

The WHO clinical, molecular and pathological (WHO-CMP) classification of five distinct clonal myeloproliferative neoplasms caused by JAK2, MPL and CALR driver mutations

Key words: essential thrombocythemia, polycythemia vera, myelofibrosis, bone marrow pathology, JAK2 mutation, MPL mutation, calreticulin mutation

Abstract

The WHO Clinical, Molecular and Pathologic (WHO-CMP) criteria the JAK2^{V617F} positive ET patients distinguishes five distinct clonal MPNs: JAK2^{V617F}-positive ET and PV; JAK2 exon 12 PV; JAK2 wild type ET and MF carrying the MPL⁵¹⁵; JAK2 wild type ET caused by somatic mutations in the calreticulin (CALR) gene. Bone marrow features in JAK2^{V617F} mutated ET and PV are similar and featured by medium sized to large (pleomorphic) megakaryocytes with only a few giant forms. Bone marrow histology in MPL⁵¹⁵ positive ET and MF show clustered small and giant megakaryocytes with hyperlobulated stag-horn-like nuclei, in a normocellular bone marrow with no features of PV. Bone marrow histology in newly diagnosed CALR mutated ET and MF patients is dominated by dense clusters of large immature dysmorphic megakaryocytes with bulky (cloud-like) hyperchromatic nuclei, which are described in primary megakaryocytic granulocytic myeloproliferation (PMGM), and never seen in JAK2^{V617F}, JAK2 exon 12 and MPL⁵¹⁵ mutated MPN. The WHO CMP classification distinguishes two JAK2 mutated clonal MPNs of JAK2^{V617F}-positive ET, PV and MF vs JAK2 exon 12 PV and three JAK2 wild type clonal MPNs: JAK2 wild type ET and MF carrying one of the MPL⁵¹⁵ or CALR mutations. A small proportion of MPN is triple negative for the JAK2, MPL and CALR mutation. The WHO-CMP classification much better delineate and stage the broad spectra MPN disease of various molecular etiology as a sound base for targeted treatment options.

Introduction

The clinical and pathological features for prodromal, erythrocythemmic and polycythemic stages of PV are variable and featured by increased erythrocytes above $6 \times 10^{12}/L$, increased leukocyte alkaline phosphatase (LAP) score (increased CD11b expression), normal or increased platelets, leukocytes and spleen size, and by characteristic bone marrow features with increased pleomorphic large megakaryocytes and erythropoiesis (table 1, Michiels et al 1996, 2000, Michiels & Thiele 2002, Michiels et al 2006). The clinical and bone marrow histology features of “true” ET has been recognized by Thiele *et al* in 1988 and 2005 as clearly distinct from PV. The peripheral blood findings in “true” ET are featured by high platelet counts, normal values for haemoglobin, haematocrit, erythrocyte, white blood cells, LAP score, LDH and no or minor splenomegaly despite platelet counts above $1000 \times 10^9/L$. According to Michiels & Thiele (2002) the megakaryocytes in “true” ET are larger than in PV (table 1). Normocellular “true” ET is a distinct MPN entity different from PV and hypercellular ET associated with prefibrotic primary myelofibrosis (pPMF) or primary megakaryocytic granulocytic myeloproliferation (PMGM, table 1, Thiele et al 1988, 1996, 2005a, 2005b, 2011, Michiels & Thiele 2002). Bone marrow histology in PV is typically featured by large pleomorphic megakaryocytes with hyperploid nuclei in a hypercellular bone marrow due to increased erythropoiesis or increased trilinear erythrocytic, megakaryocytic and granulocytic myeloproliferation. Georgii *et al* (1980, 1990) first discovered the third entity of primary myeloproliferative disease (MPD) characterized by chronic megakaryocytic granulocytic myelosis (CMGM) in the absence of reticulin or collagen fibrosis in bone marrow biopsy material (figure 1). The Hannover Bone Marrow Classification of the MPDs distinguished three primary prefibrotic MPDs ET, PV and CMGM from the advanced fibrotic stages of MPD (Georgii et al 1990, Michiels 1987). As myelofibrosis (MF) is a secondary event in all variants of MPD, the labeling of chronic idiopathic myelofibrosis or primary myelofibrosis (CIMF or PMF) is a contradiction of terms, Georgii et al (1990, 1996) replaced the term PMF by CMGM and used grading of myelofibrosis (MF) for staging of early, overt and advanced stage of MF in patients with ET, PV and CMGM. Michiels et al (2013, 2015) used the term primary MGM (PMGM) as the third JAK2 wild type MPN entity without features of PV or CML. In this study we produce good evidence that hypercellular JAK2/MPL wild type ET and MF associated with PMGM is the third CALR mutated MPN entity dominated by an increase of clustered atypical dysmorphic megakaryocytes due to increases of cellular and nuclear size and bulky nuclei

with clumsy lobuli and irregular roundish shaped form (so-called cloud-like nuclei), which are never described in JAK2 mutated ET and PV.

