The Hematology Journal (2004) 5, 93–102

www.nature.com/thj

REVIEW

Bone marrow histopathology and biological markers as specific clues to the differential diagnosis of essential thrombocythemia, polycythemia vera and prefibrotic or fibrotic agnogenic myeloid metaplasia

Jan Jacques Michiels*[1,2]

[1]*Goodheart Institute, MPD Center Europe, Rotterdam, The Netherlands;* [2]*Department of Hematology, University Hospital Antwerp, University of Antwerp, Belgium*

Clinical, hematological and morphological peripheral blood and bone marrow characteristics, in particular, megakaryopoiesis and bone marrow cellularity, reveal diagnostic clues and pathognomonic features, which enable a clear-cut distinction between essential thrombocythemia (ET), polycythemia vera (PV) and prefibrotic and fibrotic agnogenic myeloid metaplasia (AMM). The characteristic increase of enlarged mature megakaryocytes with mature cytoplasm and multilobulated nuclei and their tendency to cluster in a normal or slightly increased cellular bone marrow represent the hallmark of ET. The characteristic increase and clustering of enlarged mature and pleiomorphic megakaryocytes with multilobulated nuclei and proliferation of erythropoiesis in a moderate to marked hypercellular bone marrow with hyperplasia of dilated sinuses are the specific diagnostic features of untreated PV. ET may precede PV for many years to more than one decade. Prefibrotic and fibrotic AMM appears to be a distinct dual proliferation of abnormal megakaryopoiesis and myelopoiesis. The histopathology of the bone marrow in prefibrotic and fibrotic AMM is dominated by atypical enlarged and immature megakaryocytes with cloud-like immature nuclei, which are not seen in ET and PV at diagnosis and during follow-up. Myelofibrosis is not a feature of ET at diagnosis and during long-term follow-up. Myelofibrosis, which is secondary to the megakaryocytic/granulocytic myeloproliferation, and extramedullary myeloid metaplasia constitute a prominent feature and usually progress more or less rapidly during the natural history of PV and AMM. Life expectancy is normal in ET, normal in the first and decreased in the second decade of follow-up in PV, but significantly shortened in thrombocythemia associated with prefibrotic AMM as well as in the various fibrotic stages of AMM. These clinical and pathological characteristics of the Ph-negative MPDs, by including bone marrow histopathology, enable a clear-cut distinction between ET, PV and prefibrotic and fibrotic AMM. The use of established and new biological markers of MPDs, like spontaneous EEC, PRV-1 gene expression etc, should be validated in large prospective multicenter studies of newly diagnosed and previously treated MPD patients using the proposed European clinical and pathological (ECP) criteria as the only gold standard available for the proper diagnosis and differential diagnosis of ET, PV and AMM.
The Hematology Journal (2004) **5,** 93–102. doi:10.1038/sj.thj.6200368

Keywords: essential thrombocythemia; polycythemia vera; agnogenic myeloid metalasia; idiopathic myelofibrosis; prefibrotic myeloid metaplasia; bone marrow histopathology

Introduction

In 1951, Dameshek presented a unifying theory that the various diseases such as chronic myeloid leukemia (CML), polycythemia vera (PV), agnogenic myeloid metaplasia (AMM), megakaryocytic leukemia or essential thrombocythemia (ET) and erythroleukemia were variable manifestations of myeloproliferative disorders due to a hypothecical stimulus that may affect the bone marrow cells diffusely or irregularly, resulting in the various distinct and overlapping syndromes.[1] However, erythroleukemia and chronic myeloid leukemia turned out to be two separate and independent malignancies distinct from the chronic myeloproliferative disorders (MPDs). Erythroleukemia AML-M6 is an unstable

*Correspondence: JJ Michiels, Goodheart Institute, MPD Center Europe, Erasmus Tower, Veenmos 13, Rotterdam, The Netherlands; Tel: +31 1 2867508; Fax: +31 10 2867539;
E-mail: postbus@goodheartcenter.demon.nl

condition clearly between myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), which originates from trilinear MDS and usually rapidly evolves to frank AML.[2–4] In the early 1970s, Gilbert[5] and Laszlo[6] of the Polycythemia Vera Study Group (PVSG) reviewed the spectrum and typical patterns of cellular involvement seen in the various MPDs ET, PV, AMM and CML with particular emphasis on the characteristic abnormalities that occur in leukocyte alkaline phosphatase (LAP) activity, bone marrow morphology and histopathology and karyotype. According to strict morphological, biochemical and cytogenetic criteria, chronic myeloid leukemia is a separate malignant and individual disease entity, whereas ET, PV and AMM form the three main different manifestations of the chronic MPDs.[7] The Philadelphia chromosome (Ph^+), the low LAP score and an obligate transition into AML M1, 2, 3 or 6 or ALL are typical for CML.[7] Both Ph^+ ET and Ph^+ thrombocythemia associated with CML can be regarded as early manifestations of the chronic stable phase of Ph^+ diseases.[8]

The morphological distinction between Ph^+ ET and thrombocythemia associated with Ph^+ CML versus the Ph-negative thrombocythemias in various MPDs is primarily based upon conspicuous differences in the form and size of megakaryocytes in bone marrow smears and sections of bone marrow aspirates.[7,9] This difference is obvious and also reproducible in bone marrow biopsies that enables pathologists to distinguish between small megakaryocytes with round nuclei in Ph^+ diseases versus enlarged megakaryocytes with hyperlobulated nuclei in Ph-negative MPDs.[10–12] The present review further extends the clinical and pathological characteristics of the Ph-negative MPDs, in particular megakaryopoiesis, by including bone marrow histopathology, which enables a clear-cut distinction between ET, PV and prefibrotic and fibrotic AMM.

PVSG criteria and biological markers of ET, PV and AMM

PV is a clonal stem cell disorder resulting in hyperproliferation of erythroid and in most cases also megakaryocytic and granulocytic lineages. A substantial proportion of ET patients appear to have polyclonal hematopoiesis, and by this criterion, ET is a heterogenous group of disorders.[13,14] The diagnostic criteria of ET as mandated by the PVSG exclude either thrombocythemia associated with other MPDs, CML, MDS or reactive thrombocytosis, but does not differentiate between true ET and thrombocythemia as the presenting feature of early-stage PV or prefibrotic AMM.[15] The PVSG developed the diagnostic criteria of PV that are widely used and cited, but have never been evaluated in prospective studies.[16] As no single marker is diagnostic for PV, the PVSG used a combination of major and minor criteria for PV, which proved to be very robust. The main major criterion of increased red cell mass for the diagnosis of PV is arbitrary and crude, a situation that is also found in idiopathic erythrocytosis, in primary erythrocytosis and in secondary erythrocytosis (Table 1). A significant number of latent phases of symptomatic patients with red cell mass in the upper range of normal but with persistent slight increases of platelet counts and/or slight splenomegaly are overlooked as true examples of early-stage MPD.

