ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 10, pp.6539-6556, October, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

HANNOVER BONE MARROW CLASSIFICATION OF CHRONIC MYELOPROLIFERATIVE DISORDERS AND THE 2008 EUROPEAN CLINICAL, MOLECULAR AND PATHOLOBIOGICAL (2008 ECMP) CRITERIA FOR CLASSIFICATION AND STAGING OF MYELOPROLIFERATIVE NEOPLASMS: PROGNOSTIC FACTORS AND THERAPEUTIC IMPLICATIONS 1950-2015

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ARTICLE INFO

Article History:

Received 06th July, 2015
Received in revised form 14th August, 2015
Accepted 23rd September, 2015
Published online 28st October, 2015

Key words:

Myeloproliferative neoplasm, essential thrombocythemia, polycythemia vera, myelofibrosis, JAK2^{V617F} mutation, MPL^{515} mutation, bone marrow pathology, World Health Organisation

ABSTRACT

Bone marrow histology is a pathognomonic clue for hematopathologists to accurately distinguish the *BCR/ABL* negative chronic Myeloproliferative Disorders(CMPD) Essential thrombocythemia (ET) and polycythemia vera (PV) from *BCR/ABL* positive chronic myeoid leukemia (CML) and ET, and from thrombocythemia associated with myelodyspastic syndromes in RARS-T and 5q-minus syndrome. The 2008 European Clinical Molecular and Pathological (2008 ECMP) classifications distinguish three distinct clonal myeloproliferative neoplasms (MPN) of JAK2^{V617F} mutated ET, JAK2 wild type MPL mutated ET and JAK2/MPL wild type ET. The 2008 ECMP criteria could delineate three prefibrotic stages JAK2^{V617F} mutated ET as normocellular ET, ET with features of early PV (prodromal PV), and ET with hypercellular megakaryocytic granulocytic myeloproliferation (EMGM) and 6 clinical stages of PV, which have important prognnostic and therapeutic implications. Spontaneous EEC and low serum erythropoietin (EPO) levels are highly specific for JAK2^{V617F} mutated ET, prodromal PV, masked PV and classical PV. The quantitation of JAK2^{V617F} mutation allele burden plays a key-role in the diagnostic work-up and staging of ET, PV and MF patients. The JAK2^{V617F} mutation allele burden in heterozygous mutated ET is low but high in combined heterozygous - homozygous or homozygous mutated PV and EMGM. The combined use of JAK2V617F mutation load, spleen size are of major prognostic significance in terms of critical care medicine on top of pre-treatment bone marrow histopathology. This has important therapeutic implications for the first, second and third line treatment options in prodromal, classical and masked PV.
JAK2 wild type ET carrying the MPL^{515} mutation is a separate and distinct MPN entity without features of PV in blood (normal serum EPO) and bone marrow at diagnosis and during follow-up. JAK2/MPL wild type ETis associated with primary megakaryocytic granulocytic myeloproliferation (PMGM) and appears to be the third distinct MPN entity first definde as chronic granulocytic megakarycytic myeloproliferation in the 1990 Hannover Bone Marrow classification of CMPD. Myelofibrosis (MF) is not a disease but a secondary response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in MPNs of various molecular etiology. Large Prospective Unmet Need (PUN) studies of treated and newly diagnosed MPN patients are warranted to delineate the natural history and outcome of MPN of various molecular etiology during long-term or life long follow-up.

INTRODUCTION

In the 19th century polycythemia vera (PV) have been discovered as a primary distinct disease entity by Vaquez and Osler[1-4]. Dameshek and Henthell proposed in 1940 a set of symptoms, signs and laboratory tests for the diagnosis of PV and believed that the following minimal data should be present: plethoric appearance, splenomegaly, definitely elevated

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erythrocyte count above $6x10^{12}$/L, elevated platelet count, and elevated hematocrit. The bone marrow is pathognomonic diagnostic showing a panmyelosis (increased trilinear hematopoiesis) and large megakaryocytes obviating the need to measure blood volume[4]. In 1950, Dameshek correctly defined PV as a trilinear myeloproliferative disorder (MPD) of the bone marrow characterized by excessive production of nucleated red cells, granulocytes and megakaryocytes, peripheral blood erythrocytosis, leukocytosis and thrombocytosis[5].

Some PV cases however show pronounced elevation of erythrocytes or extreme degree of thrombocytosis, while in others the leukocyte counts may be at or close to leukemic levels, with only slight increase in red cells or platelets[5]. Dameshek proposed in 1950 two highly speculative possibilities regarding etiology of PV: first, the presence of excessive bone marrow stimulation by an unknown factor or factors, and second, a lack or a diminution in the normal inhibitory factor or factors (Figure 1 left)[5]. Vainchenker confirmed in 2005 the one cause hypothesis of Dameshek that PV indeed appeared to be a trilinear PV by the discovery of the $JAK2^{V617F}$ mutation as the driver cause of trilinear MPN by the demonstration that loss of inhibitory activity of the JH2 pseudokinase part on the JH1 kinase part of JAK2 leads to enhanced activity of the normal JH1 kinase activity thereby inducing constitutively activated JAK2V617F mutated hematopietic progenitor cells as the cause of trilinear MPNs essential thrombocythemia (ET), PV and myelofibrosis (MF) (Figure 1 left)[6]

In 1951 Dameshek illogically lumped such apparently dissimilar diseases as polycythemia vera, erythroleukemia, idiopathic or agnogenic myeloid metaplasia, megakaryocytic leukemia as myeloproliferative diseases (MPD) and proposed an unifying theory that all these variable manifestations represent myeloproliferative activity of bone marrow cells due to one hypothetical stimulus for each or all variants of MPDs (Table 1, Figure 1 right)[7]. Lumping erythroleukemia with PV, and putting together chronic granulocytic or myeloid leukemia (CML) with PV appeared to be without scientific foundation (Figure 1 right, Table 1 upper part). In 1960 Nowell and Hungerford described the presence of a minute chromosome in leukemic cells of patients with CML[8]. This minute chromosome was called Philadelphia (Ph) chromosome after the city of discovery[8]. Using banding techniques Janet Rowley (1973) discovered that the Ph chromosome originated from a translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11)[9].

The Polycythemia Vera Study Group (PVSG 1973, 1975) reviewed the spectrum and typical patternsof cellular involvement seen in the myeloproliferative disorders (MPD) with particular emphasis on the characteristic abnormalities that occur in leukocyte alkaline phosphatase (LAP) activity , bone marrow histology and karyoctype (Table 1)[10,11]. The Ph-chromosome, the low LAP score and an obligate transition into acute myelomonoblastic, lymphoblastic or megakaryoblastic leukemia became typical for Ph-chromosome positive CML[12].

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

Three Dutch scientific investigators Heisterkamp, Groffen & Grosveld of the Erasmus University Rotterdam discovered that a hybrid gene is generated by the translocation consisting of the *BCR* gene on chromosome 22 and the *ABL* oncogene originating from chromosome 9[13-15], which results in a *BCR/ABL* fusion gene with high tyrosine kinase activity and CML-transformation capacity[16,17]. Ninety-five percent of all CML patients are Ph^+; 90% are $Ph^+/BCR/ABL^+$, 5% are $Ph^-/BCR/ABL^+$, and 5% are $Ph^-/BCR/ABL^-$, the latter group usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia[18]. According to strict morphological, biochemical, cytogenetic and molecular criteria including the Ph^+ chromosome and bcr/abl fusion gene and protein, Michiels *et al* (1987) defined *BCR/ABL*-positive CML and ET as a malignant disease with an obligate transition into acute leukemia, whereas *BCR/ABL*-negative ET, PV and agnogenic myeloid metaplasia (AMM) or primary megakaryocytic granulocytic myeloproliferation (PMGM) form the *BRC/ABL* negative chronic myeloproliferative disorder (CMPD) featured by a benign proliferation of the three hematopoietic cell lines[19].

The morphological distinction between BCR/ABL^+ (Ph-positive) CML and ET versus BCR/ABL^- (Ph-negative) thrombocythemia in various MPDs is primarily based upon conspicuous differences in the form and size of megakaryocytes in bone marrow smears and sections of bone marrow biopsy[19]. This difference observed by Michiels and co-workers in 1987 is reproducible in bone marrow biopsies (figure 2), which enables pathologists to distinguish between small megakaryocytes in Ph-positive thrombocythemia in CML versus enlarged megakaryocytes with more or less pronounced hyperlobulated nuclei in Ph-negative thrombocythemia of various MPDs[19]. This original observation by Michiels on the diagnostic differentiation at the bone marrow level between Ph-positive CML and Ph-negative MPD and the subclassification of the MPDs into ET, classic PV and prefibrotic agnogenic myeloid metaplasia (AMM)[19] was reproduced and worked out by German pathologists Thiele and Georgii[20-28]. Georgii *et al* inventively defined in 1990[24] Hannover Bone Marrow classification of the MPDs, which had the great advantage to pick up the early stages of prefibrotic MPD ET, PV chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM, Figure 1, right, Tables 1 and 2), which are overlooked by the PVSG investigators. With the improvement of bone marrow biopsy and tissue processing in the 1980s and 1990s, Georgii and Thiele defined the pathological features of ET, PV and CIMF or AMM on bone marrow histopathological morphology[20-27]. Georgii regarded myelofibrosis (MF) as a reactive feature secondary to progressive disease[24] seen in AMM, PV and CML. Georgii reasoned that the terms agnogenic myloid metaplasia (AMM) or chronic idiopathic myelofibrosis (CIMF) lack accuracy since they are applied to both the prefibrotic hypercellular and advanced fibrotic stages[24]. As myelofibrosis (MF) is not a disease and not a primary but a secondary complication of MPD, Georgii *et al*

used the term MF for grading of the MPD disease burden based on the degree of reticulin fibrosis (RF) and reticulin-collagen myelofibrosis (MF) (Table 2)[24-26]. Accordingly, Georgii *et al* replaced the terms AMM and CIMF by chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM or PMGM). The Hannover Bone Marrow classification in table 2 have defined ET by persistent increase of platelets in excess of 400 $x10^9$/l without the Ph^+ chromosome together with monolinear proliferation of mature enlarged megakaryocytes in the bone marrow with normal cellularity, normal erythropoiesis and normal granulopoiesis (Table 3, normocellular ET, Figure 3)[24-26]. The Hannover Bone Marrow classification in table 2 have defined PV as a trilinear proliferation of megakaryopoiesis, erythropoiesis and granulopoiesis in which the erythropoiesis was most prominent together with variable degrees of increased platelets, erythrocytes and granulocytes in the peripheral blood in the absence of the Ph^+ chromosome (trilinear PV, Table 4, Figure 4)[24-26]. The diagnosis of prefibrotic CMGM or PMGM[24-26] seems to be consistent with hypercellular ET (Megakaryocyte Leukemia followed by MMM according to Dameshek in 1951 (Table 1 upper part) as AMM in the PVSG (Table 1 lower part)[10,11] and as chronic idiopathic myelofibrosis (CIMF) in the PVSG 2001 WHO classifications)[28]. The diagnosis of CMGM according to the 1990 Hannover Bone Marrow Classification (Table 2)[18,19] and PMGM according to ECP[24] criteria (Table 4)[29-31] are based on three specific bone marrow histology criteria: 1) the presence of large dysmorphic megakaryocytes with immature cytoplasm and immature cloud-like nuclei not seen in ET and PV, 2) increased granulopoiesis but never disturbed in maturation and 3) no features of PV with relatively decreased erythropoiesis (figures 5 and 6).

Thiele persisted to use the term CIMF instead of CMGM and introduced the WHO bone marrow features on top of PVSG criteria to defined the World Health Organization (2001 WHO MPD) classification[27,28]. The PVSG and the 2001 WHO criteria to classify the MPDs as ET, PV and CIMF are suboptimal, crude and overlook the early prefibrotic stages by definition[27,28]. The Hannover Bone Marrow classification appeared to be suprerior as the pathognonomic clue to each of the MPDs to pick up all early prefibrotic stages of ET, PV and CMGM/PMGM[24-26]. Since 1999 we have incorporated the Hannover Bone Marrow criteria into the European Clinical and Pathological (ECP) criteria to define and distinguish the three types of MPD. ET, PV and CMGM/PMGM[29-31]. In the present manuscript we could integrate the PVSG[10,11], the 2006 ECMP[32] and the 2007 WHO criteria into the 2008 ECMP classification in tables 3 and 4 by including bone marrow pathology and the use of specific laboratory features and molecular markers for diagnostic differentiation of each of the latent (masked), early and overt MPDs ET and PV.

