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Endothelial dysfunction and thrombosis in polycythemia vera

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Abstract

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm with an increased risk of thrombotic events. Endothelial dysfunction is a pathogenetic mechanism contributing to thrombus formation in PV. The presence of the *JAK2 V617F* mutation is associated with an increased risk of thrombosis due to changes in endothelial homeostasis mediated by overexpression of pro-adhesive and proinflammatory agents. Cytoreductive treatment that decreases the *JAK2* allele burden and inhibits the *JAK/STAT* signaling pathway is potentially more effective in thrombosis prevention than drugs that are less effective in the reduction of the *JAK2* allele burden.

This review aims to present the spectrum of endothelial dysfunction and the impact of cytoreductive treatment on the condition of endothelial cells and thrombosis risk in patients suffering from polycythemia vera.

Keywords: polycythemia vera, endothelium, endothelial dysfunction, thrombosis, thrombotic complications

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Introduction

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm characterized by increased red blood cell mass and blood viscosity. Thrombotic events are observed in c.40% of patients, and are the leading cause of morbidity and mortality in PV [1, 2]. Arterial thrombosis comprises 60–70% of all thromboembolic events in PV [3]. Of these, the most frequent are cerebrovascular events (stroke and transient ischemic attack), which constitute 25.2% and 24.5% of all arterial events, respectively [4]. Arterial occlusion less frequently involves coronary arteries and peripheral arteries [3, 4]. Venous thrombosis includes pulmonary embolism, deep vein thrombosis, cerebral vein thrombosis, and splanchnic venous thrombosis i.e. thrombosis located in the portal vein, splenic vein, porto-mesenteric vein, or hepatic vein (Budd-Chiari syndrome) [5, 6]. Venous events in PV often occur in atypical locations, most frequently in splanchnic veins, which constitute 45% of all venous events [7]. Thrombosis may be the first manifestation of PV, which is observed in 12–25% of patients [1, 7, 8]. In a group of patients under the age of 25 diagnosed with PV and treated with cytoreduction, the reason for treatment implementation in 24.6% of cases was thrombotic events [9]. highest rates of thrombosis in PV usually occur before, or at the time of, diagnosis, and decrease over time. This is probably due to the effects of cytoreductive treatment (64% of thrombotic events at the time of diagnosis or before vs. 36% during follow-up) [1]. However, an increased risk of developing thrombotic events persists throughout the patient's lifetime [10]. The rate of thromboembolic events in PV after diagnosis is c.2.6% per year [11, 12]. Thrombosis after diagnosis occurs in 22% of PV patients [7], and 16% of PV patients develop at least one thrombotic event after

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treatment initiation, which occurs more frequently in high-risk PV patients compared to low-risk [13]. The 20-year rate of thrombotic events in PV is 26%. Contrastingly, the 20-year rates of two other life-shortening complications, i.e. fibrotic transformation and leukemic transformation, are 16% and 4%, respectively [14]. Thus, the prevention of thrombotic complications is one of the main treatment goals in PV.

The pathogenesis of thrombosis in PV is complex and multifactorial, and involves both blood and vascular cells. Increased risk of thrombosis is associated with elevated blood cell count, *JAK2 V617F* allele burden, and activation of platelets, leukocytes, and endothelial cells [15, 16]. Tumor-mutated clone and circulating malignant cells induce an inflammatory host response which leads to generalized inflammation and promotes a prothrombotic state in PV. Malignant cells produce cytokines responsible for the prothrombotic phenotype of vascular endothelial cells [17]. Endothelial dysfunction is one of the factors associated with a high propensity for thrombosis in PV. Physiologically, the endothelium participates in the maintenance of vascular integrity and generates an antithrombotic surface. The dysfunction of endothelial cells observed in PV causes a series of changes that create pro-adhesive and prothrombotic blood conditions represented by an activation and adhesion of platelets, leukocytes, and erythrocytes to the endothelium, the formation of circulating platelet-leukocyte aggregates, neutrophil extracellular traps and microparticles, the generation of thrombin and the release of pro-inflammatory cytokines [5, 18, 19].