In vitro assays for culture of erythroid progenitors colony-forming unit-erythroid (CFU-E) and burst forming unit-erythroid (BFU-E) have been available for over 25 years.[17] The addition of erythropoietin (EPO) to the culture medium is essential to colony growth of normal erythroid progenitors. EPO-independent progenitors can form erythroid colonies in the absence of exogenous EPO. This phenomenon is called spontaneous or endogenous erythroid colony formation (EEC) and was first described in 1974 by Prchal and Axelrad in patients with PV.[17] In 26 studies altogether,

Table 1 Diagnostic differentiation of benign erythrocytoses from myeloproliferative polycythemia vera by histopathology from bone marrow biopsy sections[7–12,46–51]

Raised cell mass, RCM	
Raised RCM = absolute erythrocytosis	Normal RCM = apparent erythrocytosis does not exclude MPD!
Bone marrow histopathology differentiates between	
Polycythemia vera (PV)[7–12,40–44] and benign erythrocytosis	Increase and clustering of enlarged megakaryocytes with hyperploid nuclei is a diagnostic clue to polycythemia vera
Benign erythrocytosis in its purity[46,47,48]	
Size, morphology and distribution of megakaryocytes are normal	
Classification of erythrocytoses:	
Congenital erythrocytosis	
Primary: Truncated EPO receptor[49,50]	
Disruption oxygen homeostasis in Chuvash polycythemia[51]	
High oxygen affinity hemoglobinopathy	
Congenital autonomous EPO production?	
Acquired eythrocytosis:	
Secondary: EPO accumulation in renal diseases	
Autonomous EPO production by tumor cells	
Hypoxia	
Idiopathic erythrocytosis	

nearly 500 patients have been reviewed concerning EEC in PV.[18] With a few exceptions all PV patients have shown spontaneous EEC.[18] In about 100 patients with secondary erythrocytosis and in normal controls, no spontaneous EEC has been observed. Spontaneous EEC has been seen as often in cultures of blood as in those of bone marrow progenitors. In most studies, spontaneous EEC was present in overt and in latent PV regardless of the disease state, stage and duration, clinical manifestations or previous cytotoxic treatment, although reduction or disappearance of spontaneous EEC after chemotherapy or interferon has occasionally been described.[18] During follow-up, in most patients with latent PV and spontaneous EEC, the course of the disease was compatible with PV, or the diagnostic criteria proposed by the PVSG were fulfilled or became apparent. In patients with secondary erythrocytosis without spontaneous EEC, no complications occurred and the blood cell counts remained unchanged. These findings in nearly 500 PV patients from 26 studies strongly suggest that spontaneous EEC might have a near 100% diagnostic specificity for overt and latent PV.[18] Spontaneous EEC may even unmask PV from patients with idiopathic thrombocytosis and appears to be very helpful and reliable to differentiate between PV and secondary erythrocytosis.[18] However, the spontaneous EEC technique is demanding, needs further standardization and is unlikely to be available in most routine laboratories. The reliability of spontaneous EEC from blood and bone marrow progenitors was investigated in a recent prospective multicenter study of 80 PV, six idiopathic erythrocytosis (IE), 51 secondary erythrocytosis (SE) patients and 10 healthy controls.[19] Spontaneous EEC was true positive for at least 75% of PV patients when a single EEC assay was performed and 94% of PV patients when both peripheral blood and bone marrow EEC were performed.[19] For all IE, SE and healthy controls, spontaneous EEC was negative.[19]

In a review of 252 ET patients and 98 patients with reactive thrombocytosis (RT) from 12 studies, spontaneous EEC was found to be a reliable and specific marker for ET, with only one reported EEC-positive case of RT.[18] However, the proportion of ET patients who exhibited spontaneous EEC varied widely from 30 to 90% with a diagnostic sensitivity of 65%.[18] With regard to EEC, ET and PV are closely related MPDs with a significant overlap indicating the need for additional tools to differentiate between ET and PV.

A review of 16 studies showed that the majority of ET patients as well as some with PV have CFU-Mk capable of generating endogenous megakaryocytic colonies (EMC) in the absence of TPO.[18] Most of these studies have found spontaneous EMC (EMC+) assays entirely specific for ET and negative in RT. There are a few reports of EMC+ in RT and normal subjects indicating the need for careful validation and control of methods when being used for diagnostic purposes. In two studies where the frequency of EMC+ ET patients was modest (63–69%), diagnostic sensitivities rose to 77 and 92% when EMC+ results were combined with EEC+.[20,21] In a recent study of 154 ET patients, 114 (74%) had either spontaneous EEC+ or EMC+, 86 (57%) EEC+, 91 (59%) EMC+, 65 (42%) EEC+ and EMC+, 26 (17%) EMC+ only and 23 (15%) EEC+ only.[22]

In 1991, Emmanual *et al.*[23] found that granulocyte/macrophage (GM) progenitor cells in juvenile chronic myelogenous/myelomonocytic leukemia were found 10 times as sensitive to the cytokine GM-CSF as normal progenitor cells in this lineage. Apart from spontaneous EEC, PV cells are hypersensitive to insulin growth factor-1. IGF-1 sensitivity ratios reached as high as 20 000 times the normal, and this level of cytokine hypersensitivity in the erythroid lineage of PV is specific to IGF-1.[24–26] In addition, PV cells are hypersensitive, but to a less extent, to other hematopoietic growth factors including IL-3, GM-CSF and SCF. Spontaneous EMC (CFU-Meg) in the absence of exogenous growth factors has been described in ET-patients.[18,20–22] However, under strictly serum-free conditions CFU-Meg from ET were TPO-dependent, but hypersensitive towards TPO in 18/20 (90%) patients with ET and in none of the 22 healthy controls.[27] 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 progenitor cells to normal endogenous TPO in ET. This hypothesis was tested with hematopoietic progenitor cells from patients with IMF/AMM; an MPD involving at least two cell lineages of megakaryocytic and myeloid proliferation.[28] Clear evidence of hypersensitivity was found for SCF, a cytokine active in several different cell lineages, while hypersensitivity was not seen for GM-CSF.[28] Thus, Axelrad proposed a model that the clinicopathological phenotypes of the clonal MPDs are related to, and perhaps determined by specific hypersensitivities of their progenitor cells to normal endogenous cytokines: EEC-IFG-1 hypersensitivity for PV, TPO-hypersensitivity for ET and SCF-hypersensitivity for IMF/AMM.

