Symbiosis

ISSN Online: 2639-7986
www.symbiosisonlinepublishing.com

Review Article International Journal of Hematology and Blood Disorders Open Access

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

Jan Jacques Michiels*

Multidisciplinary Internist and Scientific Investigator, Good heart Institute in Nature Medicine, Freedom of Science & Education, Erasmus Tower Rotterdam, The Netherlands and European Working Group on Myeloproliferative Neoplasms (EWG.MPN)

Received: July 12, 2019; Accepted: August 6, 2019; Published: August 21, 2019

***Corresponding author:** Jan Jacques Michiels, Good heart Institute Rotterdam, Erasmus Tower, Veenmos 13, 3069 AT Rotterdam; Email: goodheart-center@outlook.com*

Abstract

The PVSG classification (1975) distinguished the Philadelphia (**Ph**^{1}) chromosome positive chronic myeloid leukemia (CML) from the **Ph**$^{1-}$ 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 JAK2^{V617F} mutation, indicating prodromal PV when the European Clinical, Molecular and Pathological (ECMP) criteria are applied. The positive predictive value of a JAK2^{V617F} 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 JAK2^{V617F} 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 masked PV). Bone marrow histology in JAK2^{V617F}-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 JAK2^{V617F} mutated ET, prodromal PV, masked PV (ET.MGM) and PV, and also not in JAK2 wild type normocellular ET carrying the MPL^{515} Mutation.

Keywords: Myeloproliferative Disorders; Essential Thrombocythemia; Polycythemiavera; Essential Megakaryocytic Granulocytic Myeloproliferation; Primary Myelofibrosis; JAK2^{V617F} Mutation; MPL^{515} Mutation; JAK2 Wild Type; Myeloproliferative Neoplasm; Bone Marrow Pathology

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

In his seminal article in 1950, Dameshek (1900-1969) described polycythemiavera (PV) as a trilinear 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 trilinear dysplastic features and gradual transition into a mixed erythroblasti/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 trilinear myelodysplastic syndrome (MDS) followed by transformation into acute leukemia mainly M2 or M4,5,6. Consequently, the Di Gugliemo 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 trilinear 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), polycythemiavera (PV) and agnogenic myeloid metaplasia (AMM) are characterized by a benign myeloproliferation of trilinear 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^1 chromosome karyotype (discovered by Nowell and Hungerford in 1960), which is present in CML, and absent in the MPDs ET, PV and agnogenic myeloid metaplasia (AMM) [9]. The PVSG used in 1975 the Ph^1-chromosome to distinguish the Ph^1- negative MPDs from the Ph^1-positive ET and chronic myeloid leukemia (CML) with various degrees of thrombocythemia and myelofibrosis [9-11].

Discovery of *BCR/ABL* in Ph^1-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^{1+}) [14]. Using improved banding techniques, Janet Rowley (1973) [15] showed that the Ph^{1+} chromosome in CML represents a deletion of the long arm of chromosome 22 (22q-) resulting in the minute Ph^{1+}. 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^{1+}-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 Cytogentics EMC), they found c-ABL moved to the Ph^1 -chromosome. Using hybrid cell lines containing the segregated Philadelphia translocation products (generated by Dr. Ad Geurts van Kessel, EUR), Groffen, Heisterkamp and Gerard Grosveld investigated whether cABL moved from the long arm of chromosome 9 to the long arm of the Ph^1 chromosome. A Southern blot confirmed this possibility [18]. Indeed c-ABL was found to translocate to the Ph^1-chromosome even in patients with complex chromosomal translocations but not in Ph^1-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 including 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^1-positive CML patients was predicted to have functional significance for the disease [20]. There was no serendipity in the discovery of the BRC/*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^1-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 thrombocythemia 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^{1+}/BCR/ABL+, 5% are Ph^{1}-/*BCR/ABL*+, and 5% are Ph^{1}-/BCR/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^{1}-chromosome and BRC/*ABL* translocation, and could detect (using the method of Groffen et al) the *BCR/ABL* transcript in a case of Ph^{1}-positive essential thrombocythemia [25, 26]. According to existing strict morphological, biochemical, cytogenetic and molecular criteria, including the Ph^{1} chromosome and *BCR/ABL* fusion gene and protein, CML is a malignant disease with an obligate transition into acute myeloid, lymphatic or megakaryoblast leukemia, whereas ET, PV and agnogenic myeloid metaplasia (AMM) or chronic idiopathic myelofibrosis (CIMF) form the Ph^{1}-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^{1+} and BCR/ABL+ ET and thrombocythemia associated CML versus the Ph^{1}- and BCR/ABL-negative thrombocythemias in various 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^{1+} CML diseases versus enlarged pleomorph megakaryocytes with hyperlobulated nuclei in Ph-negative MPDs (Hannover Bone Marrow Classification of Ph^{1}-positive CML and the prefibrotic Ph^{1}-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 erythrocythemia 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 erythrocythemia, thrombocythemia, granulocythemia 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, thrombocythemia is frequent and may be pronounced with bizarre and giant platelets, and granulocytic leukocytosis (granulocythemia) [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 erythrocythemic 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].

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 polycythemiavera 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 $JAK2^{V617F}$ mutation as a clue to the 3 PVSG defined variants of myleproliferative 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 the1975 PVSG 01 studyrevealed 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 marked 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 hypercllular 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 trilinear 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 erythrocythemic PV (idiopathic erythrocytosis) by definition44. 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 0.50, 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 chorambucil 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 ris ET and PV.

The PVSG 01 randomized clinical trial confirmed the hypothesis of Dameshek1 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 Weitherley-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 anno 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 1975 PVSG Criteria for Essential Thrombocythemia

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).

3. Absence of the Philadelphia chromosome to exclude CML
4. Absence of significant reticulin fibrosis (myelofibrosis) with dry tap on bone marrow aspiration, and no signs of preleukemia (table 1).

In the first prospective evaluation of PVSG defined ET, 37 evaluable ET patients had platelet counts between 1000 to 2650x10^9/L [66,67]. Thrombohemorrhagic events at presentation included mild bleedings in 5 epistaxis in 5, ecchymoses in 2, pelvic, buccal, fundal and urinary tract hemorrhage in 2, 2, 2 and 1 respectively, melena with a fall in hemoglobin of 7 gm/dl in 1 and massive postoperative bleeding in 1 case. Eleven ET patients experienced acroparesthesias (numbness), including burning sensations, usually in hand or feet (suggestive for erythromelalgia), 9 had dizziness, light-headedness or syncope, 7 had visual disturbances such as scotomas and transient dimming or blurred vision [66, 67]. Catastrophic complications (severe hemorrhages, myocardial infarction, stroke) in 6 (16%) [54,55]. 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. T***he 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 myelopoiesis 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)[50]	
1 A. The 1978 RCP major (A) and confirmative (B) criteria for prefibrotic ET[1]	
A1	Persistent platelet count in excess of 400x10^9/L.
A2	Increase and clustering of enlarged megakaryocytes in bone marrow biopsy.
A3	No or slight increase of reticulin fibers (RF 0 or RF 1)
B1	Presence of large platelets in a peripheral blood smear
B2	Absence of any underlying disease for reactive thrombocytosis and normal ESR.
B3	No or slight splenomegaly on palpation or scan (<15 cm)
B4	Increase of LAP-score and no signs of fever or inflammation
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]	
A1	Raised red cell mass. Male >36 ml/kg, female >32 ml/kg10 consistent with erythrocyte count of >6x1012/L (Dameshek & Henthel 1940 [32], Michiels table 6)
A2	Absence of primary or secondary erythrocytosis by clinical and laboratory tests.
A3	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 erythrocytosis[19].
No or presence of reticuline fibers and no collagen fibers (no dry tap)	
B1	Thrombocythemia, persistant increase of platelet >400x10^9/L
B2	Leukocytosis, leucocyte count >10^9/L and low erythrocyte sedimentation rate (ESR)
B3	Raised leukocyte alkaline phosphatase (LAP) score >100, absence of fever or infection
B4	Splenomegaly on palpation or on isotope/ultrasound scanning
A1+ A3 plus one of B establishes PV and excludes any variant of erythrocytosis.	

