [42], reviewed by Michiels in 1996 [13]. In the late 1960s Wasserman addressed the question whether or not the ordinary dose of P32 used in the treatment of polycythemia vera was harmful or productive of leukemia-46 [44-46]. Wasserman founded the Polycythemia Vera Study Group (PVSG) and performed between 1968 and 1985 within the context of the PVSG a large randomized clinical trial directly comparing phlebotomy alone versus P32 and chlorambucil, the PVSG 01 study [43-45]. In 1971 Wasserman defined the inclusion criteria for PV patients eligible for inclusion in the PVSG 01 study [43]. These inclusion criteria are used since 1975 the world wide used PVSG major and minor criteria for the diagnosis of PV patients (Berlin)[44] until the introduction in 2008 of revised PVSG/WHO criteria by adding the JAK2V617F mutation as a clue to the 3 PVSG defined variants of myeloproliferative neoplasms ET, PV and myelofibrosis (MF) [46-49]. Characteristic histology findings in bone marrow biopsies of 155 evaluable PV patients with a documented increased RCM in the 1975 PVSG 01 study revealed a broad spectrum of no, slight, moderate to marked (>80%) increase of bone marrow cellularity from 50 to 60% in 10 cases, from 60 to 80% in 45 cases, and from 80 to 100% in 100 cases [44]. Reticulin fiber content was normal (RF-0 and 1 = prefibrotic) in 94 cases, slightly increased (RF-2 = earlyfibrotic) in 40 cases, and moderately to markedly increased (RF-3 and 4) in 21 cases. Comparing the grades of reticulin content with bone marrow cellularity the bone marrow histology in the PVSG-01 study could readily be interpreted as a normocellular ET picture in 10, mixed ET/PV picture in 45, a typical hypercellular PV picture in 70 and a PV/RF-3 or 4 picture in 13 PV patients [50]. The cohort of 431 PV patients in the PVSG 01 study consisted of clearly defined PV patients with various degrees of MPD disease burden: early PV with a ET or ET/PV bone marrow picture or overt prefibrotic PV with a typical trilineal PV picture in the majority and a MF picture with RF grade 3 and 4 in a minority. The 1975 PVSG criteria exclude stage 1 pure erythrothromic PV (idiopathic erythrocytosis) by definition [44]. PV patients stage 2 and 3 in the PVSG 01 study were randomized for phlebotomy in 134, chlorambucil in 141 and P32 in 156[34, 44, 51]. In the phlebotomy arm aiming at a haematocrit below 0.40 according to Dameshek (1946, 1950) [1,38] and aiming at a hematocrit below 0.45 in males and below 0.42 in females according to Wasserman and Bassen (Berlin 1975, figure 7). In the phlebotomy and aiming at a haematocrit below 0.50, there was a significant loss of deceased PV patients due to major thrombotic complications during the first 3 years, but not in the two myelosuppressive arms [34, 51]. There was a striking increased incidence of malignant complications in PV patients after 5 years during long-term treatment (10 to 15 years) with P32 or with chlorambucil as compared to the phlebotomy-treated PV patients [34, 51, 52]. In retrospect, PV patients included in the PVSG 01 study were exposed to the leukemogenic agents (P32, chlorambucil) in their early overt PV stages with no or minor signs of myeloid metaplasia and myelofibrosis[50-52]. In the randomized clinical trial in 293 PV patients of the European Organization on Research and Treatment of Cancer (EORTC), the first remission duration of one course of busuphan (BU) versus one course of P32 was 4 years versus 2 years respectively [53].

The overall survival of repeated courses of BU versus P32 was 70% and 55% respectively after a mean follow-up of 8 years. Messinezy et al treated in the 1970 and early 1980s 65 PV patients with low dose BU to keep the platelets around 400x10^9/L and kept thehematocrit below 0.45 by additional phlebotomy [54]. At a median survival of 11.1 years from diagnosis, the vascular causes of death were only a little bit higher than expected and death from acute leukemia and myelofibrosis was twice that expected for the general population [54]. Van de Pette treated 37 symptomatic ET patients with low dose BU for periods up to 25 years [55]. Reduction of platelet count to less than 400x10^9/L resolved rapid vascular occlusive symptoms including erythromelalgia, digital ischemia and atypical neurologic, ocular and cardiac ischemic manifestation [55]. With a median survival of 9.8 years the number of death was 2.1 times higher, with deaths from myelofibrosis markedly increased and no death from leukemia. Progression of ET into myelofibrosis occurred in 24% and 9% became polycythemic and might represent the natural history of PVSG defined ET. As the extension of t. The EORTC comparing BU and P32 in PV53, the ET and PV studies from London by Wetherley-Mein clearly indicate that low dose BU in elderly patients above the age of 65 to 70 years is far superior and easier to control platelet counts in low and intermediate risk ET and PV.

The PVSG 01 randomized clinical trial confirmed the hypothesis of Dameshek in 1950 [36-39] that P32 is leukemogenic as a first line treatment option in PV. The majority of PV patients in the PVSG 01 study treated with P32 were in the early stage MPD disease before developing significant myeloid metaplasia of the spleen. According to current categorisation of MPD disease a large group of patients with low risk PV included in the PVSG 01 study would have been treated with low dose aspirin/phlebotomy alone [56], and myelosuppressive treatment would have been postponed according to improved guidelines proposed in the1990s by the PVSG [51-52]. A primary rigid venesection regimen aiming at a hematocrit of 0.40 according to Dameshek (1946, 1950) [1,38] and aiming at a hematocrit below 0.45 in males and below 0.42 in females according to Pearson Wetherley-Mein 1978 [57,58] on top of low dose aspirin according to Michiels [59-65] is still the treatment of choice in early stage low risk PV patients [56]. This non-leukemogenic approach in the treatment of low risk PV ann 2013 will reduce the cumulative incidence of minor and major thrombosis from above 50% to less than 2% per patient/year during long-term follow-up [63-65].

The PVSG Criteria for Essential Thrombocytemia

The Polycythemia Vera Study Group proposed in 1975 simple but rather crude inclusion and exclusion criteria for the diagnosis of hemorrhagic or essential thrombocythemia [10]:

1. A platelet count in excess of 1000x10^9/L and a bone marrow smear which shows marked megakaryocytic hyperplasia and abundant platelet clumps.

2. Absence of polycythemia vera as defined by the PVSG (Wasserman 1971 Berlin 1975, figure 7).
Increased bone marrow cellularity due to increase and clustering of enlarged megakaryocytes in bone marrow biopsy. Slight, moderate or marked increase in bone marrow biopsy of clustered, enlarged pleomorphic leukocytosis, leucocyte count >10^9/L. Presence of large platelets in a peripheral blood smear. Thrombocythemia, persistent increase of platelet count >400x10^9/L. Splenomegaly on palpation or on isotope/ultrasound scanning. No or slight increase of reticulin fibers (RF 0 or RF 1). Absence of any underlying disease for reactive thrombocytosis and normal ESR. Raised red cell mass. Male >36 ml/kg, female >32 ml/kg. Consistent with erythrocyte count of >6x10^12/L (Dameshek & Henthel 1940 [32], Michiels table 6).

In this study of 37 untreated ET patients, bone marrow cellularity was normal in 11%, greater than 90% in 11% and increased between 50 to 90% in 78% (Table 1B). Two-thirds of biopsies showed marked megakaryocyte hyperplasia with atypical large megakaryocytes. Reticulin content was essentially normal in 90% indicating prefibrotic MPD. The megakaryocytes in PVSG defined PV and ET were identical in appearance and the condition PV versus ET cannot be distinguished on megakaryocyte histology grounds [44, 66, 67]. Increased bone marrow cellularity due to increased erythropoiesis and/or myelopoesis in PVSG defined PV and ET is identical. The PVSG concluded that the condition PV versus ET cannot be distinguished on the basis of bone marrow histopathology. Leukocytosis is common in ET and PV [44, 66, 67]. LAP scores over 100 were seen in 42% of ET, and in 70% of PV patients. Pruritis was observed in 14% in ET and 43% in PV patients (PVSG study). The spleen was palpable in 38% of ET and 70% of PV patients, and when enlarged in ET the spleen was only 2 to 4 cm below the costal margin.

