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REVIEW

European *vs* 2015-World Health Organization clinical molecular and pathological classification of myeloproliferative neoplasms

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Abstract

The *BCR/ABL* fusion gene or the Ph[1]-chromosome in the t(9;22)(q34;q11) exerts a high tyrokinase acticity, which is the cause of chronic myeloid leukemia (CML). The 1990 Hannover Bone Marrow Classification separated CML from the myeloproliferative disorders essential thrombocythemia (ET), polycythemia vera (PV) and chronic megakaryocytic granulocytic myeloproliferation (CMGM). The 2006-2008 European Clinical Molecular and Pathological (ECMP) criteria discovered 3 variants of thrombocythemia: ET with features of PV (prodromal PV), "true" ET and ET associated with CMGM. The 2008 World Health Organization (WHO)-ECMP and 2014 WHO-CMP classifications defined three phenotypes of $JAK2^{V617F}$ mutated ET: normocellular ET (WHO-ET), hypercelluar ET due to increased erythropoiesis (prodromal PV) and ET with hypercellular megakaryocytic-granulocytic myeloproliferation. The $JAK2^{V617F}$ mutation load in heterozygous WHO-ET is low and associated with normal life expectance. The hetero/homozygous $JAK2^{V617F}$ mutation load in PV and myelofibrosis is related to myeloproliferative neoplasm (MPN) disease burden in terms of symptomatic

splenomegaly, constitutional symptoms, bone marrow hypercellularity and myelofibrosis. JAK2 exon 12 mutated MPN presents as idiopathic eryhrocythemia and early stage PV. According to 2014 WHO-CMP criteria JAK2 wild type MPL^{515} mutated ET is the second distinct thrombocythemia featured by clustered giant megakaryocytes with hyperlobulated stag-horn-like nuclei, in a normocellular bone marrow consistent with the diagnosis of "true" ET. JAK2/MPL wild type, calreticulin mutated hypercellular ET appears to be the third distinct thrombocythemia characterized by clustered larged immature dysmorphic megakaryocytes and bulky (bulbous) hyperchromatic nuclei consistent with CMGM or primary megakaryocytic granulocytic myeloproliferation.

Key words: Myeloproliferative disorders; Essential thrombocythemia; Primary megakaryocytic granulocytic myeloproliferation; Myelofibrosis; JAK2^{V617F} mutation; MPL^{515} mutation; Calreticulin mutation; JAK2 wild type; Myeloproliferative neoplasm; Bone marrow pathology; Polycythemia vera

Core tip: The 2015 World Health Organization-Clinical Molecular and Pathological criteria define three phenotypes of JAK2^{V617F} mutated myeloproliferative neoplasms (MPNs) essential thrombocythemia (ET), prodromal polycythemia vera (PV), prodromal PV, hypercellular megakaryocytic-granulocytic myeloproliferation and classical PV *vs* the JAK2 exon 12 mutated idiopathic eryhrocythemia and PV. MPL^{515} mutated JAK2 wild type ET and myelofibrosis is a distinct thrombocythemia without features of PV in blood and bone marrow. Calreticulin mutated JAK2/MPL wild type ET and myelofibrosis is the third thrombocythemia entity with characteristic features of primary megakaryocytic granulocytic myeloproliferation in the bone marrow, which are not seen in JAK2 and MPL mutated MPNs. MPN disease burden is best reflected by the degree of anemia and splenomegaly on top of mutation allele burden, bone marrow cellularity and increase of reticulin fibrosis.

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INTRODUCTION

Dameshek *et al*[1] proposed in 1940 a set of symptoms, signs and laboratory tests for the diagnosis of polycythemia vera (PV) based on the description of 20 cases with PV seen between 1928 and 1937. Dameshek believed that the following minimal data should be present before a definite diagnosis of PV can be made: plethoric appearance, splenomegaly, definitely elevated erythrocyte count above 6 × 10^{12}/L, elevated platelet count, and elevated hematocrit. The bone marrow is pathognomonic diagnostic showing a panmyelosis (increased trilinear hematopoiesis) and large megakaryocytes[1,2]. In a doubtful case, the procedure of blood volume estimation may be helpful[1-3]. Between 1940 and 1950, Dameshek[2] considered the majority of PV patients as fundamentally normal[3]. The PV patient frequently has a long life span and every attempt should be made to keep the treatment of PV as physiologic as possible by venesection aiming at hematocrit of 0.40 as a satisfactory method resulting in a state of iron deficiency[1-3]. Red cell count remains elevated above 6 × 10^{12}/L due to microcytosis of red cells, but hematocrit and hematocrit levels remain low for periods of months to years. During of complete remission of PV by phlebotomy alone the patient is reasonable asymptomatic indicating that the best index of phlebotomy therapy is the hematocrit value and hematocrit concentration[2,3]. During the state of chronic iron deficiency and normal values of haematocrit (0.40), the patient himself frequently becomes completely asymptomatic. On this program it is possible to control PV patients for several up to ten to fifteen years and such PV are in as good health as comparable persons of the same age group. Dameshek hesitated to use a potentially dangerous radioactive material in an individual with a relatively long life span and questioned whether the acute leukemic states in some cases are due to the potentially leukemogenic drug P^{32} or may be part of the natural history of PV. In the experience of Dameshek in about 50 reasonably well followed cases of polycythemia, acute leukemia developed in only 1 (2%) instance without previous roentgen ray or radioactive phosphor therapy[3-5].

Dameshek[2] (1900-1969, Figure 1) defined in 1950 PV as a total marrow disorder in which peripheral blood erythrocytosis, leukocytosis and thrombocytosis are all simultaneously present and the bone marrow is featured by a trilinear myeloproliferative disease (MPD) of erythrocythemia, thrombocythemia and granulocythemia[2,3]. PV is complicated by primary myeloid metaplasia of the spleen with increasing degree of splenomegaly, myelofibrosis and the development of anemia in about one third of the cases after long-term follow-up of about 15 to 30 years. Dameshek[2] proposed in 1950 the one cause hypothesis cause for PV as a trilinear MPD due to the presence of excessive bone marrow stimulation by an unknown factor or the lack or diminution of an inhibitory factor[2,3]. The one cause hypothesis of Dameshek for trilinear PV has been confirmed by Vainchenker in France by the discovery in 2005 of the acquired somatic JAK2^{V617F} mutation as the cause of three phenotypes of MPD essential thrombocythemia (ET), PV and myelofibrosis (MF)[2,3].

In this historical appraisal of the MPDs and myelo-

Table 1 The 1980 Rotterdam clinical and pathological criteria for essential thrombocythemia and polycythemia vera

1A The 1980 RCP major (A) and confirmative (B) criteria for prefibrotic ET		
A1 Persistent platelet count in excess of 400 × 10^9/L		
A2 Increase and clustering of enlarged megakaryocytes in bone marrow biopsy		
A3 No or slight increase of reticulin fibers (RF 0 or RF 1)		
B1 Presence of large platelets in a peripheral blood smear		
B2 Absence of any underlying disease for reactive thrombocytosis and normal ESR		
B3 No splenomegaly (< 12 cm) or slight splenomegaly on palpation or scan (< 15 cm)		
B4 Increase of LAP-score and no signs of fever or inflammation		
Exclusion criterion		
Ph^+ chromosome and any other cytogenetic abnormality in blood or bone marrow cells		
1B The 1980 RCP major (A) and minor (B) criteria for prefibrotic PV		
A1 Raised red cell mass. Male > 36 mL/kg, female > 32 mL/kg consistent with erythrocyte count of > 6 × 10^{12}/L (Dameshek[1,2])		
A2 Absence of primary or secondary erythrocytosis by clinical and laboratory tests		
A3 Slight, moderate or marked increase in bone marrow biopsy of clustered, enlarged pleomorphic megakaryocytes with hyperlobulated nuclei and moderate to marked increase cellularity of megakaryopoiesis/erythropoiesis or typically trilinear mega-erythro-granulopoiesis. A typical PV bone marrow excludes erythrocytosis. No or presence of reticuline fibers and no collagen fibers (no dry tap)		
B1 Thrombocythemia, persistant increase of platelet > 400 × 10^9/L		
B2 Leukocytosis, leucocyte count > 10^9/L and low erythrocyte sedimentation rate		
B3 Raised leukocyte alkaline phosphatase score > 100, absence of fever or infection		
B4 Splenomegaly on palpation or on isotope/ultrasound scanning		
A1 + A3 plus one of B establishes PV and excludes any variant of erythrocytosis		
1C Grading of bone marrow biopsy content of RF according to Ellis *et al*[41], Georgii *et al*[35,36] and Wilkins *et al*[171] and WHO grading of MF[98-101]		
Grading RF[41]	Grading MF 2008 WHO[91]	Description of RF and reticulin/collagen fibers in MF as a secondary event in MPN
Normal RF 0	N MF 0	No reticulin fibers, occasional individual fibers or focal areas with tiny amount of reticulin fiber network
Slight increase RF 1	+ MF 0	Fine reticulin fiber network throughout much of section and no course reticulin fibers
Moderate increase RF 2	++ MF 1	Diffuse fine reticuline network with focal collections of thick course reticulin fibers and no collagenisation
Marked increase RF 3	+++ RCF = MF 2	Diffuse and dense increase in reticulin with extensive intersections, and presence of collagen fibers and no or minor O
OS Dry tap RF 4	Sclerotic RCF and O = MF 3	Diffuse and dense reticulin with with coarse bundles of collagen associated with significant O

MF: Myelofibrosis; MPN: Myeloproliferative neoplasms; O: Osteosclerosis; RCP: Rotterdam clinical and pathological; ET: Essential thrombocythemia; RF: Reticulin fibrosis; PV: Polycythemia vera.

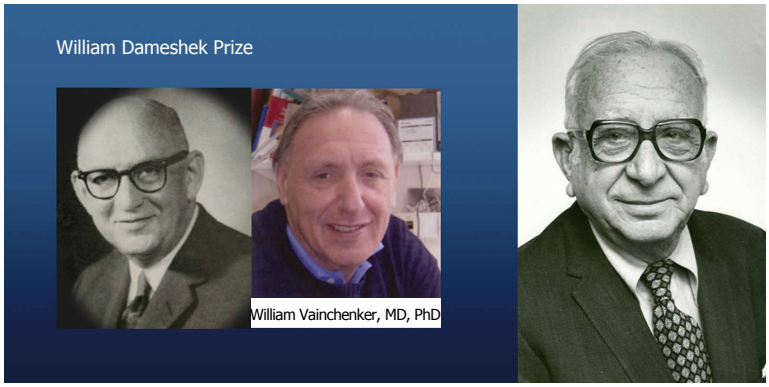


Figure 1 Dameshek 1900-1969. Description of trilinear PV in 1950. "Photo courtesy of the American Society of Hematology (ASH) 2012." Louis Wasserman 1912-1999. Founder of the polycythemia vera study group 1967. Photo by Jerry Soalt. "Courtesy of the ASH" 2012. William Vainchenker discoverer of the JAK2V617F mutation in 2005 as the cause of the trilinear MPNs ET, PV and MF. MF: Myelofibrosis; PV: Polycythemia vera; ET: Essential thrombocythemia; MPN: Myeloproliferative neoplasms.

proliferative neoplasm (MPN) the Rotterdam, Hannover, Cologne and European clinical and bone marrow criteria *vs* the polycythemia vera study group (PVSG) and World Health Organization (WHO) classifications (Tables 1-7) are compared against the 2015 WHO-Clinical Molecular and Pathological (CMP) criteria for the diagnosis and staging of the masked and manifest MPNs ET, PV and MF (Tables 8-12).

MYELOPROLIFERATIVE DISORDERS *VS* CHRONIC MYELOID LEUKEMIA

In 1951 Dameshek[6] illogically proposed an unifying theory that erythroleukemia, chronic myeloid leukemia (CML), PV, idiopathic or agnogenic myeloid metaplasia (AMM) of the spleen, megakaryocytic leukemia or primary represent one myeloproliferative activity of bone

Table 2 Polycythemia vera study group criteria for polycythemia vera[10] and diagnostic differentiation of polycythemia vera from all variants of primary and secondary erythrocytoses by bone marrow histology[17]

Major criteria PV	Minor criteria PV
A1 RCM, males > 36 mL/kg females > 32 mL/kg. Hemoglobin 18.5 g/dL male and > 16.5 g/dL females (PVSG, WHO)	B1 Thrombocytosis Platelet count > 400 × 10^9/L
A2 Normal arterial oxygen saturation > 92%	B2 Leukocytosis > 12 × 10^9/L
A3 Splenomegaly on palpation	B3 Raised neutrophil alkaline phosphatase score > 100 or raised B12 (> 900 ng/L) or raised unsaturated B12 binding capacity (> 2200 ng/L)
	B3 is replaced by spontaneous EEC as a specific clue to PV
Benign erythrocytosis: 1980 RCP criteria	Myeloproliferative PV: 1980 RCP criteria
RCM, males > 36 mL/kg females > 32 mL/kg or increased erythrocytes above 6 × 10^{12}/L	Increased red cell counts > 6 × 10^{12}/L or increased RCM and increase of clustered large megakaryocytes with hyperlobulated nuclei is a pathognomonic diagnostic clue to PV
Normal platelet and leukocyte counts	Normal RCM = inapparent erythrocytosis is not associated with splenomegaly and shows normal bone histology, whereas IPV is associated with splenomegaly and show typical features of PV bone marrow histology
Normal bone marrow histopathology: normal cellularity and erythropoiesis, and normal size, morphology and distribution of megakaryocytes	
Classification of erythrocytoses[74]	Notes anno 1980-1999[74]
Congenital or primary erythrocytosis including mutation truncated EPO recepotor, disrupted oxygen homeostasis in Chuvash erythrocytosis, high oxygen affinity hemoglobinopathy, and congenital autonomous EPO production	Increased RCM does not distinguish between PV and primary erythrocytosis
	Increased RCM does not distinguish between PV and IPV. IPV is featured by advanced PV with normal hb, Ht and erythrocyte count due to splenomegaly and hypersplenism and with increase of reticulin fibrosis with typical PV bone marrow features
Secondary erythrocytosis due to autonomous EPO production in renal diseases or by tumour cels or due to hypoxia	In IPV the values of hemoglobin hematocrit and erythrocytes are normal but RCM is increased due to splenomegaly with absence of hypervolemic symptoms
Idiopathic erythrocytoses	

Diagnostic criteria for polycythemia vera (PV) proposed in 1975 by the polycythemia vera study group (PVSG)[10] and used in the 2001 and 2008 World Health Organization Classifications of MPD and MPN[75,76]. Diagnosis of PV is acceptable if the following combinations are present: A1 + A2 + A3 or A1 + A2 + any two from category B. Diagnostic differentiation of benign erthrocytosis from myeloproliferative polycythemia vera by histopathology from bone marrow sections according to the Rotterdam clinical and pathological (1980 RCP criteria for PV) (Table 1)[73,74]. RCM does not distinguish between PV and primary or secondary erythrocytosis. RCM does not distinguish between PV and inapparent PV with splenomegaly in splanchnic vein thrombosis (IPV, Table 3). In IPV RCM is increased due to splenomegaly with absence of hypervolumemic symptoms. Bone marrow histology distinguishes PV from all variants of erythrocytosis with a sensitivity and specificity of 100%. RCM: Raised red cell mass; EEC: Erythroid colony formation; IPV: Inapparent PV.

Table 3 Comparion of clinical and laboratory features between polycythemia vera study group defined polycythemia vera (group A) and inapparent polyctemia vara (group B)

Clinical feature	Group A PV	Group B IPV	*P*-value
No. of cases	85	18	
Age (range)	61 (27-83)	52 (28-82)	NS
Sex male/female	56/42%	39/61%	NS
Splenomegaly	44 (52%)	15 (83%)	< 0.005
Leukocytes > 12 × 10/L	31 (36%)	5 (28%)	NS
Platelets > 500 × 10/L	40 (47%)	10 (56%)	NS
Red cell counts × 10/L: males	6.2 (4.9-7.4)	5.2 (4.7-5.9)	< 0.0002
Red cell mass males	48.2 (36-60)	43.3 (41-61)	NS
Red cell counts females	6 (4.2-7.3)	4.7 (3.7-5.5)	< 0.003
Red cell mass females	40.1 (32-59)	37.3 (34-46)	NS
Plasma volume PV *vs* IPV			
Increase/theoretical norm (%)	10 (-11, 61)	36 (20, 98)	< 0.00001

Lamy *et al*[44]. Conclusion: inapparent polycythemia vara (IPV) is featured by normal values of haemoglobin, haematocrit and erythrocyte counts, in the absence of hypervolumic symptoms and in IPV red cell mass is increased related to the degree of increased spleen size. NS: Not significant.

marrow cells due to one hypothetical stimulus[6,7] on the basis of which the PVSG defined in 1975 the authorative criteria for the disgnosis of PV, AMM and primary hemorrhagic thrombocythemia (PHT)[8-10]. PMF or AMM is a clinicopathological entity not preceded by any other PVSG defined MPD PHT or ET, PV, CML or preleukemia with myelodysplastic features. CML is leukemia or neoplasia that destroy normal hematopoiesis whereas ET, PV and AMM form a benign proliferation of trilinear hematopoietic proliferation in the bone marrow (myeloproliferation) and extramedullary hematopoiesis in the spleen[8].

Lumping of erythroleukemia with PV, and putting together chronic granulocytic or myeloid leukemia with PV was without scientific foundation[6,7,11]. Dameshek *et al*[11] and Dameshek[12] (1990-1969) separated in 1969 erythroleukemia from CML and PV by describing that all variations of the chronic and acute erythroleukemias form a distinct entity, the Di Guglielmo syndrome[11,12]. When running its full course, the Di Guglielmo syndrome appeared to pass through three stages of refractory anemia with predominant erythroid hyperplasia and maturation arrest with development of dysplastic features and gradual transition into a mixed erythroblastic myeloblastic leukemia. According to a prospective clinical basic research study by Michiels[13] and Michiels *et al*[13,14], the sequential preleukemic stages of the Di Gugliemo syndrome appeared to be a continuum of trilinear myelodysplatic syndrome (MDS), refractory anemia with excess of blasts (RAEB) and acute myeloid leukemia (AML)[13,14].

DISCOVERY OF BCR/ABL IN PHILADELPHIA-POSITIVE CHRONIC MYELOID LEUKEMIA

Nowell *et al*[15] discovered a disease specific minute cytogenetic marker in patients with CML, labelled after

Table 4 Clinical and hematological findings in thrombocythemia of various myeloproliferative diseases polycythemia vera, primary myelofibrosis and essential thrombocythemia in 395 myeloproliferaive disease patients from the Cologne Institute of Pathology 1980-1989

Diagnosis cologne criteria	PV	PMF	True ET	NV
No. of patients	55	250	40	
Thrombocythemia > 500 × 10^9/L (%)	48	48	100	< 350
Thrombocythemia > 1000 × 10^9/L (%)	6	17	65	
Age (median years)	63	66	58	
Male/female	20/35	58/62	14/26	
Platelets, × 10^9/L mean ± SD	808 ± 288	960 ± 361	1386 ± 541	< 350
Erythrocytes, × 10/L mean ± SD	6.7 ± 0.2	4.5 ± 0.1	4.6 ± 0.7	< 6.0
Hemoglobin, g/dL	17.7 ± 0.4	12.8 ± 0.2	13.7 ± 2	
Leukocytes	17 ± 1	15 ± 9	13 ± 5	
Leukocyte alkaline phosphatase score	164 ± 91	98 ± 83	57 ± 43	< 10
Spleen size increase on palpation (cm)	2.0 ± 3.3	2.6 ± 3.1	0.4 ± 0.8	NP
Observed 10 yr survival (mo)	106	85	170	
Specific loss of life expectancy (%)	19	22	3	
Bone marrow histopathology	PV	PMF	True ET	RT
Megakaryocytes	Pleomorph	Immature giant	Staghorn	N
Frequency/mm hematopoietic area	123/27	112/37	157/45	98/39
Size (μm^2)	385 ± 102	386 ± 197	425 ± 117	328 ± 84
Erythropoiesis × 10	44 ± 8	9 ± 4	22 ± 5	27 ± 4
Granulopoiesis × 10	65 ± 12	58 ± 27	47 ± 15	57 ± 18
Reticulin fibers × 10 (mm)	21 ± 11	97 ± 41	15 ± 7	15 ± 6
No. of patients	120	40		
LAP score	110 ± 60	57 ± 43	< 100	
Spleen size	1.7 ± 1.4	0.4 ± 0.8	NP	
Observed survival (mo)	77	170	-	
Specific loss in life expectancy (%)	53	3	0	

Data from Thiele *et al*[90]. MPD: Myeloproliferaive disease; PV: Polycythemia vera; PMF: Primary myelofibrosis; ET: Essential thrombocythemia; LAP: Leukocyte alkaline phosphatase; RT: Reactive thrombocytosis; NV: Normal value; NP: Not palpable; N: Normal.

the city of discovery the philadelphia (Ph^1). With the advent of the Philadelphia chromosome (Ph^1) as a disease specific marker for CML patients, Gilbert[16] of the PVSG separated in 1973 Ph^1-positive CML from the Ph^1-negative MPDs PV, ET and AMM[16,17]. Using improved banding techniques, Janet Rowley (1973, Figure 2) showed that the Ph^{1+} chromosome in CML represents a deletion of the long arm of chromosome 22 (22q-) resulting in the minute Ph^{1+} chromosome (Figure 3)[18]. Additional studies showed that a large part of 22q was translocated to 9q, and that a small part of 9q was translocated to 22q resulting in the translocation (t) t(9;22)(q34;q11)[19,20].

