

blood

Prepublished online Apr 9, 2008;
doi:10.1182/blood-2007-12-128454

The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis and primary myelofibrosis: an alternative proposal

Jerry L. Spivak and Richard T. Silver

Information about reproducing this article in parts or in its entirety may be found online at:
http://bloodjournal.hematologylibrary.org/misc/rights.dtl#repub_requests

Information about ordering reprints may be found online at:
<http://bloodjournal.hematologylibrary.org/misc/rights.dtl#reprints>

Information about subscriptions and ASH membership may be found online at:
<http://bloodjournal.hematologylibrary.org/subscriptions/index.dtl>



The revised World Health Organization diagnostic criteria for polycythemia
vera, essential thrombocytosis and primary myelofibrosis: An alternative
proposal

Jerry L. Spivak and Richard T. Silver

Johns Hopkins University School of Medicine and Weill Cornell Medical College

Short title: Diagnostic criteria for myeloproliferative disorders

Corresponding Author
Jerry L. Spivak, MD
Johns Hopkins University School of Medicine,
Traylor 924
720 Rutland Avenue
Baltimore MD 21205
410-955-5454
410-614-0854 (FAX)
jlspivak@jhmi.edu

Scientific heading: Myeloproliferative Disorder

In its August 15th issue, *Blood* published a proposal for revision of the World Health Organization (WHO) diagnostic criteria for the chronic myeloproliferative disorders (MPD) polycythemia vera (PV), essential thrombocytosis (ET) and primary myelofibrosis (PMF) ¹. Algorithms based on these diagnostic criteria were subsequently published in *Leukemia* ². Ostensibly prompted by newly described MPD molecular abnormalities, the proposed revision was both timely and appropriate. The initial WHO diagnostic criteria for these disorders, published in 2001 ^{3,4}, were never prospectively evaluated and subsequently were invalidated ⁵. The discovery of JAK2 ⁶⁻⁶ and MPL ⁷⁻⁹ gene mutations not only provided new insights into the molecular basis of the MPD, but also new molecular approaches to their diagnosis. Unfortunately, the proposed guideline revision and the attendant algorithms not only recapitulated all the faults of the initial WHO diagnostic criteria, but also failed to capitalize on the biological insights and opportunities offered by these newly discovered mutations to improve diagnostic accuracy. This was because the proposed revision eschewed the fundamental tenets of evidence-based medicine ¹⁰⁻¹². The purpose of this review, therefore, is to offer an alternative perspective of the diagnostic approach to PV, ET and PMF to enable clinicians to select the appropriate diagnostic tests for a particular MPD within the context of their own practices.

The Back Story

The MPD are neither new nor rare diseases but they continue to confound physicians diagnostically for reasons that are many and cogent. While not rare, the MPD are sufficiently uncommon that most physicians see few such patients, and since disease duration for the MPD is typically measured in decades, physicians rarely have the opportunity to observe their full natural history. Importantly in this regard, the initial clinical manifestations of the MPD are

highly variable and their clinical phenotypes are also subject to change with time. These disorders not only mimic each other phenotypically but many other benign and malignant blood disorders as well. For example, PV can present as isolated erythrocytosis¹³, leukocytosis¹⁴, thrombocytosis^{15,16} (**Figure 1a**) or even myelofibrosis^{17,18}, while isolated thrombocytosis is the presenting feature in approximately 20 % of PMF patients¹⁹. In addition, myelofibrosis is a well-recognized feature of PV²⁰⁻²² and erythrocytosis can develop in PMF during the course of the illness²³ (**Figure 1b**).

William Osler was not the first to identify PV as a distinct clinical entity²⁴, but he was the first to recognize its capacity for phenotypic mimicry and devised diagnostic criteria that addressed the problem²⁵. The Polycythemia Vera Study Group (PVSG) subsequently expanded Osler's diagnostic criteria²⁶ and, as new knowledge was obtained, other groups formulated diagnostic criteria for PV^{27,28}, ET²⁹ and PMF³⁰, but to date there has been no uniformly agreed upon set of diagnostic criteria, or at least not one, according to recent surveys^{31,32}, to which most clinicians strictly adhere.

In 2001, the WHO attempted to fill this void with a new MPD classification, grouping PV, ET and PMF together with chronic myelogenous leukemia (CML), chronic neutrophilic leukemia, chronic eosinophilic leukemia, the hypereosinophilic syndrome and unclassifiable myeloproliferative disorders under the rubric of "the chronic myeloproliferative diseases"³². The rationale was to apply to the MPD the WHO REAL (Revised European-American Lymphoma) classification paradigm that had been successfully employed for lymphoid and myeloid neoplasms. The REAL scheme combines morphology, genotype, immunophenotype and clinical phenotype to define distinct clinical entities³. The principles espoused by the WHO

were both laudable and appropriate, but only to the extent that the REAL classification paradigm used for other hematologic neoplasms, was applicable to the MPD; unfortunately, this proved to be limited. The WHO also provided diagnostic criteria for the MPD and these soon proved to be problematic ⁵.

The Problem

The WHO MPD diagnostic classification was based on the shared features of “myeloproliferation” with relatively normal maturation, and a tendency to extramedullary hematopoiesis that characterize the various MPD ³. The shared feature of myeloproliferation, however, was more apparent than real, since with respect to the involved stem cell, PV, ET and PMF are actually disorders of myeloaccumulation, not myeloproliferation ³³⁻³⁶. In this regard, in contrast to the other “myeloproliferative” disorders, survival with PV, ET or PMF, even with supportive therapy alone, is usually measured in decades, and transformation to acute leukemia is much less common and often treatment-related. Beyond these characteristics, PV, ET and PMF share more in common genotypically and phenotypically with each other than they do with the other myeloproliferative disorders with which they have been classified ⁴, and on this basis alone they merited a separate classification. This contention was solidified recently by the discovery of JAK2 ³⁷⁻⁴⁰ and MPL gene mutations ⁷⁻⁹ in MPD patients. Indeed, considering their genotypic similarities and the tendency for each disorder to acquire the phenotypic characteristics of the others, it is worth asking if PV, ET and PMF are separate diseases, different manifestations of the same disease or a combination of both, and current molecular evidence supports the last possibility ^{19,36,41}.

The REAL paradigm, while useful for distinguishing and classifying lymphoid and many

myeloid neoplasms, was not appropriate for PV, ET and PMF. These three disorders share in common the following features: origin in a multipotent hematopoietic progenitor cell, relatively normal cellular maturation, phenotypic and genotypic mimicry, and a tendency to evolve into each other or develop myelofibrosis. They also do not have unique immunophenotypes. Therefore, none of the REAL paradigm tenets, alone or together, were diagnostically useful.

Nevertheless, the WHO based their diagnostic criteria on morphology, and with respect to PV, as a surrogate for red cell mass and plasma volume studies, substituted hemoglobin values of >18.5 gm/dL in men and >16.5 gm/dL in women, or > 99th percentile of the chosen method-specific reference range for age, sex and altitude of residence⁴. However, no data were offered to support the substitution of specific hemoglobin values as a surrogate for direct measurement of the red cell mass. Ostensibly, the hemoglobin reference standards were for those physicians who lacked access to a nuclear medicine facility but proof that such values were clinically meaningful was not provided.

Considering the many phenotypic similarities between PV, ET and PMF, what should have been most important was defining their differences. With respect to laboratory characteristics, only erythrocytosis sets PV apart from its companion MPD, while during the early stages of the disease, an increase in circulating CD34+ cells is characteristic for PMF, although this is not uniformly so^{42,43}. Importantly, ET has no specific clinical or laboratory characteristics that distinguish it from PV. Therefore, considering that trilineage involvement is the ultimate possible phenotype for an MPD arising in a multipotent hematopoietic stem cell, recommendation of an accurate method for detecting absolute erythrocytosis should have been mandatory. Unfortunately, the WHO alternatives to direct measurement of the red cell mass proved to be inadequate because of the erroneous assumption that in an MPD patient, a

normal hemoglobin or hematocrit level signified that the red cell mass was normal.

In 2005, Johansson and colleagues challenged the WHO assertion that only hemoglobin levels greater than 18.5 gm/dL (hematocrit > 55.5 %) in a man or greater than 16.5 gm/dL (hematocrit > 49.5 %) in a woman (or the equivalent > 99th percentile of the method-specific reference value) established the presence of absolute erythrocytosis. They applied the WHO criteria to 77 PV patients and 66 patients with apparent erythrocytosis, all of whom had direct red cell mass and plasma volume measurements⁵. They found that the WHO criteria identified absolute erythrocytosis in only 35% of the male PV patients and 63 % of the women, while 14 % of the men and 35 % of the women without erythrocytosis were branded as having it (**Figure 2**).

Moreover, the degree to which the WHO hemoglobin criteria failed was actually worse than it appeared, since by direct measurement, the red cell mass is not considered elevated *unless it is 125 % of normal*⁴⁴. This not only enhances the specificity of the test, it also indicated that the WHO recommendations lacked sensitivity. Surprisingly, these objective data were omitted in the revised WHO diagnostic recommendations, which remain unchanged¹. The specificity of the WHO PV diagnostic criteria was also challenged by the British Committee for Standards in Haematology⁴⁵.