The 1978 Rotterdam Clinical and Pathological (RCP) criteria for ET and PV

Focusing since 1975 on the causal relation between erythromelalgia and thrombocythemia in ET and PV patients, we were able to document the very early stage of ET by the use of the Rotterdam Clinical and Pathological (RCP) criteria for ET and PV (table 1)[50, 59, 68]. The 1978 RCP criteria of ET and PV were determined by careful prospective documentation of peripheral blood and bone marrow smears and bone marrow

Table 1A: The 1978 Rotterdam Clinical and Pathological (RCP) criteria for Essential Thrombocythemia (ET)9

<table>
<thead>
<tr>
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<th>A Criteria</th>
<th>B Criteria</th>
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<tr>
<td>A1</td>
<td>Persistent platelet count in excess of 400x10^9/L</td>
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<tr>
<td>A2</td>
<td>Increase and clustering of enlarged megakaryocytes in bone marrow biopsy.</td>
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<tr>
<td>A3</td>
<td>No or slight increase of reticuline fibers (RF 0 or RF 1)</td>
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<tr>
<td>B1</td>
<td>Presence of large platelets in a peripheral blood smear</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>Absence of any underlying disease for reactive thrombocytsis and normal ESR.</td>
<td></td>
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<tr>
<td>B3</td>
<td>No or slight splenomegaly on palpation or scan &lt;15 cm</td>
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<tr>
<td>B4</td>
<td>Increase of LAP-score and no signs of fever or inflammation</td>
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Exclusion criterion

Ph+ chromosome and any other cytogenetic abnormality in blood or bone marrow cells

Table 1 B: The 1978 RCP major (A) and minor (B) criteria for prefibrotic PV [50]

<table>
<thead>
<tr>
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<th>A Criteria</th>
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<tr>
<td>A1</td>
<td>Raised red cell mass. Male &gt;36 ml/kg, female &gt;32 ml/kg10 consistent with erythrocyte count of &gt;6x10^12/L, (Dameshek &amp; Henthel 1940 [32], Michiels table 6)</td>
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<tr>
<td>A2</td>
<td>Absence of primary or secondary erythrocytosis by clinical and laboratory tests.</td>
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<tr>
<td>A3</td>
<td>Slight, moderate or marked increase in bone marrow biopsy of Clustered, enlarged pleomorphic megakaryocytes with hyperlobulated nuclei and moderate to marked increase cellularity of megakaryopoiesis/erythropoiesis or typically trilinear mega-erythro-granulopoiesis. A typical PV bone marrow excludes erythrocytosis19.</td>
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No or presence of reticuline fibers and no collagen fibers (no dry tap)

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<th>B Criteria</th>
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<tbody>
<tr>
<td>B1</td>
<td>Thrombocythemia, persistent increase of platelet count &gt;400x10^9/L.</td>
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<tr>
<td>B2</td>
<td>Leukocytosis, leucocyte count &gt;10^9/L, and low erythrocyte sedimentation rate (ESR)</td>
</tr>
<tr>
<td>B3</td>
<td>Raised leukocyte alkaline phosphatase (LAP) score &gt;100, absence of fever or infection</td>
</tr>
<tr>
<td>B4</td>
<td>Splenomegaly on palpation or on isotope/ultrasound scanning</td>
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A1+ A3 plus one of B establishes PV and excludes any variant of erythrocytosis.
biopsy material. Platelets in excess of 400x10^9/L, and an increase of clustered enlarged megakaryocytes in a bone marrow biopsy material was found to be diagnostic for ET and excluded reactive thrombocytosis. On top of the clinical PVSG criteria for PV [45] we introduced in 1978 bone marrow histopathology and erythrocyte count above 6x10^12/L proposed by Dameshek & Henthel in 1940 [10] as specific clues to the diagnosis of PV to clearly differentiate PV from all variant of primary and secondary erythrocytosis [45, 50]. The 1978 RCP modifications of the 1975 PVSG criteria for PV include four main changes (table 1). First, the major criterion O2-saturation of >92% is replaced by absence of primary or secondary erythrocytosis by clinical and laboratory tests. Second; splenomegaly is replaced by bone marrow histology as a major criterion (A3). Third, the 1978 RCP diagnostic set used splenomegaly as a minor criterion (table 1). Fourth, we skipped raised B12 (>900 ng/L) or raised B12 binding capacity (>2200 ng/L) as completely irrelevant for the diagnosis of early and overt stage PV.

European Clinical, Molecular and Pathology (2007/2008 ECMP) MPD Criteria for ET and PV

In 1997 we extended the RCP proposed by the Thrombocytopenia Vera Study Group (TVSG) for the diagnosis of early PV and ET, and to pick up masked cases of primary myeloproliferative disorders [68-70]. Bone marrow histology features according to the Hannover Bone Marrow criteria for Ph^1-negative MPD and Ph^1-positive CML (Georgii et 1990) [71-73] and according to the Cologne Clinical and Pathological (1999 CCP) of thrombocytosis in various MPDs defined by Michiels & Thiele, [74, 75] revealed a broad spectrum of MPD disease in PVSG defined ET ranging from typical ET, typical ET/PV, typical prefibrotic PV and ETMGm pictures with progression to reticulin (RF) grade 1,2, and 3 and transformation of RF in collagen fibrosis (table 2). During the first European MPD Workshop on MPD we reached in 1999 an European consensus towards diagnostic criteria of ET, PV and EMGM or idiopathic myelofibrosis (IMF) by including bone marrow histopathology according to the 1990 Hannover Bone Marrow Classification and the 1999 CCP criteria for the diagnosis and staging of myelofibrosis in ET, PV and chronic megakaryocytic granulocytic myeloproliferation (CMGM, Georgii), chronic idiopathic myelofibrosis (CIMF, Thiele) or ET with hyper cellular MGM bone marrow (EMGM, Michiels) as the third MPD entity (table 2) [70]. In 1999 Michiels et al introduced the term ET associated with hyper cellular essential/primary megakaryocytic granulocytic myeloproliferation (EMGM/PMGM to replace CMGM and CIMF, table 2) with various degrees of myelofibrosis and various degrees of clinical and laboratory features of primary myeloid metaplasia of the spleen [70]. Within the context of the European Working Group on MPD (EWG.MPD) Michiels could improve the 1978 RCP and the 1999 European consensus criteria for ET and PV by including expert bone marrow histology in collaboration with the pathologists Thiele and Kvasnicka to define the European Clinical and Pathological (2002-2005 ECP) [75, 76] criteria (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) and to criticize the shortcomings of the 2001 WHO MPD criteria for ET, PV and prefibrotic CIMF or EMGM [76].

In 2005 Thiele left the ECP and joined the WHO to define the 2008 WHO classification of the myeloproliferative neoplasms ET, PV and primary myelofibrosis (PMF) [77]. Michiels extended the ECP and defined in 2007 the European Clinical, Molecular and Pathology (ECMP) [78, 79] criteria for prefibrotic ET and PV and ET associated with primary dysmegakaryocytic granulocytic (PDGM) by including the JAK2V617F mutation screening as a pathognomic clue to distinguish JAK2V617F mutated trilinear
Splenomegaly on palpation, or >11 cm on ultrasound scan or CT. Raised red cell mass: RCM. Spontaneous erythroid colony (EEC) and/or spontaneous megakaryocyte colony formation (CFU-Meg).

Increase and clusters of mature giant megakaryocytes with hyperploid nuclei in bone marrow biopsies.

Normal or slightly increased cellularity and no or minimal reticulin fibrosis in bone marrow biopsies.

Platelet count in excess of 400 x 10^9/L and no known cause of reactive thrombocytosis.

Increase and clusters of mature giant megakaryocytes with hyperploid nuclei in bone marrow biopsies.

No preceding or allied other subtype of myeloproliferative disorders or myelodysplastic syndrome.

Normal or elevated leukocyte alkaline phosphatase (LAP) score, normal ESR, and no fever.

Normal or slightly increased cellularity and no or minimal reticulin fibrosis in bone marrow biopsies.

Splenomegaly on palpation, or >11 cm on ultrasound scan or CT.

Spontaneous erythroid colony formation (EEC) and/or spontaneous megakaryocyte colony formation (CFU-Meg).

Diagnosis: Primary masked myeloproliferative disease: PMD

Table 2a and b: Towards a European Consensus on the Diagnostic Criteria of Essential Thrombocytopenia (ET), Polycythemia Vera (PV) according to Michiels et al. 1999-2019

<table>
<thead>
<tr>
<th>Diagnostic (A) and confirmative (B) PVSG Criteria of ET by including bone marrow histopathology according to Georgii et al. 2013, 2019 and Thiele et al. 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A1</strong></td>
</tr>
<tr>
<td><strong>A2</strong></td>
</tr>
<tr>
<td><strong>A3</strong></td>
</tr>
<tr>
<td><strong>B1</strong></td>
</tr>
<tr>
<td><strong>B2</strong></td>
</tr>
<tr>
<td><strong>B3</strong></td>
</tr>
<tr>
<td><strong>B4</strong></td>
</tr>
</tbody>
</table>

A2 plus one of B: primary masked myeloproliferative disease: PMD

<table>
<thead>
<tr>
<th>Diagnostic (A) and confirmative (B) PVSG Criteria of PV by including bone marrow histopathology according to Georgii et al. 2013, 2019 and Thiele et al. 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A1</strong></td>
</tr>
<tr>
<td><strong>A2</strong></td>
</tr>
</tbody>
</table>
| **A3** | Histopathology of bone marrow biopsy: 
  a) increase and clusters of pleomorphic megakaryocytes with hyperploid nuclei 
  b) increased cellularity: panmyelosis 
  c) reticulin fibers (optional) |
| **B1** | Thrombocytopenia platelet count >400 x 10^9/L |
| **B2** | Granulocytes >10 x 10^9/l and/or raised LAP score in the absence of fever or infection |
| **B3** | Splenomegaly on palpation or >11 cm on ultrasound scan or CT |
| **B4** | Spontaneous erythroid colony formation in the absence of Epo and low plasma Epo level |

