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MYELOPROLIFERATIVE DISEASES

Clinical, pathological and molecular features of the chronic myeloproliferative disorders: MPD 2005 and beyond

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Abstract

The combined use of bone marrow histopathology, biomarkers and clinical features has the potential to diagnose, stage and distinguish early and overt stages of ET, PV and idiopathic myelofibrosis, that has an important impact on prognosis and treatment of MPD patients. As the extension of the PVSG and WHO for ET, PV and agnogenic myeloid metaplasia (AMM), a new set of European clinical and pathological (ECP) criteria clearly distinct true ET from early or latent PV mimicking true ET, overt and advanced polycythemia vera (PV), and from thrombocythemia associated with prefibotic, early fibrotic stages of chronic megakaryocytic granulocytic metaplasia (CMGM) or chronic idiopathic myelofibrosis (CIMF). Cases of atypical MPD and masked PV are usually overlooked by clinicians and pathologists. Bone marrow biopsy will not differentiate between post-PV myelofibrosis versus so-called classical agnogenic myeloid metaplasia. The recent discovery of the JAK2 V617F mutation can readily explain the trilinear megakaryocytic, erythroid and granulocytic proliferation in the bone marrow, but also the etiology of the platelet-mediated microvascular thrombotic complications at increased platelet counts and red cell mass in essential thrombocythemia and polycythemia vera.

Introduction

In the 1980s and 1990s, the German pathologists Burkhardt et a1. Georgii et al. [2–4] and Thiele et al. [5–10] have described typical histopathological features from bone marrow biopsy marterial for the diagnosis and classification of each of the 3 different Ph-negative MPDs ET, PV and AMM. In 1997, we introduced histopathology from bone marrow biopsy as a diagnostic clue and pathognomonic feature of each of the MPDs ET, PV and megakaryocytic granulocytic myeloid metaplasia (CMGM) [11–13]. Thiele & Kvasnicka extended the Rotterdam criteria for ET, PV and added the Cologne criteria for MGM or chronic idiopathic myelofibrosis (CIMF) [14,15]. This concept was taken over by the WHO criteria for true ET, classical PV, and prefibrotic and fibrotic stages of MGM or CIMF [16]. To overcome the shortcomings of the PVSG and WHO criteria, a new set European clinical and pathological (ECP) criteria for the diagnosis ET, PV and CIMF was proposed by Michiels and Thiele [17], which allows to differentiate between the early stages of ET, PV and MGM, to detect prefibrotic and various degrees of fibrosis in PV and MGM and to classify the early, overt and advanced stages of PV and MGM or CIMF, that have major prognostic and therapeutic implications.

Diagnosis and classification of the myeloproliferative disorders: MPD

The inclusion and exclusion criteria for the diagnosis of true ET according to WHO [55] and ECP [17] are identical except platelet count of $>600 \times 10\ l^{-1}$ according the WHO, and $>400 \times 10^9\ l^{-1}$ according the ECP (Table I).

A typical bone marrow picture for true ET affects mainly of the megakaryocytic cell lineage and shows increased numbers of loosely clustered enlarged, mature megakaryocytes with hyperploid staghorn-like nuclei together with normal cellularity, erythropoiesis, and granulopoiesis, no increase of reticulin fibrosis, and there is no peripheral blood or bone marrow and cytogentic evidence of CML, PV, MGM, CIMG, MDS or reactive thrombocytosis.

The Rotterdam criteria of PV proposed by the Thrombocythemia Vera Study Group (TVSG) extend the PVSG criteria by including histopathology from bone marrow biopsies (Figure 1) [14].A typical

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Table I. The Rotterdam, the ECP and the WHO criteria for the diagnosis of true ET

Platelet count	$>400\times10^{9}\,l^{-1}$	Rotterdam & ECP
	$>600\times10^{9}\,l^{-1}$	WHO
Bone marrow	Increase of dispersed or loosely clustered, predominantly enlarged mature mega-karyocytes with hyperlobulated nuclei and mature cytoplasm, normal cellularity, no or borderline increase of reticulin	Rotterdam & WHO & ECP
	No proliferation or immaturity of granulo- or erythropoiesis	ECP
Exclusion	No peripheral blood, bone marrow and cytogenetic evidence of PV, CML, CIMF, MDS or reactive thrombocytosis	Rotterdam & WHO & ECP