Blood Volume Physiology

Most discussions of the blood volume emphasize the important direct and exponential relationship between hematocrit and blood viscosity as it occurs in large vessels^{46,47}.

However, the emphasis should really be on the behavior of blood flow in arterioles, capillaries and venules. In these small vessels, the ratio of vessel surface area to its volume is greatest and the exposure of the blood to the frictional drag of the vessel wall is maximal. Thus, the flow of plasma nearest the vessel wall is retarded compared with the flow of red cells at the vessel center⁴⁸. Because of this, there are always fewer red cells in these small vessels and as a consequence, the volume of distribution of red cells in the circulation differs from that of plasma. Since the microvasculature comprises almost 20 % of the circulatory system⁴⁹, the hematocrit of blood taken from a peripheral artery or vein will not accurately reflect the total body hematocrit⁵⁰.

From a practical perspective, this has important ramifications with respect to the potential for organ-specific thrombosis when the red cell mass is increased. First, the hematocrit is not uniform in all organs, being highest in the spleen and liver and lowest in the brain, bowel and kidneys⁴⁹. Second, normally, whenever there is an hypoxia-induced increase in erythropoiesis, there is a reciprocal decrease in the plasma volume⁵¹⁻⁵⁴. This is also true when red cell transfusions are given⁵⁵ and has the effect of maintaining a normal blood volume at the expense of an increase in peripheral vascular resistance (**Table 1**). In PV, however, the plasma volume usually does not shrink with the development of erythrocytosis and may even expand, particularly in women (**Table 1**), masking the absolute increase in red cell mass^{35,56-58}. Thus, it is not surprising that the WHO hemoglobin or hematocrit guidelines were invalid.

There are also other important implications of red cell mass and plasma volume determinations not addressed by the latest WHO recommendations. First, a high hematocrit is not synonymous with erythrocytosis any more than a normal hematocrit is synonymous with

the absence of erythrocytosis when PV is a diagnostic consideration³⁵. A high hematocrit can be simply due to plasma volume contraction (**Table 1**). Indeed, unless the hematocrit is $\geq 60\%$ in a man or woman, it is not possible to distinguish plasma volume contraction from absolute erythrocytosis⁵⁹. It was for this reason that the PVSG stipulated that direct determination of the red cell mass and plasma volume should be an integral part of the evaluation of a high hematocrit²⁶. The clinical problem of plasma volume contraction is not trivial⁶⁰⁻⁶³, and there is no excuse for ignoring this group of patients.

We recognize the WHO concern that some physicians may not have access to red cell mass and plasma volume measurements because of economic or geographic considerations. In this regard, the assumption that JAK2 mutation assays are not currently subject to the same constraints is also erroneous. From our perspective, in developed countries, it is a deviation from standard of care if these tests are not available, at the very least, in major academic centers. For other circumstances, we offer some alternatives. First, microcytic erythrocytosis is an important clue to the presence of an increased red cell mass (**Figure 3**)⁶⁴. Second, since it is necessary for the red cell mass to be greater than 125 % of normal to qualify for absolute erythrocytosis, phlebotomy can be diagnostic as well as therapeutic. If absolute erythrocytosis is suspected, there should be a minimum excess of ~ 700 mL of red cells in either a man or woman. If reduction of the hematocrit to less than 45 % in a man or less than 42 % in a woman requires two or more phlebotomies, absolute erythrocytosis can be assumed.

A further important PVSG stipulation was that after such a phlebotomy trial, the hematocrit should increase by at least 10 % within 3 months in the absence of iron deficiency⁶⁵. These criteria should ensure that physicians without access to a nuclear medicine facility can achieve diagnostic accuracy; they should not, however, be used as surrogates for direct red cell mass

and plasma volume measurements when available, since not all patients with a high hemoglobin or hematocrit have an elevated red cell mass, while many MPD patients with a supposedly normal hematocrit or hemoglobin level do ^{56,57}. Finally, it should be emphasized that red cell mass and plasma volume determinations only establish the presence of erythrocytosis, *not its cause*.

JAK2 V617F

The discovery of the JAK2 V617F mutation ⁶⁻¹⁰ was the most important advance in the study of the MPD since the demonstration thirty years ago that these were clonal disorders involving a multipotent hematopoietic stem cell ^{66,67}. JAK2 is the cognate tyrosine kinase of the erythropoietin and thrombopoietin receptors and also the obligate chaperone responsible for their cell surface expression ^{68,69}; The substitution of phenylalanine for valine (V617) in the regulatory JH2 domain of JAK2 leading to constitutive kinase activation, occurs in approximately 95 % of PV patients and in approximately 50 % of PMF and ET patients ^{41,70,71}. Indeed, this mutation explains many of the clinical and laboratory features shared by these three disorders, although it does not appear to be the initiating mutation ^{36,72-74}.

How one mutation could be responsible for three different clinical phenotypes is still unresolved, but in vitro clonal assays ³⁴, animal models ⁷⁵, and studies quantifying the JAK2 V617F neutrophil allele burden in MPD patients ^{41,76,77} indicate that both gene dosage and gender have roles. In ET, in which females predominate, the JAK2 V617F neutrophil allele burden is usually low ⁴¹ and isolated thrombocytosis is the rule, whereas in PV, the higher neutrophil allele burden was associated with higher hematocrit and leukocyte counts, a lower platelet count, splenomegaly and pruritus ⁷⁸. As a corollary, some ET patients with a rising

neutrophil allele burden transform over time to PV (**Figure 1a**) or PMF, although JAK2 V617F expression is not mandatory for this to occur⁷⁷. Importantly, ET patients expressing JAK2 V617F also appear to have a “PV-like” phenotype compared to their JAK2 V617F-negative counterparts, even to the extent of having an increased incidence of venous thrombosis⁷⁹. **Figure 4** illustrates quantitative neutrophil JAK2 V617F allelic burdens in ET, PV and PMF patients⁴¹, demonstrating not only their important differences but also the significant overlap that occurs, presumably accounting in part for their phenotypic mimicry.

Given these data, since PV, PMF and ET all arise in a multipotent hematopoietic stem cell and the JAK2 V617F mutation appears to cause a “PV-like” phenotype in ET patients, it is illogical not to consider the possibility of PV when evaluating a patient with thrombocytosis. Indeed, based on a recent publication by Cassinat et al.,⁸⁰ the new WHO dictum that red cell mass and plasma volume studies *are not required* in the evaluation of isolated thrombocytosis¹ is invalid. These authors found that red cell mass and plasma volume determinations identified erythrocytosis in 46 % of patients initially considered to have ET by the WHO hemoglobin criteria, but when the JAK2 V617F mutation was present, the proportion rose to 64 %⁸⁰. An example of such a patient is provided in **Table 1**.

Although the WHO correctly concluded that a JAK2 V617F assay is an important diagnostic test in the evaluation of an MPD, no guidance was provided with respect to test sensitivity and specificity. For example, in the Cassinat study cited above⁸⁰, the JAK2 V617F assay had a sensitivity of 86 % when the red cell mass was elevated but its specificity was only 64 %. It is now clear that a positive JAK2 V617F assay is not diagnostic for a particular MPD, and assay-positive patients with isolated erythrocytosis have been identified, who have not evolved to classical PV^{9,81}. Finally, since JAK2 V617F is responsible for many of the biochemical

abnormalities associated with the MPD, it is unclear why the WHO still requires a serum erythropoietin level or the iconic but usually unobtainable endogenous erythroid colony assay for diagnostic purposes if JAK2 V617F is present ².

Bone Marrow Morphology as a Diagnostic Test

It is a stated WHO concern that bone marrow morphology was not optimally used as a diagnostic tool by the PVSG ³. However, this concern ignores a careful PVSG analysis of bone marrow morphology in 281 PV patients followed for over 9 years ¹⁷. In this study 13 % of patients did not have increased marrow cellularity at diagnosis; megakaryocyte hyperplasia correlated with marrow cellularity, and 11 % of patients had a moderate to marked increase in reticulin early in their disease that had no bearing on prognosis. Over 90 % of a parallel series of untreated ET patients had marrow cellularity greater than 50 % together with megakaryocyte hyperplasia ⁸². Thus, the PVSG concluded that marrow morphology was an inadequate tool for distinguishing PV from ET or PMF and stipulated the use of a red cell mass determination for this purpose ²⁶.

In 2001, the WHO also endorsed the concept of prefibrotic PMF and provided diagnostic criteria for it ^{3,4} but no prospective study validating these criteria was ever conducted. Indeed, the proposed morphologic criteria were based entirely on retrospective studies claiming that marrow histology could distinguish the “prefibrotic cellular phase” of PMF from “true” ET ⁸³. Interestingly, when these criteria were applied in an unblinded fashion to 116 ET patients, 70 % were reclassified as having PMF, an epidemiologically implausible outcome ⁸⁴ that led to concern about the usefulness of marrow morphology as a diagnostic tool ⁸⁵. Nevertheless,

marrow morphology was inexplicably given a dominant role in the latest WHO diagnostic algorithms^{1,2}

The need to assess 17 different histologic features was a major but unstated problem of the WHO prefibrotic PMF morphologic criteria, and one not easily translated to routine pathology practice, violating the WHO REAL paradigm³. These criteria have now been refuted by a study demonstrating substantial interobserver variability for 16 of the 17 histologic features, a correlation of marrow cellularity with JAK2 V617F status, and no difference in clinical phenotype and prognosis amongst ET patients with respect to the presence or absence of a “prefibrotic myelofibrosis” histology⁸⁶.