A3 plus one of B: primary masked myeloproliferative disease: PMD

Citation: Jacques Michiels J (2019) Changing concepts on the myeloproliferative disorders/neoplasms (MPD/MPNs), chronic myeloid leukemia and thrombocytopenia in various MPDs: From Dameshek 1950 to Vainchenker 2005 and Michiels 2012 in view of the ECMP criteria for the diagnosis, classification and staging of MPNs. Int J Hematol Blo Dis 4(2) 1-22
**Table 2c:** Towards European consensus on criteria of chronic megakaryocytic granulocytic myelosis CMGM proposed by the Hannover MPD Pathology Study Group\[^{28,29}\]*, or the Cologne criteria for chronic idiopathic myelofibrosis CIMF\[^{30,31}\]*, or essential thrombocythemia associated with a hypercellular essential or primary MGM bone marrow (EMGM/PMGM) according the Rotterdam MPD Study Group\[^{70}\]

<table>
<thead>
<tr>
<th>Clinical and hematological features[^{31,32}]</th>
<th>Diagnostic criteria[^{28-31}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A No preceding or allied other subtype of myeloproliferative disorders or MDS</td>
<td>EMGM/PMGM: Histopathology: megakaryocytic granulocytic myeloproliferation (MGM) atypical giant to large megakaryocytes dysplaying defects of multilobulated nuclei and definitive maturation defects of cytoplasm and nuclei</td>
</tr>
<tr>
<td>B Abnormal clustering and increase of Thrombocytopenia, platelets &gt;400 x10^9/l and/or raised LIP or raised PRV-1 expression in the absence of fever or infection</td>
<td>Myelofibrosis (MF): MF 0. no reticulin fibrosis MF 1. slight (early) reticulin fibrosis MF 2. marked increase (density) in reticulin and/or collagen fibrosis MF 3. Advanced collagen fibrosis and osteosclerosis, endophytic bone formation</td>
</tr>
<tr>
<td>C Splenomegaly on palpation or &gt;11cm on ultrasound scan or CT</td>
<td></td>
</tr>
<tr>
<td>D Anemia, hemoglobin &lt;12 g/dl</td>
<td></td>
</tr>
<tr>
<td>E Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** 2007 ECMP criteria for the diagnosis of Polycythemia Vera: PV

<table>
<thead>
<tr>
<th>Clinical and Molecular criteria</th>
<th>Pathological criteria (WHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major PV criteria</strong></td>
<td><strong>P1. Bone marrow pathology: increased cellularity due to trilinearity of erythropoiesis, megakaryopoiesis and granulopoiesis and clustering of small to giant (pleomorphic) megakaryocytes with hyperlobulated nuclei. Absence of stainable iron. No pronounced inflammatory reaction (plasmacytosis, cellular debris)</strong></td>
</tr>
<tr>
<td>A0. Early PV. Hematocrit in the upper limit of normal: Ht: 0.45 to 0.51 in male and 0.43 to 0.48 in female[^{23}]</td>
<td></td>
</tr>
<tr>
<td>A1. Classical WHO defined PV: Hematocrit &gt;0.51/&gt;0.48 in male/female</td>
<td></td>
</tr>
<tr>
<td>A2. Presence of JAK2V617F or exon 12 Mutation (sensitivity 98%)</td>
<td></td>
</tr>
<tr>
<td>A3. Low serum EPO level and/or spontaneous endogenous erythroid colony (EEC) formation</td>
<td></td>
</tr>
<tr>
<td><strong>Minor MPD criteria</strong></td>
<td><strong>P2. Selective increase of erythropoiesis, normal granulopoiesis and megakaryocytes of normal size, morphology and no clustering of megakaryocytes in primary or secondary erythrocytosis</strong></td>
</tr>
<tr>
<td>B1. Persistent increase of platelet count: grade I: 400-1500, grade II: &gt;1500</td>
<td></td>
</tr>
<tr>
<td>B2. Granulocytes &gt;10 x10^9/l or Leukocytes &gt;12 x10^9/l and/or raised LIP or increased PRV-1 expression in the absence of fever or infection</td>
<td></td>
</tr>
<tr>
<td>B3. Splenomegaly on palpation or on ultrasound echogram (&gt;12 cm length in diameter)</td>
<td></td>
</tr>
</tbody>
</table>

**Citation:** Jacques Michiels J (2019) Changing concepts on the myeloproliferative disorders/neoplasms (MPD/MPNs), chronic myeloid leukemia and thrombocythemia in various MPDs: From Dameshek 1950 to Vainchenker 2005 and Michiels 2012 in view of the ECMP criteria for the diagnosis, classification and staging of MPNs. Int J Hematol Blo Dis 4(2) 1-22
Table 4: 2007 ECMP criteria for the diagnosis of 3 phenotypes of JAK2V617F mutated Essential Thrombocythemia (ET): important to differentiate because the natural history may differ

<table>
<thead>
<tr>
<th>Clinical and molecular criteria</th>
<th>WHO bone marrow criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ET stage 1</strong></td>
<td>Predominant proliferation of enlarged pleomorphic megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or borderline increase in reticulin. RF0-1</td>
</tr>
<tr>
<td>1. Platelet count of &gt;350 x 10^9/l and the presence of large platelets in a blood smear (also stage 2 and 3)</td>
<td></td>
</tr>
<tr>
<td>2. Presence of JAK2V617F mutation</td>
<td></td>
</tr>
<tr>
<td>3. Normal hematocrit: male &lt;51%, female &lt;48% erythrocytes&lt; 6x10^12/L</td>
<td></td>
</tr>
<tr>
<td><strong>ET stage 2</strong></td>
<td>Prodomal PV</td>
</tr>
<tr>
<td>1. Platelet count of &gt;350 x 10^9/l and hematocrit in normal or upper normal range: male &lt;51%, female &lt;48% erythrocytes&lt; 6x10^12/L</td>
<td>Increased cellularity with trilineage myeloproliferation (i.e. pancytosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes. No pronounced inflammatory reaction (plasmacytosis, cellular debris). Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis. No or borderline increase in reticulin. RF0-1</td>
</tr>
<tr>
<td>2. Presence of JAK2V617F mutation</td>
<td></td>
</tr>
<tr>
<td>3. Low serum EPO level and/or increased LAP score</td>
<td></td>
</tr>
<tr>
<td>4. Spontaneous EEC.</td>
<td></td>
</tr>
<tr>
<td><strong>ET stage 3</strong></td>
<td>ET MGM</td>
</tr>
<tr>
<td>1. Platelet count of &gt;350 x 10^9/l and no signs of leuko-erythroblastosis</td>
<td>Increased cellularity due to megakaryocytic granulocytic myeloproliferation (MGM). Normal or reduced erythropoiesis. Loose to dense clustering of enlarged pleomorphic megakaryocytes with hyperploid nuclei and the presence of pleiomorphic megakaryocytes with with clumsy dysmorphic lobulated nuclei.</td>
</tr>
<tr>
<td>2. No or slight splenomegaly on ultrasound</td>
<td></td>
</tr>
<tr>
<td>3. No anemia with Hb and Ht in the normal or lower range of normal: &gt;12g/dl</td>
<td></td>
</tr>
<tr>
<td>4. Presence of JAK2V617F mutation</td>
<td></td>
</tr>
<tr>
<td>5. No preceding or allied of CML, PV, RARS-T or MDS</td>
<td>No or borderline increase in reticulin RF0-1</td>
</tr>
</tbody>
</table>

Source Poster P-0025. Fourth International Congress on MPD/MDS New York, 2007
Changing concepts on the myeloproliferative disorders/neoplasms (MPD/MPNs), chronic myeloid leukemia and thrombocythemia in various MPDs: From Dameshek 1950 to Vainchenker 2005 and Michiels 2012 in view of the ECMP criteria for the diagnosis, classification and staging of MPNs

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**Table 5A:** Clinical and molecular features of 10 JAK2\textsuperscript{V617F} positive MPN patients with essential thrombocythemia (ET) in six and polycythemia vera (PV) in four according to clinical criteria according to TVSG/PVSG criteria\textsuperscript{1,3,4}, the 2008 WHO\textsuperscript{5} and the European Clinical Molecular and Pathological (ECMP) classifications\textsuperscript{7,8} according to the French approach without the use of bone marrow histopathology