The discovery that the translocation t(9;22) in the Ph^{1+} chromosome resulted in the *BCR/ABL* translocation in the early 1980s originates from the search by three Dutch investigators Nora Heisterkamp, John Groffen and Gerard Grosveld (Figure 2)[21-24]. John Groffen and Nora Heisterkamp obtained their Drs degree in Groningen and moved to the United States in 1981 and worked in John Stephenson's lab in Frederick to study viral oncogenes. Gerard Grosveld was working at the Erasmus University in Rotterdam on a project to identify the Ph^{1+}-chromosome breakpoint. They worked together at the Erasmus University Rotterdam (EUR) and Erasmus Medical Center (EMC), Rotterdam, and at the National Health Institute in Frederick, MD United States (personal communications Gerard and Frank Grosveld 2008-2012). The *BCR/ABL* discovery runned through a three step scientific process: (1) John Groffen learned to make cosmid libraries in Dick Flavell's lab in the MRC in London and took the technique along to the United States. There John Groffen and Nora Heisterkamp cloned parts of the human *ABL* gene and in collaboration with Walter Bodmer's group in the United Kingdom localized *ABL* to chromosome 9. Using a v-abl probe Heisterkamp and Groffen had localized *ABL* on human chromosome 9[21]; (2) Groffen and Heisterkamp contacted Gerard Grosveld mediated by Frank Grosveld and collaborated. Using somatic cell hybrids made by Anne Hagemeijer, (chief of Medical Cytogentics EMC), they found *c-ABL* moved to the Ph^1 -chromosome. Using hybrid cell lines containing the segregated Philadelphia translocation products (generated by Dr. Ad Geurts van Kessel, EUR), Groffen, Heisterkamp and Gerard Grosveld investigated whether *cABL* moved from the long arm of chromosome 9 to the long arm of the Ph^1 chromosome by Southern blot analysis[22]. Indeed c-*ABL* was found to translocate to the Ph^1-chromosome even in patients with complex chromosomal translocations but not in Ph^1-negative CML patients with apparently normal karyotypes[23]; and (3) John Groffen and Nora Heisterkamp cloned more to the 5' of *ABL* and discovered and cloned a breakpoint fragment from a CML patient DNA. Subsequent chromosome walking upstream from *ABL* identified a probe that

Table 5 The 2002 European Clinical and Pathological criteria for the diagnosis of "true" essential thrombocythemia and chronic idiopathic myelofibrosis or primary megakaryocytic granulocytic myeloproliferation according to Michiels *et al*[91]

Clinical ECP criteria of "true" ET	Pathological ECP criteria of "true" ET
A1 Persistent increase of platelet count grade 1 400-1500 × 10^9/L, grade 2 > 1500 × 10^9/L	B1 Predominant proliferation of enlarged to giant megakaryocytes wit hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicious cytological abnormalities
A2 Normal spleen or only minor splenomegaly on echogram	B2 No proliferation or immaturity of granulopoisis or erythropoiesis
A3 Normal LAP score, normal ESR and increased MPV	B3 No or only borderline increase in reticulin fibers
A4 Spontaneous megakaryocyte colony formation (CFU-Meg)	The combination of A1 and B1 + B2 establish "true" ET. Any other criterion confirms ET
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 ECP criteria of CIMF or PMGM	Pathological ECP criteria of CIMF or PMGM
A1 No preceding or allied other subtype of MPN, PV, CML or MDS	B1 PMGM 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
Early clinical stage	Staging of myelofibrosis: MF in ET, PV and PMGM
Normal hemoglobin, or anemia grade 1: Hemoglobin > 12 g/dL, slight or moderate splenomegaly on palpation or > 11 cm on ultrasound or CT. Thrombocythemia around 1000 × 10^9/L	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)
Intermediate clinical stage	
Anemia grade 2, hemoglobin > 10 g/dL, definitive leuko-erythroblastic blood picture and/or tear-drop erythrocytes. Splenomegaly on palpation, no adverse signs	
Advance clinical stage	
Anemia grade 3, hemoglobin < 10 g/dL, significant splenomegaly and one or more adverse signs	

ECP: European Clinical and Pathological; CIMF: Chronic idiopathic myelofibrosis; PMGM: Primary megakaryocytic granulocytic myeloproliferation; ET: Essential thrombocythemia; LAP: Leukocyte alkaline phosphatase; ESR: Erythrocyte sedimentation rate; MPV: Mean platelet volume; MPN: Myeloproliferative neoplasm; PV: Polycythemia vera; MDS: Myelodysplastic syndrome; RF: Reticulin fibrosis; CML: Chronic myeloid leukemia.

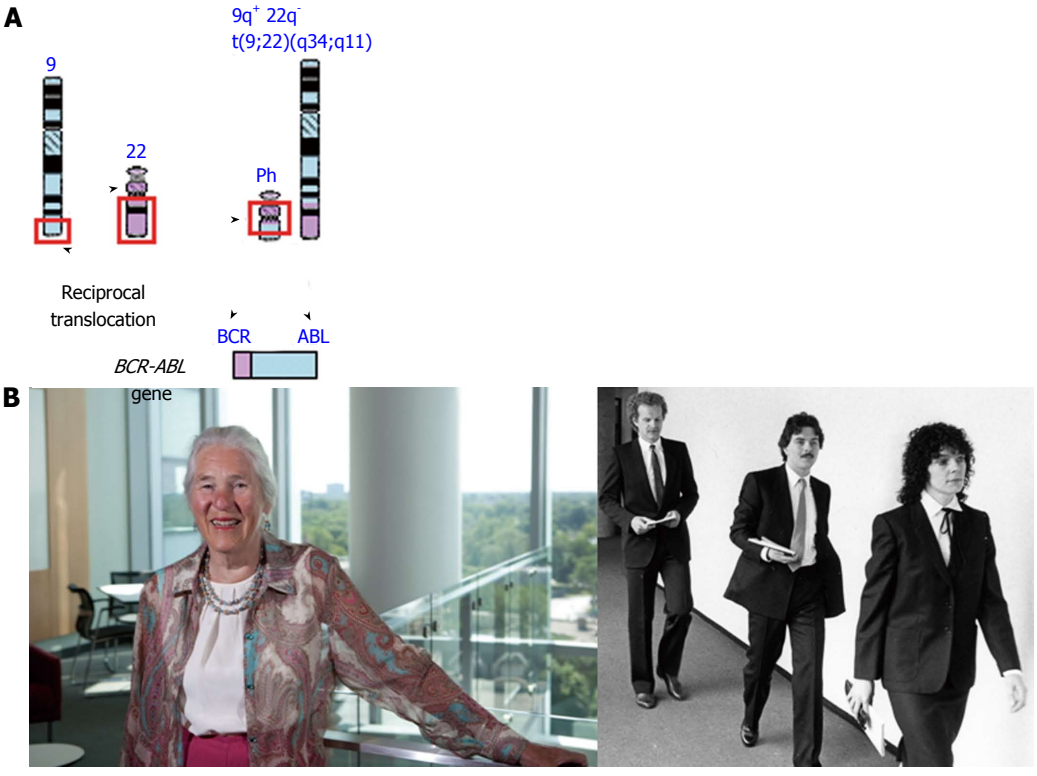


Figure 2 Reciprocal translocation t(9;22)(q34;q11) creates a novel breakpoint cluster region/ABL fusion gene with high tyrosinase activity as the cause of chronic myeloid leukemia. A: Dr. Janet Rowley, discoverer of t(9;22)(q34;q11) in Ph1chromosome positive chronic myeloid leukemia (CML)[19,20], and winner the Beutler Prize 2011, American Society of Hematology (ASH). Photo courtesy of ASH 2012; B: The discoverers of *BCR/ABL* fusion gene as the cause of Ph+ CML[21-24]. From left behind to right Gerard Grosveld, John Groffen and Nora Heisterkamp.

Table 6 The 2000 European Clinical and Pathological criteria for the diagnosis of polycythemia vera defined by Michiels[73] in 1997[9,74], and in 2000[93]

Clinical ECP criteria of PV	Pathological ECP criteria of PV
A1 Increased erythrocytes > 6 × 10^{12}/L. Raised RCM: RCM (optional) male > 36 mL/kg, female > 3.2 mL/kg or increased red cell counts above 6 × 10^{12}/L	B1 Thrombocytheia: platelet count > 400 × 10^{9}/L
A2 Absence of any cause of primary or secondary erythrocytosis by clinical and laboratory investigations	B2 Granulocytes > 10 × 10^{9}/L and raised LAP score in the absence of fever or infection
A3 Histopathology of bone marrow biopsy	B3 Splenomgaly on palpation or on echogram > 11 cm
(1) Increase and clusters of pleomorph large megakaryocytes with hyperploid nucei	B4 Spontaneous erythroid colony formation in the absence of EPO and low plasma or serum EPO level
(2) Increased cellulartity due to increased erythropoiesis or erythropoiesis and granulopoiesis (panmyelosis)	Staging of MF related to reticulin fibrosis
(3) No or slight increase of reticulin fibers	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

Diagnosis of idiopathic erythrocythemia (IE), early prodromal polycythemia vera (PV), classical PV or latent primary myeloproliferative disease (subclinical PV) are acceptable when[73,74,93]: A1 + A2 + A3 and none of B (except B4) is consistent with early erythrocythemic PV, labelled as idiopathic erythrocythemia: IE; A3 + B1 + B4 is consistent with prodromal PV with PV features in the bone marrow; A1 + A2 + A3 + plus one of B1 to 3 is consistent with classical PV; B4 confirms all variants of IE, prodromal PV and classical PV; A3 + B3 and none of the others is consistent with latent subclinical primary myeloproliferative diease (PMD) usually preceding masked cases of PV or MF during long term follow-up. ECP: European Clinical and Pathological; MF: Myelofibrosis; RF: Reticulin fibrosis; EPO: Erythropoetin; RCM: Red cell mass; LAP: Leukocyte alkaline phosphatase.

Table 7 2015 World Health Organization Clinical Molecular and Pathological criteria for the diagnosis of prodromal, masked and classical JAK2 mutated polycythemia vera *vs* primary or secondary erythrocytoses[77,78]

CM criteria	Bone marrow pathology (P) criteria (WHO)
Major criteria for PV	P1 Bone marrow pathology: increased cellularity (60%-100%) due to trilinear increase of erythropoiesis, megakaryopoiesis and granulopoiesis and clustering of small to giant (pleomorph) megakaryocytes with hyperlobulated nuclei
A1 Hematocrit > 0.51/> 0.48 in male/female Erythrocytes > 5.8 × 10^{12}/L males > 5.6 × 10^{12}/L females	Absence of stainable iron. No pronounced inflammatory reaction
A2 Presence of heterozygous and/or homozygous JAK2V617F or JAK2 exon 12 mutation	P2 Erythrocytosis. Normal erythropoiesis, normal granulopoiesis and megakaryocytes of normal size, morphology and no clustering
A3 Low serum Epo level	Grading of RF and MF
Minor	Prefibrotic: RF-0/1 = MF-0
B1 Persistent increase of platelet count × 10^{9}/L: grade 1: 400-1500, grade 2: > 1500	Early fibrotic: RF-2 = MF-1
B2 Granulocytes > 10 × 10^{9}/L or Leukocytes > 12 × 10^{9}/L and raised LAP-score or increased CD11b expression in the absence of fever or infection	Fibrotic: RCF 3 = MF-2
	Post-PV MF: RF 4 = MF-3
B3 Splenomegaly on ultrasound echogram (> 12 cm length in diameter) or on palpation	
B4 Spontaneous EEC formation (optional)	

2015 WHO-CMP criteria for staging of prodromal, erythrocythemic, and advanced polycythemia vera (PV). A2 + B1 + P1 establish early PV (mimicking ET) prodromal PV CMP stage 0; A1 + A2 + A3 + P1 and none of B establish idiopathic erythrocythemia (IE) or stage 1 PV; A1 + A2 + A3 + P1 and one or more of B establish classic stages of PV stage 2 and 3; A2 + B3 + P1 detect masked cases of PV with splenomegaly and hypersplenism to be labelled as inapparent PV (IPV) frequently seen Budd-Chiari syndrome or splanchnic vein thrombosis. CM: Clinical and molecular; RF: Reticulin fibrosis; MF: Myelofibrosis; ET: Essential thrombocythemia; WHO-CMP: World Health Organization clinical molecular and pathological; EEC: Endogenous erythroid colony.

recognized the chromosome 9 breakpoint in the DNA of a CML patient. Cloning of this fusion fragment provided probes of the breakpoint cluster region on chromosome 22, which detected the Philadelphia breakpoints in almost all CML patient samples including those with complex cytogenetic translocations. In CML patients, the chromosomal breakpoints were clustered within a limited region on chromosome 22, the "breakpoint cluster region": *BCR*[24]. The specific molecular *BCR/ABL* translocation on chromosome 22 in the t(9;22) of Ph^{1}-positive CML patients was predicted to have functional significance for the disease[24]. There was no serendipity in the discovery of the *BRC/ABL* translocation t(9;22) and "they all were very lucky to have had their unique collaboration" (personal communication Nora Heisterkamp 2012).

The sequential discoveries of the Ph^{1}-chromosome in the t(9;22)(q34;q11), and the *BCR/ABL* fusion gene on chromosome 22 became the cause of a clearly defined human neoplasia, *BCR/ABL* positive CML (Figure 3).

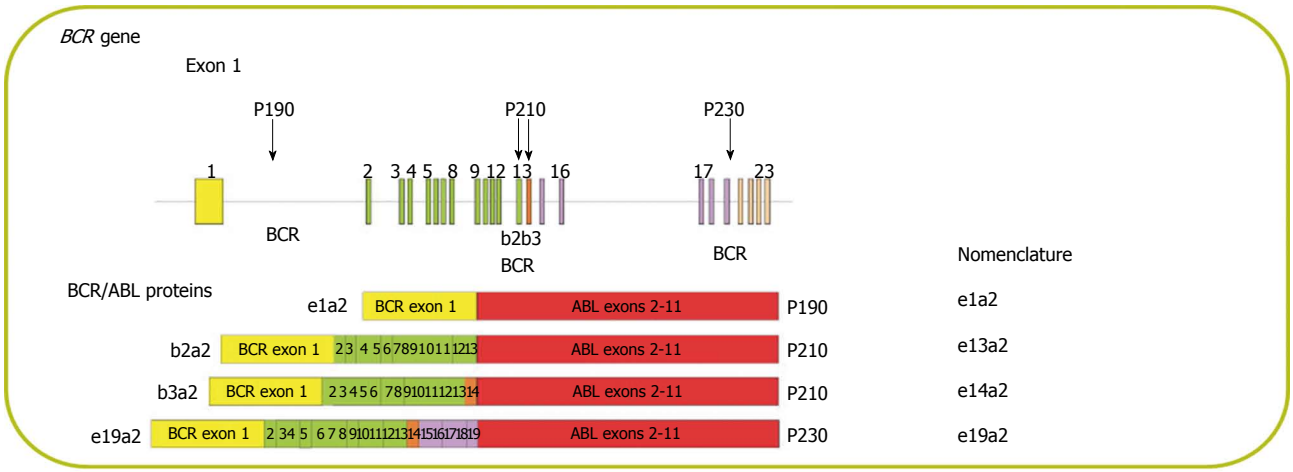


Figure 3 Locations of the breakpoint regions in the *BCR* gene and nomenclature of breakpoint cluster region/ABL proteins. BCR: Breakpoint cluster region.

Table 8 2015 World Health Organization clinical molecular and pathobiological criteria for diagnosis of JAK2^{V617F} mutated essential thrombocythemia[77,78]

CM criteria	Bone marrow pathology (P) criteria (WHO)
ET	Normocellular ET
(1) Platelet count of > 350 × 10^9/L and the presence of large platelets in a blood smear	Proliferation and clustering of enlarged mature pleomorphic megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities
(2) Heterozygous JAK2^{V617F} mutation, and low JAK2 allele mutation load	Normocellular bone marrow (< 60%) and no proliferation or immaturity of granulopoiesis or erythropoiesis
(3) Normal erythrocytes < 5.8 × 10^{12}/L males, < 5.6 × 10^{12}/L females	RF 0 or 1
(4) Hb and ht normal or in the upper range of normal	
Prodromal PV	ET with bone marrow features of PV
(1) Platelet count of > 350 × 10^9/L Hb and Ht normak or in the upper range of normal, normal erythrocyte < 5.8 × 10^{12}/L males, < 5.6 × 10^{12}/L females	Increased cellularity (60%-80%) due to increased erytropoiesis or trilineage myeloproliferation (*i.e.*, panmyelosis). Proliferation and clustering of medium sized to large (pleomorphic) mature megakaryocytes
(2) Presence of JAK2^{V617F} mutation and variable JAK mutation load	Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis
(3) Low serum EPO level and increased LAP score	RF 0 or 1
(4) Spontaneous EEC	
Prefibrotic hypercellular ET	EMGM
(1) Platelet count of > 350 × 10^9/L	Hypercellular ET due to chronic megakaryocytic and EMGM and normal or reduced erythroid precursors
(2) Presence of JAK2^{V617F} mutation and high JAK2 mutation load	Loose to dense clustering of more pleiomorphic megakaryocytes with hyperploid or clumpsy nuclei (not or some cloud-like)
(3) Slight or moderate splenomegaly on ultrasound or on palpation	
(4) No preceding or allied CML, PV, PMGM, RARS-T or MDS	
Clinical stage 1: No anemia with Hb and Ht in the normal or low normal range: hb > 12 g/dL, normal LDH and CD34$^+$	Grading of reticulin fibrosis and MF in EMGM
Clinical stage 2: Slight anemia Hb < 12 to > 10 g/dL, LDH↑, and splenomegaly	Prefibrotic: RF 0/1 = MF 0, no/minor splenomegaly
Clinical stage 3: Anemia, Hb < 10 g/dL, LDH↑↑, CD34$^+$, leukoerythroblastose and, tear drop	Early fibrotic EMGM: RF 2 = MF 1 and minor or moderate splenomegaly
	Fibrotic EMGM: RF 3, RCF = MF 2 and overt splenomegaly
	Post-ET MF: RF 3/4 = MF 2/3 (WHO criteria)

ET: Essential thrombocythemia; MF: Myelofibrosis; EPO: Erythropoetin; CM: Clinical and molecular; Hb: Hemoglobin; ht: Hematocrit; WHO: World Health Organization; PV: Polycythemia vera; EPO: Erythropoetin; EEC: Endogenous erythroid colony; CML: Chronic myeloid leukemia; PMGM: Primary megakaryocytic granulocytic myeloproliferation; MDS: Myelodysplastic syndrome; EMGM: ET with hypercellular megakaryocytic-granulocytic myeloproliferation; RF: Reticuline fibrosis; LDH: Lactodehydrogenase; RCF: Reticulin collagen fibrosis.

The *BCR/ABL* fusion gene is detectable in hematopoietic bone marrow cells but not in fibroblasts of CML patients indicating that reticulin fibrosis in Ph-positive CML and ET is a reactive and secondary process. The *BCR/ABL* fusion gene produces a *BCR/ABL* protein, which has a high tyrosine kinase activity and CML-transformation capacity

Table 9 2015 WHO Clinical Molecular and Pathological criteria for the diagnosis of normocelular essential thrombocythemia carrying one of the MPL^{515} mutations[78]

CM JAK2 wild type ET	Bone marrow pathology (P) criteria (WHO)
(1) Platelet count > 350 × 10^9/L and presence of large platelets in blood smear	P1 Proliferation of large to giant mature megakaryocyte with hyperlobulated, staghorn-like nuclei in a normocellular bone marrow (< 65%)
(2) Hemoglobin, haematocrit and erythrocyte count in the normal range	No increase of erythropoiesis, and no increase or immaturity of granulopoiesis or erythropoiesis, no or slight increase in reticulin RF 0/1
(3) Presence of MPL^{515} mutation and JAK2 wild type	ET → MF
(4) Normal serum EPO	Increased reticulin fibrosis around dense clustered megakaryocytes in a normocellular bone marrow and reduced erythropoiesis. Follow-up data of RF and MF related to splenomegaly in MPL^{515} ET transltional states to MF are lacking. Grading of RF and MF similar as described for PV
(5) Normal LAP score and CD11b expression	
(6) No or slight splenomegaly	
(7) No leukoerythroblastosis	
(8) No preceding or allied CML, PV, RAS-T or MDS	

This entity is identical to "true" ET as defined in 2002 by Michiels and Thiele in Table 5[91]. ET: Essential thrombocythemia; CM: Clinical and molecular; RF: Reticulin fibrosis; MF: Myelofibrosis; EPO: Erythropoetin; LAP: Leukocyte alkaline phosphatase; CML: Chronic myeloid leukemia; PV: Polycythemia vera; MDS: Myelodysplatic syndrome; WHO: World Health Organization.

Table 10 World Health Organization-clinical molecular and pathological criteria for hypercellular essential thrombocythemia associated with primary megakaryocytic, granulocytic myeloproliferation caused by calreticulin mutations[78]

Clinical CM criteria JAK2 wild type PMGM	Pathological ECP criteria of CALR MGM
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 1000 × 10^9/L	P1 PMGM 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
A2 Presence of CALR mutation and JAK2 wild type	
C Clinical stages of CALR MGM	MF Grading RF, MF
C1 Early clinical stage: Hb > 12 g/dL, slight to moderate splenomegaly, thrombocytosis around or above 1000 × 10^9/L, normal LAP score	MF 0 Prefibrotic CALR MGM, 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 MGM 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 MGM increase RF grade 3 and slight to moderate collagen fibrosis
	MF 3 Advanced fibrotic CALR MGM with collagen fibrosis-osteosclerosis

The combination of A1 + A2 and P1 establishes calreticulin (CALR) ET and various clinical stages(C1, C2, C3) related to the degree of myelofibosis (MF). This entity has been defined as CMGM in the 1990 Bone Marrow Classification by Georgii *et al*[35] as the third MPD entity and as chronic idiopathic myelofibrosis false ET or prefibrotic or primary megakaryocytic granulocytic myeloproliferation in 2002 by Michiels and Thiele (Table 5)[91]. PMGM: Primary megakaryocytic, granulocytic myeloproliferation; RF: Reticulin fibrosis; CM: Clinical and molecular; PV: Polycythemia vera; MDS: Myelodysplatic syndrome; LAP: Leukocyte alkaline phosphatase; CML: Chronic myeloid leukemia; Hb: Hemoglobin; LDH: Lactodehydrogenase; MGM: Megakaryocytic granulocytic myeloproliferation.

in animal models[25-27]. Ninety percent of all CML patients are Ph^{1+}/*BCR/ABL*$^+$, 5% are Ph^{1-}/*BCR/ABL*$^+$, and 5% are Ph^{1-}/*BCR/ABL*$^-$, the latter group usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia[28]. The current molecular diagnosis of CML is made by peripheral blood reversed transscript PCR for the B*CR/ABL* fusion gene. Possible BCR fusion transscripts include P190, two variants of P210, and P230 and to be labeled as e1a2, e13a2, e14a2 and e19a2 (Figure 3). More than 98% of CML patients have P210 BCR transscript, of which one third e13a2 en two third 3 e14a2, and only 1% to 2% P190 BCR transscript having a poorer prognosis. The European LeukemiaNet recommendations for the diagnosis staging and management of CML patients are recently reviewed in great detail[29].