Overexpression of the mRNA of a novel member of the uPAR receptor superfamily, designated PRV-1, was identified by Pahl *et al.* in granulocytes of all PV patients investigated so far, in normal granulocytes after exposure to granulocyte colony-stimulating-factor (G-CSF), in granulocytes of an occasional case of idiopathic myelofibrosis (IMF), but not in mononuclear cells from patients with secondary erythrocytosis, CML or AML.[29,30] In healthy volunteers, PRV-1 is selectively expressed in the bone marrow and not in a large variety of other tissues.[29,30] Immunohistochemical staining localized PRV-1 expression to early erythroblasts, as well as megakaryocytes, promyelocytes and myelocytes.[29,30] These cells represent the precursor cells of all three hyperproliferating lineages in PV. PRV-1 gene expression has also been detected in ET patients who also demonstrated spontaneous EEC.[31] There appears to be a 100% concordance between spontaneous EEC and overexpression of the PRV-1 gene in PV and ET patients as well as EEC negativity and normal PRV-1 expression in ET patients.[31,32] Thus, Pahl *et al.* have

postulated the existence of an EEC/PRV-1 positive disease, and propose that the two markers will concur in most MPD patients.[31,32] The EEC/PRV-1-positive disease may present clinically either as an ET that will ultimately develop into PV, as PV or as a variant of IMF having quickly passed the hypercellular polycythemic stage. In this concept, EEC/PRV-1 positive ET and IMF represent variants of PV, whereas ECC/PRV-1-negative ET and IMF represent true forms of ET and IMF.[32] Expanding the model to include growth factor hypersensitivity described by Axelrad, Pahl proposed a model for the molecular development of MPDs[32] According to this model, the EEC + /PRV-1 + /IgF-1-sensitive ET, PV and IMF, versus the EEC-/PRV-1-/TPO-sensitive ET and the EEC-/PRV-1-/SCF-sensitive IMF may represent 3 different entities at the biological and molecular level.[32] A recent study could demonstrate an overexpression of PVR-1 in patients with ET, and could not detect any PVR-1 expression in the granulocytes of patients with secondary erythrocytosis, secondary thrombocytosis, CML, IMF and MDS.[33] This intriguing concept has to be confirmed in prospective multicenter studies of newly diagnosed ET, PV and IMF/AMM and may have important implications. First, ET patients that do not have a clonal disorder may be EEC-/PVR-1- and at a lower risk of thrombotic complications.[14] Second, EEC-positive ET patients are likely to be clonal and tend to have higher hematocrits during the course of their disease (latent PV), and consequently may be at higher risk of minor and major thrombotic complications. Third, it may solve the yet unsettled role of reduced expression of MPL in ET, PV and IMF/AMM patients.

Platelet cMPL expression is dysregulated in ET, but appears not to be a primary pathogenic event in ET as it is also evident in other MPDs.[34] Moliterno *et al*.[35] reported a reduction of cMPL expression in 34 PV and in 13/14 IMF/AMM patients but not in 15 ET patients. The reason for these conflicting findings in ET remains elusive. MPL expression and its response to TPO in the megakaryocytes and platelets of PV patients were defective due to an as yet undefined impairment of the post-translational glycosylation that became more profound with disease duration and extent.[36] ET patients with spontaneous EEC also appear to have invariantly increased expression of PRV-1 in granulocytes and decreased expression of MPL protein in platelets (EEC + /PRV-1 + /MPL-).[37] It remains to be determined whether this correlation holds up when a large group of ET patients is tested. In a recent study, Kralovics *et al*.[38] compared EEC, PVR-1 and cMPL in 23 PV, 15 ET and six IMF patients and observed a concordance between EECs, PVR-1 in all these three subtypes of MPD patients. Interestingly, Kralovics *et al*.[38] found decreased cMPL and elevated PVR-1 but absence of EEC in the majority of eight patients with hereditary thrombocythemia with a mutation in the TPO gene as the cause of congenital thrombocythemia in this family.[38]

Little is known regarding the expression of PVR-1 in bone marrow cells and immature hematolpoiesis. Bock *et al*.[39] analyzed PVR-1 expression in bone marrow cells and demonstrated that the PVR-1 gene is constitutively expressed by bone marrow cells in all types of MPD, including PV, ET, IMF and CML, and also in reactive hyperplasia of eythrocytosis and megakaryopoiesis, and that PVR-1 expression in bone marrow cells does not discriminate PV from reactive and other MPDs. Gisslinger and Thiele studied 20 well-documented cases with advanced IMF and found that PVR-1 expression in peripheral blood cells was increased in about half of them (personal communication).

Differential diagnosis of ET, PV and AMM by bone marrow histopathology

With the advent of bone marrow biopsy and tissue processing, the German pathologists Georgii *et al*.[10,11,40,41] and Thiele *et al*.[12,42,43,44] have drawn attention to the importance of histopathology from bone sections for the diagnosis of the Philadelphia chromosome-negative (Ph^-) MPDs ET, PV and AMM. The number and size of mature megakaryocytes in bone marrow biopsies are typically increased in Ph^- ET. Enlarged megakaryocytes with mature cytoplasm and multilobulated nuclei, and the tendency of such megakaryocytes to cluster in small groups close to sinuses represent the hallmark features of ET.[7–12,40–44] The histologic background of hematopoiesis in ET is featured by normal cellularity in the early stage at platelet count in excess of 400×10^9/l. A slight-to-moderate increase of cellularity may be seen during long-term follow-up of ET with a high platelet count in excess of 1000×10^9/l. In thrombocythemia associated with the chronic stage of Ph^+ chronic granulocytic leukaemia and in Ph^+ ET, the megakaryocytes are smaller than normal with round nuclei showing little lobulations.[7–12,40–44] The megakaryocytes are increased in reactive thrombocytosis, but the morphology, size and nuclear ploidy of megakaryocytes remain normal and there is no tendency to cluster.

