New Perspectives on Polycythemia Vera

Subjects: Pathology

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Polycythemia vera (PV) is mainly characterized by elevated blood cell counts, thrombotic as well as hemorrhagic predisposition, a variety of symptoms, and cumulative risks of fibrotic progression and/or leukemic evolution over time. Major changes to its diagnostic criteria were made in the 2016 revision of the World Health Organization (WHO) classification, with both hemoglobin and hematocrit diagnostic thresholds lowered to 16.5 g/dl and 49% for men, and 16 g/dl and 48% for women, respectively. The main reason leading to these changes was represented by the recognition of a new entity, namely the so-called “masked PV”, as individuals suffering from this condition have a worse outcome, possibly owing to missed or delayed diagnoses and lower intensity of treatment. Thrombotic risk stratification is of crucial importance to evaluate patients’ prognosis at diagnosis. Currently, patients are stratified into a low-risk group, in the case of younger age (<60 years) and no previous thromboses, and a high-risk group, in the case of patients older than 60 years and/or with a previous thrombotic complication. Furthermore, even though they have not yet been formally included in a scoring system, generic cardiovascular risk factors, particularly hypertension, smoking, and leukocytosis, contribute to the thrombotic overall risk. In the absence of agents proven to modify its natural history and prevent progression, PV management has primarily been focused on minimizing the thrombotic risk, representing the main cause of morbidity and mortality. When cytoreduction is necessary, conventional therapies include hydroxyurea as a first-line treatment and ruxolitinib and interferon in resistant/intolerant cases. Each therapy, however, is burdened by specific drawbacks, underlying the need for improved strategies. Currently, the therapeutic landscape for PV is still expanding, and includes several molecules that are under investigation, like long-acting pegylated interferon alpha-2b, histone deacetylase inhibitors, and murine double minute 2 (MDM2) inhibitors.

Keywords: polycythemia vera ; risk factors ; target therapy ; hydroxyurea ; ruxolitinib ; interferon ; givinostat ; idasanutlin

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1. Introduction

Polycythemia vera (PV), together with essential thrombocythemia (ET) and myelofibrosis (MF), belongs to the so-called “classic” *BCR-ABL1*-negative myeloproliferative neoplasms (MPN), a heterogeneous group of diseases, characterized by the clonal expansion of an abnormal hematopoietic stem/progenitor cell. Its incidence has been estimated to be 2.3–2.8 per 100,000 persons/year, with a median age at diagnosis of about 60 years and a male/female ratio of 1.2:1 [1]. It is

mainly characterized by elevated blood cell counts, especially red blood cells; thrombotic as well as hemorrhagic predisposition; a variety of symptoms; and cumulative risks of progression to MF and/or transformation over time into acute myeloid leukemia.

The understanding of MPN pathophysiology dramatically improved following the description of recurrent molecular abnormalities. In particular, compared with both ET and MF, PV is molecularly more homogeneous, being driven by *JAK2* mutations in virtually all cases [2]; about 97% of such mutations are represented by *JAK2*V617F, which results from a somatic G to T mutation involving *JAK2* exon 14, leading to a nucleotide change at position 1849 and the substitution of valine to phenylalanine at codon 617 [3]. *JAK2*V617F-negative PV occurs in 1–3% of patients and mostly involves *JAK2* exon 12 [4]. Mouse models and clinical studies have both revealed phenotypic differences between *JAK2* exon 12 and *JAK2*V617F-mutated PV, the former being characterized by erythroid-dominant myeloproliferation, subtler tri-lineage hyperplasia in the bone marrow (BM), and younger age [5][6]. Concerning the V617F allele burden, in MPN patients, it correlates with both hematologic characteristics and clinical features[7]. Specifically in PV, *JAK2*V617F homozygosity seems to be associated with a stimulated erythropoiesis and myelopoiesis, lower platelet count, a higher incidence of splenomegaly, a larger spleen size, and a greater proportion of patients requiring cytoreductive therapy, as well as with a higher incidence of pruritus. On the contrary, the rate of major thromboses is not increased in homozygous PV patients compared with heterozygous subjects [8][9]. More recently, other *JAK2* variants have been identified, with *JAK2*V625F and *JAK2*F556V being gain-of-function mutations[10].