Hannover Bone Classification and WHO-CMP criteria for ET, PV and PMGM

Michiels *et al* (1987) defined strict morphological, biochemical, and cytogenetic criteria for *BCR/ABL*-positive ET and CML as a separate malignant and individual entity, whereas ET, PV and CMGM form a chronic proliferation of three hematopoietic cell lines. The 1990 Hannover Bone Marrow Classification of MPD (table 1) separated the Philadelphia (Ph) chromosome positive or *BCR/AB*-positive CML and ET from the Ph- or *BCR/ABL*-negative MPDs ET, PV and CMGM based on distinct bone marrow histology findings for each of the three MPDs ET, PV and CMGM (Georgii *et al* 1990, 1996, Michiels *et al* 1999). The difference in size and morphology of small monolobulated megakaryocytes in Ph-positive CML and ET from the large pleomorphic megakaryocytes in the Ph-negative MPDs ET and PV is so obvious that cytologists and pathologists can easily distinguish. The Hannover Bone Marrow Classification of the MPDs distinguished the three MPDs ET, PV and chronic megakaryocytic granulocytic myeloproliferation (CMGM) from advanced fibrotic stages of MPD (figure 1, Georgii *et al* 1990, 1996). As myelofibrosis (MF) is a secondary event in all variants of MPD and the terms chronic idiopathic myelofibrosis (CIMF) or primary myelofibrosis (PMF) are a misconception, Georgii consequently replaced the term CIMF and PMF by CMGM and used grading of myelofibrosis (MF, table 1) for staging of the early, overt and advanced MPDs ET, PV and CMGM (Georgii *et al* 1990, Michiels 1997, Thiele *et al* 1996, 2005b). Prefibrotic CMGM is the third MPD entity without features of ET, PV or CML and its diagnosis is based on the presence of loose to dense clustering of large megakaryocytes with immature cytoplasm and cloud-like nuclei not seen in ET, PV and CML (Georgii *et al* 1990, 1996, Thiele *et al* 1996, Michiels *et al* 1999). The term CMGM of the Hannover Bone Marrow Classification has been labeled as PMF in the 2008 WHO classification (Swerdlow 2008) (figure 1). The diagnosis of prefibrotic PMGM is based on the association of hypercellular ET with the presence of large immature megakaryocytes with immature cytoplasm and cloud-like nuclei not seen in ET and PV (table 1, figure 1) (Michiels 1997, Michiels *et al* 2006, 2013). The PV criteria of the Polycythemia Vera study Group (PVSG) exclude stage 1 idiopathic erythrocythemia (IE) and do not recognize prodromal PV by definition (Berlin 1975). The PV experts in the USA, UK and France did not use bone marrow biopsy for the diagnostic differentiation between PV and primary or secondary

erythrocytosis and therefore overlooked stage 1 erythremic PV by definition (Pearson & Wetherley-Mein 1979, Najean et al 1981). Idiopathic erythrocythemia (IE) has been defined by increased RCM and not meeting the A and B criteria of the PVSG (Berlin 1975). IE is clinically defined by an increased RCM, normal platelet count and leukocyte count, no splenomegaly and absence of any cause for secondary erythrocytosis by good clinical and laboratory practice. IE represent a significant number of early stage erythrocythemic PV of about 10% to 15% at time of PV presentation (Pearson & Wetherley-Mein 1979). The diagnostic difficulties regarding the original PVSG criteria to differentiate IE from erythrocytosis without the use of pathognomonic bone marrow pathology can partially solved by the combination of the PVSG criteria with low serum erythropoietine (EPO) levels and spontaneous endogenous erythrocyte colony formation (EEC) as specific features of PV (Pearson 1998).

According to ECP (Michiels et al 1999, Michiels & Thiele 2002, Michiels et al 2006, Table 1) and ECMP criteria IE is featured by increased red cell mass, normal spleen size, normal leukocyte and platelet counts and no clinical or laboratory evidence of primary or secondary erythrocytosis and a PV bone marrow histology (Michiels et al 2000, 2014). ECMP defined bone marrow histology has a specificity and sensitivity near to 100% to differentiate between the MPDs ET and PV from reactive thrombocytosis and all variants of primary or secondary erythrocytosis (table 1) (Michiels et al 2000, 2013, 2014). A low serum EPO level has also been described about half of ET patients, which are to be regarded as prodromal phases (*forme frusta*) of PV (Michiels et al 2006). Standardized and easy-to-perform commercial serum EPO assays provide a reliable and accurate criterion in support of the diagnosis of either erythrocytosis or PV and ET (Mossuz et al 2004, 2006). In a multicenter study on 241 patients, Mossuz *et al* identified two thresholds, allowing a specific and direct diagnosis of 65.6% (65-99) of untreated PVSG-defined PV (EPO <1.4 U/L) and 19.7% (13 of 66) of SP (EPO >13.7 U/L) (Mossuz et al 2004). About 50% of patients with absolute erythrocytosis could unequivocally diagnosed as PV or erythrocytosis by the combination of increased red cell mass (RCM) and serum EPO levels (Mossuz et al 2004, 2006).

JAK2^{V617F} mutated trilinear MPNs in ET and PV: Dameshek-Vainchenker's Disease

Dameshek (1950) proposed two highly speculative possibilities as the cause of trilinear PV (erythrocythemia, thrombocythemia, granulocythemia: either excessive bone marrow stimulation by an unknown factor, or the lack or diminution of an inhibitory factor

(Michiels et al 2013). This hypothesis of PV as a trilinear MPD has been proven to be correct by Vainchenker and his team by the discovery of the somatic JAK2^{V617F} mutation as the driver cause of trilinear MPNs ET, PV and MF (James et al 2005). On position 617 of the JAK2 JH2 domain Valine (V) is replaced by Phenylalanine (F) in the JAK2^{V617F} mutation and induces a loss of inhibitory activity of the JH2 pseudokinase part on the JH1 kinase part of JAK2, leading to enhanced activity of the normal JH1 kinase activity of JAK2. The JAK2^{V617F} makes the mutated hematopoietic stem cells hypersensitive to hematopoietic growth factors TPO EPO, IGF1, SCF and GCSF, resulting in PV as a trilinear MPN (table 1). Detection of JAK2^{V617F} has become the first intention diagnostic test for erythrocytosis and the prevalence of the JAK2^{V617F} mutation in PVSG defined PV is 95% and about 50% in ET and MF (Michiels et al 2006). The JAK2^{V617F} mutation load is usually low in ET, less than 10 to 50% of the granulocytes are JAK2^{V617F} positive (heterozygous) and either low or high in PV with less than 50% (heterozygous homozygous) or high between 50 to 100% (homozygous) of the granulocytes positive for the JAK2^{V617F} mutation (Antonioli et al 2005, Passamonti et al 2006, Vannucchi et al 2007). Patients with hypercellular ET and PV homozygous for the JAK2^{V617F} mutation patients are at high risk for myeloid metaplasia of the spleen with splenomegaly and bone marrow transformation into myelofibrosis (MF) and the percentage of JAK2^{V617F} positive granulocytes in PV may range from rather low to 100% for JAK2^{V617F} during the long-term follow-up (Antonioli et al 2005, Passamonti et al 2006, Vannucchi et al 2007). The 2005 concept according to Vainchenker & Michiels is that heterozygous JAK2^{V617F} mutation leading to constitutively activated megakaryocytes with increased sensitivity to TPO and EPO is enough to induce ET with the production of constitutively activated (hypersensitive) platelets (table 2, James et al 2005, Michiels et al 2006). Two MPN basic research groups from the UK and USA indeed demonstrated that so-called heterozygous PV with allele load less than 50% are hetero/homozygous for the JAK2^{V617F} mutation at the EEC level in blood and bone marrow, whereas ET patients are heterozygous with a maximal JAK2^{V617F} mutation load of 50% (Scott et al 2006, Moliterno et al 2008). The second molecular hit of the loss of 9p heterogeneity (9p LOH) is due to the amplification of the JAK2^{V617F} locus through mitotic amplification resulting in chromosome 9p loss of heterogeneity (9pLOH) indicating homozygous JAK2^{V617F} mutation (table 2). Godfrey *et al* (2012) studied the JAK2 mutation status of BFU-E grown in low erythropoietin conditions in 77 patients with PV or ET. Using microsatellite PCR to map loss-of-heterozygosity breakpoints within individual colonies, homozygous mutant colonies were absent or present in low percentages in heterozygous ET,