The diagnostic features of untreated PV include a prominent increase in the number and size of clustered and enlarged megakaryocytes, comparable to ET, and a moderate-to-marked increased cellularity. Increase of large megakaryocytes with mature cytoplasm and multilobulated nuclei gives the diagnostic feature, which is even more conspicuously altered in PV than in ET.[7–12,40–44] The megakaryocytes in PV may have a rather pleiomorphic appearance with wide ranges of sizes including many small ones (the so-called micromegakaryocytes) and unusually giant forms as demonstrated in immunostained bone marrow biopsies using monoclonal antibodies against platelet glycoproteins. The characteristic increase and clustering of large megakaryocytes and proliferation of erythropoiesis with hyperplasia of dilated sinuses is the diagnostic hallmark of untreated PV to distinguish it from secondary erythrocytosis, from thrombocythemia associated with myelodysplastic syndromes,[45] from Ph^+ CML and Ph^+ ET and most importantly also from AMM even in its

very early stage without myelofibrosis.[7–12,40–44] Bone marrow histopathology in AMM is dominated by atypical immature megakaryocytes, which are conspicuously large due to an increase of nuclear as well as cellular size. The nuclei of megakaryoctes in AMM are bulky with lobuli becoming clumsy. The lightly stained chromatin and the irregular roundish nuclear forms give rise to the so-called cloud-like nuclei, which are almost never observed in ET and PV.[7–12,40–44] In primary and in secondary erythrocytosis, in which increased cellularity of usually the erythroid cell line may be present, the number, size, morphology and distribution of megakaryocytes in bone marrow smears and biopsies remain normal (Table 1).[46–51]

European clinical and pathological criteria of ET, PV and AMM

ET

The PVSG criteria for the diagnosis of ET in 1975 were a platelet count in excess of 1000×10^9/l and megakaryocytic hyperplasia and abundant platelets in bone marrow smears in the absence of any other MPD.[6] The main criterion of a minimum platelet count of 1000×10^9/l resulted in the exclusion of a significant number of patients with apparent characteristic of ET. This prompted the PVSG in 1986 to adjust the minimum platelet count for the diagnosis of ET to 600×10^9/l.[15] Diagnostic criteria proposed by the Thrombocythemia Vera Study Group (TVSG) are a platelet count in excess of 400×10^9/l and an increase of clustered large and megakaryocytes with mature cytoplasm and hyperploid nuclei in bone marrow biopsy material in the absence of any cause of reactive thrombocytosis and no preceding or allied other subtype of MPD.[52] The PVSG[15] and TVSG[52] clinical and the WHO[53] pathological criteria for the diagnosis of ET are equally considered and brought together in a new set of clinical and pathological criteria for the diagnosis of true ET (thrombocythemia vera) (Table 2).[54] According to elaborate morphometric evaluations increase of enlarged-to-giant megakaryocytes with mature cytoplasm and deeply lobulated nuclei represent the diagnostic hallmark of true ET. This feature discriminates true ET from the presence of small and abnormal megakaryocytes often described in smears and histological sections derived from patients with a rather ill-defined entity, the so-called Ph^1 + or BCR-ABL + ET.[7,8] Similar diagnostic restrictions regard patients with the so-called ET and ringed sideroblasts that are occasionally assumed to present transitional stages between MDS and MPDs and are closely related to the $5q^-$ syndrome.[43,45] Myelofibrosis (MF) is rare in true ET and very few ET patients develop MF during long-term follow-up, indicating that true ET is the most benign variant of MPD with a normal-to-near-normal life expectancy.[40,52]

PV

Wasserman[55] proposed in 1954 a concept on the course of PV and distinguished five sequential stages in the natural history of PV stage 1, pure erythrocythemia; stage 2, erythrocythemia and thrombocythemia; stage 3, myeloid metaplasia with overlap syndromes of different grades of reticulin and collagen fibrosis of the bone marrow, splenomegaly and leukocytosis; stage 4, spent phase polycythemia with leukoerythrocytosis; and stage 5, acute leukemia.[55] The PVSG failed to add histopathology from bone marrow biopsies as a specific diagnostic aid to a proper diagnosis of PV. The TVSG[48] extended the PVSG[16] criteria for PV by including bone marrow morphology as a specific clue to PV and as a pathognomonic guideline to distinguish clearly between PV and secondary erythrocytosis (SP).[46,47] The TVSG and PVSG criteria for PV are further improved by a new set of the clinical and WHO[56] pathological PV criteria as outlined in Table 3.[54] In contrast to SP, overt PV is always characterized by a conspicuous hypercellularity of the bone marrow provided age-related changes are regarded. Furthermore, proliferation of all three hematopoietic cell lineages with predominance of erythroid precursors (panmyelosis) is a major finding. In this context, megakaryopoiesis is an invaluable clue to the diagnosis of PV, because loose clusters of megakaryocytes and a dislocation towards the paratrabecular area are frequently encountered together with a peculiar cytology, including a pleiomorphic appearance but no significant maturation defect.[7–12,16,40–43,52] These specific

Table 2 ECP criteria for the diagnosis of ET or TV[54]

Clinical criteria	*Pathological criteria*
(A1) Persistent increase of platelet count: in excess of 400×10^9/l	(B1) Predominant proliferation of enlarged megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous cytological abnormalities. No proliferation or immaturity of granulopoiesis or erythropoiesis
(A2) Normal spleen or only minor splenomegaly on echogram	(B2) No or only borderline increase in reticulin
(A3) Normal or increased LAP-score, normal ESR and increased mean platelet volume (MPV)	
(A4) Spontaneous megakaryocyte colony formation (CFU-Meg)	
(A5) No signs or cause of reactive thrombocytosis (RT)	
(A6) No preceeding or allied other subtype of MPD, CML or MDS	
(A7) Absence of the Philadelphia chromosome	

The combinations of (A1) and (B1) + (B2) establish (true) ET—any other additional A criterion confirms ET.
ET or TV: grade I: platelet counts between $400 = \times 10^9$ and 1500×10^9/l, grade II: platelet counts $> 1500 \times 10^9$/l.

features are not expressed in SP, in particular, concerning the inconspicuous megakaryopoiesis in this disorder.[47] In PV opposed to SP, there are usually no iron-laden macrophages or prominent inflammatory reaction of the interstitial bone marrow compartment observable.[47]