Furthermore, *JAK2*V617F mutation has been proven to play a crucial role in thrombotic complications. In detail, the pathogenesis of blood clotting activation in this disease is multifactorial, and involves various anomalies of platelets, erythrocytes, and leukocytes, as well as dysfunction of endothelial cells [11]. Indeed, abnormalities of blood cells arising from the clonal hematopoietic stem cells' proliferation also involve qualitative changes that characterize the switch of these cells from a resting to a procoagulant phenotype [12]. Prothrombotic features include blood cells' expression of procoagulant and proteolytic properties, inflammatory cytokines secretion, and the expression of adhesion molecules. Specifically concerning platelets, different studies showed that, in MPN patients, they circulate in an activated status, as assessed by the detection of increased expression of surface P-selectin and tissue factor [13][14][15] and by the increased fraction of platelets phagocytosed by circulating neutrophils and monocytes [16]. In addition, the thrombin generation induced by platelets was found to be increased and associated with platelet activation, particularly in *JAK2*V617F-mutated cases [17]. Interestingly, immature platelets, the newly formed platelets that show a higher hemostatic activity [18], are more elevated and more reactive than their mature counterpart, and positively correlate with the presence of the *JAK2*V617F mutation [19]. With regards to red blood cells, an abnormal adhesion of the latter to the sub-endothelial protein laminin, owing to the phosphorylation of Lu/BCAM by *JAK2*V617F pathway, has been demonstrated [20]. Moreover, neutrophils play a crucial role in the inflammatory response and in the blood coagulation system activation [21]. In particular, the release of proteolytic enzymes (i.e., elastase, cathepsin G) and reactive oxygen species (ROS) and the increased expression of CD11b on their surface can activate/damage platelets and endothelial cells and impair some coagulation proteins [22][23]. The adhesion of platelets to leukocytes and the formation of platelet–leukocyte aggregates mediate the crosstalk between platelets, neutrophils, and monocytes [24], suggesting that aspirin may inhibit the interaction between neutrophils and platelets . Finally, several factors, such as ROS and intracellular proteases, may perturb the physiological state of endothelium in MPN patients and turn it into a pro-adhesive and procoagulant surface [24].

Splenomegaly is estimated to affect 30% to 40% of PV patients and is usually associated with an advanced disease. In addition, spleen enlargement during follow-up has been shown to be significantly associated with an increased risk of fibrotic transformation and/or leukemic evolution [25][26].

Cytogenetic abnormalities can be detected in 14–20% of patients at the time of the initial diagnosis of PV [27][28][29][30], with del(20q), + 8, + 9, and + 1q being the most commonly reported [31][32][33]. The low frequency of abnormal karyotypes has made prognostication of PV patients using cytogenetic data challenging. While some studies have not shown a prognostic difference based on cytogenetic characteristics, other investigations, including one by the International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT), have recorded a higher risk of disease progression and a worse outcome in patients with an abnormal karyotype [34][35].

More recently, the occurrence and prognostic relevance of DNA sequence variants/mutations other than *JAK2/CALR/MPL* in PV were described [36][37][38]. In particular, a myeloid neoplasm-relevant 27-gene panel was used for next-generation sequencing (NGS) in 133 Mayo Clinic PV patients, revealing that 53% of them harbored one or more sequence variants/mutations other than *JAK2/CALR/MPL*; the most frequent were *TET2* and *ASXL1*. Adverse variants/mutations, in terms of overall (OS), leukemia-free (LFS), or myelofibrosis-free survival, included *ASXL1*, *SRSF2*, and *IDH2*, with a combined prevalence of 15%. The latter were also associated with an inferior OS (median, 7.7 versus 16.9 years) and their effect was independent of other conventional prognostic models. These observations were then validated in 215

Italian PV patients. In both the Mayo Clinic and Italian cohorts, leukemic or fibrotic progression, but not the thrombotic risk, was also predicted by adverse variants/mutations. Furthermore, the mutations number did not provide additional prognostic information [38].