but prevalent and common in patients with JAK2^{V617F}-positive PV and JAK2 exon 12-mutated PV. PV was distinguished from ET by expansion of a dominant homozygous subclone, the selective advantage of which is likely to reflect additional genetic or epigenetic lesions (Godfrey et al 2012). Combined heterozygous homozygous or homozygous JAK2^{V617F} mutation is associated with pronounced constitutively activation and genetic instability of megakaryopoiesis, erythropoiesis and granulopoiesis in the bone marrow as the cause of hypercellular trilinear PV with a high risk of myelofibrotic progression.

According to WHO (Swerdlow et al 2008, Tefferi 2014a, 2014b, Barbui et al 2014) and WHO-CMP (Michiels et al 2013, 2014, 2015) criteria for JAK2^{V617F} mutated MPN (tables 3, and 4), heterozygous JAK2^{V617F} positive ET is defined by a normocellular with slight increase of erythropoiesis in the bone marrow (Table 3, figure 2) or with a hypercellular bone marrow due to increased erythropoiesis (prodromal PV, Table 3 figure 3). JAK2^{V617F} mutated WHO defined PV typically shows a hypercellular bone marrow histology with increased trilinear hematopoiesis of megakaryopoiesis, erythropoiesis and granulopoiesis defined as panmyelosis by Dameshek (1950) with no or slight increase of reticuline fibers (Table 4 Figure 4) (Michiels et al 2013, 2014, 2015). The UK MPN Study Group assessed the clinical features in the cohort of 806 PVSG defined ET patients subdivided in 414 JAK2^{V617F} positive and 362 JAK2 wild type ET and evaluated the bone marrow features in 393 ET patients (Campbell et al 2005, Wilkins et al 2008). JAK2^{V617F} positive ET patients had multiple features of PV such as a significantly higher hemoglobin, lower serum EPO and ferritin, higher neutrophils, bone marrow erythrocytosis and granulocytosis (figures 2 and 3), more venous thrombosis and a higher rate of polycythemic transformation. PVSG defined JAK2 wild type ET had significant higher platelet counts (962, range 668-1535x10⁹/L) than JAK2^{V617F}-positive ET (846, range 632-1222x10⁹/L) (Campbell et al 2005). In the UK Primary Thrombocythemia 1 (PT-1) study, bone marrow trephine of 209 JAK2^{V617F} positive and 184 JAK2 wild type ET was independently assessed by 3 blinded hematopathologists who did not know the JAK2 mutation status (Wilkins et al 2008). The overall cellularity was significantly increased in JAK2^{V617F} mutated ET as compared to JAK2 wild type ET, indicating that increased erythroid and granulocytic cellularity appears to be a main feature of prodromal PV, masked PV and classical PV (Campbell et al 2005, Wilkins et al 2008, Michiels et al 2014).

JAK2 exon 12 mutated Polycythemia Vera

The finding of typical MPN bone marrow findings (figure 5) in WHO defined JAK2 exon 12 mutated PV in patients with JAK2^{V617F} negative PV or idiopathic erythrocytosis further confirms the strong association between the JAK2 mutations and MPN (Scott et al 2007, Pardani et al 2007, Lakey et al 2010). The 5% PV patients negative for JAK2^{V617F} are frequently heterozygous for exon 12 JAK2 mutations and usually present with early stage PV with a favourable outcome and normal life expectancy. The UK MPN Study Group identified JAK2 exon 12 mutations in 10 idiopathic erythrocytosis (IE) patients with increased red cell mass but negative for the JAK2^{V617F} mutation, which could be diagnosed as PV in 6 and IE in 4 cases (Scott et al 2007). Bone marrow biopsies in JAK2 exon 12 mutated IE or PV showed characteristic normoblastic erythroid hyperplasia with morphological abnormalities of the megakaryocyte clearly different from primary or secondary erythrocytosis. In another study, 5 cases of JAK2^{V617F} negative PV carrying exon 12 mutation (F537-K539delins or N542-E534del) were diagnosed as IE with increased hemoglobin and hematocrit, low serum EPO, normal platelet and leukocyte counts, no or palpable spleen and a typical hypercellular bone histopathology predominantly due to erythroid hyperplasia and clusters enlarged megakaryocytes with hyperploid nuclei (Pardani et al 2007). The bone marrow histology in 7 cases of JAK2 exon 12 mutated MPN (IE in 4, PV in 2, MF in 1) in the study of Lakey et al (2010) revealed hyperplasia of atypical small to medium-sized large megakaryocytes was present in all (figure 5), which differs from JAK2^{V617F} mutated ET and PV (figures 2, 3 and 4). At diagnosis, exon 12 IE or PV patients presented aquagenic pruritis and/or erythromelalgia in 3 and microvascular events including headache, dizziness, blurred vision and distal extremity numbness (aspirin responsive platelet thrombophilia) in 4 at platelet counts between 152 and 790x10⁹/L (of whom 5 below and 2 above 300x10⁹/L) (Pardani et al 2007, Lakey et al 2010). The hemoglobin ranged from 18.3 to 22 g/dL and leukocytes were below 10,5x10⁹/L in 6 of the 7 JAK2 exon 12 PV cases consistent with the diagnosis of idiopathic erythrocytosis (IE). Serum EPO levels were low and conventional karyotyping was normal in all 7 JAK2 exon12 MPN cases. The JAK2 exon 12 MPN cases lack the prominent clusters of large megakaryocytes with hyperlobulated nuclei that characterize JAK2^{V617F}-positive prodromal and classical PV. A spectrum of small to medium sized megakaryocyte is seen in JAK2 exon 12 PV bone marrows with a predominance of smaller forms with atypical nuclei with various degrees of monolobulation to hyperlobulation and abnormal chromatin distribution (figure 5) (Lakey et al 2010). Bone marrow histology is featured by prominent

