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2. To explain practical diagnostic and treatment algorithms.
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ANNUAL CLINICAL UPDATES IN HEMATOLOGICAL MALIGNANCIES:
A CONTINUING MEDICAL EDUCATION SERIES

Polycythemia vera and essential thrombocythemia: 2013 update on diagnosis, risk-stratification, and management

Ayalew Tefferi*

Disease Overview: **Polycythemia Vera (PV) and essential thrombocythemia (ET) are myeloproliferative neoplasms (MPN) primarily characterized by erythrocytosis and thrombocytosis, respectively. Other disease features include leukocytosis, splenomegaly, thrombohemorrhagic complications, vasomotor disturbances, pruritus and a small risk of disease progression into acute myeloid leukemia or myelofibrosis.**

D*iagnosis:* **Almost all patients with PV harbor a *JAK2* mutation. When PV is suspected, the presence of a *JAK2* mutation highly suggests the diagnosis and its absence, combined with normal or increased serum erythropoietin level, excludes the diagnosis. Differential diagnosis of ET should include reactive thrombocytosis, chronic myeloid leukemia, prefibrotic myelofibrosis and RARS-T (refractory anemia with ring sideroblasts associated with marked thrombocytosis). A *JAK2* mutation is found in 50–70% of patients with ET, myelofibrosis or RARS-T and is capable of distinguishing reactive from clonal thrombocytosis.**

Risk Stratification: **Current risk stratification in PV and ET is designed to estimate the likelihood of thrombotic complications: high-risk is defined by the presence of age >60 years or presence of thrombosis history; low-risk is defined by the absence of both of these two risk factors. Recent data considers *JAK2*V617F and cardiovascular (CV) risk factors as additional risk factors for thrombosis. Presence of extreme thrombocytosis (platelet count >1,000 $\times$ 10^9/L) might be associated with acquired von Willebrand syndrome (AvWS) and, therefore, risk of bleeding. Risk factors for shortened survival in both PV and ET include advanced age, leukocytosis, and history of thrombosis.**

Risk-Adapted Therapy: **Survival is near-normal in ET and reasonably long in PV. The 10-year risk of leukemic/fibrotic transformation is <1%/1% in ET and <3%/10% in PV. In contrast, the risk of thrombosis exceeds 20%. The main goal of therapy is therefore to prevent thrombohemorrhagic complications. In low risk patients, this is effectively and safely accomplished by the use of low-dose aspirin in both PV and ET and phlebotomy (hematocrit target of <45%) in PV. In high risk patients, treatment with hydroxyurea is additionally recommended, although not mandated in older patients without *JAK2*V617F or CV risk factors. Treatment with busulfan or interferon-α is usually effective in hydroxyurea failures. Screening for clinically significant AvWS is recommended before administrating aspirin in the presence of extreme thrombocytosis.**

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Disease Overview

Myeloproliferative neoplasms (MPN) constitute one of five categories of myeloid malignancies, according to the World Health Organization (WHO) classification system for hematopoietic tumors (Table I) [1]. "*BCR-ABL1*-negative MPN" is an operational sub-category of MPN that includes polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [2]. All three disorders are characterized by stem cell-derived clonal myeloproliferation but their disease-causing mutations remain unidentified despite a plethora of mutations described beginning in 2005 [3].

Almost all patients with PV harbor a *JAK2* (Janus kinase 2; 9p24) mutation; approximately 96% and 3% displaying somatic activating mutations in exon 14 (*JAK2*V617F) and exon 12 of *JAK2*, respectively [4,5]. *JAK2*V617F also occurs in ET and PMF with respective mutational frequencies of 55% and 65% [3]. *JAK2* exon 12 mutations are rare in ET or PMF [6]. *MPL* (myeloproliferative leukemia virus oncogene; 1p34) mutations occur in approximately 4% of ET patients, 8% of PMF patients, and rarely in PV [7]. *MPL* mutations cluster in exon 10, the most frequent being *MPL*W515L/K [8–10]. *MPL*S505N is both a germline (hereditary thrombocythemia) [11,12] and somatic (ET) mutation [10]. Hereditary thrombocytosis has also been reported with germline *JAK2* mutation (*JAK2*V617I) and associated with vascular events but not fibrotic/leukemic progression [13]. Both *JAK2*V617F and *MPL* mutations also occur infrequently in other myeloid malignancies [3]. Conversely, other mutations involving *TET2*, *IDH*, *ASXL1*, or *DNMT3A* are occasionally seen in PV and ET [14–17].

*JAK2*V617F presence or increased allele burden does not appear to affect survival or leukemic transformation in PV or ET [5,18,19]. In ET, the presence of *JAK2*V617F has been associated with an increased risk of arterial thrombosis and a lower risk of post-ET MF [19,20]. In PV, a higher *JAK2*V617F mutant allele burden has been associated with pruritus and fibrotic transformation [18]. In general, *JAK2*V617F clusters with older age, higher hemoglobin level, leukocytosis, and lower platelet count [5]. *JAK2* exon

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TABLE I. World Health Organization (WHO) Classification of Myeloid Malignancies

Acute myeloid leukemia (AML) and related precursor neoplasms[a]
Myeloproliferative neoplasms (MPN)
Classic MPN
Chronic myelogenous leukemia, *BCR-ABL1* positive (CML)
Polycythemia vera (PV)
Primary myelofibrosis (PMF)
Essential thrombocythemia (ET)
Non-classic MPN
Chronic neutrophilic leukemia (CNL)
Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
Mastocytosis
Myeloproliferative neoplasm, unclassifiable (MPN-U)
Myelodysplastic syndromes (MDS)
Refractory cytopenia[b] with unilineage dysplasia (RCUD)
Refractory anemia (ring sideroblasts $<$ 15% of erythroid precursors)
Refractory neutropenia
Refractory thrombocytopenia
Refractory anemia with ring sideroblasts (RARS; dysplasia limited to erythroid lineage and ring sideroblasts $\geq$ 15% of bone marrow erythroid precursors)
Refractory cytopenia with multilineage dysplasia (RCMD; ring sideroblast count does not matter)
Refractory anemia with excess blasts (RAEB)
RAEB-1 (2–4% circulating or 5–9% marrow blasts)
RAEB-2 (5–19% circulating or 10–19% marrow blasts or Auer rods present)
MDS associated with isolated del(5q)
MDS, unclassifiable
MDS/MPN
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia, *BCR-ABL1* negative
Juvenile myelomonocytic leukemia (JMML)
MDS/MPN, unclassifiable
Provisional entity: Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T)
Myeloid an lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA,*[c] *PDGFRB,*[c] or *FGFR1*[c]
Myeloid and lymphoid neoplasms with *PDGFRA* rearrangement
Myeloid neoplasms with *PDGFRB* rearrangement
Myeloid and lymphoid neoplasms with *FGFR1* abnormalities

[a] Acute myeloid leukemia-related precursor neoplasms include "therapy-related myelodysplastic syndrome" and "myeloid sarcoma."

[b] Either mono- or bi-cytopenia: hemoglobin level $<$10 g/dL, absolute neutrophil count $<1.8 \times 10^9$/L, or platelet count $<100 \times 10^9$/L. However, higher blood counts do not exclude the diagnosis in the presence of unequivocal histological/cytogenetic evidence for myelodysplastic syndrome.

[c] Genetic rearrangements involving platelet-derived growth factor receptor α/β (*PDGFRA/PDGFRB*) or fibroblast growth factor receptor 1 (*FGFR1*).

12 mutation-positive patients usually present with predominantly erythroid myelopoiesis, subnormal serum erythropoietin level and younger age at diagnosis, but were prognostically similar to *JAK2*V617F [21]. *MPL* mutations have been inconsistently [22] associated with older age, female gender, lower hemoglobin level and higher platelet count [10,23,24] no associations with survival or leukemic transformation have been reported [10,23].