picture in the bone marrow diagnostic for classical PV is featured by increase of clustered enlarged mature megakaryocytes comparable to ET, and a moderate to marked increased cellularity, erythropoiesis and granulopoiesis, the so-called trilinear myelopriliferation [16,17]. The megakaryocytes in PV may have a rather pleiomorphic appearance with wide ranges of sizes including small and giant forms. In our experience a typical PV picture of the bone marrow is seen in classical PV, in erythrocythemia or idiopathic erythrocytosis, in early or latent PV and in masked PV [14–22]. The combination of a typical PV picture, increased red cell mass, high hematocrit and one of the B criteria is consistent with classical PV according the WHO [16] and PVSG criteria. A typical PV picture of the bone and increased red cell mass, high hematocrit but normal platelet count and spleen size is consistent with stage 1 erythrocythemia, is not consistent with PV according to WHO and will be overlooked by the PVSG criteria. A typical PV bone marrow picture with normal red cell mass and hematocrit but with increased platelet count is consistent with ET [13]. This prompted Thiele to define this entity as early (latent) PV (Figure 1 bottom right) [14]. In our experience, early or latent PV usually presents with microvascular disturbances at platelet count in excess of $400\times10\,l^{-1}$, increased LAF score, low serum EPO, spontaneous EEC and no or slight splenomegaly. The combination of a typical PV picture, normal red cell mass, normal platelet but slowly progresssive splenomegaly, granulocytosis or even slight anemia is not consistent with either ET, PV but with atypical or unclassifiable MPD or masked PV [13]. Such cases may present with thrombotic complications including splanchnic vein thrombosis (Budd-Chiari syndrome, portal, splenic or mesenteric vein thrombosis) [24,25], or masked PV [26,27], which all typically show spontaneous EEC as the clue to the atypical presentation of MPD. Such cases of atypical MPD and masked PV are overlooked by clinicians and pathologists and may comprises about one quarter of patients with initially a typical PV picture of the bone marrow and usually will progress to so-called classical CIMF without overt PV. At time of classical CIMF bone marrow biopsy will not differentiate between post-PV myelofibrosis versus so-called classical agnogenic myeloid metaplasia. In this regard, we reported a case of primary myelofibrosis with splenomegaly in 1971 in a 61-year old man [28]. The spleen size had progressed to 7 and 13 cm below the costal margin (18 and 23 cm length diameter on scan) respectively associated with progressive anemia increase of platelet count from normal to $811\times10\,l^{-1}$ and increase of leukocytes from normal to $24\times10\,l^{-1}$ during a follow-up period of 18 years. Sequential bone marrow biopsies showed normal cellularity, fine reticuline fibers grade 1 according to Baumeister [29], and an increase of clustered mature megakaryocytes with hyperploid nuclei in 1971, 1978 and 1982, but hypercellularity, coarse reticulin and collagen fibrosis (dry tap) and increased clustered megakaryocytes in 1985 and 1989 [28].

The Rotterdam Criteria of Polycythemia Vera Proposed by the Thrombocythemia Vera Study Group (TVSG)

A1 Raised red cell mass male >36 mL/kg Female >32 mL/kg	B1 Thrombocytosis Platelet count >400 × 10^9/L
A2 Absence of any cause of secondary erythrocytosis by clinical and laboratory investigations	B2 Granulocytes >10 × 10^9/L and/or raised neutrophil alkaline phosphatase score of >100 in the absence of fever or infection
A3 Histopathology of bone marrow biopsy increase of: a. cellularity, panmyelosis b. enlarged megakaryocytes with hyperploid nuclei; clusters of megakaryocytes c. reticulin fibers (optional)	B3 Splenomegaly on palpation or isotope/ultrasound scan
	B4 Erythroid colony formation in absence of EPO: spontaneous EEC

A1 + A2 + A3 is consistent with early stage PV (so-called "idiopathic erythrocytosis")
A1 + A2 + A3 + any one from category B establishes overt PV
A3 + B1 is consistent with essential thrombocythemia
A3 + B3 and/or B4 is consistent with a primary myeloproliferative disorder

Figure 1.

Bone marrow biopsy clearly differentiates between PV and congenital or secondary erythrocytosis with a sensitivity and specificity of more than 95% near to 100% as recently by Thiele et al. [18,19]. In congenital and secondary erythrocytosis, in which increased erythropoiesis is present, the number, size, morphology and distribution of megakaryocytes in bone marrow smears and biopsies remain normal [19,20,22].

The third category of chronic myeloproliferative disorders (CMPD) is usually termed agnogenic myeloid metaplasia or chronic idiopathic myelofibrosis (CIMF), but various other designations have been used such as primary myelofibrosis, myelofibrosis with myeloid metaplasia (MMM) etc [30–33]. AMM, CIMF or MMM is generally defined as a clinicopathological entity not preceded by any other MPD, CML or MDS and characterized by various degrees of anemia, splenomegaly, a leuko-erythroblastic blood picture with tear drop-shaped erythrocytes and various degrees of bone marrow fibrosis or osteosclerosis, and thus by definition disregarding the early non-fibrotic stage of the disease [16,30–33]. In the late 1970s and early 1980s Thiele et al. [5,6] and Georgii et al. [2] drew attention to an authentic dual megakaryocytic granulocytic myeloproliferation (CMGM) as a separate pathological entity among the MPDs. This condition has been labeled in 1996 as chronic megakaryocytic granulocytic myeloproliferation (CMGM) by Georgii et al.[4] and described in detail as prefibrotic CIMF by Thiele et al. [34–39] to distinguish this entity from ET. Prefibrotic CMGM or CIMF according to the Cologne [14,15], WHO [16] and ECP [17] criteria is a mixed proliferation of increased granulopoiesis and megakaryopoiesis without or with early fibrosis but dominated with immature giant megakaryocytes which are conspiciously enlarged due to increase of nuclear as well as cellular size with bulky and irregular roundish-shaped nuclei, so-called cloud-like nuclei is typical of CMGM (Table II) [34–39]. The prefibrotic and early fibrotic stages of CMGM or CIMF are frequently associated with pronounced thrombocythemia, without a leuko-erythroblastic blood picture, normal or increased LAF-score and no or minimal splenomegaly (Table II) [34–39], which according to the PVSG criteria are to be diagnosed as ET [38].