increased erythropoiesis (figure 5) and reticulin fibrosis was normal or slightly increased in 6, and one case evolved 15 years after initial diagnosis into post-PV myelofibrosis with reticulin fibrosis grade 3 and associated osteosclerosis (Lakey et al 2010).

Bone marrow histology in MPL⁵¹⁵ mutated Essential Thrombocythemia

Bone marrow histology from a patient with JAK2 wild type ET carrying the MPL^{W515L} mutation displayed clusters large megakaryocytes with a greater number of giant megakaryocytes with hyperlobated stag-horn nuclei in a normal cellular bone marrow and no increase of erythropoiesis (Table 5, figure 6). We recently described the essential differences in bone marrow histopathology features of differential diagnostic significance between patients with MPL⁵¹⁵ mutated (N=12) versus JAK2^{V617F} mutated MPN (Michiels et al 2015). **First**, The presence of clustered small and giant megakaryocytes with deeply lobulated stag-horn like nuclei (Figure 6, Table 5) in ET carrying the MPL⁵¹⁵ mutation are not seen in JAK2^{V617F} positive ET, prodromal PV, and classical PV. The pleomorphic medium to large megakaryocytes in JAK2^{V617F} mutated ET and PV in bone marrow smears and bone marrow biopsy were comparable regarding size and degree of pleomorphy (figures 2, 3 and 4). **Second**, there was local increase of erythropoiesis in areas of loose clustered pleomorphic megakaryocytes in normocellular JAK2^{V617F} mutated ET and prodromal PV, which is not seen in MPL⁵¹⁵ mutated ET (figure 6). **Third**, JAK2 wild type MPL⁵¹⁵ mutated ET have no clinical, laboratory and bone marrow features of prodromal PV at diagnosis (Table 5), do not evolve into PV during follow-up, and have normal LAP score, serum EPO and ferritin levels. Laboratory and bone marrow histology evaluations have the diagnostic potential to separate the JAK2^{V617F} mutated ET and prodromal PV with increased LAP score, low serum EPO and pleomorphic megakaryocyte morphology (Tables 3 and 4) from MPL⁵¹⁵ mutated ET with normal LAP score and serum EPO and giant megakaryocytes with staghorn-like nuclei similar to “true” ET (table 5) (Michiels et al 2015).

Bone marrow histology in CALR mutated ET and MF

In six consecutive newly diagnosed CALR positive ET cases in 2014 we found consistent bone marrow characteristics of hypercellular ET as the presenting feature of prefibrotic and early fibrotic stages of PMGM (Table 5). Five of these six CALR mutated ET patients presented with aspirin sensitive microvascular disturbances of erythromelalgic, cerebral and ocular ischemic manifestations (Sticky Platelet Syndrome) as the specific presenting

manifestations of myeloproliferative thrombocythemia. Bone marrow histology in typical prefibrotic CALR ET (Table 5) and in early fibrotic CALR myelofibrosis (Table 5) show dysmorphic megakaryocytes with definite abnormalities of maturation with bulky (bulbous) hyperchromatic nuclei and some disturbances of the nuclear cytoplasmic ratio consistent with CALR mutated PMGM (figure 7), which are not seen in MPL⁵¹⁵ mutated ET (figure 6) and also not in JAK2^{V617F} mutated ET, prodromal PV and classical PV (figures 2, 3 and 4).

Discussion

With the advent of the JAK2^{V617F} mutation all latent, masked, early and overt stages of PV will be picked up more than 5 to 10 years earlier by the WHO- CMP criteria as compared to the PVSG and WHO criteria. Heterozygous JAK2^{V617F} mutated ET and hetero/homozygous JAK2^{V617F} mutated PV and post-ET MF or post-PV MF represent different phenotypes of a single distinct MPN. JAK2 wild type ET and MF carrying one of the MPL⁵¹⁵ mutations is the second distinct MPN without features of PV at diagnosis and during follow-up (figure 8). Pretreatment bone marrow biopsy will allow clinicians and pathologists to diagnose the early stages of thrombocythemia in various MPNs on top of the JAK2^{V617F}, MPL⁵¹⁵ or CALR mutation status. The WHO and WHO-CMP criteria classify JAK2 mutated ET as normocellular ET, early PV mimicking ET (prodromal PV); hypercellular ET associated with prefibrotic (RF-0 or RF-1 = MF 0) megakaryocytic granulocytic myeloproliferation (EMGM) and splenomegaly without features of leukoerythrocytosis (masked PV, Barbui et al 2014) and post-ET or post-PV myelofibrosis MF-1, 2 and 3 and features of leukoerythroblastosis (Tefferi et al 2014b). The WHO and the WHO-CMP criteria distinguish thrombocythemia in various MPNs from thrombocythemia associated with Ph¹-chromosome and *bcr/abl* positive chronic myeloid leukemia (CML) or myelodysplastic syndromes (MDS) including the so-called 5q-syndrome and refractory anemia with ringed sideroblasts and significant thrombocytosis (RARS-T) (Michiels & Thiele 2002, Michiels et al 2006, Barbui et al 2014, Tefferi et al 2014a, 2014b Michiels et al 2013, 2014, 2015). 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 hematopoietic cellularity of the bone marrow in biopsy material, indicating early thrombocythemic PV mimicking ET (“forme fruste” PV, stage 1 PV, Campbell et al 2005, Wilkins et al 2010, Rumi et al 2014a, Michiels et al 2015). JAK2 wild type ET patients carrying one of the MPL⁵¹⁵ or CALR mutation represent two

distinct category of MPN disease without features of PV. CALR mutated ET had significantly higher platelet counts, lower erythrocyte counts haemoglobin and hematorit levels, normal serum EPO levels, a typical bone marrow picture of ET with no features of early PV, and are at lower risk for the development of major thrombotic complications (Campbell et al 2005, Klampf et al 2013, Nanglia et al 2013, Rumi et al 2014a, 2014b).