<table>
<thead>
<tr>
<th>Case</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Clinical data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) and sex (F/M)</td>
<td>56/M</td>
<td>60/M</td>
<td>66/F</td>
<td>47/F</td>
<td>40/F</td>
<td>31/F</td>
<td>50/M</td>
<td>43/F</td>
<td>47/F</td>
<td>63/M</td>
</tr>
<tr>
<td>Platelets at onset x10(^9)/L</td>
<td>575</td>
<td>814</td>
<td>544</td>
<td>553</td>
<td>425</td>
<td>576</td>
<td>397</td>
<td>405</td>
<td>924</td>
<td>384</td>
</tr>
<tr>
<td>Duration of symptoms, years</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>&lt;1</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;10</td>
</tr>
<tr>
<td>JAK2\textsuperscript{V617F} *</td>
<td>+/-</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>Normal</td>
<td>zero</td>
<td>low</td>
<td>low</td>
<td>NT</td>
<td>NT</td>
<td>zero</td>
<td>low</td>
<td>zero</td>
<td>zero</td>
</tr>
<tr>
<td>Leukocytes x 10(^9)/L</td>
<td>6.7</td>
<td>5.3</td>
<td>12.9</td>
<td>8.2</td>
<td>6.1</td>
<td>6.2</td>
<td>7.3</td>
<td>14.3</td>
<td>13.1</td>
<td>8.0</td>
</tr>
<tr>
<td>LAP score(N &lt;=100)</td>
<td>.</td>
<td>160</td>
<td>197</td>
<td>N</td>
<td>N</td>
<td>186</td>
<td>163</td>
<td>263</td>
<td>232</td>
<td>284</td>
</tr>
<tr>
<td>Hemoglobin g/dl</td>
<td>13.6</td>
<td>15.5</td>
<td>14.2</td>
<td>14.4</td>
<td>13.4</td>
<td>14.0</td>
<td>18.6</td>
<td>17.3</td>
<td>16.3</td>
<td>12.8</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.40</td>
<td>0.45</td>
<td>0.44</td>
<td>0.44</td>
<td>0.40</td>
<td>0.41</td>
<td>0.63</td>
<td>0.52</td>
<td>0.53</td>
<td>0.60</td>
</tr>
<tr>
<td>Erythrocytes x10(^12)/L</td>
<td>.</td>
<td>5.3</td>
<td>.</td>
<td>4.8</td>
<td>4.6</td>
<td>5.9</td>
<td>6.3</td>
<td>6.1</td>
<td>7.4</td>
<td>6.7</td>
</tr>
<tr>
<td>EEC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Red cell mass</td>
<td>.</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Spleen, echogram cm</td>
<td>.</td>
<td>13</td>
<td>16</td>
<td>13</td>
<td>16.5</td>
<td>11.8</td>
<td>13.7</td>
<td>13</td>
<td>14.3</td>
<td>16</td>
</tr>
<tr>
<td><strong>Clinical Diagnosis</strong></td>
<td>ET</td>
<td>ET</td>
<td>ET</td>
<td>ET</td>
<td>ET</td>
<td>ET</td>
<td>PV</td>
<td>PV</td>
<td>PV</td>
<td>PV</td>
</tr>
</tbody>
</table>

JAK2\textsuperscript{V617F}: +/1 is heterozygous, +/- is homozygous

**Table 5B:** Clinical and molecular and pathological features of 10 patients with either essential thrombocythemia (ET), ET followed by slow onset polycythemia vera (PV), or rapid onset PV, diagnosed according to TVSG/PVSG criteria\textsuperscript{1,3,4}, the 2008 WHO\textsuperscript{5} and the European Clinical Molecular and Pathological (ECMP) classifications\textsuperscript{7,8} by including bone marrow histopathology according to ECMP criteria

<table>
<thead>
<tr>
<th>BM Histology</th>
<th>Case 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BM Cellularity</strong></td>
<td>65%</td>
<td>60%</td>
<td>90%</td>
<td>75%</td>
<td>80%</td>
<td>75%</td>
<td>80%</td>
<td>75%</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>M:E  ratio</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>4</td>
<td>0.7</td>
<td>0.7</td>
<td>1</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td><strong>Megakaryocytes</strong></td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
<td>MPN</td>
</tr>
<tr>
<td><strong>Myeloid lineage</strong></td>
<td>N</td>
<td>N</td>
<td>Increase</td>
<td>N</td>
<td>Increase</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td><strong>Erythroid lineage</strong></td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>N</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
<td>MF-0</td>
</tr>
</tbody>
</table>

**C. Diagnosis/clinicalvs BM**

| Clinical no BM | ET | ET | ET | ET | ET | ET | PV | PV | PV | PV |
| BM pathology | ET | ET | PV | PV | ET.MGM | PV | PV | PV | PV | PV |
| 2008-WHO | ET | ET | ET/U | ET/U | MPN | ET/U | PV | PV | PV | PV |
| 2008-ECMP | ET-1 | ET-1 | ET-2 | ET-2 | ET-3 | ET-2 | PV | PV | PV | PV |
| Follow-up years | 4 | >12 | >10 | 10 | >11 | 8 | 4 | 1 | 5 | >15 |

US: unstainable, U: unclassifiable. Details of BM histology related to the clinical features are shown in figures 9
ECMP criteria in view of the WHO revisions of the MPNs of various molecular etiology

The 2005 concept of JAK2*V617F* mutated trilinear MPN

As to the etiology of trilinear MPD in patients with PV Dameshek proposed in 1950 two highly speculative possibilities: either excessive bone marrow stimulation by an unknown factor, or the lack or diminution of an inhibitory factor. This hypothesis has been confirmed vainchenker & Constantinescu in 2005 by the discovery of the JAK2*V617F* mutation [81, 82], which was rapidly confirmed by three other groups [83-85]. On position 617 of the JAK2 JH2 domain Valine (V) is replaced by Fenyalalanine (F). The JAK2*V617F* mutation induces a loss of inhibitory activity of the JH2 pseudokinase part on the JH1 kinase activity of JAK2 [81-85]. The JAK2*V617F* makes the mutated hematopoietic stem cells hypersensitive to hematopoietic growth factors TPO, EPO, IGF1, SCF and GCSF, resulting in PV as a trilinear MPD. The prevalence of the JAK2 V617F mutation in PV is 95% and about 50% in ET and MF [86]. The JAK2*V617F* mutation load is usually low in ET, less than 10 to 50% (heterozygous) of the granulocytes are JAK2*V617F* positive (heterozygous) [83-96]. The JAK2*V617F* mutation load in PV is either low with less than 50% (heterozygous/ homozygous) or high with 50 to 100% (homozygous) of the granulocytes positive for the JAK2*V617F* mutation. The percentage of JAK2*V617F* positive granulocytes in PV may range from rather low to 100% for JAK2*V617F*, thereby reflecting the natural history of various degree of progressive disease (PV stage 1 to 5 of Wasserman, 1954) [33, 34] during the long-term follow-up. Scott from the laboratory of Green elegantly demonstrated that so-called heterozygous PV with allele load less than 50% in fact are hetero/homozygous at the EEC blood and bone marrow level for the JAK2*V617F* mutation [91], which has been confirmed by Moliterno & Spivak from the MDP expert center Baltimore USA [96]. In contrast, ET patients are usually heterozygous with a maximal JAK2*V617F* mutation load of 50% [91, 96]. Normocellular ET (WHO-ET) in contrast to JAK2-wild type prefibrotic primary myelofibrosis (pPMF) or JAK2*V617F* mutated hyper cellular ET (MGM) (masked PV) is known to be associated with a low incidence of progressive disease and a normal life expectancy [98-101]. The few ET patients homozygous for the JAK2*V617F* mutation patients are at higher or high risk for myeloid metaplasia of the spleen (splenomegaly) and myelofibrotic transformation [90]. JAK2*V617F* allele burden in PV above 50% represent a main risk factor for progression to myelofibrosis [102], and grading of bone marrow reticulin fibrosis has a significant impact on survival in PV patients [103].

Personal observations in newly diagnosed ET and PV patients

Between 1997 and 2007 we studied 10 JAK2*V617F* mutated patients with early stage ET or newly diagnosed PV who presented with migraine-like micro vascular cerebral ischemic attacks (MIA) and were referred from the Benelux (N=5), Europe (N=3) and the USA (N=2) to the Antwerp University Hospital from various European countries between January 2000 and August 2007 for expert evaluation and treatment recommendation. We prospectively studied blood and bone marrow features in 10 MPN patients carrying the with JAK2*V617F* mutation. The clinical diagnoses are ET in 6 and PV in 4 without the use of bone marrow histopathology (table 10). The 6 ET were heterozygous for the JAK2*V617F* mutation and had an erythrocyte count below 6x10¹²/L. Three PV patients were homozygous for the JAK2*V617F* mutation (case 7, 9 and 10, table 10). As shown in (table 11), bone marrow cellularity of the 10 JAK2*V617F* mutated MPN patients (6 ET and 4 PV) was increased (range 60% to 90%). There was an increased erythrocytosis in 8/10 and increased granulopoiesis in 4/10 patients. Interestingly, 3 ET with early features of PV (prodromal PV) fulfilled the bone marrow features of prodromal PV and 4 cases presented with rapid onset PV (cases 7, 8, 9 and 10) according to ECMP bone marrow criteria (table 3). The three ET patients with prodromal PV developed overt PV after long-term follow-up of 8, 9 and more than 10 years (slow onset PV, table 11). Myelofibrosis (MF) was scored according to Thiele et al [21] as MF-0 (RF-0/1) in 8 (5 ET, 3 PV), and MF-1 (RF-2) in 2 (1 ET, 1 PV) MPN patients. ET case 5 showed increased megakaryocytic-granulocytic myeloproliferation (MGM) bone marrow histology consistent with the JAK2*V617F* mutated masked PV (ETMG, table 2).