The semiqualitative grading of reticulin and collagen fibrosis has recently been improved and standardized. In each of the MPDs, myelofibrosis (MF) can be graded from 0 to 3 [40]. Myelofibrosis is not a feature of true ET according to the WHO or ECP. Very few ET patients will develop myelofibrosis during long-term follow-up [4]. Myelofibrosis is present in only a minority of PV patients at time of diagnosis, but all stages of myelofibrosis have been observed during long-term follow-up [43]. There is a conflict of opinion and observer disagreement with regard to the classification and natural history of prefibrotic and early CMGM or CIMF and its differentiation from true ET [41]. Those cases within prefibrotic CMGM or CIMF with slight maturation defect of enlarged megakaryocytes are featured by a rather slowly progressive myelofibrosis with slight splenomegaly or anemia and a life expectancy near to normal similar as in PV. Prefibrotic MGM or CIMF with slight dysmegakaryopiesis may precede classical CIMF for 5 to more than 10 years [42]. Discussions between clinicians and pathologists reveal that diagnostic differentiation between true ET and thrombocythemia as the presenting feature of prefibrotic CMGM (or CIMF) with slight maturation defect of enlarged clustered megakaryocytes and no or slight increased cellularity is subjective with a high inter-observer disagreement between pathologists.

Clinical, laboratory and pathological features of the MPDs in 2005 and beyond

Comparing the WHO/ECP with the PVSG criteria for the diagnosis of ET show that the PVSG criteria fail to distinguish ET from early PV mimicking ET and fail to distinguish ET from early stages of thrombocythemia associated with CMGM or CIMF (Table III) [42–44]. The diagnostic guidelines of the World Health Organisation (WHO) explicitly include bone marrow pathology as a positive criterion for the distinction of true ET, early and overt PV and prefibrotic or early fibrotic myeloid metaplasia or CIMF. In consideration of disease-related complications occurring at low platelet counts, the arbitrarily chosen limit for platelet count ($>600 \times 10^{-1}$) by the PVSG and WHO has been reduced to $400 \times 10[9]^{-1}$ by the European clinical and pathological (ECP) criteria for ET. The PVSG clinical criteria for ET, when compared to the WHO and ECP criteria, include true ET, early PV mimicking ET and thrombocythemia as the presenting feature of prefibrotic or early fibrotic MGM or CIMF (Table III) [42–44]. The ECP criteria clearly differentiate PV from SP, and ET from reactive thrombocytosis, initial PV and prefibrotic MGM or CIMF. The PVSG and the WHO criteria for the diagnosis of PV in Table IV used increased red cell as the main requisite, which is a very crude and overlook by definition early PV mimicking true ET (stage 0) and the erythrocythemic phase (stage 1) of PV, formerly labeled as idiopathic erythrocytosis. It became apparent that spontaneous endogenous erythroid colony formation (EEC) and PRV-1 expression are the hall mark of PV, and about 50% of ET patients are EEC positive or PRV-1 positive. The reports on EEC/PRV-1 positive ET according to the PVSG very likely represents early or initial stages of PV because of a typical PV bone marrow picture. The cohorts of early stage 0 and 1 PV

Table II. The Cologne, the WHO and the European clinical and pathological (ECP) criteria for the diagnosis and staging of MGM, CIMF or MMM-AMM

	Clinical criteria		Pathological criteria
A1	No preceding or allied other subtype of myeloproliferative disorders CML or MDS. Main presenting feature is pronounced thrombocythemia and no dry tap on bone marrow aspiration.	**B1**	Megakaryocytic and granulocytic myeloproliferation (MGM) and no or relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant to medium sized megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects.
C	Clinical stages	**MF**	**Staging of myelofibrosis (MF)**
C1	Early clinical stages		
	Normal hemoglobin or slight anemia, grade I: hemoglobin >12 g/dl	**MF-0**	prefibrotic stage CIMF: no reticulin fibrosis
	Slight or moderate splenomegaly on ultrasound scan or CT	**MF-1**	early CIMF: slight reticulin fibrosis
	Thrombocytosis, platelets in excess of 400, 600 or even $1{,}000 \times 10^{9-1}$		
	Normal or increased LAF-score		
	No leuko-erythroblastose		
C2	Intermediate clinical stage		
	Anemia grade II: hemoglobin >10g/dl	**MF-1**	early CIMF: slight reticulin fibrosis
	Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes Increased LDH	**MF-2**	manifest CIMF: marked increase in reticulin and slight to moderate collagen fibrosis
	Splenomegaly		
C3	Advanced clinical stage		
	Anemia grade III: Hemoglobin <10g/dl	**MF-3**	overt CIMF: advanced collagen fibrosis with optional osteosclerosis
	Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes		
	Splenomegaly, thrombocytopenia, leukocytosis, leukopenia		

The combinations of A1 + B1 establish CIMF-any other criterion confirms CIMF/AMM

and the overt stage 2 PV patients are featured by spontaneous EEC, positive PRV-1, low serum EPO levels and a typical PV bone marrow picture. The cohorts of early stage 0 and 1 PV and the overt stage 2 PV patients are featured by EEC and PRV-1 positivity, low serum EPO levels and a typical PV bone marrow picture (Table IV) [18–27]. Both the early (stage 0 and 1) and overt (stage 2) PV patients are at high-risk for potential minor and major vascular complications, because they present with elevated platelet count mimicking true ET [21–23]. About 50% of ET patients, diagnosed according to the PVSG criteria, have elevated levels of PRV-1 expression together with low serum erythropoietine (Epo) levels, and Epo independent erythroid colony formation (EEC) [45–48]. EEC/PRV-1 positive ET according to PVSG criteria is associated with a higher risk of developing microvascular and major thrombotic com-

Table III. Clinical, pathological and molecular features of true ET, initial PV mimicking ET and false ET when the PVSG and WHO/ECP criteria are compared