In a prospective study of 59 JAK2^{V617F} positive ET and 44 JAK2 wild ET cases, Piche *et al* (2012) described that JAK2^{V617F} mutated ET patients have PV-like morphological bone marrow changes of medium sized to large pleomorphic megakaryocytes similar to our findings in newly diagnosed JAK2^{V617F} mutated ET, prodromal PV patients and PV patients. JAK2^{V617F} positive ET and prodromal PV patients usually have low serum EPO, increased LAP score, and slight to moderate increased bone marrow cellularity due to increased erythropoiesis. Increase of bone marrow erythropoiesis, granulopoiesis and serum LDH levels and spleen size are more pronounced in advanced JAK2^{V617F} mutated ET (masked PV) at higher JAK2^{V617F} mutation allele burden. Clustered large and giant megakaryocyte with hyperlobulated ‘staghorn’ nuclei are rare in JAK2 mutated MPN, but typically present in MPL⁵¹⁵ mutated ET patients with no features of PV in the bone marrow consistent with the diagnosis of ‘true’ ET and normal blood values for serum EPO, ferritine levels and LAP score (Michiels & Thiele 2002, Vannucchi et al 2008, Beer et al 2008). The prevalence of MPL⁵¹⁵ mutated ET or MF patients ranges from 5 to 10% of the JAK2 wild type MPN population (Vannucchi et al 2008, Beer et al 2008). In the large collaborative European study of 176 MPN cases with the MPL⁵¹⁵ mutations W515L and W515K were detected in 110 and in 58 respectively (Jones et al 2010). The overall MPL mutation allele levels in granulocytes were lower (25%) in W515L (N=106) than in W515K (37%, N=32). Of the 138 cases (ET, N=99; MF, N=36), the median W515L mutation allele levels were significantly lower (21%) in ET than those (46%) in MF (Jones et al 2010). The molecular etiology of JAK2/MPL wild type ET and MF remained elusive until two groups independently discovered in 2013 the calreticulin (CALR) mutations in MPN patients with non-mutated JAK2 (Klampf et al 2013, Nanglia et al 2013). Dr Kralovics and his team in Vienna Austria first described the occurrence of calreticulin (CALR) mutation in 78 of 311 (25%) ET patients and in 72 of 203 (35%) MF patients and in none of 382 PV patients (Klampf et al 2013). CALR mutations are mutually exclusive with both JAK2^{V617F} and MPL⁵¹⁵ mutations: 195 (67%) of 289 JAK2 wild type ET and 105 (80%) of 120 195 carried one of the CALR mutations. Green and his team in the UK independently found somatic CALR mutations in 110 of 158 JAK2 and MPL wild

type MPN, including 80 of 112 (70%) ET patients, 18 of 32 (56%) MF patients (Nanglia et al 2013). CALR exon 9 mutations were found in 26 of 31 (84%) patients with JAK2/MPL wild type MF. CALR exon 9 mutations were absent in all 120 patients who had JAK2 or MPL mutations. CALR mutations were identified in 10 of 120 (8%) MDS patients (RA in 5 of 53, RARS in 3 of 27 and RAEB-T in 2 of 27), and in one patient each with CMML and atypical CML. The bone marrow findings in the present study of CALR mutated ET and MF in figures 3 and 4 are consistent with prefibrotic JAK2 wild type PMGM, which clearly differs from the pleomorphic megakaryocytes in JAK2^{V617F} mutated ET and PV and from giant megakaryocytes and hyperlobulated staghorn-like nuclei in MPL⁵¹⁵ mutated ET. Recently, the JAK2 wild type CALR-mutated ET and MF has become the third distinct MPN entity without features of PV at time of diagnosis and during follow-up (Klampf et al 2013, Nanglia et al 2014, Rumi et al 2014a, 2014b). The JAK2/MPL wild type but CALR-positive ET and MF patients appeared to become the third distinct MPN entity without features of PV (figure 8). Absence of clinical, laboratory and bone marrow features of PV and no polycythemic transformation has been observed in CALR mutated patients (Rumi et al 2014a, 2014b, Tefferi et al 2014a, 2014b). The evolution of ET to MF belong to the natural history of all molecular variants of the MPNs. Life expectancy was significantly longer in CALR mutated MF patients as compared to those with a JAK2^{V617F} or MPL⁵¹⁵ mutation. The overall survival of JAK2/MPL wild type MF carrying one of the CALR mutations was 23 years as compared to 14.4 years of MF patients with the JAK2^{V617F} or MPL⁵¹⁵ mutation MF (Rumi et al 2014a, 2014b). Patients with JAK2^{V617F} mutated ET and PV had a similar high risk of major thrombosis, which was twice that of thrombocythemia patients with CALR mutation (Rumi et al 2014a, 2014b). The lower incidence of major thrombotic complications in JAK2/MPL wild type ET is very likely related to the fact that CALR-positive ET and MF patients had normal or low normal hemoglobin and leukocyte counts as compared to those in JAK2^{V617F} mutated ET patients. In the Mayo Clinic study of 254 WHO-defined MF patients the JAK2-, MPL- and CALR-mutations were detected in 58%, 8.3 and 25% respectively and 8.7% were triple negative (Tefferi et al 2014a). The median overall survival (OS) among 253 WHO-defined PMF patients in 83 CALR-, 21 MPL-, and 147 JAK2-mutated cases and in 22 triple negative cases was 8.2, 4.1, 4.3 and 2.5 years. As compared to CALR wild type MF, CALR-mutated MF patients were younger, had higher platelet count, lower leukocyte count, higher hemoglobin (less anemic) and lower DIPSS-plus score. CALR-mutated MF patients had a favorable impact on median survival as compared to CALR-negative MF patients whether