PVSG, ECP and 2007 WHO diagnostic criteria for ET, PV and AMM or PMGM

In 1986, the PVSG reduced the platelet count from 1000 to 600 $x10^9$/l as the arbitrary minimum for the diagnosis of ET[33]. Since 1980 the Rotterdam Clinical and Pathological (RCP), the 2000 ECP and the 2006 ECMP criteria combined a typical ET histological bone marrow picture with platelet counts in excess of 400 $x10^9$/l for ET and latent or prodromal PV[32]. Comparing WHO and PVSG MPD criteria according to Thiele & Kvsnicka reveals that PVSG-defined ET comprises three phenotypes of prefibrotic early MPD: normocellular ET (table 3); ET with features of PV early or latent = prodromal PV (table 3); and hypercellular not preceded or followed by PV but associated with prefibrotic primary megakaryocytic granulocytic myeloproliferationn (PMGM labelled as CIMF by Thiele) and subsequent early fibrotic stages of MF-0 and MF-1 and advanced fibrotic stages of MF-2 and MF-3 (table 4) [34-36]. The minimum of $600x10^9$/l platelets according to the 1986 PVSG and 2001 WHO excluded early (masked) ET at platelet count below 600 $x10^9$/l in 29% of 143 ET cases[37]. In this study, 97% of all 143 ET patients showed a typical bone marrow histology of increase and clustering of enlarged megakaryocytes diagnostic for MPD[37]. This was associated with normal cellularity in 52% consistent with true ET, with increased erythropoiesis in 17% consistent with early PV, and with increased cellularity due to pronounced granulopoiesis in 45% consistent with prefibrotic MPD[37]. The 2007 WHO revision of the PVSG and 2001 WHO classifications decided to lower the platelet counts from 600 to around $450x10^9$/l for the diagnosis of ET and to change the term MPD into myeloproliferative neoplasia (MPN) ET, PV and primary myelofibrosis (PMF)[38]. The PVSG[10,11] and 2007 WHO[38] definitions are rather crude in particular for PV and PMF and consequently overlook latent and early stages of MPN patients with: 1) initial ET with a typical ET bone marrow but platelet count below 450 $x10^9$/l; 2) initial PV with a typical PV bone marrow, platelet count less than 400 $x10^9$/l, low serum erythropoietin (EPO), normal red cell mass and hematocrit less than 0.51; 3) initial masked MPD with splenomegaly and normal or slightly increased platelet count (<$450x10^9$/L) no or slight anemia (haemoglobin between 12 to 14 g/dL) the so-called idiopathic or agnogenic myeloid metaplasia of the spleen (see case description Table 5). The 2006-2007 ECMP criteria[32,39,40] in fact could overcome the shortcomings of the 2007 WHO revision[38] of the MPNs by the extension of the 2008 ECMP criteria in tables 3 and 4 for the diagnoses of three distinct clonal MPNs: $JAK2^{V617F}$ mutated ET and PV with various degrees of MF; JAK2 wild type ET and MF carrying the MPL^{515} mutation; and JAK2/MPL wild type PMGM with features of hypercellular ET and various degrees of MF and splenomegaly.

Laboratory markers for the diagnosis of ET and PV

In PV, RCM measurement was already found by Dameshek & Henthel in 1940 to be without additional diagnostic value since all PV patients show a typical PV bone marrow histology and have erythrocyte counts above $6x10^{12}$/L obviating the need to measure RCM[4]. Red Cell Mass (RCM) measurement as the gold standard for the diagnosis of PV is cumbersome, time consuming, costly, and not specific for MPD/MPN. Increased RCM in patients with erythrocytosis does not distinguish early erythrocythemic PV from congenital polycythemia (CP) or secondary erythrocytosis (SE). RCM is increased in Iapparent PV (IPV=masked PV) normal values for haemoglobin, haematocrit and erythrocytes due to pronounced splenomegaly with hypersplenism[41]. In a consecutive cohort of 105 polycythemic patients, RCM had a sensitivity of 76% for the diagnosis of PV and a specificity of 79% in distinguishing PV and non-clonal polycythemia[42]. Masked PV patients with increased RCM may have normal hemoglobin and hematocrit

because of associated iron deficiency and/or significant splenomegaly, erythrocyte count is always increased. with values above $6x10^{12}$/L. RCM is a crude mathematical concept to measure blood volume in erythrocytoses and does not directly reflect the biology of the underlying disease. In a series of 77 consecutive patients (31 males and 46 females) with PVSG defined PV in the study of Johansson *et al*, only 35% of male and 63% of female PV patients had WHO defined Hb values above 18.5 and 16.5 g/dL respectively[43]. Bone marrow histology, spleen size and blood cell counts reveal specific manifestation as pathognomonic clues to $JAK2^{V617F}$ mutated and JAK2 wild type MPN.

Spontaneous endogenous erythroid colony (EEC) formation[44,45] and low serum EPO levels[46-50] are specific confirmative criteria for the diagnosis of PV, but have insufficient diagnostic sensitivity as isolated parameters to differentiate between PV, CP, CE, SE, ET and normal controls[49,50]. About 50% of PVSG defined ET patients show spontaneous EEC and low serum EPO levels[51-53] indicating that EEC positive ET comprises a biologically distinct subgroup of ET patients reflecting early PV ("forme fruste" PV) that is at risk for progression to overt PV[53]. In the latter study of 170 PVSG-defined ET patients, spontaneous EEC was seen in all 11 (6.5%), who later developed PV, and in 60% of 159 patients with stable ET during a median follow-up of 29 months (12-138 months)[53].

Acquired $JAK2^{V617F}$ somatic mutation as the driver cause of trilinear MPN

Dameshek-Vainchenker's Disease: 1950[5]-2005[6] In 2005 Vainchenker confirmed the one cause hypothesis of Dameshek that PV appeared to be a trilinear PV by the discovery of the $JAK2^{V617F}$ mutation as the driver cause of trilinear MPN by the demonstration that loss of inhibitory activity in exon 14 of the JH2 pseudokinase part on the JH1 kinase part of JAK2 gene leads to enhanced activity of the normal JH1 kinase activity thereby inducing constitutively activated $JAK2^{V617F}$ mutated hematopietic progenitor cells as the cause of trilinear MPNs ET, PV and MF (Figure 1 left, Figures 7)[5,6,54,55]. The sequential stages of heterozygous and homozygous JAK2V617 mutations (Figure 7, Table 5) due to mitotic recombination render the TPO and EPO receptors constitutively activated and hypersensitive to minimal amounts of hematopoietic growth factors thrombopoietin (TPO), erythropoietin (EPO) and granulocyte colony stimulating factor (GCSF), resulting in trilinear MPN (ET, PV and MF)[5,6,54]. Detection of $JAK2^{V617F}$ has become the first intention diagnostic test to differentiate between PV and myeloproliferative idiopathic erythrocythemia (IE) from erythrocytosis with a sensitity of 95% and specificity of 100%[32]. The $JAK2^{V617F}$ mutation renders the receptors on mutated hematopoietic progenitor cells hypersensitive to the cytokines erythropoietin (EPO), thrombopoietin (TPO), and granulocyte colony stimulating factor (G-CSF), thereby resulting in growth advantage of the mutated over the non-mutated trilinear hematopoietic cells present in the bone marrow. The $JAK2^{V617F}$ mutation is detectable in $CD34^{+}$ hematopoietic bone marrow cells, erythroblasts, in cells of spontaneous EEC, blood platelets and granulocytes (Table 5). Applying allele-specific polymerase chain reaction (PCR) analysis in PVSG-defined MPD patients, a high frequency of the $JAK2^{V617F}$ mutation of 95% (92-97%) is described in PV, and a lower frequency of 53% (49-57%) in ET and 52% (44-55%) in MF[32]. Only 3 to 4% of ET, 24 to 27% of PV and 6 to 18% of MF patients are homozygous for the $JAK2^{V617F}$ mutation[32]. Representative pretreatment bone marrow features of normocellular ET, prodromal PV and homozygous PV are shown in figures 2, 3 and 4.

Based on animal studies and different mutation states of $JAK2^{V617F}$ in MPN patients, two hypotheses have been proposed to explain why three different phenotypes of MPN are caused by the same $JAK2^{V617F}$mutation: the "dosage" hypothesis and the "additional events" hypothesis[6,54]. According to the "dosage" hypothesis the level and duration of $JAK2^{V617F}$ directly contribute to the phenotypic diversity of trilinear MPNs (figure 8, Table 5)[32,54]. This hypothesis is based on different densities of TPO receptors (TPOR or MPL) and EPO receptors (EPOR) on hematopoietic progenitor cells and on differences of response of TPOR and EPOR to various levels of $JAK2^{V617F}$ activity. TPOR/MPL is expressed at high levels in megakaryocytic cells where it controls physiological TPO levels. It seems that minute amounts of TPO to consitutively activation TPO receptors in heterozygous $JAK2^{V617F}$ mutated normocellular ET is sufficient to send a signal to bone marrow megakaryocytic cells to induce spontaneous growth of large clustered megakaryocytes[6,32]. A slight increase in numbers of mutated large (giant) megakaryocytes and platelets (about 100 $x10^{9}$/l mutated platelets) might be sufficient to produce platelet-mediated microvascular circulation disturbances (Table 5). Conversely, EPOR is expressed at low levels on hematopoietic progenitor cells and therefore high levels of $JAK2^{V617F}$ may be required to activate EPOR and generate a PV-like phenotype[6,54,55]. Sustained high levels of $JAK2^{V617F}$ mutation load per hematooietic stem cell in hetro/homozygous or homozygous PV during long-term follow-up subsequently may lead to a high level activation of activation of EPOR and GCSF receptor (GCSFR) leading to a trilinear hypercellular bone marrow, extramedullary hematopoiesis in the spleen (splenomegaly) and cytokine mediated secondary myelofibrosis (figure 7, Table 5)[54-56]. The percentage of $JAK2^{V617F}$ positivity and progression from heterozygous to homozygous is strongly correlated with the ability to form spontaneous EEC formation and with progressive post-PV myelofibrosis. Scott *et al*. showed that BFU-e colonies are already homozygous for the $JAK2^{V617F}$ mutation in PV patients with a heterozygous pattern of $JAK2^{V617F}$ in their peripheral blood granulocytes of less than 50% (Figure 8)[57]. In contrast, the BFU-E colonies from heterozygous patients with ET did not contain a subpopulation of $JAK2^{V617F}$ homozygous cells[57]. French investigators studied a large group of $JAK2^{V617F}$ positive PV (N=159, 36% homozygous) and ET (N=147, 4% homozygous), and genotyped BFU-E colonies in in 20 PV and 6 ET patients[58]. They showed that $JAK2^{V617F}$ positive ET patients usually harbour heterozygous BFU-E clones, some PV patients have a purely heterozygous profile, and most PV patients have a mixture of heterozygous and homozygous BFU-E clones.

Mutated erythroid progenitors are more sensitive to EPO than normal progenitors, and most homozygous progenitors are EPO independent. In this cohort of 306 $JAK2^{V617F}$ positive MPD patients, PV was associated with significantly lower platelet counts and higher hematocrit and granulocyte values than ET patient. The highest platelet count was associated with low $JAK2^{V617F}$ levels in PV, whereas high JAK^{V617F} levels correlated with high hemoglobin and high granulocyte counts in ET[58]. Tefferi *et al* detected $JAK2^{V617F}$ in 75% of ET (n=60) and in 97% of PV patients (n=62), whereas allelic ratios exceeding 50% $JAK2^{V617F}$ indicating homozygosity were found in 70% of PV at diagnosis but never in ET[59]. Transition from heterozygosity to homozygosity for the $JAK2^{V617F}$ mutation represents a very important step in the progression from early classic PV, advanced PV and masked PV (IPV) and subsequent post-PV myelofibrosis (Figure 8, Table 5)[32]. Comparing $JAK2^{V617F}$ heterozygous and homozygous PV patients showed that homozygote $JAK2^{V617F}$ PV patients displayed significantly higher hemoglobin at time of diagnosis, increased incidence of pruritus, and a higher rate of fibrotic transformation[59]. Sex appears to be a powerful genetic background modifier in $JAK2^{V617F}$-positive MPDs as ET is more common in females and PV in males.

Mechanisms other than mitotic recombination such as duplication of the mutated allele is observed in a proportion of PV and MF patients displaying a gain of 9p, mostly due to trisomy 9[60,61]. Campbell *et al* reported that the $JAK2^{V617F}$ mutation was associated with trisomy 9 with all 10 MPN patients investigated. Scott *et al* identified JAK2 exon 12 mutations in 10 erythrocytosis patients with increased red cell mass but no $JAK2^{V617F}$, of which according to PVSG criteria 6 could be diagnosed as PV and 4 as idiopathic erythrocytosis[63]. Pre-treatment bone marrow biopsies in 5 JAK2 exon 12 mutated PV patients showed a characteristic MPN pattern of erythroid hyperplasia without morphological abnormalities of the megakaryocyte and normal granulocyte lineages[63]. These observations are in support of an overlap between "dosage" and "additional molecular events" hypotheses in patients with PV[54,55].

