Review Article

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Change of the 2008/2016 WHO Criteria into European Clinical, Laboratory Molecular and Pathological (ECMP/CLMP) Classification of *BRC/ABL* Negative Myeloproliferative Neoplasms Caused by JAK2, MPL and CALR Driver Mutations

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Abstract

Ninety five percent of Polycythemia Vera (PV) patients and half of patients with Essential Thrombocythemia (ET) patients have low serum Erythropoietin (EPO) levels, and carry the $JAK2^{V617F}$ mutation. The change of 2008/2016 WHO criteria for ET into the European Clinical, Molecular and Pathologic (ECMP) classification of the $JAK2^{V617F}$ positive ET patients refers to three phenotypes of ET. First, heterozygous $JAK2^{V617F}$ mutated normocellular ET life-long. Second, hypercellular ET due to increased erythropoiesis (prodromal PV) preceding classical PV. Third, hypercellular and ET with erythrocythic-megakaryocytic-granulocytic bone marrow proliferation not meeting the WHO criteria for PV (masked PV). $JAK2^{V617F}$ mutation load is low and stable around 30% in heterozygous normocellular ET but high and increasing from below 50% to 80-100% homozygous $JAK2^{V617F}$ mutated PV and masked PV. $JAK2^{V617F}$ mutation load is related to MPN disease burden in terms of leucocytosis, splenomegaly, constitutional symptoms and myelofibrosis. Five distinct $JAK2^{V617F}$ trilinear MPN stages can be distinguished: $JAK2^{V617F}$ heterozygous positive ET, $JAK2^{V617F}$ homozygous positive PV; benign $JAK2^{exon\ 12}$ PV; MPL^{515} mutated ET without features of PV and CALR mutated ET without features of PV. CALR mutated thrombocythemia has been recognized and described in great detail by Georgii & Michiels

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between 1990 and 1997 as Primary Megakaryocytic Granulocytic Myeloproliferation (PMGM).

Bone marrow features in JAK2^{V617F} mutated ET and PV are similar characterized by medium sized to large (pleomorphic) megakaryocytes with hyperlobulated nuclei. Bone marrow histology in MPL^{515} positive ET show clustered large to giant megakaryocytes with hyperlobulated stag-horn-like nuclei, in a normocellular bone marrow. Bone marrow histology in CALR mutated ET patients is dominated by dense clusters of large immature dysmorphic megakaryocytes with bulky (cloud-like) hyperchromatic nuclei, which are never seen in JAK2^{V617F} , JAK2^{exon12} and MPL^{515} mutated MPN. The 2008/2016 WHO defined terminology of ET and PMF can easily be replaced by ECMP defined CALR-, MPL^{515}-and JAK2^{V617F}-mutated thrombocythemia and secondary myelofibrosis (MF) with various degrees of splenomegaly, hypersplenism and myelofibrotic transformation of the bone marrow.

Keywords: Bone marrow pathology; Essential thrombocythemia; JAK2 mutation; MPL mutation, calreticulin mutation; Myelofibrosis; Polycythemiavera; Primary megakaryocytic granulocytic myeloproliferation; Reticulin fibrosis

Introduction

The clinical and pathological features for prodromal, erythrocythemic and polycythemic stages of PV are variable and featured by increased erythrocytes above 6x10^{12}/L, increased Leukocyte Alkaline Phosphatase (LAP) score (increased CD11bexpression), normal or increased platelets, leukocytes and spleen size, and by characteristic bone marrow features with increased pleomorphic large megakaryocytes and erythropoiesis (Table 1) [1-4]. The clinical and bone marrow histology features of PVSG = WHO defined ET has been recognized by Thiele *et al.* in 1988 and 2005 as clearly distinct from PV [5,6]. The peripheral blood findings in JAK2 wild type ET carrying the MPL or CALR mutation are featured by high platelet counts, normal values for haemoglobin, haematocrit, erythrocyte, white blood cells, LAP score, serum EPO, LDH and no or minor splenomegaly despite platelet counts above 1000x10^9/L^7. The megakaryocytes in JAK2 wild type ET carrying the MPL or CALR mutation are much larger to giant than the medium to large seized megakaryocytes in PV (Table 1) [5-7].

Bone marrow histology in JAK2^{V617F} mutated PV is typically featured by medium sized to large pleomorphic megakaryocytes with hyperploid nuclei in a hypercellular bone marrow in a hypercellular bone marrow due to increased erythropoiesis or increased trilinear-erythrocytic, megakaryocytic and granulocytic myeloproliferation [2-4]. Georgii *et al* discovered a third entity of primary Myeloproliferative Disease (MPD) characterized by Chronic or Primary Megakaryocytic Granulocytic Myelosis (CMGM/PMGM) in the absence of reticulin or collagen fibrosis in bone marrow biopsy material (Figure 1) [8,9].