Table 1C: Grading of reticulin fibrosis Ellis (PVSG 1975)[44], and reticulin/collagen grading according to Thiele et al 2005[31]

Grading reticulin fibrosis (RF=+)[44]	Grading MF Thiele et al[31]	Description of reticulin fibers (RF)[18] and reticulin/collagen fibers (RCF) in myelofibrosis (MF)[21] as a secondary event in myeloproliferative neoplasms (MPN)
Normal RF-0	N MF 0	No reticulin fibers, occasional individual fibers or focal areas with tiny amount of reticulin fiber network
Slight increase RF 1	+ MF 0	Fine reticulin fiber network throughout much of section and no course reticulin fibers
Moderate increase RF 2	+ + MF 1	Diffuse fine reticuline network with focal collections of thick course reticulin fibers and no collagenisation
Marked increase BM dry tap RF 3 = RCF	+++ MF 2	Diffuse and dense increase in reticulin with extensive intersections, and presence of collagen fibers and no or minor osteosclerosis
OS Dry tap RF 4 = RCF&O	Sclerotic MF 3	Diffuse and dense reticulin with coarse bundles of collagen associated with significant osteosclerosis (O)

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[1-4].

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

In 1997 we extended the RCP proposed by the Thrombocythemia 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 thrombocythemia 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 ET.MGM 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 idiopatisc 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 Pathologic (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 $JAK2^{V617F}$ mutation screening as a pathognomic clue to distinguish $JAK2^{V617F}$ mutated trilinear

MPN from JAK2 wild type MPN (tables 3, 4, 6 ,7 and 8). The publication of the ECMP [78, 79] criteria preceded for several months the publication of the PVSG revised WHO criteria in 2007 [46-48] .The PVSG [45], the 2001 WHO [46,47], and the 2008 WHO revised criteria [48, 49, 77] detect the same cohort of PV patients simple because of one common major inclusion criterion: increased red cell mass (female >32 ml/kg and male >36 ml/kg) or equivalent high cut-off levels for, haemoglobin (>16.5 and male >18.5 g/dL), or hematocrit (female >48%, male >51%). About 20 to 25% of PV patients do not meet the cut off levels for haemoglobin and haematocrit, but do have erythrocyte counts above $6x10^{12}$/L and to be diagnosed as PV according to the 2008 ECMP criteria (table 4) [50]. As compared to RCM and the $JAK2^{V617F}$ mutation screening, erythrocyte count at a cut-off level of below or above $6x10^{12}$/L (ECP) better splits ET and PV (tables 4 and 5) [50] . Bone marrow biopsy histopathology evaluations and JAK2 mutation screening have a sensitivity and specificity of near to 100% and 95% respectively to differentiate to separate erythrocytoses with increased RCM into erythrocythemic stage 1 PV versus congenital or secondary erythrocytosis, to differentiate masked and overt PV from primary or secondary erythrocytoses, and todifferentiatebetween reactive thrombosis and ET with a sensitivity of 100% and about 50% respectively[47, 50, 68-70, 74-76, 78,79]. A bone marrow biopsy is mandatory for grading reticulin and collagen fibrosis in cases with advanced PV with MF, post-ET myelofibrosis, post-PV myelofibrosis and in patients with AMM of the spleen meeting the criteria of primary myelofibrosis (PMF) as defined by the WHO classification for myeloproliferative neoplasms77. The bone marrow histopathologic picture in PVSG defined (latent, prefibrotic) PV, which presented with thrombocythemia at platelet counts between 600 and $1260x10^9$/L mimicking ET clearly showed a mixed ET/PV picture of clustered distribution of pleomorphic enlarged megakaryocytes with moderate increase of trilinear hematopoietic cellularity (60-80%) [80]. The laboratory data of these 23 PV cases did not yet not meet the PVSG levels for hemoglobin and/or hematocrit required for diagnosing PV according to 1975 PVSG/2008 WHO classification, but did have increased erythrocyte count above $6x10^{12}$/L in men and above $5.5x10^{12}$/L in female [80] according to ECP and ECMP criteria (tables 4 and 5). The 2002/2005 ECP (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) and the 2007 ECMP criteria for the prefibrotic MPDs ET, prodromal PV, PV, EMGM/ PMGM (prefibrotic PMF) are original and preceded the formulation and publication of the 2007/2008 WHO criteria for myeloprilferativeneolasms (MPN).

Table 2a and b: Towards a European Consensus on the Diagnostic Criteria of Essential Thrombocythemia (ET), Polycythemia Vera (PV) according to Michiels et al 1999[70]

Diagnostic (A) and confirmative (B) PVSG Criteria of ET by including bone marrow histopathology according to Georgii et al[28,29] and Thiele et al[30,31]

A1	Platelet count in excess of 400 $x10^9$/l and no known cause of reactive thrombocytosis.
A2	Increase and clusters of mature giant megakaryocytes with hyperploid nuclei in bone marrow biopsies.
A3	No preceding or allied other subtype of myeloproliferative disorders or myelodysplastic syndrome.
B1	Normal or elevated leukocyte alkaline phosphatase (LAP) score, normal ES, and no fever.
B2	Normal or slightly increased cellularity and no or minimal reticulin fibrosis in bone marrow biopsies.
B3	Splenomegaly on palpation, or >11cm on ultrasound scan or on computer tomogram (CT).
B4	Spontaneous erythroid colony (EEC) and/or spontaneous megakaryocyte colony formation (CFU-Meg).

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

Diagnostic (A) and confirmative (B) PVSG Criteria of PV by including bone marrow histopathology according to Georgii et al[28,29] and Thiele et al[30,31]

A1	Raised red cell mass: RCM male >36 ml/kg, female >32 ml/kg	B1	Thrombocythemia platelet count >400 $x10^9$/l
A2	Absence of any cause of secondary erythrocytosis by clinical and laboratory investigations	B2	Granulocytes >10 $x10^9$/l and/or raised LAP score in the absence of fever or infection
A3	Histopathology of bone marrow biopsy: a) increase and clusters of pleiomorphic megakaryocytes with hyperploid nuclei b) increased cellularity: panmyelosis c) reticulin fibers (optional)	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[70]

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]

Clinical and hematological features[31,32]		**Diagnostic criteria[28-31]**
A	No preceding or allied other subtype of myeloproliferative disorders or MDS	**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
B	Abnormal clustering and increase of Thrombocythemia, platelets >400 x10^9/l	
C	Splenomegaly on palpation or >11cm on ultrasound scan or CT	**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.
D	Anemia, hemoglobine<12 g/dl	
E	Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes	
Proceedings of the Rotterdam MPD Workshop, March 13-14, 1998. Michiels & Thiele[70]		

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

Clinical and Molecular criteria	**Pathological criteria (WHO)**
Major PV criteria	**P1.** Bone marrow pathology: increased cellularity due to trilinear increase of erythropoiesis, megakaryopoiesis and granulopoiesis and clustering of small to giant (pleomorph) megakaryocytes with hyperlobulated nuclei. Absence of stainable iron. No pronounced inflammatory reaction (plasmacytosis, cellular debris)
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]	**P2.** Selective increase of erythropoiesis, normal granulopoiesis and megakaryocytes of normal size, morphology and no clustering of megakaryocytes in primary or secondary erythrocytosis
A1. Classical WHO defined PV: Hematocrit >0.51/>0.48 in male/female	**P3. Grading of reticulin fibrosis (RF) Post PV-MF-1, RF-2 and RF-3**
A2. Presence of JAK2V617F or exon 12 Mutation (sensitivity 98%)	
A3. Low serum EPO level and/or spontaneous endogenous erythroid colony (EEC) formation	
Minor MPD criteria	
B1. Persistent increase of platelet count: grade I: 400- 1500, grade II: >1500	
B2. Granulocytes >10 x10^9/l or Leukocytes >12 x10^9/l and/or raised LAP-score or increased PRV-1 expression in the absence of fever or infection	
B3. Splenomegaly on palpation or on ultrasound echogram (>12 cm length in diameter)	