We proposed to distinguish six clinicopathological stages as already suggested by Wasserman *et al.*[55] during the natural history of newly diagnosed PV patients (Table 4).[57] The finding of increased cellularity due to panmyelosis (hyperplasia of all marrow elements) and the typical increase of clustered enlarged megakaryocytes with hyperploid nuclei were the characteristic features in bone marrow sections of untreated PV patients.[54] According to the new clinical and pathological criteria, the latent stage 0, latent PV and the erythremic stage 1, the so-called idiopathic erythrocytosis are overlooked by the PVSG criteria with the consequence that the majority of PV patients at presentation have stage 2 and 3 disease (Table 4). Idiopathic or pure eythrocytosis was defined by an increased red cell mass, normal platelet and leukocyte counts and no splenomegaly in the absence of any cause for secondary erythrocytosis.[58] Patients defined as idiopathic or pure erythrocytosis are in fact early-stage PV and comprise 15–22% of the PV cases at the time of diagnosis.[59,60] The diagnostic difficulties of 'idiopathic or pure erythrocytosis' can easily be solved by including bone marrow histopathology as a specific guide to diagnose this very early stage of PV and to differentiate

Table 3 ECP criteria for the diagnosis of PV[54]

Clinical criteria	*Pathological criteria*
(A1) Increase in packed cell volume (PCV) with hemoglobin (male >18.5 g/dl, female >16.5 g/dl) and hematocrit (male $>51\%$, female $>48\%$)	(B1) Increased cellularity with trilineage myeloproliferation (ie panmyelosis) Proliferation and clustering of small to giant (pleiomorphic) megakaryocytes Absence of stainable iron No pronounced inflammatory reaction (plasmacytosis, cellular debris)
Raised red cell mass (optional) RCM: male >36 ml/kg, female >32 ml/kg	(B2) Spontaneous erythroid colony formation
(A2) Persistent increase of platelet count: grade I: 400–1500, grade II: >1500	*Grading of myelofibrosis in PV*
(A3) Splenomegaly on palpation or on ultrasound or CT (>11 cm length in diameter)	MF 0 *prefibrotic stage PV*
(A4) Granulocytes $>10\times10^9$/l and/or raised LAP-score in the absence of fever or infection	MF 1 *early fibrotic stage PV*
(A5) Absence of any cause of secondary erythrocytosis	MF 2 *manifest myelofibrosis in PV*
(A6) Low plasma Epo level	MF 3 *advanced myelofibrosis in PV*
	MF >3 *osteosclerosis, decreased cellularity*

The combinations of A1 + B1 + B2 establish PV—any other criterion confirms PV. For grading MF see Table 3.

Table 4 Six clinicopathologic stages of PV according to Wassermann[55] and Michiels et al.[57]

Stage	*0 ET*	*1 PV*	*2 PV*	*3 PV PVMF*	*4*	*5 Spent phase*
Hemoglobin (mmol/l)	N/↑	↑	↑	↑	↑	*N*/↑
Erythrocytes $>6\times10^{12}$/l	N/↑	↑	↑	↑	↑	N/↑
Red cell mass:						
male >36 ml/kg	N	↑	↑	↑	↑	—
female >32 ml/kg	N	↑	↑	↑	↑	—
Hematocrit	N/↑	↑	↑	↑	↑	N/↑
Platelets $\times10^9$/l	$<400/>400$	<400	>400	>1000	Variable	N/↑
Leukocytes $\times10^9$/l	N	N	N	>15	Variable	>20
Leukoerythroblastosis	—	—	—	±	+	++
Spleen length diameter (cm) on scan or echogram	<12 12–15 cm	<12	<15	>15	large	
Bone marrow biopsy:						
Cellularity	N/↑	N/↑	↑↑	↑↑	↑↑	↑↑
Megakaryocytes	↑	↑	↑↑	↑↑	↑↑	↑
Myelofibrosis (MF) grade	0	0	0/1	1/2	2/3	3
Spontaneous EEC	+	+	+	+	+	+
PVR-1 gene	+	+	+	+	+	+

N = normal, − = absent, + = present, ↑ = increased, ↑↑ = pronounced increased.
Six clinicopathological stages of PV according to Wasserman[55] and Michiels[57]: 0: latent or subclinical prefibrotic PV; 1: the so-called erythremia of Wasserman[55] the or so-called idiopathic erythrocytosis of Pearson;[58] 2: early stage PV with no significant or slight splenomegaly and no or early MF; 3: overt plethoric stage of PV with various degrees of pronounced splenomegaly and MF; 4: advanced myelofibrotic or spent phase PV; 5: transformation to MDS and/or AML.

it from primary and secondary erythrocytosis (Tables 1 and 3). Histopathology from bone marrow biopsy material constitutes a specific criterion for the diagnosis of PV and its differentiation from SP,[46,47] and is also a powerful tool to stage PV patients at the time of presentation and during follow-up.[47,54,57] Such a simple approach for the correct diagnosis and staging of PV is suitable for clinical practices in both general and teaching hospitals.

Prefibrotic and fibrotic AMM

The clinical and pathological diagnostic features of classical AMM or idiopathic myelofibrosis (IMF) are characterized by anemia, splenomegaly, leukoerythroblastic blood picture, tear drop erythrocytes and varying degrees of bone marrow fibrosis and osteosclerosis.[61] Different sets of prognostic factors in classical AMM/IMF have been proposed.[62–70] One set of prognostic factors is statistically derived from one series of retrospectively studied AMM/IMF-patients but not applicable to another large series of AMM/IMF-patients. These conflicting results of studies on prognostic factors in AMM/IMF can be explained by differences in diagnostic criteria and lack of uniform criteria for prognosis prediction. This makes selection of AMM/IMF patients for new therapeutic strategies very problematic. However, all AMM/IMF-studies agree on the clinical scoring system shown in Table 5.[62–70]