The Hannover Bone Marrow Classification of the MPDs distinguished three primary prefibrotic MPDs ET, PV and CMGM/PMGM from the advanced fibrotic stages of MPD[8]. 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 [8,9]. Georgii replaced the term CIMF and 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 [8,9]. Michiels replaced in 1997 the term CMGM by essential or primary MGM (EMGM or PMGM) as the third JAK2 wild type MPN entity without features of PV or CML [10-12]. EMGM or PMGM (hypercellular JAK2 wild type ET) is the third MPN entity caused by the CALR driver mutation. ET associated with prefibrotic PMGM is dominated by an increase of clustered atypical dimorphic megakaryocytes due to increases of cellular and nuclear size and

Clinical criteria JAK2V617F ET and PV

A1 Erythrocyte count below in ET and above 6x10^{12}/L in PV (ECMP). Hemoglobin. 18.5 g/dL male and >16.5 g/dL females. Raised red cell mass (RCM) male .36 ml/kg, female >32 ml/kg (PVSG, WHO)
A2 Persistent increase of platelet count grade I 400-1500, grade II >1500x10^9/L
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 MPL515 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

Clinical criteria CALR PMGM

A1 Early thrombocythemia stage with increased platelets due to megakaryocytic (M) proliferation
A2 Early clinical hypercellular due to dual megakaryocytic granulocytic (MG) proliferation 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
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

Pathological criteria JAK2V617F PV

B1 Increase of large pleiomorph megakaryocytic (M) proliferation in a normal cellular bone marrow: ET and Increased cellularity due to increased erythropoiesis or due to trilinearmyeloproliferation of erythropoiesis megakaryopoiesis, and granulopoiesis (EMG) in PV. Proliferation of small medium sized and large (pleomorphic) megakaryocytes. Absenceofstainable iron, No or slight increase of reticulin fibers.
B2 Sponataneouserythroid colony (EEC) formation

A1 + B1 is idiopathic erythrocythemia: IE
A2 + B1 is ET with features of PV (prodromal PV)
A3 and B1 is primary MPD or latent PV
A1 + B1 plus one of A2 to A6 or B2 is overt classical PV

Pathological criteria MPL515 ET

B1 Predominant proliferation of enlarged to giant megakaryocytes wit hyperlobulatedstag-horn-like nuclei and mature cytoplasm, lacking conspicious 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 establishMPL 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;
Pathological criteria CALR PMGM
B1 Megakaryocytic (M) followed by dual megakaryocytic granulocytic (MG) myeloproliferation 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) clumpsyhypo/hyperlobulated nuclei and definitive maturation defects

Staging of myelofibrosis: MF in ET, 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 1: European Clinical, Molecular and Pathological (EMCP) criteria3 for diagnosis of myeloproliferative disorders (MPD) and myeloproliferative neoplasms (MPN) JAK2 polycythemia Vera (PV) [2-4], MPL ET [5-7] and CALR primary megakaryocytic granulocytic myeloproliferation (PMGM) [8-10]. Michiels, Georgii, Thiele, Schwarz & Penka [2-10].

bulky nuclei with clumsy lobuli and irregular roundish shaped form (so-called cloud-like nuclei), which are never described in JAK2^{V617F} mutated ET and PV when the European Clinical Laboratory, Molecular and Pathological (ECMP/CLMP) criteria for *BRC/ABL* negative Myeloproliferative Disorders or Neoplasms (MPD/MPN, Tables 1 to 7) are applied [1-51].

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

Molecular	Molecular	Molecular
Step 1 V617F+	Step 1→ 2 V617F++ 9p LOH	Step 1→ 2 V617F++ 9p LOH
↓	↓ ↓↓	↓ ↓↓
Spontaneous CFU-MK/EEC BM: ET picture	**Spontaneous EEC/CFU-MK ET-PV picture**	**PVR-1 =↑ LAP score↑ Granulocytes growth/activation Myeloid metaplasia: PMGM**
↓	↓ ↓↓	↓ ↓↓
Increase of enlarged hypersensitive platelets Platelet > 400 x 10^9/L	Increase of hematocrit to above 0.45–0.50: PV Platelets > 400 x 10^9/L	**Leukocythemia**/cytokines↑↑ Fatigue, splenomegaly, masked PV, MPD, MF
↓ ↓↓	↓ ↓↓	↓↓↓
Clinical step 1 →	Clinical step 2 →	Clinical step 3
Microvascular → Thrombosis	Macrovascular → Thrombosis	Secondary MF: 30% Constitutional symptoms
Treatment		
Aspirin sensitive	Aspirin/phlebotomy	IFN/hydroxyurea

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.

Table 2: The Dameshek one cause hypothesis of trilinear PV and Vainchenker's discovery of heterozygous and homozygous JAK2V617F somatic mutation as the driver cause of trilinear MPN with clinical and pathological manifestations of Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Myelofibrosis (MF).

Clinical, Laboratory, Molecular and Pathological (CLMP) classification and staging of JAK2^{V617F} ET and PV (red) versus CALR (blue) and MPL^{515} (black) ET and MF according to Georgii et al 1990 and De Raeve and Michiels 2018

1975 PVSG 2008 WHO clinical criteria MPN ↓ translation Bone Marrow → 2006/08 ECMP criteria	ET ↓ *3 stages of ET*		PV ↓ *4 stages of PV*	Hypercellular ET PMF 1996 ↓ Hypercellular ET	PMGM 1990
2018 CLMP phenotype/stage	stage 1	ET stage 2	PV stage 3	ET stage 4	PMGM 1990
Bone marrow proliferation	*M*	*EM*	*MG*	*EMG/MG*	*M or MG*
Red JAK2^{V617F} Blue CALR	normocellular	prodromal PV	trilinear	Masked PV	
Bone marrow histology picture 2018 CLMP	ET picture	ET/PV picture	PV picture	MGM	CALR-M/MG
Cellularity %	<60%	60-80% prodromal PV	80-100%	80-100%	60-100%
Megakaryocytes	Mature Giant MPL 2006	Pleomorph	Pleomorph	Pleomorphic	Dysmorphic Cloudy nuclei
Large/clusters	↑↑↑	+/↑	+/↑↑	+/↑↑	+/↑↑
Erythropoiesis	N/N *N*	↑	↑↑	N/↓	↓/↓↓
Granulopoiesis	N/N *N*	N/↑	↑↑	↑↑	↑↑
Erythrocytes N = <6x10^{12}/L Dameshek/Michiels	N/N *N*	N<6x10^{12}/L	>6x10^{12}/L	N	N
Platelets >400x10^9/L	+ / + ++	+	+	+	>1000
JAK2^{V617F} 2005 CALR 2013	+ / - neg	+	+/++	-/+/++	CALR ET/MF

