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Mechanisms of Thrombogenesis in Polycythemia Vera

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Abstract

Thrombotic and cardiovascular events are among the leading causes of death for patients with polycythemia vera (PV), and thrombosis history is a key criterion for patient risk stratification and treatment strategy. Little is known, however, about mechanisms of thrombogenesis in patients with PV. This report provides an overview of thrombogenesis pathophysiology in patients with PV and elucidates the roles of conventional and nonconventional thrombosis risk factors. In addition to several conventional risk factors for thrombosis, clinical data have implicated increased hematocrit and red blood cell adhesiveness, activated platelets, leukocytosis, and elevated *JAK2*^{V617F} allele burden in patients with PV. Furthermore, PV-related inflammation may exacerbate thrombogenesis through varied mechanisms, including endothelial damage, inhibition of natural anticoagulant pathways, and secretion of procoagulant factors. These findings suggest a direct link between myeloproliferation and thrombogenesis in PV, which is likely to provide new opportunities for targeted antithrombotic interventions aimed at decreasing PV-related morbidity and mortality.

Keywords

Janus kinase 2; polycythemia vera; thrombosis

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1 Introduction

Thrombosis is an important complication among patients with polycythemia vera (PV) and thrombotic events reflect disease burden and impact clinical outcomes. Roughly one fifth of patients with PV are diagnosed with an arterial or venous thrombotic event as a presenting feature,[1, 2] and fatal cardiovascular events contribute to the increased mortality rate among patients with PV.[1–4] Furthermore, healthcare utilization and cost are higher for patients with PV compared with age- and gender-matched persons in a large US health insurance claims database[5] and this is driven in part by arterial and venous thrombotic events.

Thrombotic events are so inextricably tied to the pathogenesis of PV that they are used in guiding patient risk stratification and treatment recommendations. Patients with no history of thrombotic events and who are <60 years of age are categorized as low risk,[6, 7] whereas patients with a history of thrombotic events or are over the age of 59 years are categorized as high risk.[6, 7] Current treatment recommendations suggest antiplatelet treatment with low-dose aspirin and phlebotomy as first-line therapy for low-risk patients.[6, 7] For high-risk patients, recommendations also include the use of cytoreductive agents (eg, hydroxyurea or interferon-α [IFN-α]).[6–9] However, none of the currently available treatment options for PV are designed to target the active Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, which over the last 10 years has been identified as the molecular etiology of PV.[10–13] Nearly all patients (99%) have an activating mutation in exon 12 or 14 of *JAK2*, which most commonly is *JAK2V617F*.[6, 13, 14] Constitutive activation of JAK2 has been associated with unregulated hematopoietic proliferation,[11–13, 15] and $JAK2^{V617F}$ allele burden is significantly correlated with markers of inflammation.[16] Inflammation is an important thrombotic risk factor.[16]

The risk of thrombotic events and their associated morbidity and mortality remain a primary concern in patients with PV[6–8] despite recent advances in understanding how JAK/STAT pathway dysregulation influences disease origin and progression. The goal of this report is to provide an overview of mechanisms of thrombogenesis in patients with PV by considering both conventional and nonconventional thrombosis risk factors, including correlates of an overactive JAK/STAT pathway.

2 Thrombosis in Patients With Polycythemia Vera

In 1856, Rudolf Virchow proposed what would later be termed *Virchow's triad*, which postulates that venous thromboses occur because of abnormalities in interactions between the blood vessel wall, blood constituents, and blood flow.[17, 18] Mechanisms underlying arterial and venous thrombogenesis have since been elucidated and include complex biochemical pathways regulating fibrin accumulation and the participation of several cell types, including platelets, endothelial cells (ECs), and leukocytes.[17, 19–21] Perhaps of most importance is the concept that mechanisms of thrombosis differ depending on the rheology of the circuit. Arterial thrombosis, because it develops in the high-resistance, high-flow vasculature, is triggered by platelet adhesion, activation, and aggregation.[22] In contrast, venous thrombosis, which develops in a high-capacitance, low-flow vascular circuit, is

triggered by the activation of the soluble coagulation system and the generation of insoluble fibrin.[22]