Clinical symptoms and Diagnosis in 497 Dutch MPN patients

The results from the 2008 MPN Questioaires of the Dutch MPN Patient Foundation are the reflection of ECMP criteria for the diagnosis, classification and staging of MPN and treatment recommendations of ET and PV patients in The Netherlands between 2000 and 2008[64]. Low dose aspirin in ET and phlebotomy on top is effective in the majority of ET and in two third of PV patients with low or mild MPN disease burden. Low dose pegylated interferon is recommended in PV with mild to moderately increased MPN disease like leukocytosis, itching and mild to moderate splenomegaly to postpone the use of hydroxyurea as long as possible (Figure 9). The collected Dutch MPN data were published in PUR SANG in 2010 based on 497 filled forms by MPN patients: 271 females (54%) and 212 males (43%), mean age at diagnosis 57 years (range 20 to 84 years)[64]. Since 2000 and 2006, diagnosis of the MPDs followed the ECP[29-31] or ECMP[39,40] criteria for ET, PV and MF. The diagnoses of 497 MPN patients were ET in 181 (36%), PV in 244 (50% of whom 18 as ET/PV), MF in 67 (13%), and MPN unclassifiable in 5 (1%). The detection of MPN disease 115 Dutch and Belgian hospitals was related to MPN specific complaints in 55%, coincidental (eg routine laboratory investigation for other reasons) in 30% and after significant delay of disease specific complications 15%. Diagnosis of MPN was confirmed by bone marrow aspiration from the sternum in 235 (47%), bone marrow biopsy from the iliac crest in 475 (96%) and both in 50% of 497 MPN patients[64]. Red Cell Mass (RCM) measurement to diagnose PV and to distinguish ET from PV was performed in 31%. PCR test for the $JAK2^{V617}$ mutation anno 2008 was performed in 230 (46%) MPN patients and found positive in 74% (ET n=52, PV n=103, MF n=14) and negative in 26%. Sixty percent of ET, 91% of PV and 52% of MF patients were $JAK2^{V617F}$ positive, thereby confirming the data in the literature (reviewed by Michiels *et al* 2006)[32]. After primary diagnosis 144 (25%) MPN patients (ET n= 38, PV n= 49, MF n=27) were referred for a second opinion. The second expert evaluation led to a change in diagnosis in 8% and a change in treatment in 28% (n=29). The second treatment option in 29 (28%) proved to be superior to the initial treatment. A change of diagnosis during follow-up occurred in 60 MPN patients, from ET into PV in 16 (9% of PV), from PV into MF in 15 (6% of PV), and from ET into MF in 10 (6% of ET).

MPN related signs and symptoms in 497 Dutch ET, PV and MF patients

Based on the Dutch MPN questionaire including 36 questions the top 20 complaints at time of diagnosis in 399 out of 497 (81%) MPN patients is shown in table 6[64] . The most frequent complaint is fatigue (81%) equally high in ET, PV and MF patients. Apart from variable severity of fatigue a specific pattern of signs and symptoms could be retrieved. The signs and symptoms in ET are mainly featured by aspirin responsive tingling and prickling sensations in footsoles, handpalms, toes and fingers (erythromelalgia), and aspirin responsive cognitive concentration and visual disturbances (Table 6)[64]. PV patients presented with similar signs and symptoms but on top of that both aspirin resistent itching (PV 58% vs ET 30%) and fatigue were much more prominent in PV. A second most frequent complaint were various degrees of night sweats related to splenomegaly in about half of the MPN patients. About one third of MPN patients suffered from bone pain (Table 6). MF patients suffered more frequently from constitutional symptoms of prominent fatigue and night sweats related to pronounced splenomegaly. Before the MPN diagnosis was made the complaints were ascribed by doctors in 173 (35%) patients to other causes including stress, burned out or overstrained in 41 (24%), to depression or hystery in 14 (8%), migraine of unknown origin (and therefore not treated with aspirin) in 13 (8%) and to rheuma, hypertension or fibromalgia in a few[64].

Treatment and adverse events in Dutch MPN patients 2003-2008

Treatment in 497 MPN patients was started with low dose aspirin or calcium carbasalate (Ascal) in 70% and phlebotomy in 42% (mainly PV 91%), hydroxyurea in 29%, and pegylated interferon-alpha2a in 7%, wait and see in 8% (n=42 of whom 26 with MF) of MPN patients at time of diagnosis (Figure 9)[64]. The treatment changed during follow-up in 294 (60%) of MPN patients: ET in 64% (n=115), PV in 59% (n=143) and MF in

49% (n=33). Out of 459 evaluable adverse drug reactions or side effects were recorded in one third (N= 168 =35%) of MPN patients. Out of the 168 recorded side effects were related to HU in 41% (n=69) and to IFN in 28% (n=47) of all side effects. Most frequent side effects of HU were skin and mucocutaneous complaints including dry skin, skin lesions, skin ulcers, itching, skin carcinoma, brittle nails, aphtous ulcers and hair loss[64]. Most frequent side effects of IFN were flue-like symptoms, fatigue and mood disturbances [64]. Low dose aspirin or Ascal induced gastritic complants in 11% for which treatment with metronazol was usually indicated[64].

Vannucchi *et al* assessed in 2007 the incidence of major thrombosis related to the JAK2 allele burden in a large retrospective Italian study of 962 MNP patients with thrombocythemia (MNP-T) subdivided in 323 PV and 639 ET patients[65]. Aspirin responsive platelet thrombophilia or microvascular symptoms due to microvessel disorder including migraine-like headache, acral paresthesia, erythromelalgia, transient neurological and visual disturbances (Sticky Platelet Syndome) were excluded from evaluation by definition and overlooked in this retrospective analysis because of unfamiliarity[65]. Only major thrombotic events were assessed as could be objectively documented from the medical records including ischemic stroke, transient ischemic attacks, myocardial infarction, angina pectoris, deep vein thrombosis abdominal vein thrombosis, and pulmonary embolism occuring at diagnosis or follow-up when not on aspirin. One hundred seventy-six patients (18.3%) had a major thrombotic event at diagnosis with a similar frequency in PV (19.2%) and ET (17.8%). The overall incidence of major thrombotic events during 10 years follow-up while not on aspirin was about 20% in ET heterozygous for the $JAK2^{V617F}$ mutation and in about 10% for JAK2 wild type ET. Hemorrhages during follow-up was recorded in 45 (4.7%) ET/PV patients. In this retrospective study, a total of 214 patients were treated with phlebotomy, 58% of 219 PV and 4% of 257 ET patients. Myelosuppressive chemotherapy was administered to Italian MPN patients (52%) including 59% of 219 PV and 48% of 257 ET patients. For comparison, in the Dutch 2008 survey of 363 MPN (123 ET, 190 PV and 50 MF) patients 93% of PV, 71% of ET and 37% of MF were on aspirin mainly because of acral paresthesia, erythromelalgia, and transient neurological and visual disturbances[64]. Phlebotomy was the first line treatment in 6% of ET, 78% of PV and 9% of MF (Figure). Because of advanced or symptomatic MNP disease burden 31% of ET, 29% of PV and 30% of MF were on treatment with hydroxyurea and 16% of ET, 16% of PV and 4% of MF were on treatment with pegylated interferon ($Pegasys^{R}$) (Figure 9). The 20% difference of HU use of 50% HU use in Italian MNP-T patients versus 30% in Dutch MNP-T patients can readily be ascribed to significant differences between the Italian IPSET recommendations[66-69] based on high thrombotic risk versus the Dutch guidelines based on MNP disease burden in ET and PV patients[30,32,36]. The Dutch guidelines treat to ET with aspirin and PV with aspirin/phlebotomy in PV as the first line and recommend low dose Pegasys to postpone the use of hydroxyurea as long as possible[30,32,36]. The Italian and WHO guidelines[67,68] used high thrombotic risk (age 60 years and previous major thrombosis) as the indication of hydroxyurea treatment in ET and PV for the secondary thrombosis prevention (2012 International Prognostic Score of Thrombosis in ET (IPSET)[66-69]. This strategy leads to significant overtreatment with hydroxyurea simple because about half of the ET and PV patients at the age of 65 in fact have low MNP disease burden, and a low thrombotc risk when on low dose asprin. According to the world wide used IPSET guidelines in review papers there is a global overtreatment of ET and PV patients with hydroxyurea[67,68].

Acquired MPL gain of function mutations as the driver cause of normocellular ET

The JAK2 kinase activity in MPNs is not only dependent on the amount of heterozygous and homozygous $JAK2^{V617F}$ mutant protein, but may also be influenced by the various steps upstream or downstream the signalling pathways including MPL, JAK2, STAT-3. This has been demonstrated in animal models overexpressing c-MPL[70]. MPL transgenic mice manifested with typical features of ET with a four fold increase of platelet count, increased colony formation of megakaryocytes, and increase of clustered enlarged megakaryocytes in the bone marrow. The ET animals appeared healthy, had a very slight decrease of hematocrit (0.39 versus 0.42 in controls) and survived normally with no evidence of myelofibrosis in the bone marrow[70]. The acquired MPL^{W515L} and MPL^{W515K} gain of function mutations have been discovered as the underlying driver cause in ET patients[71,72]. Screening of 1182 PVSG-defined MPD patients (318 ET, 242 PV, and 290 IMF) and 64 controls for MPL^{515} mutations resulted in the detection of MPL mutations either MPL^{W515L} (n=17) or MPL^{W515K} (n=5) in 20 MPN patients (MF in 4%, ET in 4 = 1%, and post-ET myelofibrosis in 1), but not in the 242 PV patients and controls[71]. Six cases carried both MPL^{W515L} and $JAK2^{V617F}$ alleles indicating that these alleles have functional complementation in MF. MPL^{515} mutated ET and MF represent a distinct entity of JAK2 wild type MPN without features of $JAK2^{V617F}$ mutated PV. These observations are in line with the "additional events" hypothesis, indicating that alternative or additional molecular abnormalities, like $JAK2^{V617}$ mutation alone, combinations of JAK^{V617F} and MPL^{515} mutations or other combinations of still unknown mutations contribute to distinct phenotypes of MPN.

Vannucchi *et al* studied 30 ET patients carrying the MPL^{515} mutation (18 MPL^{W515L} and 12 MPL^{W515K}), 9 males and 21 females, age 22-84 (mean 56) years[73]. In this study 8 MPL positive ET patients (26%) also carried the $JAK2^{V617F}$ mutation. The clinical presentation at diagnosis and follow-up was remarkable with a high incidence of major arterial event, 23%, venous thrombosis, 10%, microvessel disturbances, 60%, and major hemorrhage, 7%. The only abnormal laboratory finding was increased platelet counts, $956\pm331x10^{9}$/L together with hemoglobin values in the lower range of normal (13.4 ± 1.3 g/l), normal white blood cells ($8.8\pm3.1x10^{9}$/L), slight increase of LDH (459 ± 182 U/L) and splenomegaly in only 5 (17%). Mutation allele burden was greater than 50% in half of MPL^{W515K} patients compared to 17% of MPL^{W515L} mutated ET

patients. MPL^{515} and $JAK2^{V617F}$ mutations coexisted in 3 with MPL^{W515L} and in 5 with MPL^{W515K} allele mutations. General features of bone marrow reports revealed significantly reduced erythropoiesis and decreased cellularity in $MPL^{W515L/K}$ patients, which associated with increased number of clustered small and large to giant megakaryocytes and no significant increase in reticulin fibrosis in a normaocellular bone marrow (Figure 10) without features of PV or PMGM. In 2008 we discovered that JAK2/MPL wild type hypercellular ET associated with a PMGM bone marrow is consistent with the CMGM of the Hannover Bone MarrowClassificatio as the third distinct MPN entity (Figures 5and 6).

2008 ECMP diagnostic criteria for the classification and staging of ET, PV and PMGM

The 2008 ECMP criteria separate $JAK2^{V617F}$ mutated ET patients into three phenotypes of prefibotic MPNs at the bone marrow level: normocellular ET, early PV mimicking ET (prodromal PV and ET with MGM (MF-0) bone marrow without features of leuko-erytroblastosis in the peripheral blood (Tables 3 and 4). These three ECMP defined JAK2V 617F mutated ET phenotypes do not differ significantly with regard to peripheral blood features, thrombocythemia related clinical presentation or laboratory findings and are to be treated equally based on clinical risk stratification for thrombotic and bleeding complications, irrespective of bone marrow features. The prognostic importance of recognition of hypercellular ET associated with PMGM (MF-0) as compared to normocellular ET is demonstrated in a large retrospective study of 476 PVSG-defined ET patients (platelet count >600 $x10^9$/l), who were reclassified according to the WHO bone marrow criteria: normocellular ET in 167, hypercellular ET MF-0 in 174 and early fibrotic ET MF-1 in 135[74,75]. Mean age of normocellular ET patients was 59 years, which is 8 to 10 years younger compared to patients with hypercellular ET MF-0, 67 years, and early fibrotic ET MF-1, 69 years. The differences in relative 10 years survival rates: 99 ± 7.8% for normocellular ET, 81 ± 11.7% for hypercellular ET MF-0, and 67 ±17.8% for early fibrotic ET MF-1 patients, are significant. The majority of hypercellular ET MF-0 patients have early stage disease without features of leuko-erythroblastosis (table 4), whereas early fibrotic ET MF-1 patients are a mixture of early and intermediate stage disease without and with leuko-erythroblastosis. Progression of normocellular ET into myelofibrosis (MF) was not seen five years after diagnosis in two other studies, but data on very long-term follow-up are lacking. The overall survival in two studies of PVSG-defined ET was similar to an age-matched control population in the first decade, but significantly worse beyond the first decade of the disease[76,77].

