

BCR::ABL1 negative myeloproliferative neoplasms: A review focused on essential thrombocythemia and polycythemia vera

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ABSTRACT

The classical myeloproliferative neoplasms are divided into chronic myeloid leukemia, and the Philadelphia negative polycythemia vera, essential thrombocythemia and primary myelofibrosis. These are heterogeneous diseases, originating from the clonal proliferation of myeloid stem cells, resulting in increased mature cell numbers in one or more myeloid lineages. The most commonly seen mutations in the Philadelphia negative myeloproliferative neoplasms include those in Janus kinase, myeloproliferative leukemia protein and the calreticulin genes. Philadelphia negative myeloproliferative neoplasms occur infrequently, with a combined annual incidence of 2.58 per 100,000. There are many overlapping symptoms of Philadelphia negative MPNs, such as fatigue, night sweats, hepatosplenomegaly and circulatory symptoms due to increased cell numbers. Total Symptom Score of the MPN Symptom Assessment Form is used to assess symptom burden on patients. The most worrisome complications are thrombo-hemorrhagic events, and risk stratification is especially important as treatment of disease is based on their category. Phlebotomy and aspirin are the mainstay of treatment in low-risk polycythemia vera and essential thrombocythemia patients, whereas high-risk disease calls for additional cytoreduction, usually with hydroxyurea.

KEYWORDS

myeloproliferative neoplasms, polycythemia vera, essential thrombocythemia, primary myelofibrosis, hematological malignancies

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DEFINITION & CLASSIFICATION OF MYELOPROLIFERATIVE NEOPLASMS

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of blood disorders affecting the myeloid stem cells, leading to their clonal and excessive proliferation of one or more types of mature myeloid cell lineages [1].

These myeloid stem cell disorders have been classically separated based on whether the BCR::ABL1 fusion gene is present or not. The BCR::ABL1 fusion gene is typically a product of reciprocal translocation between chromosome 9, hosting the ABL1 gene, and chromosome 22, hosting the BCR gene, giving rise to the now known, Philadelphia chromosome [2]. This process is strongly implicated with the disease entity of chronic myeloid leukemia (CML), and when this translocation is present, an abnormal tyrosine kinase is produced, allowing for the continued proliferation and survival of the myeloid stem cell [3]. In fact, almost all patients with CML have been shown to have one of the following: the Philadelphia chromosome, the BCR-ABL1 fusion gene, or the mRNA for the BCR-ABL fusion gene. Hence, we usually refer to CML as Philadelphia chromosome positive MPN or BCR::ABL1+ MPN [4]. In CML, this clonal proliferation leads to the mass production of mature granulocytes, namely neutrophils, though the number of eosinophils and basophils may also be elevated.

On the other side, there are the Philadelphia Chromosome negative, or more recently referred to as, the BCR::ABL1- MPNs, which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These diseases share many features with significant overlap, yet each has slightly different diagnostic criteria to help categorise them to their individual entities. PV is suspected when there is an otherwise unexplained increase of hematocrit/hemoglobin and red blood cell mass, ET to be considered as a diagnosis of exclusion for autonomous thrombocytosis, and PMF by the presence of bone marrow fibrosis, not attributed to secondary causes, such as infection, chronic inflammatory or autoimmune disease, neoplasms or toxic myelopathies [4]. These criteria are further detailed in Table 1. Due to the high likelihood for these diseases to possess the Janus Kinase 2 (JAK2) valine to phenylalanine mutation at position 617 (V617F), they have been further classified into their positivity or negativity for this mutation. Together with CML, these are referred to as the classical MPNs. There are also rarer entities within the realm of BCR::ABL1- MPNs, such as chronic neutrophilic leukemia, and chronic eosinophilic leukemia, though due to their rarity, their discussion will not be prompted here.

Although these diseases have specific criteria to help categorise them, it has also been theorized that these MPNs, together with myelodysplastic syndrome (MDS), comprise a spectrum of hematological diseases rather than their own distinct entities. This theory suggests these diseases are descendants from the so-called clonal hematopoiesis of indeterminate potential (CHIP). CHIP is the presence of a clonally expanded hematopoietic stem cell caused by a leukemogenic mutation in individuals without evidence of hematological malignancy, dysplasia or cytopenia. Many patients with CHIP may never progress to hematological disease, though some may progress to overt acute leukemia, or to MDS, MPNs or other types of myeloid or lymphoid neoplasms [5]. This could also explain why certain cases are difficult to categorise and hence referred to as MPN-unclassifiable or MDS/MPN unclassifiable. Additionally, it may also help to explain why they share many overlapping mutations and features, and why they all have the capability to transform to acute myeloid leukemia (AML).



Table 1. Criteria required to establish diagnosis of PV, ET or MF, as adopted from the 2016 WHO guidelines [4]

		Polycythemia Vera	Essential Thrombocythemia	Primary Myelofibrosis
Major Criteria	1a	Hemoglobin >16.5 g dL ⁻¹ men >16.0 g dL ⁻¹ women	Platelet Count ≥450 × 10 ⁹ L ⁻¹	Not meeting WHO diagnostic criteria for BCR-ABL positive CML, PV, RT, MDS or any other myeloid neoplasm
	1b	Hematocrit >49% men >48% women	AND Not meeting diagnostic criteria for BCR-ABL positive CML, PV, PMF,	
	1c	Increased red cell mass >25% above mean normal predicted value	MDS, or any other myeloid neoplasm	
	2 Biopsy	Bone marrow biopsy positivity	Bone marrow biopsy positivity	Bone marrow biopsy positivity
Minor Criteria	3 Peripheral blood mutation screening	Presence of JAK2 V617F or JAK2 exon 12 mutation	Presence of JAK2, CALR, or MPL mutation	Presence of JAK2, CALR, MPL, mutation OR if absent, presence of another clonal marker, OR exclusion of other causes of Bone marrow fibrosis
	4	Subnormal serum erythropoietin level	Presence of a clonal marker or absence of evidence for reactive thrombocytosis	At least one must be present on 2 consecutive occasions: - Anemia without identifiable cause - Leukocytosis >11 × 10 ⁹ L ⁻¹ - Palpable splenomegaly - LDH increased to above upper normal limit of institutional reference range - Leukoerythroblastosis
Criteria Combinations allowing for diagnosis		1a/b/c + 2 + 3 1a/b/c + 2 + 4 Biopsy not required if: 1a/b + 3 + 4	1 + 2 + 3 1 + 2 + 4	1 + 2 + 3 + 4



GENETIC BACKGROUND OF BCR::ABL1 NEGATIVE MPNS

Polycythemia vera

PV refers to the clonal proliferation of myeloid stem cells, primarily involving the erythropoietic process. Arber et al. [4] defines the peripheral blood mutations of JAK2 V617F, or the JAK2 exon 12 mutation as one of the criteria for helping to establish the diagnosis of PV.