In patients with PV, thromboses have been reported throughout the arterial (transient ischemic attack, stroke, acute myocardial infarction, and peripheral arterial thrombosis) *and* venous (deep vein thrombosis, superficial thrombophlebitis, and pulmonary embolism) systems (Table 1).[23] Thrombotic events also may occur in the splanchnic vasculature in patients with myeloproliferative neoplasms (MPNs) and/or *JAK2* mutation. A recent meta-analysis found that 8% to 53% of patients with hepatic vein thrombosis (Budd-Chiari syndrome) or portal vein thrombosis who did not have cirrhosis or a biliary malignancy had a MPN, which was PV in the majority of cases.[24]

The rate of nonfatal thrombosis in patients with PV has been estimated at 3.8 per 100 patient-years.[23] Thrombotic events often occur before diagnosis and increase in frequency in the years immediately preceding diagnosis.[1] Patient sex may influence the type of thrombotic event; arterial thrombosis has been reported to be more common in men (18% vs 14%; P=0.02), whereas venous thrombosis has been reported to be more common in women (9.3% vs 5.4%; P<0.01).[2]

Thrombotic events, in particular arterial thrombosis, may contribute to an increased mortality risk in patients with PV. A retrospective Italian study of 1213 patients reported that 30% of 145 arterial thrombotic events and 11% of 87 venous thrombotic events were fatal, accounting for 24% and 6% of all deaths, respectively (median follow-up, 5.3 years [range, 0–32.9 years]).[1] A retrospective international study of 1545 patients reported thrombotic complications as the cause of death in 9% of patients (median follow-up, 6.9 years [range, 0–39.3 years]).[2] Patients with a history of thrombotic events were at further risk for mortality compared with those who had no such history (hazard ratio [HR], 1.9; 95% CI, 1.2–3.0; P=0.007).[2] These results are consistent with data from the European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) study showing that a previous thrombosis doubles PV-associated mortality.[23]

3 Thrombosis Risk Factors

There are few studies elucidating molecular mechanisms of thrombogenesis in PV. Several clinical studies, however, have identified risk factors associated with thrombosis in patients with PV. There are multiple risk factors, including both conventional risks and PV-specific risks such as *JAK2*^{V617F} allele burden. These latter elements may someday inform the design of future studies investigating better treatments of thrombogenesis in this setting.

3.1 Conventional Risk Factors

3.1.1 Thrombotic Event History—Patients with PV share several thrombosis risk factors with otherwise healthy individuals (Table 2).[25] Perhaps of greatest importance is a history of thrombotic events. The prospective ECLAP study reported increased risk of cardiovascular events among patients with a history of thrombosis (HR, 2.09; 95% CI, 1.55–2.81)[23] and that risk for major thrombosis was associated with varied prior thrombotic events (major thrombosis [HR, 1.69; 95% CI, 1.21–2.36], arterial thrombosis [HR, 1.66;

95% CI, 1.20–2.32], venous thrombosis [HR, 1.74; 95% CI, 1.06–2.87]), prior stroke or transient ischemic attack (HR, 1.81; 95% CI, 1.27–2.57), and prior deep vein thrombosis (HR, 2.04; 95% CI, 1.22–3.39).[26] Large retrospective studies have also reported significantly greater risk for thrombotic events in patients with a history of thrombosis.[1, 27]

3.1.2 Erythrocytosis—A recent prospective randomized clinical trial analyzed 365 patients with PV, and identified a lower incidence of cardiovascular events among patients who maintained hematocrit <45% compared with 45% to 50% (4.4% vs 10.9%; *P*=0.02) (Table 2).[28] Furthermore, the lower hematocrit group was associated with a lower death rate from cardiovascular causes or major thrombosis (1.1 vs 4.4 per 100 person-years). This important prospective clinical study clarified the target hematocrit level for clinicians to pursue in everyday practice. The issue was previously debated because two retrospective analyses reported no correlation between patient hematocrit and subsequent arterial or venous thrombotic events.[27, 29] Further research is necessary to untangle the complex mechanisms by which small differences in hematocrit, which have little effect on viscosity and blood fluidity,[30] could influence thrombosis in patients with PV.