According to strict morphological, biochemical, cytogenetic and molecular criteria, Ph-positive CML is a malignant disease with an obligate transition into acute myeloid, lymphoblastic or megakaryoblastic leukemia, whereas ET, PV and AMM or chronic primary myelofibrosis (PMF) form the *BRC/ABL* negative MPDs featured by a benign proliferation of the three hematopoietic cell lines with a low incidence of leukemic transformation in PV and AMM[30-32]. In the Rotterdam cohort of 50 MPD patients seen between 1975 and 1985, Michiels and Hagemeijer found that all MPD patients diagnosed as ET, PV and MF were negative for the Ph^1-chromosome and *BRC/ABL* translocation, and could detect the *BCR/ABL* transcript in cases of Ph^1-positive essential thrombocythemia[29-31]. Ph^{1+} and *BCR/ABL*$^+$ ET and thrombocythemia associated CML

Table 11 Staging of JAK2^{V617F} positive prodromal polycythemia vera, erythrocythemic polycythemia vera, classical polycythemia vera, early myelofibrosis, inapparent polycythemia vera, spent phase polycythemia vera and post-polycythemia vera myelofibrosis according to 2015 World Health Organization-Clinical Molecular and Pathological criteria related to therapy

PV: WHO-ECMP stage	0	1	2	3	4	5	6
WHO-ECMP	Prodromal	Erythrocythemic PV	Early PV	Manifest PV	PV early MF	Inapparent	Spent PV
Clinical diagnosis	PV			Classical PV	Masked PV	PV	Post-PV MF
LAP-score	↑	↑	↑	↑	↑/↑↑	↑	Variable
EEC	+	+	+	+	+	+	+
Serum EPO	N/↓	N/↓	↓	↓	↓	↓	Variable
Erythrocytes × 10^{12}/L	> 5.8	< 5.8	> 5.8	> 5.8	> 5.8	Normal < 5.5	Decreased
Leukocytes × 10^{9}/L	< 12	< 12	< or > 12	< or > 15	> 15	N or ↑	> 20
Platelets × 10^{9}/L	> 400	400	< or > 400	> 400	< or >1000	N low or ↑	Variable
WHO-ECMP bone marrow	Early PV	Early PV	Early PV	Trilinear PV	Trilinear PV	Prilinear PV	Myelofibrosis
Bone marrow cellularity (%)	50-80	50-80	60-100	80-100	80-100	60-100	Decreased
Grading reticulin fibrosis: RF	RF 0-1	RF 0-1	RF 0-1	RF 0/1	RCF1/2/3	RCF 1/2/3	RCF 3/4
Grading myelofibrosis: MF[57]	MF 0	MF 0	MF 0	MF 0	MF 0/1	MF 0/2	MF 2/3
Splenomegaly on palpation	No/+	No	No/+	+	++/+++	++/+++	/large
Spleen size, echogram (cm)	< 12-15	< 13	12-15	12-16	18 > 20	16 > 20	> 20
Spleen size on palpation (cm)	0-3	NP	0-3	4-6	> 6	> 6	> 8
JAK2^{V617F} in granulocytes %	Low	Low	Moderate < 50	High > 50	High > 50	Mod/High	High > 50
JAK2^{V617F} in BFU-e (exon 12)	+(++)	+(++)	+(++)	++	++	+	++
Risk stratification → therapeutic implications anno 2014	Low risk	Low risk	Low risk	Intermediate risk PV	High risk PV early MF	Wait/see IFN JAK2	Post-PV MF Spent phase PV
First line Aspirin/Phlebotomy	Aspirin	Aspirin	Phlebotomy	Phlebotomy	If IFN resistant →	If IFN	JAK2
Second line IFN *vs* HU	Phlebotomy	Phlebotomy	Aspirin	Aspirin	HU or JAK2	Resistent	Inhibitor →
Third line JAK2 inhibitor			Low dose IFN → responsive	IFN → resistant → HU	inhibitor	JAK2 inhibitor	Bone marrow transplant

↑: Increased; ↓: Decreased; N: Normal; +: Present or heterozygous; ++: Homozygous; MF: Myelofibrosis; EPO: Erythropoetin; PV: Polycythemia vera; WHO-ECMP: World Health Organization European Clinical Molecular and Pathological; RF: Reticulin fibrosis; LAP: Leukocyte alkaline phosphatase; EEC: Endogenous erythroid colony; BFU-e: Burst forming units erythropoiesis; RCF: Reticulin collagen fibrosis; IFN: Interferon; HU: Hydroxy urea.

Table 12 2015 update on the molecular landscape findings in the chronic phase of essential thrombocythemia, polycythemia vera and myelofibrosis and during blast phase of myeloproliferative neoplasms transformation[121]

Gene	Chronic phase ET, PV and MF	Blast phase/AML
JAK2^{V617F}	PV: 95%-98%; ET and MF: 50%-60%	
MPL	ET: 1.5%; MF: 5%-10%	
TET2	PV: 7%-16%; ET: 4%-11%; MF: 8%-17%	
ASXL	PV: 2%; ET: 5%-8%; MF: 7%-17%	19%
DNMT3A	PV: 7%; ET: 3%; MF: 7%-15%	17%
CBL	MF: 6%	
LNK	PV, ET, MF: < 5%	10%
IDH 1/2	MF: 4%	21%
IKZF		19%
EZH2	MPNs: 5%-13%	
P53		27%
SRSF2		19%

MF: Myelofibrosis; PV: Polycythemia vera; ET: Essential thrombocythemia; AML: Acute myeloid leukemia.

vs the Ph^{1}- and *BCR/ABL*-negative thrombocythemias showed conspicuous differences in the form and size of megakaryocytes in bone marrow smears and sections of bone marrow biopsy. This difference of megakaryocyte histopathology observed in the Rotterdam cohort of CML and MPD patients described by Michiels *et al*[30] in 1987 appeared to be reproducible in bone marrow biopsies by the German pathologists Thiele *et al*[33,34] (1988, 1989) and Georgii *et al*[35,36] (1990, 1996) to distinguish between small mono or binucleated megakaryocytes as diagnostic for Ph^{1+} CML and ET *vs* large pleomorph megakaryocytes with hyperlobulated nuclei seen in the Ph-negative MPDs[37].

THE 1975 PVSG CRITERIA FOR ESSENTIAL THROMBOCYTHEMIA

PHT has already been defined in 1960 by Gunz[38] as clinical syndrome of recurrent spontaneous hemorrhages often preceded by thromboses, extremely high platelet count in excess of 1000 × 10^{9}/L, frequently splenomegaly, and hypochromic anemia with a tendency towards polycythemia between hemorrhages. Mucocutaneous bleeds from nose gums and gastrointestinal tract were most frequent followed by bruises and bleedings after trauma or surgery[38]. Accordingly, the PVSG used from 1975 to 1986 a minimum platelet count of 1000 × 10^{9}/L for the diagnosis for PHT without features of PV or AMM[8,38-40]. The PVSG inclusion and exclusion criteria in 1975 for the diagnosis of PHT or ET were very crude[8]: (1) A platelet count in excess of 1000 × 10^{9}/L and a bone marrow smear which shows marked megakaryocytic hyperplasia and abundant platelet clumps; (2) Absence of PV as defined by the PVSG normal red cell mass (RCM)[10]; (3) Absence of the

Philadelphia chromosome to exclude CML[16]; and (4) Absence of significant reticulin fibrosis (myelofibrosis) with dry tap on bone marrow aspiration, and no signs of preleukemia (erythroleukemia or trilinear myelodysplastic syndrome)[8,39,40].

PVSG defined PHT[8] labeled as ET[39,40] is featured by platelet counts between 1000 and 3000 × 10^9/L, splenomegaly in about 80%, autoinfarction of the spleen in 20%, and iron deficient microcytic anemia in 60%[39,40]. In 40% of PHT patients gastrointestinal roentgenograms suggest duodenal ulcer caused by small infarcts in the duodenal mucosa resulting from the high platelet count[40]. In the first prospective evaluation of PVSG defined PHT, 37 evaluable ET patients with platelet counts between 1000 to 2650 × 10^9/L suffered from thrombohemorrhagic events at presentation including mild bleedings in 5 epistaxis in 5, ecchymoses in 2, pelvic, buccal, fundal or urinary tract hemorrhage in 6, melena with a fall in hemoglobin of 7 gm/dL in 1 and massive postoperative bleeding in 1 case[39,40]. Eleven ET patients experienced acroparesthesias (numbness), including burning sensations, usually in hand or feet (suggestive for erythromelalgia), 9 had dizziness, light-headedness or syncope, 7 had visual disturbances such as scotomas and transient dimming or blurred vision. Catastrophic complications (severe hemorrhages, myocardial infarction, stroke) in 6 (16%) were observed. In this study of 37 untreated PHT or ET patients, bone marrow cellularity was normal in 11%, increased between 50% to 90% in 78% and greater than 90% in 11%[39,40]. Two-thirds of diagnostic bone marrow biopsies showed marked megakaryocyte hyperplasia with atypical large megakaryocytes. Reticulin content was essentially normal in 90% indicating prefibrotic MPD. MPD features in ET and PV were quiete similar: leukocytosis was common in PHT (ET) and PV, leukocyte alkaline phosphatase (LAP) scores over 100 were seen in 42% of PHT, and in 70% of PV patients; pruritis was observed in 14% in PHT and 43% in PV patients; the spleen was palpable in 38% of PHT and 70% of PV patients, and when enlarged in PHT the spleen was palpable 2 to 4 cm below the costal margin[8]. Since 1975 we discovered a causal relation between erythromelalgic microvascular disturbances and thrombocythemia in early early stage MPD disease at platelet counts above 400 × 10^9/L in symptomatic ET and PV patients with persistent increased platelet count in excess of 400 × 10^9/L and recognized that the increase of clustered large pleomorphic megakaryocytes in bone marrow biopsies was a pathognomonic clue to myeloproliferative thrombocythemia in ET and PV (Table 1).

IMPACT OF PVSG CRITERIA FOR PV ON DIAGNOSIS: RCM *VS* BONE MARROW HISTOLOGY

The PVSG established in 1975 clinical criteria (Table 2) for the diagnosis PV that are relatively simple to implement but rather crude thereby overlooking prodromal and masked PV when bone marrow histology as a pathognomonic clue to PV and ET is not considered or performed. The laboratory findings of 325 PV patients in the PVSG 01 study all PV patients had increased red cell mass by definition and showed an increase in hematocrit > 0.52 in 92%, white cells > 12 × 10^9/L in 43%, platelets > 400 × 10^9/L in 63%, LAP score > 100 in 70% and increased spleen size on palpation (splenomegaly due to myeloid metaplasia) in 70%[10]. Absence of splenomegaly was noted in about 30% of cases and leukocytosis and platelet counts can remain normal in early stage PV with typical PV bone marrow histology[10,41]. Such masked cases of PV with normal platelets, leukocytes and spleen were labeled in 1979 as idiopathic erythrocythemia (IE) by Pearson *et al*[42]. The 1975 PVSG criteria for PV in Table 2 exclude per definition IE (stage 1 PV, Table 11)[10,41]. 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[42]. LAP scoring[8,17], *in vitro* cultures of erythroid progenitors (EEC)[43] and radioimmunoassay of erythropoetin (EPO) do contribute to differentiate PV from all variants erythrocytosis. These assays are useful when there is only isolated elevation of the red cell mass and all the usual causes of secondary polycythemia have been excluded. The characteristic histology findings in bone marrow biopsies of 155 evaluable PV patients with a documented increased RCM in the PVSG 01 study revealed a broad spectrum of bone marrow cellularity from 50% to 60% in 10 cases, from 60% to 80% in 45 cases, and from 80% to 100% in 100 cases (Figure 4)[41]. Silver stained reticulin fiber content was normal (RF-0 and 1 = prefibrotic) in 94 cases, slightly increased (RF-2 = early fibrotic) in 40 cases, and moderately to marked increased (RF-3) in 21 cases. The bone marrow histology diagnoses in the PVSG-01 study[41] could roughly be interpreted as typical for normocellular ET in 10, for PV (hypercellular 60%-80%) in 45, for trilinear PV in 70 and for PV/RF-3 or 4 in 13 PV patients (Figure 4). The bone marrow histology data of megakaryopoiesis in PVSG PV and PHT studies were identical in appearance, and the condition PV *vs* ET could not be distinguished on megakaryocyte histology[39-41]. Increased bone marrow cellularity due to increased erythropoiesis and/or myelopoesis in PVSG defined PV and PHT or ET were identical. The PVSG concluded that the condition PV *vs* ET could not be distinguished on the basis of bone marrow histopathology. Consequently, the PVSG only used increase RCM and did not include bone marrow histology as the determinative major inclusion criterion for the diagnosis of PV, and to separate ET from PV[10,39-41]. RCM is insensitive and not specific for the diagnostic differentiation of PV, IE, SE and inapparent PV (Table 2)[17]. In contrast, EEC and bone marrow histology are specific clues for the diagnosis of PV since the early 1970s[1-3,41-43].

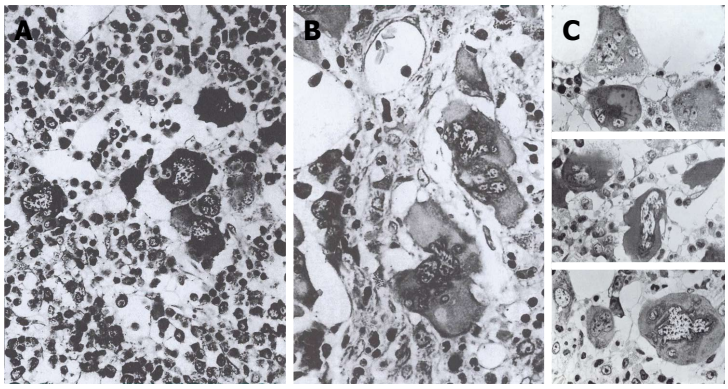


Figure 4 Cluster of medium and large immature megakaryocytes (350 × left) and large megakaryocytes with immature cytoplasm and immature clumpsy cloudlike nuclei (pseudolobulation) (right 900 ×) and very slight reticulin fibrosis in chronic megakaryocytic granulocytic myeloproliferation, and characteristics of megakaryocytes in prefibrotic myeloproliferative disorders: myeloproliferaive disease, 900 × plastic embedding. A: Small and large and immature cloud-like nuclei in chronic megakaryocytic granulocytic myelosis or primary megakaryocytic granulocytic myeloproliferatio; B: Pleomorp large megakaryocytes in Polycythemia Vera; C: Deep hyperlobulated nuclei in essential thrombocythemia. Georgii *et al*[35,36].

From 1988 to 1994, Lamy *et al*[44] measured RCM in 103 consecutive patients seen in a single center diagnosed in 85 cases (83%) as PVSG defined PV patients with increased hemoglobin (Hb) and hematocrit (Ht) defined, respectively, by Hb > 18 g/dL, Ht > 0.52 in males and Hb > 16 g/dL, Ht > 0.47 in females and as inapparent PV (IPV) in 18 patients (17%) (Table 3)[44]. IPV was defined by a normal Hb and Ht value at diagnosis. In the IPV group, the reasons to perform RCM were as follows: splenomegaly associated with increased platelets and/or leucocytes counts (n = 8), portal vein thrombosis (n = 5), increased platelets or leukocytes counts without splenomegaly (n = 3), and isolated splenomegaly (n = 2)[44]. The two groups were balanced in terms of age, sex, leukocyte, serum iron, and platelet level. Hemoglobin, Ht levels, red cell counts, and plasma volumes were significantly different between the two groups (Table 3). Red cell mass was increased in the two groups due to hypervolumemia in PV, but caused by splenomegaly in cases with IPV, whereas the erythrocyte counts were increased in the majority of 85 PV patients, but completely normal in 18 IPV patients except one (Table 3). Consequently IPV with normal or decreased hemoglobin, hematocrit and erythrocytes in Table 3 cannot become candidates for phlebotomy because of absence of hypervolemic symptoms. Treatment will hydroxyurea carry the great danger of inducing relative anemia and acceleration of myelofibrosis. In the context of splanchnic vein thrombosis (portal or splenic vein thrombosis), masked and overt Budd Chiari syndrome RCM and blood volume are increased due to splenomegaly as the cause of IPV[45]. We observed in 1990 a case with post-PV myelofibrosis and gigantosplenomegaly with increased RCM, 37 mL/kg, increased plasma volume (hypersplenism) at a hemoglobin level of 2.1 mmol/L (6.0 g/dL) erythrocytes 2.9 × 10^{12}/L and platelet count of 35 × 10^{9}/L. After splenectomy (spleen weight 5000 g) resulted in correction of the hemoglobin to 7.8 mmol/L (12.5 g/dL) and a postsplenectomy hemorrhagic thrombocythemia with platelet counts of 3000 to 4000 × 10^{9}/L associated with the paradoxical occurences of platelet medidiated erythromelalgia and spontaneous hemorrhages (bruises, nose bleedings) caused by severe acquired von Willerand disease type 2A VWF: Ag 10.8 U/mL VWF:RCo 0.48 U/mL, VWF:CB 0.27U/mL with the absence of large and intermediate VWF multimer. Such occult and overt cases of splanchnic vein thromboses secondary to hepatic, portal or splenic vein thrombosis is related to splenomegaly and hypersplenism keeping the blood counts of platelets and erythrocytes normal or decreased inducing masked stage PV with typical EEC and bone marrow histology[2,17,41-44]. In our analysis in 1997 of the series by De Stefano *et al*[45] 13 out of 33 patients with splanchnic vein thrombosis had spontaneous EEC and could be diagnosed according to classic PVSG criteria as PV in 5, ET in 1, MF in 1 and masked primary MPD in 6[45]. The high frequencies of gastric ulcers and gastritis estimated at 20% to 30% in patients with Budd-Chiari syndrome or splanchnic vein thrombosis result in iron deficiency accompanied by hypochromia and microcytosis, which do explain a substantial additional decrease in hemoglobin levels as compared to still normal erythrocyte counts as could be observed in the study of Lamy on IPV (masked PV). On behave of the European Working Group on Myeloproliferative Disorders (EWG.MPD) Briere and Michiels introduced in the late 1990s bone marrow histology as a pathognomonc clue to MPD in patients with splanchnic vein thrombosis (SVT)[45,46]. In a single-center retrospective study of 128 patients with SVT, clusters of abnormal megakaryocytes in bone marrow biopsy combine with EEC were used as reference standard for the diagnosis of MPD (including PV, ET and masked MPD)[46]. In the group of 129 SVT patients 31 had definitive MPD positive for both BMB and EEC, 63 had no MPD with a negative result of BMB and EEC and 34 were positive for either BMB or EEC[46]. Kiladjian *et al*[47] assessed the diagnostic and prognostic value of JAK2 and MPL^{515} mutations in 241 SVT patients

(104 BCS, 137 PVT). JAK2^{V617F} was found in 45% of BCS and 34% of PVT, while JAK2 exon 12 and MPL^{515} mutations were not detected[47]. JAK2^{V617F} was found in 96.5% of patients with BM changes specific for MPD and EEC, but also in 58% of those with one feature (BM or EEC), and in 7% of those with neither feature indicating the superiority of JAK2 screening for detection of MPD in SVT patients[47]. In the meta-analysis of Smalberg *et al*[48], JAK2^{V617F} screening in SVT patients without typical WHO defined MPN features identified masked MPN disease in 17.1% and 15.4% who usually did have typical features of MPN on bone marrow histology evaluation[48].