The clinical diagnosis of the 10 JAK2*V617F* positive patients without the use of bone marrow histology data was ET in 6 and PV in 4 cases (table 10). The diagnosis of the 10 JAK2*V617F* positive MPN patients based on bone marrow histology picture alone, as blindly judged by pathologists, and was consistent with ET in 3 and PV in 7 cases (table 11). The 3 ET patients diagnosed as PV bone marrow histology evaluation had very low serum EPO levels and EEC, but erythrocyte counts less than 6x10¹²/L consistent with the diagnosis of prodromal PV (tables 10 and 11). The diagnoses according to 2008 WHO criteria [24] were ET in 5, MPN unclassifiable in 1 and PV in 4 due to the complete lack of criteria to stage ET and PV patients. The diagnoses according to the ECMP criteria for diagnosis and staging of ET and PV patients (tables 2 and 3) were normocellular ET in 2 cases, prodromal PV (ET with low serum EPO, the presence of EEC and normal erythrocyte counts) in 3 cases, ET with masked PV (ETMG table 2) with no leuko-erythroblastosis in 1 case, and acute onset PV in 4 patients (cases 7, 8, 9 and 10, tables 10 and 11). Examples of bone marrow histopathology of JAK2*V617F* mutated normocellular ET, prodromal PV, masked PV (ETMG) and PV are shown in figures 5, 6, 7, and 8 demonstrating that bone marrow histopathology alone cannot distinguish JAK2*V617F* mutated ET, prodromal PV, classical PV and masked PV (ETMG).

Among WHO defined ET carrying the JAK2*V617F* mutation 10 MPN patients at time of diagnosis (table 1) we could distinguish ET stage 1, ET stage 2 with features of early PV and ET stage 3 with PMF-0 bone marrow features. Interestingly, the three JAK2*V617F* mutated ET patients with features of early PV in blood and bone marrow indeed transformed into PV during follow-up as documented by increased erythrocyte counts above 6x10¹²/L obviating the need of red cell mass measurement. Treatment of PV by phlebotomy alone corrects both hematocrit values to normal (around 0.40) due to iron deficiency, but the number
Table 6: 2007/2008 ECMP criteria for JAK2 wild type and MPL515 mutated primary thrombocythemia (PT). Whether PT-MGM remains to be evaluated

<table>
<thead>
<tr>
<th>Clinical and molecular criteria</th>
<th>WHO bone marrow criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>Predominant proliferation of enlarged mature pleiomorphic megakaryocytes with hyperlobulated nuclei lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or slight increase in reticulin: RF 0-1</td>
</tr>
<tr>
<td>1. Platelet count &gt;350-400x10⁹/l</td>
<td>Presence of large platelets in blood smear</td>
</tr>
<tr>
<td>2. JAK-2 wild type or MPL515 mutation</td>
<td>Presence of enlarged megakaryocytes in bone marrow smear</td>
</tr>
<tr>
<td>3. No anemia, Hb &gt;12.8 g/dl</td>
<td>No or slight increase in reticulin: RF 0-1</td>
</tr>
<tr>
<td>4. Normal serum EPO</td>
<td>Predominant proliferation of enlarged mature pleiomorphic megakaryocytes with hyperlobulated nuclei lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or slight increase in reticulin: RF 0-1</td>
</tr>
<tr>
<td>5. No or slight splenomegaly on ultrasound</td>
<td>No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or slight increase in reticulin: RF 0-1</td>
</tr>
<tr>
<td>6. No leukoerythroblastosis</td>
<td>No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or slight increase in reticulin: RF 0-1</td>
</tr>
<tr>
<td>7. No dry tap on bone marrow biopsy</td>
<td>No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or slight increase in reticulin: RF 0-1</td>
</tr>
<tr>
<td>8. No preceding or allied of CML, PV, RARS-T or MDS</td>
<td>No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or slight increase in reticulin: RF 0-1</td>
</tr>
</tbody>
</table>

Table 7: 2007/2008 WHO criteria for the diagnosis of primary advanced myelofibrosis or agnogenic myeloid metaplasia (AMM), and post ET/PV myelofibrosis

**Diagnosis PAMM requires 2 minor and all 4 major criteria**

**Minor criteria, manifest clinical features**

1. Left shifted white blood cell differential count and/or leukoerythroblastosis
2. Increase in serum lactate dehydrogenase (LDH) above borderline levels.
3. Anemia, hemoglobin <12.8 g/dl.
4. Enlarged spleen on echogram or palpable spleen.

**Major criteria (bone marrow histopathology and clonal markers)**

1. Presence of dual dysmegakaryocytic and granulocytic proliferation (hypercellular bone marrow) with dense clusters of small to large dysmature megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei (cloud-like), usually accompanied by various degrees of reticulin fibrosis (RF)
   or
   A typical hypercellular bone marrow (80-100%) due to PDGM with relative reduction of erythroid precursors, and dominated by dense clustering and increase in atypical giant to medium sized megakaryocytes containing clumsy (cloud-like) lobulated nuclei and definitive maturation defects with no or slightly increased reticulin fibrosis (RF 0-1).
2. No Ph-chromosome or any other fusion gene specific for CML, CEL/HES etc and no evidence of previous MDS, ET, PV or other myeloid or lymphoid neoplasm, no cancer or inflammation.
3. JAK2 wild type or MPL mutation versus JAK2<sup>V617F</sup> post-ET/PV MF
4. Presence of cytogenetic abnormalities including del (20q) and other cytogenetic abnormalities as has been described for AMM and post-PV MF

Source Poster P-0025. Fourth International Congress on MPD/MDS New York, 2007
Table 8:

2012 ECMP criteria for the diagnosis, clinical staging and grading of myelofibrosis (MF) in JAK2-MPL wild type primary dysmegakaryocytic, granulocytic myeloproliferation: PDGM

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>No preceding or allied subtype of myeloproliferative disorders CML or MDS. Main presenting feature is pronounced thrombocythemia and no dry tap on bone marrow aspiration.</td>
<td>B1 Dysmegakaryocytic and granulocytic myeloproliferation (DGM) and no or relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant to medium sized megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects.</td>
</tr>
<tr>
<td></td>
<td>JAK2 and MPL wild type (2008 ECMP)</td>
<td>Staging of myelofibrosis (MF)</td>
</tr>
<tr>
<td>C</td>
<td>Clinical stages of PMF or PMGM or PDGM</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>Early clinical stages Normal hemoglobin or slight anemia, grade I: hemoglobin &gt; 12 g/dl Slight or moderate splenomegaly on ultrasound scan or CT Thrombocytosis, platelets in excess of 400, 600 or even 1,000 × 10^9/L Normal or increased LAF-score No leuko-erythroblastosis</td>
<td>MF-0 prefibrotic stage PMF or PDGM no reticulin fibrosis: RF 0 or 1</td>
</tr>
<tr>
<td></td>
<td>MF-1 early PMF or PDGM slight reticulin fibrosis: RF 2</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>Intermediate clinical stage: PDGM Anemia grade II: hemoglobin &gt; 10g/dl Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes Increased LDH Splenomegaly</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>Advanced clinical stage: PDGM Anemia grade III: Hemoglobin &lt; 10g/dl Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes Splenomegaly, thrombocytopenia, leukocytosis, leukopenia</td>
<td></td>
</tr>
</tbody>
</table>

The combinations of A1+B1 establish PMF or PMGM: ary other criterion C or MF contributes to staging

Table 9: Low, intermediate and high thrombohemorrhagic risk stratification of thrombocythemia in ET and PV patients: a flexible approach towards therapeutic implications with reference to platelet counts for the indication of low dose aspirin and the need for platelet count reduction by anagrelide, pegylated interferon or hydroxyurea

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Platelets</th>
<th>Platelets</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 - 1500 × 10^9/L</td>
<td>400 - 1000 × 10^9/L</td>
<td>400 - 1000 × 10^9/L</td>
<td>&gt; 1500 × 10^9/L</td>
</tr>
<tr>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
<td>High risk</td>
</tr>
<tr>
<td>Completely asymptomatic</td>
<td>Microvascular disturbances only</td>
<td>Major thrombosis, and/or bleeding</td>
<td>&gt;1000 × 10^9/L and minor thrombosis and/or bleeding</td>
</tr>
<tr>
<td>No vascular risk</td>
<td>No vascular risk</td>
<td>Vascular risk</td>
<td>No vascular risk</td>
</tr>
<tr>
<td>No bleeding risk</td>
<td>No bleeding risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin uncertain</td>
<td>Low dose aspirin</td>
<td>Platelet reduction to normal or near</td>
<td>Platelet reduction to normal</td>
</tr>
<tr>
<td>Wait and see?</td>
<td>50 to 100 mg/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very low?</td>
<td>Intermediate risk</td>
<td>Continuous aspirin</td>
<td>Add aspirin</td>
</tr>
</tbody>
</table>

Aspirin primary prevention? ET patients and their physician usually prefer the use of low dose aspirin

Microvascular disturbances and platelet count between 1000 and 1500 × 10^9/L with clear indication aspirin, side effects platelet reduction
### Table 10: Clinical Staging of PV according to ECMP criteria related to therapy, anno 2012