The conclusion from these observations together with what is known clinically about the three MPD is clear: PV, ET and PMF are not static illnesses but evolve over time and their marrow morphology will reflect the particular stage in their evolution. Since the clonal burden in these disorders expands with time, and is driven by JAK2 V617F in most PV patients and approximately 50 % of ET and PMF patients, MPD marrow morphology is not only a moving target but also nonspecific with respect to phenotype, in contrast to lymphomas, and acute myeloid malignancies.

Serum Erythropoietin

With the discovery of erythropoietin, it was expected that the serum erythropoietin level would permit differentiation between hypoxic and autonomous erythrocytosis. Unfortunately, because

erythropoietin is metabolized by its target cells⁸⁷ and erythropoietin production is suppressed by erythrocytosis⁸⁸ or an increase in blood viscosity⁸⁹, an increase in the red cell mass is associated with down regulation of erythropoietin production unless hypoxia is severe⁸⁸. Thus, many patients with hypoxic erythrocytosis have a normal serum erythropoietin level³⁵. While it is true that the lowest serum erythropoietin levels are found in PV⁹⁰, not only is the level often normal, but ET patients can have a similarly low serum erythropoietin level for the same hematocrit level⁹¹. Thus, a normal serum erythropoietin level does not exclude PV as a diagnosis and a low serum erythropoietin level is not specific for it⁹². Since erythrocytosis is a consequence of JAK2 V617F expression, a positive assay for this mutation in the presence of documented erythrocytosis makes the serum erythropoietin assay redundant for the diagnosis of PV.

Endogenous Erythroid Colony Formation (EEC)

The seminal discovery that PV erythroid progenitors could proliferate in vitro in the absence of erythropoietin defined the autonomous nature of this disorder⁹³. While EEC are not limited to PV^{94,95}, their presence in association with erythrocytosis confirms its autonomous nature⁹⁵. Unfortunately, this test was never standardized for clinical use, which is not a trivial issue with respect to its interpretation⁹⁶, and it is usually only available in research laboratories. Given the fact that EEC formation in PV is not dependent on JAK2 V617F expression⁷⁴, this assay provides no additional information, and since EEC can be observed in ET patients^{97,98}, the assay is not specific and should be considered a research tool of historic interest.

Alternatives to the WHO Diagnostic Guidelines and Algorithms

Given the considerations discussed above, we now wish to individually address the proposed WHO diagnostic guidelines and algorithms for PV, ET and PMF and offer alternative guidelines based on physiologic principles. There are several issues central to the diagnosis of these disorders not considered by the WHO. First, given greater access to medical care and the use of electronic cell counters, MPD patients are being recognized at a younger age and earlier in the course of their disease, challenging the classical clinical and laboratory phenotypes. Second, there is no single or simple method for establishing the diagnosis of an MPD because their clinical manifestations are so pleomorphic and overlapping. Therefore, the diagnostic approach must be tailored to the patient yet not be so complex to tax physician resources.

Polycythemia Vera

There are five major considerations with respect to the diagnosis of PV: first, it is the most common of the MPD, presumably because it represents the ultimate phenotype of activating JAK2 mutations^{37,80}. Second, absolute erythrocytosis is the hallmark of PV, and without it the diagnosis cannot be established, nor can PV be distinguished from its companion MPD; a positive JAK2 mutation assay in this situation only proves the presence of an MPD, not necessarily PV. As a corollary, *when PV is a diagnostic consideration, a red cell mass and plasma volume determination or a phlebotomy trial is desirable*. Third, plasma volume expansion, even in the absence of splenomegaly, can mask the true increase in red cell mass in PV^{35,56,58} (**Table 1**). As a consequence, hemoglobin or hematocrit values alone cannot be used to establish the presence of erythrocytosis; only red cell mass and plasma volume determinations can. Fourth, the presentation of PV is sufficiently pleomorphic that *all* laboratory clues need to be utilized (**Table 2**). Finally, not mentioned by the WHO but implicit in any

evidence-based approach, is the need in a given practice situation to define pre-test probabilities with respect to the frequency with which different forms of erythrocytosis or apparent erythrocytosis are encountered. For example, in one study, idiopathic erythrocytosis and secondary erythrocytosis were 4-7 times more common than PV⁹⁹.

Table 2 lists the presenting blood counts and prevalence of splenomegaly from two older series^{13,100} documenting the phenotypic diversity of PV. Any combination of a high red cell count and leukocytosis, thrombocytosis or splenomegaly establishes the diagnosis. In the absence of a high red cell count, red cell mass and plasma volume studies are required. Surprisingly and inexplicably, the WHO omits blood counts and splenomegaly from its PV diagnostic criteria.

Figure 5 illustrates a diagnostic algorithm for patients who present without leukocytosis, thrombocytosis or palpable splenomegaly, which is also useful in situations of plasma volume contraction and when the hematocrit appears to be normal, because these are the patients who trouble us most and for whom the WHO provides no guidance. Bone marrow aspiration and biopsy are not required, and a JAK2 mutation assay, while desirable, is also not mandatory.

Finally, we take exception to the WHO contention that missing occult PV is clinically insignificant¹, particularly when there is no excuse for doing so. Contrary to the WHO claim, the treatment of “high-risk” MPD patients is not the same regardless of diagnosis because there is as yet no drug therapy proven to prevent venous thrombosis in PV¹⁰¹⁻¹⁰⁴ (and **Figure 3**), while phlebotomy to a gender-specific hematocrit¹⁰⁵ rapidly alleviates the hyperviscosity

symptoms unique to PV, as well as reducing the red cell contribution to thrombosis by lowering blood viscosity⁴⁶ and diminishing red cell nitric oxide scavenging¹⁰⁶. Moreover, It would be inappropriate to treat an ET patient for PV; the assumption of PV when that disease was not present has been associated with the iatrogenic induction of acute leukemia^{107,108}.

Essential Thrombocytosis

ET is the only MPD without a specific phenotype. Since isolated thrombocytosis can be the initial clinical manifestation of PV, PMF or CML, ET is not only a diagnosis of exclusion, it should also not be considered a single disease entity¹⁰⁹. The degree of female predominance in ET is also unique amongst the MPD as is its natural history, which is compatible with a normal lifespan^{110,111}. The JAK2 V617F mutation provides an opportunity to identify approximately 50 % of patients with isolated thrombocytosis as possibly having ET and importantly, also further distinguishes them as possibly having PV^{41,79,80}. Since arterial thrombosis is more common in ET, while venous thrombosis is more common in PV¹¹², this is not a trivial issue.

In addition, it is also important to consider the pre-test probabilities with respect to the frequency with which clonal forms of thrombocytosis are encountered. For example, in one 4 year survey of 732 patients with a platelet count greater than 500,000/ μ L, only 89 (12.3 %) had a clonal disorder and of these, only 40 (5 %) had ET¹¹³. As a corollary, while the height of the platelet count is usually not diagnostic for clonally-derived thrombocytosis, the higher the platelet count, the greater the degree of diagnostic specificity¹¹⁴.

What also appears to be important is not the chosen threshold platelet count but whether the thrombocytosis is persistent. In an epidemiologic study of 99 patients with platelet counts greater than 400,000/ μ L, only 8 had still thrombocytosis eight months later⁹⁹. Thus, prior blood counts, if available, can provide valuable information. Finally, context is also important. While ET patients can have leukocytosis and splenomegaly, if these are not extremely modest, and if extramedullary hematopoiesis is present in the form of a leukoerythroblastic reaction, another MPD should be considered because these findings suggest a clonal burden larger than usually encountered in ET (**Figure 4**).

Considering all these facts together, we offer the following ET diagnostic guideline (**Table 3**) from the perspective that since the WHO diagnostic criteria for PV are unsatisfactory, and since exclusion of PV is mandatory in the differential diagnosis of ET, the WHO PV criteria corrupt the other MPD diagnostic guidelines.

Primary Myelofibrosis

The recent change in nomenclature from idiopathic myelofibrosis to primary myelofibrosis¹¹⁵ illustrates the lack of exactness with which the MPD are considered. Biologically, there is no such thing as “primary” myelofibrosis. Marrow fibrosis is reactive and reversible, if the underlying cause can be eradicated¹¹⁶, and not a disease per se. PMF is the least common of the MPD¹¹⁷ and the hardest to define because of its phenotypic mimicry of a wide variety of hematologic and nonhematologic illnesses, and the particular burden marrow fibrosis imposes on histologic evaluation.