Diagnosis	Hereditary ET	True ET	Initial PV mimicking ET	ET-MGM Prefibrotic Early Fibrotic CIMF
Incidence (%)	<0.001	20–30	20–30	50–60
Serum EPO	Normal	Normal	Decreased ◆	Normal
Thrombocytes	◆/◆◆	◆/◆◆	◆/◆◆	◆/◆◆
Erythrocytes	N	N	*N/t*	
Hematocrit	N	N	N/*	N/#
Bone marrow:	ET picture	ET picture	PV picture	MGM picture
Cellularity myelopoise	N	N	◆/◆◆	◆/◆◆
Erythropoiesis	N	N		
Megakaryocytes				
Megakaryocytes	Normal	enlarged / giant and mature	megakaryocytes	abnormal
Clonality	polyclonal	poly/monoclonal	monoclonal	monoclonal
JAK2V617F	–	+	+	+ versus –
EEC	–	+	++	–
PVR-1		+	++	+

Table IV. Diagnosis of Poiycythemia Vera (PV): therapeutic implications

Poiycythemia Vera	Evolution		Manifestation	
Staging of PV New concept	ECPO Aspirin/ phlebotomy A/P	ECP 1 Aspirin/ phlebototm A/P	ECP 2 PVSG WHO A/P	ECP3 PVSG WHO IFN/HU
	initial PV mimicking ET	Erythrocythemic PV	Thrombo/erythro/leuko cythemic PV	
Estimated incidence (%)	20–25	20–25		40–60
Hemoglobin g/dl	N/*			
Serum EPO	◆			
Hematocrit	male 0.43– <0.51 Female0.42– <0.48	male >0.51 female >0.48		
Red cell mass	N			
Thrombocytes (x 10^9/l)	400–600 >600	<400	>400	>1,000
Leukocytes (x 10^9/l)	N	N	N	>15
Spleen on echogram (cm)	N-15	N	N-15	>15
Bone marrow:	PV picture	PV picture	PV picture	PV MF picture
JAK2V617F	+	+		++/LOH
EEC	+	+	+	+
PRV-1	+	+	+	+
LAF score				
Myelofibrosis (MF)	0	0	0/1	1/2

plication as compared to EEC/PRV-1 negative ET [47,48]. PRV-1-positive ET comprises a pathophysiologically distinct subgroup of ET patients that is at risk for the development of thrombotic complications and for emergence of PV that may reflect early or initial PV [48]. Early or initial PV according to WHO [16] and ECP [17] criteria is typically featured by a PV picture in the bone marrow, positive results for EEC and PRV-1, and/or low serum Epo levels, and a much higher thrombotic risk, which is related to increase of hypersensitive platelet counts (thrombocythemia) and slightly increased values for hematocrit up to 0.50, and therefore candidates for low dose aspirin and phlebotomy (Table II).

The clonal and molecular etiology of ET, PV and MGM

The MPDs represent clonal proliferation of the hematopoietic stem cells [49–53]. The clonal nature is found in nearly all PV and CIMF patients, while a variable proportion of ET patients are polyclonal. A key observation since 1974 [19] is the spontaneous growth of EEC as a hall mark of PV, but also found in about half of ET patients and in a proportion of CIMF patients [24–27,45,55]. Apart from spontaneous EEC, PV bone marrow cells are hypersensitive to insulin growth factor-1 (IGF-1) [56–58]. IGF-1 sensitivity ratios reached as high as 20.000 times the no and this level of cytokine hypersensitivity in the erythroid lineage of PV is specific to IGF-1. In addition, PV bone marrow cells are hypersensitivity but to a less extent, to other hematopoietic growth factors including IL-3, GM-CSF and SCF. Spontaneous endogenous megakaryocyte colonies (EMC) (CFU-Meg) in the absence of exogenous growth factors has been described in ET patients [59–62]. The median TPO sensitivity ratios were more than 50 times the normal and this was highly specific with respect to cytokine, disease and cell lineage, suggesting a lineage restricted hypersensitivity of hematopoietic progenitors to normal endogenous TPO in thrombocythemia (ET and early PV mimicking ET) [62]. In patients with AMM/CIMF clear evidence of hypersensitivity was found for SCF, a cytokine active in several different cell lineages [63]. These results prompted Axelrad to propose a model that the clinicopathological phenotypes of the clonal MPDS (ET, PV and CIMF) are related to, and perhaps determined by specific hypersensitivities of their progenitor cells to normal endogenous cytokine: EEC-IFG-1 hypersensitivity for PV, TPO-hypersensitivity for thrombocythemia (ET and early PV mimicking ET) and GM-CSF and SCF hypersensitivity for granulocytosis in PV and for CIMF/AMM respectively. In the studies of Pahl et al. PVR-1 gene expression has been detected in nearly all PV and in about half of the ET patients, who also demonstrated spontaneous EEC, and there was a near to 100% concordance between EEC and PRV-1 positivity in PV and ET patients as well as EEC negativity and normal PRV-1 expression in ET patients [64–67]. The EEC/PRV-1 positive disease may present clinically as an ET (early PV mimicking PV), that will develop into PV, as PV or as a variant of CIMF having quickly passed the hypercellular polycythemic stage (Tables III and IV).