There may also be qualitative changes in the red blood cells of patients with PV that promote thrombosis. Recent data suggest that erythrocytes from patients with PV are more adhesive because $JAK2^{V617F}$ drives phosphorylation of the red blood cell adhesion receptor Lutheran/basal cell-adhesion molecule (Lu/BCAM), which enhances red blood cell binding to subendothelial laminin.[31]

3.1.3 Leukocytosis—In recent years, leukocytosis has emerged as a potential risk factor for thrombosis in patients with PV. An unadjusted analysis of ECLAP study data identified an increased risk for myocardial infarction during follow-up in patients with elevated white blood cell (WBC) counts (>15×10^9/L vs 10.1 to 15×10^9/L; *P*=0.015) that was confirmed in multivariate analyses adjusted for age, sex, disease duration, prior thrombosis, prior hemorrhage, conventional risk factors, number of comorbidities, or cytoreductive and antithrombotic treatment.[26] In agreement, a prospective study of 187 patients with PV or essential thrombocythemia (ET) reported increased risk of thrombosis among patients with a WBC count >9.5×10^9/L (median) at baseline (*P*=0.03)[32] and a nonsignificant trend toward increased thrombotic risk among patients with leukocytosis in the PV subgroup (*P*=0.07). Finally, a retrospective study of 459 patients with PV reported a significant association between baseline leukocyte count ≥15×10^9/L and venous thrombotic events during a median follow-up of 64 months (range, 0–562 months; *P*=0.005) (Table 2).[27]

The association between leukocyte count and recurrent thrombotic risk may be limited to patients aged <60 years. A retrospective study of 494 patients with PV (n=235) or ET (n=259) reported increased risk for recurrent thrombosis in patients with leukocyte count in the highest versus lowest quartile (HR, 3.55; 95% CI, 1.02–12.25) only for patients aged <60 years.[33]

3.1.4 Other Conventional Risk Factors—Several other conventional risk factors have also been reported in patients with PV. Analysis of the ECLAP study data identified older age (>65 years; HR, 2.89; 95% CI, 1.98–4.22) and smoking (HR, 1.55; 95% CI, 0.99–2.43)

as risk factors for major thrombosis.[26] A retrospective study of 459 patients with PV reported that hypertension and tobacco use were associated with arterial thrombosis ($P \leq 0.02$), and diabetes mellitus was associated with venous thrombosis ($P = 0.002$).[27]

3.2 Nonconventional Risk Factors

3.2.1 *JAK2^{V617F}* Allele Burden—The genetic ratio of the *JAK2^{V617F}* mutation to *JAK2* wild-type in granulocytes, termed the *JAK2^{V617F}* allele burden,[34, 35] may also be prognostic for thrombotic risk (Table 2). Patients with a *JAK2^{V617F}* allele burden >75% compared with ≤25% have a seven-fold greater risk for thrombosis according to a prospective study that followed 173 patients with PV for a median of 24 months. Although a more recent prospective study (n=338 patients with PV followed for a median of 3.2 years) was unable to confirm an association between *JAK2^{V617F}* allele burden and thrombotic risk, this analysis compared patients with an allele burden >50% versus ≤50%.[14] Taken together, these results suggest that a *JAK2^{V617F}* allele burden threshold between 50% and 75% may identify patients at high risk for thrombotic events.