ASXL1-negative or positive. Among 181 WHO-defined CALR-negative MF patients, the median overall survival was 2.3 years in 55 CALR-negative/ASXL1-positive as compared to 5.6 years in 126 CALR-negative/ASXL1-negative MF patients. Among 146 CALR-positive MF cases the median survival was 7 years in 20 CALR-positive/ASXL1-positive MF patients as compared to 9.6 years in 126 CALR-positive/ASXL1-negative MF patients (Tefferi et al 2014a). The etiology and bone marrow histology of triple JAK/MPL/CALR negative MF remains elusive whether they represent MPN or MDS. The awareness of the molecular heterogeneity of the MPNs ET and PV including JAK, MPL and CALR mutation on top of epigenetic factors reflects the funeral of the term primary myelofibrosis (PMF) (Silverstein 1977) and confirms the Hannover Bone Marrow Classification that primary myelofibrosis (PMF) is a misconception (Georgii et al 1990, 1996). According to the WHO-CMP classification PMF does not exist as a distinct disease entity anymore (Michiels et al 2013, 2015). The WHO defined term PMF (Swerdlow et al 2008) can easily be replaced by three distinct disease entities of CALR-, MPL⁵¹⁵-and JAK2^{V617F}-mutated ET and myelofibrosis (MF) with various degrees of splenomegaly, hypersplenism and myelofibrotic transformation of the bone marrow (Barbui et al 2014, Tefferi et al 2014b, Michiels et al 2015).

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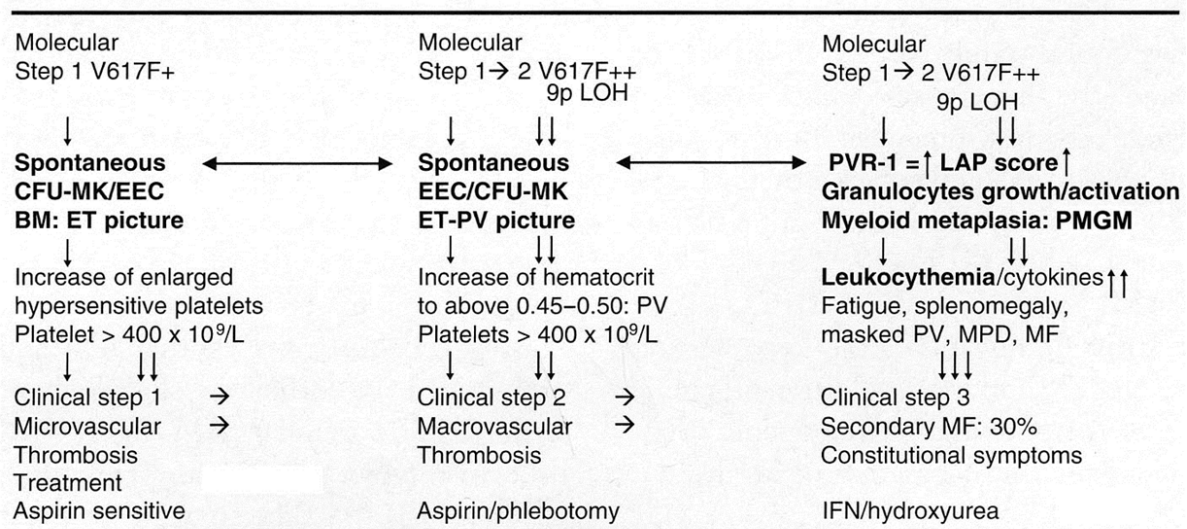
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Table 1. The 2000-2004 European Clinical and Pathological (ECP) criteria³ for the diagnosis of polycythemia vera (PV, Berlin 1975), ‘true’ET (Thiele et al 1988, 2005) and chronic megakaryocytic granulocytic myeloproliferation (CMGM, Georgii et al 1980, 1990) or primary megakaryocytic granulocytic myeloproliferation (PMGM, Michiels et al 2013). Modified according to Michiels & Thiele 2002

ECP Clinical criteria PV A1 Erythrocyte count above $6 \times 10^{12}/L$, 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 $>1500 \times 10^9/L$ A3 Splenomegaly on ultrasound or CT (>12 cm) or splenomegaly on palpation A4 Granulocytes $>10 \times 10^9/L$ or leukocytes $>12 \times 10^9/L$ 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 ECP Clinical criteria ‘true’ ET A1 Persistent increase of platelet count grade I 400-1500, grade II $>1500 \times 10^9/L$ 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 ECP Clinical criteria CMGM or PMGM A1 No preceding or allied other subtype of MPN, PV, CML or MDS A2 Early clinical stage Normal hemoglobin, or anemia grade I: hemoglobin >12 g/dL, slight or moderate splenomegaly on palpation or >11 cm on ultrasound or CT. Thrombocythemia $>400 \times 10^9/L$ A3 Intermediate clinical stage Anemia grade II, hemoglobin >10 g/dL, definitive leuko-erythroblastic blood picture and/or tear-drop erythrocytes. Splenomegaly on palpation, no adverse signs A4 Advance clinical stage Anemia grade III, hemoglobin <10 g/dL, significant splenomegaly and one or more adverse signs	ECP 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 medium sized and large (pleomorphic) megakaryocytes. Absence of stainable iron, No or slight increase of reticulin fibers. B2 Spontaneous erythroid colony (EEC) formation <i>A1 + B1 and none of the others is idiopathic erythrocythemia: IE</i> <i>A2 + B1 and none of the others is ET with features of PV (prodromal PV)</i> <i>A3 and B1 and none of the others is primary MPD or latent PV</i> <i>A1 + B1 plus one of A2 to A6 or B2 is overt classical PV</i> ECP Pathological criteria ‘true’ ET B1 Predominant proliferation of enlarged to giant megakaryocytes with hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicuous cytological abnormalities B2 No proliferation or immaturity of granulopoiesis 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; ECP Pathological criteria CMGM or 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 nuclei and definitive maturation defects Staging of myelofibrosis: MF in ET, PV & PMGM (Table 6) 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)
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Table 2. The Dameshek one cause hypothesis of trilinear PV⁴³³ and Vainchenker's discovery of heterozygous and homozygous JAK2^{V617F} somatic mutation¹⁷ as the driver cause of trilinear MPN with clinical and pathological manifestations of Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Myelofibrosis (MF).