JAK2 is a cytokine-driven receptor involved in activation of several downstream elements. Upon binding of the appropriate ligand, JAKs become phosphorylated, and promote the phosphorylation and activation of “Signal Transducers and Activators of Transcription” (STATs) [6]. As their name suggests, after phosphorylation, STATs dimerize, and they are able to translocate to the nucleus, activating transcription. Moreover, JAKs have also been associated with the activation of the growth-signalling pathway, through mitogen-activated protein kinases and through protein kinase B activation. It has been shown through knock-out studies of JAKs, inactivation of these proteins, either leads to early death, or severe disease [7–9]. In the case of JAK2, absence of this receptor has been shown to result in complete lack of erythropoiesis, Interferon- γ insensitivity and post-coital death by day 13 in knock-out mice [8]. However, JAK2 receptors are not only mediators in erythropoiesis, they are also involved in several other processes within the body. Other cytokines and chemokines may also bind and activate JAK2 receptors, allowing the regulation of thrombopoiesis, inflammation, the immune response and growth [7].

In PV, the JAK2 V617F is the most commonly observed mutation, seen in more than 90% of patients. This mutation leads to both the uncontrolled activation, as well as the hypersensitivity of JAK2 receptors to ligand binding [10]. The other more prevalent mutation is the JAK2 exon 12 mutation, seen in about 2% of PV patients that do not exhibit the JAK2 V617F mutation [11]. The JAK2 exon 12 mutation has been reported to present with similar phenotype presentations to that of the V617F on exon 14, though these patients may exhibit higher hemoglobin, with lower platelet and leukocyte numbers to their counterpart [12]. The remaining proportion of PV patients may exhibit mutations in ASXL1, DNMT3A, SF3B1, TP53, TET2 and many more [13–16]. These rarer mutations have also been observed in patients with other types of MPNs, CHIP, MDS, AML and the rest of the spectrum that these diseases have been theorized to exist on, as well as sometimes co-occurring with the three main driver mutations that are described later in this paper [7, 17].

Essential thrombocythemia & primary myelofibrosis

The JAK2 V617F mutation is not only associated with PV, but is also seen in ET and PMF. This mutation is also present in about 50–60% of patients with ET and PMF [18]. Similar to the hypersensitivity of the JAK2 receptor to erythropoietin (EPO), this mutation allows for the hypersensitivity to thrombopoietin, resulting in higher mobility of megakaryocytes, increased thrombocyte formation and increased chance of thrombus formation [19]. This is of course consistent with the treatment modality primarily used in BCR::ABL1- MPNs, allowing for the routine use of aspirin to prevent thrombotic complications. Additionally, the JAK-mutated platelets showed a relative deficiency of Glycoprotein VI, and potentially decreased von-Willebrand factor multimers, possibly explaining the acquired von Willebrand disease seen with the extremely high thrombocytosis in the BCR::ABL1- MPNs [20].



The other 2 more specific mutations seen in ET and PMF include those of the thrombopoietin receptor: MPL, and calreticulin: CALR. The MPL gene mutation is similarly oncogenic to the JAK2 mutation mechanism, through constitutive activation of the thrombopoietin receptor. This mutation is only found in about 3% and 5–8% of patients with ET and PMF, respectively [21]. Interestingly, MPL-mutated ET has also been found to be associated with higher rates of fibrotic progression, probably representing the prefibrotic stage of PMF, when morphologically reviewed [22]. This further highlights the difficulty in distinguishing certain cases and strengthens the theory of continuity in MPNs. The CALR gene is another that is commonly mutated in 15–32% of patients with ET and 25–35% of patients with PMF [23]. There are more than 50 distinct CALR mutations, yet they seem to occur only when patients show negative JAK2 and MPL gene mutations [24]. Consistent with JAK2 signalling, mutations in CALR have also been shown to operate through its constitutive activation via MPL, yet is unable to induce cytokine independence when co-expressed with EPO receptor or with the granulocyte-colony stimulating factor receptor [24, 25]. This could explain why mutations in CALR are not seen in PV patients and why those with JAK2 mutated ET display higher neutrophil counts than those with CALR mutant ET patients [24]. Further, CALR mutations in ET and PMF patients seem to have higher platelet counts, higher incidence of aspirin-induced hemorrhage, and a lower incidence of thrombosis compared to their respective counterparts with the JAK2 V617F mutation [24, 26]. When not displaying mutations in either JAK2, CALR or MPL, ET and PMF are referred to as ‘triple negative’. Triple negative ET displays a younger median age, lower hemoglobin, lower leukocyte count and lower incidence of thrombosis, compared with JAK2 mutated cases. Similarly, triple negative PMF patients were shown to have lower hemoglobin and platelet counts, compared to JAK2 mutated cases [23]. In such cases, triple negative ET seems to have a more benign course, with favourable outcomes, while triple negative PMF has substantially reduced survival when compared to the other mutational forms [27].