IMPACT OF PVSG CRITERIA FOR PV ON TREATMENT

In the PVSG 01 study of 431 PV patients randomized for phlebotomy in 134, chlorambucil in 141 and P^{32} in 156, there was a significant loss of survival of PV patients due to major thrombotic complications during the first 3 years in the phlebotomy arm due to uncontrolled thrombocythemia and aiming at a too high haematocrit just below 0.50[9,10,49,50]. In retrospect this would not be the case with the recommendation of phlebotomy aiming at a haematocrit of 0.40 according to Dameshek and Pearson *et al*[42] on top of aspirin in the United Kingdom and The Netherlands since 1985[51]. There was a striking increased incidence of overall malignant complications in PV patients with P^{32} and chorambucil as compared to the phlebotomy-treated PV patients during long-term treatment[49,50]. The overall incidence of leukemia/lymphoma and cancer after 10 to 11 years follow-up was 25% in the phlebotomy arm, 40% in the P32 arm and 67% in the clorambucil arm. The increased incidence of malignancies of bone marrow, lymphoid tissue, skin, and gastrointestinal tract highlights mutagenic effects of chronic myelosuppressive agents in particular when treatment is already started in newly diagnosed early and overt stages of PV. The PVSG 01 trial confirmed the hypothsis of Dameshek[3,50,51] that P^{32} is leukemogenic when used as the first line myelosuppressive treatment in early and overt stage PV indicating the need to postpone myelosuppressive therapy in PV as long as possible[52-55]. A large group of low risk PV patients included in the PVSG 01 study were exposed to the leukemogenic agents P^{32} and chlorambucil. The PVSG 01 investigators recommended around 1990 to replace P^{32} by hydroxyurea as the first treatment option in PV patients[52-55]. The final analysis of the French PVSG study compared hydroxyurea (HU) (n = 136) *vs* pipobroman (n = 149) as first-line therapy in 285 newly diagnosed PV patients younger than 65 years (27 patients were older than 65 years)[56]. During follow-up 42 patients (31%) switched from HU to pipobroman because of HU toxicity and 19 (13%) from pipobroman to HU[56]. According to the intention to treat, the median survival was 17 years for the whole cohort, 20.3 years for the HU arm, and 15.4 for the pipobroman arm (P = 0.008). At 10, 15 and 20 years, the cumulative incidence (probability) of AML/MDS was 6.6%, 16.5% and 24% in the HU arm *vs* 13%, 34% and 52% in the pipobroman arm. The cumulative incidence (probability) of MF at 10, 15 and 20 years was 15%, 24% and 32% in the HU arm *vs* 5%, 10% and 21% in the pipobroman arm (P = 0.02)[56]. Results from PV patients who received only one treatment during the entire period (HU n = 94, Pipobroman n = 130) the cumulative incidence of AML/MDS at 10, 15 and 20 years was 7.3%, 10.7% and 16.6% for HU *vs* 14.6%, 34% and 49.4% for pipobroman[56].

The baseline risk of leukemic transformation in 459 PV and 605 ET patients treated in a single institution retrospective study without cytoreductive therapy or hydroxyurea alone was 3.3% and 7.4%, respectively[57,58]. A primary rigid venesection regimen according to the London PV study group in the late 1970[59-61] aiming at a hematocrit around to below 0.40 irrespective of gender on top of low dose aspirin introduced by Michiels *et al*[51] in 1985[62-64] will reduce the cumulative incidence of minor circulatory ischemic events and major thrombosis from above 50% to less than 2% per patient/year during long-term follow-up[59-64]. Hydroxyurea has to be postponed in early and intermediate stage PV (Table 11) as long as possible by phlebotomy on top of low dose aspirin[17,65]. Current risk stratification in PV and ET should not anymore be based on age above 60 years and history of thrombosis[55], but on real life MPN disease burden using objective parameters including the degree of leucocytosis, splenomegaly, JAK2 allele burden, itching and constitutional symptoms[65]. HU and JAK2 inhibitors are indicated according to not yet clearly defined recommendations in hyperproliferative PV with increased to high MPN disease burden (Table 11) althought targeted recommendations on the use of JAK2 inhibitors will be further clarified and defined soon[66].

THE 1990 HANNOVER BONE CLASSIFICATION OF THE PH-NEGATIVE MPD

In 1987 Georgii and Michiels discussed their experiences that bone marrow histology features of Ph-positive CML *vs* the Ph-negative MPDs reflect distinct disease entities simple because the differences in megakaryocyte size and morphology is so obvious that cytologists and pathologists can easily distinguish the small monolobulated megakaryocytes in Ph-positive CML and ET from the large pleomorphic megakaryocytes in the Ph-negative MPDs ET and PV[14,17,67,68]. The PVSG distinguished four distinct categories of myeloproliferative disorders (CML, ET, PV and AMM or PMF) and a fifth category of unclassifiable MPD. The concept of this traditional classification by the PVSG and the WHO in 1979 has been revised by Georgii *et al*[35,36] in his Hannover Bone Marrow classification of the MPDs as

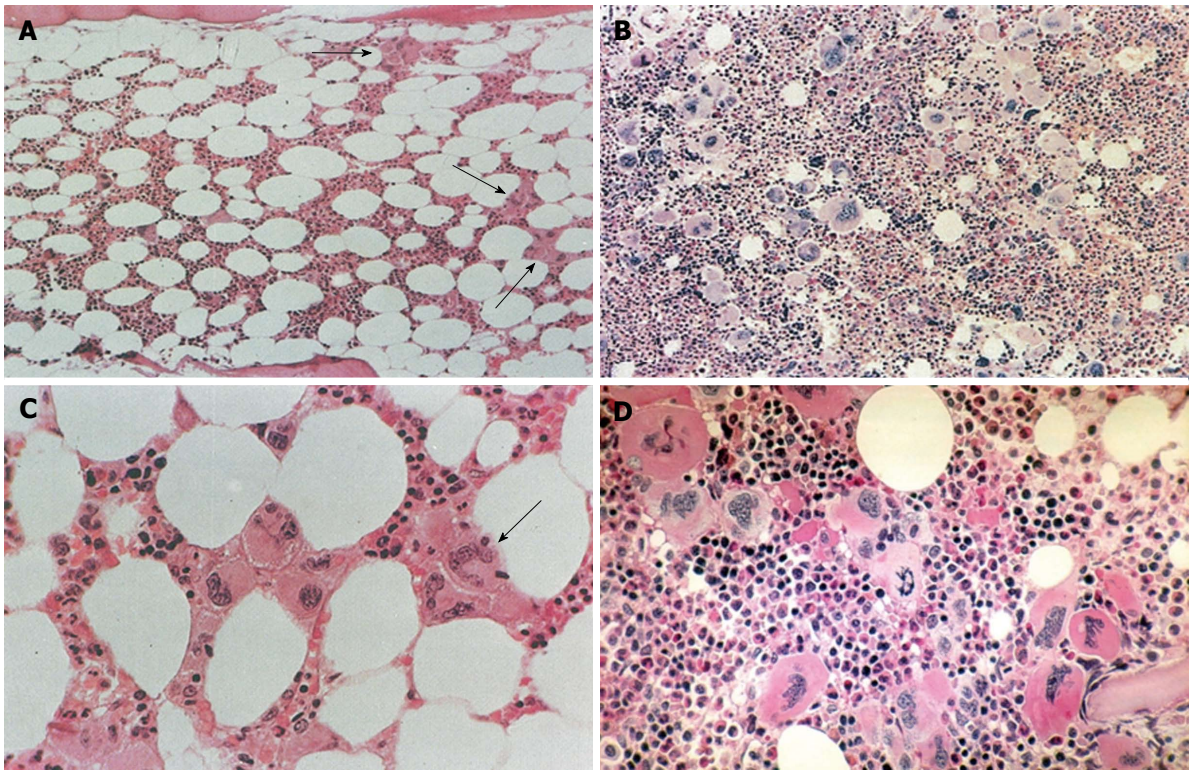


Figure 5 Spectrum of essential thrombocythemia and polycythemia vera bone marrow features in 155 bone marrow biopsies with clustered large pleomorphic megakaryocytes in polycythemia vera patients from the polycythemia vera study group 01 study. ET bone marrow histology (A and C): arrows indicate clustered pleiomorphic megakaryocytes. PV (B) with trilinear megakaryo/eryhtro/granulocytic hyperplasia. Source Ellis *et al*[41] polycythemia vera study group 1975 and Wasserman *et al*[49]. An ET picture was observed in 10 PV. An ET/PV bone marrow picture with increased cellularity (60%-80%, D) was detected in 45 PV. A hypercellular (80%-100%, B) PV picture was recorded in 90 bone marrow biopsies of 155 PV patients the PVSG 01 study. PV: Polycythemia vera; ET: Essential thrombocythemia.

follows: primary diseases are CML, PV, thrombocythemia, chronic megakaryocytic granulocytic myeloproliferation (CMGM) and unclassifiable MPD[35-37]. Reticulin and collagen fibrosis is a reactive feature consecutive to myeloproliferation[35]. Georgii *et al*[35] distinguished three bone marrow histology types of *BCR/ABL*-positive CML: CML of common type with a predominance of granulopoiesis (CML.CT), CML with megakaryocyte increase (CML.MI), and CML with megakaryocytes predominance (CML.MP)[35]. The blood and bone marrow presentation of BCR/ABL-positive ET without features of CML is similar as has been described for CML with megakaryocyte predominance (CML.MP)[30-32,37]. The reliable distinction within the three Ph-negative MPDs and its variations appears to be problematic caused by an overlapping cytomorphology of megakaryocyte and within the three MPDs thrombocythemia, PV and CMGM According to Georgii and Michiels an MPD classification system should be focused cytomorphology of megakaryocytes from bone marrow smears and histopathology from bone marrow biopsies[30-32,35,36]. Megakaryocyte morphology and bone marrow histology had become a hallmark of distinction for the diagnostic differentiation of thrombocythemia (ET), PV *vs* CMGM in the Hannover Institute of Pathology since 1980[67,68]. For the understanding of MPD classification bone marrow histology should distinguish between primary prefibrotic and advanced diseases[35,36]. Primary prefibrotic MPDs according to the Hannover Bone Marrow Classification in 1990 include PV, normocellular thrombocythemia and hypercellular thrombocythemia associated with CMGM without any feature of PV (Figure 5)[35,36]. Results from Adamson *et al*[69], Fialkow *et al*[70,71] and many others revealed the reactive nature of reticulin and collagen fibrosis within the MPDs[69-74]. Consequently, the term of chronic idiopathic myelofibrosis (CIMF) or PMF can not be considered as correct in the Hannover Bone Classification of the MPDs since it has to be considered as a consequence of an underlying myeloproliferative disease[35-37,73,74]. In the 1990 Hannover bone marrow classification, Georgii *et al*[35,36] omitted the terms AMM, CIMF and PMF and introduced the entity of CMGM as the third distinct entity without features of PV and ET. The diagnosis of prefibrotic CMGM is mainly based on the presence of large immature megakaryocytes with immature cytoplasm and cloud-like nuclei not seen in ET and PV (Figure 5)[35,36]. The term CMGM has illogically been changed into CIMF under the influence of Thiele and Vardiman in the 2001 WHO classification[75] and as PMF in the 2008 WHO classification by Tefferi and Thiele[76]. With the advent JAK2 and MPL mutations as driver causes of clonal ET and PV Michiels *et al*[77,78] recognized CMGM as the third distinct MPD entity and replaced the term CMGM by JAK2/MPL wild type primary

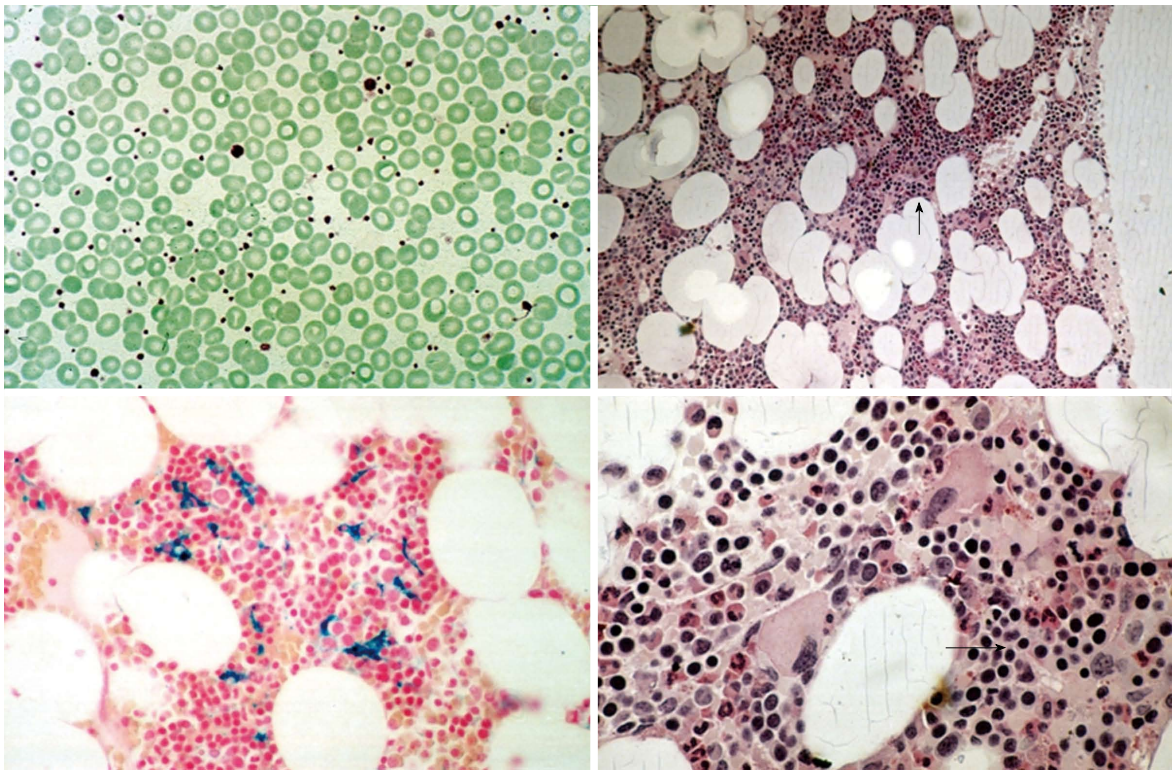


Figure 6 Presence of large platelets in peripheral blood smear (left top), increase of clustered enlarged megakaryocytes in a normocelluar essential thrombocythemia bone marrow with stainable iron (left bottom). Local increase of erythropiesis (long arrow) in areas of loose clustered pleiomorphic megakaryoctyes in patients with normocellular essential thrombocythemia: essential thrombocythemia bone marrow histology.

megakaryocytic granulocytic myeloproliferation (PMGM) in the 2015 WHO-CMP classification (Table 11)[77,78].

THE 1980 ROTTERDAM CLINICAL AND PATHOLOGICAL CRITERIA FOR ET AND PV

Since 1976 the German pathologists Georgii[79] and Burkhardt *et al*[80] had clearly defined the pathological features of ET, PV and CMGM[67-80] on the basis of which we could developed the Rotterdam clinical and pathological (RCP, Table 1) criteria for ET and PV[81,82]. As the 1975 PVSG criteria for ET (PTH) are crude, we could recognize since 1975 the existence of erythromelalgic thrombotic thrombocythemia (ETT) in early stage MPD with a persistent increase of platelet count in excess of 400 × 10^9/L[17,81,82]. The trephine biopsy in these cases showed a proliferation of large mature megakaryocytes in a normal cellular bone marrow with normal erythropoiesis and granulopoiesis (Table 1 and Figure 6). At that time we followed since 1975 the definition of PV according to Dameshek[2] as a trilinear MPD[3]. In PV increased erythropoiesis was most prominent[76] together with variable degrees of increased platelets (> 400 × 10^9/L)[10], erythrocytes (> 6 × 10^{12}/L)[2,3] and granulocytes in the peripheral blood in the absence of the Ph-chromosome (Table 1) and could document distinct bone marrow features as the pathognomonic clue to very early stage of ET (Figure 6). The 1980 RCP criteria of ET and PV were determined by careful prospective documentation of peripheral blood and bone marrow smears and bone marrow biopsy material (Table 1). Platelets in excess of 400 × 10^9/L, and an increase of clustered enlarged megakaryocytes in a bone marrow biopsy material was found to be pathognomonic diagnostic for ET and excluded reactive thrombocytosis. The combination of bone marrow histology and erythrocyte count above 6 × 10^{12}/L according to Dameshek[1,2] appeared to be specific clues to the diagnosis of PV clearly different from all variant of primary and secondary erythrocytosis as documented by Kurnick *et al*[83] in 1972 and by Vykoupil *et al*[67] in 1980. The 1980 RCP modifications of the 1975 PVSG criteria for PV include 4 main changes (Table 1). First, the major criterion O_2-saturation of > 92% is replaced by absence of primary or secondary erythrocytosis by clinical and laboratory tests. Second; splenomegaly is replaced by bone marrow histology as a major criterion (A3). Third, the 1980 RCP diagnostic set used splenomegaly as a minor criterion (Table 1). Fourth, we skipped raised B12 (> 900 ng/L) or raised B12 binding capacity (> 2200 ng/L) as completely irrelevant for the diagnosis of early and overt stage PV (Table 1).

Between 1975 and 1980, we prospectively evaluated the RCP criteria in 30 consecutive early prefibrotic stage patients, who presented with ETT, 14 ET and 16 PV patients[84]. The mean age of 30 ETT patients (ET and PV)

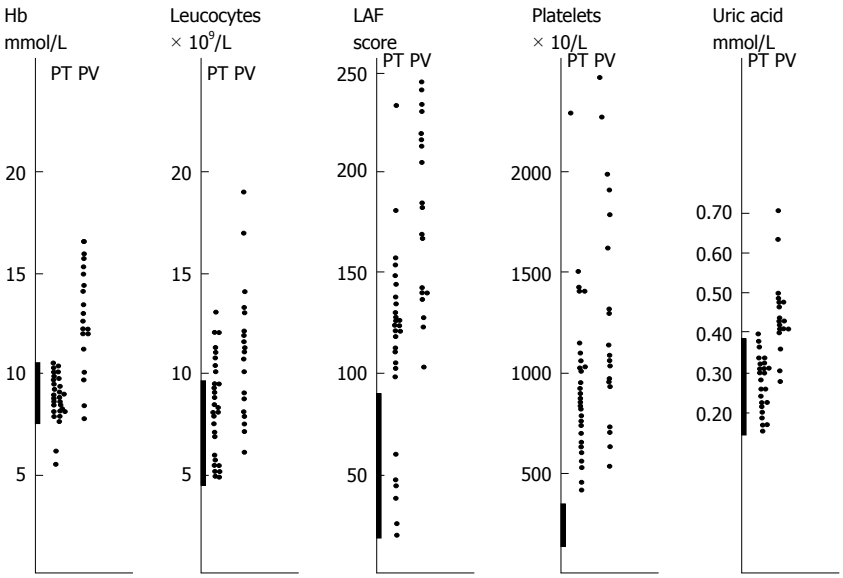


Figure 7 Laboratory findings at time of presentation in symptomatic with thrombocythemia subdivided in 30 primary thrombocythemia (= essential thrombocythemia) and 20 polycythemia vera patients seen between 1975 and 1985. Hb levels are normal in PT and elevated in PV[16]. Leukocytes were normal or elevated in PT and PV. Leukocyte alkaline phosphatase score was much more elevated in PV than in PT with normal values in 6 PT patients. Platelet counts were were between 400 and 1000 × 10/L in the majority of the 30 PT patients. Serum uric acid levels were normal in PT and usually elevated in PV. PT: Primary thrombocythemia; LAP: Leukocyte alkaline phosphatase; PV: Polycythemia vera.

was 56.7 (range 33-96) years. Eleven of 14 ET patients had platelet counts below 1000 × 10^9/L, in whom the diagnosis of ET would have been overlooked by the crude PVSG criteria at that time between 1975-1985. Spleen size on scan was slightly increased in 5 of 14 ET and in 13 of 16 PV patients. Leukocyte count counts was increased (> 10 × 10^9/L) in 5 out of 14 patients with ET and in 14 of 16 PV patients. LAP score was increased (> 100) in 12 out of 14 ET and in all PV patients. All PV patients with erythrocytes above 6 × 10^{12}/L had increased red cell mass (manuscript in preparation). Increased erythrocyte counts above 6 × 10^{12}/L and increase of large pleomorphic megakaryocytes in bone marrow smear (Dameshek)[2,3] and biopsy is diagnostic for PV[17]. Erythrocyte count at a cutoff level of 6 × 10/^{12}L differentiates ET from PV on top of a typical MPD bone marrow histology obviating the need to measure RCM (Table 1). Increased erythrocytes above 6 × 10^{12}/L persists in PV in a comple hematological remission by repeated venesection[2,3,77,78].

The presence of clustered large pleomorph megakaryocytes in bone marrow smears and biopsies is diagnostic clue for ET and PV (Table 1, Figures 4 and 6). An ET bone marrow picture with increase of clustered pleomorphic megakaryocytes and no increase of cellularity (Figure 5) was seen in 7 of 14 ET and only in 1 of 16 PV patients[83,84]. A moderate increase of cellularity (60%-80%) in the bone marrow due to increased erythropoiesis (= decreased M/E ratio) consistent with early stage PV was seen in 3 ET and 4 PV patients. A typical PV hypercellular (80%-100%) bone marrow due to megakaryo/erythro/granulocytopoiesis (panmyelosis according to Dameshek[1]) was seen in one ET patients and in the majority of PV[83,84]. These results indicate that bone marrow histopathology on its own is characteristic for MPN but not fully reliable to differentiate between ET and PV (Figures 4 and 6)[77,78]. The peripheral blood findings in 30 ET [primary thrombocythemia (PT)] and 20 PV seen between 1975 and 1985 are shown in Figure 7[84]. Hemoglobin levels are normal in ET and elevated in PV, leukocytes were normal or elevated in ET and PV[84]. Out of 30 ET patients 24 had an increased and 6 had a normal LAP scores (Figure 7). Platelet counts were were between 400 × 10^9/L and 1000 × 10^9/L in the majority of the 30 ET patients. Serum uric acid levels were normal in ET and frequently elevated in PV. Increased LAP score in the absence of infection and normal erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) is indicative for MPN PV or ET (erf). LAP score was much more elevated in all 20 PV than in ET[84]. As leukocyte alkaline phosphatase (LAP) scoring is becoming increasingly rare in common practice, it can easily be replaced by CD11b neutrophil expression[85]. In the setting of SVT patients PV patients showed higher CD11b values of 190 (CI: 151-238) in PV *vs* 111 (CI: 81-153) in non-PV patients with SVT[85]. In routine practice CD11b expression in the MPNs PV, ET of various molecular etiology *vs* all variants of erythrocytosis should be assessed aganist CD11b expression in controls who have normal ESR and CRP in order to kick out false positive due to underlying infectious or systemic diseases.