Diagnosis

Diagnosis of PV and ET is currently according to WHO criteria and based on a composite assessment of clinical and laboratory features (Table II) [25]. Figure 1 provides a practical diagnostic algorithm that begins with peripheral blood mutation screening for *JAK2*V617F. The laboratory detection of *JAK2*V617F is highly sensitive (97% sensitivity) and virtually 100% specific for distinguishing PV from other causes of increased hematocrit [26,27] the possibility of false positive or false negative mutation test result is effectively addressed by the concomitant measurement of serum erythropoietin (Epo) level, which is expected to be subnormal in more than 85% of patients with PV [28]. A subnormal serum Epo level in the absence of *JAK2*V617F mandates additional mutational analysis for *JAK2* exon 12 mutation in order to capture some of the approximately 3% of PV patients who are *JAK2*V617F-negative [4]. Bone marrow examination is not essential for the diagnosis of PV because patients who otherwise fulfill the diagnostic criteria for PV are labeled as having PV even if they display substantial bone marrow fibrosis (Table II) [25].

When evaluating thrombocytosis, the detection of *JAK2*V617F confirms the presence of an underlying MPN but its absence does not rule out the possibility since up to 40% of patients with ET might be *JAK2*V617F-negative [29]. It is also important to note that other *JAK2*V617F-positive MPN (or MDS/MPN) can mimic ET in their presentation; these include prefibrotic PMF [30] and refractory anemia with ring sideroblasts with marked thrombocytosis (RARS-T) [31]. Therefore, bone marrow examination is often necessary to make an accurate morphologic diagnosis of ET and distinguish it from other myeloid neoplasms, especially from prefibrotic PMF; megakaryocytes in ET are large and mature-appearing whereas those in prefibrotic PMF display abnormal maturation with hyperchromatic and irregularly folded nuclei [32]. A recent large international study confirmed the prognostic relevance of distinguishing ET from pre-fibrotic PMF [19]. In the absence of *JAK2*V617F, the possibility of CML is readily addressed by *BCR-ABL1* mutation screening but it is also to be noted that megakaryocytes in CML (small and hypolobulated) are easily distinguished from those of ET [33]. The diagnosis of post-PV or post-ET MF should adhere to criteria recently published by the International Working Group for MPN Research and Treatment (IWG-MRT) (Table III) [34].

Risk Stratification

Current risk stratification in PV and ET is designed to estimate the likelihood of thrombotic complications and not necessarily survival or risk of leukemic/fibrotic transformation (Table IV) [37,38]. In a recent international study of 891 patients with true WHO-defined ET, after a median follow-up of 6.2 years, 109 (12%) patients experienced arterial ($n = 79$) or venous ($n = 37$) thrombosis. In multivariable analysis, predictors of arterial thrombosis included age more than 60 years, thrombosis history, cardiovascular risk factors including tobacco use, hypertension, or diabetes mellitus, leukocytosis ($>$ 11 $\times$ 10(9)/L), and presence of JAK2V617F [20]. In contrast, only male gender predicted venous thrombosis. Interestingly, platelet count more than 1,000 $\times$ 10(9)/L was associated with a lower risk of arterial thrombosis. This new information does not necessarily change how we currently risk stratify patients with PV or ET; age $\geq$60 years and history of thrombosis are the two risk factors used to classify patients with PV or ET into low (0 risk factors) and high (1 or 2 risk factors) risk groups (Table IV). However, it refines our treatment approach by giving us some flexibility on management, as will be discussed below [38]. In addition, because of the potential risk for bleeding, low-risk patients with extreme thrombocytosis (platelet count $>1{,}000 \times 10^9$/L) are considered separately (Table IV) [39].

Risk factors for shortened survival in both PV and ET include history of thrombosis, leukocytosis, and advanced age [40–43]. During a recent international study of over 1,000 patients with ET, the prognostically detrimental effect (on survival, leukemic transformation, and fibrotic progression) of prefibrotic morphology was demonstrated and the study also identified age $>$60 years, leukocyte count $>11 \times 10^9$/L, anemia, and thrombosis history as additional independent risk factors for survival [19]. The study also identified older age, anemia and absence of *JAK2*V617F as risk factors for fibrotic progression and history of thrombosis and extreme thrombocytosis as risk factors for leukemic

TABLE II. World Health Organization (WHO) Diagnostic Criteria for Polycythemia Vera, Essential Thrombocythemia, and Primary Myelofibrosis

	2008 WHO Diagnostic Criteria		
	Polycythemia vera[a]	Essential thrombocythemia[a]	Primary myelofibrosis[a]
Major criteria	1 Hgb > 18.5 g/dL (men); >16.5 g/dL (women); or[b]	1 Platelet count ≥ 450 × 10^9/L	1 Megakaryocyte proliferation and atypia[c] accompanied by either reticulin and/or collagen fibrosis, or[d]
	2 Presence of *JAK2*V617F or *JAK2* exon 12 mutation	2 Megakaryocyte proliferation with large and mature morphology. 3 Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm 4 Demonstration of *JAK2*V617F or other clonal marker or no evidence of reactive thrombocytosis	2 Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm 3 Demonstration of *JAK2*V617F or other clonal marker or no evidence of reactive marrow fibrosis
Minor criteria	1 BM trilineage myeloproliferation 2 Subnormal serum Epo level 3 EEC growth		1 Leukoerythroblastosis 2 Increased serum LDH level 3 Anemia 4 Palpable splenomegaly

[a] PV diagnosis requires meeting either both major criteria and one minor criterion or the first major criterion and two minor criteria. ET diagnosis requires meeting all four major criteria. PMF diagnosis requires meeting all three major criteria and two minor criteria.

[b] Hgb or Hct > 99th percentile of reference range for age, sex, or altitude of residence or red cell mass >25% above mean normal predicted or Hgb >17 g/dL (men)/>15 g/dL (women) if associated with a sustained increase of ≥2 g/dL from baseline that cannot be attributed to correction of iron deficiency

[c] Small to large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.

[d] In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis (i.e. pre-fibrotic PMF).

BM, bone marrow; Hgb, hemoglobin; Hct, hematocrit; Epo, erythropoietin; EEC, endogenous erythroid colony; WHO, World Health Organization; CML, chronic myelogenous leukemia; PV, polycythemia vera; PMF, primary myelofibrosis; MDS, myelodysplastic syndromes; LDH, lactate dehydrogenase.

transformation. Using age ≥60 years, hemoglobin below normal value and leukocyte count >15 × 10^9/L, one study demonstrated a median survival of >20 years in the absence of all 3 risk factors and ~9 years in the presence of two of the three risk factors [42].

In PV, median survivals were ~23 and 9 years, in the absence of advanced age and leukocytosis or presence of both risk factors, respectively [40]. Leukocytosis has also been associated with leukemic [40] and *JAK2*V617F allele burden with fibrotic [18] transformation in PV. Such observations were further validated by a recent population-based study of 327 patients with PV where multivariate analysis identified age >70 years, leucocyte count >13 × 10(9)/L and thrombosis at diagnosis, as risk factors for poor survival [44]. Patients with two or three of these factors had a 10-year RS of 26%, compared with 59% and 84% in patients with one and no risk factors, respectively. The relationship between thrombosis and leukocytosis [45,46], thrombosis and *JAK2*V617F [5] or pregnancy-associated complications and *JAK2*V617F [47–49] have been examined by different groups of investigators with findings that were conflicting and inconclusive.