2018 CLMP MPN classification and staging separate JAK2^{V617F} positive classical trilinear PV and ET stage 1, 2 and 3 (in red) and masked PV (red) from CALR mutated ET (blue) and MPL^{515} mutated normocellular ET (black)

Figure 1: Hannover Bone Marrow Classification of MPD by Georgii1990[21] versus the 1975 PVSG, the 2008 WHO and the 2013 WHO CMP criteria 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).

Hannover Bone Classification and WHO Criteria for the MPDS ET, PV and PMGM

According to strict morphological, biochemical, and cytogenetic criteria for *BCR/ABL*-positive ET and CML is a separate malignant and individual entity, whereas ET, PV and PMGM form a chronic proliferation of three hematopoietic cell lines [13]. The Hannover Bone Marrow Classification of MPD (Table 1) [8,9,13] separated the Ph-positive or *BCR/AB* -positive CML and ET from the Ph- or *BCR/ABL*-negative MPDs ET, PV and PMGM based on distinct bone marrow histology findings for each of the three MPDs ET, PV and PMGM [8-12]. Increased of clustered large megakaryocytes in bone marrow histology from biopsies is a pathognomonic clue to the diagnosis of in Ph-negative ET PV and PMGM. 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 [13,14]. The Hannover Bone Marrow Classification distinguished the three primary prefibrotic MPDs ET, PV and PMGM from advanced fibrotic stages of MPD (Figure 1) [8,9,34,51]. Prefibrotic PMGM is the third distinct entity of primary MPD in the absence of reticulin or collagen fibrosis in bone marrow biopsy material [15]. Myelofibrosis (MF) is a secondary event in all variants of MPD. Consequently, the WHO defined Chronic Idiopathic Myelofibrosis (CIMF) or Primary Myelofibrosis (PMF) are a misconception. Georgii replaced the term CIMF and PMF by PMGM and used grading of Myelofibrosis (MF) (Table 1) for staging of the early, overt and advanced MPDs ET, PV and PMGM [8,9]. Prefibrotic PMGM 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 [8-15]. The term PMGM of the Hannover Bone Marrow Classification of the MPDs is illogically replaced in the 2001 WHO classification into chronic idiopathic myelofibrosis (CIMF) [16,17] and has been labeled as PMF in the 2008 WHO classification (Figure

1) [18]. 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). The ECMP classification of myeloproliferative neoplasms (MPN) took over the CMGM concept and used the term primary megakaryocytic granulocytic myeloproliferation (PMGM, Table 1, Figure 1) [19,34,51].

The 1975 PVSG and the 2008/2016 classification of MPD and MPN exclude stage 1 Idiopathic Erythrocythemia (IE) and do not recognize prodromal PV by definition [20]. According to ECP 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. ECP 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) [2,3]. The PV experts in the UK and France modified the PVSG criteria by including laboratory screening for serum EPO, spontaneous Erythroid Colony Formation (EEC) as clues for latent and classical PV but did not use bone marrow biopsy for the diagnostic differentiation between PV and primary or secondary erythrocytosis and therefore overlooked stage 1 erythrocythemic PV by definition [21,22]. In 1979 Pearson defined idiopathic erythrocythemia (IE) by increased RCM and not meeting the A and B criteria of the PVSG [21]. IE appeared to be an early stage of PV in about 10% to 15% at time of PV presentation [21-23]. A low serum EPO level has also been described about half of PVSG defined ET patients, which are to be regarded as prodromal phases (forme frusta) of PV [17,23]. 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 [24,25]. In a large study of 241 patients, Mossuz *et al.* identified two thresholds, allowing a specific and direct diagnosis of 65.6% (65-99) of untreated PVSG-defined PV with very low serom EPO levels (EPO <1.4 U/L) and 19.7% (13 of 66) versus Primary Or Secondary Erythrocytosis (PSE) with increased serum EPO levels (EPO >13.7 U/L) [25]. About 50% of patients with absolute erythrocytosis could unequivocally diagnosed as PV or PSE by the combination of increased Red Cell Mass (RCM) and serum EPO levels.

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

In 1950, Dameshek (1900-1969) 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 [26]. This hypothesis of PV as a trilinear MPD has been proven to be correct by Vainchenker and Constantinescu by the discovery of the somatic JAK2^{V617F} mutation as the driver cause of trilinear MPNs ET, PV and MF[27]. On position 617 of the JAK2 JH2 domain Valine (**V**) is replaced by Fenylalanine (F) in the JAK2^{V617F} mutation and induces a loss of inhibitory activity of the JH2 pseudo-kinase part on the JH1 kinase part of JAK2, leading to enhanced activity of the normal JH1 kinase activity of JAK2[27]. 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 [17]. The prevalence of the JAK2 ^{V617F} mutation in PVSG defined PV is 95% and about 50% in ET and MF[17]. 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 between50 to 100% (homozygous) of the granulocytes positive for the JAK2^{V617F} mutation. 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 [28-30,51]. At the bone marrow hematopoietic stem cell level, ET patients are heterozygous and PV patients hetero/homozygous or homozygous for the JAK2^{V617F} mutation (Table 2, 3 and 4) [17,34,51].