JAK2^{V617F} may also identify patients specifically at risk for splanchnic vein thromboses. A meta-analysis of patients with splanchnic vein thromboses reported the presence of the *JAK2^{V617F}* mutation in 26% to 70% of patients with Budd-Chiari syndrome and 10% to 46% of patients with portal vein thrombosis.[24]

3.2.2 Inflammation—Inflammation may predict thrombosis in patients with PV. A retrospective study identified patients with elevated inflammation using the serum biomarker C-reactive protein (CRP). Patients with high serum CRP concentration had significantly greater risk for thrombosis ($P = 0.01$),[16] which was confirmed in a multivariable analysis that adjusted for age, sex, ET or PV diagnosis, cardiovascular risk factors, *JAK2^{V617F}* mutation status, and hydroxyurea treatment (all $P \leq 0.045$).

3.2.3 Blood Cell Activation—Laboratory data suggest that activation of leukocytes, platelets, and ECs may promote a prothrombotic state;[19, 36–41] markers for activation of these cell types have been observed in patients with PV. A prospective study of 37 patients with ET and 34 with PV reported significant elevations in markers for leukocyte activation (CD11b, leukocyte alkaline phosphatase, elastase activity, plasma elastase, and plasma myeloperoxidase; $P < 0.01$) and EC activation (von Willebrand factor [vWF] and thrombomodulin; $P < 0.01$) compared with healthy adults with no history of thrombohemorrhagic events.[42] The aggregation of leukocytes with platelets is a marker for platelet activation[43] and is thought to be a key component of leukocyte-driven thrombosis.[44] A follow-up study that included 34 patients with PV reported increased levels of markers for leukocyte-platelet aggregates (CD11b/CD42b, CD11b/CD62P; $P \leq 0.001$) and a higher percentage of circulating activated platelets than controls (CD62P; $P \leq 0.001$).[43] In patients with PV, treatment with hydroxyurea did not influence marker levels for leukocyte activation, endothelial activation, or hypercoagulability.[44] More studies are needed to understand whether these markers can be used to guide treatment decision making, either with cytoreduction, JAK2 inhibition, or with anticoagulation.

A connection between *JAK2*^{V617F} and platelet activation in human disease is suggested by a mouse model in which a megakaryocyte-specific knock-in of *JAK2*^{V617F} resulted in the production of platelets that were hyperreactive to thrombin and collagen.[45] Paradoxically, these platelets were hyporesponsive to adenosine diphosphate, suggesting that purinergic blockade may be less effective than aspirin for preventing acute arterial thrombosis in PV.[45]

3.2.4 Microparticles—Serum microparticles are small membrane vesicles that are derived from activated or apoptotic cells, including platelets, monocytes, erythrocytes, and ECs.[46] Among patients with PV, serum microparticles originating from platelets, erythrocytes, granulocytes, and ECs are elevated compared with healthy controls.[47, 48] In particular, patients with MPNs that had the *JAK2*^{V617F} mutation had significantly higher plasma concentrations of tissue factor–positive microparticles and erythrocyte microparticles ($P<0.05$).[48] Among patients with MPNs, serum concentrations of platelet, erythrocyte, and EC microparticles were higher in patients with thrombotic complications ($P<0.05$).[48]

3.2.5 Coagulation System Activation—Patients with PV have demonstrated significantly higher levels of plasma coagulation markers (prothrombin fragment F1+2, thrombin-antithrombin complexes, and D-dimers) than controls ($P<0.05$).[42] Furthermore, the combined coagulant activity of microparticles, erythrocytes, and platelets is increased in patients with PV, as evidenced by a significantly reduced clotting time ($P<0.01$).[47]