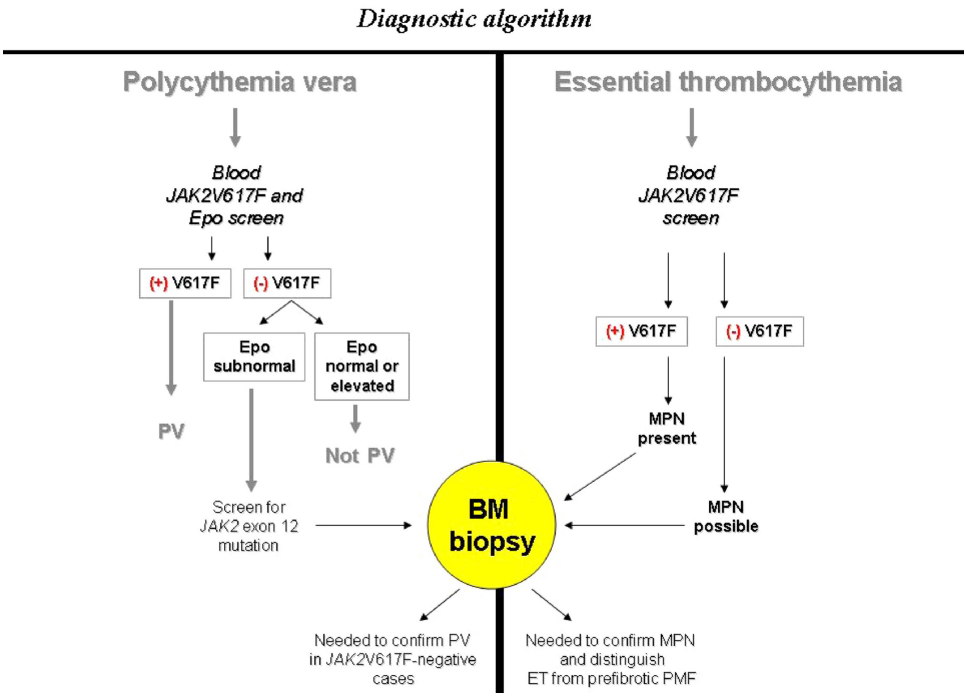

Figure 1. Diagnostic algorithm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III. International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) Recommended Criteria for Postpolycythemia Vera and Postessential Thrombocythemia Myelofibrosis [34]

Criteria for postpolycythemia vera myelofibrosis
Required criteria
Documentation of a previous diagnosis of polycythemia vera as defined by the WHO criteria (see Table II)
Bone marrow fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale) (see footnote for details)
Additional criteria (two are required):
Anemia or sustained loss of requirement for phlebotomy in the absence of cytoreductive therapy
A leukoerythroblastic peripheral blood picture
Increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly
Development of ≥1 of three constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)
Criteria for postessential thrombocythemia myelofibrosis
Required criteria
Documentation of a previous diagnosis of essential thrombocythemia as defined by the WHO criteria (see Table II)
Bone marrow fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale) (see footnote for details)
Additional criteria (two are required):
Anemia and a ≥2 g/dL decrease from baseline hemoglobin level
A leukoerythroblastic peripheral blood picture
Increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly
Increased lactate dehydrogenase
Development of ≥1 of three constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)

Grade 2–3 according to the European classification [35]: diffuse, often coarse fiber network with no evidence of collagenization (negative trichrome stain) or diffuse, coarse fiber network with areas of collagenization (positive trichrome stain). Grade 3–4 according to the standard classification [36]: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis or diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis.

Risk-Adapted Therapy

Because survival in strictly WHO-defined ET is near-normal (15-year survival of ~80%) and the 10-year risk of AML or MF less than 1% [19], it would be inappropriate to suggest that any current treatment modifies the natural history of the disease. Similarly, in WHO-defined PV, the 10-year projected rates for survival, leukemic transformation and fibrotic progression were >75%, <5%, and <10%, respectively [50]. In contrast, the risk of thrombosis, in both PV and ET, exceeds 20% and a substantial proportion of patients experience vasomotor disturbances (e.g. headaches, lightheadedness, acral paresthesias, erythromelalgia, atypical chest pain) [51], and in case of PV, pruritus [52]. Also, in both PV and ET, some patients may develop acquired von Willebrand syndrome (AvWS), especially in the presence of extreme thrombocytosis (platelets >1,000 $\times$ 10^9/L), and be at risk for aspirin-associated bleeding [53]. Accordingly, the goal of current therapy in PV and ET is primarily to prevent thrombohemorrhagic complications, without increasing bleeding risk, and secondarily to control the aforementioned symptoms. In this regard, treatment is tailored to individual patients according to their risk for thrombosis or bleeding (Table IV).

Management of Low-Risk PV or ET, in the Absence of Extreme Thrombocytosis

Controlled studies have confirmed the anti-thrombotic value of low-dose aspirin in PV, among all risk categories [54]. In a retrospective study, aspirin use has also been reported to be beneficial in *JAK2*V617F-positive low-risk ET, in preventing venous thrombosis, and also in patients with cardiovascular risk factors, in preventing arterial thrombosis [55]. There is now both controlled [56] and uncontrolled [57] evidence that supports phlebotomy for all patients with PV. In a recent randomized study, 365 adult patients with PV were treated with a target hematocrit of <45% or 45 to 50% [56], after a median follow-up of 31 months, the primary end point of thrombotic events or deaths from cardiovascular causes was recorded in 5 of 182 patients in the low-hematocrit group (2.7%) and 18 of 183 patients in the high-hematocrit group (9.8%) ($P = 0.007$), supporting the current practice of keeping the hematocrit below 45% in patients with PV.

Low-dose aspirin therapy has also been shown to be effective in alleviating vasomotor (microvascular) disturbances associated with ET or PV [58]. Vasomotor symptoms in ET constitute headaches, lightheadedness, transient neurologic or ocular disturbances, tinnitus, atypical chest discomfort, paresthesias, and erythromelalgia (painful and burning sensation of the feet or hands associated with erythema and warmth). These symptoms are believed to stem from small vessel-based abnormal platelet-endothelial interactions [59]. Histopathological studies in erythromelalgia have revealed platelet-rich arteriolar microthrombi with endothelial inflammation and intimal proliferation accompanied by increased platelet consumption that is coupled with abundant VW factor deposition [59–61]. In regards to aspirin therapy in PV or ET, a recent report suggested that twice-a-day aspirin may work better than once daily dose in apparently aspirin-resistant cases [62].

Aspirin therapy is also considered to be adequate, and potentially useful in preventing complications during pregnancy, especially in *JAK2*V617F-positive cases [48,49,63]. First-trimester spontaneous miscarriage rate in ET or PV (>30%) [64–66] is significantly higher than the 15% rate expected in the control population and does not appear to be influenced by specific treatment [64]. Late obstetric complications as well as maternal thrombohemorrhagic events are relatively infrequent and platelet count usually decreases substantially during the second and third trimesters [67]. Neither platelet count nor cytoreductive therapy

TABLE IV. Risk Stratification in Polycythemia Vera and Essential Thrombocythemia and Risk-Adopted Therapy

Risk categories	Essential thrombocythemia	Polycythemia vera	Management during pregnancy
Low-risk without extreme thrombocytosis (age <60 years and no thrombosis history)	Low-dose aspirin	Low-dose aspirin + phlebotomy	Low-dose aspirin + phlebotomy if PV
Low-risk with extreme thrombocytosis (platelets >1,000 $\times$ 10^9/L)	Low-dose aspirin provided ristocetin cofactor activity >30%	Low-dose aspirin provided ristocetin cofactor activity >30% + phlebotomy	Low-dose aspirin provided ristocetin cofactor activity >30% + phlebotomy if PV
High-risk (age ≥60 years and/or presence of thrombosis history)	Low-dose aspirin + hydroxyurea	Low-dose aspirin + phlebotomy + hydroxyurea	Low-dose aspirin + phlebotomy if PV + Interferon-α
High-risk disease that is refractory or intolerant to hydroxyurea	Low-dose aspirin + interferon-α (age <65 years) or busulfan (age ≥65 years)	Low-dose aspirin + phlebotomy + interferon-α (age <65 years) or busulfan (age ≥65 years)	Low-dose aspirin + phlebotomy if PV + interferon-α

appears to affect either maternal morbidity or pregnancy outcome. Therefore, cytoreductive treatment is currently not recommended for low-risk women with ET that are either pregnant or wish to be pregnant.