THE COLOGNE CLINICAL AND PATHOLOGICAL CRITERIA FOR PH^{1-} NEGATIVE MPD

In the 1980s Thiele *et al*[86,87] described two variants of ET. The histopathology of "true" ET as compared

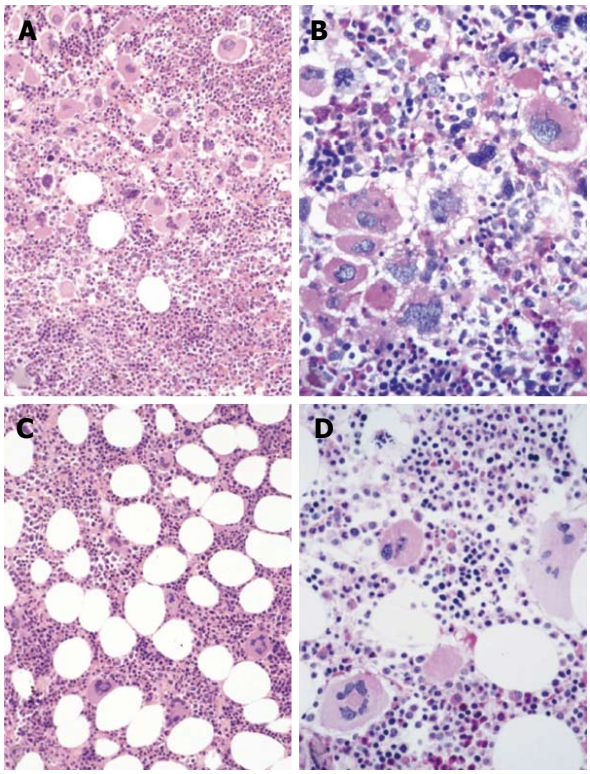


Figure 8 Hypercellular false and normocellular true essential thrombocythemia. A and B: Hypercellular false ET or prefibrotic PMF according to Thiele (Table 6) consistent with CMGM according to Georgii *et al*[35] (1990), or primary megakaryocytic granulocytic myeloproliferation (PMGM122) featured by dense clustered immature megakaryopoiesis and the predominance of immature cloud-like nuclei (Kvasnicka), which are not seen in JAK2V617F positive ET and PV; C and D: Normocellular True ET (World Health Organization-ET, Table 5) according to Thiele and Michiels 1988-2006[59,62,107] with the presence of large to giant megakaryocytes larger than in JAK2^{V617F} positive ET and PV. True ET as defined by Thiele seems to our experience most consistent with normocellular JAK2 wild type ET carrying the MPL^{515} mutation featured by clustered large and giant megakaryocytes in a normocellular bone marrow, which usually runs a very benign course. ET: Essential thrombocythemia; PV: Polycythemia vera; CMGM: Chronic megakaryocytic granulocytic myeloproliferation.

to PV in bone marrow biopsy is characterized by uniform appearance of mature large and giant mature megakaryocytes with hyperlobulated nuclei (staghorn) in a normal cellular bone marrow dispersed among normal granulopiesis and erythropoiesis (Figure 8)[86-88]. At the bone marrow level classical PV was featured by a marked pleomorphism of the megakaryocyt (small, medium and large megakaryocytes) in ET and PV in combination with an increased erythropoiesis with or without increased granulopiesis, whereas the megakaryocytes were of normal size in controls and reactive thrombocytosis[86-90].

In 1999 Thiele *et al*[90] introduced the Cologne Clinical and Pathological (CCP) criteria and defined the bone marrow features of normocellular "true" ET, hypercellular trilinear PV, and hypercellular ET associated with prefibrotic chronic idiopathic myelofibrosis (false ET or prefibrotic CIMF). The CCP criteria of thrombocythemias in various MPDs were based on a retrospective clinico-pathological study of 395 PVSG defined MPD patients with platelet count above 500 × 10^9/L (Table 4)[81]. For comparison 35 patients with reactive thrombocytosis were enrolled in this study. The 395 MPD patients PV (*n* = 55), CIMF = PMF (*n* = 250 prefibrotic and fibrotic), prefibrotic PMF (*n* = 120) and "true" ET according to the CCP criteria (Tables 3 and 4). In 2002 Michiels and Thiele defined "true" ET and differentiated "true" ET from ET associated with prefibrotic CIMF (Table 5, Figure 8)[82]. In "true" ET megakaryocytes display large to giant megakaryocytes showing hyperlobulated staghorn-like nuclei in a normocellular bone marrow (Figure 8)[78,80]. PV was typically featured by large pleomorphic megakaryocytes with hyperploid nuclei in a hypercellular bone marrow due to increased erythropoiesis or increased erythrocytic-megakaryocytic-granulocytic myeloproliferation. Interestingly the megakaryocytes in "true" ET were larger than in PV (Table 5, Figure 8)[1]. Hypercellular ET associated with prefibrotic CIMF ("false" ET = CMGM = PMGM, Figure 7) is 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, Figures 7 and 8), which are never described in ET and PV[2]. Normocellular "true" ET according to the 2002 European clinical pathological (ECP) criteria is featured by normal LAP scores (normal CD11b neutrophil expression), higher platelet counts and large to giant megakaryocytes with multilobulated stag-horn like nuclei in a completely normocellular bone marrow (Table 5)[90,91]. In "true" ET the values for hemoglobin, erythrocytes, and LAP scores (CD11b neutrophil expression) were completely normal (Table 5). In contrast, the RCP defined prefibrotic normocellular ET and prodromal PV were associated with increased LAP score (Figure 7), the presence of pleomorphic megakaryocytes (Figure 6) low serum EPO levels, slight splenomegaly, and spontaneous EEC[17,84]. This point to the existance of at least two phenotypes of normocelluar ET: ET with features of early PV (prodromal PV) *vs* "true" ET without features of PV. These differences in RCP defined ET (Table 1) criteria and CCP defined "true" ET and false ET (Table 3) are related to a selection bias of patients. In 1988, Thiele as a pathologist selected 25 cases with 1975 PVSG defined normocellular ET (minimum platelet count of 1000 × 10^9/L) who had pronounced thrombocythemia and normocellular bone marrow without PV features and this was associated with normal LAP score (CD11b neutrophil expression)[87]. Focussing on aspirin-sensitive erythromelalgic inflammatory and ischemic complications as pathognomonic presenting symptoms of early ET or PV patients, the Rotterdam MPD Working group studied a selected and biased group of early stage myeloproliferative ET and PV at platelet counts above 400 × 10^9/L[84]. Only symptomatic ET and PV patients were included in our 1975-1985 studies because of erythromelalgic ischemic digital circulation disturbances[84].

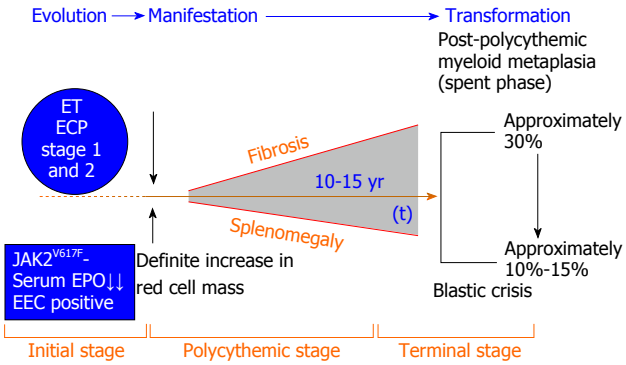


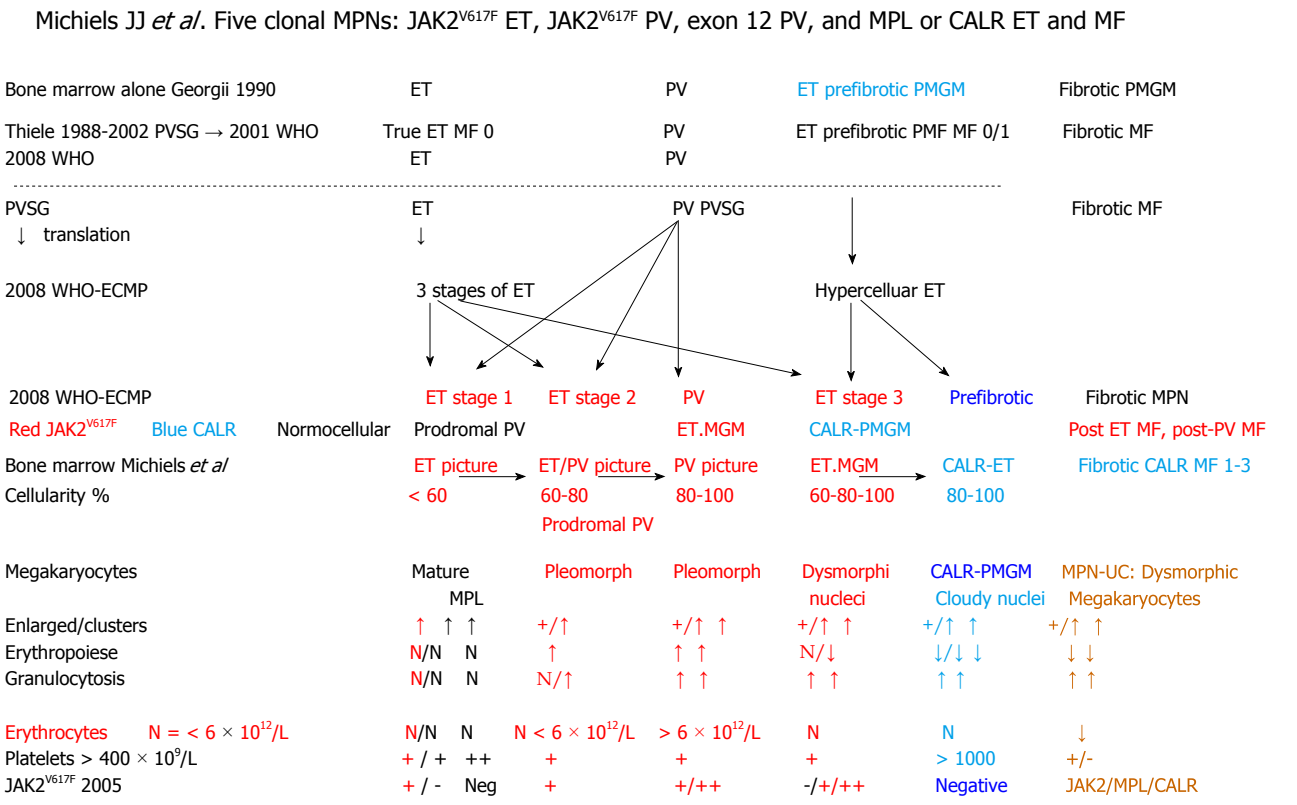
Figure 9 Prodromal polycythemia vera with normal red cell mass (red cell mass, Tables 3 and 7) and erythrocytes (< 6 × 10^{12}/L, Tables 8 and 9), prefibrotic idiopathic erythrocythemia and polycythemia vera with increased erythrocytes (> 6 × 10^{12}/L) and red cell mass, and evolution into masked PV with splenoegaly, spent phase polycythemia vera with myelofibrosis (Table 12) and post-polycythemia vera-myelofibrosis according to European Clinical and Pathological criteria defined by Michiels and Thiele (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) and according to the 2006-2007 World Health Organization-European Clinical Molecular and Pathological criteria for JAK2 mutated stages of prodromal overt and advanced polycythemia vera[71-73].

THE 2006-2008 EUROPEAN CLINICAL, MOLECULAR AND PATHOLOGY CRITERIA FOR JAK2^{V617F} MUTATED AND JAK2 WILD TYPE MPN

Myelofibrosis is not specific for a disease and can be observed in patient with hairy cell leukemia, Ph-positive CML and in the Ph-negative MPDs, CMGM, PV, ET and many other conditions as well[35-37,92,93]. An increase of reticulin in the silver impregnation stain of bone marrow biopsies can be graded as fine or coarse fibers and is a secondary event relevant for prognosis assessment[94]. The use of CIMF and PMF in the 2001 and 2008 WHO classifications[75,76] is misleading and scientifically not recommended[94]. We persisted to use this term in the 2006/2007 WHO-ECMP classification[94-96] for reasons of compliance with peer reviewers, but we convincely eliminated the terms CIMF and PMF in the 2013-2014 WHO-CMP classifications (Tables 7-11) for the MPNs of JAK2^{V617F} positive ET, PV, JAK2-negative ET and hypercellular ET associated with PMGM[77,78]. MF itself is the reflection of MPN disease progression but not a disease entity on its own because reticulin and collagen fibrosis are produced by polyclonal fibroblasts in response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in both PV and ET of various molecular etiology[69-74]. The presence of reticuline fibrosis is well documented in all variants of ET, PV, PMGM, CML and in many other conditions. An increase of reticulin fibrosis is rare in WHO normocellular ET will occur in about one third of PV and will occur in the majority of patients with PMGM during long-term follow-up[35,36,89].

The WHO bone marrow features and the variable phenotypes of thrombocythemias according to the 2002 ECP (Tables 5 and 6) and 2006/2007 ECMP criteria for ET (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) (Tables 6, 7 and 8) do in fact distinguish within the JAK2^{V617F} mutated MPN normocellular (WHO-ET, < 60%) and hypercellular ET (prodromal PV, 60%-90%) due to increased erythropoiesis, from JAK2 wild type normocellular ET and separate JAK2 mutated MPN from JAK2 wild type hypercellular ET associated with a granulocytic myeloproliferation (PMGM, Figures 9 and 10)[94-96]. JAK2^{V617F} mutated normocellular ET and prodromal PV patients had increased LAP score similar as in PV (Figure 7). Bone marrow histopathology on its own was not reliable to differentiate between JAK2^{V617F} positive ET, prodromal PV and classical PV (Table 6), whereas the 2002 ECP criteria had defined "true" ET with normal LAP score and giant megakaryocytes with staghorn-like nuclei in a normocellular bone marrow (Table 5) and prefirotic CIMF = PMF = PMGM (table) very likely belonging to the JAK2 wild type MPNs (Table 5 and Figure 10)[91]. According to Michiels *et al*[91], WHO bone marrow biopsy histopathology evaluations have a sensitivity and specificity near to 100% to differentiate all variants of ET in various MPD/MPNs from thrombocythemia in CML, from myelodysplastic syndromes and from reactive thrombocytosis[94-96]. The distinction of prefibrotic thrombocythemias in the MPNs of various molecular etiology at the bone marrow level is a topic of research for the Dutch and Belgian hematopathologists[77,78]. The ECP, ECMP and WHO bone marrow features separate myeloproliferative PV from all variants of primary congenital erythrocytoses and secondary erythrocytoses[77,78]. Bone marrow histology separates idiopathic erythrocytosis with increased RCM from early erythrocythemic stage 1 PV, and do detect "masked" ET, "masked" PV overlooked by the PVSG and WHO criteria with a sensitivity and specificity of 100% if the trephine biopsy is of good quality (Table 6 and Figure 10)[77,78]. A bone marrow biopsy is mandatory for grading cellularity in prefibrotic stages and for grading reticulin and collagen fibrosis (Table 1) in hypercellular MPN advanced PV and ET (post-ET myelofibrosis, post-PV myelofibrosis, Figure 9).

In 2007 Thiele left the European Working Group on MPD and joined again the WHO investigators to define the 2008 WHO classification of the myeloproliferative neoplasms ET, PV and PMF[76,97,98]. The formulation of the WHO-ECMP criteria in 2006/2007 by Michiels *et al*[94-96] (Tables 7 and 8, Figure 11) for ET prodromal PV and PV preceded the publication of the 2007/2008 revision of the WHO classification[96-98]. Thiele *et al*[99] validated the 2008 WHO classification of MPN in JAK2^{V617F} positive and JAK2 wild type MPN from large series of PVSG defined MPD patients diagnosed in the past and persisted to use the terms "true" ET *vs* "false" ET or PMF[100-102]. The 2008 WHO investigators were persistently confronted with unsolved problems and pitfalls regarding in ET of various MPNs and did not recognize the distinct differences in bone marrow histology between JAK2 mutated ET and PV and JAK2 wild type ET without features



Conclusion: for clarity the WHO-ECMP MPN classification and staging separate JAK2^{V617F} positive classical trilinear PV and ET stage 1, 2 and 3 (in red) *vs* JAK2 wild type (blue) ET stage 3 = PMGM, and fibrotic endstage of PMF.

Figure 10 Charaterization of polycythemia vera study group defined essential thrombocythemia and polycythemia vera by applying the 2006 European Clinical Molecular and Pathological criteria and 2001 World Health Organization bone marrow features; JAK2^{V617F} mutated normocellular essential thrombocythemia, prodromal polycythemia vera, classical polycythemia vera and myelofibrosis *vs* JAK2 wild type normocelluar essential thrombocythemia and chronic idiopathic myelofibrosis or Primary megakaryocytic granulocytic myeloproliferation. WHO: World Health Organization; PV: Polycythemia vera; ECMP: European clinical molecular and pathological; MF: Myelofibrosis; ET: Essential thrombocythemia; MPN: Myeloproliferative neoplasm; PMGM: Primary megakaryocytic granulocytic myeloproliferation; PVSG: Polycythemia vera study group; CALR: Calreticulin.

of PV. The 2008 WHO recognized the differences in megakaryocyte morphology in CML, MDS as compared to PV but did not pick up the potential importance of significant differences of megakaryocyte morphology between JAK2, MPL and CALR mutated ET and MF[78]. The 2015 WHO-CMP classification of the prefibrotic MPNs distuinguishes JAK2^{V617F} mutated trilinear ET and PV (Tables 7-9, Figure 11) from exon 12 PV and based on distinct megakaryocyte morphology features to differentiates JAK2^{V617F} mutated ET and MF from JAK2 wild type "true" ET carrying the MPL mutation (Table 10) and from JAK2/MPL wild type hypercelluar ET carrying the calreticulin (CALR) mutation associated with PMGM (Table 11, Figure 8).

2015 WHO-CMP CLASSIFICATION OF FIVE DISTINCT CLONAL MPNS

JAK2^{V617F} mutated trilinear PV and ET: From Dameshek to Vainchenker

The one cause hypothesis of trilinear PV proposed by Dameshek[2] in 1950[3] has been confirmed by Vainchenker in 2005 (Figure 1) by the discovery of the JAK2^{V617F} mutation as the driver cause of ET, PV, masked PV and MF[103,104]. Detection of JAK2^{V617F} has become the first intention diagnostic test to differentiate between PV and myeloproliferative IE from erythrocytosis with a sensitity of 95% and specificity of 100%[105-116]. The prevalence of the JAK2^{V617F} mutation in PVSG defined PV is 95% and about 50% in ET and MF[7,105-107]. The JAK2^{V617F} mutation load is low in ET and ranges from less than 10% to 50% of the granulocytes, either low less than 50% or high between 50% to 100% (homozygous) of the granulocytes positive for the heteroxygous/homozygous JAK2^{V617F} mutation in PV (Figure 12)[110-112]. A group of JAK2^{V617F} positive normocellular ET with a very low percentage of heterozygous mutant JAK2 can maintain as a non-progessive subpopulation in the bone marrow without a tendency to evolve into prodromal PV or hypercellular ET during long term follow-up[115]. The few patients with hypercellular ET homozygous for the JAK2^{V617F} mutation patients are at high risk for anemia on one hand and myeloid metaplasia of the spleen (splenomegaly) with secondary myelofibrosis on the other hand (Figure 9)[112]. 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. Hetero/homozygous or homozygous JAK2^{V617F} mutation is associated with pronounced constitutively activation of megakaryopoiesis, erythropiesis and granulopoiesis in the bone marrow as the cause of hypercellular trili-

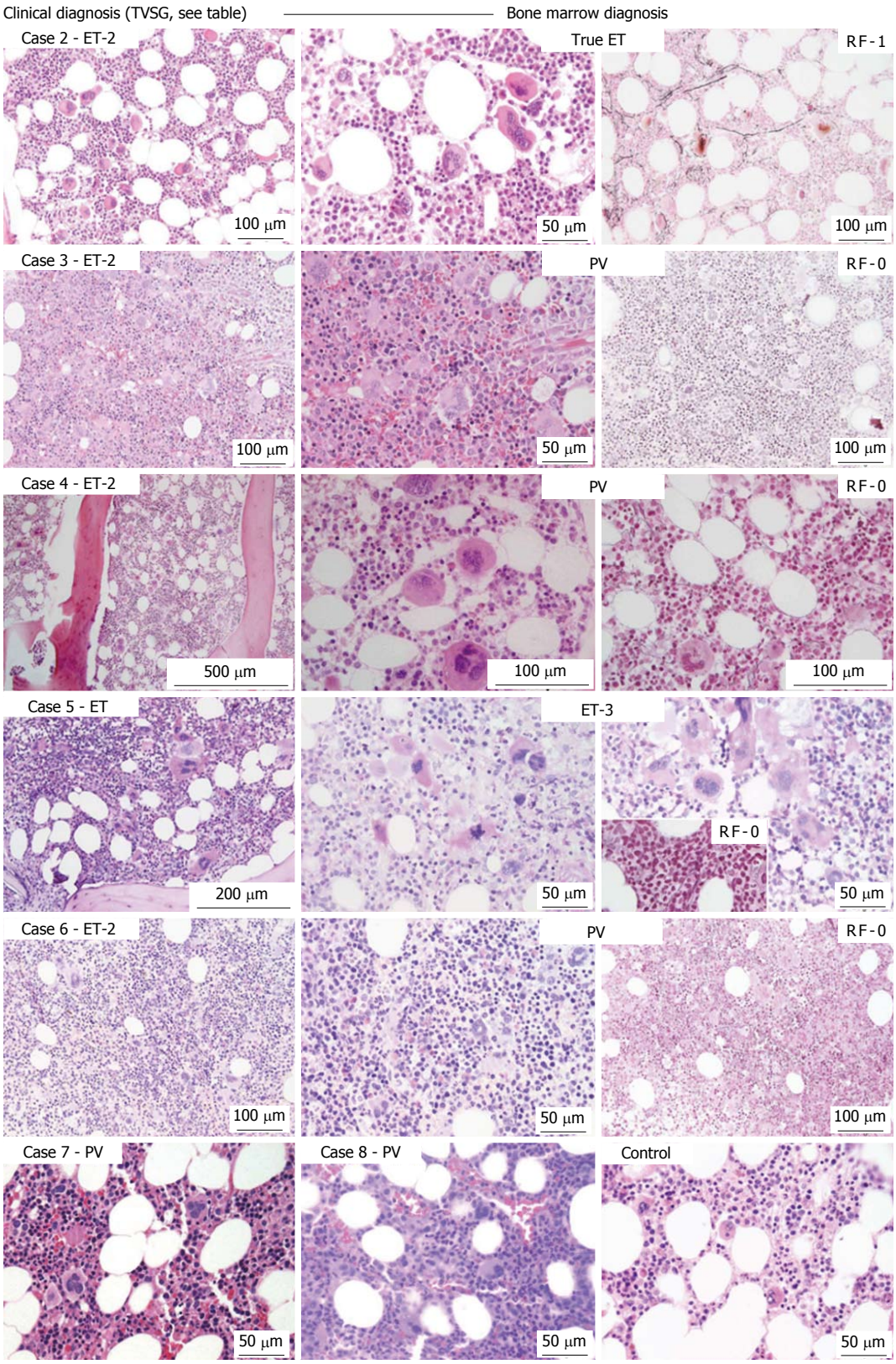


Figure 11 The clinical features of four JAK2^{V617F+} essential thrombocythemia and two JAK2^{V617F+} polycythemia vera cases refer to the numbering of cases in Figure 10. Case 2: Clinically JAK2^{V617F} ET 2 (low serum erythropoetin) and a normocellular ET [World Health Organization (WHO)-ET] bone marrow wit pleomorph small and large megakaryocytes and reticulin fibers (RF) grade 1; Case 3 and 4: Clinically JAK2^{V617F} ET 2 with a trilinear hypercellular PV bone marrow and RF 0 in case 3: ET 2 with increased cellularity due to increased erythropoiesis RF grade 0 in case 4; Case 5: Clinically JAK2^{V617F} ET with moderate splenomegaly and a hypercellular megakaryocytic granulocytic myeloproliferation (ET.MGM = ET 3), with dysmorphic megakaryocytes (not cloud-like) and RF grade 0 in case 5; Case 6: Clinically JAK2^{V617F} ET 2 with a trilinear PV bone marrow picture1 and RF grade 0; Case 7: Clinically JAK2^{V617F} PV with a 65% hypercellular ET/PV bone marrow picture in between "normocellular ET" (WHO-ET) and trilinear hypercellular (90%-100%) PV picture in case 7 and 8 with PV with increased RCM and erythrocytes above 6 × 10^{12}/L. In 2007, we concluded that bone histology alone does not differentiate between JAK2 mutated ET and PV, as compared to control, morphology of pleiomorph megakaryocytes in JAK2^{V617F} mutated ET and PV are similar Source Poster P-0025. Fourth International Congress on myeloproliferaive disease/ myelodysplatic syndrome New York, 2007. ET: Essential thrombocythemia; PV: Polycythemia vera.