On the basis of these data, the same group of authors have recently examined the possibility of integrating genetic information for predicting survival in PV. In a large cohort of 906 molecularly-annotated patients, including 404 with PV, adverse mutations occurred in 8 (2%) PV patients and multivariable analysis identified spliceosome mutations (*SRSF2*) to adversely affect OS. Furthermore, they also suggested the independent survival effect of adverse mutations, age >67 years, leukocyte count ≥15 × 10^9/L, and previous thromboses in PV. A subsequent hazard ratio (HR)-based risk point allocation allowed the development of a three-tiered mutation-enhanced international prognostic system (MIPSS) whose performance was shown to be superior to other conventional scoring systems [39].

2. Diagnostic Criteria

Major changes to the PV diagnostic criteria were made in the 2016 revision of the World Health Organization (WHO) classification (Table 1) [2]. In particular, the diagnostic thresholds for hemoglobin (Hb) and hematocrit (Hct) were both lowered to 16.5 g/dl and 49% for men, and 16 g/dl and 48% for women, respectively. The dismissal of the endogenous erythroid colony formation “in vitro” as a minor diagnostic criterion should also be mentioned; indeed, although highly specific for *JAK2*V617F-mutated, erythropoietin-independent erythroid progenitors [40], it suffers from being technically demanding and expensive, and it is available only in a very limited number of research laboratories.

Furthermore, as we have previously reported, the use of the WHO 2016 revised diagnostic criteria led to a higher number of PV diagnosis among those cases that would have been formerly classified as MPN, unclassifiable owing to the lack of all required diagnostic criteria [41].

Table 1. Diagnostic criteria for polycythemia vera according to the World Health Organization (WHO) classification.

	2008 WHO Classification	**2016 WHO Classification**
Major Criteria	1. Hb > 18.5 g/dL in men/Hb > 16.5 g/dL in women or other evidence of increased RCM; 2. Presence of *JAK2*V617F or other functionally similar mutation such as *JAK2* exon 12 mutation	1. Hb > 16.5 g/dL in men/Hb > 16.0 g/dL in women, or Hct > 49% in men/Hct > 48% in women, or increased RCM; 2. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature, megakaryocytes (differences in size); 3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation
Minor Criteria	1. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation; 2. Subnormal serum EPO level; 3. Endogenous erythroid colony formation in vitro	Subnormal serum EPO level
Criteria required for diagnosis	All 2 major and 1 minor or the first major and 2 minor criteria	All 3 major or the first 2 major and the minor criterion

Abbreviations: Hb: hemoglobin; **Hct**, hematocrit; **RCM**, red cell mass; **BM**, bone marrow; **EPO**, erythropoietin.

The main reason for these changes was represented by the recognition of a new entity, namely the so-called “masked PV”; indeed, individuals suffering from this condition have a worse outcome [42][43], possibly owing to missed or delayed diagnoses and, as a consequence, a lower intensity of treatment [44]. In addition, BM biopsy has been included among the major criteria for PV diagnosis; first of all, it can be helpful to distinguish between PV and *JAK2*-positive ET [43], and it also enables the assessment of BM fibrosis grade at diagnosis, thus identifying a more aggressive disease [45][46]. Indeed, increased BM reticulin fibrosis (≥1) at diagnosis has been found in 20–51% of patients [47][48][49]. In a previous report by the IWG-MRT, mostly mild BM reticulin fibrosis (grade ≥ 1 of a three-graded score system) at diagnosis was associated with both a lower risk of thrombosis during the clinical course and a higher risk of fibrotic progression, while OS or LFS were not affected[50]. In a more recent study, about 127 (48%) out of 262 PV patients displayed grade ≥ 1 reticulin fibrosis at the time of diagnosis, without significant differences in presenting clinical and laboratory features. In univariate analysis, BM fibrosis had no significant impact on OS, LFS, or thrombosis-free survival, whereas a significant association was recorded for myelofibrosis-free survival (HR 2.9; 95% confidence interval (CI) 1.32–6.78, $p = 0.009$) [51].