Table 4: 2007 ECMP criteria for the diagnosis of 3 phenotypes of JAK2^{V617F} mutated Essential Thrombocythemia (ET): important to differentiate because the natural history may differ

Clinical and molecular criteria	WHO bone marrow criteria
ET stage 1	**ET**
1. Platelet count of >350 x10^{9}/l and the presence of large platelets in a blood smear (also stage 2 and 3) 2. Presence of JAK2-^{V617F} mutation 3. Normal hematocrit: male <51 %, female <48 % erythrocytes< 6x10^{12}/L	Predominant proliferation of enlarged pleiomorphic 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. RF 0-1
ET stage 2	**Prodromal PV**
1. Platelet count of >350 x10^{9}/l and hematocrit in normal or upper normal range: male <51 %, female <48, erythrocytes< 6x10^{12}/L 2. Presence of JAK2-^{V617F} mutation 3. Low serum EPO level and/or increased LAP score 4. Spontaneous EEC.	Increased cellularity with trilineage myeloproliferation (i.e. panmyelosis). 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. RF 0-1
ET stage 3	**ET MGM**
1. Platelet count of >350 x10^{9}/l and no signs of leuko-erythroblastosis 2. No or slight splenomegaly on ultrasound 3. No anemia with Hb and ht in the normal or lower range of normal: >12g/dl 4. Presence of JAK2-^{V617F} mutation 5. No preceding or allied of CML, PV, RARS-T or MDS .	Increased cellularity due to megakaryocytic granulocytic myeloproliferation (MGM). Normal or reduced erythropoiesis. Loose to dense clustering of enlarged pleiomorphic megakaryocytes with hyperploid nuclei and the presence of pleiomorphic megakaryocytes with with clumsy dysmorphic lobulated nuclei. No or borderline increase in reticulin RF 0-1

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

Table 5A: Clinical and molecular features of 10 JAK2^{V617F} positive MPN patients with essential thrombocythemia (ET) in six and polycythemia vera (PV) in four according to clinical criteria according to TVSG/PVSG criteria1,3,4, the 2008 WHO5 and the European Clinical Molecular and Pathological (ECMP) classifications [7,8] according to the French approach without the use of bone marrow histopathology

Case	1	2	3	4	5	6	7	8	9	10
A. Clinical data										
Age (years) and sex (F/M)	56/M	60/M	66/F	47/F	40/F	31/F	50/M	43/F	47/F	63/M
Platelets at onset x10^9/L	575	814	544	553	425	576	397	405	924	384
Duration of symptoms, years	4	11	8	6	8	<1	1	<1	<1	>10
JAK2^{V617F} *	+/-	+/-	+/-	+/-	+/-	+/-	+/-+/+	+	++	++
Serum EPO	Normal	zero	low	low	NT	NT	zero	low	zero	zero
Leukocytes x 10^9/l	6.7	5.3	12.9	8.2	6.1	6.2	7.3	14.3	13.1	8.0
LAP score(N =<100)	.	160	197	N	N	186	163	263	232	284
Hemoglobin g/dl	13.6	15.5	14.2	14.4	13.4	14.0	18.6	17.3	16.3	12.8
Hematocrit	0.40	0.45	0.44	0.44	0.40	0.41	0.63	0.52	0.53	0.60
Erythrocytes x10^{12}/L	.	5.3	.	4.8	4.6	5.9	6.3.	6.1	7.4	6.7
EEC	+	+	+	.	.	+.	+	+	+	+
Red cell mass	.N	N.	N	N	.	N.	↑.	↑	↑	↑
Spleen, echogram cm	.	13	16	13	16.5	11.8	13.7	13	14.3	16
Clinical Diagnosis	ET	ET	ET	ET	ET	ET	PV	PV	PV	PV
JAK2^{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 polycythemiavera (PV), or rapid onset PV, diagnosed according to TVSG/PVSG criteria1,3,4, the 2008 WHO5 and the European Clinical Molecular and Pathological (ECMP) classifications7,8 by including bone marrow histopathology according to ECMP criteria

BM Histology	Case 1	2	3	4	5	6	7	8	9	10
Cellularity	65%	60%	90%	75%	80%	75%	80%	75%	80%	80%
M:E ratio	1	1	1	0,5	4	0,7	0,7	1	1.5	-
Megakaryocytes	MPN	MPN	MPN	MPN	MPN	MPN	MPN	MPN	MPN	MPN
Myeloid lineage	N	N	Increase	N	Increase	N	N	N	Increase	Increase
Erythroid lineage	Increase	Increase	Increase	Increase	N	Increase	Increase	Increase	Increase	Increase
Iron	Stainable	Stainable	-	US	US	US	US	US	US	US
Fibrosis	MF-0	MF-1	MF-0	MF-0	MF-0	MF-0	MF-0	MF-0	MF-1	MF-0
C.Diagnosisclinicalvs 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	MPNU	ET/U	PV	PV	PV	PV
2008-ECMP	ET-1	ET-1	ET-2 →PV	ET-2 →PV	ET-3	ET-2 →PV	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 Fenylalanine (F). The JAK2^{V617F} mutation induces a loss of inhibitory activity of the JH2 pseudokinase part on the JH1 kinase part of JAK2, leading to enhanced activity of the normal JH1 kinase activity of JAK2 [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 PVSG defined 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 MPD 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 (maskedPV) 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 $6x10^{12}$/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 erythropoiesis 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 (ET.MGM, 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 $6x10^{12}$/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 (ET.MGM 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 (ET.MGM) 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 (ET.MGM).

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 $6x10^{9}$/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 is similar to or analogue to ET.MGM remains to be evaluated

Clinical and molecular criteria	WHO bone marrow criteria
PT	**PT**
1. Platelet count >350- 4 00 x10^9/l Presence of large platelets in blood smear Presence of enlarged megakaryocytes in bone marrow smear 2. JAK - 2 wild type or MPL^{515} mutation 3. No anemia, Hb >12.8 g/dl 4. Normal serum EPO 5. No or slight splenomegaly on ultrasound 6. No leukoerythroblastosis 7. No dry tap on bone marrow biopsy 8. No preceding or allied of CML, PV, RARS - T or MDS	Predominant proliferation of enlarged mature pleiomorphic megakaryocytes with hyperlobulated nuclei lacking conspicuous morphological a bnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or slight increase in reticulin: RF 0- 1 **PT MM** In crease of large to giant msture megakaryocytic myeloprloliferation (MM) in a normocellular bone marrow with normal erythropiesis or reduced erythoid precursor cells. The magakaryocytes are large to gian with hyperlobulated staghorn-like nuclei not seen in PDGM, CMGM or PMGM. No or slight increase in reticulin :RF 0-1

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 $JAK2^{V617F}$ 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

European clinical and pathological (ECP) criteria for the diagnosis and staging of PMF or PMGM and AMM (Michiels&Thiele 2002,2005)

J. J. Michiels	Clinical criteria 2005		Pathological criteria 2005/2008
A1	No preceding or allied other subtype of myeloproliferative disorders CML or MDS. Main presenting feature is pronounced thrombocythemia and no dry tap on bone marrow aspiration. **JAK2 and MPL wild type (2008 ECMP)**	**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.
C	Clinical stages of PMF or PMGM or PDGM	**MF**	**Staging of myelofibrosis (MF)**
C1	Early clinical stages Normal hemoglobin or slight anemia, grade I: hemoglobin >12 g/dl Slight or moderate splenomegaly on ultrasound scan or CT Thrombocytosis, platelets in excess of 400, 600 or even $1{,}000 \times 10^{9-1}$ Normal or increased LAF-score No leuko-erythroblastose	**MF-0** **MF-1**	prefibrotic stage PMF or PDGM no reticulin fibrosis: RF 0 or 1 early PMF or PDGM slight reticulin fibrosis:RF 2
C2	Intermediate clinical stage:PDGM Anemia grade II: hemoglobin >10g/dl Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes Increased LDH Splenomegaly	**MF-1** **MF-2**	PMF or PDGM : slight reticulin fibrosis:RF 2 manifest AMM: marked increase in reticulin and slight to moderate collagen fibrosis: RCF 3
C3	Advanced clinical stage: PDGM Anemia grade III: Hemoglobin <10g/dl Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes Splenomegaly, thrombocytopenia, leukocytosis, leukopenia	**MF-3**	overt PDGM: advanced collagen fibrosis with optional osteosclerosis