Clinical and molecular (CLM) criteria	Bone marrow pathology (P) criteria[51]
Prefibrotic ET	**Normocellular ET [51]**
1. Platelet count above 350 x10^9/l 2. Heterozygous JAK2-^{V617F} mutation, and low JAK2 allele mutation load 3. Erythrocytes below 5.8x10^{12}/L males, below 5.6 x10^{12}/L females	Normocellular bone marrow (<60%), Megakaryocytic (**M**) proliferation of clustered of medium sized to large (pleomorphic) mature megakaryocytes in a normocellular bone marrow (<60%), no proliferation of erythropoiesis and granulopoiesis. Reticuline fibrosis (RF) 0 or 1
Prefibrotic prodromal PV	**ET with bone marrow features of PV[51]**
1. Platelet count above 350 x10^9/l. 2. Erythrocyte count below 5.8x10^{12}/L males, below 5.6x10^{12}/L females. 3. JAK2-^{V617F} mutation and variable JAK mutation load	Increased cellularity (60-80%) due to increased erythrocytic, megakaryocytic (EM) proliferation or trilinear-erythrocytic, megakaryocytic, granulocytic (EMG) proliferation. Increase of clustered medium sized to large (pleomorphic) mature megakaryocytes. Increased LAP score Spontaneous EEC. RF 0 or 1
Prefibrotic hypercellular ET and masked PV	**Masked PV and advanced PV[51]**
1. Platelet count above 350 x10^9/l, 2. Presence of JAK2-^{V617F} mutation and high JAK2 mutation load 3. Moderate myeloid neoplasia of the spleen → splenomegaly 4. No preceding or allied CML, PMGM, RARS-T or MDS. --- **Clinical stage 1**: Hb in lower range of normal: hb below 14 g/dl and above 12 g/dl. **Clinical stage 2**: anemia Hb below 12 to below 10 g/dL, LDH↑, and splenomegaly **Clinical stage 3**: severe anemia, Hb below 10 g/dL, LDH↑↑, circulating CD34+, leuko erythroblastose, tear drop erythrocytes, and large spleen	Hypercellular ET due to increased Erythrocytic, Megakaryocytic and Granulocytic Myeloproliferation (EMG, masked PV, prefibrotic) or increased Megakaryocytic, Granulocytic (MG, fibrotic) proliferation with relative reduced erythroid precursors. Loose to dense clustering of more pleiomorphic megakaryocytes with hyperploid or clumpsy nuclei **Grading of reticulin fibrosis and MF in advanced PV[51]** 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.**

Table 3: European Clinical, Laboratory, Molecular and Pathobiological (ECMP/CLMP) criteria for diagnosis of JAK2^{V617F} mutated ET, prodromal PV and masked PV [19,34,51]. ECMP criteria designed by Michiels 2005/2006

ECMP/CLMP criteria	Bone marrow pathology (P) criteria[51]
Major criteria for PV **A 1.** Erythrocytes above 5.8x10^{12}/L males and above 5.6x10^{12}/L females. **A 2.** Heterozygous and/or homozygous JAK2^{V617F} or JAK2 exon 12 mutation **A 3.** Low serum EPO level **Confirmative criteria** **B 1.** Persistent increase of platelet count x10^9/L: grade I: 350-1500, grade II: above1500. **B 2.** Granulocytes >10 x10^9/l or Leukocytes >12 x10^9/l and raised LAP-score or increased CD11b expression in the absence of fever or infection **B 3.** Myeloid neoplasia of the spleen → splenomegaly on ultrasound echogram (>12 cm length in diameter) or on palpation. **B 4**. Spontaneous endogenous erythroid colony (EEC) formation (optional)	**PV**. Increased cellularity (60-100%) due to increased erythrocytic, megakaryocytic (**EM**) proliferation or trilinearerythrocytic, megakaryocytic and granulocytic (**EMG**) proliferation. Increase of clustered medium to large, pleomorphic megakaryocytes with hyperlobulated nuclei. Absence of stainable iron. **Erythrocytosis.** Normal erythropoiesis, normal granulopoiesis and megakaryocytes of normal size, morphology and no clustering Grading of secondary reticulin fibrosis (RF), collagen fibrosis (RCF) and myelofibrosis (MF)[51] Prefibrotic: RF-0/1 = MF-0 Early fibrotic: RF-2 = MF-1 Fibrotic: RCF 3 = MF-2 Post-PV MF: RCF 4 = MF-3

Table 4: European Clinical, Laboratory, Molecular and Pathological (ECMP/CLMP) criteria for the diagnosis of prodromal, masked and classical JAK2 mutated polycythemia Vera (PV) versus primary or secondary erythrocytoses [19,34,51]. ECMP criteria designed by Michiels 2005/2006.