Diagnostic work-up of patients with ET in various MPNs 1980-2008

Clinical manifestations of thrombocythemia in masked and overt MPNs consist of aspirin responsive microvascular circulation disturbances including atypical and typical TIAs, ocular ischemic attacks, erythromelalgia and splanchnic vein thrombosis[78-82]. Sustained increase of platelet counts (>400 $x10^9$/l) associated with slight splenomegaly on echogram (>12cm), increased leukocytes (>12 $x10^9$/l), or LAP score with normal ESR is highly suspicious of PVSG-defined ET or thrombocythemia in various MPN with the absence of any cause for reactive thrombocytosis (figure 11). The presence of giant platelets in a peripheral blood smear is indicative for MPN and excludes reactive thrombocytosis. $JAK2^{V617F}$ mutation screening as a first intention diagnostic test is very helpful in the diagnostic work-up of patients with suspected thrombocythemia in various MPDs, but only half of ET and MF patients carry this mutation (sensitivity 50-60%). Pretreatment bone marrow biopsy will allow clinicians and pathologists to diagnose the early stages of thrombocythemia in various MPNs irrespective of $JAK2^{V617F}$ or MPL^{515} mutation status (Figure 11). The 2008 ECMP criteria classify JAK2 mutated ET as: normocellular ET (table 3); early PV mimicking ET ([prodromal PV, table 3); ET associated with MGM (RF-0 or RF-1 (EMGM. Table 3) without features of leukoerythrocytosis and extramedullary hematopoiesis (table 1); and post-ET MGM with MF-1, 2 and 3 and features of leucoerythroblastosis. The 2008 ECMP criteria distinguish thrombocythemia in various MPNs from thrombocythemia associated with Ph^1-chromosome and *bcr/abl* positive chronic myeloid leukemia (CML)[83] or myelodysplastic syndromes (MDS) including the so-called 5q-syndrome, which clearly differs from refractory anemia with ringed sideroblasts and significant thrombocytosis (RARS-T)[84-87]. Among 9 RARS-T patients, 6 showed the presence of $JAK2^{V617F}$ mutation[86,87]. As compared to JAK2 wild type ET, $JAK2^{V617F}$ positive ET is characterized by higher values for hemoglobin, hematocrit, neutrophil counts, LAP score, by lower values for serum EPO levels, serum ferritin and MCV, and by increased cellularity of the bone marrow in biopsy material[88,89], indicating early thrombocythemic PV mimicking ET ("forme fruste" PV, stage 1 PV, .Tables 3 and 4). JAK2 wild type ET patients represent a distinct category who had significantly higher platelet counts, normal serum EPO levels, a typical bone marrow picture of ET, no features of early PV, and are at lower risk for the development of thrombotic complications.

Diagnostic work-up of patients with polycythemia vera 1980-2008

PV patients frequently present with dull headaches and aspirin responsive migraine like atypical neurlogic or ocular ischemic attacks (MIAs), erythromelalgia (figure 12), splanchnic vein thrombosis[78-82], and sometimes microcytosis of erythrocytes due to iron deficiency[88-89]. Characteristic PV features include increased hematocrit (>0.51), increased erythrocytes (>6 $x10^{12}$/l), slight splenomegaly, increased leukocytes (>12 $x10^9$/l) or LAP score with normal ESR, increased platelets (>400 $x10^9$/l). PV patients usually show the presence of large platelet in peripheral blood smear. Patients with congenital erythrocytosis with a gain of function mutation in the EPOR or acquired erythrocytosis lack the clinical, laboratory, molecular and bone marrow features of MPN and are usually asymptomatic. The detection of $JAK2^{V617F}$ in granulocytes with sensitive PCR techniques plays a key-role as a first intention diagnostic test for erythrocytosis, because it simplifies the diagnostic work-up of PV (figure 12). In the context of erythrocytosis (hematocrit >0.51 in males and >0.48 in females) the presence of the $JAK2^{V617F}$ mutation has a sensitivity of 95% and positive predictive value of 100% for the diagnosis of PV, and excludes congenital and secondary

erythrocytosis (figure 10). EEC and low serum EPO significantly contribute but are not sensitive enough to diagnose the broad spectrum of PV phenotypes (table 5). Bone marrow histology assessment is a gold standard for the diagnosis of masked, overt and advanced $JAK2^{V617F}$ mutated and exon 12 mutated PV.

Grading of myelofibrosis in myeloproliferative disorders 1980-2008

Myelofibrosis (MF) itself is not a disease because reticulin and collagen fibrosis are produced by polyclonal fibroblasts in response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in both PV and MF (Table 1)[24-26]. Transformation to myelofibrosis is rare in ET and does occur in about one third of PV and in the majority of patients with ET associated with PMGM (MF-0) during long-term follow-up[25,26]. The grading of reticulin fibrosis (RF) and myelofibrosis (MF) in bone marrow biopsies was developed by pathologists[,26]. A scoring system based on morphometric analysis (point intersection with an ocular grid) and quality of fibers (reticulin and collagen fibers) and the bone marrow fiber density (fine or course reticulin and some or course bundles of collagen) has been proposed by European consensus for grading of MF[,90]. According to defined standardized semiquantitative grading of reticulin and collagen fibrosis in the bone marrow, MF can reliably be graded at the pathological bone marrow level as 0 in prefibrotic, as 1 in early fibrotic, as 2 in classical fibrotic and as 3 in classical sclerotic MF[24-26,90].

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

Spivak and his co-workers assessed the burden of $JAK2^{V617F}$ mutation and PVSG-defined MPD in 84 ET, 92 PV, and 19 fibrotic MF patients[91]. The $JAK2^{V617F}$ mutation was detected in 92% of PV, in 45% of ET, and in 42% of fibrotic MF patients. The median burden of $JAK2^{V617F}$ alleles was significantly lower ET (47%) than in PV (67%) patients (p=<0.001) when stratified for disease duration, a $JAK2^{V617F}$ burden of 100% (homozygosity) was present in only 15% of PV less than three years from diagnosis compared to 40% of PV three to 10 years since diagnosis, but none in ET patients during very long-term follow-up (figure 13)[91]. Passamonti *et al* studied the burden of $JAK2^{V617F}$ in WHO-defined MPD ET, PV, prefibrotic MF (p-MF), fibrotic MF (f-MF) and 16 post-PV myelofibrosis (figure 14)[56]. The $JAK2^{V617F}$ mutation was detected in 92% of 25 PV, in 53% of 19 ET, in 58% of 12 MF-0 (ET associated with MGM) in 56% of 18 fibrotic MF, and in 100% of 16 post-PV myelofibrosis patients[56]. Interestingly, ET and p-MF patients had significantly lower percentage of mutated alleles than patients with PV (p=0.01), whereas patients with fibrotic MF (f-PMF)had much higher values than prefibrotic MF (p-MF)or ET (p=0.0008) (figure 14). Circulating CD34 positive circulating cells were normal all patients with PV (N=25), ET (N=19) and p-CIMF (N=12) and 6 out of 18 f-MF patients had normal ($<10x10^6$/L) circulating CD34 cells (figure 14). Conversely, 12 out f 18 f-MF and all post-PV MF (16) had increased CD34 circulating cells (figure 13). These data indicate that ET and p-MF are not different at the molecular ($JAK2^{V6217F}$) and biological (CD34 cells) level. Post-PV myelofibrosis had the highest percentages of mutant alleles approaching 100% homozygosity (figure 8). PV and MF patients with a high mutation burden (granulocytes mutant alleles in excess of 50%) have leukocytosis, splenomegaly, increased LDH levels, increased circulating CD34-positive cells, a worse event free survival and a compromised overall survival as compared with those with lower mutation burden (granulocyte mutant alleles of 1-50%) mainly seen in ET and early stage PV[56].

The use of the 2008 ECMP criteria clearly show that JAK2 wild type ET and MF lack specific PV laboratory and pathological features at diagnosis and during follow-up. This has been demonstrated for MPL^{515} mutated (ET/MF) and for JAK2/MPL wild type hypercellular ET in PMGM. The flexible use of the 2008 ECMP criteria should serve as pathognomonic diagnostic clues to each of the prefibrotic MPNs, will distinguish early and overt PV MPN disease from primary or secondary erythrocytosis. The 2008 ECMP criteria can be applied to document the natural history of myeloproliferative and fibrotic disease in JAK2V617F, MPL515 and JAK2 wild type ET and MF patients, which has important therapeutic implications (Table 7). The 2008 ECMP were the critical responses on the shortcomings of the 2007 WHO criteria and were conceptualised before the publication of the final 2008 WHO classification[92]. Between 2008 and 2015 we could further improve and integrate the 2008 ECMP into the updated 2014/2015 WHO-CMP for the five distinct clonal MPNs caused by the JAK2V617F, exon 12 JAK2, MPL515 and CALR driver mutation leaving a small group of triple negative group of MPN[93-95].

Therapeutic implications in PV and ET in view of critical care medicine 1950-2015.

The prognosis of PV patients treated by phlebotomy alone is compromised by the high incidence of major vascular complications. The major major occlusive thrombosis of five large retrospective series of PV patient did occur in about one-third of the cases involving the peripheral, cerebral and coronary circulation (Table 8)[12]. The incidence of thrombotic events in 4 large series of PV patients decreased significantly by treatment with P32 probaly due to the correction of erythrocyte, platelet and leuocyte counts (Table 8)[12]. PV patients treated in the phlebotomy arm of the PVSG 01 study experienced a high incidence of early major thrombotic events as compared to either chlorambucil and P32 often fatal or disabling cerebrovascular accidents with significant loss of life expectance during the first three years on study[96,97]. These were especially common in elderly patients and those with a previous history of major thrombotic events at hematocrits between 0.45 and 0.50. PV patients treated in the PVSG 01 study treated with either chlorambucil and P32 had a statistically significant increased risk of developing acute leukemia, malignant lymphoma or carcinomas as compared to phlebotomy alone. In the randomized EORTC phase III busulfan (mean dose 301 mg) treatment was significantly better

than P32 with regard to duration of first remisson and overall survival..

The 10-year survival in the busulphan and P32 groups were 74% and 55% respectively[98]. This difference was mainly due to a much higher frequency of lethal vascular accidents in the P32 arm (25 of 149 PV patients) as compared to busulphan (8 of 145 PV patients). A primary rigid venesection regime in the treatment of newly diagnosed PV patients keeping the hematocrit at 0.40 significantly reduced the incidence of major thrombotic event but the incidence microvascular ischemic events related to associated thrombocythemia is rather high[5,99,10].

The platelet-mediated microvascular ischemic syndrome of thrombocythemia in ET and PV patients is best controlled by low dose aspirin[100]. Phlebotomy on top of low dose aspirin in PV reduces the incidences of major and microscular events in PV from about 60 to 80% to less than than 3%. Consequently clinicians are reluctant to postpone the use of myelosupprssive agents as much as possible in ET and PV who do have a low to intermediate MPN disease burden. This comprises about half to two-third of the ET, PV patients (Figure 9)[30-32].

The risk stratification for thrombosis as low, intermediate and high thrombotic risk by Cortelazzo *et al* in 1990[101] has been derived from a historical cohort of 100 ET patients related to age not treated with aspirin in the retrospective Bergamo cohort of 100 patients were the following:

Age	number of patients	Patient/years	Events number	Events % pt/yrs
<40 year	34	118	2	1.7%
40-60 years	37	112	7	6.3%
>60 years	29	73	11	15%
Total events in 20 of 100 ET patients (not on aspirin)				=20%

The type and number of 25 arterial and 3 venous thrombotic episodes in 20 out of a historical cohort of 100 untreated ET patients in the 1990 Bergamo study were mainly microcirculatory events including digital ischemia, transient ischemic attacks, superficial thrombophlebitis, ususual site of DVT, no stroke, and major thrombosis only in 4, myocardial infarction in 3 and femoral DVT in 1[101].

It has been demonstrated at that time that such microcirculatory disturbances, superficial thrombophlebitis and TIAs are highly sensitive to low dose aspirin but not to coumarin[12,32,100]. If left untreated symptomatic ET patients with microcirculatory disturbances including erythromelalgia and atypical TIAs or visual symptoms are supposed to be at very high risk for digital ischemia, TIAs, stroke or acute coronary ischemic syndromes[1]. The stratification in low, intermediate and high thrombotic risk in subsequent validation studies define low thrombotic risk ET and PV for the indication of low dose aspirin and define high thrombotic risk ET and PV patients as a clear indication for hydroxyurea treatment.

The so-called high thrombotic risk ET and PV are defined by a history or presentation of major thrombosis at time of diagnosis or by reaching the age 60 to 65 years. In our analysis and experiences this definition of high thrombotic risk in the 2012 International Prognostic Score for ET (IPSET)[67,68] with the indication to treat ET disease with hydroxyurea when reaching the age of 60 years alone leads to significant overtreatment of ET and PV patients since about half of the ET and PV patients at and above 60 to 65 do have low or intermediate MPN and are not at risk for thrombosis when on low dose aspirin (Figure 9).

Hydroxyurea is not an innocent drug and should be used with caution and withheld as long as phlebotomy and low dose aspirin are effective in the treatment of early and intermediate plethoric PV stages 1and 2. In the 1980 French PV study in PV patients under the age of 65 years, toxicity of hydroxyurea was observed within 5 years in 29% of 133 PV patients, which was limited to *dry* skin and acne in 7%, gastric pain and diarrhea in 9%, aphthous ulcers in the mouth in lO%, and leg ulcers in 9%[102]. Leg ulcers only healed after discontinuation of hydroxyurea; these complications generally appear late within 5 years or more after initial treatment.