DEMOGRAPHICS OF BCR::ABL1- MPNS

Due to their rarity, there are few studies reporting the incidence and prevalence of MPNs [1]. Additionally, due to their significant overlap, ability for transformation, and diagnostic criteria, and lack of cancer registries in many countries, there is a wide variation in the epidemiological data for BCR::ABL1- MPNs. A meta-analysis performed by Titmarsh et al. [28], using a total of 14 different studies in the European region showed an incidence rate of 1.05 per 100,000 (95% CI: 1.03–1.07) for PV. This was in comparison to the incidence rate of 0.94 per 100,000 (95% CI: 0.92–0.96) in North America, showing no significant difference between the regions. Similarly, analysis from 8 studies in Europe showed an annual incidence rate of 1.60 per 100,000 (95% CI: 1.53–1.68) for ET and 0.46 per 100,000 (95% CI: 0.42–0.49) for PMF. It is also worth mentioning that there is difficulty in distinguishing early pre-PMF from ET, and PMF from secondary myelofibrosis (sMF), hence the incidence rate for PMF should be interpreted carefully [28]. The incidence ratios did not differ significantly between male and female, with the exception of ET, showing a tendency to occur in higher frequency in males [28, 29].

In a study carried out representing 455 MPN patients from the German registry, the median ages of patients with PV, ET and PMF were 60, 54 and 61.5 years, respectively [30]. In the USA, 826 patients with MPN at Mayo Clinic showed that the median age for PV, ET and PMF were



64, 55, and 63 years, respectively [23]. There have been cases where MPNs have been reported in children, though the annual incidence is as low as 0.003 per 100,000 and reflects the extreme rarity in this subgroup [31].

There have been few studies detailing the genetic predisposition of developing MPNs. On a population level, individuals with a JAK2 V617F positive MPN were 3.7 more likely to have the JAK2 46/1 allele compared to those without the allele [32]. This is further supported by the fact that the V617F mutation specifically arises on the 46/1 allele in most cases. The TERT gene also has a germline variant that further predisposes individuals to MPNs [33]. Other studies have also noted that individuals with MPNs were more likely to be of Jewish, especially of Ashkenazi descent [34, 35]. In addition to predisposition at a population level, are germline variants in families. These include ATG2B and GSKIP duplications [36], as well as RBBP6-R1569H [37], though they are seldom found and described. The MPNs described in these families seem to be no different from the MPNs developed in sporadic cases.

Moreover, environmental and lifestyle factors also appear to play a role in the development of MPNs. MPNs have also been associated with people working in agriculture, petroleum refineries, cooks and waiters, and those working with electrical devices [29]. Additionally, exposure to benzene may well be a risk factor to the development of MPNs. In a study of more than 2.5 million Israeli adolescents, obesity was associated with an increased incidence of developing a MPN [38]. Another study conducted by Kroll et al. showed that smoking was also associated with a 42% increased risk to develop a MPN/MDS [39]. Though in the analysis conducted by Podoltsev et al. [40], smoking was only found to increase the risk of MPN in women, and not men. Regardless, smoking seems to have a detrimental effect by reducing both overall survival and molecular response [41]. Further reported in the study by Podoltsev et al. [40] was that caffeinated coffee consumption seemed to be protective against PV.

CLINICAL ASPECTS AND COMPLICATIONS OF BCR::ABL1- MPNS

Patients with MPNs may present with a variety of symptoms, with significant overlap in those symptoms between the BCR::ABL1- MPNs. In one of the first studies conducted in 2006, survey results from 1,179 patients with MPNs were collected about their demographics, comorbidities, as well as their MPN and their symptoms [42]. The results showed that the symptoms that appear most frequently are: fatigue (80.7%), pruritus (53%), night sweats (50%), bone pain (44%), fever (14%) weight loss (13%), and pain under the left rib cage due to spleen enlargement (6%). As patient results for the phase 1 trial of ruxolitinib were reported, it was seen that there was no standardized manner in reporting patient outcomes with respect to their symptoms, and this was especially true for PMF [43]. This led to the development of the myelofibrosis symptom assessment form, which was validated in 2009 [44]. This was a 20-item survey, including the Brief Fatigue Inventory, as well as questions about symptoms associated with splenomegaly, and the catabolic and proliferative symptoms of MPNs [43]. Since then, there has been several developments such as including those with ET and PV, as well as refinement to help capture a wider range of symptomatology, eventually leading to the development of the MPN Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) [45]. This questionnaire has ten total items, where patients with MPNs could rank each item on a scale of 0–10, with 0 being absence of symptom and 10 being the worst imaginable. The MPN-SAF TSS, or MPN10 is now widely



used to assess the quality of life, and the evolution of symptoms in patients with MPNs [46, 47]. The ten symptoms most commonly seen in MPNs, are the same ones that are described in the MPN10 and are illustrated in Table 2.

Thrombosis & bleeding risk

The complications for ET, PV and PMF primarily consist of thrombohemorrhagic events, transformation to AML, or to sMF [48, 49]. As a result, several prognostic models have been developed to assess the risk of thrombotic events, or transformation to leukemia, or sMF. It has been described that across all BCR::ABL1- MPNs, there is an increase in risk of both arterial and venous thrombotic events in patients. Consequently, patients are classified according to this risk, and treatment is based on the group they lie in. Another consideration is the fact that venous thrombotic events frequently occur in unusual places, such as the hepatic, portal, splenic, mesenteric, or cerebral venous systems. In such cases, this may be the leading clinical sign that helps the subsequent diagnosis of a MPN [50].

In the case of PV patients, conventional risk stratification is primarily based on 2 categories: age >60 years and thrombotic history [51]. This was confirmed in the multicenter prospective European Collaborative Low-dose Aspirin Polycythemia Vera (ECLAP) study, showing these two risk factors were the most important [52]. In the case of high-risk, either risk factor can be present, or in the case of low risk, neither are. Additional risk factors that have been suggested to increase thrombotic risk include leukocytosis, V617F allele burden, and frequency of phlebotomy. In fact, in a meta-analysis performed by Carobbio et al. [53] involving more than 30,000 patients, proposed leukocytosis was implicated in an increased risk of arterial thrombosis in both PV and ET, with a higher risk in those with ET. However, there have been concerns regarding the validity of some of the studies included and others have failed to find such a relationship [49]. Similarly, the allelic burden and frequency of phlebotomy have also shown to have contradictory evidence between studies for thrombotic risk [49, 54]. As such, it currently stands that the thrombotic history and age remain as the primary risk factors in determining risk classification for PV patients [49, 55].