<table>
<thead>
<tr>
<th>PV, ECMP stage</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Michiels ECMP Clinical Diagnosis</strong></td>
<td>Erythrocytic PV</td>
<td>Prodromal PV</td>
<td>polycythemic PV prefibrotic</td>
<td>Classic PV prefibrotic</td>
<td>Advanced PV PMF stage</td>
<td>Post-PV MF AMM Neoplastic</td>
</tr>
<tr>
<td>LAP-score and/or PRV-1</td>
<td>N/↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑/↑↑</td>
<td>variable</td>
</tr>
<tr>
<td>Red cell mass (RCM)</td>
<td>↑</td>
<td>N</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>variable</td>
</tr>
<tr>
<td>Serum EPO</td>
<td>N/1</td>
<td>N/1</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>variable</td>
</tr>
<tr>
<td>Leukocytes x10⁹/l</td>
<td>&lt;12</td>
<td>&lt;12</td>
<td>&lt;12</td>
<td>N-&gt;12</td>
<td>&gt;15</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Platelets x10⁹/l</td>
<td>&lt;400</td>
<td>&gt;400</td>
<td>&lt;400</td>
<td>&gt;400</td>
<td>&lt; or &gt;1000</td>
<td>variable</td>
</tr>
<tr>
<td>Hemoglobin g/dl (mmol/l)</td>
<td>&gt;16 (10)</td>
<td>&gt;0.51</td>
<td>&gt;6</td>
<td>&gt;16 (10)</td>
<td>&gt;0.51</td>
<td>&gt;16 (10)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>&gt;16 (10)</td>
<td>&gt;0.51</td>
<td>&gt;6</td>
<td>&gt;16 (10)</td>
<td>&gt;0.51</td>
<td>&gt;16 (10)</td>
</tr>
<tr>
<td>Erythrocytes x10¹²/L</td>
<td>&gt;6</td>
<td>&lt;6</td>
<td>12</td>
<td>12-15</td>
<td>12-18</td>
<td>++/+++</td>
</tr>
<tr>
<td><strong>ECMP bone marrow</strong></td>
<td>Early PV</td>
<td>Early PV</td>
<td>Early PV</td>
<td>Trilinear PV</td>
<td>Trilinear PV</td>
<td>Trilinear / MF Decreased RCF 2/3</td>
</tr>
<tr>
<td>Bone marrow cellularity (%)</td>
<td>50-80</td>
<td>50-80</td>
<td>60-100</td>
<td>80-100</td>
<td>80-100</td>
<td>RCF 1/2</td>
</tr>
<tr>
<td>Grading myelofibrosis</td>
<td>RF 0-1</td>
<td>RF 0-1</td>
<td>RF 0-1</td>
<td>RCF 1/2</td>
<td>RCF 1/2</td>
<td>RCF 1/2</td>
</tr>
<tr>
<td>Splenomegaly on palpation</td>
<td>no</td>
<td>No/+</td>
<td>No/+</td>
<td>+</td>
<td>++/+++</td>
<td>/large</td>
</tr>
<tr>
<td>Spleen size, echogram cm</td>
<td>&lt;12</td>
<td>&lt;12-15</td>
<td>12-15</td>
<td>12-18</td>
<td>18-&gt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Spontaneous EEC+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JAK2V617F in Granulocytes and BFU-e (exon 12)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>Therapeutic implications Anno 2012/2013</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Intermediate risk PV</td>
<td>High risk PV</td>
<td>Post-PV MF</td>
</tr>
<tr>
<td>First line treatment option MPN reductive treatment JAK2 inhibitor</td>
<td>Aspirin</td>
<td>Aspirin</td>
<td>Phlebotomy</td>
<td>Phlebotomy</td>
<td>Phlebotomy*</td>
<td>If IFN resistant</td>
</tr>
<tr>
<td></td>
<td>Phlebotomy</td>
<td>Aspirin</td>
<td>Low dose IFN</td>
<td>Aspirin</td>
<td>Aspirin</td>
<td>IFN</td>
</tr>
<tr>
<td></td>
<td>Low dose IFN?</td>
<td>Phlebotomy</td>
<td>Complete response</td>
<td>IFN</td>
<td>IFN</td>
<td>HU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*↑ = increased, ↓ = decreased, N = normal, + = present or heterozygous; ++ = homozygous*
of erythrocytes remain above 6.0 x10¹²/L in PV and are below 6.0x10¹²/L in ET patients (table 2)[13, 16, 28]. The use of bone marrow histopathology in combination with clinical and molecular markers are powerful tools for the characterization and staging of JAK2V617F mutated thrombocythemia into ET stage 1, ET stage 2 (prodomal PV), and ET stage 3 at time of presentation. An erythrocyte count at a cut-off of 6.0x10¹²/L in JAK2V617F mutated MPN is a relevant diagnostic criterion to distinguish ET type 2 ("forme fruste PV") from PV in remission by phlebotomy alone. Phlebotomy relieves PV-related "hypervolumenic" symptoms, [28-31] but not the erythroelagic and MIA's because of persisting thrombocythemia [4, 5, 16, 18]. Low dose PegasysR did induce a complete haematological and even molecular remission with correction of platelet and erythrocyte counts and bone marrow morphology to normal [32]. Adequate use of low dose aspirin and a non-leukemogenic platelet lowering agent if indicated but not coumadin in JAK2V617F mutated ET and PV [16, 18, 31] will prevent the erythroelagic and MIA's and prevent major thrombotic complications during long-term follow-up when adequately monitored and managed for vascular risk factors.

**JAK2V617F positive ET and PV reflects a broad spectrum of trilinear MPN**

The 2006 concept according to Vainchenker; Green & Michiels is that heterozygous JAK2V617F mutation leading to constitutively activated megakaryocytes with increased sensitivity to TPO and EPO is enough to induce ET with the production of constitutively hyper reactive platelet as the cause of platelet mediated arteriolar inflammation (essential platelet thrombophilia according to Dameshek 1940 and Michiels 1985-2006 (table 8)[47,86]. Platelet-mediated thrombophilia is associated with normalcellular ET (cases 1 and 2, table 5), prodomal PV (cases 3 and 4, table 5) and has been recognized in 1940 by Dameshek as main presenting features of PV. A group of JAK2V617F positive normalcellular ET (WHO-ET) with a very low percentage of heterozygous mutant JAK2V617F can maintain as a non-progressive sub population in the bone marrow without a tendency to evolve into prodomal PV or masked PV (ET.MGM) during long-term follow-up. Hetero/ homozygous or homozygous JAK2V617F mutation with pronounced constitutively activation of megakaryopoiesis, erythropoiesis and granulopoiesis is associated with hyper cellular MPN with a trilinear PV (cases 7, 8, 9, 10 , table 5), or hyper cellular masked PV (ET.MGM, case 5, table 5) [97]. The sequential occurrence of heterozygous and homozygous JAK2V617F mutation can readily explain the evolution of prodomal PV into slow onset PV, and of ET into ET.MGM with subsequent evolution into post-PV and post-ET myelofibrosis and increasing splenomegaly due to expansion of primary myeloid neoplasia in the spleen, already starting from the very beginning that the acquired JAK2V617F mutation had occurred in hematopoietic progenitor cells of the bone marrow. There is a broad spectrum of transitional states in between JAK2V617F mutated ET, prodomal PV, masked PV (ET.MGM) and PV, their early reticulin fibrotic stage of ET or PV, and their subsequent advanced reticulin/collagen fibrotic stage of PV97 not meeting the 2008 WHO criteria for primary myelofibrosis (PMF) or primary advanced myeloid metaplasia (PAMM, table 7). Such transitional states not meeting the 2008 WHO criteria for advanced post-ET and post-PV myelofibrosis may elapse for 10 years to even more than 25 years of follow-up (table 12) [9, 7]. The prefibrotic, early fibrotic and fibrotic stages of bone marrow histopathology in PV and in masked PV (ET.MGM) is correlated to the degree of splenomegaly due to expansion of hematopoietic neoplasm in the spleen (MPN spleen burden), the JAK2V617F mutation load, degree of reticulin fibrosis and reticulin/collagen fibrosis of the bone marrow, increased circulating CD34+ cells, and LDH. The ECMP criteria for the classification of the JAK2V617F positive ET, prodomal PV, masked PV and PV reflect the molecular mutation load and objective clinical MPN burden [90, 97, 102].

The PVSG and WHO criteria do overlook the early stages of ET and PV when TVSG, ECP, and ECMP criteria are applied (table 3). Masked ET or PV in the setting of splanchic vein thrombosis (SVT, Budd-Chiari syndrome or portal vein thrombosis) in 241 patients, platelet counts were between 238 and 456x10¹²/L [mean 333] in 74 patients carrying the JAK2V617F mutation and between 104 and 258x10¹²/L [mean 159] in 147 JAK2 wild type SVT patients and none of them carried the MPL515 mutation [104]. In patients with a first episode of splanchic vein thrombosis (SVT), analysis of any venous thromboembolic risk factors as well as a JAK2V617F mutation status indicative for MPD is warranted [104-106]. Administration of heparin followed by oral anticoagulation with vitamin K antagonists is the treatment of choice in patients with SVT. Anticoagulation therapy combined with low-dose aspirin and proper treatment of the MPD is recommended in patients with SVT associated with the JAK2V617F mutation [106,107]. Myelodysplastic variants of megakaryocytic myeloproliferations in the literature include thrombocythemia as a manifestation of 5q minus thrombocythemia, thrombocythemia associated with RARS (RARS-T). RARS-T appears to be a distinct MDS subgroup, which rather frequently carries the JAK2V617F mutation [47, 50,108]. The finding of the JAK2 exon 12 mutations in patients with JAK2V617F negative PV or idiopathic erythrocytosis, but not in ET further confirms the strong association between the JAK2 mutations and MPD [109, 110]. The 5% PV patients negative for JAK2V617F are frequently heterozygous for exon 12 JAK2 mutations and usually present with early stage PV with a favourable outcome and normal life expectancy.