PMF is the one MPD for which a bone marrow biopsy is essential for diagnostic purposes but it is important to remember that the presence of myelofibrosis does not exclude PV¹⁷. While there is undoubtedly a prefibrotic phase of PMF, it is not possible at present to identify this morphologically⁸⁶. Moreover, based on most large clinical series to date, (reviewed in reference¹¹⁸), the absence of splenomegaly or other evidence of extramedullary hematopoiesis also makes the diagnosis of PMF suspect. As a consequence, we do not consider splenomegaly a minor criterion. This is in keeping with the high JAK2 V617F clonal burden in PMF at the time of diagnosis (**Figure 4**). **Table 4** lists an alternative diagnostic approach for PMF.

Conclusions

JAK2 V617F expression is responsible for the most prominent clinical features of the MPD but its impact on disease phenotype and disease progression is clearly dependent on the total allelic burden of the mutation^{76,78} and other genetic influences, of which gender is a major contributor¹¹⁹. Since clonal dominance is time-dependent³³, the MPD are not static disorders and their clinical phenotype is subject to change over time. As consequence, no single diagnostic test and, frequently, no combination of diagnostic tests is sufficient to establish the diagnosis of a particular MPD or even to distinguish the MPD from the other benign and malignant hematologic disorders that they mimic. MPD diagnosis is still a clinical exercise and, in this regard, it is important to remember that the “apparent absence of evidence is not evidence for its absence”. It is for these reasons that we advocate the time-tested diagnostic principles described in this review.

Acknowledgments: The authors thank Dr. Alison R. Moliterno for permission to present the patient illustrated in Figure 1b.

JLS drafted and wrote the manuscript. RTS drafted and wrote the manuscript. The authors declare no competing financial interests.

Reference List

- (1) Tefferi A, Thiele J, Orazi A et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood*. 2007;110:1092-1097.
- (2) Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia*. 2007.
- (3) Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001.
- (4) Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292-2302.
- (5) Johansson PL, Safai-Kutti S, Kutti J. An elevated venous haemoglobin concentration cannot be used as a surrogate marker for absolute erythrocytosis: a study of patients with polycythaemia vera and apparent polycythaemia. *Br J Haematol*. 2005;129:701-705.
- (6) Zhao R, Xing S, Li Z et al. Identification of an acquired JAK2 mutation in polycythemia vera. *J Biol Chem*. 2005;280:22788-22792.
- (7) Pikman Y, Lee BH, Mercher T et al. MPLW515L Is a Novel Somatic Activating Mutation in Myelofibrosis with Myeloid Metaplasia. *PLoS Med*. 2006;3:e270.
- (8) Pardanani AD, Levine RL, Lasho T et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*. 2006;108:3472-3476.
- (9) Williams DM, Kim AH, Rogers O, Spivak JL, Moliterno AR. Phenotypic variations and new mutations in JAK2 V617F-negative polycythemia vera, erythrocytosis, and idiopathic myelofibrosis. *Exp Hematol*. 2007;35:1641-1646.
- (10) Shekelle PG, Woolf SH, Eccles M, Grimshaw J. Clinical guidelines: developing guidelines. *BMJ*. 1999;318:593-596.

- (11) Oosterhuis WP, Bruns DE, Watine J, Sandberg S, Horvath AR. Evidence-based guidelines in laboratory medicine: principles and methods. *Clin Chem*. 2004;50:806-818.
- (12) Woolf SH. Do clinical practice guidelines define good medical care? The need for good science and the disclosure of uncertainty when defining 'best practices'. *Chest*. 1998;113:166S-171S.
- (13) Berglund S, Zettervall O. Incidence of polycythemia vera in a defined population. *Eur J Haematol*. 1992;48:20-26.
- (14) Taylor KM, Shetta M, Talpaz M et al. Myeloproliferative disorders: usefulness of X-linked probes in diagnosis. *Leukemia*. 1989;3:419-422.
- (15) Janssen JW, Anger BR, Drexler HG, Bartram CR, Heimpel H. Essential thrombocythemia in two sisters originating from different stem cell levels. *Blood*. 1990;75:1633-1636.
- (16) Shih LY, Lee CT. Identification of masked polycythemia vera from patients with idiopathic marked thrombocytosis by endogenous erythroid colony assay. *Blood*. 1994;83:744-748.
- (17) Ellis JT, Peterson P, Geller SA, Rappaport H. Studies of the bone marrow in polycythemia vera and the evolution of myelofibrosis and second hematologic malignancies. *Semin Hematol*. 1986;23:144-155.
- (18) Barosi G, Cazzola M, Frassoni F, Orlandi E, Stefanelli M. Erythropoiesis in myelofibrosis with myeloid metaplasia: recognition of different classes of patients by erythrokinetics. *Br J Haematol*. 1981;48:263-272.
- (19) Cervantes F, Alvarez-Larran A, Talam C, Gomez M, Montserrat E. Myelofibrosis with myeloid metaplasia following essential thrombocythaemia: actuarial probability, presenting characteristics and evolution in a series of 195 patients. *Br J Haematol*. 2002;118:786-790.
- (20) Bouroncle B, Doan CA. Myelofibrosis Clinical, Hematologic and Pathologic Study of 110 patients. *American Journal of the Medical Sciences*. 1962;243:697-715.
- (21) Pitcock JA, Reinhard EH, Justus BW, Mendelsohn RS. A Clinical and Pathological Study of Seventy Cases of Myelofibrosis. *Ann Intern Med*. 1962;57:73-84.
- (22) Pettit JE, Lewis SM, Nicholas AW. Transitional myeloproliferative disorder. *Br J Haematol*. 1979;43:167-184.
- (23) Barosi G, Baraldi A, Cazzola M et al. Polycythaemia following splenectomy in myelofibrosis with myeloid metaplasia. A reorganization of erythropoiesis. *Scand J Haematol*. 1984;32:12-18.

- (24) Cervantes F, Barosi G, Demory JL et al. Myelofibrosis with myeloid metaplasia in young individuals: disease characteristics, prognostic factors and identification of risk groups. *Br J Haematol.* 1998;102:684-690.
- (25) Osler W. Chronic Cyanosis, With Polycythemia and Enlarged Spleen: A new clinical entity. *American Journal of the Medical Sciences.* 1903;126:176-201.
- (26) Wasserman L. The Management of Polycythemia Vera. *British Journal of Hematology.* 1971;21:371-376.
- (27) McMullin MF, Bareford D, Campbell P et al. Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. *Br J Haematol.* 2005;130:174-195.
- (28) McMullin MF, Reilly JT, Campbell P et al. Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis. *Br J Haematol.* 2007;138:821-822.
- (29) Weber FP. Polycythaemia, Erythrocytosis and Erythraemia. *Q J Med.* 1908;2:85-134.
- (30) Barosi G, Ambrosetti A, Finelli C et al. The Italian Consensus Conference on Diagnostic Criteria for Myelofibrosis with Myeloid Metaplasia. *Br J Haematol.* 1999;104:730-737.
- (31) Streiff MB, Smith B, Spivak JL. The diagnosis and management of polycythemia vera in the era since the Polycythemia Vera Study Group. *Blood.* 2002;99:1144.
- (32) Johansson P, Andreasson B, Safai-Kutti S et al. On the diagnosis of polycythaemia vera as assessed in the health and medical care in the Vastra Gotaland region, Sweden. *J Intern Med.* 2002;251:348-354.
- (33) Golde DW, Koeffler HP, Adamson JW. Polycythemia vera: mechanisms and management. *Ann Intern Med.* 1981;95:71-87.
- (34) Dupont S, Masse A, James C et al. The JAK2 617V>F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. *Blood.* 2007;110:1013-1021.
- (35) Spivak JL. Polycythemia vera: myths, mechanisms, and management. *Blood.* 2002;100:4272-4290.
- (36) Bellanne-Chantelot C, Chaumarel I, Labopin M et al. Genetic and clinical implications of the Val617Phe JAK2 mutation in 72 families with myeloproliferative disorders. *Blood.* 2006;108:346-352.
- (37) James C, Ugo V, Le Couedic JP et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature.* 2005;434:1144-1148.

- (38) Baxter EJ, Scott LM, Campbell PJ et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*. 2005;365:1054-1061.
- (39) Kralovics R, Passamonti F, Buser AS et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;352:1779-1790.
- (40) Levine RL, Wadleigh M, Cools J et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005;7:387-397.
- (41) Moliterno AR, Williams DM, Rogers O, Spivak JL. Molecular mimicry in the chronic myeloproliferative disorders: reciprocity between quantitative JAK2 V617F and Mpl expression. *Blood*. 2006;108:3913-3915.
- (42) Barosi G, Viarengo G, Pecci A et al. Diagnostic and clinical relevance of the number of circulating CD34+ cells in myelofibrosis and myeloid metaplasia. *Blood*. 2001;98:3249-3255.
- (43) Passamonti F, Vanelli L, Malabarba L et al. Clinical utility of the absolute number of circulating CD34-positive cells in patients with chronic myeloproliferative disorders. *Haematologica*. 2003;88:1123-1129.
- (44) Pearson TC, Guthrie DL, Simpson J et al. Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on Radionuclides of the International Council for Standardization in Haematology. *Br J Haematol*. 1995;89:748-756.
- (45) Turkington RC, Arnold EC, Percy MJ et al. Comparison of diagnostic criteria for polycythaemia vera. *Hematology*. 2007;12:123-130.
- (46) Wells RE, Merrill EW. Influence of flow properties of blood upon viscosity-hematocrit relationship. *Journal of Clinical Investigation*. 1962;41:1591-1598.
- (47) Pearson TC. Hemorheologic considerations in the pathogenesis of vascular occlusive events in polycythemia vera. *Semin Thromb Hemost*. 1997;23:433-439.
- (48) Freis ED, Stanton JR, Emerson CP. Estimation of relative velocities of plasma and red cells in the circulation of man. *Am J Physiol*. 1949;157:153-157.
- (49) Gibson JG, Seligman A, Peacock WC et al. The Distribution of Red Cells and Plasma in Large and Minute Vessels of the Normal Dog, Determined by Radioactive Isotopes of Iron and Iodine. *J Clin Invest*. 1946;848-857.
- (50) Chaplin H, Mollison P, Vetter H. The Body/Venous Hematocrit Ratio: Its Constancy Over a Wide Hematocrit Range. *Journal Clinical Investigation*. 1953;1309-1316.