As for ET, patients are divided into 4 categories according to their age, JAK2 mutational status and thrombotic history [56]. Table 3 summarises the different category-risk groups for ET and PV. It should be noted that cardiovascular risk factors were once part of the risk-stratification criteria for PV, though were later removed, probably due to inconsistent findings. A study conducted by Santoro et al. [57], demonstrated that the presence of cardiovascular risk factors is

Table 2. The most common symptoms described by patients with MPNs that are part of the MPN10. Each symptom is given a score of 0–10, where 10 is the most severe. The total score is calculated by addition of the scores for the individual items. Information retrieved and adapted from Emanuel et al. [45]

Myeloproliferative Neoplasms 10 most common symptoms	
Fatigue	Early satiety
Abdominal discomfort	Inactivity
Concentration problems	Night sweats
Itching	Bone pain
Fever	Weight loss



Table 3. Current criteria for classification of thrombotic risk for ET and PV patients

	ET (rIPSET-T) [56]	PV (ELN) [51]
Very Low Risk	-Age ≤60 AND Wild-type JAK2 AND No thrombotic history	N/A
Low Risk	-Age ≤60 AND Mutated JAK2 AND No thrombotic history	Age ≤60 AND No thrombotic history
Intermediate	-Age >60 AND Wild-type JAK2 AND No thrombotic history	N/A
High	-Age >60 AND Mutated Jak2 OR Thrombotic history	Age >60 OR thrombotic history

also a strong determinant of poor prognosis in patients with ET. Similar findings were reported by Mancuso et al. [58], showing the deteriorating survival rates of PV patients with increasing cardiovascular risk factors. However, conflicting studies showing the negligible impact of cardiovascular risk factors, such as that by Krečák [59], may have contributed to the lack of addition to current risk stratification criteria.

The ECLAP study also presented the rates of hemorrhage and major bleeding in ET and PV. These were shown to be 2.9 events per 100 persons per year in the ET cohort, more than 3 times the risk of those with PV, at 0.8 events per 100 persons per year [52]. Bleeding events may range from petechiae and mucosal bleeding, to other more severe, yet rare outcomes, such as major hemorrhage and intracranial bleeding [50]. Hemorrhagic complications are more likely to appear in patients with extreme thrombocytosis ($1,000 \times 10^9 \text{ L}^{-1}$) and/or those with an acquired von Willebrand syndrome (AvWS). Extreme thrombocytosis has been theorised to predispose to AvWS, though it should also be noted that AvWS may also present in PV and ET patients with normal platelet counts. Although almost all PV, and most ET patients are eligible for aspirin therapy as per the current guidelines, care should still be taken when using aspirin in patients with risk factors for bleeding, and it may be of value to screen certain patients for AvWS [60].

Progression & transformation

The BCR::ABL1 MPNs are generally considered indolent conditions with a good overall prognosis [61]. The median survival for those with ET, PV and PMF was found to be approximately 20 years, 14 years and 6 years respectively [23]. However, in some cases, they may develop to acute leukemia, whereby >20% of blasts are present in the blood stream or bone marrow. Though, this is uncommon, and the leukemic transformation rate at 20 years is estimated at <10% for PV and <5% for ET [62]. In such cases, the overall survival is severely reduced and although some patients may be rescued, it is not frequent [10]. In patients with such transformations, the frequency of TP53 and IDH 1/2 mutations were higher than those who did not progress to leukemia [10].

Additionally, in about 15% of cases with ET or PV, progression to myelofibrosis can also occur, referred to as post-ET MF, post-PV MF, or sMF [63]. Interestingly, patients with post-ET



MF have better overall survival rates as well as lower rates of arterial thrombosis compared to patients with PMF [64]. Contrastingly, patients with post-PV MF showed a much higher arterial thrombotic risk compared to post-ET MF or PMF patients [64]. Patients who progress to sMF have higher frequency of mutations in the above-mentioned genes, TP53 and IDH $\frac{1}{2}$, than those who don't progress to sMF [10].

DIAGNOSTIC ALGORITHM

PV is suspected when there is an acquired increase in one or more of the red blood cell parameters (hemoglobin, hematocrit, red cell mass). In such cases, peripheral blood screening for characteristic mutations should follow, and measurement of serum EPO may also be requested to address issues of uncertainty [65]. If the patient displays JAK2 mutational status and subnormal EPO, the diagnosis of PV can be established without bone marrow sampling. In such cases, the required hematocrit should be 55.5% or more in men, or 49.5% or more in women [4, 66]. Diagnosis of primary erythrocytosis is additionally supported by the presence of hepatosplenomegaly, and elevation of the white blood cell and thrombocyte number [67].

The anamnesis of patients with suspected MPNs is important, and cases of unusual thrombosis, constitutional and/or vasomotor symptoms and/or splenomegaly should direct the physician to a possible MPN [27]. The symptoms that raise suspicion of MPNs are also the same symptoms that are included in the MPN10 and this may serve as a basis to help screen those with suspicion of MPNs [47]. Due to the increased amount of circulating blood volume, hypertension, plethora, cyanosis and conjunctival hyperemia and erythromelalgia may be present.

In case of negative JAK2 mutational status, and a continued clinical suspicion of a MPN, genetic investigation of MPL, CALR, and BCR::ABL1 are usually done to rule out other types of MPNs [27]. In these cases, where a diagnosis still has not been made, and JAK2 negative PV is still a clinical suspicion, then a BM biopsy must be performed to establish the diagnosis [65]. Only the hematocrit or hemoglobin may be used here, and a positive biopsy with a subnormal EPO is required to make the diagnosis. Bone marrow examination not only helps in diagnosis, but also to determine the degree of accumulation of fibrotic tissue at this stage of disease. This may have some prognostic significance during follow-up of the patient [27].

In cases where EPO levels are normal/elevated and a person displays negative JAK2 mutational status, then investigation of other causes of secondary erythrocytosis usually ensue, looking at hypoxia-associated polycythemia or tumor-associated polycythemia for example [65].