Pruritus occurs in the majority of patients with PV (and a substantial number with PMF) [68] and is often exacerbated by hot bath [52]. In the low-risk disease setting, management should start with simple non-drug measures, such as avoidance of precipitating conditions, dry skin and temperature control of one's environment and water used for bathing. Etiology of PV-associated pruritus remains to be determined and treatment responses to antihistamines have been both unpredictable and variable [52]. In contrast, recent studies have suggested a greater than 50% response rate in PV-associated pruritus treated with paroxetine (20 mg/day), which is a selective serotonin reuptake inhibitor [69]. Other treatment modalities that have been reported to be useful in PV-associated pruritus include JAK inhibitors [70], IFN-α [71], and narrow-band ultraviolet B phototherapy [72].

Recommendations. I recommend the use of low-dose aspirin (81 mg/day; range 40–100 mg/day) in all patients with low-risk PV or ET, provided there are no major contraindications; the latter include clinically significant (ristocetin cofactor activity of $<$20–30%) AvWS that might be associated with extreme thrombocytosis (i.e. platelet count over 1 million/micL). In PV patients, I prefer a hematocrit target of 45%. I manage, pregnant patients or women of child-bearing potential, in the same general manner and I do not use platelet-lowering agents or heparin therapy in the setting of low-risk disease. In the presence of aspirin-resistant symptoms, it is reasonable to utilize a twice-daily rather than once-daily regimen of low dose aspirin or alternative antiplatelet agents such as clopidogrel (75 mg/day) alone or in combination with aspirin [73], as long as patients are monitored closely for drug side effects. One might also consider platelet-lowering agents (e.g. hydroxyurea) in such aspirin-refractory cases, but the target platelet count in this instance should be the level at which relief of symptoms is observed, and not necessarily 400 $\times$ 10^9/L. I no longer use anagrelide for the treatment of PV or ET because of its reported association with increased risk of arterial thrombosis, major bleeding and fibrotic progression [74]. Based on preliminary data from ongoing anti-JAK2 clinical trials, I suspect that JAK inhibitors might become the most effective agents for the treatment of MPN-associated pruritus [70,75].

Management of Low-Risk PV or ET Patients with Extreme Thrombocytosis or Abnormal Bleeding Diathesis

Bleeding diathesis in ET or PV is currently believed to be multifactorial in etiology. Laboratory evidence of AvWS occurs in the majority of patients with ET or PV and is characterized by the loss of large von Willebrand factor multimers, linked to their increased proteolysis by the ADAMTS13 cleaving protease, in a platelet count-dependent fashion [39,76–79]. This results in a functionally more relevant defect that may not be apparent when measuring VWF:Ag and FVIII levels alone [39,80] and requires the use of assays that assess VWF function (e.g. ristocetin cofactor activity; VWF:RCoA) [81–83]. Other causes of platelet dysfunction in ET or PV include acquired storage pool deficiency, increased platelet activation, decreased adrenergic receptor expression, impaired response to epinephrine, and decreased platelet membrane glycoprotein receptor expression [84–92].

Based on the above, the use of aspirin in both PV and ET requires caution, especially in the presence of extreme thrombocytosis (platelet count $>$1,000 $\times$ 10^9/L), which promotes the development of AvWS. However, clinically-relevant AvWS can occur even when the platelet count is well below 1,000 $\times$ 10^9/L, and that laboratory evaluation of AvWS must be performed in the presence of abnormal bleeding, regardless of platelet count [93].

Recommendations. In patients with PV or ET and extreme thrombocytosis, the use of aspirin can lead to bleeding complications because of AvWS; therefore, in the presence of platelets $>$1,000 $\times$ 10^9/L, screening for ristocetin cofactor activity is advised and consideration be given to withhold aspirin therapy if the result shows $<$30% activity. On the other hand, extreme thrombocytosis neither defines high-risk disease nor warrants the use of cytoreductive therapy.

Management of High-Risk PV or ET

Summary of randomized studies in PV

In the first controlled study in PV, the PV study group (PVSG) randomized 431 patients, between 1967 and 1974, to treatment with either phlebotomy alone or phlebotomy with either oral chlorambucil or intravenous radioactive phosphorus (P32) [94]. The results significantly favored treatment with phlebotomy alone with a median survival of 12.6 years compared with 10.9 and 9.1 years for treatment with radiophosphorus and chlorambucil, respectively. The difference in survival was attributed to an increased incidence of AML in patients treated with chlorambucil or radiophosphorus compared to those treated with phlebotomy alone (13.2% vs. 9.6% vs.1.5% over a period of 13–19 years) [95]. Furthermore, 3.5% of the patients treated with chlorambucil developed large cell lymphoma and the incidence of gastrointestinal and skin cancer was increased in those patients treated with either chlorambucil or radiophosphorus.

The European Organization for Research on Treatment of Cancer (EORTC) randomized 293 patients between 1967 and 1978 to treatment with either radiophosphorus or oral busulfan [96]. The results favored busulfan in terms of both first remission duration (median, 4 years vs. 2 years) and overall survival (10-year survival rates of 70% vs. 55%). At a median follow-up period of 8 years, there was not significant difference in the risk of leukemic transformation (2% vs. 1.4%), non-hematologic malignancy (2.8% vs. 5%), vascular complications (27% vs. 37%), or transformation into post-PV MF (4.8% vs. 4.1%) between the two arms.

Other randomized studies in PV have compared hydroxyurea against pipobroman (the first report showed a significant difference favoring pipobroman in the incidence of transformation into post-PV MF but no difference in survival, incidence of thrombosis, or the rate of leukemic conversion; however, a longer-term follow-up revealed a shorter survival, an increased risk of leukemic transformation, and a lower risk of post-PV MF, associated with pipobroman therapy) [97,98], radiophosphorus alone or with HU (no difference in survival, incidence of thrombosis, or risk of transformation into post-PV MF but radiophosphorus alone was associated with significantly less incidences of both acute leukemia and other cancers) [99], and radiophosphorus plus phlebotomy against phlebotomy plus high-dose aspirin (900 mg/day) in combination with dipyridamole (225 mg/day) (the addition of antiplatelet agents provided no benefit in terms of thrombosis prevention but increased the risk of gastrointestinal bleeding) [100].

The lack of anti-thrombotic value from anti-platelet agents in the above-mentioned PVSG-aspirin study may have been influenced by the fact that 27% of the patients randomized to the phlebotomy-aspirin-dipyridamole arm had a prior history of thrombosis compared with 13% in the other arm. This contention was confirmed by the most recent study from the European collaboration study on low-dose aspirin in polycythemia (ECLAP) [54]. The study enrolled 518 patients with PV in a double-blind randomized trial to low-dose aspirin (100 mg daily) or placebo. Treatment with aspirin did not increase the incidence of major bleeding and instead reduced the risk of combined endpoints for "nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes" and "nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes." [101]. Finally, a randomized study comparing hematocrit targets of $<$45% or 45 to 50%, using phlebotomy with or without hydroxyurea, was recently published and revealed decreased thrombotic events with the lower hematocrit target (see above) [56].