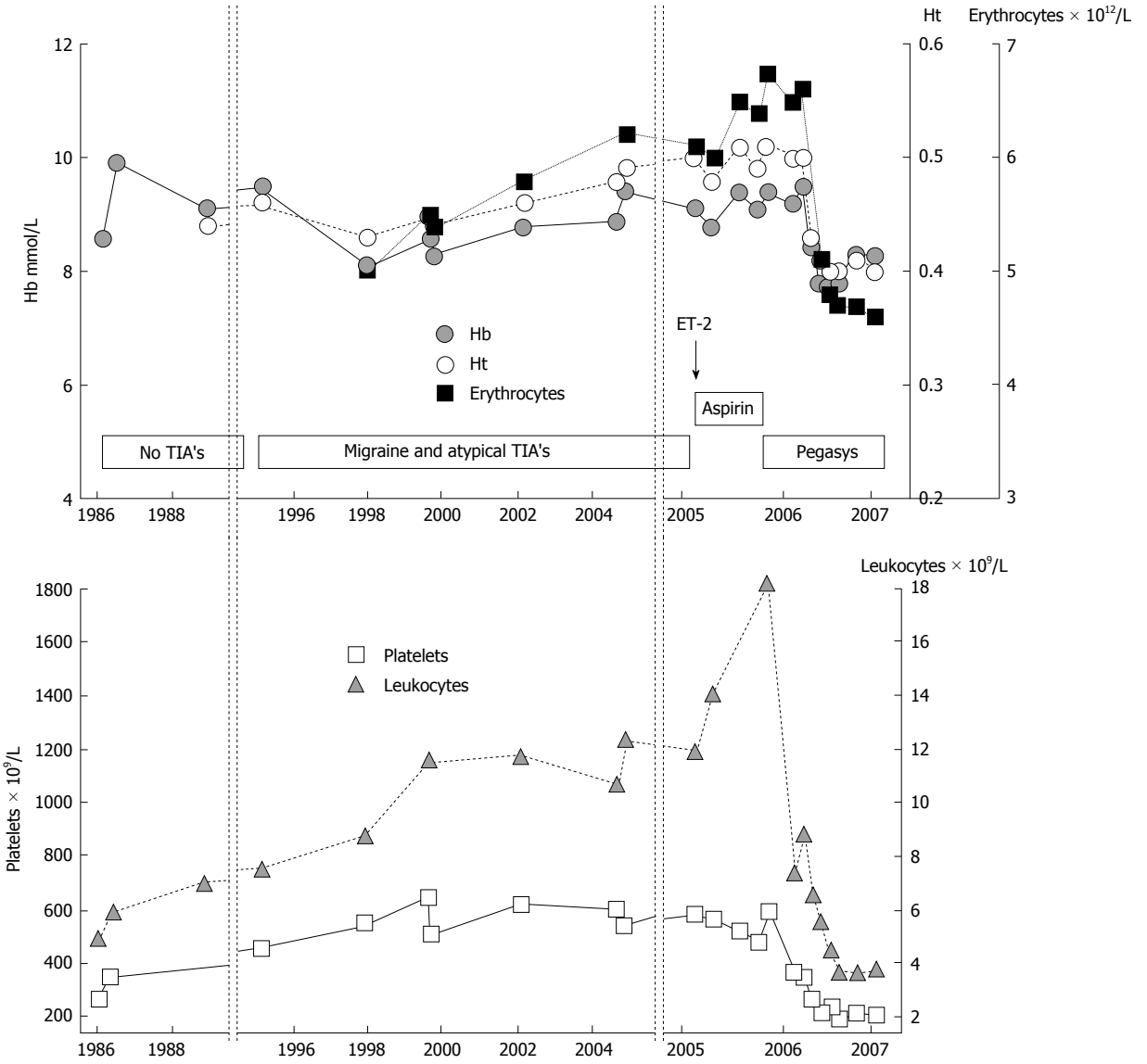


Figure 12 Sequential stages of essential thrombocythemia 1995-2004, prodromal polycythemia vera 2004-2005 and overt polycythemia vera 2006, with a complete hematological response (complete hematological responses, case 6, Table 9, Figure 11) and subsequent complete hematological responses of interferon for 6 years at age of 72 years anno 2013.

near PV[2,3,103,104]. Scott *et al*[113] and Moliterno *et al*[114] demonstrated that so-called heterozygous PV with allele load less than 50% are hetero/homozygous at the EEC level in blood and bone marrow for the JAK2^{V617F} mutation, whereas ET patients are heterozygous reflecting a maximal JAK2^{V617F} mutation load of 50%[113,114]. Heterozygous JAK2^{V617F} mutation leading to constitutively activated megakaryocytes with increased sensitivity to TPO is enough to induce ET and to produce constitutively activated, hypersensitive, sticky platelets responsive to aspirin (aspirin-responsive Sticky Platelet Syndrome)[116,117]. Godfrey *et al*[118] studied the JAK2 mutation status of burst forming unit-erythropiesis grown in low erythropoietin conditions in 77 patients with PV or ET[113]. 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[118]. In this study of Godfrey *et al*[118], PV has been distinguished from ET by expansion of one dominant homozygous subclone, the selective advantage of which is likely to reflect additional cytogenetic[119], genetic or epigenetic alterations (Table 12)[120-123]. Such additional, acquired background biological factors on top of the JAK2, MPL and CALR driver mutations of MPN will become of huge importance for the understanding of differences in prognosis and outcome.

Prospective evaluation of WHO-CMP criteria in JAK2^{V617F} mutated MPN

Bone marrow cellularity, increased erythropoiesis or granulopoisis and the morphology of pleomorphic megakarocytes are not different in JAK2^{V617F} mutated ET and PV (Figure 11). Normocellular ET had stable ET disease without any progression during life long follow-

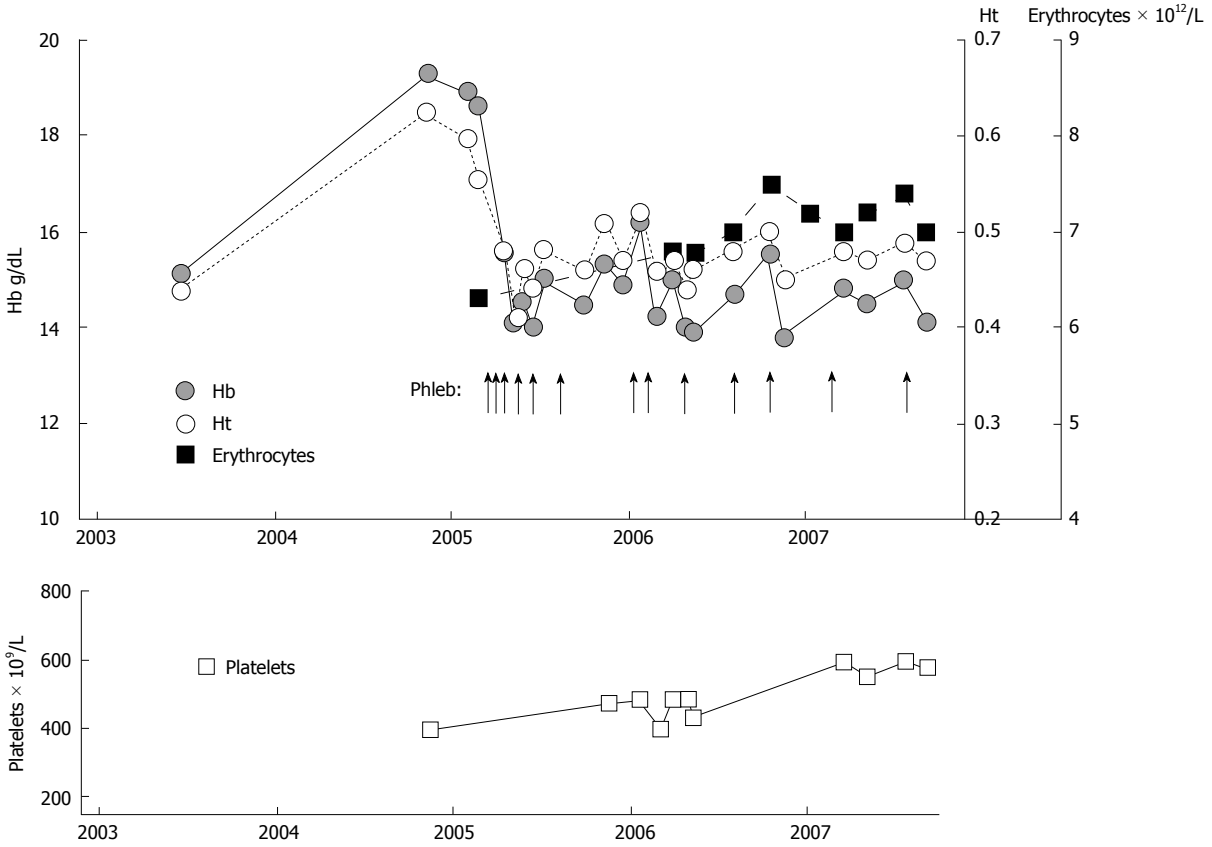


Figure 13 Clinical course in case 8 (Table 9, Figure 11) with erythrocythemic polycythemia vera treated with venesections (arrows). The development of microcytic hypochromic erythrocytes due to iron deficiency was associated with persistent increased red cell count (> 6 × 10^{12}/L), which is diagnostic for polycythemia vera. The iron deficient state and the low normal values for haemoglobin (Hb) and hematocrit (Ht) was associated with relief of hypervolumic symptoms with phlebotomy on top of low dose aspirin.

up (Figures 13 and 14). As shown in Figure 13 the sequential evolution of ET, prodromal PV and overt PV is predicted to response to pegylated inteferon (Pegasys^{R}) treatment. PVSG defined ET patients frequently had a typical hypercellular PV bone marrow picture due to increased erythropoiesis (prodromal PV, cases 3, 4, 5 in Figure 11) similar as observed in newly diagnosed PV patients (cases 7, and 8 in Figure 11). As shown in Figure 14 stage 1 JAK2^{V617F} mutated pure erythrocythemia or idiopathic erythrocytosis according to PVSG criteria presented with a typical PV bone marrow histology (case 8, Figure 11) and persistant increased erythrocyte counts above 6 × 10^{12}/L. After correction of haemoglobin and hematocrits to to around 0.40 by repeated venasections (Figure 14) the erythrocyte counts remained above 6 × 10^{12}/L whereas the JAK^{V617F} mutation load increased in this case raised from heterozygous 25% to homozygous 65% after 5 years follow-up.

Detection of JAK2^{V617F} mutation and serum EPO measurement have become the first step in the diagnostic work-up of erythrocytosis with erythrocyte counts above the upper limit of normal (> 5, 6 × 10^{12}/L)[124-126]. Vannucchi *et al*[111] employed quantitative assays for JAK2^{V617F} allele levels in granulocytes in a prospective study of 175 PV patients at time of diagnosis[111]. The JAK2 mutant allele burden could be quantified as 1%-25%, 25% to 50%, 50%-75% and 75%-100% in 57, 50, 34 and 32 PV patients respectively at time of investigation[111]. The burden of JAK2^{V617F} allele was directly correlated with abnormally increased levels of hematocrit, white cell and neutrophil count, LDH and LAP score, spleen size on echogram and with decreased values for serum ferritin, and erythropoietin[111]. The JAK2^{V617F} mutation load in this study nicely correlated with a progressively higher relative risk for aquagenic pruritus, spleen size on echogram, total thrombosis and the need for receiving myelosuppressive.

Among patients with SVT (Budd-Chiari syndrome or portal vein thrombosis) are MPN in an early phase, the so called "masked" ET or PV which not yet meet the 2008 WHO criteria[47,48]. In the Kiladjian *et al*[47] study, 241 of such SVT patients of whom 74 had platelet counts between 238 and 456 × 10^{9}/L (mean 333) who carried the JAK2^{V617F} mutation. In 147 SVT patients JAK2 wild type masked MPN the platelet count varied between 104 and 258 × 10^{9}/L (mean 159)[47]. Interestingly, none of the 241 SVT cases carried the MPL^{515} mutation, whereas congenital factors for venous thrombosis like Factor V Leiden were rather frequent[47]. In the meta-analysis of Smalberg *et al*[48] the JAK2^{V617F} mutation was identified in 276 of 1002 (28.5%) patients with Budd-Chiari syndrome and in 173 of 855 (19.4%) patients with portal vein thrombosis. A total of 268 SVT patients were tested for JAK2 exon 12 and 305 for

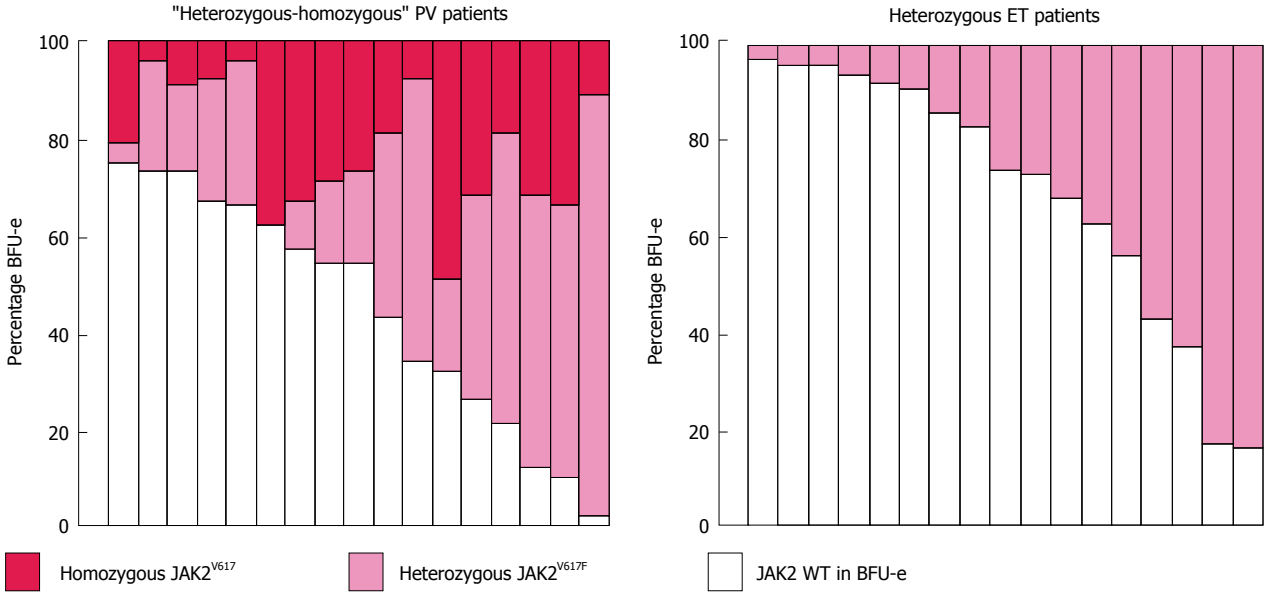


Figure 14 Genotype of individual BFU-E in polycythemia vera and essential thrombocythemia with granulocytes heterozygous for the JAK2^{V617F} mutation (less than 50% JAK2^{V617F} mutation load) show that polycythemia vera patients are heterozygous/homozygous and essential thrombocythemia patients heterozygous for the JAK2^{V617F} somatic mutation. ET: Essential thrombocythemia; PV: Polycythemia vera; WT: Wild type; BFU-e: Burst forming units erythropoiesis.

MPL^{515} mutations. Three patients were found to carry the MPL^{515} mutation and JAK2 exon mutation was not detected in any of these SVT patents[48]. Three studies concluded that JAK2^{V617F} mutation can be considered pathognomonic for MPN in patients with splanchnic vein thrombosis[47,48,127]. These SVT patients with portal hypertension, splenomegaly and hypersplenism suppress and consequently mask the elevated blood cell counts, which can be expect in active MPN disease[47,48,127]. Masked cases of early stage ET and PV in patients with splanchnic vein thrombosis are overlooked by the 2008 WHO classification[128] and nowadays easily detected by combining JAK2^{V617F} mutation screening and WHO-CMP criteria[77,78]. The 2015 WHO-CMP classification recognizes the existence a broader spectrum of JAK2 mutated normocellular ET, prodromal PV, IE, early PV, overt PV, masked PV (not meeting the WHO criteria), advanced PV with splenomegaly and post-PV myelofibrosis, which will has significant therapeutic implications (Table 11)[77,78].