According to the classical diagnostic criteria of AMM/IMF for entry in the various studies on prognostic factors, the early hypercellular prefibrotic stages of prefibrotic AMM/IMF are neglected and overlooked in all these studies.[62–70] Thiele *et al*.[43] recently introduced the Cologne Criteria for the diagnosis and clinicopathological staging of the hypercellular prefibrotic stage, the early fibrotic stage, the manifest collagen fibrotic stage and the advanced myelofibrotic stages of AMM/IMF. Prefibrotic and early fibrotic stage AMM/IMF is typically featured by a dual megakaryocytic and granulocytic myeloproliferation with relative reduction of erythropoiesis and usually associated with pronounced thrombocythemia (Table 6).[43,54] Bone marrow histopathology in the prefibrotic and early fibrotic stage of AMM/IMF is dominated by atypical immature megakaryocytes, which are conspicuously large due to an increase of nuclear as well as cellular size. The nuclei of megakaryocytes are bulky with lobuli becoming clumsy, and the irregular roundish nuclear forms give rise to the so-called cloud-like nuclei, which are almost never observed in ET and PV (Table 6).[40,41,43,54] The Cologne criteria of IMF (AMM) have been established with the intention to introduce bone marrow morphology as a specific diagnostic guideline for the unequivocal distinction between thrombocythemia as the presenting feature of prefibrotic or early IMF and true ET.[12,43] A new set of clinical and pathological criteria for the prefibrotic and the various clinical and fibrotic stages of IMF/AMM is given in Table 6.[54] The clinicopathological staging in Table 6 surely will not be congruent with the prognostic clinical scoring system in Table 5. Prefibrotic stages account for about 20–25% of all IMF/AMM cases.[67,71] A significant number of patients with early IMF/AMM (ie without or only minor reticulin fibrosis) may present with pronounced thrombocythemia in excess of 600×10^9–1000×10^9/l and therefore by using the PVSG clinical criteria alone could display signs and symptoms in accordance with those for the diagnosis of ET (false ET).[12,67,71] Comparing the PVSG[14,15] and the pathological WHO[54] criteria (according to Table 6), ET was diagnosed in 483 patients according to the PVSG, but on applying the WHO criteria only 162 could be diagnosed as true ET, and 321 were diagnosed as prefibrotic or early fibrotic IMF.[71] It is noteworthy that the differentiation between true ET (thrombocythemia vera) and thrombocythemia as the presenting feature of prefibrotic or early fibrotic AMM (false ET) exerts a significant impact on prognosis, since loss in life expectancy was minor (8.9%) in true ET, but ranged up to about 32.3% in false ET as the initial presentation of AMM/IMF accompanied by pronounced thrombocythemia.[71]

In conclusion, the proposed European clinical and pathological (ECP) criteria for the proper diagnosis and differential diagnosis of ET, PV and prefibrotic AMM/IMF (Tables 2, 3 and 6) surely will avoid possible diagnostic pitfalls in the discrimination of true ET from thrombocythemias associated with or accompanying PV, IMF/AMM, Ph^+ CML or MDS. In particular, bone marrow histopathology unequivocally separates true ET (Table 2) from thrombocythemia associated with prefibrotic stages of AMM/IMF (Table 6). The use of biological markers, like spontaneous EEC, PRV-1 expression, etc. should be validated in large prospective multicenter studies of newly diagnosed and previously treated MPD patients using the proposed ECP criteria as the only gold standard currently available for the proper diagnosis and differential diagnosis of ET, PV and IMF/AMM.

Table 5 The Rotterdam prognostic clinical scoring system of patients with AMM or IMF[48]

Prognosis Group	*Criteria*	*Score*	*Clinical stage*
Very favorable	Hemoglobin normal >12 g/dl, no anemia., no fibrosis of the bone marrow: EMGM	Score 0	Very early
Favorable	Hemoglobin >10 g/dl, slight anemia	Score 1	Early
Intermediate	Hemoglobin <10 g/dl, anemia but no other adverse sign[a]	Score 2	Overt
Unfavorable	Two adverse signs[a]:	Score 3	Advanced
Very unfavorable	More than two adverse signs[a]	Score 4	Endstage

[a]Hemoglobin <10 g/dl, blast PB >1%, precursors WBC in PB >10%, serum LDH >3 times upper limit, leukopenia <3, leukocytose >20 or >30, thrombocytopenia $<100 \times 10^9$/l, constitutional symptoms, massive splenomegaly, cytogenetic abnormality.

Table 6 ECP criteria for the diagnosis of AMM or IMF[54]

Clinical criteria	*Pathological criteria*
(A1) No preceding or allied other subtype of myeloproliferative disorders CML or MDS.	B1 Megakaryocytic and granulocytic myeloproliferation and relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant to medium sized megakaryocytes containing clumsy (cloud-like) lobulated nuclei and definitive maturation defects.
C:Clinical stages	Staging of idiopathic myelofibrosis (IMF)
(C1) Early clinical stages	MF 0: *Prefibrotic stage IMF:* no reticulin fibrosis
Normal hemoglobin or slight anemia, grade I: hemoglobin >12 g/dl	MF 1: *Early IMF:* slight reticulin fibrosis
Slight or moderate splenomegaly on palpation or >11 cm on ultrasound scan or CT	MF 2: *Manifest IMF:* marked increase in reticulin and slight to moderate collagen fibrosis
Thrombocythemia, platelets in excess of 400, 600 or even 1000×10^9/l	MF 3: *Overt IMF:* advanced collagen fibrosis
(C2) Intermediate clinical stage	MF >3: *Osteosclerosis (endophytic bone formation) and decreased cellularity*
Anemia grade II: hemoglobin >10 g/dl	
Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes Splenomegaly	
No adverse signs[a]	
(C3) Advanced clinical stage	
Anemia grade III: hemoglobin <10 g/l plus	
One or more adverse signs[a]	

[a]Age >70 years, hemoglobin <10 g/dl, myeloblasts PB $>2\%$, erythro-normoblasts PB $>2\%$, leukocytosis $>20 \times 10^9$/l, thrombocytopenia $<300 \times 10^9$/l, severe constitutional symptoms, massive splenomegaly, cytogenetic abnormalities.
[1]The combinations of A1 + B1 establish IMF – any other criterion confirms IMF/AMM.
[2]Four clinicopathological stages of AMM/IMF: (1) prefibrotic AMM/IMF: A1, B1, C1 + MF 0, (2) early AMM/IMF: A1, B1, C1 + MF 1, (3) manifest AMM/IMF: A1, B1, C2 + MF 2 or 3 (4) full-blown, endstage AMM/IMF: A1, B1, C3 + MF 3 or >3.