3.3 Proposed Mechanism for Thrombogenesis in Polycythemia Vera

Mechanisms of thrombogenesis in patients with PV remain unclear but appear to be complex, involving multiple components (Figure 1). Statistically significant correlations of *JAK2*^{V617F} allele burden with leukocytosis and elevated hematocrit suggest that several drivers of thrombogenesis may be unique to patients with PV.[34] Elevation in hematocrit pushes platelets closer to the vessel wall and has been shown to increase the probability of adhesive wall collisions mediated by platelets binding to vWF and collagen.[49, 50] Furthermore, under higher shear conditions, as exist with elevated hematocrit and in mid-sized arteries affected by atherothrombotic narrowing, platelets are activated directly[51] and indirectly through leukocyte-platelet interactions mediated by cathepsin G[41] and CD62P (P-selectin).[52] In addition, constitutively active *JAK2* signaling may directly drive activation of platelets and granulocytes,[53, 54] and indirectly lead to endothelial activation via platelet and leukocyte binding to ECs.[39, 55, 56] Such interactions may provoke endothelial injury,[36, 37] endothelial expression of tissue factor,[57] and endothelial release of procoagulant factors such as vWF.[38–40] Furthermore, activated leukocytes, platelets, and ECs secrete microparticles,[46] which express procoagulant factors important for forming the fibrin clot.[46, 58] Finally, inflammation in PV may exacerbate the thrombotic state through varied mechanisms that include endothelial damage resulting in EC expression of tissue factor, the inhibition of EC-derived natural anticoagulant pathways, and the secretion of procoagulant factors, particularly vWF.[59]

3.4 Treatment Options

Current treatment recommendations for patients with PV are supported by significant reductions in the risk of cardiovascular death and nonfatal thrombotic events observed in

patients receiving antiplatelet therapy (HR, 0.72; 95% CI, 0.53–0.97; *P*=0.0315) and significantly reduced risk of death from cardiovascular or thrombotic events (HR, 3.91; 95% CI, 1.45–10.53; *P*=0.007) among patients who maintained hematocrit <45% with phlebotomy, aspirin, and/or cytoreductive agents.[28] Hydroxyurea and IFN-α are considered first-line therapeutic agents for patients in a need of cytoreduction.[60]

Second-line chemotherapeutic agents, including busulfan or radioactive phosphorus, are rarely used because of their leukemogenic potential.[61, 62] Consensus recommendations should also be considered to support aggressive control of conventional thrombotic risk factors like diabetes, hypertension, hyperlipidemia and, of course, abstinence from smoking.[7, 25] In women, avoiding hormone-based contraception likely reduces the rate of venous thromboembolism,[25] although clinical trials would be needed to address this hypothesis prospectively. Although these interventions address the conventional thrombosis risk factors, current treatment options do not target the underlying molecular cause of PV (ie, dysregulation of the JAK/STAT pathway) or PV-related inflammation.

The JAK1/JAK2 inhibitor ruxolitinib is currently in phase 3 trials. The RESPONSE trial compared ruxolitinib with best available therapy in patients with PV who were intolerant of or resistant to hydroxyurea. Initial results of the study suggest reduced thrombotic risk in patients randomized to ruxolitinib compared with best available therapy (0.9% of patients randomized to ruxolitinib had a thrombotic event after 32 weeks vs 5.4% randomized to best available therapy).[63] The JAK1/JAK2 inhibitor momelotinib (ClinicalTrials.gov identifier, NCT01998828) is currently in phase 2 clinical trials for patients with ET or PV. Finally, pegylated (PEG)–IFN-α2a and PEG–IFN-α2b have been evaluated in phase 2 clinical trials for patients with PV. One study (n=37) reported no thrombotic events after a median follow-up of 31 months[64]; two other studies (n=40 or 43) indicated that 70% to 76% of patients achieved complete response (a composite endpoint that included absence of thrombosis) after a median follow-up time of 21 or 42 months, respectively.[65, 66] Further studies will be required to confirm if/how these developing treatment options affect thrombotic-related outcomes in patients with PV.

4 Future Directions

Although many risk factors for thrombosis have been identified for PV, several mechanistic questions remain regarding the causal relationship. Of particular interest is the role of the JAK/STAT pathway and understanding the molecular mechanisms of activated JAK2 on blood cell and vascular activation. It will be important to clarify how thrombogenesis is affected by blood and vascular cell activation, and how serum microparticles, inflammation, and elevated blood cell counts interact with these factors to promote thrombosis. Such data could have important implications for guiding future treatment recommendations and for determining if targeting activated JAK2 is useful for preventing and/or treating PV-related arterial and venous thrombosis. If JAK2-dependent vascular cell activation or inflammation is the trigger of thrombogenesis in patients with PV, inhibition of JAK2 activity could be associated with greater thrombotic benefit than nontargeted treatment options.