Summary of randomized studies in ET

Unlike the case with PV, the PVSG did not carry out large scale randomized studies in ET. In one of the very few controlled studies in ET, Cortelazzo et al. randomized 114 mostly high-risk patients to hydroxyurea ($n = 56$) or not ($n = 58$) [102]. After 27 months of follow-up, the incidences of thrombotic complications were 3.6% for hydroxyurea and 24% for no hydroxyurea, although the "thrombotic" episodes in two patients in the non-hydroxyurea arm constituted superficial thrombophlebitis. This is the only study, to-date, which randomized patients with ET to a drug versus no drug.

More recently, two studies randomized ET patients to hydroxyurea or anagrelide. In the earlier study [74], 809 high-risk patients were given low-dose aspirin plus either anagrelide or hydroxyurea. Hydroxyurea was better in terms of reducing the risk of arterial thrombosis, major bleeding, and fibrotic progression. Anagrelide performed better in preventing venous thrombosis. In addition, adverse dropout rate was significantly higher in the anagrelide arm. In the second study [103], anagrelide was compared to hydroxyurea in WHO-classified ET in 259 high-risk ET patients; during the total observation time of 730 patient-years, there was no significant difference between the anagrelide and hydroxyurea group regarding incidences of major arterial (7 vs. 8) and venous (2 vs.6) thrombosis, severe bleeding events (5 vs. 2), minor arterial (24 vs. 20) and venous (3 vs. 3) thrombosis and minor bleeding events (18 vs. 15), or discontinuation rates (adverse events 12 vs. 15 or lack of response 5 vs. 2); incidences of leukemic or fibrotic transformations were not reported.

Overview of single arm alkylating therapy in PV and ET

In a non-randomized study by the PVSG, treatment with hydroxyurea was associated with a lower incidence of early thrombosis compared with a historical cohort treated with phlebotomy alone (6.6% vs 14% at 2 years). Similarly, the incidence of AML in patients treated with hydroxyurea, compared to a historical control treated with either chlorambucil or radiophosphorus, was significantly lower (5.9 % vs. 10.6% vs. 8.3%, respectively, in the first 11 years of treatment) [104]. Other studies have confirmed the low incidence of AML in PV patients treated with hydroxyurea (1–5.6%) [105–107].

Many studies have reported on the use of pipobroman as a single agent in PV [108,109]. In one of these studies involving 163 patients, the drug was effective in more than 90% of the patients and median survival exceeded 17 years [108]. In the first 10 years, the incidences of thrombotic events, acute leukemia, post-PV MF, and other malignancies were 16%, 5%, 4%, and 8%, respectively. A similar retrospective study in 164 patients with ET treated with pipobroman as first-line therapy (starting dose 1 mg/kg/day) and followed for a median of 100 months, AML occurred in 5.5% of the cases [110]. In another study of 33 young patients ($<$50 years of age) with ET treated with pipobroman only and followed for a median of almost 16 years, the complete remission rate was 94% and only one patient (3%) developed AML whereas no patient experienced thrombotic complications [111]. However, as mentioned earlier, the final analysis of a French PV study comparing hydroxyurea to pipobroman has revealed a shorter survival, an increased risk of leukemic transformation, and a lower risk of post-PV MF, associated with pipobroman therapy [97,98].

Favorable outcome has also been reported in single arm studies using oral busulfan [112,113]. In 65 busulfan-treated patients with PV followed between 1962 and 1983, median survival was 19 years in patients whose disease was diagnosed before age 60 years [112]. Only two patients (3.5%) treated with busulfan alone developed acute leukemia. A similar percentage (3%) developed the complication in another study involving ET patients [114]. These figures were well within the baseline risk that is intrinsic to the diseases and no different than those seen with hydroxyurea [114]. The safety and efficacy of busulfan treatment in ET was recently underlined by a long-term study of 36 patients above age 60 years of age [115]; no instances of AML or other malignancies were documented after a median follow-up of 72 months.

Interferon therapy

It is now well established that IFN-α can control erythrocytosis or thrombocytosis in the majority patients with PV or ET (usual dose is 3 million units SC three times-a-week) [116]. A similar degree of benefit is appreciated in terms of reduction in spleen size or relief from pruritus. Two recent studies of pegylated INF-α ($\sim$90 μg SC weekly) in PV and ET reported hematologic remissions of $\sim$80% accompanied by decreases in *JAK2*V617F allele burden (complete molecular remission rate of 5–10%) [117,118]. In one of the two studies [117], 77 cases were evaluable after a median follow-up of 21 months and 76% and 70% of patients with ET or PV, respectively, achieved a complete hematologic remission, mostly in the first 3 months; side effects were recorded in 96% of the patients and 22% had discontinued treatment. Controlled studies are needed to clarify the advantage (or disadvantage) of IFN therapy in PV, compared to hydroxyurea therapy.

There is no hard data that implicates hydroxyurea or busulfan as being leukemogenic in PV or ET

There are, to date, no controlled studies that implicate either hydroxyurea or busulfan as being leukemogenic in either ET or PV. Similarly, the two largest non-controlled studies in ET [42] and PV [107] do not support the concern that leukemia might arise from the use of hydroxyurea and there is additional evidence to that effect from long-term studies of patients receiving hydroxyurea for sickle cell disease [119]. The evidence for busulfan leukemogenicity in the context of treatment for PV or ET is equally weak and inappropriately extrapolated from older patients with advanced phase disease and exposed to multiple

cytoreductive drugs. The recurrent flaw in data interpretation, when it comes to examining the relationship between leukemic drugs and leukemic transformation, is best illustrated by the largest prospective/retrospective study, to date, in PV ($n = 1,638$) [107]. At a median follow-up of 8.4 years from diagnosis, only 1.3% of the patients developed AML. When the authors compared the patients who transformed to those who did not, the former were older and more likely to have leukocytosis (known risk factor for leukemic transformation) at time of diagnosis or registration to the central database. They also had significantly longer disease duration and were more likely to have been treated with multiple drugs. In other words, exposure to alkylating agents other than hydroxyurea probably selects patients who are at a higher risk of leukemic transformation because of older age, longer disease duration and intrinsic aggressive disease biology. This, in my opinion, is the reason for the apparent association in some studies between leukemic transformation and drug therapy in PV or ET.

Recommendations. In addition to low-dose aspirin (and phlebotomy to a hematocrit target of 45% in case of PV), high-risk patients with PV or ET should receive hydroxyurea in order to minimize their risk of thrombosis (starting dose 500 mg BID). The dose of hydroxyurea is titrated to keep platelet count in the normal range and leukocyte count $>2 \times 10^9$/L. However, it is to be noted that the recommended platelet target is not based on controlled evidence. PV or ET patients who are either intolerant or resistant to hydroxyurea are effectively managed by INF-α (pegylated preparations preferred) or busulfan. Among these two second-line drugs, I prefer the use of INF-α for patients younger than age 65 years and busulfan in the older age group, although there is no controlled evidence to support or refute such a strategy. Busulfan is started at 4 mg/day, withheld in the presence of platelets $<100 \times 10^9$/L or WBC $<3 \times 10^9$/L, and the dose is reduced to 2 mg/day if the corresponding levels are $<150 \times 10^9$/L and $<5 \times 10^9$/L. I usually start pegylated IFN-α at 45 μg once-a- week and titrate up to 180 μg once-a-week if tolerated. Finally, recent data suggests that it might not be inappropriate to withhold cytoreductive drugs in older patients with ET (age >60 years) in the absence of both JAK2V617F and cardiovascular risk factors [38].