The 2005 concept of Vainchenker & Michiels is that heterozygous JAK2^{V617F} mutation is enough to constitutively activate TPO mediated megakaryopoiesis to induce ET with the production of constitutively activated (hypersensitive) platelets (Table 2) [17]. Homozygous JAK2^{V617F} mutation is needed to more pronouncedly stimulate EPO mediated erythropoiesis compared to TPO mediated megakaryopoiesis. PV with allele load less than 50% indeed are hetero/homozygous at the EEC level in blood and bone marrow for the JAK2^{V617F} mutation, whereas ET patients are heterozygous with a maximal JAK2^{V617F} mutation load of 50% [31,32]. 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^{V616F} mutation (Table 2). Godfrey *et al* studied the JAK2^{V617F} mutation status of BFU-E grown in low erythropoietin conditions in 77 patients with PV or ET[33]. Using microsatellite PCR to map loss-of-heterozygosity breakpoints within individual colonies, homozygous mutant colonies were absent or present in low percentages in JAK2^{V617F} heterozygous ET, but prevalent and common in patients with homozygous JAK2^{V617F}-positive 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 [33,34,51]. Combined heterozygous homozygous or homozygous JAK2^{V617F} mutation is associated with pronounced constitutively activation of trilinear proliferation of megakaryopoiesis, erythropoiesis and granulopoiesis in the bone marrow as the cause of hyper cellular trilinear PV with a high risk of myelofibrotic progression.

According to WHO and ECMP criteria (Tables 3 and 4) [19,34,51], heterozygous JAK2^{V617F} positive ET is defined by a normocellular with slight increase of erythropoiesis in the bone marrow or with a hypercellular bone marrow due to increased erythropoiesis (Figures 2 and 3).

JAK2^{V617F} mutated WHO defined PV uniformly reveal a hyper cellular bone marrow histology due to increase of trilinear hematopoiesis of megakaryopoiesis, erythropoiesis and granulopoiesis (panmyelosis [1,26;51]) and no or slight increase of reticuline fibers (Figures 3 and 4, Table 4) [4,19,34,51].

The UK MPN Study Group assessed the clinical features in the cohort of 806 PVSG = 2008/2016 WHO defined ET patients [51] subdivided in 414 JAK2^{V617F} positive and 362 JAK2 wild type ET and evaluated the bone marrow features in 393 ET patients [35,36]. 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, more venous thrombosis and a higher rate of transformation into PV. PVSG = 2008/2016 WHO defined JAK2 wild type ET[51] had significant higher platelet counts (962, range 668-1535x10^9/L) than JAK2^{V617F}-positive ET (846, range 632-1222x10^9/L) [35]. 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 pathologists who did not know the JAK2 mutation status[36]. 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 amain feature of prodromal PV, masked PV and classical PV [4,17,19,34,51].

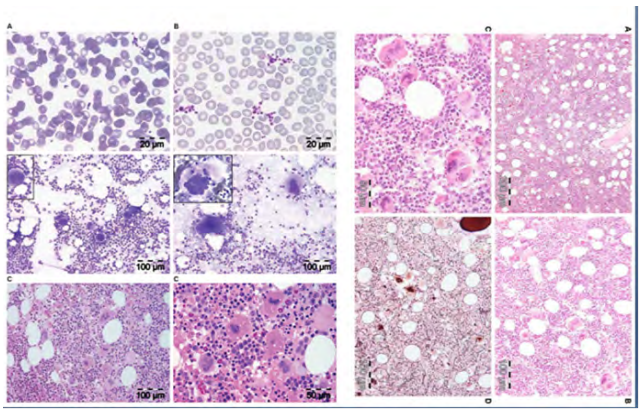

Figure 3: Bone marrow features of JAK2^{V617F} heterozygous positive essential thrombocythemia (ET, left) and JAK2^{V617F} homozygous acute onset polycythemia vera (PV, right). Contribution of Drs Michiels and De Raeve, University Hospital Antwerp, Belgium.

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 435x10^9/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: Hypercellur bone marrow due to increased megakarypoiesis, erythropoiesis and granulopoiesis in a case with rapid onset trilinear JAK2V617F homozygous positive PV.

JAK2$^{exon\ 12}$ Mutated PV negative for the JAK2^{V617F}mutation

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 [37-39].

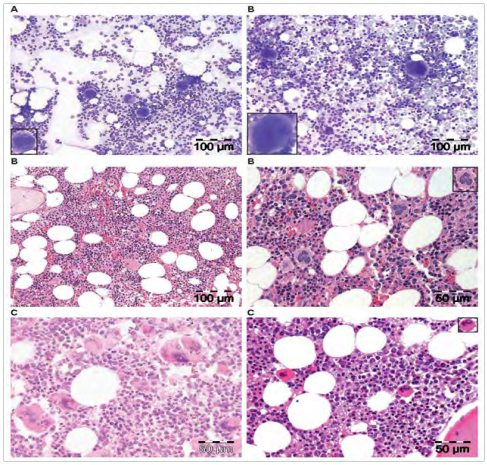

Figure 4: Morphology of bone marrow smears in control and polycythemia vera (PV) and bone marrow histology in PV versus idiopathic erythrocytosis. Upper panels. Bone marrow smears of PV with homozygous rapid onset PV show large megakaryocytes and hypercellularity (upper right) as compared to normal sized megakaryocytes and normal cellularity in a control Bmsmear (Upperleft).

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 acase with classical PV with a hypercellular trilinear bone marrow proliferation (left bottum).