- (51) Sanchez C, Merino C, Figallo M. Simultaneous measurement of plasma volume and cell mass in polycythemia of high altitude. *J Appl Physiol.* 1970;28:775-778.
- (52) Verel D. Blood Volume Changes in Cyanotic Congenital Heart Disease and Polycythemia Rubra Vera. *Circulation.* 1961;23:749-753.
- (53) Weil JV, Jamieson G, Brown DW et al. The Red Cell Mass-Arterial Oxygen Relationship in Normal Man. *The Journal of Clinical Investigation.* 1968;47:1627-1639.
- (54) Johansson P, Safai-Kutti S, Lindstedt G, Suurkula M, Kutti J. Red cell mass, spleen size and plasma erythropoietin in polycythaemia vera and apparent polycythaemia. *Acta Haematol.* 2002;108:1-7.
- (55) Neff MS, Kim KE, Persoff M, Onesti G, Swartz C. Hemodynamics of uremic anemia. *Circulation.* 1971;43:876-883.
- (56) Lamy T, Devillers A, Bernard M et al. Inapparent polycythemia vera: an unrecognized diagnosis. *Am J Med.* 1997;102:14-20.
- (57) Spivak JL, Moliterno AR, Silver RT. Case 15-2006: the Budd-Chiari syndrome and V617F mutation in JAK2. *N Engl J Med.* 2006;355:737.
- (58) Hassoun H, Pavlovsky M, Mansoor S, Stopka T. Diagnosis of polycythemia vera in an anemic patient. *South Med J.* 2000;93:710-712.
- (59) Pearson TC, Botterill CA, Glass UH, Wetherley-Mein G. Interpretation of measured red cell mass and plasma volume in males with elevated venous PCV values. *Scand J Haematol.* 1984;33:68-74.
- (60) Isbister JP. Contracted plasma volume syndromes. *Int J Microcirc Clin Exp.* 1984;3:93-108.
- (61) Blum A, Zbar M. Relative Polycythemia. *Arch Intern Med.* 1959;104:385-389.
- (62) Biswas M, Prakash PK, Cossburn M, Myers K, Hanna F. Life-threatening thrombotic complications of relative polycythaemia. *J Intern Med.* 2003;253:481-483.
- (63) Burge PS, Johnson WS, Prankerd TA. Morbidity and mortality in pseudopolycythaemia. *Lancet.* 1975;1:1266-1269.
- (64) Bessman DJ. Microcytic Polycythemia: Frequency of Nonthalassemic Causes. *J A M A.* 1977;238:2391-2392.
- (65) Berk PD, Goldberg JD, Silverstein MN et al. Increased incidence of acute leukemia in polycythemia vera associated with chlorambucil therapy. *N Engl J Med.* 1981;304:441-447.

- (66) Jacobson RJ, Salo A, Fialkow PJ. Agnogenic myeloid metaplasia: a clonal proliferation of hematopoietic stem cells with secondary myelofibrosis. *Blood*. 1978;51:189-194.
- (67) Fialkow PJ, Faguet GB, Jacobson RJ, Vaidya K, Murphy S. Evidence that essential thrombocythemia is a clonal disorder with origin in a multipotent stem cell. *Blood*. 1981;58:916-919.
- (68) Huang LJ, Constantinescu SN, Lodish HF. The N-terminal domain of Janus kinase 2 is required for Golgi processing and cell surface expression of erythropoietin receptor. *Mol Cell*. 2001;8:1327-1338.
- (69) Royer Y, Staerk J, Costuleanu M, Courtoy PJ, Constantinescu SN. Janus kinases affect thrombopoietin receptor cell surface localization and stability. *J Biol Chem*. 2005;280:27251-27261.
- (70) Jones AV, Kreil S, Zoi K et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood*. 2005;106:2162-2168.
- (71) Vizmanos JL, Ormazabal C, Larrayoz MJ, Cross NC, Calasanz MJ. JAK2 V617F mutation in classic chronic myeloproliferative diseases: a report on a series of 349 patients. *Leukemia*. 2006;20:534-535.
- (72) Kralovics R, Teo SS, Li S et al. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood*. 2006;108:1377-1380.
- (73) Rumi E, Passamonti F, Pietra D et al. JAK2 (V617F) as an acquired somatic mutation and a secondary genetic event associated with disease progression in familial myeloproliferative disorders. *Cancer*. 2006;107:2206-2211.
- (74) Nussenzveig RH, Swierczek SI, Jelinek J et al. Polycythemia vera is not initiated by JAK2V617F mutation. *Exp Hematol*. 2007;35:32-38.
- (75) Lacout C, Pisani DF, Tulliez M et al. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. *Blood*. 2006;108:1652-1660.
- (76) Vannucchi AM, Antonioli E, Guglielmelli P et al. Clinical profile of homozygous JAK2V617F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood*. 2007;110:840-846.
- (77) Pemmaraju N, Moliterno AR, Williams DM, Rogers O, Spivak JL. The quantitative JAK2 V617F neutrophil allele burden does not correlate with thrombotic risk in essential thrombocytosis. *Leukemia*. 2007;21:2210-2212.

- (78) Vannucchi AM, Antonioli E, Guglielmelli P et al. Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. *Leukemia*. 2007;21:1952-1959.
- (79) Campbell PJ, Scott LM, Buck G et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet*. 2005;366:1945-1953.
- (80) Cassinat B, Laguillier C, Gardin C et al. Classification of myeloproliferative disorders in the JAK2 era: is there a role for red cell mass? *Leukemia*. 2007.
- (81) Percy MJ, Jones FG, Green AR, Reilly JT, McMullin MF. The incidence of the JAK2 V617F mutation in patients with idiopathic erythrocytosis. *Haematologica*. 2006;91:413-414.
- (82) Iland HJ, Laszlo J, Peterson P et al. Essential thrombocythemia: clinical and laboratory characteristics at presentation. *Trans Assoc Am Physicians*. 1983;96:165-174.
- (83) Thiele J, Kvasnicka HM, Zankovich R, Diehl V. Relevance of bone marrow features in the differential diagnosis between essential thrombocythemia and early stage idiopathic myelofibrosis. *Haematologica*. 2000;85:1126-1134.
- (84) Gianelli U, Vener C, Raviele PR et al. Essential thrombocythemia or chronic idiopathic myelofibrosis? A single-center study based on hematopoietic bone marrow histology. *Leuk Lymphoma*. 2006;47:1774-1781.
- (85) Tefferi A. Is bone marrow biopsy essential for the diagnosis of essential thrombocythemia? *Leuk Lymphoma*. 2006;47:1724-1725.
- (86) Wilkins BS, Erber WN, Bareford D et al. Bone marrow pathology in essential thrombocythemia: inter-observer reliability and utility for identifying disease subtypes. *Blood*. 2007;IN PRESS.
- (87) Cazzola M, Guarnone R, Cerani P et al. Red blood cell precursor mass as an independent determinant of serum erythropoietin level. *Blood*. 1998;91:2139-2145.
- (88) Milledge JS, Cotes PM. Serum erythropoietin in humans at high altitude and its relation to plasma renin. *J Appl Physiol*. 1985;59:360-364.
- (89) Glasser RM, Walker RI. Transitions among the myeloproliferative disorders. *Ann Intern Med*. 1969;71:285-307.
- (90) Mossuz P, Girodon F, Donnard M et al. Diagnostic value of serum erythropoietin level in patients with absolute erythrocytosis. *Haematologica*. 2004;89:1194-1198.