References

1 Dameshek W. Some speculations on the myeloproliferative disorders. *Blood* 1954; **6:** 372–375.
2 Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton D, Gralnick HR *et al*. Proposal for the classification of the acute leukemias. *Br J Haematol* 1976; **33:** 451–459.
3 Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton D, Gralnick HR *et al*. Proposal revised criteria for the classification of acute leukemias. *Ann Intern Med* 1985; **103:** 626–629.
4 Michiels JJ. Erythroleukemia and myelodysplastic syndromes: a historical appraisal and a personal view. *Leuk Lymph* 1993; **9:** 27–34.
5 Gilbert HS. The spectrum of myeloproliferative disorders. *Med Clin North Am* 1973; **57:** 355–393.
6 Laszlo J. Myeloproliferative disorders (MPD). *Semin Hematol* 1975; **12:** 409–432.
7 Michiels JJ, Prins ME, Hagemeijer A, Brederoo P, van de Meulen J, van Vliet HHDM. Philadelphia chromosome positive essential thrombocythemia and megakaryoblast leukemia. *Am J Clin Pathol* 1987; **88:** 645–652.
8 Michiels JJ, Berneman ZN, Schroyen W, Kutti J, Swolin B, Ridell B. Philadelphia (Ph) chromosome positive thrombocythemia without features of chronic myeloid leukemia in peripheral blood: natural history and diagnostic differentiation from Ph^- negative essential thrombocythemia. *Ann Hematol*. (in press).
9 Lundin PM, Ridell B, Weinfeld A. The significance of bone marrow morphology for the diagnosis of polycythemia vera. *Scand J Haematol* 1972; **9:** 271–282.
10 Georgii A, Vykoupil KF, Thiele J. Chronic megakaryocytic granulocytic myelosis – CMGM. *Virch Arch A Path Anat Histol* 1980; **389:** 253–268.
11 Georgii A, Vykoupil KF, Buhr Th, Choritz H, Döhler U, Kaloutsi V *et al*. Chronic myeloproliferative disorders in bone marrow biopsies. *Pathol Res Pract* 1990; **186:** 3–27.
12 Thiele J, Kvasnicka HM, Werden C, Zankovich R, Diehl V, Fischer R. Idiopathic primary osteo-myelofibrosis: a clinico-pathological study on 208 patients with special emphasis on evolution of disease features, differentiation from essential thrombocythemia and variables of prognostic impact. *Leuk Lymph* 1996; **22:** 303–317.
13 Yan L, Elkassar N, Gardin C, Briere J. Clonality assays and megakaryocyte culture techniques in essential thrombocythemia. *Leuk Lymph* 1996; **22:** 31–40.
14 Harrison CH, Gale RE, Machin SI, Linch DC. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. *Blood* 1994; **83:** 744–748.
15 Murphy S, Iland H, Rosenthal D, Laszo J. Essential thrombocythemia: an interim report from the Polycythemia Vera Study Group. *Semin Hematol* 1986; **23:** 177–182
16 Berlin NI. Diagnosis and classification of polycythemia. *Semin Hematol* 1975; **12:** 339–351.
17 Prchal JF, Axelrad AA. Bone marrow responses in polycythemia vera. *N Engl J Med* 1974; **290:** 1382.
18 Westwood NB, Pearson TC. Diagnostic applications of haematopoietic progenitor culture techniques in polycythaemias and thrombocythaemias. *Leuk Lymph* 1996; **22** (Suppl 1):95–103.
19 Dobo I, Donnard M, Girodon F, Mossuz P, Boiret N, Boukhari R *et al*. Standardization and comparison of endogenous erythroid colony assays performed with bone marrow and blood progenitors for the diagnosis of polycythemia vera. *Hematol J*. (in press).
20 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. *Br J Haematol* 1993; **83:** 192–197.

21 Florensa L, Bessis C, Woessner S, Sole F, Acin P, Pedro C *et al*. Endogenous megakaryocyte and erythroid colony formatio from blood in essential thrombocythemia. *Leukemia* 1995; **9:** 271–273.
22 Jantunen R, Juvonen E, Ikkala E, Oksanen K, Anttila P, Jormila P *et al*. Essential thrombocythemia at diagnosis: causes of diagnostic evaluation and presence of positive diagnostic findings. *Ann Hematol* 1998; **77:** 101–106.
23 Emmanual PD, Bates JL, Castleberry RP, Gualtieri RJ, Zuckerman KS. Selective hypersensitivity to granulocyte–macrophage colony-stimulating factor by juvenile chronic myeloid leukemic hematopoietic progenitors. *Blood* 1991; **77:** 925–929.
24 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.
25 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.
26 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.
27 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.
28 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** (Suppl 1): 2866.
29 Temerinac S, Klippel S, Strunck E, Röder S, Lübbert M, Lange M *et al*. Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. *Blood* 2000; **95:** 2569–2576.
30 Pahl HL. Towards a molecular understanding of polycythemia rubra vera. *Eur J Biochem* 2000; **267:** 3395–3401.
31 Klippel S, Strunck E, Termerinac S, Bench AJ, Meinhardt G, Mohr U *et al*. Quantification of PRV-1 mRNA distinguishes polycythemia vera from secondary erythrocytosis. *Blood* 2003; **102:** 3569–3574.
32 Pahl HL. Molecular markers in MPDs. Proceedings first Como workshop on the molecular pathogenesis and genetics of myeloproliferative disorders. September 2002 Como, Italy.
33 Teofili L, Martini M, Luongo M, Di Mario A, Leone G, De Stefano V. Overexpresion of the polycythemia vera rubra-1 gene in essential thrombocythemia. *J Clin Oncol* 2002; **20:** 4249–4264.
34 Harrison CN, Gale RE, Pezella F, Mire-Sluis A, Machin SJ, Linch DC. Platelet c-mpl expression is dysregulated in patients with essential thrombocythemia but this is not of diagnostic value. *Br J Haematol* 1999; **107:** 139–147.
35 Moliterno AR, Hankins WD, Spivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. *N Engl J Med* 1998; **338:** 572–580.
36 Moliterno AR, Spivak JL. Posttranslational processing of the thrombopoietin receptor is impaired in polycythemia vera. *Blood* 1999; **94:** 2556–2561.
37 Skoda RC. Molecular pathogenesis of polycythemia vera and essential thrombocythemia. *Hematol J* 2002; **3** (Suppl): 11–14.
38 Kralovics R, Buser AS, Teo SS, Coers J, Tichelli A, van der Maas APC *et al*. Comparison of molecular markers in a cohort of patients with chronic myeloproliferative disorders. *Blood* 2003; **102:** 1869–1871.
39 Bock O, Serinsoz E, Neusch M, Schlué J, Kreipe H. The polycythaemia rubra vera-1 gene is constitutively expressed by bone marrow cells and does not discriminate polycythaemia vera from reactive and other chronic myeloproliferative disorders. *Br J Haematol* 2003; **123:** 472–474.
40 Georgii A, Buhr T, Buesche G, Kreft A, Choritz H. Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. *Leuk Lymph* 1996; **22** (Suppl 1): 15–29.
41 Georgii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. *Ballieres Clin Haematol* 1998; **11:** 721–749.
42 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.
43 Thiele J, Kvasnicka HM, Diehl V, Fischer R, Michiels JJ. Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disorders by histopathology, histochemistry and immunostaining from bone marrow biopsies. *Leuk Lymph* 1999; **33:** 207–218.
44 Thiele J, Kvasnicka HM, Zankovich R, Diehl V. Relevance of bone marrow features in the differential diagnosis between essential thrombocythemia and early stage myelofibrosis. *Haematologica* 2000; **85:** 17–21.
45 Schmitt-Graeff A, Thiele J, Zuk I, Kvasnicka HM. Essential thrombocythemia with ringed sideroblasts: a heterogeneous spectrum of diseases, but not a distinct entity. *Haematologica* 2002;**87:**392–399.
46 Kurnicke JE, Ward HP, Block MH. Bone marrow biopsy in the differential diagnosis of polycythemia. *Arch Pathol* 1972; **94:** 489–499.
47 Thiele J, Kvasnicka HM, Muehlhausen K, Walter S, Zankovich R, Diehl V. Polycythemia vera versus secondary polycythemia: a clinicopathological evaluation of distinctive features in 199 patients. *Pathol Res Pract* 2001; **197:** 77–84.
48 Michiels JJ, Kutti J, Stark P, Bazzan M, Gugliotta L, Marchioli R *et al*. on behave of the European Working Group on Myeloproliferative Disorders (EWG.MPD). Diagnosis, pathogenesis and treatment of the myeloproliferative disorders essential thrombocythemia, polycythemia vera and essential megakaryocytic granulocytic metaplasia and myelofibrosis. *Neth J Med* 1999; **54:** 46–62.
49 Juvonen E, Ikkala E, Fyhrquist F, Ruutu T. Autosomal dominant erythrocytosis caused by increased sensitivity to erythropoietin. *Blood* 1991; **78:** 3066–3069.
50 De la Chapelle A, Träskelin AL, Juvonen E. Truncated erythropoietin receptor causes dominantly inherited benign human erythrocytosis. *Proc Natl Acad Sci USA* 1993; **90:** 4495–4499.
51 Ang SO, Chen H, Hirota K, Gordeuk V, Jrlinek J, Guan Y *et al*. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat Genet*, November 2002, on-line.
52 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.