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

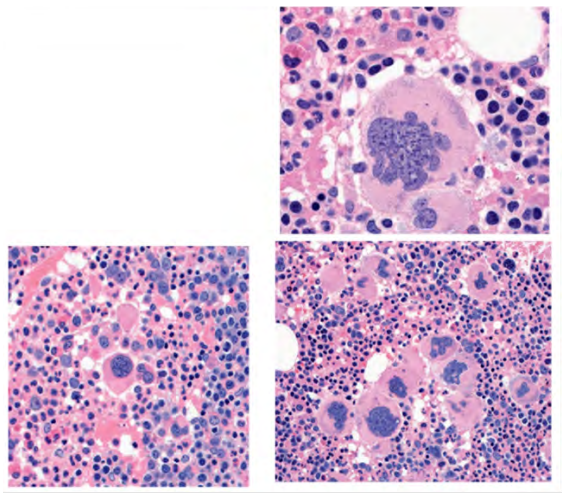

Figure 5: Bone marrow histology in PV patients with JAK2^{exon12} mutations are featured by prominent erythroid hyperplasia meeting the criteria for Idiopathic Erythrocythemia (IE) or classical PV and megakaryocytic hyperplasia of atypical small to medium-sized large megakaryocytes with various degrees of monolobation to hyperlobation and abnormal chromatin distribution [39].

JAK2^{exon12} mutations in 10 Idiopathic Erythrocytosis (IE) patients showed increased red cell mass and could be diagnosed in 6 patients as PV in 6 and IE in 4 cases [37]. Bone marrow biopsies in 5 JAK2^{exon12} positive patients showed characteristic erythroid hyperplasia with some morphological abnormalities of the megakaryocyte and normal granulopoiesis in bone marrow biopsy specimens clearly different from primary or secondary erythrocytosis. The bone marrow histology in 7 cases of JAK2^{exon12} mutated MPN (IE in 4, PV in 2, MF in 1) revealed hyperplasia of atypical small to medium-sized large megakaryocytes was present in all (Figure 5) [38,39]. Which differs from JAK2^{V617F} mutated ET and PV (Figures 2, 3 and 4). At diagnosis, JAK2^{exon12} 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 or sticky platelet syndrome) in 4 at platelet counts between 152 and 790x10^9/L (of whom 5 below and 2 above 300x10^9/L) [38,39]. The JAK2^{exon12} 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^{exon12} PV bone marrows with a predominance of smaller forms of megakaryocytes with atypical nuclei with various degrees of monolobulated, normolobulated to hyperlobulated nuclei and abnormal chromatin distribution (Figure 5) [39].

Bone Marrow Histology in MPL^{515} Mutated Essential Thrombocythemia

Within the JAK2 wild type MPN, the prevalence of the MPL^{515} mutation as the cause of ET is 3% in the Vannucchi study [41], and 8.5% in the UK studies [42,43]. In the study of Vannucchi et al [41], patients with JAK2 wild type ET carrying the MPL^{515} mutation present with typical microvascular erythromelalgic acrocyanosis and migraine-like ocular or cerebral ischemic events (Sticky Platelet Syndrome) but have no clinical, laboratory and bone marrow features of prodromal PV at diagnosis, do not evolve into overt PV during follow-up, have normal serum EPO, normal ferritin levels, absence of spontaneous endogenous erythroid colonies (EEC). 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 hyperlobulated stag-horn nuclei in a normal cellular bone marrow and no increase of erythropoiesis (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 JAK2V617F mutated MPN [34,51]. First, The presence of clustered small and giant megakaryocytes with deeply lobulated stag-horn like nuclei (Figure 6, Table 5). In ET carrying the MPL^{515} 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 was comparable regarding size and degree of pleomorphy (Figures 2, 3 and 4). Second, there was local increase of erythropoiesis in areas of loose clustered pleiomorphic megakaryoctyes in normocelluar JAK2^{V617F} mutated ET and prodromal PV, which is not seen in MPL^{515} mutated ET (Figure 6).Third, JAK2 wild type MPL^{515} mutated ET have no clinical, laboratory and bone marrow features of prodromal PV at diagnosis (Table 1), 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 from MPL^{515} mutated ET with normal LAP score and serum EPO and giant megakaryocytes with staghorn-like nuclei similar to “true” ET (Table 1) [34,51].

ECMP/CLMP criteria MPL^{515} Thrombocythemia	Bone marrow pathology (P) MPL^{515} MPN^{51}
Platelet count above 350x10^{9}/L and presence of large platelets in blood smear Normal erythrocyte count below 5.8x10^{12}/L males, below 5.6 x10^{12}/L females Presence of MPL^{515} mutation Normal serum EPO Normal LAP score (CD11b) No or slight splenomegaly No preceding or allied CML, PV, PMGM, RAS-T or MDS Clinical staging similar as in CALR thrombocythemia based on the degree of anemia, splenomegaly and myelofibrosis	**Megakaryocytic (M) p**roliferation in a normocellular (<60%) bone marrow featured by large to giant mature megakaryocyte with hyperlobulated, staghorn-like nuclei. No increase of erythropoiesis, and granulopoiesis[51] No or slight increase in reticulin RF 0/1 Grading of reticulin fibrosis (RF) and myelofibrosis (MF) similar as described for JAK2^{V67F} and CALR Thrombocythemia (Tables 3 and 6)

Table 5a: European Clinical Laboratory, Molecular and Pathological (ECMP/CLMP) criteria for the diagnosis of nrmocellular ET carrying one of the MPL^{515} mutations [34,51]. ECMP criteria designed by Michiels between 2006 and 2014.