ET is made when thrombocytosis is observed and other secondary causes can be ruled out. As it has been previously mentioned with PV, clinical suspicion of MPNs is an important aspect of diagnosis, as signs and symptoms of MPNs help to direct the investigation. If there is suspicion of MPN with a patient presenting with thrombocytosis, then peripheral blood should be screened for JAK2, CALR and MPL mutations [65]. In the case of positive mutational status, a bone marrow biopsy is necessary to establish diagnosis. This is because, ET is established as a diagnosis of exclusion and patients with prefibrotic or overt myelofibrosis may also present with the same mutations and/or thrombocytosis. In the case of triple negative mutational status, diagnosis may become difficult as it requires further examination ruling out causes of reactive thrombocytosis (bleeding, iron deficiency, inflammation and cancer) or showing the presence of clonal markers, as well as a bone marrow biopsy showing ET morphology [27, 65].



Similar to ET, to establish a diagnosis of PMF, a bone marrow biopsy is required. PMF may present similarly to PV and ET, though may characteristically show teardrop-shaped cells, also known as dacrocytes, on blood smear [63].

OTHER LABORATORY PARAMETERS

Due to high cell turnover, MPN patients usually present with elevated LDH and uric acid levels [67]. Additionally, potassium levels may be elevated, and thus should be regularly checked to prevent the development of severe arrhythmias [68]. Although the erythrocyte sedimentation rate is not a specific marker, it is usually fastened in case of ET and PMF [69]. In PV patients, the sedimentation rate is often 1–2 mm h⁻¹ [70]. The laboratory examinations that may be useful in diagnosis, or to differentiate between other diseases are shown in Table 4.

RISK-ADAPTED THERAPY IN PV AND ET

Very low- & low risk disease

As previously mentioned, the stratification of risk for patients with PV and ET is imperative to guide treatment. For all patients with PV, lowering the hematocrit levels to less than 45% in males and 42% in females is recommended by the European Leukemia Network [51]. In 2011, Marchioli et al. [71] reported findings in the CYTO-PV study, comparing the rates of thrombosis at maintained hematocrit values of <45% vs 45%–50%. Results showed that there was a significantly lower risk for thrombosis at hematocrit levels of <45% for PV patients. This trial set the hematocrit control threshold for PV patients receiving phlebotomy. Phlebotomy is advised to be initiated by withdrawing 250–500 mL of blood daily, or every second day, until the threshold is met. For the elderly, and those with cardiovascular disease, blood withdrawal is recommended to be between 200 and 300 mL and performed twice weekly. Once the hematocrit has stabilized, it is recommended to perform regular blood counts every 4–8 weeks in order to determine the regularity of future phlebotomies [72].

The ECLAP study demonstrated that for all patients with PV, aspirin is beneficial for reducing risk of non-fatal myocardial infarction, stroke, pulmonary embolism, venous thrombosis or death from other cardiovascular causes. This reduction in risk was also paired with the fact that the use of aspirin did not significantly increase the risk of bleeding in these patients [73]. Low-dose aspirin has also shown to lessen the vasomotor/microcirculatory symptoms associated with both PV and ET [74]. As a result, low-dose aspirin seems to be the mainstay of treatment in those without a contraindication, such as in AvWS. However, as ET is associated with a greater risk of bleeding, aspirin is only indicated in very-low risk patients with cardiovascular risk factors that may show a benefit in its use [62]. For those with ET that fall in the very low risk category and are otherwise healthy, protocol recommends observation until patient status changes.

Furthermore, several studies have shown that twice or thrice daily dosing of aspirin may be better at preventing thrombosis than once daily. This has been studied by measuring the *in vivo* marker of platelet activation, serum thromboxane B2, which seemed to be lower with multi-dosing than single dosing aspirin [74–78]. As such, in the presence of treatment-resistant



Table 4. Laboratory Examinations that may help in diagnosis of ET or PV, or to differentiate from other diseases

Laboratory Examinations	ET	PV	Differential Diagnosis
Complete blood count [including hemoglobin, hematocrit, platelets, leukocytes]	Presents with elevated platelet counts. Repeated measurement may help to confirm the level of thrombocytosis.	Requires elevated hematocrit/hemoglobin levels, though platelets, leukocytes and other cell types may also be increased. Repeated measurement helps to track the progression.	Looking at overall result may help to exclude ET and PV by suggesting other disease processes i.e. anemia to rule out secondary thrombocytosis.
Peripheral Blood Smear	May show giant platelets.	Usually normochromic normocytic red cells, (can be microcytic and hypochromic in iron deficiency).	May help to visualise Howell-jolly bodies in asplenia or to rule out hematological conditions (i.e., leukemia or MDS).
Renal Function Parameters [including GFR, creatinine]	Decreased renal function, i.e. decreased GFR or increased creatinine, may indicate presence of MPN glomerulopathy.		Renal function is usually unimpaired in reactive causes of erythrocytosis. Renal function may also decrease in other hematological conditions (i.e. multiple myeloma)
Oxygen Saturation	—	Normal oxygen saturation helps to rule out hypoxic causes of polycythemia.	Oxygen saturation is usually decreased in other causes of secondary erthrocytosis.
Serum EPO levels	—	EPO levels are expected to be normal or subnormal in PV.	Elevated EPO levels could indicate hypoxia, renal artery stenosis, or tumor
Leukocyte Alkaline Phosphatase (ALP)	—	Acts as a marker for the last stages of myeloid differentiation and may be elevated.	Elevated alkaline phosphatase is common in leukocytosis. CML patients usually have a low level.
Uric Acid levels	Uric acid levels may be elevated in MPNs due to the high turnover rate.		Uric acid levels may also be elevated in other diseases with high cell turnover, i.e. leukemia.
Potassium levels	Pseudohyperkalemia may occur due to the high cell numbers and increased cell fragility [68].		—

(continued)