The prothrombotic phenotype of endothelial cells with the *JAK2* mutation is reflected in the overexpression and increased secretion of inflammatory, pro-adhesive, and endothelial cell activating factors: von Willebrand factor, P-selectin, E-selectin, soluble thrombomodulin, vascular cell adhesion protein 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), endothelial leukocyte adhesion molecule 1 (ELAM-1), vascular endothelial growth factor (VEGF), IL-6 and IL-33 receptor [20–25].

The molecular basis of endothelial dysfunction in PV includes a *JAK2 V617F* expression promoting thrombosis by inducing endothelial P-selectin expression, which may be reversed by cytoreductive therapy [23]. Molecules that participate in the adhesion of erythrocytes to the endothelium in PV are the Lutheran/basal cell adhesion receptor (Lu/BCAM) phosphorylated by the *JAK2 V617F* mutation and the endothelial alpha-5-laminin chain [26]. *JAK2* mutation expression in the endothelium causes tight adhesion of leukocytes to endothelial cells [21]. *JAK2* is also involved in collagen-induced platelet activation [27]. Inhibition of *JAK/STAT* signaling limits the overexpression of several genes that are involved in venous stenosis and thrombosis due to pro-inflammatory and pro-adhesive properties [22]. Inhibition of *JAK/STAT* signaling prevents thrombogenesis in PV.

Observational studies have confirmed the involvement of the *JAK2 V617F* mutation in the increased risk of thrombosis. *JAK2* variant allele frequency (VAF) >50% correlates with the risk of venous thrombotic events. This is an important risk factor for venous thrombosis, and also in conventionally low-risk patients [28].

Therefore, a reduction in the *JAK2* mutant allele burden seems to be a priority in the treatment of PV patients. The second, and equally important, goal of modern PV therapy should be to extinguish chronic inflammation.

Effect of treatment on endothelial function in PV

Thrombosis history and age over 60 are the two crucial criteria for the stratification of risk in patients and the choice of treatment regimen [29]. Cytoreductive treatment is indicated in patients with PV conventionally defined as high-risk, and in some specific situations in the low-risk patient group, such as inadequate hematocrit (Hct) control with phlebotomies (i.e. a need for 6+ phlebotomies per year), symptomatic splenomegaly, severe pruritus, persistence of constitutional symptoms, leukocytosis $> 15 \times 10^9$/L, thrombocytosis $\geq 1{,}500 \times 10^9$/L, and relevant cardiovascular risk (hypertension, ischemic heart disease, diabetes mellitus) [29, 30].

The three main goals of PV treatment are the prevention of thrombotic complications, improving quality of life by releasing or alleviating constitutional symptoms, and inhibiting the progression of fibrosis in the bone marrow. Good disease control is reflected in the maintenance of Hct below 45%. PV patients with Hct <45% have a significantly lower rate of cardiovascular death and of major thrombotic events [31]. Historically, the primary method of maintaining target Hct values has been phlebotomy. But nowadays it is known that phlebotomies alone do not decrease the risk of PV progression to the fibrotic phase; therefore, they are mainly used as adjunctive therapy in order to rapidly lower Hct in high-risk patients or periodically in low-risk patients [29, 32]. In the large cohort of PV patients included in the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) study, the maintenance of Hct <45% at 12 months was achieved in more than half of patients treated with HU but in only 31% of patients treated with phlebotomy alone. Phlebotomy was also less effective in controlling leukocytosis. The advantages of HU over phlebotomy were seen in reduced overall mortality, cardiovascular events, and myelofibrosis transformation [4].