ECMP/CLMP criteria CALR thrombocythemia (ET)	Pathological (P) criteria of CALR MPN[51]
A1 No preceding or allied other subtype of myeloproliferative neoplasm PV, CML, MDS. The main presenting features is pronounced isolated thrombocythemia with platelet count around or above 1000x10^{9}/L	Dual megakaryocytic granulocytic (**MG**) proliferation and relative or absolute reduction of erythropoiesis and erythroid precursors. Abnormal dense clustering and increase in atypical medium sized, large to giant immature megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects No features of PV in blood and bone marrow
A2 CALR mutation and JAK2 wild type	
C Clinical stages of CALR Thrombocythemia	**MF Grading reticulin fibrosis (RF), myelofibrosis (MF)** [51]
C 1. Early clinical stage: Hb>12g/dL, slight to moderate splenomegaly, thrombocytosis around or above 1000x10^{9}/L, normal LAP score	MF 0 Prefibrotic CALR MG, no reticulin fibrosis RF 0/1
C2. Intermediate clinical stage: slight anemia Hb<12 to >10 g/dL, decreasing platelet count, splenomegaly, increased LDH and definitive tear drop erythrocytes	MF 1 Early fibrotic CALR MG slight reticulin fibrosis RF 2
C3. Advanced stage: anemia Hb<10 g/dL, tear drop erythrocytes, increased LDH, increased CD34+ cells, pronounced splenomegaly, normal or decreased platelet counts, leucocytosis or leukopenia.	MF 2 Fibrotic CALR MG increase RF grade 3 and slight to moderate collagen fibrosis (RCF) MF 3 Advanced fibrotic CALR MG with RF 3 and collagen fibrosis (RCF) plus osteosclerosis

Table 5b: European Clinical Laboratory, Molecular and Pathological (ECMP/CLMP) criteria for hypercellular ET associated with primary megakaryocytic, granulocytic myeloproliferation (PMGM) or JAK2 wild type Thrombocythemia caused by calreticulin (CALR) mutations[19,34,51]. ECMP criteria designed by Michiels between 2006 and 2014.

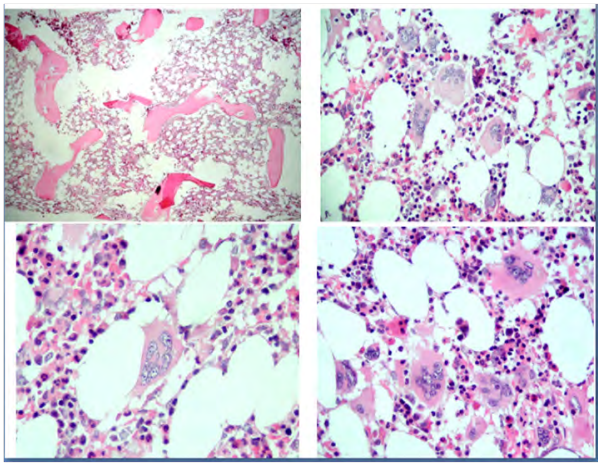

Figure 6: Bone marrow histology in essential thrombocythemia (ET) consistent with normocellular ET carrying the MPL^{W515L} mutation showing giant megakaryocytes with hyperlobulated stag-horn like nuclei charateristic for ET the MPLW515L/K mutation. Courtesy of Dr Schwarz, University Hospital Prague,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

Clinical, lzaboratory Features and Bone Marrow Histology in CALR Mutated ET and MF

Dr Kralovics and his team discovered Calreticulin (CALR) mutationin 78 of 311 (25%) ET patients, in 72 of 203 (35%) MF patients, in none of 382 PV patients [44]. 195 (67%) of 289 JAK2 wild type ET and 105 (80%) of 120 wild type MF carried one of the CALRmutations. Green and his team of MPN investigators in the UK 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 [45]. CALR exon 9 mutations were found in 26 of 31 (84%) patients with JAK2/MPL wild type MF, were absent in all 120 patients who had JAK2 or MPL mutations, were present 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.CALR, JAK2^{V617F} and MPL^{515} mutated MPN mutually exclude each other [44,45,51].

In eight newly diagnosed CALR positive ET cases in 2014 Michiels & De Raeve found consistent bone marrow characteristics of hypercellular ET as the presenting feature of prefibrotic and early fibrotic stages of PMGM. Bone marrow histology in typical prefibrotic CALR ET and in early fibrotic CALR myelofibrosis (MF) (Figure 7) 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, which are not seen in MPL^{515} mutated ET (Figure 6) and also not in JAK2^{V617F} mutated ET, prodromal PV and classical PV (Figures 2, 3 and 4). The JAK2/MPL wild type but CALR-mutated ET and MF patients appeared to become the third distinct MPN entity with typical characteristics of PMGM without features of PV when the ECMP criteria are applied (Figure 8). Absence of clinical, laboratory and bone marrow features of PV and no transformation into PV has been observed in CALR mutated patients [46-48,51].