- (91) Messinezy M, Westwood NB, El Hemaidi I et al. Serum erythropoietin values in erythrocytoses and in primary thrombocythaemia. *Br J Haematol.* 2002;117:47-53.
- (92) Casadevall N. Determination of serum erythropoietin. Its value in the differential diagnosis of polycythemia. *Nouv Rev Fr Hematol.* 1994;36:173-176.
- (93) Prchal JF, Axelrad AA. Letter: Bone-marrow responses in polycythemia vera. *N Engl J Med.* 1974;290:1382.
- (94) Casadevall N, Lacombe C, Varet B. Erythroid cultures and erythropoietin assay. Clinical and diagnostic value. *Nouv Rev Fr Hematol.* 1990;32:77-81.
- (95) Liu E, Jelinek J, Pastore YD et al. Discrimination of polycythemia and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-E response to erythropoietin. *Blood.* 2002;101:3294-3301.
- (96) Zwicky C, Theiler L, Zbaren K, Ischi E, Tobler A. The predictive value of clonogenic stem cell assays for the diagnosis of polycythemia vera. *Br J Haematol.* 2002;117:598-604.
- (97) Vannucchi AM, Grossi A, Pancrazzi A et al. PRV-1, erythroid colonies and platelet Mpl are unrelated to thrombosis in essential thrombocythaemia. *Br J Haematol.* 2004;127:214-219.
- (98) Westwood NB, Pearson TC. Diagnostic applications of haemopoietic progenitor culture techniques in polycythemia and thrombocythaemia. *Leuk Lymphoma.* 1996;22 Suppl 1:95-103.
- (99) Ruggeri M, Tosetto A, Frezzato M, Rodeghiero F. The rate of progression to polycythemia vera or essential thrombocythemia in patients with erythrocytosis or thrombocytosis. *Ann Intern Med.* 2003;139:470-475.
- (100) Berlin NI. Diagnosis and Classification of the Polycythemia. *Seminars in Hematology.* 1975;12:339-351.
- (101) Najean Y, Rain J. Treatment of Polycythemia Vera: The use of Hydroxyurea and Pipobroman in 292 Patients Under the Age of 65 Years. *Blood.* 1997;90:3370-3377.
- (102) Harrison CN, Campbell PJ, Buck G et al. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *N Engl J Med.* 2005;353:33-45.
- (103) Bachleitner-Hofmann T, Grumbeck E, Gisslinger H. Oral anticoagulants as secondary prophylaxis of thrombosis in patients with polycythemia vera: a retrospective analysis of 15 patients. *Thromb Res.* 2003;112:229-232.

- (104) Gruppo Italiano Studio Policitemia. Polycythemia vera: the natural history of 1213 patients followed for 20 years. *Ann Intern Med.* 1995;123:656-664.
- (105) Pearson TC, Weatherly-Mein G. Vascular occlusive episodes and venous haematocrit in primary proliferative polycythaemia. *Lancet.* 1978;2:1219-1221.
- (106) Chen X, Jaron D, Barbee KA, Buerk DG. The influence of radial RBC distribution, blood velocity profiles, and glycocalyx on coupled NO/O₂ transport. *J Appl Physiol.* 2006;100:482-492.
- (107) Bagby GC, Jr., Richert-Boe K, Koler RD. 32P and acute leukemia: development of leukemia in a patient with hemoglobin Yakima. *Blood.* 1978;52:350-354.
- (108) Najfeld V, Price TH, Adamson JW, Fialkow PJ. Myelofibrosis with complex chromosome abnormality in a patient with erythrocytosis due to hemoglobin Rainier and treated with 32P. *Am J Hematol.* 1978;5:63-69.
- (109) Campbell PJ, Green AR. The myeloproliferative disorders. *N Engl J Med.* 2006;355:2452-2466.
- (110) Rozman C, Giralt M, Feliu E, Rubio D, Cortes MT. Life expectancy of patients with chronic nonleukemic myeloproliferative disorders. *Cancer.* 1991;67:2658-2663.
- (111) Passamonti F, Rumi E, Pungolino E et al. Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. *Am J Med.* 2004;117:755-761.
- (112) Elliott MA, Tefferi A. Thrombosis and haemorrhage in polycythaemia vera and essential thrombocythaemia. *Br J Haematol.* 2005;128:275-290.
- (113) Griesshammer M, Bangerter M, Sauer T et al. Aetiology and clinical significance of thrombocytosis: analysis of 732 patients with an elevated platelet count. *J Intern Med.* 1999;245:295-300.
- (114) Buss DH, Cashell AW, O'Connor ML, Richards F, Case LD. Occurrence, etiology, and clinical significance of extreme thrombocytosis: a study of 280 cases. *Am J Med.* 1994;96:247-253.
- (115) Mesa RA, Verstovsek S, Cervantes F et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): Consensus on terminology by the international working group for myelofibrosis research and treatment (IWG-MRT). *Leuk Res.* 2007;31:737-740.
- (116) Thiele J, Kvasnicka HM, Dietrich H et al. Dynamics of bone marrow changes in patients with chronic idiopathic myelofibrosis following allogeneic stem cell transplantation. *Histol Histopathol.* 2005;20:879-889.

- (117) McNally RJ, Rowland D, Roman E, Cartwright RA. Age and sex distributions of hematological malignancies in the U.K. *Hematol Oncol.* 1997;15:173-189.
- (118) Varki A, Lottenberg R, Griffith R, Reinhard E. The syndrome of idiopathic myelofibrosis. A clinicopathologic review with emphasis on the prognostic variables predicting survival. *Medicine (Baltimore).* 1983;62:353-371.
- (119) Videbaek A. Polycythemia Vera. Course and Prognosis. *Acta Med Scand.* 1950;138:179-187.

Table 1 **Examples of the Diagnostic Value of Red Cell Mass (RCM) and Plasma Volume (PV) Determinations**

Initial Diagnosis:	Secondary Erythrocytosis	Essential Thrombocytosis	Budd-Chiari Syndrome
Final Diagnosis:	Renal disease with renal cysts and plasma volume contraction	Polycythemia vera with plasma volume expansion	Polycythemia vera with plasma volume expansion
Age, y/sex,M/F	37/F	61/ F	30/F
BSA	2.01	1.64	1.57
Hemoglobin, g/dL	17.1	14.9	13.5
Hematocrit, %	54.4	45.9	40.3

	Expected*	Observed	Expected*	Observed		Expected*	Observed
RCM, ml	1724	1956	1240	2010		1323	2845
PV, ml	2818	2250	1984	2459		2190	3507
TBV, ml	4542	4206	3224	4469		3513	6352

These examples illustrate the utility of red cell mass and plasma volume determinations in establishing the presence of true erythrocytosis or plasma volume contraction in clinical situations in which this information could not be obtained in any other way. The ET patient was a JAK2 V617F heterozygote with stainable marrow iron, a normal serum ferritin and red cell MCV and no splenomegaly. The PV patient was having recurrent intra-abdominal venous thrombosis despite therapeutic anticoagulation.

* The expected values were derived from the Tables in reference 44.

Table 2

The presenting blood counts and prevalence of palpable splenomegaly in two series of polycythemia vera patients at the time of diagnosis.

	<u>PVSG</u> ¹⁰⁰	<u>Malmö</u> ¹³
Erythrocytosis alone	0	17 %
Erythrocytosis and:		
Leukocytosis	13 %	29 %
Thrombocytosis	30 %	16 %
Leukocytosis and thrombocytosis	57 %	38 %
Splenomegaly (palpable)	70 %	58 %
Splenomegaly and:		
Leukocytosis	ND*	66 %
Thrombocytosis	ND	54 %

*ND, not determined

Table 3

Diagnostic Criteria for Essential Thrombocytosis

- Persistent thrombocytosis > 400,000/ μ l in the absence of a reactive cause*
- Absence of iron deficiency (normal serum ferritin for gender)
- JAK2 V617F assay (peripheral blood) (expression establishes the presence of an MPD *but not its type; the absence of JAK2 V617F does not exclude an MPD*)
- Hemoglobin <16gm % in a man or <14 gm % in woman (Hematocrit < 47% in a man or <44% in a woman) in the absence of splenomegaly. Otherwise, red cell mass and plasma volume determinations are mandatory if a JAK2 V617F assay is positive
- Negative Bcr-Abl FISH (peripheral blood) if a JAK2 V617F assay is negative
- If there is anemia or macrocytosis or leukopenia, or evidence of extramedullary hematopoiesis (i.e. circulating nucleated erythrocytes, immature myelocytes or splenomegaly), a bone marrow examination (including flow cytometry and cytogenetics) is mandatory regardless of JAK2 V617F expression status

*As indicated in the text, MPD patients represent only a minority of thrombocytosis patients in general but constitute most of those with persistent thrombocytosis in the absence of a definable cause.

Table 4

Diagnostic Criteria for Primary Myelofibrosis

- Leukoerythroblastic blood picture
- Increased marrow reticulin in the absence of an infiltrative or granulomatous process
- Splenomegaly
- JAK2 V617F assay (peripheral blood)(expression establishes the presence of an MPD *but not its type; the absence of JAK2 V617F does not exclude an MPD*)
- Increased circulating CD34 + cells ($>15 \times 10^6/L$) and no increase in marrow CD34+ cells by in situ immunohistochemistry
- Characteristic cytogenetic abnormalities (peripheral blood: del(13q), 9p, del(20q), del(12p), partial trisomy 1q, trisomy 8, and trisomy 9)
- Absence of Bcr-Abl, AML or MDS cytogenetic abnormalities by FISH (peripheral blood)

Figure Legends

Figure 1a. Erythrocytosis developing in a 60 year old man with essential thrombocythemia six years after diagnosis. The increase in the JAK2 V617F neutrophil allelic burden with time is also shown. The hemoglobin (Hgb) level was reduced by phlebotomy.