The combinations of A1+B1 establish PMF or PMGM : -any 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, peglyated interferon or hydroxyurea[76]

Platelets	**Platelets**	**Platelets**	**Platelets**
400 - 1500 × 10^9/L	400 - 1000 × 10^9/L	400 - 1000 × 10^9/L	> 1500 × 10^9/L
Low risk	Low risk	High risk	High risk
Completely asymptomatic	Micro vascular disturbances only[1]	Major thrombosis, and/or bleeding	>1000 × 10^9/L and minor thrombosis and/or bleeding
No vascular risk	No vascular risk	Vascular risk	No vascularrisk
No bleeding risk	No bleeding risk		
Aspirin uncertain	Low dose aspirin	Platelet reduction to normal or near	Platelet reduction to normal
Wait and see?	50 to 100 mg/d		< 1000 × 10^9/L
Very low?	Intermediate risk	Continueaspirin	Add aspirin
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

PV, ECMP stage	**0**		**1**	**2**	**3**	**4**	**5**
Michiels ECMP Clinical Diagnosis	Erythrocy-Themic PV	Prodromal PV	polycythemic PV prefibrotic	Classic PV prefibrotic	Advanced PV PMF stage	Post-PV MF AMM Neoplastic	Spent phase 'anemic' PV AML MDS
LAP-score and/or PRV-1	N/↑	↑	↑	↑	↑/↑↑	variable	variable
Red cell mass (RCM)	↑	N	↑	↑	↑	variable	N/↓
Serum EPO	N/↓	N/↓	↓	↓	↓	variable	N/↓
Leukocytes $x10^9$/l	<12	<12	<12	N->12	>15	>20	>20
Platelets $x10^9$/l	<400	>400	<400	>400	< or >1000	variable	variable
Hemoglobin g/dl (mmol/l) Hematocrit Erythrocytes $x10^{12}$/L	>16 (10) >0.51 >6	<16 (10) <0.51 <6	>16 (10) >0.51 >6	>16 (10) >0.51 >6	>16 (10) >0.51 >6	N / >12 variable variable	<12 N↓ N/↓
ECMP bone marrow Bone marrow cellularity (%) Grading myelofibrosis[57]	Early PV 50-80 RF 0-1	Early PV 50-80 RF 0-1	Early PV 60-100 RF 0-1	Trilinear PV 80-100 RF 0/1-2	Trilinear PV 80-100 RCF 1/2	Trilinear / MF Decreased RCF 2/3	MF Decreased RCF 3
Splenomegaly on palpation Spleen size, echogram cm	no <12	No/+ <12-15	No/+ 12-15	+ 12-18	++/+++ 18->20	/large >20	large >20
Spontaneous EEC+	+	+	+	+	+	+	+
$JAK2^{V617F}$ in Granulocytes and BFU-e (exon 12)	+ +(++)	+ +(++)	+ +(++)	+/++ ++	+/++ ++	++ ++	++ ++
Therapeutic implications Anno 2012/2013[41-43]	Low risk	Low risk	Low risk	Intermediate risk PV	High risk PV	Post-PV MF	Spent PV
First line treatment option MPN reductive treatment JAK2 inhibitor	Aspirin Phlebotomy	Aspirin Phlebotomy Low dose IFN?	Phlebotomy Aspirin Low dose IFN → Complete response	Phlebotomy* Aspirin IFN→ If resistant → HU	If IFN resistant → HU or HU first line	JAK2 Inhibitor → Palliative Aspirin?	Supportive

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

of erythrocytes remain above 6.0 $x10^{12}$/L in PV and are below 6.0$x10^{12}$/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 $JAK2^{V617F}$ mutated thrombocythemias into ET stage 1, ET stage 2 (prodromal PV), and ET stage 3 at time of presentation. An erythrocyte count at a cut-off of 6.0$x10^{12}$/l in $JAK2^{V617F}$ 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 erythromelalgic and MIAs because of persisting thrombocythemia [4, 5, 16, 18]. Low dose PegasysR did induce a complete haematological and even molocular 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 $JAK2^{V617F}$ mutated ET and PV [16, 18, 31] will prevent the erythromelagic and MIAs and prevent major thrombotic complications during long-term follow-up when adequately monitored and managed for vascular risk factors.

$JAK2^{V617F}$ positive ET and PV reflects a broad spectrum of trilinear MPN

The 2006 concept according to Vainchenker, Green & Michiels is that heterozygous $JAK2^{V617F}$ 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 normocellular ET (cases 1 and 2, table 5), prodromal PV (cases 3 and 4, table 5) and has been recognized in 1940 by Dameshek as main presenting features of PV. A group of $JAK2^{V617F}$ positive normocellular ET (WHO-ET) with a very low percentage of heterozygous mutant $JAK2^{V617F}$ can maintain as a non-progressive sub population in the bonemarrow without a tendency to evolve into prodromal PV or masked PV (ET.MGM) during long-term follow-up. Hetero/homozygous or homozygous $JAK2^{V617F}$ 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 $JAK2^{V617F}$ mutation can readily explain the evolution of prodromal 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 $JAK2^{V617F}$ mutation had occurred in hematopoietic progenitor cells of the bone marrow. There is a broad spectrum of transitional states in between $JAK2^{V617F}$ mutated ET, prodromal 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) [97]. 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 $JAK2^{V617F}$ 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 $JAK2^{V617F}$ positive ET, prodromal 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 splanchnic vein thrombosis (SVT, Budd-Chiari syndrome or portal vein thrombosis) in 241 patients, platelet counts were between 238 and 456$x10^9$/L (mean 333) in 74 patients carrying the $JAK2^{V617F}$ mutation and between 104 and 258$x10^9$/L (mean 159) in 147 JAK2 wild type SVT patients and none of them carried the MPL^{515} mutation [104]. In patients with a first episode of splanchnic vein thrombosis (SVT), analysis of any venous thrombophilic risk factors as well as a $JAK2^{V617F}$ 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 $JAK2^{V617F}$ 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 $JAK2^{V617F}$ mutation [47, 50,108]. The finding of the JAK2 exon 12 mutations in patients with $JAK2^{V617F}$ 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 $JAK2^{V617F}$ 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 = MPL^{515} mutation

The JAK2 kinase activity in clonal MPD is dependent not only on the amount of heterozygous and homozygous mutant $JAK2^{V617F}$ 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 thrombopoitein 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 MPL^{W515L} and MPL^{W515K} 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 JAK2^{V617F} mutated trilinear MPN, patients with the MPL^{515} mutation have no clinical, laboratory and bone marrow features of prodromal 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 (EEC), and may show pronounced megakaryocytic proliferation of small and large (giant) megakarocytes and increased granulopoisis 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 MPL^{515} mutation kindly provided by the courtesy of Dr. Vannucchi, Florence, Italy [117]. Bone marrow histology from patients with JAK2 wild type ET carrying the MPL^{515} 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 JAK2^{V617F} mutated ET; there were significant differences on three points. The megakaryocytes in MPL^{515} mutated PT are larger than in PV, whereas the megakaryocytes in JAK2^{V617F} 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 pleiomorphic megakaryoctyes in JAK2^{V617F} mutated Et, but not in JAK2 wild type PT carrying the MPL^{515} 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 JAK2^{V617F} mutated normocellular ET, and hyper cellular prodromal PV and ET.MGM. Whether such differences in bone marrow histology features of megakaryocyte morphology in bone marrow between normocellular ET with low JAK2 mutation load and JAK2 wild type ET carrying the MPL^{515} 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 MPL^{515} mutation and also not in prefibrotic JAK2^{V617F} 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 prefibroticprimary megakaryocytic granulocytic myeloproliferation (PMGM) according to Georgiiet al 199028,32, and do not meet the criteria for AMM (fibrotic PMF) according to the 2008 WHO classification (tables7 and 8)[32]. A typical case of PDGM/PMGM with normal blood cells counts, absence of the JAK2^{V617F} 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 JAK2^{V617F} mutation, we could separate the 1999 EMGM entity (table 2) into cases of JAK2^{V617F} 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 TIAs 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 TIAs 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 Will brand disease type 2A by the combined use of von Will brand 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, peglyated 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

or 100 mg 4 times a week) (Table 6) [65].