A typical PV picture of the bone marrow:
Classical PV
Hematocriet (ht) > 0.51 male or > 0.48 female and increased platelet count >400
Idiopathic erythrocythemia
Increased ht, normal platelets and spleen
Early PV mimicking ET
Normal hematocritand increased platelets
Masked PV
Splenomegaly, leukocytosis, normal hematocrit and platelets (PVSG <600)

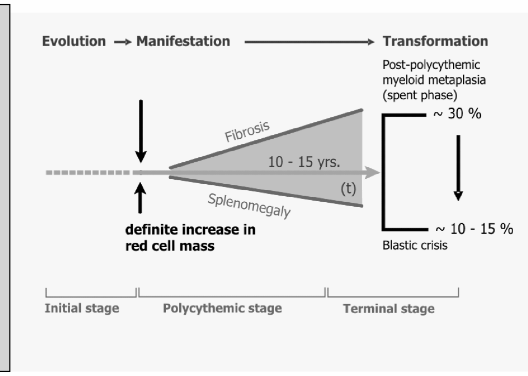

Figure 2.

The discovery of JAK2 V617F gain of function mutation by Vainchenker in June 2004 has become a real evolutionary event for a better understanding towards an unifying concept on the molecular etiology for ET, PV, and MGM, or CIMF as well as for the clinical manifestations of platelet-mediated thrombosis, for the increased red cell mass complicated by major and for secondary myelofibrosis [68]. JAK2 plays an essential role in hematopoiesis by mediating signals from several hematopoietic cytokines including EPO, TPO IL-3 G-CSF, GMSCF etc [69–71]. The JAK2 mutation makes the mutated hematopoietic progenitor cells hypersensitive for TPO, EPO. IL3, G-SCF and GM-CSF and thereby leading to growth advantage of the mutated above the normal trilinear hematopopoietic cells in the bone marrow. The discovery of the JAK2 V617F mutation by Vainchenker [68] was rapidly confirmed by several investigators [72–76]. According to the PVSG criteria, half of the ET and MF patients and the majority of PV patients have the mutated the JAK2 allele. By pooling the currently available data that were generated by DNA sequencing, the frequency of JAK2 V617F is 73% in PV, in ET, and 43% in CIMF. A much higher frequency of JAK2 in MPD (97% in PV, 57% in ET and 50% in CIMF) was described in one study that used allele-specific polymerase chain reaction (PCR) analysis in addition to sequencing. The mutation was absent in more than 600 healthy controls, in patients with Ph^{1+} CML, and in patients with reactive thrombocytosis. The mutation has been found rarely in CML, MDS, hyper-eosinophilic syndrome, chronic neutrophilic leukemia, chronic myelomonocytic leukemia, but somewhat more frequent in CML-like MDS and unclassified MPD. The acquired JAK2 V617F mutation is located on chromosome 9p. A minority of ET and about half of the PV and MF patients have both JAK2 alleles mutated, which is the consequence of mitotic recombination between homologous chromosomes 9p in a cell heterozygous for V616F and results in loss of heterozygosity of chromosome 9p (9pLOH). The 9pLOH is a second genetic event of duplication of chromosome 9p bearing the mutated JAK2 and therefore homozygous.

The JAK2 V617F mutation affects the trilinear hematopoietic bone marrow cells and is detectable in platelets, erythroblasts and granulocytes. The gain of function mutation is in line with the concept of Dameshek that all “stops” to blood production in the bone marrow seem to have been pulled out by one factor JAK2 V617F causing, due to hypersensitivity of hematopoietic progenitor cells to growth factors, trilinear myeloproliferation. The hypothesis may be that heterozygous JAK2 mutation with low activity may be enough for megakaryocyte proliferation with increase of hypersensitive platelets (ET) with no or slight increase of erythropoiesis (initial early PV), and that homozygous JAK2 mutation with moderate to high activity surely will produce pronounced trilinear megakaryocyte, erythroid or even granulocytic proliferation with the clinical pictures of PV, atypical granulocytic leukaemia, unclassifiable MPD with secondary myelofibrosis [76–78]. The sequential occurrence of heterozygous and homozygous V617F mutation can readily explain the spontaneous megakaryocyte and erythroid colony formation (EEC), and the hypersensitivity of granulocyte precursors to growth factors (increased PRV-1 expression) with the sequential production of increased hypersensitive platelets as a first step, increased hematocrit as a second step aggravating the microvascular disturbances of thrombocythemia into the macrovacsular complications of polycythemia vera. Similarly, the sequential occurrence of heterozygous and homozygous V617F mutation can also explain the dual granulocytic megakaryocytic proliferation associated with increased leukocyte activation and production (leukocythemia) and secondary myelofibrosis without features of PV because of progressive splenomegaly. Two main questions to be answered are the following. First, are cases of true ET either clonal or polyclonal positive or negative for JAK2 V617F. Second, are cases of prefibrotic or early fibrotic CMGM or CIMF JAK2 V617F positive, are additional genetic defect needed for progressive MPD disease or do independent genetic defects give rise to JAK2 negative and Philadelphia chromosome negative PV, and AMM with predicted poor prognosis (Figure 2). The genetic aetiology and natural history, as well as the pathophysiology of clinical manifestations and the haematological peripheral blood and bone marrow features of JAK2 positive and JAK2 negative MPDs have to be determined in a large prospective study of newly diagnosed and previously untreated Philadelphia chromosome negative MPD patients to be followed-up for 5 to more than 10 years [79].

References

[1] Burkhardt R, Bartl R, Jaeger K, Frisch B, Kettner G, Mahl G, Sund M. Chronic myeloproliferative disorders. Path Res Pract 1084;179:131–186.

[2] Georgii A, Vykuopli KF, Thiele J. Chronic megakaryocytic granulocytic myelosis: CMGM. Virch Arch A Path Anat Histol 1980;389:253–268.