5 Conclusions

Thrombogenesis in patients with PV is a complex process that likely involves synergistic interactions between several blood and vascular cells. Elevated blood cell counts; *JAK2*^{V617F} allele burden; high serum concentration of microparticles; and the activation of platelets, leukocytes, and endothelial cells have all been associated with increased risk of thrombotic events. Clarifying mechanisms of thrombogenesis in the PV setting is likely to offer opportunities to improve clinical outcomes for patients with PV and other myeloproliferative disorders.

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CONFLICT OF INTEREST STATEMENT

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Practice Points

- Cardiovascular and thrombotic events contribute to the increased mortality rate among patients with PV compared with the general population.
- Advanced age, a history of thrombosis, elevated hematocrit, and leukocytosis are risk factors for cardiovascular and thrombotic events in patients with PV.
- To reduce thrombotic risk, patients with PV should receive aspirin with phlebotomy and/or cytoreductive therapy to maintain hematocrit <45%.

Research Agenda

- Characterization of mechanistic links between thrombotic risk factors and hematologic proliferation in PV
- Determination of the prognostic and predictive value of nonconventional risk factors for thrombosis in patients with PV
- Determination of the clinical benefit associated with PV treatment options that target the underlying causes of PV and thrombosis

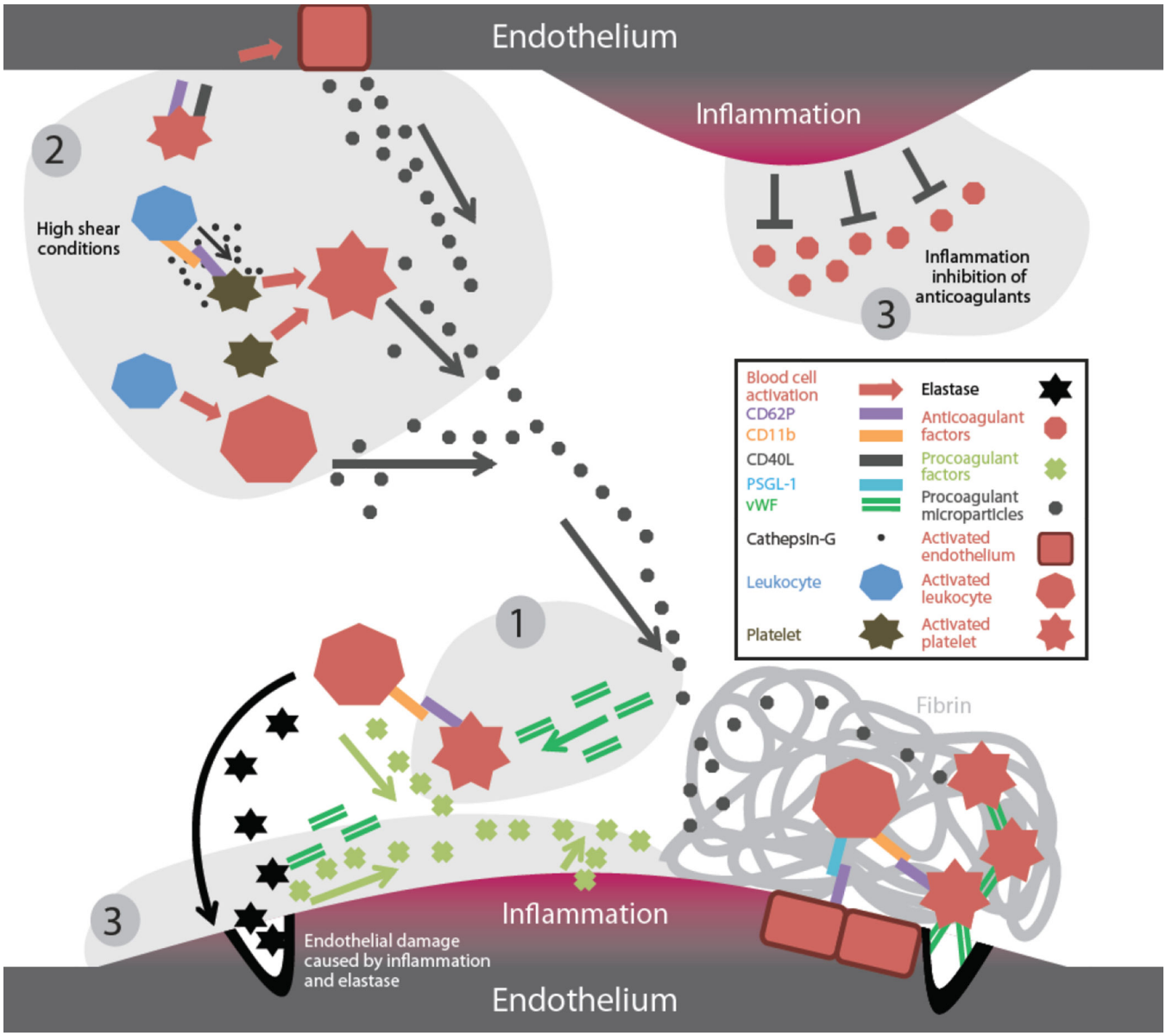

Figure 1.

Proposed Mechanism of Thrombogenesis in Patients With PV. Thrombogenesis in patients with PV is a complex process that involves many components. Future research will be necessary to confirm and refine this proposed mechanism; however, several drivers of thrombogenesis are depicted in this figure that may be unique to patients with PV. (1) Under high hematocrit conditions common in patients with PV, platelets are pushed centrifugally and are more likely to adhere to vessel wall collagen and vWF, thus initiating thrombus formation. (2) High shear conditions