Table 1 The concept on myeloproliferative disorders (MPD) according to Dameshek in 1951[7], and classification of the Philadel phiachromsome negative myeloproliferative disorders (MPD versus the Philadelphia chromosome positive chronic myelocytic leukaemia (CML) by the Polyxythemia Vera study Group (PVSG) in 1973 and 1975[10].

	Myelostimulatory Factor's				
	Bone marrow				*Potential bone marrow*
SYNDROMES	*Erythroblasts*	*Granulocytes*	*Megakaryocytes*	*Fibroblasts*	*Myeloid metaplasia of spleen and liver*
Chronic Granulocytic Leukemia	±	+++	+ to +++	+	++
Polycythemia Vera	+++	++	++ to +++	+ to +++	+ to +++
Idiopathic or Agnogenic Myeloid Metaplasia of Spleen	±	±	+++	+ to +++	+++
Megakaryocytic Leukemia	±	±	+++	+	+ to +++
Erythroleukemia (including diGuglielmo syndrome)	+++	+	±	±	+ to +++

Degrees of Proliferation: +: slight; ++: moderate; +++: marked

The concept on The Myeloproliferative Disorders according to Dameshek, Blood 1951; 6: 354. Reproduced by permission of W.B. Saunders Company.

	Erythroid Proliferation	Megakaryocytic Proliferation	Granulocytic Proliferation	Fibroblastic Proliferation	Extramedullary Hematopoiesis (Myeloid Metaplasia)	Leukocyte Alkaline Phosphatase Activity	Philadelphia Chromosome
Polycythemia Vera	↑	↑	↑	↑	↑	↑	−
Essential Thrombocythemia	N	↑	↑	↑	↑	↑N	−
Myeloid Metaplasia-myelofibrosis	↑N	↑↑	↑↑	↑	↑	↑	−
Chronic Myelocytic Leukemia	N	↑	↑	N	N	↓	+

N = Normal; ↑ = increased; ↓ = decreased; − = negative; + = positive.

Dry skin in 1, aphthous stomatitis in 4 and leg ulcers in 10 cases were the reasons for replacing hydroxyurea by pipobroman in 9%. In the update 10 years later in 1997, the incidence of leukemia was about 10% at 13 years in hydroxyurea treated PV-patients and life expectancy was 70% at 14 years as compared to 83.7% in age-matched controls[102]. The frequency of MF or spent phase PV in the update in 1997 was 17% at 10 years and 40% at 16 years. The final analysis of this 1980 French PVSG study of HU as the upfront first-line therapy in 136 evaluable PV patients younger than 65 years is published in 2011[103].

The cumulative incidence (probability) of myelofibrosis (MF) at 10, 15 and 20 years was 15%, 24% and 32% in the HU arm and the cumulative incidence of AML/MDS at 10, 15 and 20 years was 7.3, 10.7% and 16.6% for HU treated PV patients.

Clear indications for the use of myelosuppressive agents in prodromal and classical PV are uncontrolled platelet count above 1000 x10ˇ/l, thrombotic or bleeding complication while on aspirin, leukocytosis or increasing splenomegalie consistent with early post-polycythemia myelofibrosis. Hydroxyurea is an effective first choice and most frequently used cytoreductive agent to control hypercellular and advanced MPD diasease in PV, although serious doubts persist about its long-term toxicity and leukemogenicity. PEG interferon is a promising alternative as long as it causes no or minor side effects, but its serious side effects can be unbearable in about 25 to 30%[104].

A reasonable approach could be to start with low PEG interferon for PV intermediate MPN burden below the age of 70 to 75 years to test whether it works without significant side effects[93-95,100]. Hydroxyurea is a good first choice for hypercellular PV with significant increase of MPN burden in terms of leukocytosis, splenemegaly and constitutional symptoms and in older PV patients over 65 to 70 years (Table 7).

Whether PEG interferon or hydroxyurea at ages between 18 and 70 years in terms of efficacy, quality of life and life expectancy is the best option remains uncertain (Table 7) and should be addressed in large Prospective Unmet Need (PUN) cross secrtional studies of treated and untreated patients and in large PUN studies of newly diagnosed ET, PV and MF patients during long-term follow-up for 5, 10 to more than 15 years.

Explanation by Michiels 1996-2015. In 1951 Dameshek proposed the unifying theory that the myeloproliferative disorders are all somewhat variable manifestations of proliferative activity of bone marrow cells due to a hypothetical stimulus, which may affect the marrow cells diffusely or irregularly in various myeloproliferative syndromes, which are either clear-cut or transitional (Table 1, right)[7].

Dameshek (1958, 1969) and Michiels (1993, 1997) demonstrated that erythroleukemia including the DiGuglielmo

Table 2 The 1990 Hannover Bone Marrow Classification and diagnostic bone marrow features of Ph-possitive chronic myeloid leukemia and the Ph-negative negative chronic myeloproliferative disorders (CMPD) thrombocythemia, polycythemia vera and chronic or primary megakaryocytic granuocytic myeloproliferation (CMGM/PMGM) and grading of reticulin and collagen bone marrow fibrosis[24]

Classification of CMPDs comparing general terminology with own system and their occurrence among 3,933 BMB from CMPD patients

WHO	Textbooks	Hannover-Classification	%[1]
CML and subtypes	CML or CGL and subtypes	Primary diseases CML CML.CT CML.M	13.9[1]
P. VERA	P. VERA	P. VERA	17
Thrombocythemia[2]	Thrombocythemia[2]	Thrombocythemia[2]	7.1
		CMGM pre-early fibrotic	16
Agnogenic myeloid metaplasia (AMM)	Primary idiopathic myelofibrosis or agnogenic myeloid metaplasia	Advanced disease increase of blasts increase of fibers	33.3
Unclassifiable	Unclassifiable	*Unclassifiable*	12.6

[1]Percentage from 3,933. – [2]Primary or Idiopathic or Essential Thrombocythemia.

Typing of myelosclerosis and – fibrosis within the *Hannover Classification* of CMPD

Typing	Early myelo-sclerosis EMS	Myelosclerosis, myelofibrosis MS/MF	Advanced myelofibrosis AMF
Fiber quality	Reticulin	Reticulin plus Collagen	Collagen
Fiber pattern	focal, patchy	diffuse net-work with patches of Collagen	Scarring
Sinus walls	no	minor sclerosis	fibrosed and extended
Bone sclerosis	no	rare	by definition
Hematopoiesis	unchanged	mostly unchanged	mostly reduced

Table 3 The 2008 WHO Clinical Molecular and Pathobiological (WHO-CMP) criteria for the diagnosis JAK2^{V617F} mutated essential throbocythemia (ET)

Clinical and molecular criteria	Bone marrow pathology (P) criteria (WHO)
JAK2V617F ET 1. Platelet count of >350 x10^9/l and the presence of large platelets in a blood smear 2. Presence of JAK2-^{V617F} mutation 3. Normal erythrocytes <5.8x10^{12}/L males, <6 x10^{12}/L females 4. Normal haemoglobin (Hb) and hematocrit (ht)	**Normocellular ET** Predominant proliferation of enlarged mature megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. Reticuline fibrosis (RF) 0 or 1
JAK2V617F prodromal PV 1. Platelet count of >350 x10^9/l and normal ht male <0.51, female <0.48, normal erythrocyte <5.8x10^{12}/L males, < 6x10^{12}/L females is mandatory. 2. Presence of JAK2-^{V617F} mutation 3. Low serum EPO level and/or increased LAP score 4. Spontaneous EEC.	**ET with bone marrow features of PV** Increased cellularity with due to icreased erytropoiesis or trilineage myeloproliferation (i.e. panmyelosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes. Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis. RF 0 or 1
JAK2V617F hypercellular ET 1. Platelet count of >350 x10^9/l, 2. No signs of leuko-erythroblastosis 3. Slight or moderate splenomegaly on ultrasound 4. Presence of JAK2-^{V617F} mutation 5. No preceding or allied CML, PV, RARS-T or MDS. ET.MGM clinical staging: Early stage: No anemia with hb and ht in the normal low normal range: hb >13g/dl: early clinical stage Intermdiate: Hb < 13 to >12 g/dL, LDH N or ↑, no leukoerythroblastosis Advanced: Hb <10 g/dL, LDH↑↑, CD34+ , leukoerythroblastosis, tear drop	**EMGM** Hypercellular ET due to chronic megakaryocytic and granulocytic myeloproliferation (**EMGM**) and normal or reduced erythroid precursors. Loose to dense clustering of more pleiomorphic megakaryocytes with hyperploid or clumpsy nuclei **(**not or some cloud-like). RF grading PVSG, MF Georgii and Thiele Prefibrotic: RF- 0/1, MF-0, no/minor splenomegaly Bone marrow staging: Early fibrotic ET:RF 2, MF 1, splenomegaly no/minor Fibrotic ET: RF3, RCF or MF2, overt splenomegaly **Post-ET MF: RF3/4, or MF-2/3**

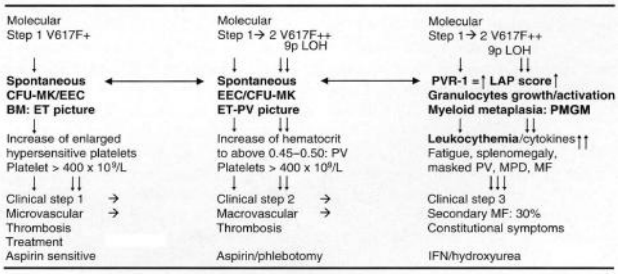
syndrome appears to be a continuum of trilinear myelodysplastic syndrome with significant erythrocytosis running through stages or refactory anemia without (RA) and with excess of blasts (RAEB) followed by leukemic transformation[12].

With the advent of the discovery of the Philadelphia chromosome as a specific and pathognomonic marker for chronic myelocytic leukemia (CML), Gilbert of the Polycythemia Vera Study Group (PVSG) reviewed in 1973 the spectrum and typical patterns of the cellular involvement in the various myeloproliferative disorders indicating the absence of the Ph-chromosome in PV, essential thrombocythemia (ET, minimal platelet count 1000x10^9/L) and myeloid metaplasia of the spleen with myelofibrosis (MMM)[10].

The PVSG separated in 1975 the Ph^1-chromosome positive chronic myeloid leukemia (CML) with various degrees of thrombocythemia and myelofibrosis from the Ph^1-negative ET, PV and idiopathic or agnogenic myeloid metaplasia (AMM) of the spleen with myelofibrosis (MMM). In retrospect, Megakaryocytic Leukemia or ET with platelet count around and above 1000x10^9/L is associated with pronounced increase of megakaryocytes in the bone marrow without features of PV and associated with minor to pronounced myeloid metaplasia of the spleen and myelofibrosis. This entity has been recognized by Georgii *et al*[24-26] as chronic or primary megakaryocytic granylocytic myeloproliferation (CMGM/PMGM) (Michiels 1996, Michiels & Thiele 2002)[2932]. CMGM/PMGM according to the Hannover Bone Marrow Classification is the third distinct MPD entity without features of PV or CML without features of PV (Tables 2 and 3)[24].

Table 5

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



MPD, myeloproliferative disorder; ET, essential thrombocythemia; PV, polycythemia vera; PMGM chronic secondary myelofibrosis; LOH, loss of heterogeneity; CFU-MK, colony-forming units megakaryocytes; EEC, endogenous erythroid colony; LAP, leukocyte alkaline phosphatase; BM, bone marrow; IFN, interferon.

Designed by Michiels *et al* 2006[32]

Microvascular acra: Tingling, prickeling sensations, redness, swelling and/or bluish discolouration of foot soles, hand palms, toes and/or fingers[78,79]. Cognitive disturbances of concentration and memory and sudden attacks of unconscienceness. Visual disturbances of scintillating scotomas, light flashes, blurred vision, transient monocular blindness, rapid spreading of visual figure disturbances[78-82].

Table 4 The 2008 European Clinical and Pathological (2008 ECMP) criteria for the diagnosis of JAK2 mutated polycythemia vera (PV), MPL^{515} mutated 'true 'ET and JAK2 wild type hypercellular ET associated with primary megakaryocytic granulocytic myeloproliferation (PMGM).