Clinical Staging of JAK2^{V617F}mutated MPN according to ECMP criteria related to therapy, anno 2012

As compared to P32 and pipobroman, 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 peglyated interferon alpha-2a [120, 121]. Clinical studies indicate that both hydroxyurea and JAK2 inhibitors are not able to eliminate the JAK2^{V617F} clone in the bone marrow. Peglyated 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 peglyated 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 PVSG defined ET (N=46), PV (N=55) and PMF (N=17) were comparable with regard to age and peripheral blood features, the JAK2^{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[124]. 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 INF (figure 1) [120]. High loading dose IFN seems to us useless and to overcome the initial hurdle of side effects starting with low dose peglyated IFN 45 ug/week in prefibrotic JAJ2V617F 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 JAK2V617 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^{V617F} mutated prodromal PV and early stage PV as well as JAK2^{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 celluar 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 PVSG defined MPDs. The 2008 WHO investigators reclassified the PVSG defined MPDs ET, PV and PAMM collected between 1975 and 2008 in retrospective studies of PVSG defined cohorts of ET, PV and MF patients. There is a compelling need to diagnose, classify and stage MPN patients (ET, PV, PDGM/PMGM 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 PMF or PAMM in routine daily practice, but do not recognize the various stages of each of the 3 primary prefbotic primary myeloproliferative disorders (PMD): 1. JAK2^{V617F} mutated trilinear MPD/MPN including normocellular ET, hyper cellular ET.MGM (masked PV), prodromal PV, slow onset and acute onset PV; 2. JAK2 wild type PT carrying the MPL^{515} 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^{V617F} mutation in 2005 as the cause of trilinear MPD/ MPN [81,81], we have split the 1999 EMGM conceptual entity in JAK2^{V617F} mutated ET.MGM (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^{515} mutation (table 6). 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 PVSG-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 (PMD) negative for the JAK2^{V617F} mutation either MPL^{515} positive or JAK2/MPL wild type PMGM should be studied separately in prospective studies. With the advent of the JAK2^{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 PVSG 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 disregarding 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 JAK2^{V617F} 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 JAK2^{V617F} mutated ET and JAK2^{V617F} mutated heterozygous/homozygous PV as two sequential molecular stages of JAK2^{V617F} 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 unclassified. 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^{9}/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 JAK2^{V617F} 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: ET.MGM (masked PV) is rather frequent but this entity is neither defined nor included in the 2007/2008 WHO classifications. **Third**, the diagnostic differentiation between JAK2^{V617F} mutated ET.MGM (masked PV) versus patients with myeloid metaplasia of the spleen (either JAK2^{V617F} mutated post-ET, post-PV versusJAK2 wild type PMF) and peripheral blood leukoerythroblastosis is clinically relevant, but not defined by the 2007/2008 WHO classification[125, 126]. JAK2^{V617F} positive ET.MGM (masjked 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 ECMP[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 JAK2^{V617F} mutated ET. MGM (masked PV) and JAK2 wild type PDGM/PMGM 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 JAK2^{V617F} 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 thrombocythemic and erythrocythemicearly 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).

Acknowledgements

Dr. Juergen Thiele was a most respected active member of the EWG.MPD (1998-2005), significantly contributed to the formulation of the ECP, WHO and ECMP criteria for the diagnosis of the MPDs ET, PV, EMGM, PDGM, and PMF, and clearly defined the clinicopathological prefibrotic and fibrotic stages of all variants of MPD.This prospective MPD evaluation study was conducted between January 2000 and August 2008 and updated in 2012 by Jan Jacques Michiels & Hendrik De Raeve at the Departments of Hematology and Pathology, University Hospital Antwerp, University of Antwerp: Chief Hematology, Prof Dr ZwiBerneman and Prof Dr Wilfried Schroyens, and Chief Pathology, Prof Dr Eric Van Mark, Pathology.

References

1. Dameshek W. Physiopathology and corse of polycythemia vera as related to therapy. JAMA. 1950;142(11):790-797. Doi:10.1001/jama.1950.02910290018005
2. Dameshek W. Some speculations on the myeloproliferative syndromes. Blood. 1951;6(4):372-375.
3. Dameshek W and Baldini M. The Di Guglielmo syndrome. Blood. 1958;13:192-195.
4. Dameshek W. The Di Guglielmo syndrome revisted. Blood. 1969;34(5):567-572.
5. Michiels JJ. Erythroleukemia and myelodysplastic syndromes: an historical appraisal and a personal view. Leukemia & Lymphoma. 1993;9(1-2):27-34.
6. Michiels JJ, Van Der Meulen J and Brederoo P. The natural history of trilinear myelodysplastic syndrome and erythroleukemia. Haematologica. 1997;82(4):452-454.
7. Glaser RM and Walker RI. Transitions among the myeloproliferative disorders. Ann Int Med. 1969;71(2):285-307.
8. Ward HP and Block MH. The natural history of agnogenic myeloid metaplasia (AMM) and a critical evaluation of its relationship with myeloproliferative syndrome. Medicine. 1971;50(5):357-420. Doi: 10.1097/00005792-197109000-00001
9. Gilbert HS. The spectrum of myeloproliferative disorders. Med Clin North Amer. 1973;57(2):355-393.