Figure 1b. Erythrocytosis developing in a 70 year old woman with classical PMF of 17 years duration, while taking hydroxyurea to control splenic enlargement (bracketed line). The hemoglobin (Hgb) level was reduced by phlebotomy.

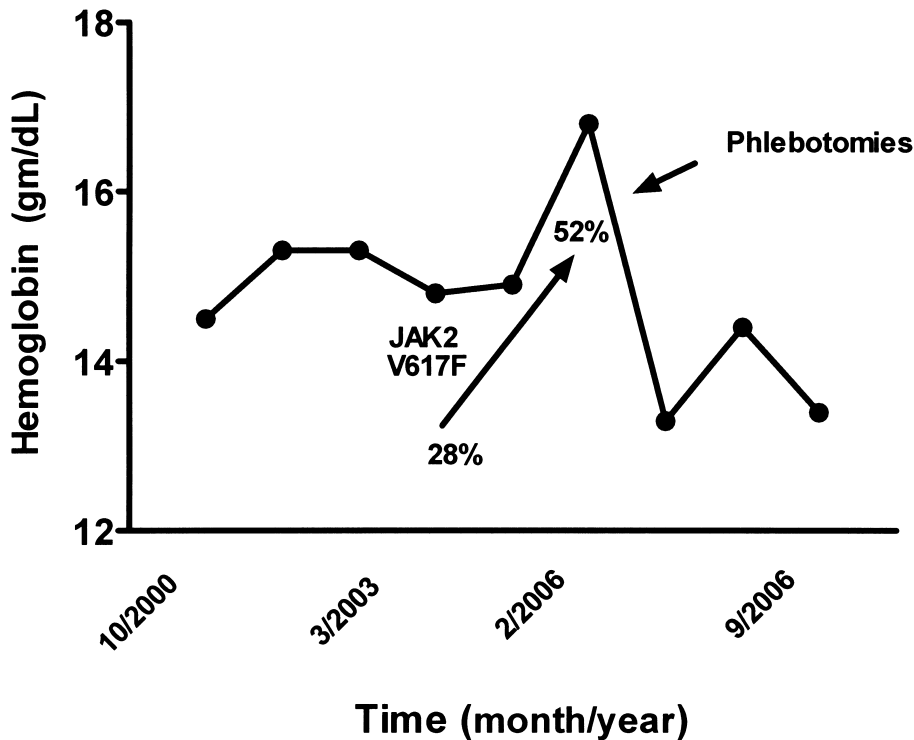
Figure 2. Correlation of the WHO hemoglobin guidelines for the diagnosis of PV with actual red cell mass and plasma volume measurements, A) Men and B) Women. Similar results were obtained if the corresponding hematocrit values were employed. The data are recalculated from Johansson et al ⁵.

Figure 3. Comparison of simultaneous hematocrits and red cell counts in a woman with PV and hepatic vein thrombosis experiencing recurrent thromboses of stents and shunts during therapeutic anticoagulation. The red cell count is above normal at the time of each thrombosis, while the hematocrit is not. The heavy lines indicate the upper and lower limits of normal for each.

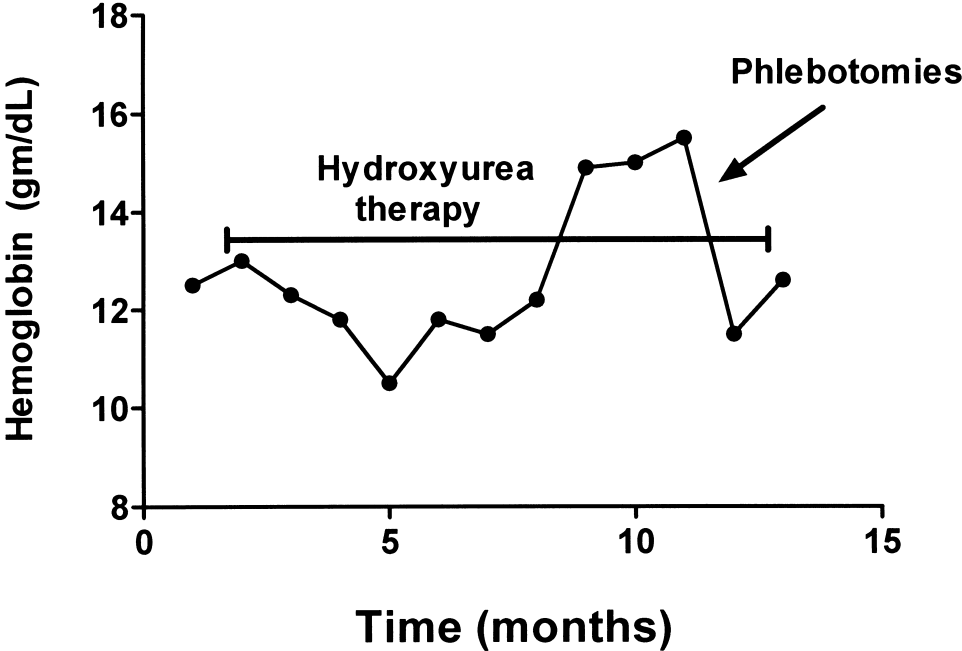
Figure 4. Comparison of the quantitative JAK2 V617F neutrophil allelic burdens in ET, PV and PMF at diagnosis, illustrating the differences in allelic burden amongst them. The degree of overlap between the neutrophil allele burden in ET and the other MPD is indicated by the horizontal bars.

Figure 5. A diagnostic algorithm for suspected erythrocytosis when leukocytosis, thrombocytosis or splenomegaly is not present. Given the likelihood that an isolated hematocrit or hemoglobin elevation will not be due to PV⁹⁹, the algorithm makes no a priori assumptions about the etiology of the abnormality.

Essential Thrombocytosis Evolving into Polycythemia Vera in a Man



Primary Myelofibrosis of 17 Years Evolving into Polycythemia Vera in a Woman

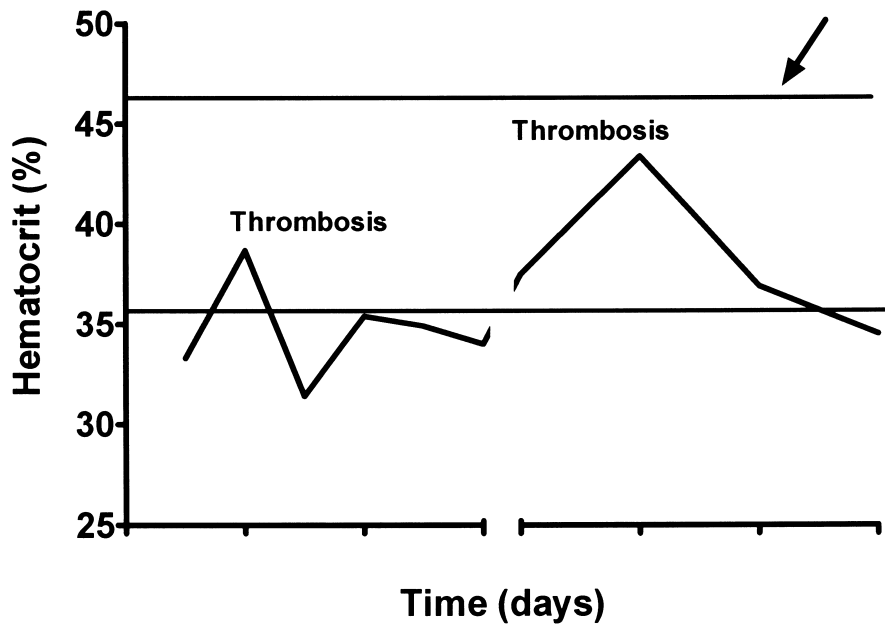
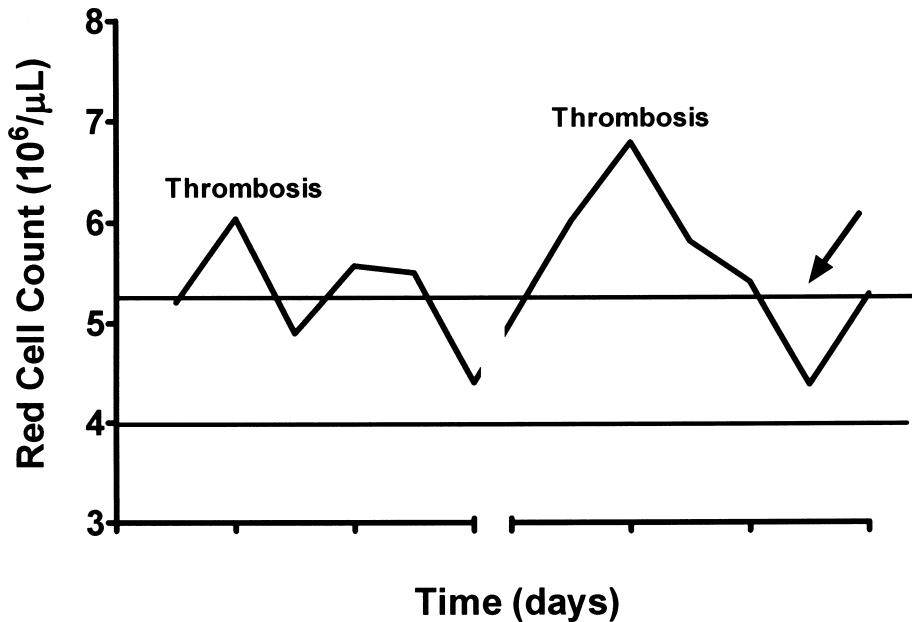