Clinical criteria JAK2 mutated PV

A1 **Erythrocyte count above 6x10^{12}/L**, hemoglobin .18.5 g/dL male and >16.5 g/dL females. Raised red cell mass (RCM optional) male .36 ml/kg, female >32 ml/kg (PVSG, WHO)
A2 Persistent increase of platelet count grade I 400-1500, grade II >1500x10^9/I
A3 Splenomegaly on ultrasound or CT (>12 cm) or splenomegaly on palpation
A4 Granulocytes >10x10^9/L or leukocytes >12x10^9/L and raised LAP score >100 in the absence of fever and no increase of ESR
A5 Absence of any cause of primary or secondary erythrocytosis
A6 Low plasma or serum EPO level

Clinical criteria MPL^{515} mutated 'true' ET

A1 Persistent increase of platelet count grade 1 400-1500, grade II > 1500x109/L
A2 Normal spleen or only minor splenomegaly on echogram
A3 Normal LAP score, normal ESR and increased MPV
A4 Spontaneous megakaryocyte colony formation (CFU-Meg)
A5 No signs or cause of reactive thrombocytosis
A6 No preceding or allied other subtype of MPN, PV, MDS or CML
A7 Absence of Philadelphia chromosome

Staging according to no, mild or severe anemia

Clinical criteria JAK2 wild type ET and PMGM

A1 No preceding or allied other subtype of MPN, PV, CML or MDS, JAK2 and MPL wildtype

Early clinical stage: no anemia
Normal hemoglobin, or anemia grade I: hemoglobin >12 g/dL, slight or moderate splenomegaly on palpation or >11 cm on ultrasound or CT. Thrombocythemia>400x10/L
Intermediate clinical stage; mild anemia
Anemia grade II, hemoglobin > 10 g/dL, definitive leuko-erythroblastic blood picture and/or tear-drop erythrocytes. Splenomegaly on palpation, no adverse signs
Advance clinical stage: severe anemia
Anemia grade III, hemoglobin<10 g/dL, significant splenomegaly and one or more adverse signs

Pathological criteria PV

B1 Increased cellularity due to increased erythropoiesis or due to trilinear myeloproliferation of megakaryopoiesis, erythropoiesis and granulopoiesis (e.g. panmyelosis). Proliferation of small medidium sized and large (pleomorphic) megakaryocytes.Absence ofstainable iron, No or slight increase of reticulin fibers.
B2 Sponataneous erythroid colony (EEC) formation

A1 + B1 and none of the others is idiopathic erythrocythemia: IE
A2 + B1 and none of the others is ET with features of PV (prodromal PV)
A3 and B1 and none of the other is primary MPD or latent PV
A1 + B1 plus one of A2 to A6 or B2 is overt classical PV

Pathological criteria MPL^{515} mutated 'true' ET

B1 Predominant proliferation of enlarged to giant megakaryocytes wit hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicious cytological abnormalities
B2 No proliferation or immaturity of granulopoisis or erythropoiesis
B3 No or only borderline increase in reticulin fibers

The combination of A1 and B1 + B2 establish 'true' ET. Any other criterion confirms ET. LAP = leukocyte alkaline phosphatase; ESR = erythrocyte sedimentation rate; MPV = mean platelet volume; MPN = myeloproliferative neoplasm; PV = polycythemia vera; MDS = myelodysplastic syndrome; CML = chronic myeloid leukemia;

Staging myelofibrosis (MF) according to MF grading

Pathological criteria JAK2 wild type PMGM

B1 Megakaryocytic and granulocytic myeloproliferation (MGM) and relative or absolute reduction of erythropoiesis (erythroid precursors. Abnormal clustering and increase of atypical immature medium-sized large to giant megakaryocyte containing (Cloud-like) hypolobulated nucle and definitive maturation defects

Staging of myelofibrosis: MF in PV and PMGM

MF 0 no reticulin fibrosis RF 0/1
MF 1 slight reticulin fibrosis RF 2
MF 2 marked increase RF grade 3 and slight to moderate collagen fibrosis
MF 3 advanced collagen fibrosis-osteosclerosis (endophytic bone formation)

Table 6 Top 20 clinical manifestations in patients with who defined myeloproliferative neoplasm (MPN) essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF) based on the Dutch MPN Questionaire 2009-2010[64]

Symptom	Top 20 MPN complaints	All MPN	MPN	ET	PV	MF
		N=497	%	%	%	%
1	Fatigue, listless	399	81	80	81	85
2	**Microvascular acra**[79]	278	57	61	56	46
3	**Cognitive disturbances**[80,81]	262	53	52	56	45
4	**Visual disturbances**[80,81]	249	51	50	52	46
5	Night sweats	236	48	44	50	52
6	Itching	220	45	30	58	36
7	Dizziness	218	44	44	46	39
8	Bruises, bleedings	211	43	40	45	43
9	**Splenomegaly constitutional symptoms**	198	40	22	43	78
10	Tinnitus	188	38	38	39	37
11	Migraine headache without visual symptoms	184	37	46	35	22
12	Bone pain	172	35	33	36	34
13	Heart arrythmias	154	31	34	31	24
14	Dysarthria, dyslexia,	151	31	31	31	30
15	Hypersensitive to sounds and noices	149	30	29	32	28
16	Paleness	145	29	30	26	40
17	Claudicatio intermittens	140	28	28	30	24
18	Hypersensitive to lights	136	28	25	32	16
19	Visual disturbances without headache	18	33	54	3	90
20	Headache without visual symptoms	24	43	43	4	90

Table 7 Staging of $JAK2^{V617F}$ positive prodromal PV, erythrocythemic PV, classical PV, early MF, inapparent PV, spent phase PV and post-PV myelofibrosis (MF) according to WHO-ECMP criteria related to therapy anno 2008 (Black) and 2014 (Red)[93-95].

PV: WHO-ECMP stage	0	1	2	3	4	5	6
WHO-ECMP Clinical Diagnosis	Prodromal PV	Erythrocy-themic PV	Early PV	Manifest PV Classical PV	PV early MF Masked PV	Inapparent PV-MF	Spent PV Post-PV MF
LAP-score	↑	↑	↑	↑	↑/↑↑	↑	variable
EEC	+	+	+	+	+	+	+
Serum EPO	N/↓	N/↓	↓	↓	↓	↓	variable
Erythrocytes $x10^{12}$/l	>5.8	<5.8	>5.8	>5.8	>5.8	Normal <5.5	Decreased
Leukocytes $x10^{9}$/l	<12	<12	<or >12	< or->15	>15	N or ↑	>20
Platelets $x10^{9}$/l	>400	,400	< or >400	>400	< or >1000	N low or ↑	variable
WHO-ECMP bone marrow	Early PV	Early PV	Early PV	Trilinear PV	Trilinear PV	Prilinear PV	Myelofibrosis
Bone marrow cellularity (%)	50-80	50-80	60-100	80-100	80-100	60-100	Decreased
Grading reticulin fibrosis: RF	RF 0-1	RF 0-1	RF 0-1	RF 0/1,	RCF1/2/3	RCF 1/2/3	RCF 3/ 4
Grading myelofibrosis: MF[57]	MF 0	MF 0	MF 0	MF 0	MF 0/1	MF 0/2	MF 2/3
Splenomegaly on palpation	No/+	No	No/+	+	++/+++	++/+++	/large
Spleen size, echogram cm	<12-15	<13	Dec-15	Dec-16	18->20	16 >20	>20
Spleen size on palpation cm	0-3	NP	0-3	04-Jun	>6	>6	>8
$JAK2^{V617F}$ in Granulocytes %	low	low	Moderate <50	High >50	High >50	Mod/High	High >50
$JAK2^{V617F}$ in BFU-e (exon 12)	+(++)	+(++)	+(++)	++	++	+	++
Risk stratification à Therapeutic implications Anno 2014	Low risk	Low risk	Low risk	Intermediate risk PV	High risk P Vearly MF	IFN JAK2	Post-PV MF Spent phase PV
First line Aspirin/Phlebotomy	Aspirin	Aspirin	Phlebotomy	Phlebotomy*	If IFN resistant à	IF IFN	JAK2
Second line IFN versus	Phlebotomy	Phlebotomy	Aspirin	Aspirin	HU or JAK2	Resistent	Inhibitor à
Hydroxyurea (HU)			Low dose IFN à responsive	IFN à resistant à HU	inhibitor	JAK2	Bone marrow
Third line JAK2 inhibitor						inhibitor	transplant

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

Treatment recommendation of Polycthemia Vera related to MPN disease burden

Attacks of migraine-like headaches followed by nausea or vomiting or loss of consciencenous or transient paresis of one extermity[78-82].

Legend to the figures

Figure 1 left. The concept of Dameshek in 1950[5] on polycythemia vera (PV) as a trilinear myeloproliferative disorder (MPD) due to an unknown excessive bone marrow stimulating factor and/or a lack or a diminution in the normal inhibitory factor has been overlooked by all PVSG and WHO investigators on myeloproliferative disorders (MPD) and myeloproliferative neoplasms (MPN). The one cause hypothesis of trilinear PV proposed by Dameshek in 1950[5] has been confirmed by Vainchenker in 2005[6] by the discovery of the heterozygous and homozygous $JAK2^{V617F}$ mutation as the driver cause of three phenotypes of the MPDs ET, PV, masked PV and MF. Figure 1 right. The PVSG and WHO classifications of the MPDs and MPN are based on the unifying MPD concept of Dameshek in 1951[7] on ET, PV, AMM, and CML, which has been splitted by the PVSG in 1975 into Ph^{1}-

positive thrombocythemia and CML and the Ph[1]-negative MPDs ET, PV and MF. With the advent of the JAK2V617F mutation in 2005[6], MPL515 mutations in 2006[71-73], the ECMP distibguished three distinct clonal MPN: trilinear MPN ET, PV and MF; JAK2 wild type MPL mutated ET/MF and JAK2/MPL wild type primary megakaryocytic granulocytic myeloprolferation (PMGM). .

Table 8 The incidence of major thrombosis in polycythemia vera (PV) in remission by phlebotomy alone and not on aspirin and in PV before and after treatment with P32 the effect[12]

Reference	Number of patients	Arterial thrombosis	Peripheral vascular occlusion	Cerebro vascular accident	Coronary artery disease
		%	%	%	%
Normal Allen 1937	98	20	3	5	6.1
Videback 1950	125	24	8	10	0.8
Burris 1953	68	22	7.4	7.4	4.4
Edwards 1970	26	61	34	23	15
Barabas 1973	200	34	12.5	19.5	5
Total	517	31.3	10	13.1	4.4

Reference	number	before	after P^{32}
Lawrence 1949	121	25 %	4.2%
Stroebel 1951	143	37.5%	4.7%
Wasserman 1959	128	24 %	5.5%
Wasserman 1976	158	15 %	1.9%

Source, Michiels 1996[12]

Figure 2, left upper.

Microplanic studies on megakaryocytes in chronic granulocytic leukemia (CGL=CML) and polycythemia vera (PV untreated) showed that the average size of megakaryocytes in CGL is less than normal. In PV the size of megakaryocytes is larger than normal[19,83].

Figure 2, left lower.

Small sized megakaryocytes with monolobulated and bilobulated nuclei in a bone marrow smear from the patients with BCR/ABL+ ET, who developed BCR/ABL+ megakakaryoblast leukemia (Michiels *et al* 1987[19])

Figure 2 right. $JAK2^{V617F}$ positive prodromal polycythemia vera (PV): Red Blood Cells $5.37x10^{12}$/L, Hemoglobin 15.8 g/dL, MCV 89, Leukocytes 12 $x10^9$/L, Platelets $517x10^9$/L, LDH 600 UI/L ($JAK2^{V617F}$ mutation allele burden 20%), Hypercellular bone marrow, with mild erythroid hyperplasia, moderate myeloid hyperplasia and marked hyperplasia of large hyperlobulated megakaryocytes and no reticulin fibrosis.

Figure 3. Normocellular essential thrombocthemia diagnosedin 2003 with a more than 20 years stable disease who was heterozygous positive for the $JAK2^{V6167F}$ mutation in 2006[32].

Figure 4. Left. JAK2V617F positive ET at platelet count of 453 x109/L. Large platelets in peripheral blood smear B as compared to control A (upper panels). Bone marrow smear:showing large megakaryocytes with multilobulated nuclei B as compared to control A (middle panels). Bone marrow biopsy hypercellular due to increased erythropoiesis and clustered large pleomorphic megakaryocytes (Lower panels

Figure 5. Right..Bone marrow features of homozygous JAK2V617F acute onset PV (right) showing hypercellular bone marrow due to increased erythro-granulo=megakaryopiesis (trilinear MPN) and increase of large pleiomorphic megakaryocytes.

Figure 5. Hypercellular ET entity presenting with **JAK2 wild type** prefibrotic (right panel) primary megakaryocytic and granulocytic myeloproliferation (PMGM, left and middle), which is characterized by a hypercellular bone marrow due to dual myeloproliferation of granulopoiesis and dense clustered enlarged immature dysmorphic megakaryocytes (left and middle panels) with bulky (bulbous) hyperchromatic nuclei (arrows) (personal observation), which are never seen in JAK2 wild type PT and also not in the prefibrotic JAK2V617F mutated ET, prodromal PV, EMGM and trilinear PV entities.

Figure 6. Clinical MF, JAK2 wild type ET: platelets $1430x10^9$/L, anemia hemoglobin 11.3 g/dL, splenomgaly and high LDH 2730 → JAK2 wild type hypercellular (65-70%) bone marrow associated with early fibrotic PMGM, reticulin fibrosis grade. (Dr De Raeve)

Figure 7. Molecular pathogenesis on the natural history of heterozygous into homozygous $JAK2^{V617F}$ mutated trilinear myeloproliferative neoplasms (MPN) and associated disease progression of the sequential occurrence of the clinical phenotypes essential thrombocythemia (ET), polycythemia vera (PV), and idiopathic myelofibrosis (IMF) related to increased JAK2/Stat kinase activity (Courtesy of Dr Villeval *et al* 2006)[55].

Figure 8. Genotype of individual BFU-E in ET and PV patients with granulocytes heterozygous for the $JAK2^{V617F}$ mutation: JAK2 allele load <50% according to Scott *et al*[85]. Reproduced by the Courtesy of Dr Green, Cambridge[57].