The literature shows that the number of phlebotomies per year correlates with an increased risk of thrombosis in PV. Patients treated with HU who also needed at least three phlebotomies per year to maintain Hct control have been shown to have a significantly higher rate of thrombosis compared to patients who required fewer venesections

(20.5% vs. 5.3% at 3-year follow up). The need for phlebotomy identifies a subgroup of PV patients with excessive cell proliferation and an increased risk of thrombosis [33]. A clinical correlation between the need for phlebotomy and the risk of thrombotic events has also been shown in the MAJIC-PV trial. The time to the first thrombotic event within the first three years was associated with the average number of venesections [34]. Phlebotomy-induced iron deficiency may be responsible for an increased risk of thrombotic events [35–37]. Iron deficiency leads to reactive thrombocytosis by increasing the expression of vascular endothelial growth factor A (VEGFA) and affecting megakaryocyte differentiation [38]. Jimenez et al. [37] analyzed a link between iron deficiency and thrombotic events in an animal model. They observed a larger diameter of arterial and venous thromboses and a faster growth of thromboses in a rat model of iron deficiency. Platelet count correlated with the final thrombus dimension. Furthermore, the plasma and platelet expression of P-selectin was higher in the iron deficiency sample. All the above changes were reversible, and normalized after iron replacement therapy [37]. Iron deficiency leads to the activation of hypoxia-inducible factors (HIFs), which results in overexpression of several prothrombotic genes [36]. Among PV patients, those with iron deficiency had higher levels of mRNA detected in granulocytes and platelets of thrombospondin 1 (*THBS1*), P-selectin (*SELP*), interleukin 1 receptor-associated kinase 1 (*IRAK1*), and plasminogen activator inhibitor-1 (*SERPINE1*). Increased expression of prothrombotic genes mediated by iron deficiency has been observed in PV, essential thrombocythemia, and Chuvash polycythemia [36].

The cytoreductive treatment for PV includes hydroxycarbamide (HU), interferon-alpha (IFN-α), and ruxolitinib.

Hydroxyurea

Despite the development of new drugs i.e. pegylated interferon alpha 2a (PEG-IFN-α), ropeg interferon alpha 2b (ropeg-IFN-α), and ruxolitinib, HU is still considered to be among the front-line therapies in high-risk PV. HU is an inhibitor of ribonucleotide reductase, the enzyme responsible for the conversion of ribonucleotides to deoxyribonucleotides. HU inhibits DNA synthesis in the S phase of the cell cycle [39].

The effect of HU on the vascular endothelium has been investigated primarily in sickle cell anemia, where HU is the only form of pharmacotherapy dedicated to this disease, which decreases the frequency and severity of vaso-occlusive crises. HU significantly reduces endothelial release of the vasoconstrictor peptide endothelin-1 (ET-1) by down-regulating ET-1 gene expression. This effect is reversible and dose-dependent [40]. HU also reduces the expression of VCAM-1 in endothelial cells [40].

However, a negative effect of HU on the endothelium has also been observed, potentially promoting thrombosis. HU has been shown to increase the synthesis of ICAM-1, which is typically expressed in endothelial cells and cells of the immune system [40]. In an analysis of sickle cell anemia patients, those treated with HU had a significantly lower level of thrombospondin-1 than those not treated with HU [40]. Thrombospondin-1 is an adhesive glycoprotein that mediates the adhesion of red blood cells and platelets to the vascular endothelium. However, the levels of a disintegrin-like metalloproteinase with thrombospondin motif type 1 member 13 activity (ADAMTS13:Act) were shown to be significantly higher in patients receiving HU, indicating that HU acts to reduce thrombus formation [41]. HU increases nitric oxide (NO) production in endothelial cells through endothelial nitric oxide synthase (eNOS) phosphorylation [42]. Under laboratory conditions, a prominent elevation in HU-induced NO levels has been observed in a human bone marrow endothelial cell line [42]. In patients with sickle cell anemia, HU treatment has been associated with a decrease in the level of the thrombin-antithrombin complex and a decrease in the plasma level of the soluble vascular cell adhesion molecule-1 (sVCAM-1), which reflects a lower prothrombotic activity [43].