10. Laszlo J. Myeloproliferative disorders (MPD): myelofibrosis, myelosclerosis, extramedullary hematopoiesis, undifferentiated MPD and hemorrhagic thrombocythemia. Semin Hematol. 1975;12(4):409-432.
11. Silverstein MN. Myeloproliferative diseases. Hematology review. Postgraduate medicine. 1977;61:206-210.
12. Heuck G. Two cases of leukemia with peculiar blood or bone marrow findings. Virch Arch. 1879;78:475.
13. Michiels JJ. The myeloproliferative disorders. An historical appraisal and personal experiences. Leukemia and Lymphoma. 1996;22(Suppl 1):1-14.
14. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic leukocytes. J Natl Cancer Inst. 1960;25(1):85-109.
15. Rowley J. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence Giemsa staining. Nature. 1973;243:290-293.
16. Rowley JD. Clin Haematol. 1980;9:55-86.
17. Heisterkamp N, Groffen J, Stephenson JR, Spurr NK, Goodfellow PN, Solomon E, Carrit B, Bodmer WF, et al. Chromosomal localization of human cellular homologues of two viral oncogenes. Nature. 1982;299:747-749.
18. De Klein A, Van Kessel AG, Grosveld G, Claus R Bartram, Anne Hagemeijer, Dirk Bootsma, Nigel K. Spurr, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukemia. Nature. 1982;300:765-767.
19. Bartram CR, de Klein A, Hagemeijer A. Translocation of the human c-abl oncogene occurs in variant Ph-positive but not Ph-negative chronic myeloid leukemia. Nature. 1983;306:277-280.
20. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal break points are clustered within a limited region, bcr on chromosome 22. Cell. 1984;36(1):93-99.
21. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr/abl oncogene products. Science. 1990;247(4946):1079-1082.
22. Kelliber MA, McLaughin J, Witte ON, Rosenberg N. Induction of a chronic myelogenous-like syndrome in mice with v-abl and BCR/ABL. Proc Nat Sci USA. 1990;87(17):6649-6653.
23. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science. 1990;247(4944):824-830.
24. Shephard PC, Ganesan TS, Galton DAG. Haematological classification of the chronic myeloid leukemias. Bailliere's Clin Haematol. 1987;1(4):887-906.
25. Michiels JJ, Prins ME, Hagemeijer A, Brederoo P, van der Meulen J, van Vliet HH, Abels J, et al. Philadelphia chromosome-positive thrombocythemia and megakaryoblast leukemia. Am J Clin Pathol. 1987;88(5):645-652.
26. Michiels JJ, ten Kate FJW. Erythromelalgia in thrombocythemia of various myeloproliferative disorders. Am J Hematol. 1992;39(2):131-136.
27. Michiels JJ, Berneman ZW, Schroyens W, Kutti J, Swolin B, Ridell B, Fernando P, Zanetto U, et al. Philadelphia (Ph) chromosome positive thrombocythemia without features of chronic myeloid leukemia in peripheral blood: natural history and diagnostic differentiation from Ph-negative essential thrombocythemia. Ann Hematol. 2004;83(8):504-512.
28. Georgii A, Vykoupil KF, Buhr T, Choritz H, Döhler U, Kaloutsi V, Werner M, et al. Chronic myeloproliferative disorders in bone marrow biopsies. Path Res Pract. 1990;186(1):3-27.
29. Georgii A, BuhrT, Buesche G, Kreft A, Choritz H. Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. Leukemia Lymphoma. 1996;22(Suppl 1):15-29.
30. Thiele J, Kvasnicka HM, Werden C, Zankovich R, Diehl V, Fischer R. Idiopathic primary osteo-myelofibrosis: a clinico-pathological study on 208 patients with special emphasis on evolution of disease features, differentiation from essential thrombocythemia and variables of prognostic impact. Leukemia Lymphoma. 1996;22(3-4):303-317.
31. Thiele J, Kvasnicka HM, Diehl V, Fischer R, Michiels JJ. Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disorders by histopathology, histochemistry and immunostaining from bone marrow biopsies. Leukemia Lymphoma. 1999;33(3-4):207-218.
32. Michiels JJ, Ten Kate FWJ, De Raeve H, Gadisseur A. Classification and natural history of Philadelphia chromosome and BCR/ABL-positive thrombocythemia and chronic myeloid leukemia as compared to BCR/ABL-negative thrombocythemia in various myeloproliferative disorders. World J Hematol. 2013.
33. Wasserman LR. Polycthemiavera, its course and treatment: relation to myeloid metaplasia and leukemia. Bull NY Acad Med. 1954;30(5):343-375.
34. Wasserman LR Berk PD and Berlin NI. Polycythemiavera and the myeloproliferative disorders. WB Saunders Philadelphia 1995.
35. Pearson TC and Wetherley-Mein G. The course and complications of idiopathic erythrocytoses. Clin Lab Haematol. 1979;1(3):189-196.
36. Erf LA. Primary polycythemia: remissions induced by therapy with radiophosphorus. Blood. 1946;1:202-208.
37. Dameshek W and Henstell HH. The diagnosis of polycythemia. Ann Intern Med. 1940;13(8):1360-1387.
38. Dameshek W. The treatment of Polycythemia. Blood. 1946;1:256.
39. Michiels JJ. Physiopathology, etiologic factors, diagnosis and course of polycythemiavera as related to therapy according to Dameshek 1940-1950. Turkish J Hematol. 2013;30(2):102–110. Doi: 10.4274/Tjh.2013.0029
40. Lawrence JH. The control of polycythemia by marrow inhibition. J Amer Med Ass. 1949;141(1):13-18.
41. Stroebel CF, Hall BE, Pease GL. The use of radiophosphor therapy in primary polycythemia. J Amer Med Ass. 1951;146:1301-1307.
42. Wassermann LR, Bassen F. Polycythemia. J Mount Sinai Hosp. 1959;1-49.
43. Wasserman LR. The management of polycythemiavera. Br J Haematol. 1971;21:371-376.
44. Ellis JT, Silver RT, Coleman M, Geller SA. The bone marrow in polycythemia vera. Semin Hematol. 12:433-444.
45. Berlin NI. Diagnosis and classification of the polycythemias.Sem Hematol. 1975;12(4):339-351.

46. 2001 WHO classification of the chronic myeloproliferative diseases (CMPD) polycythemiavera, chronic idiopathic myelofibrosis, essential thrombocythemia and CMPD unclassifiable. In: Jaffe S, Harris NL, Stein H et al, editors. WHO Classification of Tumours. Tumours of Haematopoiesis and Lymphoid Tissues. Lyon. IARC. 2001;31-42.
47. Michiels JJ, De Raeve H, Berneman Z, Van Bockstaele D, Hebeda K, Lam K, Schroyens W, et al. The 2001 World Health Organization (WHO) and updated European clinical and pathological (ECP) criteria for the diagnosis, classification and staging of the Ph1-chromosome negative chronic myeloproliferative disorders (MPD). Sem ThrombHemostas. 2006;32(4 Pt 2):307-340.
48. Tefferi A, Thiele J Orazi A, Kvasnicka HM, Barbui T, Hanson CA, Barosi G, Verstovsek S, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood. 2007;110(4):1092-1097.
49. Tefferi A and Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point of care diagnostic algorithms. Leukemia. 2008;22(1):14-22.
50. Michiels JJ and Ten Kate FWJ. Erythromelalgia and thrombocythemia vera: a causal relation of platelet-mediated arteriolar inflammation and thrombosis in patients with essential thrombocythemia (ET) and polycythemiavera (PV), and the Rotterdam Clinical and Pathological (RCP) criteria for ET and PV. Turkish J Hematol. 2013.
51. Berk PD, Goldberg JD, Silverstein MN , Weinfeld A, Donovan PB, Ellis JT, Landaw SA, et al. Increasedincidence of acute leukemia in polycythemia vera associated with chlorambucil therapy. N Eng J Med. 1981;304(8):441-447.
52. Berk PD, Goldberg JD, Donovan PD, Fruchtman SM, Berlin NI, Wasserman LR. Therapeutic recommendations in polycythemiavera based on the PVSG protocols. Sem Hematol. 1986;23(2):132-143.
53. Haanen C and Mathe G. European Organization for research on Treatment of Cancer. Brit J Cancer. 1981;44:75-78.
54. Messinezy M, Pearson TC, Prochaaka A, Wetherley-Mein G. Treatment of primary proliferative polycythemia by venesection and low dose busulphan: retrospective study from one centre. Brit J Haematol. 1985;61(4):657-666.
55. Van de Pette, Prochazka A, Pearson TC, Songer AK, Dickson ER, Wetherley-Mein G. Primary thrombocythaemiatreate with busulphan. Brit J Haematol. 1986;62:229-237.
56. Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, Harrison C, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. J Clin Oncol. 2011;29(6):761-770. Doi: 10.1200/JCO.2010.31.8436
57. Thomas DJ, du Boulay GH, Marshall J, Pearson TC, Ross Russell RW, Symon L, Wetherley-Mein G, Zilka E, et al. Effect of haematocrit on cerebral blood-flow in man. Lancet. 1977;2(8045):941-943.
58. Pearson TC and Wetherley-Mein G. Vascular occlusive episodes and venous haematocrit in primary proliferative polycythemia. Lancet. 1978;2(8102):1219-1222.
59. Michiels JJ, Abels J, Steketee J, vanVliet HHDM, Vuzevski VD. Erythromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia. Ann Intern Med. 1985;102(4):466-471.
60. Michiels JJ. Erythromelalgia and vascular complications in polycythemia vera. Sem ThrombHemostas. 1997;23(5):441-454.
61. Koudstaal PJ and Koudstaal A. Neurologic and visual symptoms in essential thrombocythemia: efficacy of low-dose aspirin. Sem ThrombHemostas. 1997;23(4):365-370.
62. Griesshammer M, Bangeter M, Van Vliet HHDM, Michiels JJ. Aspirin in essential thrombocythemia: status quo and quo vadis. Sem ThrombHemostas. 1997;23(4):371-377.
63. Van Genderen PJJ, Mulder PGH, Waleboer M, van de Moesdijk D, Michiels JJ. Prevention and treatment of thrombotic complications in essential thrombocythemia: efficacy and safety of aspirin. Brit J Haematol. 1997;97(1):179-184.
64. Landolfi R, Marchioli R, Kutti J, Gisslinger H, Tognoni G, Patrono C, BarbuiT, et al. Efficacy and safety of low-dose aspirin in polycythemiavera: results of the ECLAP trial. New Eng J Med. 2004;350:114-124. Doi: 10.1056/NEJMoa035572
65. Michiels JJ, Ten Kate FWJ, Koudstaal PJ, Van Genderen PJJ. Aspirin responsive platelet thrombophilia in essential thrombocythemia and polycythemia vera. World J Hematol 2013;2(2):20-43.
66. Iland H, Laszlo J, Peterson P, Murphy S, Briere J, Weinfeld A, Rosenthal DS, et al. Essential thrombocythemia: clinical and laboratory characteristics at presentation. Trans Assoc Am Phys. 1983;96:165-174.
67. Murphy S, Iland H, Rosenthal D, Lazslo J. Essential thrombocythemia: an interim report from the Polycythemia Vera Study Group. Semin Hematol. 1986;23(3):177-182.
68. Michiels JJ, Juvonen E. Proposal for the revised diagnostic criteria of essential thrombocythemia and polycythemia by the Thrombocythemia Vera Study Group. Sem ThrombHemostas. 1997;23(4):339-347.
69. Michiels JJ, Barbui T, Finazzi G, Fruchtman SM, Kutti J, Rain J-D, Silver RT, et al. Diagnosis and treatment of polycythemia vera and possible future study designs of the PVSG. Leukemia and Lymphoma. 2000;36(3-4):239-253.
70. Michiels JJ, Kutti J, Bazzan M, Gugliotta L, Marcioli R, Griesshammer M, Van Genderen PJJ, et al. Neth J Med. 1999;54:46062.
71. Georgii A, Vykoupil KF, Buhr H, Choritz H, Doehler U, Kaloutsi V, Werner M, et al. Chronic myeloproliferative disorders in bone marrow biopsies. Path Res Pracxt. 1990;186(1):3-27.
72. Georgii A, Buhr H, Buesche G, Kreft A, Chorotz H. Classification and staging of Ph-negative chronic myeloproliferative diseases. Leuk Lymphoma. 1996;22(suppl 1):15-29.
73. Georgii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. Bailliere's Clin Haematol. 1998;11(4):721-749.
74. Thiele J, Kvasnicka HM, Fischer R, Michiels J. Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disorders by histopathology, histochemistry and immunostaining from the bone marrow. Leukemia Lymphoma. 1999;33(3-4):207-218.