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 2005

Table 3. WHO Clinical Molecular and Pathobiological (WHO-CMP) criteria for diagnosis of JAK2^{V617F} mutated essential thrombocythemia (ET)

WHO Clinical and molecular criteria	WHO Bone marrow pathology criteria
JAK2^{V617F} ET	Normocellular ET
1. Platelet count of $>350 \times 10^9/l$ and normal erythrocytes $<5.8 \times 10^{12}/L$ males, $<5.6 \times 10^{12}/L$ females 2. Heterozygous JAK2-^{V617F} mutation, and low JAK2 allele mutation load 3. Hemoglobin (Hb) and hematocrit (ht) normal or in the upper range of normal 4. No or minor splenomegaly	Proliferation and clustering of enlarged mature pleomorphic megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. Normocellular bone marrow (<60%) and no proliferation or immaturity of granulopoiesis or erythropoiesis. Reticuline fibrosis (RF) 0 or 1
JAK2^{V617F} prodromal PV	ET with bone marrow features of PV
1. Platelet count of $\geq 350 \times 10^9/l$ Hb and Ht normal or in the upper range of normal, normal erythrocyte $<5.8 \times 10^{12}/L$ males, $<5.6 \times 10^{12}/L$ females. 2. Presence of JAK2-^{V617F} mutation and variable JAK mutation load 3. Low serum EPO, increased LAP score and/or spontaneous EEC. 4. No, minor or slight splenomegaly	Increased cellularity (60-80%) due to increased erythropoiesis or trilineage myeloproliferation (i.e. panmyelosis). Proliferation and clustering of medium sized to large (pleomorphic) mature megakaryocytes. Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis. RF 0 or 1
JAK2^{V617F} hypercellular ET	EMGM (masked PV)
1. Platelet count of $\geq 350 \times 10^9/l$, Hb ht and erythrocytes normal or in the lower range of normal 2. Presence of JAK2-^{V617F} mutation and high JAK2 mutation load 3. Slight or moderate splenomegaly on ultrasound or on palpation 4. No allied CML, PMGM or MDS . EMGM clinical staging Early stage 1: No anemia with Hb and Ht in the normal or low normal range: hb >12 g/dl, normal LDH and CD34+ Intermediate stage 2: slight anemia Hb between <12 to >10 g/dL, LDH$\uparrow$, and splenomegaly Advanced stage 3: anemia, Hb <10 g/dL, LDH$\uparrow\uparrow$, CD34+ , leukoerythroblastose and, tear drop	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 clumpy nuclei (not or some cloud-like). EMGM Grading of reticulin fibrosis and MF Prefibrotic: RF- 0/1 = MF-0, no/minor splenomegaly Early fibrotic EMGM: RF 2 = MF 1 and minor or moderate splenomegaly Fibrotic EMGM: RF3, RCF = MF2 and overt splenomegaly Post-ET MF: RF3/4 = MF-2/3 (WHO criteria)

Table 4. WHO Clinical and Pathological (WHO-CMP) criteria for the diagnosis of JAK2 mutated polycythemia vera (PV).

Clinical criteria JAK2 mutated PV	Pathological criteria PV (WHO)
A1 Erythrocyte above $5.8 \times 10^{12}/L$ males, above $5.6 \times 10^{12}/L$ females Hemoglobin variable around and above 18.5 g/dL male and 16.5 g/dL females. Presence of JAK2^{V617F} or exon 12 mutation A2 Persistent increase of platelet count grade I 400-1500, grade II $>1500 \times 10^9/L$ A3 Splenomegaly on ultrasound or CT (>12 cm) or splenomegaly on palpation A4 Granulocytes $>10 \times 10^9/L$ or leukocytes $>12 \times 10^9/L$ 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 serum EPO level and or spontaneous EEC	B1 Increased cellularity due to increased erythropoiesis or due to trilinear myeloproliferation of megakaryopoiesis, erythropoiesis and granulopoiesis (panmyelosis). Proliferation of small medidium sized and large (pleomorphic) megakaryocytes. Absence of stainable iron, No or slight increase of reticulin fibers. B2 Homozygous JAK2^{V617F} mutation of cells in EEC colonies Staging of myelofibrosis: MF (Table 6) 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 (bone 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

Staging of PV: the prefibrotic, early fibrotic, fibrotic and advanced fibrotic stages are related to the degree of splenomegaly indicating the need of myeloreductive treatment with low dose pegylated interferon, hydroxyurea and or JAK2 inhibitor in the early, fibrotic and advanced fibrotic stage respectivel (Michiels et al 2013, 2015)

Table 5. WHO Clinical and Pathological (WHO-CMP) criteria for the diagnosis of MPL⁵¹⁵ mutated ET and CALR mutated hypercellular ET associated with primary megakaryocytic granulocytic myeloproliferation (PMGM)

Clinical criteria MPL⁵¹⁵ mutated ET^{19,34} 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 Clinical Staging according to no, mild or severe anemia similar to clinical staging of CALR mutated ET -----	Pathological criteria MPL⁵¹⁵ mutated ET^{19,34} B1 Predominant proliferation of enlarged to giant megakaryocytes wit hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicuous cytological abnormalities B2 No proliferation or immaturity of granulopoiesis 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 (Table 6) -----
Clinical criteria CALR mutated ET and MF^{19,34} 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>400x10⁹/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 CALR ET and MF (PMGM)^{19,34} 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 (Table 6) 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

Table 6. Grading of reticulin fibrosis (RF) (Wilkins et al 2008) grading of myelofibrosis (MF) according to the European clinical and Pathological (ECP) criteria (Michiels & Thiele 2002, Thiele et al 2005b) in bone marrow biopsies of patients with WHO defined myeloproliferative Neoplasms (MPN, Swerdlow et al 2008).