[3] Georgii A, Vykoupil KF, Buhr Th, Choritz H, Doehler U, Kaloutsi V, Werner M. Chronic myeloproliferative disorders in bone marrow biopsies. Path Res Pract 1990;186:3–27.

[4] Georgii A, Buhr T, Buesche G, Kreft A, Choritz H. Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. Leukemia and Lymphoma 1996;22:15–29.

[5] Thiele J, Ballard AC, Georgii A, Vykoupil KF. Chronic megakaryocytic granulocytic myelosis: An electron microscopic study, Megakaryocytes and thrombocytes. Virch Arch (Path Anat Histol) 1977;373:191–211.

[6] Thiele J, Holgado S, Choritz H, Georgii A. Density distribution and size of megakaryocytes in inflammatory reactions of the bone marrow (myelitis) and chronic myeloproliferative disorders. Scand J Haematol 1983;31:329–341.

[7] Thiele J, Zankovich R, Schneider G, Kremer B, Fischer R, Diehl V. Primary (essential) thrombocythemia versus polycythemia vera rubra. A histomorphometric analysis of bone marrow features in trephine biopsies. Analyt Quat Cytol Histol 1988;10:375–382.

[8] Thiele J, Zankovich R, Steinberg T, Kremer B, Fischer R, Diehl V. Primary (essential) thrombocythemia versus hyperplastic stages of agnogenic myeloid metaplasia with thrombocytosis: a critical evaluation of clinical and histomorphological data. Acta Haematol 1989;81:192–202.

[9] Thiele J, Wagner S, Degel C, et al. Megakaryocyte precursors (pro- and megakaryoblasts) in bone marrow tissue from patients with reactive thrombocytosis, polycythemia vera and primary (essential) thrombocythemia. Virch Arch (Cell Pathol) 1990;58:295–302.

[10] Thiele J, Kvasnick HM, Werden C, Zankovich R, Diehl, Fischer R. Idiopathic primary osteo-myelofibrosis: A clinico-pathological study on 208 patients wit special emphasis on evolution of disease features, differentiation from essential thrombocythemia and variables of prognostic impact. Leukemia and Lymphoma 1996;22:303–317.

[11] Michiels JJ. The myeloproliferative disorders. An historical appraisal and personal experiences. Leukemia & Lymphoma 1997;22:1–14.

[12] Michiels JJ. Diagnostic criteria of the myeloproliferative disorders (MPD) essential thrombocythemia, polycythemia vera and chronic megakaryocytic granulocytic metaplasia. Neth J Med 1997;51:57–64.

[13] Michiels JJ, Juvonen E. Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. Semin Thromb Hemostas 1997;23:339–347.

[14] Thiele J, Kvasnicka HM, Fischer R. Histochemistry and morphometry on bone marrow biopsies in chronic myeloproliferative disorders: aids to diagnosis and classification Ann Hematol 1999;78:495–506.

[15] Thiele J, Kvasnicka HM, Diehl V, Fischer R, Michiels JJ. Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disrders by histopathology, histochemistry and immunostaining from bone marrow biopsies. Leukemia & Lymphoma 1999;33: 207–218.

[16] WHO classification of the chronic myeloproliferative diseases (CMPD) polycythemia vera, chronic idiopathic myelofibrosis, essential thrombocythemia and CMPD unclassifiable. WHO Classification of Tumours. Tumours of Haematopoiesis and Lymphoid Tissues, Lyon. IARC 2001 pp 31–42.

[17] Michiels JJ, Thiele J. Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera and idiopathic myelofibrosis (agnogenic myeloid metaplasia). Int J Hematol 2002;76:133–145.

[18] Thiele J, Kvasnicka HM, Zankovich R, Diehl V. The value of bone marrow histopathology for the differentiation between early stage polycythemia vera and secondary (reactive) polycythemias. Haematologica 2001;86:368–374.

[19] Thiele J, Kvasnicka HM, Muehlhausen K, Walter S, Zankovich R, Diehl V. Polycythemia rubra vera versus secondary polycythemias A clinicopathological evaluation of distinctive features in 199 patients. Pathology Res Pract 2001;197:77–84.

[20] Thiele J, Kvasnicka HM, Diehl V. Bone marrow features of diagnostic impact in erythrocytosis. Ann Heamatol 2005;84: 362–367.

[21] Thiele J, Kvasnicka HM, Diehl V. Initial (latent) polycythemia vera with thrombocytosis mimicking essential thrombocythemia. Acta Haematologica 2005;113:213–219.

[22] Thiele J, Kvasnicka HM. Diagnostic impact of bone marrow histopathology in polycythemia vera PV. Histol Histpathol 2005;20:317–328.

[23] Michiels JJ. Bone marrow histopathology and biological markers as specific clues to the differential diagnosis of essential thrombocythemia, polycythemia vera and prefibrotic or fibrotic myeloid metaplasia. Hematol J 2004;5:93–102.

[24] De Stefano V, Teofili L, Leone G, Michiels JJ. Spontaneous erythrid colony formation as the clue to an underlying myeloproliferative disorder in patients with Budd-Chiari syndrome or portal vein thrombosis. Semin Thromb Hemostas 1997;23:411–418.

[25] Briere BJH 2005.

[26] Liu E, Jelinek J, Pastore YD, Guan Y, Prchal JF, Prchal JT. Discrimination of polycythemias and thrombocythemia by novel, simple accurate clonality assays and comparison with PRV-1 expression and BFU:E response to erythropoietin. Blood 2003;101:3294–3301.