A**WHO Hemoglobin Guidelines (♂)****Direct
Red Cell Mass
and Plasma Volume
Measurements**

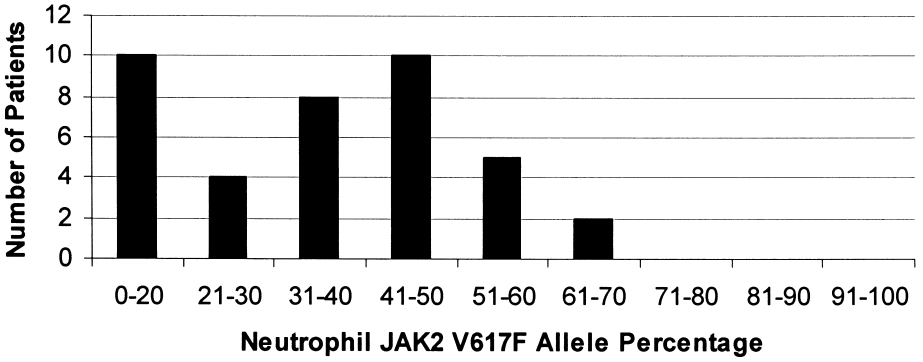
	True Erythrocytosis (Hgb > 18.5 gm%)	Apparent Erythrocytosis (Hgb < 18.5 gm%)
True Erythrocytosis	35 %	65 %
Apparent Erythrocytosis	14%	76 %

B**WHO Hemoglobin Guidelines (♀)****Direct
Red Cell Mass
and Plasma Volume
Measurements**

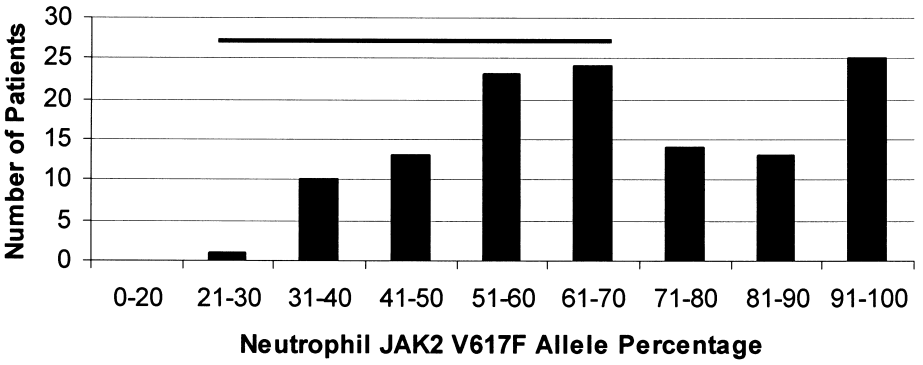
	True Erythrocytosis (Hgb > 16.5 gm%)	Apparent Erythrocytosis (Hgb < 16.5 gm%)
True Erythrocytosis	63 %	37 %
Apparent Erythrocytosis	35%	65 %



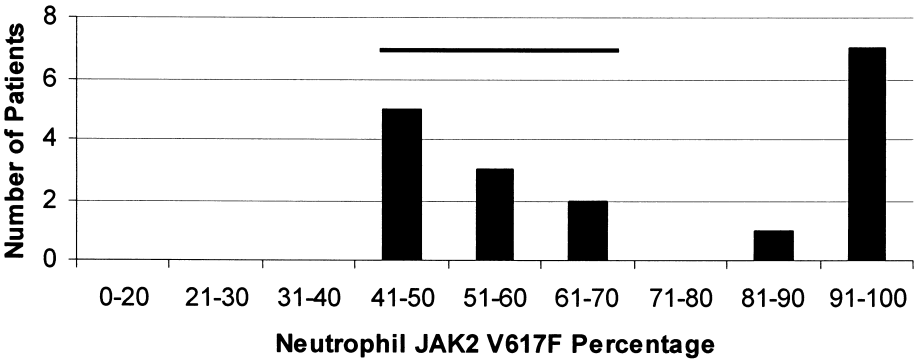
Essential Thrombocythosis

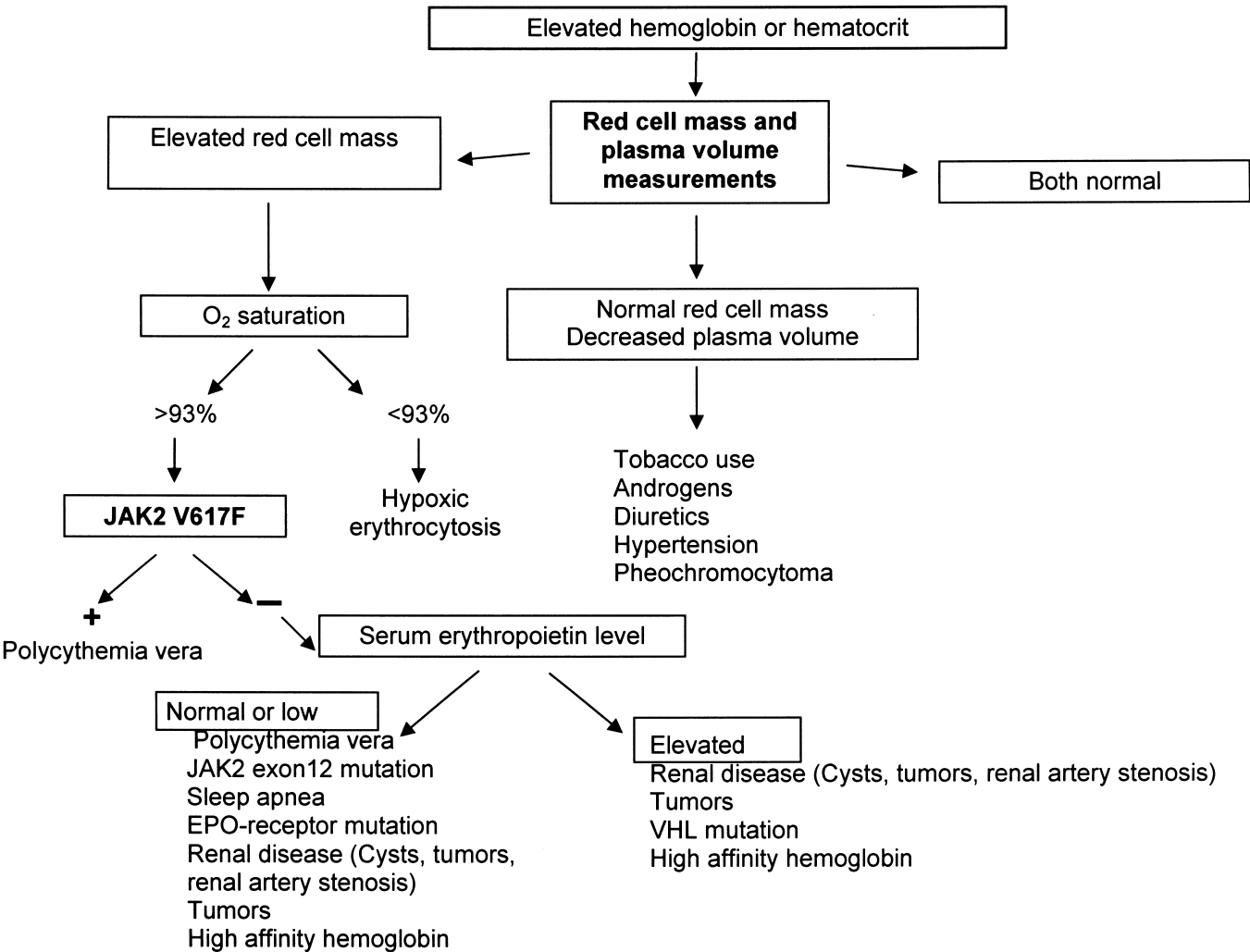


Polycythemia Vera



Primary Myelofibrosis





Elevated hemoglobin or hematocrit



Elevated red cell mass

Red cell mass and plasma volume measurements

Both normal



O₂ saturation

Normal red cell mass
Decreased plasma volume

>93%

<93%

JAK2 V617F

Hypoxic erythrocytosis

Tobacco use
Androgens
Diuretics
Hypertension
Pheochromocytoma

+

Polycythemia vera

-

Serum erythropoietin level

Normal or low

Polycythemia vera
JAK2 exon12 mutation
Sleep apnea
EPO-receptor mutation
Renal disease (Cysts, tumors,
renal artery stenosis)
Tumors
High affinity hemoglobin

Elevated

Renal disease (Cysts, tumors, renal artery stenosis)
Tumors
VHL mutation
High affinity hemoglobin