Figure 9. Dutch 2008 survey of 363 MPN (123 ET, 190 PV and 50 MF) patients 93% of PV, 71% of ET and 37% of MF were on aspirin, phlebotomy in 6% of ET, 78% of PV and 9% of MF. Because of symptomatic MNP disease burden 31% of ET, 29% of PV and 30% of MF were on treatment with hydroxyurea and 16% of ET, 16% of PV and 4% of MF were on treatment with pegylated interferon ($Pegasys^R$)[64]

Figure 10. JAK2 wild type, MPL515 mutated ET with enlarged and giant mature megakaryocytes with loose clusters of hyperlobulated, "stag-horn" hyperlobulated nuclei. Case 1, upper panel Case 2 lower panel, Bone marrow slides were kindly provided in 2008 by Dr. Vannucchi, Italy[95].

Figure 11. Algorithm for diagnostic work-up for patients with suspected thrombocythemia as the presenting feature of ET, early PV, prefibrotic CIMF-0 (ET-MGM), early fibrotic CIMF-1 or refractory anemia with increased ringed sideroblasts (RARS-T)[112]. Designed by Michiels 2008. For explanation see text.

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

Figure 13. Neutrophil JAK2^{V6217F} allele percentages (%) related to disease in 36 PVSG-defined ET and 77 PV patients (upper left)[91]. Median neutrophil JAK2^{V6217F} allele % were significantly higher in PV than those for ET, regardless of disease duration. Within PV the differences in neutrophil JAK2^{V6217F} allele % as a function of disease duration were not statistically significant. This may be indicative for good risk PV and poor risk PV with neutrophil JAK2^{V6217F} allele burden between 30 to 80% and between 80 and 100% respectively.
Neutrophil genomic DNA and platelet cDNA from the same blood samples in 13 ET and 23 PV patients (upper right)[91]. First, median neutrophil JAK2^{V6217F} allele % in PV were greater than in ET (P=<0.001). Second, median platelet JAK2^{V6217F} allele % in ET were lower than in PV (P=<0.002). Third, median neutrophil JAK2^{V6217F} allele % in ET were lower than platelet JAK2^{V6217F} allele % in ET (P=<0.001). Reproduced with the courtesy of Dr Jerry Spivak, Baltimore, USA[91].

Figure 14. Granulocyte JAK2^{V617F} mutation burden (%) in 23 PV, 10 ET, 7 prefibrotic CIMF (p-CIMF) 10 fibrotic CIMF (f-CIMF) and 16 post-PV MF patients (lower left)[56]. First, patients with PV had higher JAK2^{V6217F} % than ET (p=0.01) and p-CIMF. Second, patients with p-CIMF had much lower JAK2^{V6217F} allele % than f-CIMF. Third, patients with post-PV myelofibrosis (MF) had the highest JAK2^{V6217F} allele %.
Circulating CD34 positive cells (lower right)[56]: all patients with PV (N=25), ET (N=19) and p-CIMF, (N=12) and 6 out of 18 f-CIMF patients had normal (<10x10^6/L) circulating CD34 cells. Conversely, 12 out f 18 f-CIMF and all 16 post-PV MF patients had increased CD34 circulating cells. These data indicate that ET and p-CIMF are not different at the molecular (JAK2^{V6217F}) and biological (CD34 cells) level. This arises the question whether WHO defined JAK2^{V6217F} p-CIMF and ET are the same (PVSG-defined ET) or distinct in their natural history during long-term follow-up. Reproduced by the courtesy of Dr Francesco Passamonti, Pavia, Italy[56].

References

1. 2008 WHO criteria for polycthemia vera, primary myelofibrosis and essential thrombocythemia. Thiele *et al* In: Swerdlow SH, Campo E, Harris NL *et al*: WHO Classification of Tumours of Haematopoietic and Lympoid Tissues. Lyon France IARC Press 2008;40-50.
2. Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, Harrison C, Hasselbalch KC, Hehlmann R, Hoffman R, Kiladjian JJ, Kroeger N, Mesa R, McMullin MF, Pardanani A, Passamonti F, Vannucchi AM, Reiter A, Silver RT, Verstovsek V, Tefferi A. Philadelphia-Negative Classical Myeloproliferative Neoplasms: Critical Concepts and Management Recommendations From European LeukemiaNet. *J Clin Oncol* 2011
3. Barbui T, Finazzi G, Carobbio G, Thiele J, Passamonti F, Rumi E, Ruggeri M, Rodeghiero F, Randi ML, Bertozzi I, Gisslinger7 H, Buxhofer-Ausch V, De Stefano V, Betti S, Rambaldi A, Vannucchi AM, Tefferi A. Development and validation of a International Prognostic Score of thrombosis in World Health Organization- essential thrombocythemia (IPSET-thrombosis0. Blood 2012; 120(26):5128-5133.
4. Berk PD, Goldberg JD, Silverstein MN *et al*. Increased incidence of acute leukemia in polycythemia vera associated with chlorambucil therapy. *N Eng J Med* 1981; 304:441-447.
5. Berlin NI. Diagnosis and classification of the polycythemias. Sem Hematol 1975; 12:339-351.
6. Campbell P, green AR. The myeloproliferative disorders. *New Eng J Med* 2006; 355:2452-2466.
7. Campbell P, Scott LM, Buck G *et al*. Definition of essential thrombocythemia and relation of essential thrombocythemia to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet 2005;366: 1945-1953.
8. Campbell PJ, Baxter EJ, Beer PhA *et al*. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and the role in leukemic transformation. Blood 2006;18:3548-3555.
9. Cervantes F, Alvarez-Larran A, Talarn C, Gomez M, Montserrat E. Myelofibrosis with myeloid metaplasia following essential thrombocythaemia: actuarial probability, presenting characteristics and evolution in a series of 195 patients. *Br J Haematol* 2002;118:786-790.
10. Chen Z, Notohamiprodjo M, Guan XX *et al*. Gain of 9p in the pathogenesis of polycythemia vera. Genes Chromosomes & Cancer 1998; 22:321-324.
11. Commandeur S. 500 MPD-ers onder de loep. Resultaten MPD enquete. PUR SANG 2010; 7(1):12-15.
12. Cortelazzo S, Viero P, Finazzi G, D'Emilio A, Rodeghiero F, Barbui T. Incidence and risk factors for thrombotic complications in a historical cohort of 100 patients with essential thrombocythemia. *J Clin Oncol* 1990; 8:556-562.
13. Dameshek W, Henstell HH. The diagnosis of polycythemia. Ann Intern Med 1940:13:1360-1387.
14. Dameshek W. Physiopathology and course of polycythemia vera as related to therapy. *J Am Med Ass* 1950; 142:790-797.
15. Dameshek W. Some speculations on the myeloproliferative syndromes. Blood 1951;6:372-375.
16. De Klein A, Van Kessel AG, Grosveld G G *et al*. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukemia. Nature 1982;300:765-767.
17. Delhommeau F, Pisani DF, James C, Casadevall N, Constatinescu S, Vainchenker W. Oncogenic mechanism in myeloproliferative disorders. Cell Mol Life Sci 2006; 63:2939-2953.
18. Dobo I, Donnard M, Giridon F, Mossuz P *et al*. Standardization and comparison of endogenous erythroid colony assays performed with bone marrow or blood progenitors for the diagnosis of polycythemia vera. *Hematol J* 2004; 5:161-167.
19. Dupont S, Massé A, James C *et al*. The JAK2V617F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from

patients with polycythemia vera. Blood March 27, 2007 Ahead of Pub.

20. Gatterman N, Billiet J, Kronenwett R, Zipperer E, Germing U, Nollet F, Criel A, Selleslag D. High frequency of the JAK2 V617F mutation in patients with thrombocytosis (platelet count >600 x10^9/l) and ringed sideroblasts more than 15% considered as MDS/MPD, unclassifiable. Blood 2007; 109:1334-1335.
21. Georgii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. Bailière's Clin Haematol 1998; 11:721-749.
22. Georgii A, Buhr T, Buesche G, Kreft A, Choritz H. Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. Leukemia and Lymphoma 1996;22(Suppl 1):15-29.
23. Georgii A, Vykoupil KF, Buhr Th, Choritz H Doehler U, Kaloutsi V, Werner M. Chronic myeloproliferative disorders in bone marrow biopsies. Path Res Pract 1990; 186:3-27.
24. Gilbert HS. The spectrum of myeloproliferative disorders. Med Clin North Amer 1973; 57(2):355-393.
25. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoits are clustered within a limited region, bcr on chromosome 22. Cell 1984; 35:93-99.
26. Haanen C, Mathe G, Hayat M. treatment of polycythemia by radioactive phosphor or busulphan; a randomized trial. *Br J Cancer* 1981:44:75-80.
27. Heisterkamp N, Groffen J., Stephenson JR, Spurr NK, Goodfellow PN, Solomon E, Carrit B, Bodmer WF. Chromosomal localization of human cellular homologues of two viral oncogenes. Nature 1982;299:747-749
28. James C, Ugo V, Le Couedic PF, Staerk J, Delhommeau F, Lacout C *et al*. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythemia vera. Nature 2005; 434:1144-1148.
29. Jantunen R, Juvonen E, Ikkala E, Oksanen K, Antilla P, Ruutu T. Development of erythrocytosis in the course of essential thrombocythemia. Ann Hematol 1999; 78:219-222.
30. Johansson P, Andreason B, Safai-Kutti S, Wennstrom L, Palmqvist L, Rickson A, Lindstedt G, Kutti J. The presence of a significant association between elevated PRV-1 mRNA expression and low plasma erythropoietin concentration in essential thrombocythemia. *Eur J Haematol* 2003; 70:358-362.
31. Johansson PL, Safia-Kutti S, Kutti J. An elevated venous haemoglobin concentration cannot be used as a surrogate marker for absolute erythrocytosis: a study of patients with polycythemia vera and apparent polycythaemia. *Br J Haematol* 2005; 129:701-705.
32. 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;87:6649-6653, with correction 1990;87:9072.
33. Kiladjian JJ, Cassinat B, Turlure P, Cambier N, Roussel M, Bellucci S, Menot ML, Massonnet G, Dutel JL, Ghomari K, Rousselot P, Grange MJ, Chait Y, Vainchenker W, Parquet N, Abdelkader-Aljassem L, Bernard JF, Rain JD, Chevret S, Chomienne C, Fenaux P. High molecular response rate of polycythemia vera treated with peglyated interpheron-alpha-2a. Blood 2008; 108:1281.
34. Kiladjian JJ, Chrevet S, Dosquet C, Chomienne C, Rain JD. Treatment of Polycythemia Vera With Hydroxyurea and Pipobroman: Final Results of a Randomized Trial Initiated in 1980. *J Clin Oncol* 2011;29:3907
35. Kvasnicka HM, Thiele J. The impact of clinicopathological studies on staging and survival in ET, PV and IMF. Sem Thromb Hemostas 2006;32:362-371.
36. Lamy T, Devillers A, Bernard M, Moisan A, Grulois I, *et al*.Inapparent polycythemia vera: an unrecognized diagnosis. *Am J Med* 1997;102: 14-20.
37. Lengfelder E, Hochhaus A, Kronawitter U *et al*. Should a platelet count of 600 x10^9/l be used as a diagnostic criterion in essential thrombocythemia? An analysis of the natural course including early stages. *Br J Haematol* 1998; 100:15-23.
38. Liu E, Jelinek J, Pastore YD, Guan Y, Prchal JF. Discrimination of polycythemias and thrombocytoses by novel simple, accurate clonality assays and comparison with PRV-1expression and BFU-e responses to erythropoietin. Blood 2003; 101:3294-3301.
39. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr/abl oncogene products. Science 1990; 247:1079-1082.
40. Messinezy M, Westwood NB, El-Hemaida I, Marsden JT, Sherwood RS, Pearson TC. Serum erythropoietin values in erythrocytoses and in primary thrombocythaemia. *Br J Haematol* 2002; 117:47-53.
41. 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; 102:466-471.
42. Michiels JJ, Barbui T, Fruchtman SM, Kutti J, Rain JD, Silver RT, Tefferi A, Thiele J. Diagnosis and treatment of polycythemia vera and possible future study designs of the PVSG. Leukemia Lymphoma 2000;36:239-253.
43. 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 polycythemia vera: therapeutic implications. Sem Thromb Hemostas 2006; 32:174-207.
44. Michiels JJ, Berneman Z,Schroyens W, De Raeve H. Changing concepts of diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms: From Dameshek 1950 to Vainchenker 2005 and beyond. Acta Haematol 2015; 133:36-51.
45. Michiels JJ, Berneman Z, Schroyens W, De Raeve H. PVSG andWHO vs European Clinical, Molecular andPathological (ECMP) criteria for prefibrotic myeloproliferative neoplasms. *World J Hematol* 2013;2:71-88.
46. Michiels JJ, Berneman ZW, Schroyens W, Kutti J, Swolin B, Ridell B, Fernando P, Zanetto U.

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; 83:504-512.