The progression of ET disease burden complicated by splenomegaly and secondary myelofibrosis (MF) belong to the natural history of all molecular variants of the JAK2, MPL and CALR mutated MPNs. Life expectance was significantly longer in CALR mutated MF patients but the mean age of CALR mutated MPN was 10 years younger as compared to those with a JAK2^{V617F} or MPL^{515} mutation. This Observation is a mystification or illusion in the mind of 2008/2016 WHO investigators simple because the CALR MF patients are 10 years younger at time of presentation whereas the overall survival of CALR mutated MF was 23 years as compared to 14.4 years of MF patients

with the JAK2^{V617F} or MPL^{515} mutation MF [46,47,51]. Patients with JAK2^{V617F} mutated ET and PV had a similar high risk of aspirin responsive platelet mediated microvascular and major thrombosis as, which was twice that of thrombocythemia patients with CALR mutation [46,47,51]. The lower incidence of thrombotic complications in CALR mutated ET does indicate that CALR mutated are not constitutively activated but indolent similar as in Ph-positive ET. CALR mutated ET patients lack PV features with normal or low normal hemoglobin, normal LAP score, normal serum EPO levels and normal leukocyte counts ascompared to increased LAP score and decreased serum EPO levels in ECMP/CLMP defined JAK2^{V617F} mutated ET and prodromal PVpatients (Table 3) [34,51].

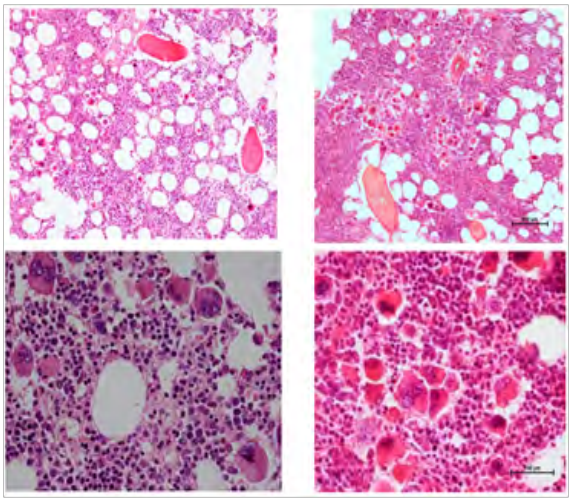

Figure 7: Bone marrow features in newly diagnosed calreticulin (CALR) mutated essential thrombocythemia (ET, left) and early stage myelofibrosis (MF, right).

Left: Clinical case of calcireticulin (CALR) positie ET who presented with normal values for hemogobin, hematocrit and erythrocytes, platelet count of 1832x10^9/L and slight splenomegaly (16 cm lenght diameter on echogram). Bone marrow histology is hypercellular with relative decrease of erythropoisis, dense cluster of immature megakaryocytes with hypolobulated nuclei consistent, and no increase of reticulin fibrosis consistent with a typical PMGM 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 9.2x10^9/L, platelets 347x10^9/L, LDH 1393 U/l, and the presence of tear drop erythrocytes, poikolocytosis and polychromasie in a peripheral bloodsmear, and hypercellular bone marrow with relative decrease of erythropoisis, dense cluster of immature megakaryocytes with hypolobulated 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 Schot and De Raeve, University Hospital, Brussels, Belgium.

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 ECMP/CLMP criteria as compared to the PVSG criteria [34,51]. 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 MPL^{515} mutations is the second distinct MPN without features of PV at diagnosis and during follow-up (Figure 8). In a prospective study of 59 JAK2^{V617F} positive ET and 44 JAK2 wild ET cases, Pich *et al.* [40] 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 MPL^{515} 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, ferritin levels and LAP score [41,42,51]. The prevalence of MPL^{515} mutated ET or MF patients ranges from 5 to 10% of the JAK2 wild type MPN population [41,42]. In the large collaborative European study of 176 MPN cases with the MPL^{515} mutations W515L and W515K were detected in 110 and in 58 respectively [43]. 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[43]. In 254 WHO-defined PMF patients the JAK2-, MPL- and CALR-mutations were detected in 58%, 8.3 and 25% respectively and 8.7% were triple negative [48]. 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. CALR-mutated MF patients were about 10 years 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 but JAK2V617F mutated MF patients whether ASXL1-negative or positive [48].

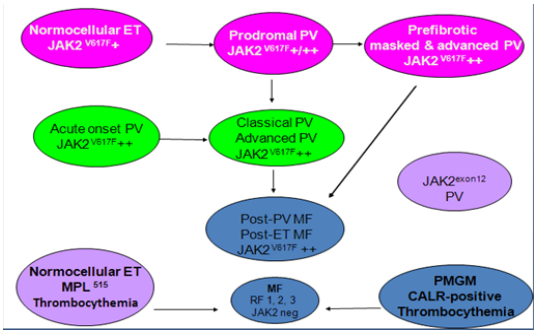

Figure 8: Classification of five distinct ECMP[34] and CLMP[51] defined clonal myeloproliferative neoplams (MPN) and translational states of JAK2^{V617F} mutated ET, prodromal PV, classical PV, Post ET and post-PV myelofibrosis (MF) and of MPL^{515} and CALR mutated Thrombocythemia and MF and triple negative MPN.

The awareness of the molecular heterogeneity of the MPNs including JAK^{V617F}, JAK2^{exonn12}, MPL and CALR mutation on top of epigenetic factors reflect the funeral of the WHO defined term primary myelofibrosis (PMF) [49-51]. The 2008/2016 WHO defined Essential Thrombocythemia (ET) is not essential and primary myelofibrosis (PMF) is not primary anymore when the ECMP classification is applied [51]. The PVSG and 2008/2016 WHO defined term ET and PMF [16-18,49,51] can easily be replaced by CALR-, MPL^{515}-and JAK2^{V617F}-mutated thrombocythemia and secondary myelofibrosis (MF) with various degrees of splenomegaly, hypersplenism and myelofibrotic transformation of the bone marrow (Figure 8) [8,9,34,51].

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