3. Prognostic Stratification for Thrombosis

As thrombotic events represent the main cause of morbidity and mortality for PV patients, with a registered rate of cardiovascular (CV) deaths and non-fatal thrombotic events of 5.5% patients/year [52], CV risk stratification is of crucial importance to evaluate patients’ prognosis at diagnosis.

In the observational, prospective ECLAP study, which enrolled 1.638 PV patients, the global incidence of both arterial and venous thromboses was significantly higher among older patients (age > 65 years) or in the case of a previous thrombosis [52]. In a subsequent study involving 1.545 PV subjects, independent predictive factors for arterial thromboses included leuko-erythroblastosis, arterial hypertension, and previous arterial thrombotic events . On the contrary, an abnormal karyotype and previous venous thromboses correlated with an increased risk of venous complications [53]. As a consequence, PV patients are currently stratified into two thrombotic risk classes: a low-risk group, in the case of younger patients (age < 60 years) with no previous thromboses, and a high-risk group, in the case of patients older than 60 years and/or with a previous thrombotic complications (Table 2).

Table 2. Risk factors for thrombosis in polycythemia vera patients: currently used and proposed ones.[54][55][56][57][58]

	Risk Factors	
	Low-risk	High-risk
Currently Used	Age < 60 years [52] and no previous thrombosis [52]	Age > 60 years [52] and/or previous thrombosis [52]
Proposed	Hypertension [54] Smoking habit Leukocytosis (>15 x 10^9/L [55] or >11 x 10^9/L [56]) Platelet count [57]	
Emerging	Abnormal karyotype [53] RDW [57,58] Lymphocyte percentage [57] Leuko-erythroblastosis	

Abbreviations: RDW: red cell distribution width.

Generic CV risk factors, particularly hypertension [59], smoking, and leukocytosis, while not yet formally included in a risk scoring system, contribute, as one can imagine, to the overall risk of thrombosis [60]. Hypertension was found to increase the annual rate of thrombosis from 0.85% patients/year to 2.05% patients/year and from 2.4% patients/year to 3.65%

patients/year in the low- and high-risk conventional category, respectively. Regarding leukocytosis, in a time-dependent multivariable analysis of the ECLAP cohort [61], a leukocyte count >15 × 10^9/L increased the HR of major thrombosis by 1.71-fold (95%CI, 1.1–2.6) compared with patients with ≤10 × 10^9/L leukocytes . Furthermore, in the prospective randomized CYTO-PV trial enrolling 365 patients with PV [62] and aimed at testing the effects of intensity of cytoreduction on thrombosis rate, a leukocyte count ≥11 × 10^9/L accounted for an HR for thrombosis of 3.9 (95%CI, 1.24–12.03) compared with the reference value (leukocytes <7 × 10^9/L) .

Concerning other new possible CV risk factors, red cell distribution width (RDW) has been investigated and higher values might represent different pathophysiological processes, including excessive erythropoiesis, more active myeloproliferation, and subclinical inflammation favoring CV disease development in MPNs . In addition, lymphocyte percentage (< 17%) and RDW (< 15%) might be predictors of vascular complications in patients without a history of thrombosis, and lymphocyte percentage (> 13%) and platelet count (>393 x 10^9/L) in the case of patients with a history of thrombosis .