75. Michiels JJ and Thiele J. Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera and idiopathic myelofibrosis (agnogenic myeloid metaplasia). Int J Hematol. 2002;76(2):133-145.
76. Michiels JJ and Kvasnicka HM Thiele J. Myeloproliferative Disorders. Current perspectives on diagnostic criteria, histopathology and treatment in ET, PV and CIMF.
77. Swerdlow S, Campo E, Harris N, Jaffe ES, Pileri SA, Stein H, Thiele J, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press. 2008.
78. Michiels JJ, Berneman Z, Schroyens, Hebeda K, Lam K, De Raeve .H, Schwarz J, et al. WHO bone marrow features and European clinical molecular and pathological criteria for the diagnosis of myeloproliferative disorders. Leukemia Research. 2007;31(8):1031-1038.
79. Michiels JJ, De Raeve H, Hebeda K, Lam K, Bot F, Berneman Z, Schroyens W, et al. Biology, Diagnosis and Classification of MPD. First International Lymphoma, Leukemia Myeloma (LLM) Congress. Turk J Hematol. 2007;24,(Suppl 1):37-53.
80. Thiele J, Kvasnicka HM, Diehl V. Initial (latent) polycythemia vera with thrombocytosis mimicking essential thrombocythemia. Acta Haematol. 2005;113(4):213-219.
81. James C, Ugo V, Le Couedic PF, Staerk J, Delhommeau F, Lacout C, Garçon L, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythemia vera. Nature. 2005;434(7037):1144-1148.
82. Vainchenker W and Constantinescu SN. A unique activating mutation in JAK2 V617F is at the origin of polycythemiavera and allows a new classification of myeloproliferative diseases. Hematology (Am Soc Hematol EducProgr). 2005;195-200.
83. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, et al. Acquired mutation of the tyrosine kinase in human myeloproliferative disorders. Lancet. 2005;365(9464):1054-1061.
84. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Eng J Med. 2005;352(17):1779-1790.
85. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, Boggon TJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis. Cancer cells. 2005;7(4):387-397.
86. Michiels JJ, Berneman Z, Van Bockstaele D, Van Der Planken M, De Raeve H, Schroyens W. Clinical and laboratory features, pathobiology of platelet-mediated thrombosis and bleeding complications and the molecular etiology of essential thrombocythemia and polycythemiavera: therapeutic implications. Sem ThrombHemostas. 2006;32(3):174-207.
87. Wolanskyj AP, Lasho TL, Schwager SM, McClure RF, Wadleigh M, Lee SJ, Gilliland DG, Tefferi A, et al. JAK2 V617 mutation in essential thrombocythaemia: clinical associations and long-term relevance. Br J Haematol. 2005;131(2):208-213.
88. Antonioli E, Guglielmelli P, Pancrazzi A, Bogani C, Verrucci M, Ponziani V, Longo G, Bosi A, Vannucchi AM, et al. Clinical implications of the JAK2 V617F mutation in essential thrombocythemia. Leukemia. 2005;19(10):1847-1849.
89. Tefferi A, Lasho TL, Schwager SM, Strand JS, Elliott M, Mesa R, Li CY, et al. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia. Cancer. 2006;106(3):631-635.
90. Passamonti F, Rumi E, Pietra D , Della Porta MG, Boveri E, Pascutto C, Vanelli L, et al. Relation between JAK2 V617F mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood. 2006;107(9):3676-3682.
91. Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F JAK2 mutation occurs in most patients with polycythemiavera, but not essential thrombocythemia. Blood. 2006;108(7):2435-2437.
92. Vanucchi AM, Antonioli E, Guglielmelli P, Rambaldi A, Barosi G, Marchioli R, Marfisi RM, et al. Clinical profile of homozygous JAK2V617F mutation in patients with polycythemiavera or essential thrombocythemia. Blood. 2007;110(3):840-846.
93. James C, Delhommeau F, Marzac C, Teyssandier I, Couédic JP, Giraudier S, Roy L, et al. Detection of JAK2 V617F as a first intention diagnostic test for erythrocytosis. Leukemia. 2006:20(2):350-353.
94. Tefferi A and Pardanani A. Mutation screening for JAK2V617F: when to order the test and how to interpret the results. Leukemia Res. 2006;108:3472-3476.
95. Girodon F, Lippert E, Mossuz P, Dobo I, Boiret-Dupre N, Levesce JF, Hermuet S, Praloran V, et al. JAK2V617F detection and dosage of serum erythropoietin: first steps of the diagnostic work-up for patients consulting for elevated hematocrit. Haematologica. 2007;92(3):431-432.
96. Moliterno AR, Williams DM, Isaacs MA, Spivak JL. Phenotypicvariabilitywithinthe JAK2V617F-positive MPD: roles of progenitor cell and neutrophil allele burden. Exp Hematol. 2008;36(11):1480-1486. Doi: 10.1016/j.exphem.2008.05.006
97. Michiels JJ, Ten Kate FWJ, Lam KH, schroyens W, Berneman Z, De RaeveH. The European Clinical, Molecular, and Pathological (ECMP) criteria and the 2007/2008 revisions of the WHO forthe diagnosis, classification, and staging of the prefibrotic myeloproliferative neoplasms carrying the JAK2V617F mutation. Turk J Hematol. 2014;31(3):239-254.
98. Michiels JJ. Normal life expectancy and thrombosis-free survival in aspirin treated essential thrombocythemia. ClinAppliedThromb/Hemostas. 1999;5(1):30-36.
99. Kvasnicka HM and Thiele J. The impact of clinicopathological studies on staging and survival in essential thrombocythemia, chronic idiopathic myelofibrosis and polycythemia rubra vera. Sem Thromb Hemostas. 2006;32(4 Pt 2):362-371.
100. Thiele J, Kvasnicka HM, muellauer L, Buxhofer-Ausch V, Gisslinger B, Gisslinger H. Essential thrombocythemia versus early primary myelofibrosis: a multicenter study to validate the WHO classification. Blood. 2011;117(21):5710-5718. Doi: 10.1182/blood-2010-07-293761
101. Barbui T, Thiele J, Passamonti F, Rumi E, Boveri E, Ruggeri M, Rodeghiero F, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphology diagnosis: an international study. J ClinOncol. 2011;29(23):3179-3184. Doi: 10.1200/JCO.2010.34.5298