[27] Shih L-Y, Lee C-T. Indentification of masked polycythemia vera from patients with idiopathic marked thrombocytosis by endogenous erythroid colony assay. Blood 1994;83:744–748.

[28] Michiels JJ, Ten Kate FJW. Erythromelalgia in thrombocythemia of various myeloproliferative disorders. Am J Hematol 1992;39:131–136.

[29] Bauermeister DE. Quantitation of bone marrow reticulin. Am J Clin Pathol 1971;56:24–31.

[30] Reilly JT. Pathogenesis of idiopathic myelofibrosis: present status and future directions. Br J Haematol 1994;88:1–8.

[31] Tefferi A, Silverstein MN, Noël P. Agnogenic myeloid metaplasia. Semin Hematol 1995;22:327–333.

[32] Barosi G, Ambrosetti A, Finelli, et al. The Italian consensus conference on diagnostic criteria for myelofibrosis with myeloid metaplasia. Br J Haematol 1999;104:730–737.

[33] Tefferi A. Myelofibrosis with myeloid metaplasia. New Eng J Med 2000;342:1255–1265.

[34] Thiele J, Kvasnicka HM, Boeltken B, Zankovich R, Diehl V, Fischer R. Initial (prefibrotic) stages of idiopathic (primary) myelogibrosis (IMF): a clinicopathological study. Leukemia 1999;13:1741–17428.

[35] Thiele J, Kvasnika HM, Zankovich R, Diehl V. Relevance of bone marrow features in the differential diagnosis between essential thrombocythemia and early stage idiopathic myelofibrosis. Haematlogica 2000;85:1126–1134.

[36] Thiele J, Kvasnicka HM, Zankovich R, Diehl V. Clinical and morphological criteria for the diagnosis of prefibrotic idipathic (primary) myelofibrosis. Ann Hematol 2001;80:160–165.

[37] Thiele J, Kvasnicka HM, Zankovich R, Diehl V. Early stage idiopathic (primary) myelofibrosis: current issues of diagnostic features. Leukemia & Lymphoma 2002;43:1036–1041.

[38] Thiele J, Kvasnicka HM. Diagnostic differentiation of essential thrombocythemia from thrombocythemia associated with chronic idiopathic myelofibrosis by discriminate analysis of bone marrow features: a clinicopathological study on 272 patients. Histol Histopathol 2003;18:93–102.

[39] Thiele J, Kvasnicka HM, Schmitt-Graeff A, Diehl V. Dynamics of fibrosis in chronic idiopathic (primary) myelofibrosis during therapy: a follow-up on 308 patients. Leukemia and Lymphoma 2003;44:949–953.

[40] Thiele J, Kvasnicka HM, Facchetti F, Franco V, Van Der Walt J, Orazi A. European consensus for grading of bone marrow fibrosis and assessment of cellularity in myeloproliferative disorders. Haematologica Hematol J 2005, in press.

[41] Michiels JJ, Kvasnicka HM, Thiele J. Myeloproliferative Disorders: current perspectives on diagnostic criteria, histopathology and treatment. 2005 ISBN 3-9898075-6-8 Verlag ME Uwe Grunwald, Munich Germany.

[42] Thiele J, Kvasnicka HM. Chronic myeloproliferative disorders with thrombocythemia: a comparative study of two classifications systems (PVSG-WHO) on 839 patients. Ann Hematol 2003;82:148–152.

[43] Thiele J, Kvasnicka HM, Diehl V. Bone marrow $CD34^+$ progenitor cells in Philadelphia chromosome-negative chronic myeloproliferative disorders: a clinicopathological study on 575 patients. Leukemia & Lymphoma 2005;46:709–715.

[44] Florena AM, Tripodi C, Iannitto E, Porcasi R, Ingrao S, Franco V. Value of bone marrow biopsy in the diagnosis of essential thrombocythemia. Haematologica 2004;89:911–919.

[45] Westwood NB, Pearson TC. Diagnostic applications of haematopoietic progenitor culture techniques in polycythaemias and thrombocythaemias. Leukemia & Lymphoma 1996; 22:95–103.

[46] Johanson P, Andreason B, Safai-Kutti S, Wennstrom L, Palmqvist L, Rickson A, Lindstedt G, Kutti J. The presence of a significant association between elevated PRV-1 mRNA expression and low plasma erythropoietin concentration in essential thrombocythemia. Eur J Haematol 2003;70:358–362.

[47] Griesshammer M, Klippel S, Strunk E, Temeric S, Mohr U, Heimpel H, Pahl HL. PRV-1 mRNA expression discriminates two types of essential thrombocythemia. Ann Hematol 2004; 83:364–370.

[48] Michiels JJ, Ten Kate FWJ, Vuzevski VD, Abels J. Histopathology of erythromelalgia in thrombocythemia. Histopathology 1984;8:669–678.

[49] Adamson JW, Fialkov PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem-cell and probable clonal origin of the disease. N EngJ Med 1976;295:913–916.

[50] Fialkov PJ, Faquet GB, Jacobson RJ, Vaida K, Murphy S. Evidence that essential thrombocythemia is a clonal disorder with origin in a multipotent stem cell. Blood 1981;58:916–919.

[51] El-Kassar N, Hetet G, Briere J, Grandschamp B. Clonality analysis of hematopoiesis in essential thrombocythemia: advantages of studying T lymphocytes and platelets. Blood 1997; 89:128–134.

[52] Harrison CN, Gale RE, Machin SJ. Linch DC, A large proportion of patients with the diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombosis. Blood 1999;93:417–424.