Concluding Remarks

In strictly WHO-defined ET, a recent study has revealed that prognosis is even better than previously assumed [19]. This has been attributed to the possibility that earlier studies unknowingly included patients with prefibrotic MF. Furthermore, disease complications in ET are effectively and safely managed by treatment with low-dose aspirin and, in case of high-risk disease, hydroxyurea. The overall scenario is similar in PV and concerns about drug leukemogenicity involving hydroxyurea or busulfan are largely based on anecdotes rather than properly executed controlled studies. Therefore, the following two things are required in order to justify the risk of unknown long-term health effects of non-conventional drug therapy such as with IFN-α or JAK inhibitors: (i) experimental or in vivo demonstration of disease-modifying activity and (ii) controlled studies to show added value.

I would also argue that hydroxyurea-refractory PV or ET is often adequately managed by treatment with busulfan or IFN-α. Therefore, there is currently no compelling evidence to support the need for JAK inhibitor therapy in the majority of patients with PV or ET, regardless of whether or not they are hydroxyurea-refractory. However, there are occasional patients who are likely to benefit from JAK inhibitor, as opposed to conventional drug, therapy, including those with intractable pruritus, severe constitutional symptoms or marked splenomegaly [70]. In this regard, it makes more sense to pursue a more specific JAK2 inhibitor, such as SAR302503 [75], rather than a less specific JAK1/2 inhibitor, such as ruxolitinib or CYT387 [70]. Even then, it is important to remember that leukemic transformation in PV or ET usually arises from *JAK2*V617F-negative progenitors and that the presence of *JAK2*V617F might actually be protective against fibrotic progression [19]. Therefore, drug-induced reduction in *JAK2*V617F allele burden might not necessarily translate into long-term benefit in ET or PV.

References

1. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood 2009;114:937–951.
2. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia 2008;22:14–22.
3. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. Leukemia 2010;24:1128–1138.
4. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia 2007;21:1960–1963.
5. Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: A critical reappraisal. Leukemia 2008;22:1299–1307.
6. Scott LM. The JAK2 exon 12 mutations: A comprehensive review. Am J Hematol 2011;86:668–676.
7. Pardanani A, Lasho TL, Finke CM, Tefferi A. Infrequent occurrence of MPL exon 10 mutations in polycythemia vera and post-polycythemia vera myelofibrosis. Am J Hematol 2011;86:701–702.
8. Boyd EM, Bench AJ, Goday-Fernandez A, et al. Clinical utility of routine MPL exon 10 analysis in the diagnosis of essential thrombocythaemia and primary myelofibrosis. Br J Haematol 149:250–257.
9. Ohashi H, Arita K, Fukami S, et al. Two rare MPL gene mutations in patients with essential thrombocythemia. Int J Hematol 2009;90:431–432.
10. Beer PA, Campbell PJ, Scott LM, et al. MPL mutations in myeloproliferative disorders: Analysis of the PT-1 cohort. Blood 2008;112:141–149.
11. Ding J, Komatsu H, Wakita A, et al. Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. Blood 2004;103:4198–4200.
12. Liu K, Martini M, Rocca B, et al. Evidence for a founder effect of the MPL-S505N mutation in eight Italian pedigrees with hereditary thrombocythemia. Haematologica 2009;94:1368–1374.
13. Mead AJ, Rugless MJ, Jacobsen SE, Schuh A. Germline JAK2 mutation in a family with hereditary thrombocytosis. New Engl J Med 2012;366:967–969.
14. Tefferi A, Lasho TL, Abdel-Wahab O, et al. IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. Leukemia 2010;24:1302–1309.
15. Tefferi A, Pardanani A, Lim KH, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia 2009;23:905–911.
16. Brecqueville M, Rey J, Bertucci F, et al. Mutation analysis of ASXL1, CBL, DNMT3A, IDH1, IDH2, JAK2, MPL, NF1, SF3B1, SUZ12, and TET2 in myeloproliferative neoplasms. Genes Chromosomes Cancer 2012;51:743–755.
17. Stegelmann F, Bullinger L, Schlenk RF, et al. DNMT3A mutations in myeloproliferative neoplasms. Leukemia 2011;25:1217–1219.
18. Passamonti F, Rumi E, Pietra D, et al. A prospective study of 338 patients with polycythemia vera: The impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. Leukemia 2010;24:1574–1579.
19. Barbui T, Thiele J, Passamonti F, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: An international study. J Clin Oncol 2011;29:3179–3184.
20. Carobbio A, Thiele J, Passamonti F, et al. Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: An international study of 891 patients. Blood 2011;117:5857–5859.
21. Passamonti F, Elena C, Schnittger S, et al. Molecular and clinical features of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations. Blood 2011;117:2813–2816.
22. Pardanani A, Guglielmelli P, Lasho TL, et al. Primary myelofibrosis with or without mutant MPL: Comparison of survival and clinical features involving 603 patients. Leukemia 2011;25:1834–1839.
23. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. Blood 2008;112:844–847.
24. Guglielmelli P, Pancrazzi A, Bergamaschi G, et al. Anaemia characterises patients with myelofibrosis harbouring Mpl mutation. Br J Haematol 2007; 137:244–247.