Table 4. Continued

Laboratory Examinations	ET	PV	Differential Diagnosis
Lactate Dehydrogenase (LDH)	LDH levels are typically in the normal range and elevation of LDH favours diagnosis of prefibrotic PMF instead of ET [65].	LDH levels are similarly raised in PV and serve as a marker of cellular proliferation.	LDH may also be elevated in other diseases with high cellular proliferation or hemolysis i.e. leukemia.
Ferritin	—	Iron deficiency may occur due to large amounts of red cell production, resulting in a low ferritin.	Elevated ferritin may indicate an inflammatory condition, while low ferritin almost always indicates iron deficiency.
C-reactive protein	May be increased and provide some prognostic information for ET and PV. In primary causes of thrombocytosis, C-reactive protein is usually much lower than in other disease processes [69].		may help to identify an infective, inflammatory or autoimmune disease as the cause of thrombocytosis or erythrocytosis
Erythrocyte Sedimentation rate	Increased in ET, but is markedly lower in primary thrombocytosis when compared to reactive causes [69]	Usually, negligible (1–2 mm h ⁻¹) [70]	ESR is much higher in secondary causes of thrombocytosis than in ET [99]

vasomotor symptoms, or patients with a higher risk of arterial thrombosis (i.e., in cardiovascular disease), it has been suggested that twice daily dosing may be beneficial [49]. These recommendations are plausible in low-risk disease for both PV and ET, and Figs 1 and 2 display these guidelines, as adapted from Tefferi and Barbui [62]. Regardless of such recommendations, multi-dose aspirin regimens are yet to be tested in clinical trials to see if this approach may indeed lower the thrombotic risk.

Another more recent consideration in low-risk PV is the potential need for cytoreduction. In 2021, Barbui et al. [79] released preliminary findings of the LOW-PV study, showing that ropeginterferon alfa-2b is more efficacious than phlebotomy alone in maintaining the hematocrit threshold for low-risk PV patients. This was the first study showing evidence of benefit of cytoreduction and led to the proposal of cytoreduction in low-risk PV. Cytoreduction has been shown to reduce vascular risk, decrease rates of transformation and increase symptom control [80]. As a result of the LOW-PV trial, experts advise that cytoreduction may be added to certain subgroups of PV patients for whom a high benefit-to-risk ratio is expected. Some instances where cytoreduction might be considered in low-risk PV include progressively increasing leukocyte and/or platelet counts, uncontrolled disease symptoms, poorly tolerated phlebotomy and enlarging spleen [51].



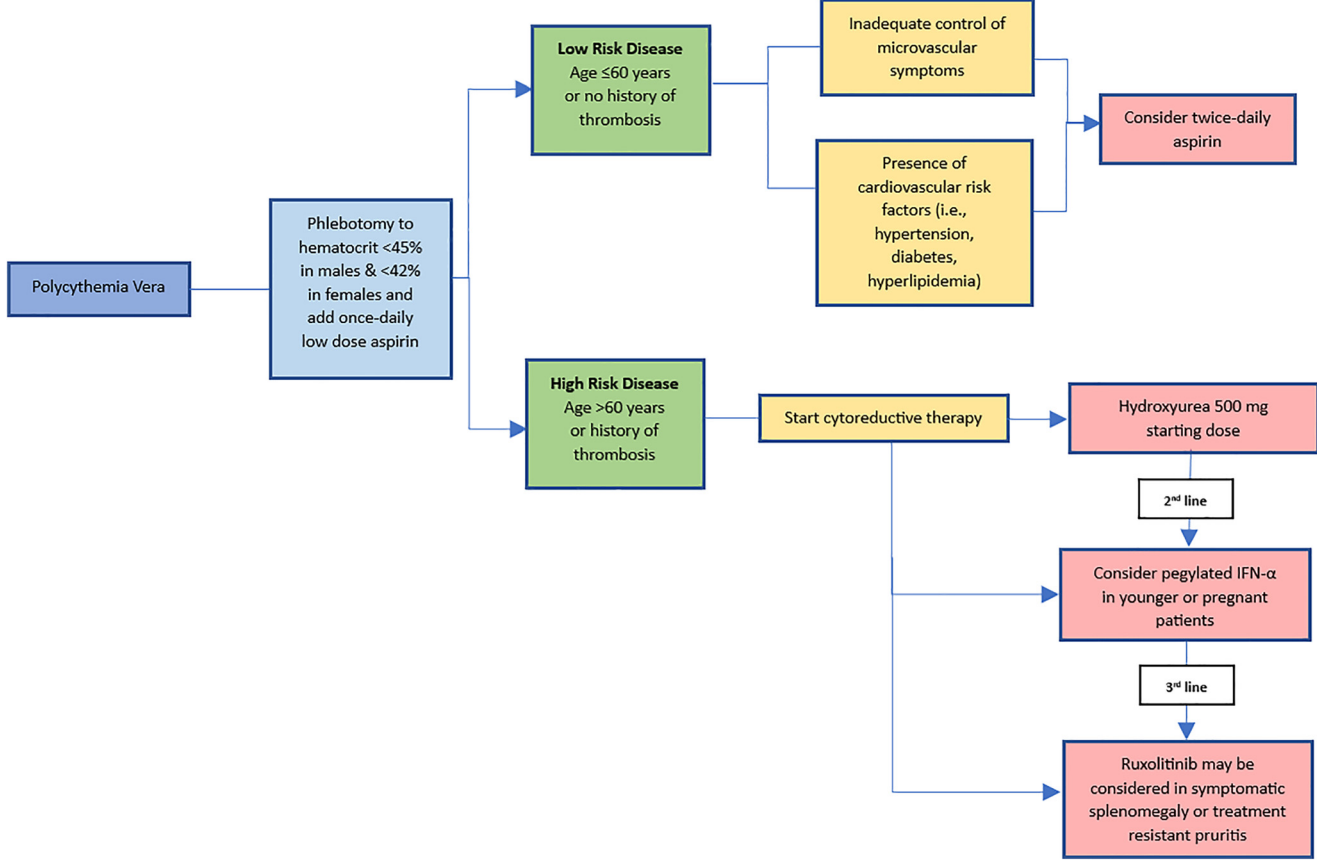


Fig. 1. Polycythemia Vera risk stratification management guidelines, adapted from Tefferi and Barbui [62]