53 Imbert M, Pierre R, Thiele J, Vardiman JW, Brunning RD, Flandrin G. Essential thrombocythemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). *WHO Classification of Tumours: Tumours of Haematopoietic and Lymphoid Tissues*. IARC Press, Lyon, France; 2001; pp 39–41.
54 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.
55 Wasserman LR. Polycythemia vera, its course and treatment. Relation to myeloid metaplasia and leukaemia. *Bull NY Acad Med Sci* 1954; **30:** 343–375.
56 Pierre R, Imbert M, Thiele J, Vardiman JW, Brunning RD, Flandrin G. Polycythemia vera. In: Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). *WHO Classification of Tumours: Tumours of Haematopoietic and Lymphoid Tissues*. IARC Press: Lyon, France; 2001; pp 32–38.
57 Michiels JJ, Barbui T, Finazzi G, Fruchtman SM, Kutti J, Rain JD *et al*. Diagnosis and treatment of polycythemia vera and possible future study designs of the PVSG. *Leuk Lymph* 2000; **36:** 239–253.
58 Pearson TC, Wetherley-Mein G. The course and complications of idiopathic erythrocytosis. *Clin Lab Haematol* 1979; **1:** 189–196.
59 Najean Y, Triebel F, Dresch C. Pure eythrocytosis: reappraisal of a study of 51 cases. *Am J Hematol* 1979; **10:** 129–136.
60 Kiladjian JJ, Gardin C, Renoux M, Bruno F, Bernard JF. Long-term outcomes of polycythemia vera patients treated with pipobroman as initial therapy. *The Haematol J* 2003; **4:** 198–207.
61 Tefferi A. Myelofibrosis with myeloid metaplasia. *N Engl J Med* 2000; **342:** 1255–1265.
62 Varki AJIT, Lottenberg R, Griffith R, Reinhard E. The syndrome of idiopathic myelofibrosis. *Medicine* 1983; **62:** 353–371.
63 Njoku OS, Lewis SM, Catovsky D, Gordon-Smith EC. Anaemia in myelofibrosis: its value in prognosis. *Br J Haematol* 1983; **54:** 79–89.
64 Visani G, Finelli C, Castelli U, Petti MC, Ricci P, Vianelli N *et al*. Myelofibrosis with myeloid metaplasia: clinical and haematological parameters predicting survival in a series of 133 patients. *Br J Haematol* 1990; **75:** 4–9.
65 Hasselbalch H, Jenssen BA. Prognostic factors in idiopathic myelofibrosis: a simple scoring system with prognostic significance. *Eur J Haematol* 1990; **44:** 172–178.
66 Dupriez B, Morel P, Demory J-L, Lai JL, Simon M, Plantier I *et al*. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. *Blood* 1996; **88:** 1013–1018.
67 Kvasnicka HM, Thiele J, Werden C, Zankovich R, Diehl V, Fischer R. Prognostic factors in idiopathic (primary) osteomyelofibrosis. *Cancer* 1997; **80:** 708–719.
68 Cervantes F, Pereira A, Esteve J, Rafel M, Cobo F, Rozman C *et al*. Identification of 'short-lived' and 'long-lived' patients at presentation of idiopathic myelofibrosis. *Br J Haematol* 1997; **97:** 635–640.
69 Cervantes F, Pereira A, Esteve J, Cobo F, Rozman C, Monserrat E. The changing profile of idiopathic myelofibrosis: a comparison of the presenting features of patients diagnosed in two different decades. *Eur J Haematol* 1998; **60:** 101–105.
70 Reilly JT, Snowden JA, Spearing RL, Fitzgerald PM, Jones N, Watmore A *et al*. Cytogenetic abnormalities and their prognostic significance in idiopathic myelofibrosis: a study of 106 cases. *Br J Haematol* 1997; **98:** 96–102.
71 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.