102. Passamonti F, Rumi E, Pietra D, Elena C, Boveri E, Arcaini L, Roncoroni E, et al. A prospective study of 338 patients with polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complication. Leukemia. 2010;24(9):1574-1579. Doi: 10.1038/leu.2010.148
103. Barbui T, Thiele J, Passamonti F, Rumi E, Boveri E, Randi ML, Bertozzi I, et al. Intial bonemarrow reticulin fibrosis in polycythemia vera exerts an impact on clinical outcome. Blood. 2012;119(10):2239-2241. Doi: 10.1182/blood-2011-11-393819
104. Kiladjian JJ, Cervantes F, Leebeek FWG, Marzac C, Cassinat B, Chevret S, Cazals-Hatem D, et al. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnicveinthrombosis: areport of 241 cases. Blood. 2008;111(10):4922-4929. Doi: 10.1182/blood-2007-11-125328
105. Smalberg JH, Arends LR, Valla DC, Kiladjian JJ, Janssen HLA, Leebeek FWG. Myeloproliferative neoplasms in Budd-Chiari syndrome and protalveinthrombosis: a meta-analysis. Blood. 2012;120(25):4921-4928. Doi: 10.1182/blood-2011-09-376517
106. Michiels JJ, Commandeur S, Hoogenboom G, Wegman JJ, Scholten L, van Rijssel RM, De Raeve H, et al. JAK2V617 postive early stage myeloproliferative disease (essential thrombocythemia) as the cause of portal vein thrombosis in two middled aged women: therapeutic implications in view of the literature. Ann Hematol. 2007;86(11):793-800.
107. Hoekstra J, BresserEL, Smalberg JH, Spaander MCW, Leebeek FWG. Long-term follow-up of patients with portal vein thrombosis and myeloproliferative neoplasms. J ThrombHaemostas. 2011;9(11):2208-2214. Doi: 10.1111/j.1538-7836.2011.04484.x
108. Schmitt-Graeff AH, Teo SS, Schaub F, Haxelmans S, Kirn A, Reinecke P, Germing U, et al. JAK2V617F mutation status indentifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombosis. Haematologica. 2008;93(1):34-40. Doi: 10.3324/haematol.11581
109. Scott LM, Wei Tong, Ross L Levine, Mike A Scott, Philip A Beer, Michael R Stratton, P Andrew Futreal, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Eng J Med. 2007;356:459-460. Doi: 10.1056/NEJMoa065202
110. Pardani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia. 2007;21(9):1960-1963.
111. Ding J, Komatsu H, Wakita A , Kato-Uranishi M, Ito M, Satoh A, Tsuboi K, et al. Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. Blood. 2004;103(11):4198-4200.
112. Pardanani A, Levine RL, Lasho TL, Pikman Y, Mesa RA, Wadleigh M, Steensma DP, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006;108(10):3472-3476.
113. Pikman Y, Lee BH, Mercher Th, McDowell E, Ebert BL, Gozo M, Cuker A, et al. MPLW515L is a novel somatic activation mutation in myelofibrosis with myeloid metaplasia. PLOS Med. 2006;3(7):e270.
114. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, Ruggeri M, et al. Characteristics and clinical correlates of MPL515W>L/K mutation in essential thrombocythemia. Blood. 2008;112(3):844-847. Doi: 10.1182/blood-2008-01-135897
115. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, Wilkins BS, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood. 2008;112(1):141-149. Doi: 10.1182/blood-2008-01-131664
116. Jones AV, Campbell PJ, Beer PA, Schnittger S, Vannucchi AM, Zoi K, Percy MJ, et al. The JAK2 46/1 haplotype predisposes to MPL-mutated myeloproliferative neoplasms. Blood. 2010;115(22):4517-4523. Doi: 10.1182/blood-2009-08-236448
117. Michiels JJ, Berneman Z, Gadisseur A, Schroyens W Ten Kate FWJ, Lam KH, De RaeveH. European Clinical, Molecular and Pathological (ECMP) criteria for the diagnosis of three distinct myeloproliferative neoplasms (MPN: JAK2V617 trilinear MPN, MPL515essential thrombocythemia, and JAK2/MPL wild type primary dysmegakaryocytic myeloproliferation (PDGM). World J Hematol. 2013;2:71-88.
118. Blorkholm M, Derolf AR, Hultcrantz M, Kristinsson SY, Ekstrand C, Goldin LR, Andreasson B, et al. Treatment-related risk factors for transformation to acute myeloid leukemia and myelodysplastic syndromes in myeloproliferative neoplasms. J Clin Oncol. 2011;29(17):2410-2415. Doi: 10.1200/JCO.2011.34.7542
119. Silver RT. Long-term effects of the treatment of polycythemia vera with recombinant interpheron alpha. Cancer. 2006;107(3):451-458.
120. Kiladjian JJ, Cassinat B, Turlure P. High molecular response rate of polycythemia vera treated with peglyated interpheron-alpha-2a. Blood. 2008;108:1281.
121. Larssen TS, Bjerrum OV, Pallisgaard N, Andersen MT, Møller MB, Hasselbalch HC. Sustained major molecular response on interferon-alpha-2b in two patients with polycythemia vera. Ann Hematol. 2008;87(10):847-850. Doi: 10.1007/s00277-008-0498-4
122. Kiladjian JJ, Mesa R, Hoffmann R. The renaissance of interferon for the treatment of myeloid malignancies. Blood. 2011;117(18):4706-4715. Doi: 10.1182/blood-2010-08-258772
123. Hasselbalch H. A new era for IFN-alpha in the treatment of Philadelphia-negative chronic myeloproliferative neoplasms. Expert Rev Hematol. 2011;4(6):637-655. Doi: 10.1586/EHM.11.63
124. Gowin K, Thapaliya P, Samuelson J, Harrison C, Radia D, Andreasson B, Mascarenhas J, et al. Experience with peglyated interferon alpha-2a in advanced myeloproliferative neoplasms in an international cohort of 118 patients. Haematologica. 2012;97(10):1570-1573. Doi: 10.3324/haematol.2011.061390
125. Buhr T, Hebeda K, Kaloutsi V, Porwit A, Van der Walt J, Kreipe H. European Bone Marrow Working group trial to discriminate essential thrombocythemia from prefibrotic primary myelofibrosis. Haematologica. 2012;97(3):360-365. Doi: 10.3324/haematol.2011.047811
126. Wilkins B, Erber WN, Bareford D, Buck G, Wheatley K, East CL, Paul B, et al. Bone marrow pathology in essential thrombocythemia: interobserver reliability and utility for identifying disease subtypes. Blood. 2008;111(1):60-70.