Observations on HU activity in sickle cell anemia studies suggest that HU may also prevent PV-related thrombotic complications due to influencing the activity of the endothelium by decreasing the expression of cell adhesion molecules, and increasing NO synthesis. However, Brusson et al. [44] showed adverse effects of HU on red blood cells and endothelial cell physiology in PV that may contribute to thrombus formation. In contrast to IFN-alpha, HU improves the adhesion of erythrocytes to endothelial laminin through increased Lu/BCAM phosphorylation [44].

Interferon

IFN-α is another cytoreductive treatment option for patients with PV. Currently, recombinant forms of IFN-α are available (rIFN-α) and are used in PV therapy: PEG-IFN-α and ropeg-IFN-α. The development of pegylated forms of IFN-α has allowed for less frequent administration, and has increased tolerance and efficacy [45]. Ropeg-IFN-α, in contrast to PEG-IFN-α, is a monopegylated rIFN-α, which extends elimination half-life, resulting in less frequent dosing and better tolerability [46]. The mechanism of action of rIFN-α in PV includes downstream activation of *JAK/STAT* signaling by binding to the interferon-alpha/beta receptor (IFNAR). Down-regulation of *JAK/STAT* signaling leads to a series of antiproliferative, antiangiogenic, pro-apoptotic, and immunomodulatory effects affecting virtually all immune cells, especially T cells, macrophages, and natural killer cells [47, 48].

rIFN-α not only normalizes elevated blood cell counts in patients with PV, but also can decrease the mutant *JAK2 V617F* allele burden [49–51]. After long-term treatment,

rIFN-α may even induce minimal residual disease, with *JAK2 V617F* VAF <1% and complete histological normalization of the bone marrow in some patients [45, 52–54]. Data from clinical trials that confirm the efficacy and safety of rIFN-α, and their ability to modulate the natural history of PV, supports the use of rIFN-α as a first-line treatment option for PV, especially for younger patients with indications for cytoreduction [32, 55, 56].

The effect of rIFN-α on the endothelium has been the subject of research in numerous diseases, including systemic lupus erythematosus (SLE). The literature shows the negative impact of type I interferon on the endothelial function in SLE [57, 58]. A link between type I interferons and decreased eNOS expression and NO bioavailability has been observed [59]. Faille et al. analyzed the impact of rIFN-α and HU on the biological hemostatic profile of patients with myeloproliferative neoplasms. They noted that rIFN-α reversibly increased endothelial activation markers [60]. In patients treated with rIFN-α, factor VIII activity, von Willebrand factor antigen concentration, and von Willebrand factor activity were all significantly higher, while protein S activity was significantly lower compared to patients treated with HU and untreated patients. The fibrinogen concentration was higher in rIFN-α-treated patients than in untreated patients, but not in HU-treated patients. After discontinuation of rIFN-α therapy, the altered hemostatic parameters were restored to normal levels: the von Willebrand factor antigen concentration decreased from 182% to 92%, the von Willebrand factor activity decreased from 172% to 92%, the factor VIII activity decreased from 158% to 112%, the fibrinogen concentration decreased from 3.7 to 3.1 g/L, and the protein S activity increased from 62% to 87% [60]. Despite the reversible changes in the biological hemostatic profile of the endothelium, rIFN-α therapy reduces the risk of thrombosis in PV.

The mechanisms responsible for thrombotic risk reduction include the inhibition of malignant cell proliferation, the stimulation of apoptosis, and increased immunogenicity against malignant clone cells [61]. rIFN-α therapy leads to the achievement of complete hematological response and a reduced *JAK2V617F* allele burden. The antithrombotic effect of rIFN-α influences two primary mechanisms of the prothrombotic tendency in PV: excessive circulating malignant cells, and chronic inflammation [62]. rIFN-α at the molecular level prevents thrombotic events. rIFN-α inhibits the generation of reactive oxygen species (ROS) in myeloproliferative neoplasms by downregulating several oxidative stress genes and upregulating antioxidative defense genes [63]. Both the *JAK2* mutation and ROS are involved in thrombosis formation [64, 65]. rIFN-α reduces genotoxic damage to hematopoietic stem cells, decreasing the risk of the appearance of additional mutations, which promote clonal evolution and disease progression to myelofibrosis and leukemic transformation [63].