**JAK2 wild type ET carrying the TpoR = MPL515 mutation**

The JAK2 kinase activity in clonal MPD is dependent not only on the amount of heterozygous and homozygous mutant JAK2V617F protein, but is also influenced by the various steps upstream or downstream the signalling pathways regulating JAK2 activity including MPL, JAK2, and STAT-3. cMPL is the thrombopoietin receptor (TpoR) on hematopoietic bone marrow cells. The first case of congenital ET due to a gain of function mutation in the cMPL = TpoR gene has been described in 2004 [111]. This has led to the discovery of the MPL515L and MPL515K mutations as the cause of ET and myelofibrosis but not PV (table 6) [112, 113]. Within the JAK2 wild type MPN, there is a small subgroup that carries an acquired gain of function mutation of the MPL = TpoR as...
the cause of ET (or primary thrombocythemia, PT, table 6): 3% in
the Vannucchi study [114], and 8.5% in the UK studies [115, 116].
In contrast to JAK2V617F mutated trilinear MPN, patients with the
MPL515 mutation have no clinical, laboratory and bone marrow
features of prodomal PV at diagnosis, do not evolve into overt PV
during follow-up, have normal serum EPO and ferritin levels, do
not show spontaneous endogenous erythroid colonies (EYC), and
may show pronounced megakaryocytic proliferation of small and
large (giant) megakaryocytes and increased granulopoiesis but no
increase of erythropoiesis in the bone marrow [114-116]. In 2008
we studied bone marrow histopathology in 12 cases with JAK2
wild type ET carrying the MPL515 mutation kindly provided by
the courtesy of Dr. Vannucchi, Florence, Italy [117]. Bone marrow
histology from patients with JAK2 wild type ET carrying the
MPL515 mutation consistently displayed clusters small and large
megakaryocytes with a greater number of giant megakaryocytes
with hyperlobulated stag-horn nuclei in a normal cellular bone
marrow and no increase of erythropoiesis [117]. As compared to
bone marrow histopathology in our patients with normocellular
JAK2V617F mutated ET, there were significant differences on three
points. The megakaryocytes in MPL515 mutated PT are larger
than in PV, whereas the megakaryocytes in JAK2V617F mutated
ET are not larger than in PV and show similar pleomorphic
megakaryocytes morphology as in PV. Second, there was local
increase of erythropoiesis in areas of loose clustered pleomorphic
megakaryocytes in JAK2V617F mutated ET, but not in JAK2 wild type
PT carrying the MPL515 mutation. Third, we observed significant
increased reticulin fibers grade 2 in a normocellular bone
marrow in areas of dense clustered megakaryocytes, which is not
seen in JAK2V617F mutated normocellular ET, and hyper cellular
prodomal PV and ETMGM. Whether such differences in bone
marrow histology features of megakaryocyte morphology in
dense normocellular ET with low JAK2 mutation load and JAK2 wild type ET carrying the MPL515 mutation can be
seen by expert hematopathologists remains to be evaluated in
prospective clinical and basic research studies.

**JAK2/MPL wild type PMGM MPN entity**

The bone marrows in prefibrotic and early fibrotic PDGM/
PMGM show dysmorphic megakaryocytes with definite
abnormalities of maturation with bulky (bulbous) hyper chromatic
nuclei and some disturbances of the nuclear cytoplasmic ratio,
which are not seen in JAK2 wild type ET carrying the MPL515
mutation and also not in prefibrotic JAK2V617F mutated ET, ET/
PV, masked PV and PV. Such bone marrow findings of PDGM
are consistent with pronounced primary thrombocythemia as
the presenting feature of prefibrotic primary megakaryocytic
granulocytic myeloproliferation (PMGM) according to Georgiet
al 199028,32, and do not meet the criteria for AMM (fibrotic
PMF) according to the 2008 WHO classification (tables 7 and
8)[32]. A typical case of PDGM/PMGM with normal blood cells
counts, absence of the JAK2V617F mutation, slight increase of
reticulin fibrosis and no splenomegaly on palpation. Similar bone
marrow features are frequently reported and classified by Thiele
as prefibrotic CIMF or PMF [30, 31, 46, 47]. With the advent of
the JAK2V617F mutation, we could separate the 1999 EMGM entity
(table 2) into cases of JAK2V617F positive ET.MGM (masked PV,
table 3) and JAK2 wild type PDGM (table 8) [117].

**Low, intermediate and high thrombotic and hemorrhagic
risk stratification in thrombocythemia of ET and PV patients**

In our experience, ET and PV patients at age around and
over 65 years without a history of thrombosis and treated with
low dose aspirin still remains a low risk for an ET patient in the
absence of vascular risk factors (http://www.mpn-stichting.nl/
doctors_brochure_2004.pdf) [76, 97]. The presence of one of the
risk factors for arterial vascular disease, such as hypertension,
hypercholesterolemia, diabetes and smoking, did not contribute
in terms of statistical evidence to an additional increased
erythromelalgic Migraine-like TIA’s in thrombocythemia [65].
Strict measures to reduce and eliminate cardiovascular risk factors
mandatory (Table 6). Increased number of platelets above 350 to
400 ×10^9/L is the main cause of micro vascular events in ET when
not on aspirin. Erythromelalgic disturbances and Migraine-like
TIA’s have never been reported in age adjusted individuals with
reactive thrombocytosis. ET and PV patients with platelet counts
above 1000 ×10^9/L are at high risk for the paradoxical occurrence
of thrombotic and bleeding complications. In that situation,
aspirin does prevent platelet mediated thrombotic events but
does increase the bleeding tendency (as well as prolongation of
the Ivy bleeding times) and, therefore, are candidates for
platelet reductive therapy with continuation of low dose aspirin
[50,63,64]. ET and PV patients with platelet counts around and
above 1000 ×10^9/L are candidates for screening for acquired
von Willbrand disease type 2A by the combined use of von
Willebrand factor antigen (VWF:Ag), VWFristocetine cofactor activity
(VWF:RCo). VWF collagen binding (VWF:CB) and a sensitive
method to demonstrate the absence of large VWF multimers. Low
dose aspirin surely does increase the risk of bleedings at platelet
counts above 1000 ×10^9/L (Table 6) [67, 97]. Asymptomatic low
risk and symptomatic (micro vascular event) low risk ET patients
with symptomatic micro vascular events have a clear indication
for low dose aspirin but do not have an indication to reduce the
platelet count in the complete absence of any vascular risk factor
and no history of bleeding and atherothrombosis at a platelet
count below 1000 × 10^9/L (Table 6). Those ET patients over the
age of 65 with no vascular risk factors, asymptomatic while on
low dose aspirin, and platelet count below 1000 ×10^9/L are to be risk
stratified as of low thrombohemorrhagic risk with no indication
for hydroxyurea to further decrease platelet number (Table 6).
In case of aspirin side effect (gastritis or aspirin allergy), the
reversible platelet COX1 inhibitor indomethacin is the alternative
treatment of choice [59, 60]. High thrombohemorrhagic risk ET
patients do not necessarily have their platelet count corrected
to normal (< 400 × 10^9/L) by anagrelide, pegylated interferon
or hydroxyurea (table 12), because they usually remain free of
thrombosis and bleeding at slight to moderate increase of platelet
count (600-800 ×10^9/L). This near to normal platelet count
strategy preferentially by one of the non-leukemogenic platelet
lowering agents, either anagrelide or pegylated interferon-alpha-2a
is recommended, on top of low dose aspirin (50 to 75 mg/day

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As compared to P32 and dipipobroman, hydroxyurea is the least leukemogenic myelosuppressive agent in long-term prospective clinical PV-studies extending observation periods of more than 10 years [32, 34, 69, 118]. Clinicians will be reluctant to postpone the use of hydroxyurea in early stage PV as long as a conservative approach using phlebotomy aiming at a hematocrit below 0.45, on top low-dose aspirin for the control platelet function, and if indicated low dose interferon for the control platelet, leukocyte and erythrocyte number is used to keep the PV patient healthy as long as possible [69]. The rational for using IFN-alpha as a first-line treatment option in newly diagnosed PV-patient include its effectiveness to abate constitutional symptoms and to induce a complete remission thereby avoiding phlebotomy, iron deficiency, and macrocytosis associated with hydroxyurea [119-124]. Recent data clearly show complete hematological and major molecular responses in prefibrotic stages of PV by relatively low dosages of pegylated interferon alpha-2a [120, 121]. Clinical studies indicate that both hydroxyurea and JAK2 inhibitors are not able to eliminate the JAK2\textsuperscript{V617F} clone in the bone marrow. Pegylated interferon alpha-2a is associated with significant side effect in about one third of PV patients. The misconception in the past was to start with too high dosages of IFN. Our preliminary experiences indicate that low dose pegylated interferon 45 ug/week or every 2 weeks for several months to one year is enough to induce complete hematological response and major molecular response without significant side effects (unpublished data).