[53] Shih LY, Lin TL, Lai CL, Dunn P, Wu JH, Wang PN, et al. Predictive value of X-chromosome inactivation patterns and clinicopathological parameters for vascular complications in female patients with essential thrombocythemia. Blood 2002; 100:1596–15601.

[54] Prchal JF, Axelrad AE. Bone marrow responses in polycythemia vera. N Eng J Med 1974;290:1382 (Letter).

[55] Dobo I, Donnard M, Girodon F, Mossuz P, Boiret N, Boukhari R, Allégraud A, Bascans E, Campos L, Pineau D, et al. Standardization and comparison of endogenous erythroid colony assays performed with bone marrow and blood progenotors for the diagnosis of polycythemia vera. Hematol J 2001;2:396–403.

[56] Correa PN, Eskinazi D, Axelrad AA. Circulating erythroid progenitors in polycythemia vera are hypersensitive to insulin-like growth factor-1. Blood 1994;84:99–112.

[57] Mirza AM, Correa PN, Axelrad AA. Increased basal and induced tyrosine phosphorylation of the insulin-like growth factor 1 receptor beta subunit in circulating mononuclear cells of patients with polycythemia vera. Blood 1995;86:877–882.

[58] Mirza AM, Ezzat S, Axelrad AA. Insulin-like growth factor binding protein-1 is elevated in patients with polycythemia vera and stimulates erythroid burst formation in vitro. Blood 1997;89:1862–1869.

[59] Juvonen E, Ikkala E, Oksanen K, Ruutu T. Megakaryocyte and erythroid colony formation in essential thrombocythemia and reactive thrombocytosis: diagnostic value and correlation to complications. Brit J Haematol 1993;83:192–197.

[60] Florensa L, Bessis C, Woessner S, Sole F, Acin P, Pedro C, Sans-Sabrafen J. Endogenous megakaryocyte and erythroid colony formatio from blood in essential thrombocythemia. Leukemia 1995;9:271–273.

[61] Jantunen R, Juvonen E, Ikkala E, Oksanen K, Anttila P, Jormila P, Jansson SF, Kekomaki R, Ruutu T. Essential thrombocythemia at diagnosis: causes of diagnostic evaluation and presence of positive diagnostic findings. Ann Hematol 1998;77:101–106.

[62] Axelrad AA, Eskinazi D, Correa PN, Amata D. Hypersensitivity of circulating progenitor cells to megakaryocyte growth and development factor (PEG-rHU MGDF) in essential thrombocythemia. Blood 2000;96:3310–3321.

[63] Axelrad AA, Eskinazi D, Amato D. Does hypersensitivity of progenitor cells to normal cytokine(s) play a role in the pathogenesis of idiopathic myelofibrosis with myeloid metaplasia? Blood 1999;94:2866.

[64] Temerinac S, Klippel S, Strunck E, Röder S, Lübbert M, Lange M, Meinhardt G, Schaefer HE, Pahl HL. Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. Blood 2000;95:2569–2576.

[65] Pahl HL. Towards a molecular understanding of polycythemia ribra vera. Eur J Biochem 2000;267:3395–3401.

[66] Klippel S, Strunck E, Termerinac S, et al. Quantification of PRV-1 expression, a molecular marker for the diagnosis of polycythemia vera. Blood 2001;98:470a.

[67] Pahl HL. Molecular markers in MPDs. Proceedings First Como Workshop on the molecular pathogenesis and genetics of myeloproliferative disorders. 2002.

[68] James C, Ugo V, Le Couedic PF, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to

constitutive signalling causes polycythemia vera. Nature 2005; 434:1144–1148.

[69] Kaushanky K. On the origin of the chronic myeloproliferative disorders: it makes all sense. Blood 2005;105:4187–4190.

[70] Goldman JM. A unifying mutation in chronic myeloproliferative disorders. New Eng J Med 2005;352:1744–1745.

[71] Cazzola M, Skoda R. Gain of function, loss of control. A molecular basis for chronic myeloproliferative disorders. Haematologica Hematol J 2005;90:871–874.

[72] Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis. Cancer cells 2005;7:387–397.

[73] Kralovics R, Paasamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Eng J Med 2005;352:1779–1790.

[74] Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas-Swanton S, et al. Acquired mutation of the tyrosine kinase Jin human myeloproliferative disorders. Lancet 2005;365:1054–1061.

[75] Zhao R, Xing S, Li Z, Fu X, Li Q, Krantz AS, et al. Identification of an acquired JAK2 mutation in polycythemia vera. J Biol Chem 2005.

[76] Jones AV, Kreil S, Xoi K, Waghorn K, Curtis C, Zhang L et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 2005; May 26[Epub ahead of print].

[77] Steensma DP, Dewald M, Lasho TL, Powell HL, McClure RF, Levine RL et al. TheJAK2 V617F activating tyrosine kinase mutation is an infrequent event in bothatypical myeloproliferative disorders and the myelodysplastic syndrome. Blood 2005 28 [Epub ahead of print].

[78] Jelinek J, Oki Y, Gharibyan V, Bueso-Ramos C, Prchal JT, Verstovsek S, Beran M, Estey E, Kantarjian HM, Issa JP. JAK2 mutation 1849G >T is rare in acute leukemias but can be found in CMML, Philadelphia-chromosome negative CML and megakaryocytic leukemia. Blood 2005 [Epub ahead of print].

[79] Tefferi A, Gilliland DG. The JAK2 V617F tyrosine kinase mutation in myeoproliferative disorders: statu report and immedate implications for diesaesclassification and diagnosis. Mayo Clin Proc 2005;80:947–958.