The outcomes of a large single-center retrospective study supported the use of rIFN-α rather than HU and phlebotomy in the treatment of both high-risk and low-risk patients with PV. In high-risk patients, the 20-year myelofibrosis-free survivals for rIFN-α, HU, and phlebotomy were 89%, 41%, and 36%, respectively, and 20-year overall survivals were 66%, 40%, and 14%, respectively. In low-risk patients, the analogic parameters were as follows: 84%, 65%, and 55% of the patients achieved 20-year survival without progression to myelofibrosis. Overall survivals were 100%, 85%, and 80%, respectively [32]. A randomized controlled trial comparing the efficacy and safety of PEG-IFN-α and HU found no significant difference between these agents in the rates of thrombotic events, although the follow-up period (12 months) was not long [66]. Final results from the 6 ± year follow-up studies PROUD-PV and its extension CONTINUATION-PV comparing ropeg-IFN-α and HU/best available treatment (BAT) showed that the probability of event-free survival was significantly higher in the ropeg-IFN-α arm compared to the HU/BAT arm (0.94 vs. 0.82, $p = 0.04$). Adverse events included thromboembolic events, progression to myelofibrosis, progression to acute leukemia, and death [56].

Ruxolitinib

Ruxolitinib is a Janus-activated kinase (JAK) inhibitor that selectively inhibits JAK1 and JAK2. Inhibition of JAK kinase results in interruption of the cytokine and growth factor signaling pathway (*JAK/STAT*), leading to a decrease in the level of pro-inflammatory cytokines and chemokines that counteract inflammatory reactions [67].

Ruxolitinib is indicated in high-risk PV patients who are resistant or intolerant to HU, especially with symptomatic splenomegaly or severe drug-resistant pruritus, and in patients with symptoms typical of post-PV myelofibrosis [29]. The benefits of ruxolitinib therapy have been confirmed in clinical trials: improving overall survival and event-free survival, and decreasing the allele burden of *JAK2 V617F* [34]. Ruxolitinib due to *JAK/STAT* inhibition has the potential to reduce endothelial prothrombotic activation and proadhesive interactions between endothelial cells and leukocytes. *JAK/STAT* inhibition reduces endothelial cell activation mediated by TNF-alpha, and decreases the secretion of endothelial proadhesive and procoagulant factors i.e. P-selectin, von Willebrand factor, IL-6, endothelial tissue factor, and urokinase plasminogen activator [68]. Analysis of the endothelialized model demonstrated a significant increase in leukocyte and platelet velocity and a reduction in cell adhesion under the influence of ruxolitinib [69]. Ruxolitinib limits the formation of extracellular neutrophil traps [70] and reduces the expression of von Willebrand factor, VCAM-1, and P-selectin [69]. However, strong evidence in favor of ruxolitinib for the prevention of cardiovascular events is still lacking [71]. Ruxolitinib is more effective than BAT

in achieving a molecular response, which correlates with a reduced risk of thrombosis [71]. In the MAJIC study, patients with a sustained complete or partial molecular response had no thrombotic complications during follow-up, compared to a thrombotic event rate of 19.1% in patients without at least a partial molecular response [72]. A meta-analysis of four randomized controlled trials: RESPONSE, RESPONSE-2, MAJIC, and RELIEF, did not show a clear advantage of ruxolitinib in preventing thrombosis. The ability of ruxolitinib to reduce the risk of thrombosis was suggested (thrombosis ratio 3.09% and 5.51% of patients per year, for ruxolitinib and BAT, respectively), but it was not statistically significant (p = 0.098) [71].