4. Therapy

In the absence of agents proven to modify its natural history and prevent progression to advance phases, PV management has primarily been focused on minimizing the risk of thrombo-hemorrhagic complications that represent the major cause of morbidity and mortality [63][64]. Typical frontline management includes a combination of phlebotomy to decrease Hct to < 45% and low-dose aspirin . Underscoring the importance of a careful management, a study by Marchioli et al. found that treating to an Hct target range of 45–50% versus stringently maintaining it at < 45% at a median follow-up of 31 months was associated with a fourfold increase in death due to CV adverse events (AEs) or major thromboses.

The anti-thrombotic efficacy and safety profile of low-dose aspirin in PV have been assessed in the ECLAP double-blind, placebo-controlled, randomized clinical trial . In this study, 518 PV patients were randomized to receive aspirin 100 mg once daily or placebo. After a follow-up of about three years, low-dose aspirin reduced the combined risk of non-fatal cardio-embolic events or cardiovascular death by 60% (relative risk, 0.40; 95%CI 0.18–0.91, p = 0.0277), with no significant increase of major bleeding episodes (relative risk, 1.62; 95%CI 0.27–9.71).

Low-dose aspirin therapy was also effective in alleviating microvascular disturbances associated with PV [65], as these symptoms are believed to stem from small vessel-based abnormal platelet–endothelial interactions [66].

Furthermore, it was suggested that twice-daily aspirin may work better than once daily dose in certain cases[67]. Accordingly, such a therapeutic approach should be considered in patients who seem to be resistant to once daily dosing or considered at higher risk of arterial thrombosis [68].

Whether these results should be re-interpreted based on recent studies failing to demonstrate risk-balanced effectiveness of aspirin for primary prophylaxis in large cohorts of normal individual without prior history of atherosclerotic cardiovascular disease [69] represents an important research issue for future studies. In the meanwhile, considering that PV patients constitute a population of subjects at intrinsically high risk of CV events, according to the European LeukemiaNet (ELN) recommendations, low-dose aspirin (81 to 100 mg daily) should be used as primary anti-thrombotic prophylaxis for all PV patients with no major contraindications to aspirin, regardless of their risk categories [69][70][71].

Management of white blood cell (WBC) and platelet counts is also an important treatment goal because the risk of major thromboses was shown to be approximately four times greater in patients with WBC counts ≥ 11 × 10^9/L versus <7 × 10^9/L (p = 0.02) [56]. On the basis of these data, in order to achieve Hct target levels and to normalize WBC and platelet counts according to the ELN recommendations, many patients require a cytoreductive treatment [69][70][71]. For this purpose, conventional therapies include hydroxyurea (HU) as first-line option, ruxolitinib and interferon (IFN) in resistant/intolerant cases, and busulfan in older subjects. Each of these drugs, however, is burdened by specific drawbacks underlying the need for improved therapeutic strategies.

Currently, the therapeutic landscape for PV is expanding. Novel agents are in development with the aim not only to reduce the thrombotic potential, but also to act directly on the malignant clone with the aim of significantly modifying disease progression. Among these, long-acting pegylated interferon (PEG-IFN) alpha-2b, histone deacetylase inhibitors, and MDM2 inhibitors should be mentioned (Table 3).

Table 3. Therapeutic landscape for polycythemia vera.

Drug	Dosage

Approved	Hydroxyurea	0.5–2 g/day
	Ruxolitinib	10 mg twice daily
	Interferon-alpha	500,000–1 million units, 3 times weekly, progressively increased to 2–3 million units, 3 times weekly
	Ropeginterferon alpha-2b	starting dose of 45 µg weekly and titrated monthly in 45 µg increments up to a maximum of 180 µg weekly
Under development	Givinostat	100 mg twice daily
	Idasanutlin	100 or 150 mg daily, for 5 consecutive days in 28-day cycles

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