A recent retrospective study of 118 MPN patients with various degrees of MPN disease burden ranging from PVS2 defined ET (N=46), PV (N=55) and PMF (N=17) were comparable with regard to age and peripheral blood features, the JAK2\textsuperscript{V617F} mutation was present in PV 91%, ET 37%, and MF 53%, and the spleen palpable (splenomegaly) in PV 25%, ET 13%, and MF 47%. Data on bone marrow histopathology, circulating CD34 cells, LDH and mutation load are lacking in this retrospective study. The complete response rate according to ELN criteria were 54% for PV and 63% for ET, but whether they reached complete responses at the bone marrow and molecular level remained elusive. It is known to hematologists that IFN may be rather effective in WHO defined prefibrotic primary myelofibrosis (PMF 0/1) but much less and usually ineffective in advanced WHO defined PMF (PMF 2/3). It has been clearly shown that complete hematologic and even significant molecular responses indeed do occur and are reached one to 3 years after initiation of 1NF (figure 1) [120]. High loading dose IFN seems to us useless and to overcome the initial hurdle of side effects starting with low dose pegylated IFN 45 ug/week in prefibrotic JAI\textsuperscript{V617F} mutated PV has two advances: first less side effects and second it offers the unique opportunity to assess the dose/response of IFN needed to reduce JAK2\textsuperscript{V617F} mutated cells and MPN disease load in that particular MPN patient during the first 6 months to year. It is of importance to give confidence to the MPN patient that IFN really works better than hydroxyurea in terms of hematological and molecular responses. The JAK2\textsuperscript{V617F} mutated prodromal PV and early stage PV as well as JAK2\textsuperscript{V617F} mutated ET patients with a hyper cellular bone marrow or prefibrotic PMF are candidates for low dose pegylated IFN to postpone the use of hydroxyurea as long as possible (table 12).

If IFN is not responsive or has been shown to elicit serious side effects, hydroxyurea becomes the second line treatment option in PV patients with the aim to improve quality of life by control or reduction of MPN disease burden (table 12). In this situation we should address the question, whether the JAK2 inhibitors as a non-leukemogenic approach is equal or superior to hydroxyurea in classical PV with a hyper cellular bone marrow (80-100%) just before its change from reticulin fibrosis RF grade 0/1 into RF grade 2 and subsequent irreversible RF grade 3 / 4 reticulin/collagen fibrosis (table 10).

Discussion

The literature on ET and PV up to the 2008 WHO classification is past history and the published results are mainly derived from survival data of large retrospective studies of PVS2 defined MPDs. The 2008 WHO investigators reclassified the PVS2 defined MPDs ET, PV and PMAM collected between 1975 and 2008 in retrospective studies of PVS2 defined cohorts of ET, PV and MF patients. There is a compelling need to diagnose, classify and stage MPN patients (ET, PV, PDGM/PMM and PAMM or PMF, table 7) based on real field findings from large prospective observational, research, intervention and outcome studies by a new generation of MPD/MPN investigators. We should realise that the 2008 WHO MPN classification do define criteria for diagnosis of ET, PV and PMAM in routine daily practice, but do not recognize the various stages of each of the 3 primary prefibrotic primary myeloproliferative disorders (PMD): 1. JAK2\textsuperscript{V617F} mutated trilinear MPD/MPN including normocellular ET, hyper cellular ET,PMGM (masked PV), prefibrotic PV, slow onset and acute onset PV; 2. JAK2 wild type PT carrying the MPL\textsuperscript{S15} mutation78; and 3. JAK2 wild type ET associated with PDGM (Table 8). Under the pressure induced by Vainchenker& Constantinescu with the discovery of the JAK2\textsuperscript{V617F} mutation in 2005 as the cause of the trilinear MPD/MPN [81,81], we have split the 1999 EMGM conceptual entity in JAK2\textsuperscript{V617F} mutated ET,PMGM (masked PV, table 4) and JAK2 wild type PMGM=PDGM (table 8). PMGM clearly differ from the description of JAK2 wild type PT carrying the MPL\textsuperscript{S15} mutation (table 7). The complete spectrum of the MPNs/MPDs of various molecular etiology and its natural history during long-term and life-long follow-up is very poorly defined for several reasons. First, the early stages are overlooked by too crude PVS2-WHO criteria to detect. Second, masked cases of ET and PV may remain asymptomatic until they manifest with overt symptoms either with thrombosis, haemorrhages and/or splenic myeloid metaplasia. Patients with primary MPD (PDM) negative for the JAK2\textsuperscript{V617F} mutation either MPL\textsuperscript{S15} positive or JAK2/MPL wild type PMGM should be studied separately in prospective studies. With the advent of the JAK2\textsuperscript{V617F} mutation all latent, early and overt stages of PV will be picked up more than 10 years earlier by the ECMP criteria as compared to the world widely used PVS2 and WHO 2008 WHO criteria.

The 2007/2008 WHO of Tefferi et al [48] by deleting red cell mass (RCM) measurements and just measuring haemoglobin...
and hematocrits disreasing erythrocyte counts do not correctly distinguish ET from PV. Increased RCM in patients with erythrocytosis does not distinguish early erythrocythemic PV from CP or SE, indicating the need of specific molecular and pathological MPN markers. Patients with JAK2V617F mutated ET stage 2 with normal haemoglobin and hematocrit do have normal erythrocyte counts (prodromal PV, table 5) whereas PV with increased erythrocyte counts above 6x10^{12}/L do frequently not meet the 2008 WHO criteria of increased hemoglobin and hematocrit levels due to iron deficiency in cases of masked PV or PV in remission by phlebotomy alone, who do have a typical PV picture on bone marrow histopathology. The shortcomings for correct diagnosis, classification, and staging of the MPDs ET and PV can be largely solved by the use erythrocyte counts below and above 6x10^{12}/L to better define and separate heterozygous JAK2V617F mutated ET and JAK2V617F mutated heterozygous/homzygous PV as two sequential molecular stages of JAK2V617F mutated MPD/MPN (tables 3 and 4). The 2007/2008 revisions of the WHO diagnostic criteria of Tefferi et al [48] for three main MPDs ET, PV, and PMF are a significant step forward as compared to the 1975 PVSG and 2007/2008 WHO diagnostic MPD criteria but still do not meet the needs in daily practice for three main reasons ref. First, the 2007/2008 WHO criteria for ET only include normocellular ET (WHO-ET), and the diagnosis of ET type 2 with features of early PV (hemoglobin<18.5 for men and <16.5 for women) with increased trilinear myeloproliferation (panmyelosis, Dameshek 1950) remain undiagnosed. This comprises a significant number of patients erythrocythemia and ET patients ET stage 2 with features of PV and normal erythrocyte count (prodromal PV). Second, spontaneous growth of erythroid colony formation (EEC) as a hall mark of PV, but it is also found in about half of ET patients consistent with “forme frusta PV” (prodromal PV). The combination of platelet counts above 400 x10^{11}/L borderline values for hemoglobin and hematocrit (0.45-0.51), normal erythrocyte counts, decreased serum EPO and/o the presence of EEC is diagnostic for JAK2V617F mutated prodromal and overt PV, which usually show an ET/PV bone marrow histology picture. There is very good evidence that PVSG-defined ET stage 3 with no leukoerythroblastosis but with increased cellularity due to increased granulopoiesis and loose clusters of slight to moderate megakaryopoiesis: ETGM (masked PV) is rather frequent but this entity is neither defined nor included in the 2007/2008 WHO classifications. Third, the diagnostic differentiation between JAK2V617F mutated ETGM (masked PV) versus patients with myeloid metaplasia of the spleen (either JAK2V617F mutated post-ET, post-PV versus JAK2 wild type PMF) and peripheral blood leukoerythroblastosis is clinically relevant, but not defined by the 2007/2008 WHO classification[125, 126]. JAK2V617F positive ETGM (masked PV) is clearly in between normocellular ET and post-ET myelofibrosis, and stages of prodromal, overt and advanced PV are better defined by the 2005 ECP and updated 2008 ECP[52, 53, 84]. The gap between the 2008 WHO defined prefibrotic ET, prodromal PV and PV on one hand and the other extreme of fibrotic AMM (PAMM) should be filled up by the intermediate stages of JAK2V617F mutated ET. MGM (masked PV) and JAK2 wild type PDGM/PMMG according to Michiels & Thiele (table 8). Fourth, the 2007/2008 revision by Tefferi et al [48] of the WHO classification disregard the importance of increased leukocytes, leukocyte alkaline phosphatase score, platelets and spleen size as typical presenting and pathognomonic features of JAK2V617F mutated ET, prodromal PV and trilinear PV. Simple tests like blood cell counts including platelets, leukocytes, hematocrit and erythrocytes, spleen size on echogram, EEC, and LAP score are even not taken into account by the 2007/2008 WHO classification to distinguish the latent (masked), early and overt thrombocytthemic and erythrocythemically stages of PV from the overt trilinear polycythemic stage of classic PV. These short comings of the 2007/2008 WHO revision of MPD criteria defined by Tefferi et al [48] prompted Michiels & De Raeve to update and clarify the origin and superiority of the ECMP diagnostic criteria for better staging of PV and ET patients, which has significant prognostic and therapeutic implications (table 12).

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