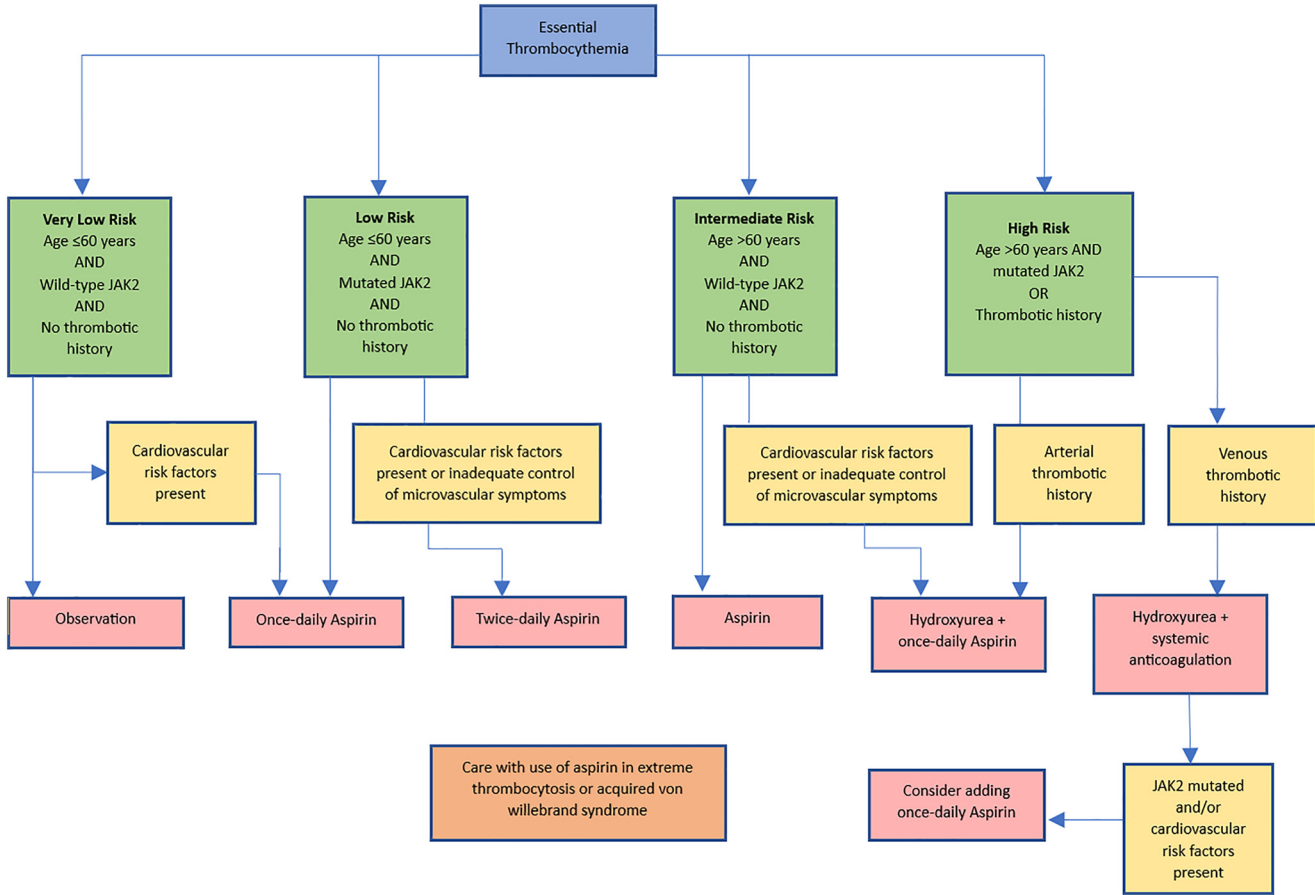


Fig. 2. Essential Thrombocythemia risk-stratification management guidelines, adapted from Tefferi and Barbui [62]



Intermediate & high-risk disease

As can be seen in Table 3, intermediate and high-risk disease refers to patients that are over 60 years old and/or with a history of thrombosis. Several studies have outlined these risk factors as the major predictors of vascular complications [63]. In high-risk disease, cytoreductive treatment is indicated for both PV and ET to reduce thrombotic risk. The first-line therapy at any age, is hydroxyurea (HU), though care should be taken when prescribing it to those less than 40 years of age. The starting dose of HU is 500–1000 mg in one dose, and regular review of blood count is used to titrate the dose to the right amount [62]. The effect of HU develops slowly, likewise, after the cessation of HU treatment, one or two weeks are needed for the recovery of the bone marrow.

In PV and for those with a history of arterial thrombosis and/or with cardiovascular risk factors, twice-daily aspirin may be considered in place of the standard daily dose. For those with history of venous thrombosis, systemic anticoagulation is suggested, and once daily aspirin may be added in select cases [62]. Of course, regular phlebotomy should be maintained in all risk categories to keep the hematocrit below the recommended threshold.

In high-risk patients with ET and history of arterial thrombosis, HU and twice daily aspirin should be considered. In history of venous thrombosis, HU and systemic anticoagulation are used. In such patients with venous thrombosis and with a JAK2 mutation, and/or with cardiovascular risk factors, once daily aspirin may be beneficial to the treatment regime. If cardiovascular risk factors are present in an intermediate risk ET patient, HU and once daily aspirin is suggested. For those with absent cardiovascular risk factors, once daily aspirin with (advised) or without HU is suggested [62].

2nd-line treatment options

For the most part, HU is well tolerated and is an acceptable treatment for cytoreduction. Though in one study of 166 patients who were taking HU for treatment of ET, as many as 22% of patients showed signs of resistance and/or intolerance [81]. Signs of intolerance include leg ulcers, mucocutaneous and gastrointestinal symptoms, pneumonitis and/or fever. The criteria for resistance are broad, though are generally related to the failure of controlling the cell counts and/or the ability to reduce massive splenomegaly [12, 27, 82]. The consistent need for phlebotomy to control hematocrit, or developing a cytopenia after starting the lowest dose of HU are also considered one of the criterions for resistance [27]. Resistance to HU also appears to be associated with worse survival and increased risk of transformation, and this effect is most prominent in patients who experienced resistance to HU via cytopenic toxicity [83]. In cases of HU intolerance, a lower dose may be trialled, and in case of failure, or in those with resistance, treatment options like anagrelide, interferon- α , or ruxolitinib may be considered.