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

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

Legend to the figures

Figure 1. Hannover Bone Marrow Classification of MPD by Georgii et al 1990 versus the 1975 PVSG, the 2008 WHO (Swerdlow et al 2008) and the 2013 WHO CMP criteria (Michiels et al 2013) for the three prefibrotic JAK2 positive ET, PV and ET.MGM (red), versus fibrotic primary myelofibrosis (PMF = PMGM = endstage MPD, black). The WHO-ECMP criteria distinguish two variants of prefibrotic hypercellular ET: JAK2^{V617F} mutated ET.MGM (red) and JAK2/MPL wild type PMGM (blue).

Figure 2.

JAK2^{V617F} positive essential thrombocythemia (ET) with long term stable essential thrombocythemia (ET) and typical ET bone marrow features 1996-2014.

JAK2^{V617F} positive ET with PV bone marrow features (prodromal PV) in 1997, who developed PV in 2004-2005 followed by a complete hematological response on pegylated interferon

Figure 3. Bone marrow features of JAK2^{V617F} heterozygous positive essential thrombocythemia (ET, left) and JAK2^{V617F} homozygous acute onset polycythemia vera (PV, right). Contribution of Drs Michiels and De Raeve, University Hospital Antwerp, Belgium.

Left. Presence of normal platelets in a peripheral blood smear (a) and normal size and morphology of megakaryocytes in a smear from an aspirated bone marrow (middle left) in a control person. Increase of platelets aggregates with the presence of large platelets in a peripheral blood smear (b) and the presence of large megakaryocytes with hyperlobulated nuclei (inset) in a smear of and aspirated bone marrow (middle right) of a case with ET featured by platelet count of $435 \times 10^9/L$ and slight splenomegaly complicated by portal vein thrombosis. Bone marrow histology showing increased cellularity (70%, c left), due to increased erythropoiesis and pronounced increase of large pleomorphic megakaryocytes with hyperlobulated nuclei and no increase of bone marrow reticulin (c right) pathognomonic diagnostic for heterozygous mutated JAK2^{V617F} ET (JAK2^{V617F} mutation load 26%).

Right. Hypercellular bone marrow due to increased megakaryopoiesis, erythropoiesis and granulopoiesis in a case with rapid onset trilinear JAK2^{V617F} homozygous positive PV.

Figure 4. Morphology of bone marrow smears in control and polycythemia vera (PV) and bone marrow histology in PV versus idiopathic erythrocytosis.

Upper panels. Bone marrow smears of PV case 8 (table 4) with homozygous rapid onset PV show large megakaryocytes and hypercellularity (upper right) as compared to normal sized megakaryocytes and normal cellularity in a control BM smear (Upper left)

Middle panels. Typical PV picture in bone marrow biopsy specimen in a case of PV with rapid onset PV, increased red cell mass, and homozygous for the JAK2V617F mutation. Please note that loose clustered pleiomorphic enlarged megakaryocytes in this case are somewhat less enlarged and have hyperlobulated nuclei (middle panels) as in a case with classical PV with a hypercellular trilinear bone marrow proliferation (left bottom).

Lower panels. Comparison of bone marrow features in a case of classical PV with typical loose clusters of pleiomorphic small and large megakaryocytes (left bottom) as compared to small isolated normal sized megakaryocytes in bone marrow biopsy of a case with idiopathic erythrocytosis (right bottom).

Figure 5. Bone marrow histology in PV patients with JAK2 exon 12 mutations are featured by prominent erythroid hyperplasia meeting the criteria for idiopathic erythrocythemia (IE) or classical PV and megakaryocytic hyperplasia of atypical small to medium-sized large megakaryocytes with various degrees of monolobation to hyperlobation and abnormal chromatin distribution (Lakey et al 2010).

Figure 6. Bone marrow histology in “true” essential thrombocythemia (ET, table 5) consistent with WHO normocellular ET carrying the MPL^{W515L} mutation showing giant megakaryocytes with hyperlobulated stag-horn like nuclei characteristic for ET the MPLW515L/K mutation. Courtesy of Dr Schwarz, University Hospital Prague, Czech Republic.

Figure 7. Bone marrow features in newly diagnosed calreticulin (CALR) mutated essential thrombocythemia (ET, left) and early stage myelofibrosis (MF, right)

Left. Clinical case of JAK2/MPL negative and calreticulin (CALR) positive ET who presented with aspirin responsive platelet thrombophilia, normal values for hemoglobin, hematocrit and erythrocytes, platelet count of $1832 \times 10^9/L$ and slight splenomegaly (16 cm length diameter on echogram). Bone marrow histology is hypercellular with relative decrease of erythropoiesis, dense cluster of immature megakaryocytes with hyperlobulated nuclei consistent, and no increase of reticulin fibrosis consistent with a typical PMGM (table 1) bone marrow. Contribution of Drs Valster, Potters and Schelfout, BRAVIS Hospital Bergen op Zoom, The Netherlands

Right. Clinical case of CALR positive myelofibrosis (MF): hemoglobin 11.2 g/dL, hematocrit 0.33, leukocytes $9.2 \times 10^9/L$, platelets $347 \times 10^9/L$, LDH 1393 U/L, and the presence of tear drop erythrocytes, poikilocytosis and polychromasia in a peripheral blood smear, and hypercellular bone marrow with relative decrease of erythropoiesis, dense cluster of immature megakaryocytes with hyperlobulated nuclei consistent, and reticulin fibrosis (RF) grade 2 consistent with bone marrow histology features similar to WHO-defined primary myelofibrosis (PMF), but distinct from JAK2^{V617F} mutated ET and PV, and distinct from MPL⁵¹⁵ mutated ET. Contribution of Drs Fostier and De Raeve, University Hospital, Brussels, Belgium.

Figure 8. Classification of five distinct clonal myeloproliferative neoplasms (MPN) and translational states of JAK2V617F mutated ET, prodromal PV, classical PV, Post ET and post-PV myelofibrosis (MF) and of MPL and CALR mutated ET and MF and triple negative MPN.