JAK2 exon 12 mutations as cause of idiopathic erythrocythemia and PV

The frequency of JAK2 exon 12 mutations among all patients with PV is estimated around 3%[129,130]. JAK2 N542-E543del is the most frequent among the different reported exon 12 mutations. The finding of the JAK2 exon 12 mutations in the 5% PV patients negative for JAK2^{V617F} usually present with early stage PV or idiopathic erthrocytosis (IE = increased red cell mass with normal values for leukocytes and platelets and no palpable spleen) with a favourable outcome and normal life expectancy[129-131]. Ten JAK2 exon 12 mutated MPN patients had increased red cell mass, were negative for the JAK2^{V617F} mutation, and could be diagnosed as PV in 6 and idiopathic erythrocytosis in 4[129]. Pre-treatment bone marrow histology in JAK2 exon 12 mutated PV or IE showed characteristic erythroid hyperplasia with minor and distinct histology changes of the megakaryocyte lineage, which are never seen in primary or secondary erythrocytoses[129]. Cases of exon 12 mutated, JAK2^{V617F} negative PV were frequently diagnosed as IE with increased hematocrit hematcrit and red cell mass, 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 of large megakaryocytes with hyperploid nuclei[129-131]. In the bone marrow histology study of Lakey *et al*[131] in 7 cases of JAK2 exon 12 mutaed PV all showed prominent erythroid hyperplasia meeting the criteria for IE in 4 and PV in 2, but hyperplasia of atypical small to medium-sized large megakaryocytes was present in all (Figure 15)[131]. Presenting features at diagnosis were aquagenic pruritis and/or erythromelalgia in 3, and microvascular events including headache, dizziness, blurred vision and distal extremity numbness in 4 at platelet counts between 152 and 790 × 10^9/L (5 below and 2 above 400 × 10^9/L consistent with aspirin responsive platelet thrombophilia[116] and the sticky platelet syndrome (SPS)[117]. Six of 7 JAK2 exon 12 MPN cases were diagnosed as IE with low serum EPO levels in all. Bone marrow pathology of the JAK2 exon 12 PV cases lacked the prominent clustering of large megakaryocytes with hyperlobulated nuclei that characterize JAK2^{V617F} mutated PV[77,78]. Bone marrow pathlogy in JAK2 exon 12 mutated MPN revealed a spectrum of small to medium sized megakaryocyte with a predominance of

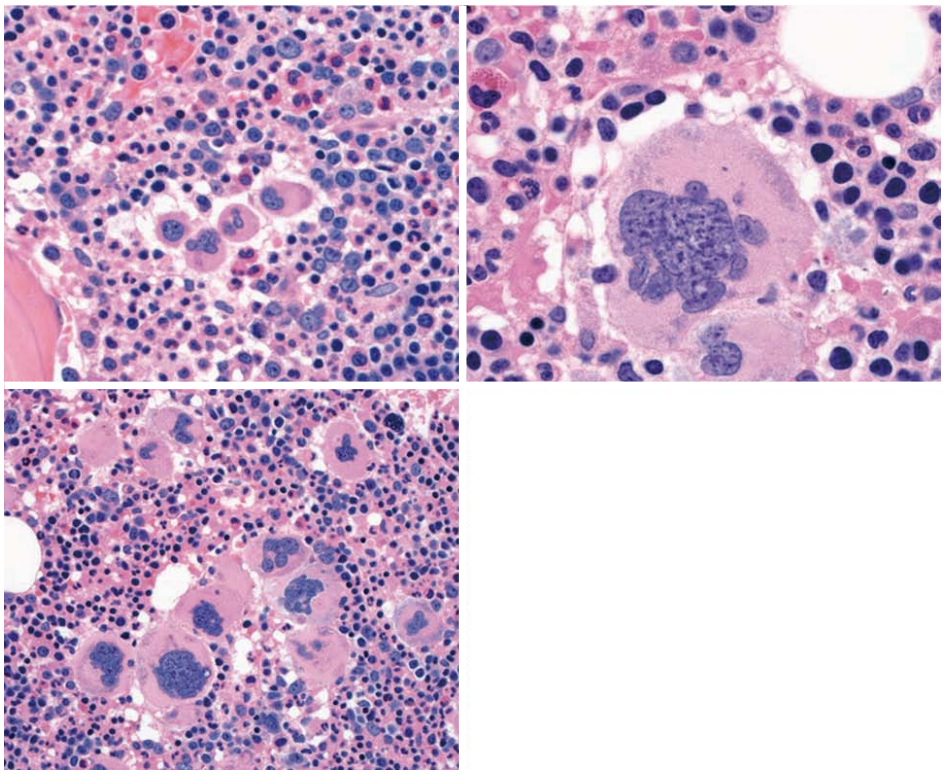


Figure 15 Bone marrow histology in JAK2 exon 12 polycythemia vera.

smaller forms. The nuclei were atypical with a varying degree of lobation comprising monolobulated and hyperlobulated forms. The chromatin was abnormally distributed (Figure 15)[131]. The bone marrow reticulin content was normal or slightly increased in 6, and one case evolved 15 years after intitial diagnosis into post-PV myelofibrosis with reticulin fibrosis grade 3 and associated osteomyelofibrosis.

Hereditary autosomal dominat essential thrombocythemia due to a gain of function mutation in the TPO and JAK2 gene

In the 1990s, studies on murine leukemia and oncogenes led to the recognition of a new member of the hematopoietin receptor super family. It was discovered as the product of the gene c-mpl, the normal cellular homologue of the oncogene v-mpl, the transforming principle of a murine myeloproliferative leukemia virus, responsible for a panmyeloid transformation[130]. This was followed by the molecular cloning and characterisation of Mpl, the human homologue of the c- and v-mpl[132-135]. The receptor MPL was then rapidly recognized as being the thrombopoietin receptor (TpoR) by the demonstration that antisense oligonucleotides of c-mpl inhibited the colony-forming of megakaryocyte progenitors by Wendling *et al*[136]. The Mpl ligand became the key to the identification of TPO and was cloned in 1994 by five independent groups[136-140]. The Mpl ligand is identical to thrombopoietin and labeled as megakaryocyte growth and development factor MGDF[139-142]. Human TPO has all the functions ascribed to MGDF, and all MGDF-like activity can be neutralized by soluble recombinant Mpl[143]. TPO stimulates hematopoietic stem cells and megakaryocyte precursors to proliferate, differentiate and mature and mature megakaryocytes form pro-platelets, which then disintegrate into platelets[143].

Two basic research studies clearly showed that continuous forced expression of TPO, (TPO^{high} mice) in mice induces megakaryocyte proliferation and differentiation and subsequently develop myelofibrosis[144-146]. TPO^{high} mice engineered to overexpress TPO in their liver and those that received transplants of marrow cells infected with a TPO containing retrovirus develop thrombocythemia due to massive hyperplasia of megakaryocytes and granulocytes and hypoplasia of erythropoiesis in the bone marrow followed by myelofibrosis and extramedullary hematopoiesis within 2 to 3 mo and die from myeloid metaplasia and myelofibrosis thereafter[144]. TGF-Beta-1 has been implicated in the pathobiology of myelofibrosis by the observation that megakaryocytes from TPO^{high} rats and mice express high levels of TGF-Beta-1 in marrow extracellular fluids and plasma[145]. In wild mice TGF-Beta-1 mRNA expression in bone marrow and spleen was barely detectable before TPO treatment, and significantly increased in both organs after TPO treatment and returned to basal levels at day 14 (Figure 9)[145]. Another growth factor produced by megakaryocytes, platelet derived growth factor, was found to be upregulated in a fashion similar to TGF-Beta-1. High levels of TGF-Beta-1 mRNA in bone marrow and spleen cells in TPO^{high} mice were associated with high levels of TGF-beta-1 protein in extracellular fluids from

these organs.

The first well documented report on autosomal dominant hereditary ET (HET) due to a gain of function mutation in the *TPO* gene was described in a large Dutch family[147-149] and in a Polish family[150]. HET due to gain of function mutation in the *TPO* gene is associated with marked increased TPO levels (530 ± 27 pg/mL *vs* controls < 62 pg/mL), and microvascular circulation disturbances including erythromelalgia and atypical transient ischemic attacks consistent with aspirin responsive platelet thrombophilia[116] and the sticky platelet syndrome[117]. Increase of large platelets and large mature megakaryocytes with hyperploid nuclei and normal cellularity in bone marrow biopsy specimens of the propositus (man born in 1934) and affected family members were diagnostic for HET, which was associated with platelet-mediated thrombophilia comparable as first discovered by Michiels *et al*[51,81,82] in 1985 and as described by Vannucchi *et al*[111] in acquired JAK2^{V617F} mutated ET and PV[110,111].

Hereditary autosomal dominant ET caused by a gain of function mutation in the *JAK2* gene R564Q and V617I produces a typical WHO-ET pictures of blood and bone marrow without features of PV[151-153] and do present with typical manifestations of the SPS[116,117]. Etheridge *et al*[151] and Mead *et al*[152] described a novel germline mutation JAK2^{V617I} in a family with autosomal dominant HET. Peripheral blood and bone marrow histology are consistent with WHO-ECMP defined ET (Figure 11)[151,152]. The authors demonstrated that JAK2^{V617I} is the sole driver in JAK2^{V617I}-positive individuals with typical peripheral blood and bone marrow features of WHO normocellular ET and completely normal values for haemoglobin, haematocrit, erythrocytes plasma TPO and serum EPO. After stimulation with granulocyte colony-stimulating factor (G-CSF), TPO and EPO of peripheral blood CD33+ myeloid and CD34^{+}stem and progenitor cells showed significant differences in congenital JAK2^{V617I} and acquired JAK^{V617F} mutated cells as compared to controls. The response to G-CSF was increased in congenital JAK^{V617I} and more prominent in acquired JAK2^{V617F} mutated HSC. In signalling and transcriptional experiments assays, congenital JAK2^{V617I} showed more activity than wild type acquired JAK2, but substantially less than JAK2^{V617F}. The responses to TPO were equal in congenital JAK2^{V617I} and acquired JAK2^{V617F} but the response to EPO was normal in congenital JAK^{V617I} and increased in acquired JAK2^{V617F}. These findings confirm the hypothesis of Vainchenker that heterozygous congenital JAK2^{V617I} mutation induces sufficient cytokine hyperresponsiveness of the HSC to TPO for the induction of a homogeneous ET phenotype in blood and bone marrow without features of PV during long term follow-up[152].

A novel heterozygous JAK2^{R564Q} mutation has been identified in another family with autosomal dominant HET[153]. The growth promoting effects of congenital JAK2^{R564Q} were much milder than those of acquired JAK2^{V617F} mutation. The authors found higher levels of STAT1 and STAT3 in cells expressing JAK2^{V617F}, compared to JAK2^{R564Q}. Total STAT1 levels were increased with JAK2^{V617F} and with JAK2^{R564Q} expression as compared to wild type JAK2 but this effect was more prominent with the somatic acquired JAK2^{V617F} mutation. An overall increase in downstream signaling in mutant JAK2^{R534Q} cells was further demonstrated by the upregulated tyrosine-phosphorylation of proteins in germline JAK2^{R564Q}-expressing cells as compared to wild type JAK2, and this was even more robust in the acquired JAK2^{V617F}-expressing somatic mutants. Similar increased signaling was observed in JAK2^{R564Q}-positive patients by the demonstration that increased phosphorylation of JAK2 protein in platelets isolated from 3 members of the family with the congenital JAK2^{R564Q} mutation as compared to a JAK2 wild type family members[153]. In the absence of TPO, and at all concentrations of TPO, the growth characteristics of congenital JAK2^{R564Q}-expressing cells showed significantly increased proliferation, compared to JAK2 wild type cells, but this was much less striking than with acquired JAK2^{V617F} mutated cells thereby explaining why the heterozygous germline JAK2^{R564Q} mutation is associated with ET without PV features[153].

JAK2 wild type MPL^{515} mutated ET

The first case of congenital ET due to a gain of function mutation in the *cMPL* gene has been described in 2004[154]. This has led in 2006 to the discovery of the MPL^{W515L} and MPL^{W515K} mutations as the driver cause of clonal MPN in JAK2 wild type ET and myelofibrosis by Pardanani *et al*[155] and Pikman *et al*[156] in the United States[149,150]. Three studies describe MPL^{W515L} and MPL^{W515K} mutations as the cause of clonal ET and myelofibrosis without features of PV (Table 9)[155,156]. Within the JAK2 wild type MPN, the prevalence of the MPL^{5155} mutation as the cause of ET (Table 9) is 3% in the Vannucchi study[157], and 8.5% in the United Kingdom studies[157,158]. In the study of Vannucchi *et al*[157], patients with JAK2 wild type ET carrying the MPL^{515} mutation present with typical Sticky Platelet Syndrome[116,117] 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 EEC[157,158]. The bone marrow is featured by pronounced megakaryopoiesis with large and giant megakarocytes and no increase of erythropoiesis[156-159]. In 2008 we studied bone marrow histopathology in 12 cases with JAK2 wild type ET carrying the MPL^{515} mutation kindly provided by the courtesy of Dr. Vannucchi, Florence, Italy[78]. Bone marrow histology from patients with JAK2 wild type ET carrying the MPL^{515} mutation consistently displayed clusters small and large megakaryocytes with a greater number of giant megakaryocytes with hyperlobulated stag-horn nuclei in a normal cellular bone marrow and no increase of erythropoiesis (Table 10). As compared to JAK2^{V617F} mutated ET in Figure 16, bone marrow histology of our case with MPL^{515} mutated ET is shown in Figure 17. The comparison of bone marrow histopathology findings in patients with normocellular

JAK2^{V617F} mutated ET (Figures 11 and 16) *vs* JAK2 wild type ET carrying the MPL^{515} mutation (Figure 17) show were significant differences on three points[78]. The megakaryocytes in MPL^{515} mutated ET are larger with hyperlobulated staghorn-like nuclei as compared to the pleomorphic megakaryocytes morphology in JAK2^{V617F} mutated ET and PV. Second, there was local increase of erythropoiesis in areas of loose clustered pleiomorphic megakaryocytes in JAK2^{V617F} mutated ET, but not in JAK2 wild type PT carrying the MPL^{515} mutation (compare Figures 16 and 17). Third, we observed increased reticulin fibers grade 2 in a normocellular bone marrow in areas of dense clustered megakaryocytes, which is not seen in JAK2^{V617F} mutated normocellular ET, hypercellular prodromal PV and EMGM[77]. Whether such differences in bone marrow histology and megakaryocyte morphology between normocellular ET with low JAK2 mutation load and normocellular JAK2 wild type ET carrying the MPL^{515} mutation can be seen by expert hematopathologists in large series of WHO-ET patients remains to be evaluated in prospective clinical and basic research studies.

PMF or AMM: From Silverstein to Tefferi

In 1975 the PVSG defined the criteria for PV and for both primary hemorrhagic thrombocythemia and PMF or AMM as the second and third variant of MPD[8]. In 1977 Silverstein[160] updated the spectrum of PVSG defined PTH *vs* AMM. AMM or PMF is a clinico-pathological entity not preceded by any other PVSG defined MPD ET, PV, CML, or preleukemia (MDS)[8,160] and characterized by various degrees of anemia, splenomegaly, leukoerythroblastosis, with tear drop-shaped erythrocytes, and dry tap on BM aspiration due to various degrees of MF or osteomyelofibrosis. PMF or AMM patients are usually of age between 50 to 80 years, have enlarged spleens, a leukoerythroblastic blood reaction, striking teardrop poikilocytosis and dry tap on bone marrow aspiration. In the studies of Thiele and Spivak the mean age of advanced PMF or AMM is above 60 and around 70 years[161,162]. According to our experiences as documented in 1992[31] this clearly indicate that masked MPD preceding PMF must have been overlooked for 10 to 15 years as the consequence of extremely crude criteria for AMM (PMF) of anemia, splenomegaly and myelofibrosis. According to Silverstein *et al*[160] a typical AMM bone marrow is fibrotic in most cases, hypocellular in 85%, normocellular in 5% and hypercellular in 10%. Anemia due to ineffective erythropoiesis developed in about 60% of AMM patients within 5 to 10 years and thrombocytopenia and leuko-penia related to hypersplenism was seen in 30% and 14% of AMM patients[160]. The diagnostic criteria PVSG defined PMF or AMM have not been changed by the 2001 WHO using the term CIMF[75] and the 2008 WHO using the term PMF[76].

In the 2012 Tefferi *et al*[163] updated one thousand consecutive patients with PMF seen at Mayo Clinic between November 4, 1977, and September 1, 2011. The international prognostic scoring system (IPSS), dynamic IPSS (DIPSS), and DIPSS-plus were applied for retrospective risk stratification. Separate analyses were included for patients seen at time of referral (n = 1000), at initial diagnosis (n = 340), and within or after 1 year of diagnosis (n = 660). Anno 2012, 592 deaths and 68 leukemic transformations (6.8%) have been documented. Parameters at initial diagnosis *vs* time of referral included median age (66 years *vs* 65 years), male sex (61% *vs* 62%), red cell transfusion need (24% *vs* 38%), hemoglobin level less than 10 g/dL (38% *vs* 54%), platelet count less than 100× 10^9/L (18% *vs* 26%), leukocyte count more than 25 × 10^9/L (13% *vs* 16%), marked splenomegaly (21% *vs* 31%), constitutional symptoms (29% *vs* 34%), and abnormal karyotype (31% *vs* 41%). Retrospective screening for mutational frequencies anno 2012 were 61% for JAK2^{V617F}, 8% for MPLW515, and 4% for IDH1/2. DIPSS-plus risk distributions at time of referral were low in 10%, intermediate-1 in 15%, intermediate-2 in 37%, and high in 37%. The corresponding median survivals of DIPSS-plus low, intermediate 1 and 2 and high were 17.5, 7.8, 3.6, and 1.8 years *vs* 20.0, 14.3, 5.3, and 1.7 years for patients younger than 60 years of age. Compared with both DIPSS and IPSS, DIPSS-plus showed better discrimination among risk groups. Five-year leukemic transformation rates were 6% and 21% in low- and high-risk patients, respectively. Tefferi *et al*[163] concluded in 2012 that prognosis in MF should be assessed after a period of clinical observation after diagnosis rather than at diagnosis. It seemed that about 50% are to be treated conventionally, about 25% are candidates for allogenic stem cell transplant and roughly 20% can be treated with a JAK2 inhibitor (Ruxolitininb)[163].

JAK2/MPL-negative CALR mutated ET and MF: From Tefferi to Green and Kralovics

The molecular etiology of JAK2/MPL wild type ET and MF remained elusive untill two groups independently discovered the calreticulin (CALR) mutations in MPN patients with nonmutated JAK2[164-166]. Klampf *et al*[164] 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. CALR mutations are mutually exclusive with both JAK2^{V617F} and MPL^{515} mutations: 195 (67%) of 289 JAK2 wild type ET and 105 (80%) of 120 195 carried one of the CALR mutations. In 150 patients with the CALR mutation for whom matched T-lymphocyte DNA was available, the CALR mutations were somatic. The CALR mutation was not found 45 CML, 73 MDS, 64 chronic myelomonocytic leukemia (CMML) and 24 RARS-T patients except that 3 SF3B1 positive RARS-T patients carried a CALR mutation. Klampf *et al*[164] detected a total of 36 types of somatic mutations (insertions and deletions) in exon 9 of the *CALR* gene encoding the C-terminal amino acids of CALR protein. Only 3 patients were homozygous. Among 1235 ET and MF patients 63.4%, 4.4% and

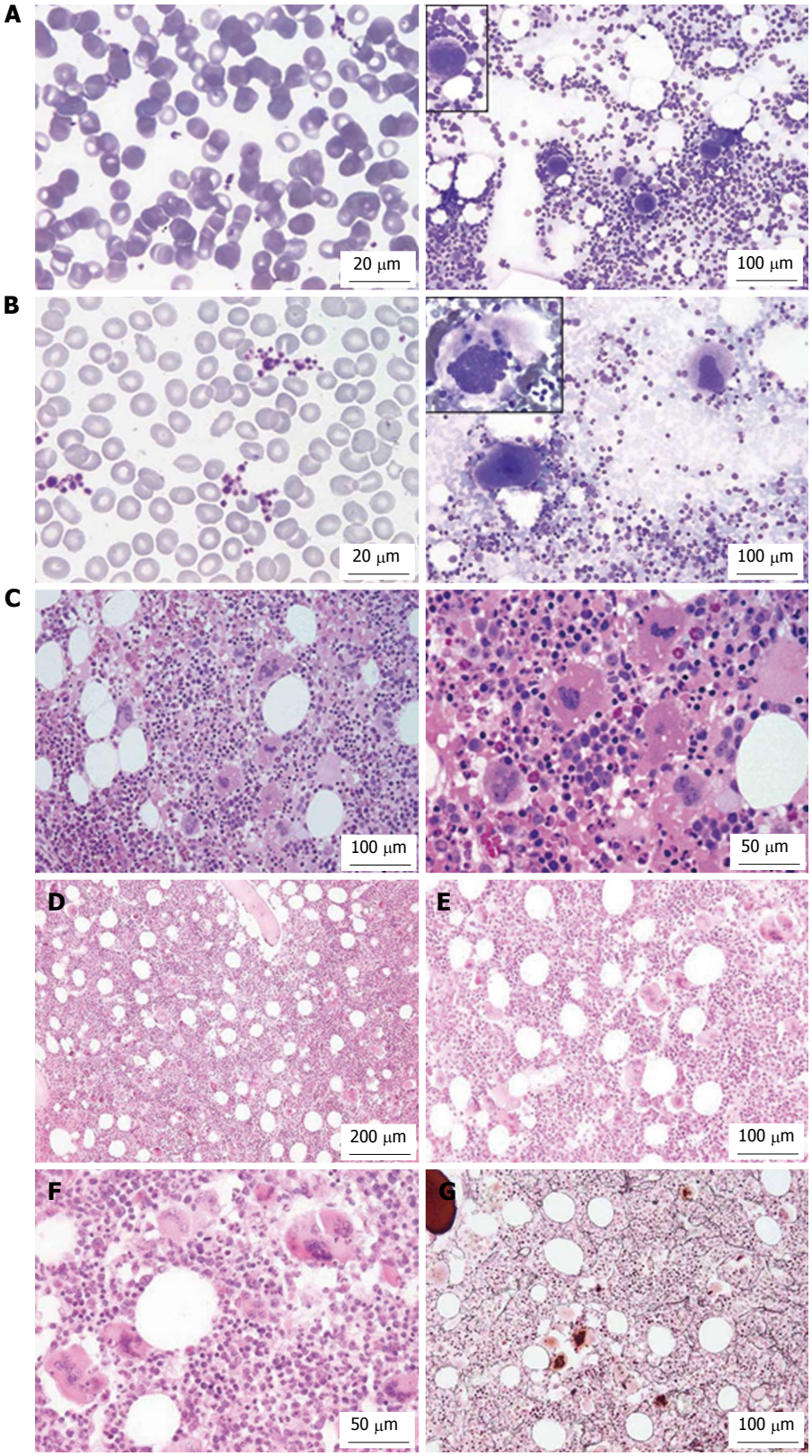


Figure 16 JAK2^{V617F} positive hypercellular essential thrombocythemia (platelets 453 × 10^9/L) in a case of portal vein thrombosis[94]. Large platelets in peripheral blood smear (B left) as compared to control (A left), bone marrow smear with large megakaryocytes with multilobulated nuclei (B right) as compared to control (A right) and hypercellular bone marrow due to increased erythropoisesis and granulopoiesis with pleomorphic megakaryocytes (C-E) similar as in typical polycythemia vera case 9 (F) with a hypercellular bone marrow picture due to increased erythropoiesis and granulopoiesis and slight increase of reticuline fibers grade 1 (G).