Anagrelide is only recommended by the European Leukemia Network as a second line agent for ET [51]. It comes around when the platelet number cannot be adjusted to the desired value without leukopenia and/or anaemia. Anagrelide's efficacy seems to be lower than that of HU in reducing arterial thrombosis, especially in V617F mutated patients [72]. Anagrelide has been associated with headache, tachycardia, edema and diarrhea in the first 3 months, though these side effects were significantly reduced and minorly reported at 1 year [72, 84].



However, anagrelide has been associated with progressive anemia and an increased rate of transformation to myelofibrosis [81, 85, 86]. As patients with ET generally have good prognosis, cytoreductive treatment with anagrelide has been a topic of concern, as its benefit-risk analysis associated with its use has been debated. A recent study published in 2022 on 48 young patients receiving anagrelide over 10 years, showed that those with CALR-mutated ET had a significantly higher risk of bone marrow fibrosis and progressive anemia, when compared to those with JAK2 mutations [85]. Further, the inotropic effect of anagrelide may warrant cardiac function monitoring and careful use in those with cardiovascular risk factors [87]. Barbui et al. [72] recommends follow-up biopsies every 2–3 years in young patients receiving anagrelide. Another study of a Japanese cohort, conducted by Ito et al. [88], where the median age of ET patients was 67 years, demonstrates that it may be safely used as a first-line therapy for cytoreduction. Although anagrelide's recommendation for 2nd line cytoreduction seems justified, careful monitoring for those taking anagrelide over a long period may be indicated, especially in the younger persons, and those with CALR mutations.

Interferon- α is the preferred 2nd line agent used in HU intolerance/resistance, and can be used as first-line treatment for cytoreduction in pregnant and young patients [66, 89]. It has shown to have several functions: it can control erythro- and thrombocytosis, has a direct inhibiting effect on bone marrow fibrosis, and can be used to control splenomegaly and MPN-associated pruritus [90]. The main problems associated with interferon- α therapy include the cost of treatment, and the short-term side effects of fever and flu-like symptoms. The long-term toxicities of interferon therapy include hair and weight loss, weakness, myalgia, and severe depression [63, 91]. Pegylated forms (PEG-Interferon-alfa-2a and PEG-Interferon-alfa-2b, and more recently, mono-pegylated forms (Ropeginterferon alfa-2b), have allowed the ability to increase the elimination half-life, allowing administration to be weekly, or fortnightly in order to improve compliance and decrease their toxicity profile [92]. Secondly, current clinical trials indicate these pegylated and mono-pegylated forms have showed similar clinical response to HU [92]. It is the only treatment that decrease the mutant clonal fraction, and thus potentially induce molecular remission [93]. In fact, as increasing evidence is supporting the use of cytoreduction, even in low-risk PV, the indications for interferon use are widening and gaining importance, and the continuing studies examining their efficacy and tolerability may see to increase their use in the field of MPNs in the near future [80].

Ruxolitinib, although less routinely used, is a JAK 1/2 inhibitor, and as such may be of some use in JAK2-mutated MPNs. It has primarily been tested in PV, due to the high frequency of JAK2 mutated cases in these patients. A meta-analysis conducted by Masciulli [94] displayed that ruxolitinib had a decreased incidence of thrombotic events when compared to the best available therapy, though this response was not globally significant. It's side effects include dizziness, headache, fatigue, and cytopenias. Further it has also been associated with an increased rate of herpes zoster infection and non-melanoma skin cancer [95]. Currently, ruxolitinib is indicated for: refractoriness or intolerance to HU, PMF or sMF, symptomatic splenomegaly and pruritus that has failed to be relieved with other drugs, [27, 49, 66].

Alkylating agents such as pipobroman and busulfan were previously used in the treatment of MPNs, though due to their toxicity profile, are seldom used in the modern era.



Emerging treatments

There are several other novel drugs that are currently in development and have shown some promise in the treatment of ET and/or PV. Some examples include inhibitors of MDM2 (KRT-232, idasanutlin), histone deacetylase inhibitors (givinostat), and lysine-specific demethylase inhibitor, bomedemstat. Idasanutlin has been shown to induce hematological remission in the majority of patients resistant/intolerant to HU, though has been associated with gastrointestinal toxicity, that has failed to be controlled with antiemetics [12]. Givinostat has shown to induce some grade of spleen reduction, symptomatic symptom relief and hematologic response in some patients. It is undergoing phase 3 clinical trials and may eventually replace HU as the first line cytoreductive choice for PV patients [96]. Bomedemstat reduced peripheral cell counts, splenomegaly, inflammatory cytokines, mutant cell burdens and improved survival in mouse models of MPNs. A phase 3 study for the use of Bomedemstat in the treatment of ET patients is currently underway [97]. Agents developed also include those that mimic or promote hepcidin, which have shown potential in PV. Development of the antisense oligonucleotide, targeting the TMPRSS6 mRNA, eventually results in increased hepcidin expression. Additionally, subcutaneous injection of the hepcidin mimetic, rusfertide, has eliminated the need for phlebotomy, increased serum ferritin, and improved symptoms in PV patients. Non-pharmaceutical options that are also being researched include the use of gene editing to correct the JAK2 V617F mutation in autologous-transplanted PV hematopoietic stem cells [98].

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ABBREVIATION MEANING

MPN	Myeloproliferative Neoplasm
CML	Chronic Myeloid Leukemia
PV	Polycythemia Vera
ET	Essential Thrombocythemia
PMF	Primary Myelofibrosis
JAK2	Janus Kinase 2
V617F	Valine to Phenylalanine mutation at position 617
MDS	Myelodysplastic Syndrome
CHIP	Clonal Hematopoiesis of Indeterminate Potential
AML	Acute Myeloid Leukemia
STATs	Signal Transducers and Activators of Transcription
EPO	Erythropoietin
MPN-SAF TSS	Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score
sMF	Secondary Myelofibrosis
ECLAP	European Collaborative Low-dose Aspirin Polycythemia Vera
AvWS	Acquired von Willebrand Syndrome
HU	Hydroxyurea



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