resulting from vascular damage and elevated blood cell counts directly enhance platelet adhesion and activation, and thereby promote leukocyte-platelet interactions. These interactions, which are principally mediated by platelet CD62P binding to leukocyte CD11b, result in neutrophil and monocyte activation. Neutrophil activation amplifies platelet activation through the release of cathepsin G, and monocyte

activation results in its expression of tissue factor, leading to the activation of the soluble coagulation system. *JAK2* mutations also promote the direct activation of leukocytes and platelets. Activated platelets and leukocytes bind to and activate ECs, and activated leukocytes, platelets, and ECs secrete procoagulant microparticles that contribute to formation of the fibrin clot. (3) PV-related blood cell and EC activation, as well as PV-related inflammation, results in endothelial injury. EC injury results in a prothrombotic state through the inhibition of EC-derived natural anticoagulant pathways, and the stimulation of several EC-derived procoagulant factors, particularly tissue factor and vWF. EC=endothelial cell; PV=polycythemia vera; vWF=von Willebrand factor.

Table 1

Incidence of Thrombotic Events in Patients With PV

Reference	Patients, n	Thrombotic event	Incidence, %
Tefferi et al 2013[2]	1545	At or before diagnosis[a]	
		Arterial	16
		Venous	7.4
		After diagnosis[b]	
		Arterial	12
		Venous	9
Marchioli et al 2005[23]	1638	Nonfatal thrombotic events during follow-up[c]	10.3
		Arterial thrombosis	5.3
		TIA	2.0
		Stroke	1.4
		Peripheral	1.2
		MI	0.9
		Venous thrombosis	5.4
		Superficial thrombophlebitis	2.8
		DVT	2.3
		Pulmonary embolism	0.8
		Fatal cardiovascular events during follow-up[c]	4.5
		Coronary heart disease	1.5
		Congestive heart failure	0.8
		Nonhemorrhagic stroke	0.8
		Pulmonary embolism	0.4
		Intracranial hemorrhage	0.1
		Other cardiac disease	0.3
		Other hemorrhage	0.3
		Other vascular death	0.3
Gruppo Italiano Studio Policitemia 1995[1]	1213	Before diagnosis[d]	14
		At diagnosis	20
		During follow-up[e]	19

DVT=deep vein thrombosis; MI=myocardial infarction; PV=polycythemia vera; TIA=transient ischemic attack.