47. Michiels JJ, Commandeur S, Hoogenboom GJ, Wegman JJ, Scholten L, van Rijssel RH, De Raeve H. JAK2V617F positive early stage myeloproliferative disease (essential thrombocythemia) as the cause of portal vein thrombosis in two middle-aged females: therapeutic implications in view of the literature. Ann Hematol 2007; 31:1031-1038.
48. Michiels JJ, De Raeve H, Berneman Z,, Van Bockstaele D,, Hebeda K, Lam K, Schroyens W. The 2001 World Health Organization (WHO) and updated European clinical and pathological (ECP) criteria for the diagnosis, classification and staging of the Ph^1-chromosome negative chronic myeloproliferative disorders (MPD). Sem Thromb Hemostas 2006; 32:307-340.
49. Michiels JJ, De Raeve H, Hebeda K, Lam KH, Berneman Z, Schroyen W, Schwarz J. WHO bone marrow features and European clinical molecular and pathlogical criteria for the diagnosis and classification of myeloproliferative disorders. Leuk Res 2007; 31:1031-1038.
50. Michiels JJ, Koudstaal P, Mulder AH, van Vliet HHDM. Transient neurologic and ocular manifestations in primary thombocythemia. Neurology 1993; 43:1107-1110.
51. Michiels JJ, Kutti J, Stark P, Bazzan M, Gugliotta L, Marchioli R, Griesshammer M Van Genderen PJJ, Brière J. Kiladjian JJ, Barbui T, Finazzi G, Berlin NI, Pearson TC, Green AC, Fruchtman SM, Silver RT, Hansmann E, Wehmeier A, Lengfelder E, Landolfi R, Kvasnicka HM, Hasselbalch H, Cervantes F, Relly JT, Demory JL, Gisslinger H, Guardiola Ph, Martyré MC, Le Bousse-Kerdiles MC, Thiele J. Diagnosis, pathogenesis and treatment of the myeloproliferative disorders essential thromboythemia, polycythemia vera and essential megakaryocytic granulocytic myeloproliferation and myelofibrosis. *Neth J Med* 1999;54:46-62.
52. Michiels JJ, Kvasnicka HM, ThieleJ. MPD Doctors Brochure. http://www.mpn-stichting.nl/doctors_brochure_2004.pdf
53. Michiels JJ, Prins ME, Hagemeijer A, Brederoo P *et al*. Philadelphia chromosome-positive thrombocythemia and megakaryoblast leukemia. *Am J Clin Pathol* 1987;88:645-652.
54. Michiels JJ, Ten Kate F, Lam KH, Schroyens W, Berneman Z, De Raeve H. The European clinical, molecula and pathological (ECMP) criteria and the 2007/2008 revisions of the World Health Organization for the diagnosis, classification and staging of the prefibrotic myeloproliferative neoplasms carrying the JAK2V617F mutation. *Turk J Hematol* 2014; 31:136-142.
55. Michiels JJ, Ten Kate FWJ, Koudstaal PJ, Van Genderen PJJ. Aspirin responsive platelet thrombophilia in essential thrombocythemia and polycythemia vera.*World J Hematol* 2013; 2:20-43.
56. Michiels JJ, Ten Kate FWJ, Vuzevski VD, Abels J. Histopathology of erythromelalgia in thrombocythemia. Histopathology 1984;8:466-471.
57. Michiels JJ, Thiele J Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera and idiopathic myelofibrosis (agnogenic myeloid metaplasia). *Int J Hematol* 2002; 76:133-145.
58. Michiels JJ, van Genderen PJJ, Lindemans J, van Vliet HHDM. Erythromelalgic, thrombotic and hemorrhagic manifestations in 50 cases of thrombocythemia. Leukemia & Lymphoma 1996;22(suppl 1):47-56.
59. Michiels JJ. The myeloproliferativedisorders. An historical appraisal and personal experiences. Leuk Lymph 1996; 22(Suppl 1):1-14.
60. Moliterno AR, Williams DM, Rogers O, Spivak JL. Molecular mimicry in the chronic myeloproliferative disorders: reciprocity between quantitative JAK2V617F and MPL expression. Blood 2006; 108:3913-3915.
61. Mossuz P, Giridon F, Latger-Cannard V, Dobo I *et al*. Diagnostic value of serum erythropoietin level in patients with absolute erythrocytosis. Haematologica 2004; 1194-1198.
62. Murphy S, Iland H, Rosenthal D, *et al*. Essential thrombocythemia: An interim report from the Polycythemia Vera Study Group. Semin Hematol 1986; 23:177–182.
63. Naifeld V, Montella L, Scalise A, Fruchtman S. Exploring polycythemia vera with fluorescence in situ hybridization: additional cryptic 9p is the most frequent abnormality detected. *Br J Haematol* 2002;119:558-566.
64. Najean Y, Rain JD. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. Blood 1997;90:3370-3377.
65. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960; 132:1497.
66. Osler W. A clinical lecture on erythraemia. Polycythemia with cyanosis, maladie de Vaquez. Lancet 1908 ; I : 143-146
67. Osler W. Chronic cyanosis with polycythaemia and enlarged spleen: a new entity. Amer J Med Scs 1903;CXXVL:187-201
68. Pardanani A, Levine RL, Lasho TL *et al*. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 2006; 108:3472-3476.
69. Passamonti F, Rumi E, Pietra D *et al*. Relation between JAK2 V617F mutation status, granulocyte activation, and constitutive mobilization of $CD34^+$ cells into peripheral blood in myeloproliferative disorders. Blood 2006; 107:3676-3682.
70. Passamonti F, Thiele J, Girodon F, Rumi E, Carobbio A, Gisslinger H, Kvasnicka HM, Ruggeri M, Randi ML,Gangat N, Vannucchi AM, Gianatti A, Gisslinger

B, Muellauer L, Rodeghiero F, d'Amore ESG, Bertozzi I, Hanson CA,Boveri E,Marino F, Maffioli M, Caramazza D, Antonioli E, Carrai V, Buxhofer-Ausch V, Pascutto C, Cazzola M, Barbui T,Tefferi A. A prognostic model in 867 patients to predictwolrd Health Organization defined essential thrombocythemia at diagnosis. Blood 2012;120(6):1197-1201

71. Pearson TC, Grimes AJ, Sender NGP,, Wetherley-Mein G. Vascular occlusive episodes and venous haematocrit in primary proliferative polycythemia. Lancet 1978;II:1219-1222.
72. Pikman Y, Lee BH, Mercher Th *et al*. MPLW515L is a novel somatic activation mutation in myelofibrosis with myeloid metaplasia. PLOS Med 2006;3:July 2006.
73. Prchal JF, Axelrad A, Crookston JH. Erythroid colony formation in plasma culture from cells of peripheral blood in myeloproliferative disorders. Blood 1974;44:912.
74. Prchal JF, Axelrad AE. Bone marrow responses in polycythemia vera. *N Eng J Med* 1974;290:1382 (Letter).
75. Rowley J. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence Giemsa staining. Nature 1973;243:290-291.
76. Schmitt-Graeff A, Thiele J, Zuk I, Kvasnicka HM. Essential thrombocythemia with ringed sideroblasts: a heterogenous spectrum of diseases, but not a distinct entity. Haematologica 2002;87:392-399.
77. Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F JAK2 mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. Blood 2006;108:2435-2437.
78. Scott LM, Tong W, Levine RL *et al*. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *New Eng J Med* 2007;356:459-468
79. Shaw GR. Ringed sideroblasts with thrombocytosis: an uncommon mixed myelodysplastic/myeloproliferative disease of older adults. *Br J Haematol* 2005131:180-184.
80. Shephard PCA, Ganesan TS, Galton DAG. Haematological classification of the chronic myeloid leukemias. Baillière's Clin Haematol 1987 ;1:887-906.
81. Shih LY, Lee CT. Identification of masked polycythemia vera from patients with idiopathic thrombocytosis by endogenous elytroid colony assay. Blood 1994;83:744-748.
82. Sirhan S, Fairbanks VG, Tefferi A. Red cell mass and plasma volume measurements in polycythemia. Cancer 2005;104:213-215.
83. Szpurka H, Tiu R, Murugesan G, Aboudola S, His ED, Theil KS, Skeres MA, Maciejewski J. Refractory anemia with ringed sideroblasts associated with matked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2V617F mutation. Blood 2006;108:2173-2181.
84. Tefferi A, Lasho TL, Schwager SM *et al*. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia. Cancer 2006;106:631-635.
85. Tefferi A, Thiele J, Orazi A, Kvasnicka HM *et al*. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Blood 2007;110:1092-1097.
86. Tefferi A. Polycythemia vera and essential thrombocythemia: 2012 update on diagnosis, risk stratification and management. *Am J Hematol* 2012;87:285-293
87. 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 and Lymphoma 1999;33:207-218.
88. Thiele J, Kvasnicka HM, Facchetti F, Franco V, Van Der Walt J, Orazi A. European consensus for grading of bone marrow fibrosis and assessment of cellularity in myeloproliferative disorders. Haematologica 2005;90:1128-1132.
89. Thiele J, Kvasnicka HM, Schmitt-Graeff A, Zankovich R, Diehl V. Follow-up examinations including sequential bone marrow biopsies in essential thrombocythemia (ET): a retrospective clinicopathological study of 120 patients. *Am J Hematol* 2002;70:283-291.
90. Thiele J, Kvasnicka HM, Werden C, Zankovich R, Diehl, Fischer R. Idiopathic primary osteomyelofibrosis: A clinico-pathological study on 208 patients wit special emphasis on evolution of disease features, differentiation from essential thrombocythemia and variables of prognostic impact. Leukemia and Lymphoma 1996;22:303-317.
91. Thiele J, Kvasnicka HM. Chronic myeloproliferative disorders with thrombocythemia: a comparative study of two classification systems (PVSG, WHO) on 839 patients. Ann Hematol 2003;82:148-152.
92. Thiele J, Schneider G, Hoeppner B, Wienhold S, Zankovich R, Fischer R. Histomorphometry of bone marrow biopsies in chronic myeloproliferative disorders with associated thrombocythosis – features of significance for the diagnosis of primary (essential) thrombocythemia. Virch Arch A Path Anat 1988;413:407-417.
93. Thiele J, Zankovich R, Schneider G, Kremer B, Fischer R, Diehl V. Primary (essential) thrombocythemia versus polycythemia rubra vera. A histomorphometric analysis of bone marrow features in trephine biopsies. Analyt Quat Cytol Histol 1988;10:375-382.
94. Thiele J, Zankovich R, Steinberg T, Fischer R, Diehl V. Agnogenic myeloid metaplasia (AMM) – correlation of bone marrow lesions with laboratory data: a longitudinal clinicopathological study on 114 patients. Hematol Oncol 1989;7:327-343.
95. Thiele J, Zankovich R, Steinberg T, Kremer B, Fischer R, Diehl V. Primary (essential) thrombocythemia versus hyperplastic stages of agnogenic myeloid metaplasia with thrombocytosis: a critical evaluation of clinical and histomorphological data. Acta Haematol 1989;81:192-202.

96. Vannucchi A, Antonioli E, Guglielmelli P, Rambaldi A, Barosi G, Marchioli R, Marfisi RM, Finazzi G, Guerini V, Fabris F, Randi ML, De Stefano V, Gaberlon S, Tafuri A, Ruggeri M, Spechia G, Liso V, Rossi E, Pogliani E, Gugliotta L, Bosi A, Barbui T. Clinical profile of homozygous JAK2V617F mutation in patients with polycythemia vera and essential thrombocythemia. Blood 2007; 110:840-846.
97. Vannucchi AM, Antonioli E, Guglielmelli P *et al*. Charateristics and clinical correlates of MPL515W>L/K mutation in essential thrombocythemia.. Blood 2008;112:844-847.
98. Vaquez MH. Sur une forme speciale de cyanose s'accompagnant d'hyperglobulie excessive et persitante. Compte Rendues des seances de la Societé de Biologie 1892 ; 44 :384-388.
99. Villeval JL, James C, Pisani DF, Casadevall N, Vainchenker W. New insights into the pathogenesis of JAK2V617F-positive myeloproliferative disorders and consequences for the management of patients. Sem Thromb Hemostas 2006; 32:341-351.
100. Wasserman LR, Berk PD, Berlin NI. Polycythemia vera and the myeloproliferative disorders. WB Saunders Philadelphia 1995, ISBN 0-7216-4213-6.
101. Westwood NB, Pearson TC. Diagnostic applications of haematopoietic progenitor culture techniques in polycythaemias and thrombocythaemias. Leukemia Lymphoma 1996;22(Suppl 1):95-103.
102. WHO classification of the chronic myeloproliferative diseases (CMPD) polycythemia vera, 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 pp 31-42.
103. Wolansky A, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors Mayo Clin Proc 2006;81:159-166.
104. Zhou W, Toombs CF, Zou T, Guo J, Robinson MO. Transgenic mice overexpression human c-mpl ligand exhibit chronic thrombocytosis and display enhanced recovery from 5-fluoruracil or antiplatelet serum treatment. Blood 1997; 89:1551-1559.

How to cite this article:

Jan Jacques Michiels *et al.,* Hannover Bone Marrow Classification Of Chronic Myeloproliferative Disorders And The 2008 European Clinical, Molecular And Patholobiogical (2008 Ecmp) Criteria For Classification And Staging Of Myeloproliferative Neoplasms: Prognostic Factors And Therapeutic Implications 1950-2015. *Int J Recent Sci Res Vol. 6, Issue, 10, pp.6539-6556, October, 2015*

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