25. 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.
26. Tefferi A, Sirhan S, Lasho TL, et al. Concomitant neutrophil JAK2 mutation screening and PRV-1 expression analysis in myeloproliferative disorders and secondary polycythaemia. Br J Haematol. 2005;131:166–171.
27. James C, Delhommeau F, Marzac C, et al. Detection of JAK2 V617F as a first intention diagnostic test for erythrocytosis. Leukemia 2006;20:350–353.
28. Mossuz P, Girodon F, Donnard M, et al. Diagnostic value of serum erythropoietin level in patients with absolute erythrocytosis. Haematologica 2004;89: 1194–1198.
29. Wolanskyj AP, Lasho TL, Schwager SM, et al. JAK2 mutation in essential thrombocythaemia: Clinical associations and long-term prognostic relevance. Br J Haematol 2005;131:208–213.
30. Thiele J, Kvasnicka HM, Vardiman JW, et al. Bone marrow fibrosis and diagnosis of essential thrombocythemia. J Clin Oncol 2009;27:e220–221; author reply e222–223.
31. Schmitt-Graeff AH, Teo SS, Olschewski M, et al. JAK2V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica 2008;93:34–40.
32. Kvasnicka HM, Thiele J. Prodromal myeloproliferative neoplasms: The 2008 WHO classification. Am J Hematol 2010;85:62–69.
33. Michiels JJ, Berneman Z, Schroyens W, et al. 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 2004;83:504–512.
34. Barosi G, Mesa RA, Thiele J, et al. Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: A consensus statement from the International Working Group for Myelofibrosis Research and Treatment. Leukemia 2008;22:437–438.
35. Thiele J, Kvasnicka HM, Facchetti F, et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 2005;90: 1128–1132.
36. Manoharan A, Horsley R, Pitney WR. The reticulin content of bone marrow in acute leukaemia in adults. Br J Haematol 1979;43:185–190.
37. Finazzi G, Barbui T. Evidence and expertise in the management of polycythemia vera and essential thrombocythemia. Leukemia 2008;22:1494–1502.
38. Barbui T, Finazzi G, Carobbio A, et al. Development and validation of an International Prognostic Score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). Blood 2012;120:5128–5133; quiz 5252.
39. Budde U, Schaefer G, Mueller N, et al. Acquired von Willebrand's disease in the myeloproliferative syndrome. Blood 1984;64:981–985.
40. Gangat N, Strand J, Li CY, Wu W, Pardanani A, Tefferi A. Leucocytosis in polycythaemia vera predicts both inferior survival and leukaemic transformation. Br J Haematol 2007;138:354–358.
41. 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.
42. Gangat N, Wolanskyj AP, McClure RF, et al. Risk stratification for survival and leukemic transformation in essential thrombocythemia: A single institutional study of 605 patients. Leukemia 2007;21:270–276.
43. Passamonti F, Rumi E, Arcaini L, et al. Prognostic factors for thrombosis, myelofibrosis, and leukemia in essential thrombocythemia: A study of 605 patients. Haematologica 2008;93:1645–1651.
44. Bonicelli G, Abdulkarim K, Mounier M, et al. Leucocytosis and thrombosis at diagnosis are associated with poor survival in polycythaemia vera: A population-based study of 327 patients. Br J Haematol 2013;160:251–254.
45. Tefferi A. Leukocytosis as a risk factor for thrombosis in myeloproliferative neoplasms-biologically plausible but clinically uncertain. Am J Hematol 2009; 85:93–94.
46. Barbui T, Carobbio A, Rambaldi A, Finazzi G. Perspectives on thrombosis in essential thrombocythemia and polycythemia vera: Is leukocytosis a causative factor? Blood 2009;114:759–763.
47. Tefferi A, Passamonti F. Essential thrombocythemia and pregnancy: Observations from recent studies and management recommendations. Am J Hematol 2009;84:629–630.
48. Passamonti F, Rumi E, Randi ML, Morra E, Cazzola M. Aspirin in pregnant patients with essential thrombocythemia: A retrospective analysis of 129 pregnancies. J Thromb Haemost 2010;8:411–413.
49. Gangat N, Wolanskyj AP, Schwager S, Tefferi A. Predictors of pregnancy outcome in essential thrombocythemia: A single institution study of 63 pregnancies. Eur J Haematol 2009;82:350–353.
50. Crisa E, Venturino E, Passera R, et al. A retrospective study on 226 polycythemia vera patients: impact of median hematocrit value on clinical outcomes and survival improvement with anti-thrombotic prophylaxis and non-alkylating drugs. Ann Hematol 2010;89:691–699.
51. Tefferi A, Elliott M. Thrombosis in myeloproliferative disorders: Prevalence, prognostic factors, and the role of leukocytes and JAK2V617F. Semin Thromb Hemost 2007;33:313–320.
52. Diehn F, Tefferi A. Pruritus in polycythaemia vera: Prevalence, laboratory correlates and management. Br J Haematol 2001;115:619–621.
53. Michiels JJ, Berneman Z, Schroyens W, et al. The paradox of platelet activation and impaired function: Platelet-von Willebrand factor interactions, and the etiology of thrombotic and hemorrhagic manifestations in essential thrombocythemia and polycythemia vera. Semin Thromb Hemost 2006;32:589–604.
54. Landolfi R, Marchioli R, Kutti J, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. N Engl J Med 2004;350:114–124.
55. Alvarez-Larran A, Cervantes F, Pereira A, et al. Observation versus antiplatelet therapy as primary prophylaxis for thrombosis in low-risk essential thrombocythemia. Blood 2010;116:1205–1210; quiz 1387.
56. Marchioli R, Finazzi G, Specchia G, et al. Cardiovascular events and intensity of treatment in polycythemia vera. New Engl J Med 2013;368:22–33.
57. Chievitz E, Thiede T. Complications and causes of death in polycythemia vera. Acta Med Scand. 1962;172:513–523.
58. Michiels JJ, Berneman Z, Schroyens W, et al. Platelet-mediated erythromelalgic, cerebral, ocular and coronary microvascular ischemic and thrombotic manifestations in patients with essential thrombocythemia and polycythemia vera: A distinct aspirin-responsive and coumadin-resistant arterial thrombophilia. Platelets 2006;17:528–544.
59. Michiels JJ, Abels J, Steketee J, van Vliet HH, Vuzevski VD. Erythromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia. Ann Int Med 1985;102:466–471.
60. van Genderen PJ, Lucas IS, van Strik R, et al. Erythromelalgia in essential thrombocythemia is characterized by platelet activation and endothelial cell damage but not by thrombin generation. Thromb Haemost 1996;76:333–338.
61. van Genderen PJ, Michiels JJ, van Strik R, et al. Platelet consumption in thrombocythemia complicated by erythromelalgia: Reversal by aspirin. Thromb Haemost 1995;73:210–214.
62. Pascale S, Petrucci G, Dragani A, et al. Aspirin-insensitive thromboxane biosynthesis in essential thrombocythemia is explained by accelerated renewal of the drug target. Blood 2012;119:3595–3603.
63. Niittyvuopio R, Juvonen E, Kaaja R, et al. Pregnancy in essential thrombocythaemia: Experience with 40 pregnancies. Eur J Haematol 2004;73:431–436.
64. Wright CA, Tefferi A. A single institutional experience with 43 pregnancies in essential thrombocythemia. Eur J Haematol 2001;66:152–159.
65. Passamonti F, Randi ML, Rumi E, et al. Increased risk of pregnancy complications in patients with essential thrombocythemia carrying the JAK2 (617V>F) mutation. Blood 2007;110:485–489.
66. Griesshammer M, Struve S, Harrison CM. Essential thrombocythemia/polycythemia vera and pregnancy: The need for an observational study in Europe. Semin Thromb Hemost 2006;32:422–429.
67. Elliott MA, Tefferi A. Thrombocythaemia and pregnancy. Best Pract Res Clin Haematol 2003;16:227–242.
68. Vaa BE, Wolanskyj AP, Roeker L, et al. Pruritus in primary myelofibrosis: Clinical and laboratory correlates. Am J Hematol, in press.
69. Tefferi A, Fonseca R. Selective serotonin reuptake inhibitors are effective in the treatment of polycythemia vera-associated pruritus. Blood 2002;99:2627.
70. Pardanani A, Vannucchi AM, Passamonti F, Cervantes F, Barbui T, Tefferi A. JAK inhibitor therapy for myelofibrosis: Critical assessment of value and limitations. Leukemia 2011;25:218–225.
71. Muller EW, de Wolf JT, Egger R, et al. Long-term treatment with interferon-alpha 2b for severe pruritus in patients with polycythaemia vera. Br J Haematol 1995;89:313–318.
72. Baldo A, Sammarco E, Plaitano R, et al. Narrowband (TL-01) ultraviolet B phototherapy for pruritus in polycythaemia vera. Br J Dermatol 2002;147:979–981.
73. Kayacioglu I, Gunay R, Saskin H, et al. The role of clopidogrel and acetylsalicylic acid in the prevention of early-phase graft occlusion due to reactive thrombocytosis after coronary artery bypass operation. Heart Surg Forum 2008;11:E152–E157.
74. 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.
75. Pardanani A, Gotlib JR, Jamieson C, et al. Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. J Clin Oncol 2011;29:789–796.
76. Budde U, Dent JA, Berkowitz SD, et al. Subunit composition of plasma von Willebrand factor in patients with the myeloproliferative syndrome. Blood 1986;68:1213–1217.
77. Lopez-Fernandez MF, Lopez-Berges C, Martin R, et al. Abnormal structure of von Willebrand factor in myeloproliferative syndrome is associated to either thrombotic or bleeding diathesis. Thromb Haemost 1987;58:753–757.
78. Tsai HM. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. Blood 1996;87:4235–4244.
79. Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001;413: 488–494.
80. Michiels JJ, Budde U, van der Planken M, et al. Acquired von Willebrand syndromes: Clinical features, aetiology, pathophysiology, classification and management. Best Pract Clin Haematol 2001;14:401–436.
81. van Genderen PJ, Budde U, Michiels JJ, et al. The reduction of large von Willebrand factor multimers in plasma in essential thrombocythaemia is related to the platelet count. Br J Haematol 1996;93:962–965.
82. van Genderen PJ, Prins FJ, Lucas IS, et al. Decreased half-life time of plasma von Willebrand factor collagen binding activity in essential thrombocythaemia: Normalization after cytoreduction of the increased platelet count. Br J Haematol 1997;99:832–836.
83. Favaloro EJ. Collagen binding assay for von Willebrand factor (VWF:CBA): Detection of von Willebrands Disease (VWD), and discrimination of VWD subtypes, depends on collagen source. Thromb Haemost 2000;83:127–135.