[a] The analysis was initiated in 2010 and included patients diagnosed with PV after 1970.

[b] The median (range) follow-up was 6.9 years (0–39.3 years).

[c] The mean (SD) follow-up was 2.7 (1.3) years.

[d] Patient histories included ≤33 years before diagnosis.

[e] The median (range) follow-up was 5.3 years (0–32.9 years).

Table 2

Risk Factors for Thrombosis in Patients With PV

Reference	Analysis Type	Patients, n	Risk Factor for Thrombosis	Comparison	*P* Value and/or HR (95% CI)[a]
Gangat et al 2007[27]	Retrospective	459	Prior thrombotic event	vs no prior thrombotic event	*P*<0.0001
Landolfi et al 2007[26]	Retrospective	1638		vs no prior thrombotic event	1.69 (1.21–2.36)
Marchioli et al 2005[23]	Prospective	1638		vs no prior thrombotic event	2.09 (1.55–2.81)[b]
Gruppo Italiano 1995[1]	Retrospective	1213		vs no prior thrombotic event	*P*=0.001
Caramazza et al 2009[32]	Prospective	99[c]	Leukocytosis	>9.5 × 10^9/L vs ≤9.5 × 10^9/L at baseline	*P*=0.03 1.8 (upper 95% CI, 3.4)
				>9.5 × 10^9/L vs ≤9.5 × 10^9/L time-dependent analysis	No significant correlation 1.3 (upper 95% CI, 2.9)
De Stefano et al 2008[33]	Retrospective	235[d]		High vs low WBC count quartile in patients aged <60 years	3.55 (1.02–12.25)
Landolfi et al 2007[26]	Retrospective	1638		>15 × 10^9/L vs 10.1 to 15 × 10^9/L	*P*=0.028 1.56 (1.05–2.30)
Gangat et al 2007[27]	Retrospective	459		≥15 × 10^9/L vs <15 × 10^9/L	*P*=0.005
Marchioli et al 2013[28]	Prospective, randomized	365	Elevated hematocrit	<45% vs 45% to 50%	*P*=0.02[e] 2.69 (1.19–6.12)
Caramazza et al 2009[32]	Prospective	99[c]		≤45% vs >45% at baseline	No significant correlation 1.3 (upper 95% CI, 1.8)
				≤45% vs >45% time-dependent analysis	No significant correlation 1.4 (upper 95% CI, 3.3)
Gangat et al 2007[27]	Retrospective	459		Not specified	No significant correlation
Di Nisio et al 2007[29]	Retrospective	1638		≤45% vs 46% to 50%	No significant correlation
					P=0.5844 0.89 (0.60–1.34)
				≤45% vs >50%	No significant correlation
					P=0.8884 1.04 (0.61–1.78)
Passamonti et al 2010[14]	Prospective	338	*JAK2*^{V617F} allele burden	<50% vs ≥50%	No significant correlation
					P=0.8 1.0 (0.9–1.0)
Vannucchi et al 2007[34]	Prospective	173		≤25% vs >75%	*P*=0.003 7.1[f] (1.6–10.1)

HR=hazard ratio; PV=polycythemia vera; WBC=white blood cell.

[a] *P* value and HR presented when available in the source material.

[b] Risk of cardiovascular events, defined as cardiovascular death and nonfatal thrombotic events.

[c] Analyses were conducted on the total patient population, which included 187 patients (PV, n=99; essential thrombocythemia, n=88).

[d] Analyses were conducted on the total patient population, which included 494 patients (PV, n=235; essential thrombocythemia, n=259).

[e] Total cardiovascular events.

[f] Relative risk for thrombosis during a median follow-up of 24 months.