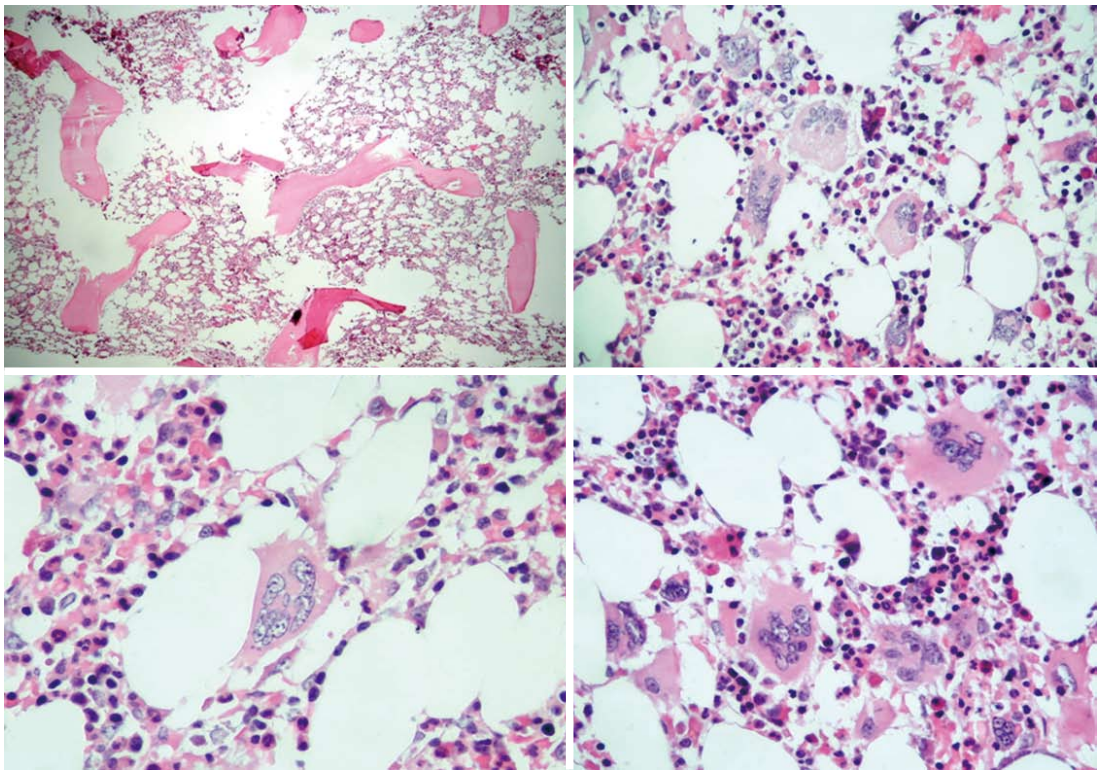


Figure 17 Bone marrow histology in normocellular essential thrombocythemia carrying the MPL^{W515L} mutation showing giant megakaryocytes with hyperlobulated stag-horn like nuclei charateristic for essential thrombocythemia the MPL^{W515L}/K mutation.

23.5% carried the JAK2^{V617F}, MPL^{515} and CALR mutation respectively, and in 8.8% none of these clonal markers (triple negative) was detected[164]. Life expectance was significantly longer in CALR mutated MF patients as compared to those with a JAK2^{V617F} or MPL^{515} mutation but the mean age at diagnosis of CALR mutated MPN patients was about 10 years younger than the JAK2V617F mutated MPN patients[164,165]. Evolution into MF during very long term follow up was equally high in CALR mutated ET as in JAK2 mutated PV (about 20% after 20 years follow up). CALR muated MPN patients had higher platelet counts, normal to low normal hemoglobin and white blood cells counts and a lower incidence of major thrombotic events simple because ist lacks PV features[164,165]. Nangalia *et al*[166] in the United Kingdom 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. 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 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. Patients with CALR mutations in the UK study had a significantly higher incidence of transformation of ET to MF than did those with JAK2 mutation (P = 0.03 Fisher's exact test)[166]. No CALR mutations were found in control samples, lymphoid cancers, solid tumors, or cell lines[111]. All CALR mutations identified in 148 patients were indels with 19 distinct variant: 14 deletions, 2 insertions and 3 complex indels, which generated a +1 base-pair frameshift, which result in a mutant protein with a novel C-terminal with the consequence that a large proportion, or almost all negatively charged amino acids and calcium binding sites are lost. More than 80% of the more than 30 identified indels involved either the type Ⅰ 52-bp deletion or the type Ⅱ 5-bp insertion. Patients with rare homozygous CALR mutation (all with the 5-bp insertion) were also identified[167]. The mechanism by which the CALR gain of function mutation selectively drives the neoproliferation of megakaryopiesis and granulopoiesis and not erythropiesis is unexplained and under investigation[167].

In the retrospective study from the Mayo Clinics Rochester United States of 254 evaluable WHO-defined MF patients the JAK2-, MPL- and CALR-mutations were detected in deep freeze sample in 58%, 8.3% and 25% respectively, and 8.7% were triple negative[168]. The retrospectively calculated median overall survival durations of 83 CALR-, 21 MPL-, and 147 JAK2-mutated MF cases and in 22 triple negative MF cases were 8.2, 4.1, 4.3 and 2.5 years respectively. As compared to CALR wild type MF, CALR-mutated MF patients were younger, had higher platelet count, lower leukocyte count, were less anemic with lower DIPSS-plus score. CALR-mutated MF patients had a favorable impact on median survival as compared to CALR-negative MF patients whether the addional sex combs like 1 (ASXL1) loss-of function mutation is negative or positive. The

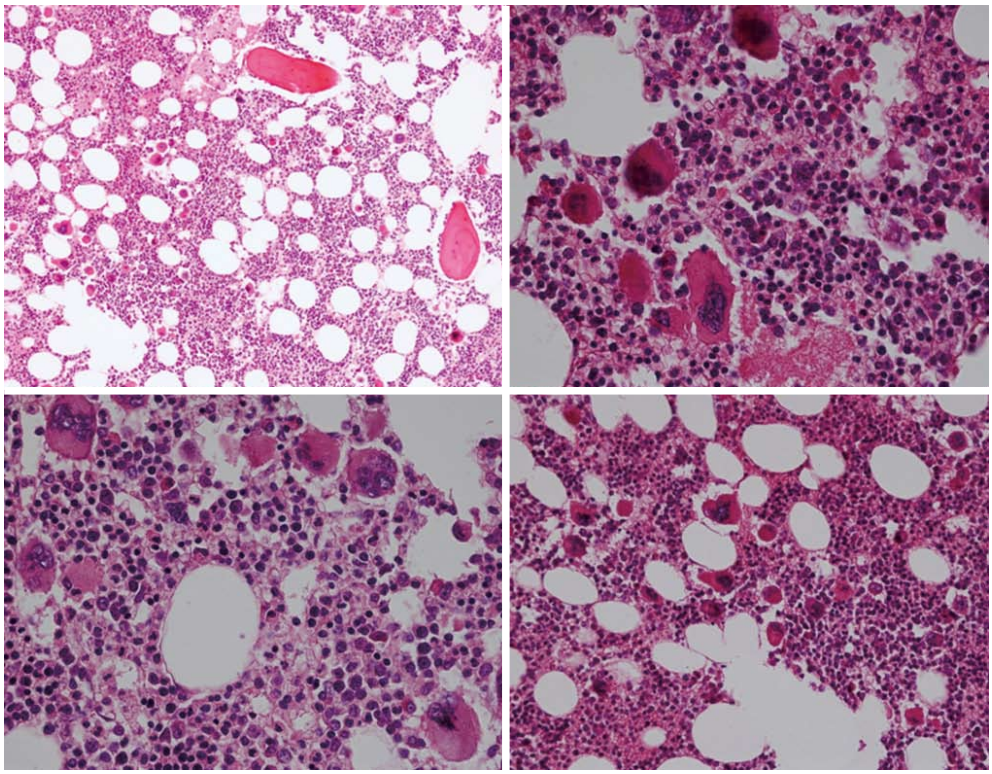


Figure 18 Clinical case of JAK2/MPL negative and calcireticulin positie essential thrombocythemia who present with aspirin responsive platelet thrombophilia, normal values for hemoglobin. Hematocrit and erythrocytes, platelet count of 1832 × 10^9/L and slight splenomegaly (16 cm lenght diameter on echogram). 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 primary megakaryocytic granulocytic myeloproliferation (Table 10) bone marrow.

median overall survival was 2.3 years in 55 CALR-negative/ASLX1-positive as compared to 5.6 years in 126 CALR-negative/ASXL1-negative MF patients. 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[168].

PMGM bone marrow histology in JAK2 wild type CALR mutated ET and MF

The bone marrow histology in 6 consecutive newly diagnosed CALR mutated ET and early MF revealed a typical PMGM picture showing dysmorphic megakaryocytes with definite abnormalities of maturation with bulky (bulbous) hyperchromatic nuclei and some disturbances of the nuclear cytoplasmic ratio (Table 11)[77,78], which are not seen in JAK2 wild type MPL^{515} mutated ET also not in prefibrotic JAK2^{V627F} mutated ET, ET/PV, EMGM[77,78]. Bone marrow findings in JAK2/MPL wild type PMGM are consistent with hypercellular ET with pronounced thrombocythemia as the presenting feature of CMGM described by Georgii *et al*[35,36] (Table 4). These features CMGM or PMGM are similar to those reported by Thiele as prefibrotic PMF in the 2008 WHO classification[97-100]. Representative bone marrow histology findings of typical cases of CALR positive ET (Figure 18) and MF (Figure 19) show dense cluster of immature megakaryocytes with the typical picture of CIMF according to 2001 WHO[75] and PMF according to 2008 WHO classification[76]. The finding of CALR mutation as the driver mutation has been confirmed in Belgium in 40 of 64 JAK2 wild type MPN (ET or MF) and 24 MPN cases were JAK2/MPL/CALR triple negative. The clinical presentation, laboratory and molecular findings and the bone marrow histology features are under investagation and will be compared with 50 cases of JAK2^{V617F} mutated ET, PV and EMGM.

2015 WHO-CMP criteria for classification and staging of MPN

The 1986 PVSG defined ET overlooks the ECMP and WHO-CMP defined ET with platelet count between 400 to 600 × 10^9/L, which comprises 30% of very early stage PV in various MPNs[169]. In 2008 the WHO reduced the minimum platelet count required for the diagnosis of ET to 450 × 10^9/L[76]. The 2015 WHO-CMP criteria clearly define JAK2^{V617F} positive normocellular ET prodromal PV, prefibrotic classical PV, early fibrotic PV, PV complicated by myelofibrosis, significant myeloid metaplasia of the spleen (splenomegaly and related constitutional symptoms) (Table 11, Figure 20). Within the JAK2^{V617F} MPN entities, the JAK2^{V617F} positive hypercellular ET is associated with clustered pleomorphic megakaryopoiesis (not cloud-like), increased granulopoiesis and relative decrease of erythropoiesis (EMGM, masked PV). The EMGM entity or masked PV is situated clearly between the normocellular ET and post-ET myelofibrosis carrying the JAK2^{V617F} mutation (Figure 20)[77,78]. Campbell *et al*[170] assessed the clinical features in the cohort of 806

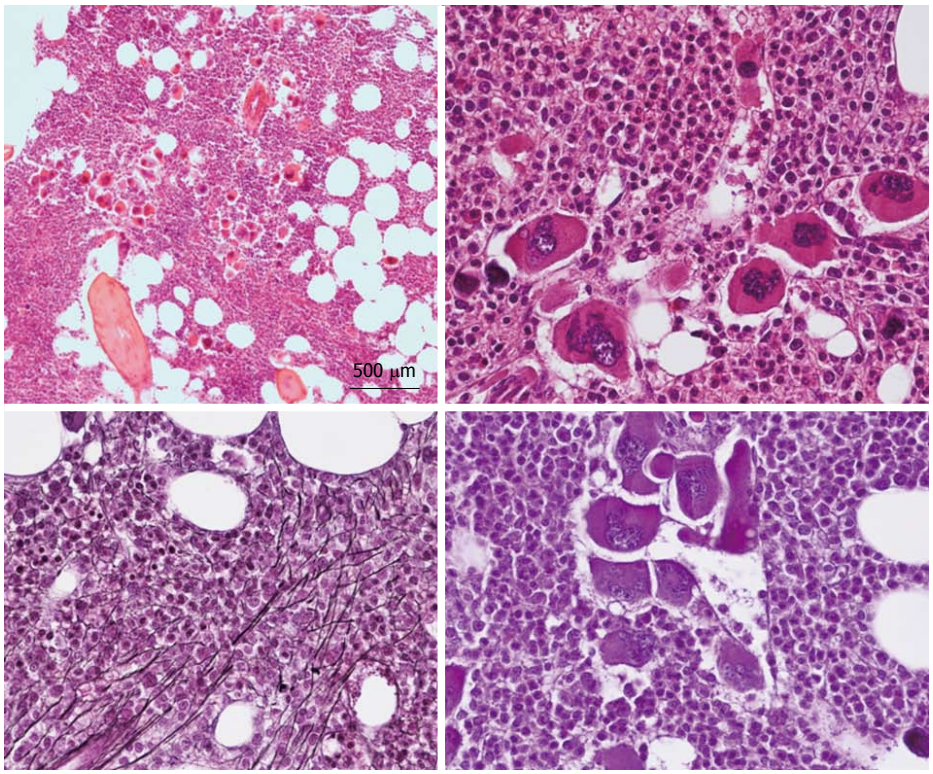


Figure 19 Clinical case of calcireticulin positive myelofibrosis: Hemoglobin 11.2 g/dL, hematocrit 0.33, leukocytes 9.2 × 10^9/L, platelets 347 × 10^9/L, lactodehydrogenase 1393 U/L, and the presence of tear drop erythrocytes, poikolocytosis and polychromasie in a peripheral bloodsmear, and hypercellular bone marrow with relative decrease of erythropoisis, dense cluster of immature megakaryocytes with hypolobulated nuclei consistent, and reticulin fibrosis grade 2 consistent with bone marrow histology features similar to World Health Organization-defined primary myelofibrosis, but distinct from JAK2^{V617F} mutated essential thrombocythemia and polycythemia vera, and distinct from MPL^{515} mutated essential thrombocythemia (Figures 17).

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[171]. JAK2^{V617F} positive ET patients had multiple features of PV with significantly higher hemoglobin, lower serum EPO and ferritin, higher neutrophils, bone marrow erythrocytosis and granulocytosis, more venous thrombosis and a higher rate of polycythemic transformation. PVSG defined JAK2 wild type ET had significant higher platelet counts (962, range 668-1535 × 10^9/L) than JAK2^{V617F}-positive ET (846, range 632-1222 × 10^9/L). In the PT-1 study, bone marrow trephine of 209 JAK2^{V617F} positive and 184 JAK2 wild type ET was independently assesed by 3 hematopathologists who did not know the JAK2 mutation status[171]. The overall cellularity was significantly increased in JAK2^{V617F} mutated ET, indicating at least in part of them an increased erythroid and/or granulocytic cellularity, which are features of PV or EMGM (masked PV).

Pich *et al*[172] prospectively analyzed histological changes in diagnostic bone marrow biopsy from 2006-2010 of 103 newly diagnosed 2008 WHO defined ET patients. Bone marrow features in 44 JAK2 wild ET cases revealed prominent clusters of large megakaryocytes with stag-horn nuclei, less micromegakaryocytes and much less erythroid hyperplasia similar to normocellular "true" ET and hypercellular "false" ET (Figure 17). In contrast, 59 JAK2^{V617F} positive ET patients (Figure 16) revealed a PV phenotype with higher hemoglobin, hematocrit, erythrocytes and bone marrow features with increased cellularity frequently some hyperplasia of erythroid and myeloid lineages and pleomorphic megakaryocytes very similar as in WHO-ECMP defined ET and PV. The mean and median JAK2^{V617F} mutation burden in WHO-ECMP defined ET was 14.4% and 8.7% respectively. Interestingly LDH (604 + 132) and spleen size (15.4 + 4.9) in 16 cases with a JAK2^{V617F} mutation load above 12.5% were significantly increased as compared to normal LDH (386 + 94) and normal speen size (11.2 + 2.1) in 37 cases with a JAK2^{V617F} mutation load below 12.5%[172].

The 2015 WHO-CMP classification and staging of patients with MPN will be very helpful in predicting and documenting prospectively the natural history of JAK2^{V617F} mutated ET, PV and EMGM patients (Table 11), *vs* MPL^{515} mutated ET, *vs* JAK2 wild type CALR mutated ET and MF associated with PMGM (Figure 20). The primary involvement of basic researchers, laboratory scientists, molecular biologists and clinicians as well as pathologists are essential to document the natural history at the clinical molecular and bone marrow level to demonstrate that scrutinized and integrated clinical, laboratory, molecular and pathological approaches and intense communications amongst clinicians, molecular biologists and pathologists are warranted in prospective diagnostic and managements studies. The 2015 WHO-

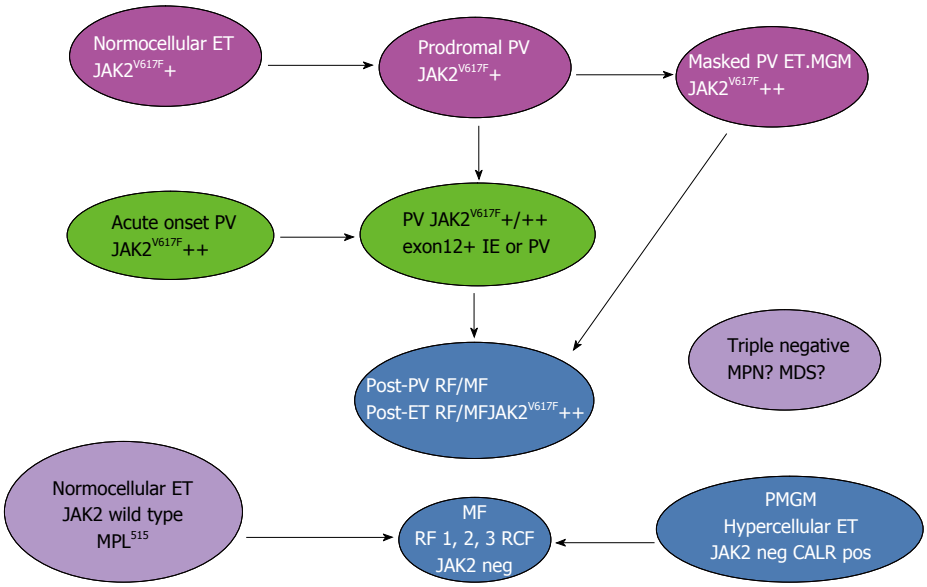


Figure 20 World Health Organization-clinical molecular and pathological myeloproliferative neoplasms classification and transitional states. ET: Essential thrombocythemia; PV: Polycythemia vera; MGM: Megakaryocytic granulocytic myeloproliferation; IE: Idiopathic erythrocythemia; RF: Reticulin fibrosis; MF: Myelofibrosis; RCF: Reticulin collagen fibrosis; CALR: Calreticulin; PMGM: Primary megakaryocytic granulocytic myeloproliferation.

CMP criteria surely will have important implications in choosing proper targeted treatment options for the management and prevention of thrombotic and bleeding complications and serious complications of progressive MPN disease burden in prodromal PV and overt PV (Table 11). Proper staging of PV in terms of JAK2^{V617F} mutation load, and MPN disease burden including splenomegaly, constitutional symptoms including itching, bone marrow histology and grading of myelofibrosis is of huge importance since it has significant implications for a non-leukemogenic or the least potential leukemogenic treatment options in low, intermediate and high risk PV patients (Table 11)[77,78]. A primary rigid venesection regimen aiming at a hematocrit around and below 0.40 seems to us better than the target of < 0.45 in males and < 0.42 in females on top of low dose aspirin for the control of activated platelets in MPN. According to our extended experiences, this strategy in stage zero, 1 and 2 PV patients (Table 11) will reduce the cumulative incidence of minor and major thrombosis from above 50% to less than 2% per patient/year during long-term follow-up.

The rational for using IFN-α as the first-line treatment option in newly diagnosed PV-patients include its effectiveness to abate constitutional symptoms and to induce a complete remission, thereby avoiding phlebotomy, iron deficiency and macrocytosis associated with hydroxyurea[173-176]. Clinicians will be reluctant to postpone the use of hydroxyurea as long as possible or even life long in early stage PV[93,116]. Two studies show IFN-induced complete hematological responses within one year, and major molecular responses (MMR) were reached after a follow-up of 2 to 3 years in PV and ET patients[173,174]. The cumulative incidence of MMR was 14% at 2 years and 30% at 4 years follow-up in the study of Kiladjian *et al*[175]. Peglyated IFN-α-2a reduced the median JAK2-allele burden from 45% to 5% in 37 PV patients in the study of Kiladjian *et al*[175] and from 64% to 12% in79 PV and ET patients in the study of Quintás-Cardama *et al*[176]. Larsen *et al*[177] demonstrated that a complete molecular response may be reached, which was associated with normalization of bone marrow histology. MPN patients and their physicians should be cautious and attentive not to become too enthusiastic since the use of pegylated IFN-α-2a or -2b may be associated with significant side effects in about one third of PV patients[93,116]. We do know that a significant proportion of around 50% of early and intermediate stage PV patients are responsive to IFN with no minor or bearable side effects[116]. The misconception in the past was to start with too high dosages of IFN[93,173]. According to current insights, low dose pegylated IFN is the treatment of choice in intermediate stage PV patients and high risk PV in terms of high JAK2^{V617F} allele burden. Patients with progressive MPN disease, splenomegaly and constitutional symptoms are candidates for myelosuppressive (hydroxyurea) or myeloreductive (JAK2 inhibtors) treatment (Table 11)[173-180]. MF transformation of thrombocythemia in MPN of various molecular etiology has to be distinguished from the expansion of one dominant homozygous subclone, the selective advantage of which is likely to reflect additional cytogenetic, genetic or epigenetic lesions (Table 12). Such additional, acquired background biological factors on top of the JAK2, MPL and CALR driver causes of MPN will become of huge importance for the understanding of differences in prognosis and outcome.

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