84. Wehmeier A, Fricke S, Scharf RE, Schneider W. A prospective study of haemostatic parameters in relation to the clinical course of myeloproliferative disorders. Eur J Haematol 1990;45:191–197.
85. Wehmeier A, Scharf RE, Fricke S, Schneider W. Bleeding and thrombosis in chronic myeloproliferative disorders: Relation of platelet disorders to clinical aspects of the disease. Haemostasis 1989;19:251–259.
86. Gersuk GM, Carmel R, Pattengale PK. Platelet-derived growth factor concentrations in platelet-poor plasma and urine from patients with myeloproliferative disorders. Blood 1989;74:2330–2334.
87. Burstein SA, Malpass TW, Yee E, et al. Platelet factor-4 excretion in myeloproliferative disease: Implications for the aetiology of myelofibrosis. Br J Haematol 1984;57:383–392.
88. Wehmeier A, Tschope D, Esser J, et al. Circulating activated platelets in myeloproliferative disorders. Thromb Res 1991;61:271–278.
89. Kaywin P, McDonough M, Insel PA, Shattil SJ. Platelet function in essential thrombocythemia. Decreased epinephrine responsiveness associated with a deficiency of platelet alpha-adrenergic receptors. New Engl J Med 1978;299: 505–509.
90. Mazzucato M, De Marco L, De Angelis V, et al. Platelet membrane abnormalities in myeloproliferative disorders: Decrease in glycoproteins Ib and IIb/IIIa complex is associated with deficient receptor function. Br J Haematol 1989;73:369–374.
91. Le Blanc K, Lindahl T, Rosendahl K, Samuelsson J. Impaired platelet binding of fibrinogen due to a lower number of GPIIB/IIIA receptors in polycythemia vera. Thromb Res 1998;91:287–295.
92. Jensen MK, de Nully Brown P, Lund BV, et al. Increased platelet activation and abnormal membrane glycoprotein content and redistribution in myeloproliferative disorders.[see comment]. Br J Haematol 2000;110:116–124.
93. Tefferi A, Smock KJ, Divgi AB. Polycythemia vera-associated acquired von Willebrand syndrome despite near-normal platelet count. Am J Hematol 2010;85:545.
94. 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.
95. Berk PD, Wasserman LR, Fruchtman SM, Goldberg JD. Treatment of polycythemia vera: A summary of clinical trials conducted by the polycythemia vera study group. In: Wasserman LR, Berk PD, Berlin NI, editors. Polycythemia Vera and the Myeloproliferative Disorders. Philadelphia: W.B. Saunders; 1995. pp 166–194.
96. Treatment of polycythaemia vera by radiophosphorus or busulphan: A randomized trial. "Leukemia and Hematosarcoma" Cooperative Group, European Organization for Research on Treatment of Cancer (E.O.R.T.C.). Br J Cancer 1981;44:75–80.
97. Najean Y, Rain JD. Treatment of polycythemia vera—The use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. Blood 1997;90: 3370–3377.
98. Kiladjian JJ, Chevret S, Dosquet C, et al. Treatment of polycythemia vera with hydroxyurea and pipobroman: Final results of a randomized trial initiated in 1980. J Clin Oncol 2011;29:3907–3913.
99. Najean Y, Rain JD. Treatment of polycythemia vera: Use of 32P alone or in combination with maintenance therapy using hydroxyurea in 461 patients greater than 65 years of age. The French Polycythemia Study Group. Blood 1997;89:2319–2327.
100. Tartaglia AP, Goldberg JD, Berk PD, Wasserman LR. Adverse effects of antiaggregating platelet therapy in the treatment of polycythemia vera. Semin Hematol 1986;23:172–176.
101. Finazzi G. A prospective analysis of thrombotic events in the European collaboration study on low-dose aspirin in polycythemia (ECLAP). Pathol Biol (Paris) 2004;52:285–288.
102. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. N Engl J Med 1995;332:1132–1136.
103. Gisslinger H, Gotic M, Holowiecki J, et al. Anagrelide compared to hydroxyurea in WHO-classified essential thrombocythemia: The ANAHYDRET Study, a randomized controlled trial. Blood, in press..
104. Fruchtman SM, Mack K, Kaplan ME, et al. From efficacy to safety—A polycythemia vera study group report on hydroxyurea in patients with polycythemia vera. Semin Hematol 1997;34:17–23.
105. West WO. Hydroxyurea in the treatment of polycythemia vera: A prospective study of 100 patients over a 20-year period. South Med J 1987;80:323–327.
106. Tatarsky I, Sharon R. Management of polycythemia vera with hydroxyurea. Semin Hematol 1997;34:24–28.
107. Finazzi G, Caruso V, Marchioli R, et al. Acute leukemia in polycythemia vera. An analysis of 1,638 patients enrolled in a prospective observational study. Blood 2005;105:2664–2670.
108. Passamonti F, Brusamolino E, Lazzarino M, et al. Efficacy of pipobroman in the treatment of polycythemia vera: Long-term results in 163 patients. Haematologica 2000;85:1011–1018.
109. Petti MC, Spadea A, Avvisati G, et al. Polycythemia vera treated with pipobroman as single agent: Low incidence of secondary leukemia in a cohort of patients observed during 20 years (1971–1991). Leukemia 1998;12:869–874.
110. De Sanctis V, Mazzucconi MG, Spadea A, et al. Long-term evaluation of 164 patients with essential thrombocythaemia treated with pipobroman: Occurrence of leukaemic evolution. Br J Haematol 2003;123:517–521.
111. Passamonti F, Rumi E, Malabarba L, et al. Long-term follow-up of young patients with essential thrombocythemia treated with pipobroman. Ann Hematol 2004;83:495–497.
112. Messinezy M, Pearson TC, Prochazka A, Wetherley-Mein G. Treatment of primary proliferative polycythaemia by venesection and low dose busulphan: Retrospective study from one centre. Br J Haematol 1985;61:657–666.
113. D'Emilio A, Battista R, Dini E. Treatment of primary proliferative polycythaemia by venesection and busulphan. Br J Haematol 1987;65:121–122.
114. Sterkers Y, Preudhomme C, Lai JL, et al. Acute myeloid leukemia and myelodysplastic syndromes following essential thrombocythemia treated with hydroxyurea: High proportion of cases with 17p deletion. Blood 1998;91:616–622.
115. Shvidel L, Sigler E, Haran M, et al. Busulphan is safe and efficient treatment in elderly patients with essential thrombocythemia. Leukemia 2007;21:2071–2072.
116. Kiladjian JJ, Chomienne C, Fenaux P. Interferon-alpha therapy in bcr-abl-negative myeloproliferative neoplasms. Leukemia 2008;22:1990–1998.
117. Quintas-Cardama A, Kantarjian H, Manshouri T, et al. Pegylated interferon alfa-2a yields high rates of hematologic and molecular response in patients with advanced essential thrombocythemia and polycythemia vera. J Clin Oncol. 2009;27:5418–5424.
118. Kiladjian JJ, Cassinat B, Chevret S, et al. Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood 2008;112:3065–3072.
119. Voskaridou E, Christoulas D, Bilalis A, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: Results of a 17-year, single-center trial (LaSHS). Blood 2010;115:2354–2363.