Discussion

Endothelial dysfunction is one of the main mechanisms that contribute to thrombosis in PV. The *JAK2 V617F* mutation does not only affect hematopoietic stem cells and multipotent progenitor cells, but is also present in the endothelial cells of patients with myeloproliferative neoplasms and Budd-Chiari syndrome [73–75]. This mutation changes the phenotype and functions of endothelial cells. It induces the overregulation of several genes and pathways involved in inflammatory processes and cellular adhesion, with consequences in terms of thrombosis formation [22, 23, 59].

Historically, phlebotomies were the basis of PV treatment. It must be emphasized that phlebotomies do not improve patient quality of life. PV patients treated with phlebotomies have been shown to have significantly more severe fatigue, abdominal discomfort, inactivity, problems with concentration, night sweats, pruritus, and bone pain [76]. Phlebotomy alone was insufficient to keep Hct at a safe level in the ECLAP study, which showed the advantage of HU over phlebotomy in the Hct control at all time points analyzed i.e. after 12, 24, and 36 months [4]. Moreover, recent studies have warned against the excessive use of phlebotomies to control Hct in PV, suggesting that phlebotomies may contribute to thrombosis by inducing the overexpression of prothrombotic genes mediated by iron deficiency [35–37].

Interesting conclusions were drawn from a study by Faille et al. [60], which showed that IFN-α changes endothelial homeostasis to a more prothrombotic one. In general, their study showed that IFN-α-induced elevation of prothrombotic biomarkers may be considered a biological side effect of this treatment [60]. The clinical significance of the above observations, which may reflect an increased susceptibility to thrombotic events, needs to be further investigated since it does not translate into a clinically increased incidence of thrombotic complications in patients treated with rIFN-α. However, particular attention may need to be paid to patients treated with IFN-α who have additional risk factors for thrombotic events. Data from observational studies and randomized controlled trials clearly shows the benefit of rIFN-α over HU in the treatment of patients with PV in terms of longer event-free survival, including thrombotic events [32, 50, 55, 56]. These studies support the use of rIFN-α in the early treatment of PV to reduce thrombotic complications, prevent fibrotic transformation, and prolong survival.

JAK2 kinase inhibitors, unlike HU, down-regulate *JAK/STAT* signaling pathway and have the greatest potential to reduce the risk of thrombosis. Given this, it is surprising that data from post-marketing reports of JAK2 kinase inhibitors efficacy shows that this drug class can increase the incidence of pulmonary thrombosis and that the use of ruxolitinib may be risky in patients with portal vein thrombosis [77]. However, randomized controlled trials do not support this observation. A meta-analysis of randomized controlled trials suggest the superiority of ruxolitinib over alternative therapies in reducing the rate of thrombotic complications (3.09% and 5.51% of patients per year, respectively), although these results were not statistically significant [71].

Conclusions

Endothelial dysfunction in PV plays a key role in the pathogenesis of thrombosis and is strongly associated with the presence of the *JAK2* mutation. *JAK/STAT* signaling promotes the overexpression of inflammatory and pro-adhesive factors. Therefore, drugs that inhibit *JAK/STAT* signaling are more effective in preventing thrombosis in patients with PV. To date, no studies have been conducted directly comparing the effectiveness of rIFN-α to that of ruxolitinib in patients with PV, also in terms of reducing the tendency to thrombotic complications. The results of such studies would be very interesting, and would allow for a better determination of the role of both these drugs in the therapy of PV.

Article information and declarations

Authors' contributions

WML — idea for article, analysis and interpretation of data, drafting article, critical revision; PK — idea for article, analysis and interpretation of data, drafting article; PL — idea for article, analysis and interpretation of data, drafting article; TS — idea for article, critical revision, final approval of version to be published.

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Conflicts of interest

The authors declare no conflict of interest.

Supplementary material

None.

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