

Thrombotic and Hemorrhagic Issues Associated with Myeloproliferative Neoplasms

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Abstract

Thrombotic and hemorrhagic complications are related to a significant rate of morbidity and mortality in patients with myeloproliferative neoplasms (MPNs), they are therefore called “thrombohemorrhagic” syndromes. Several clinical factors, such as age and presence of cardiovascular comorbidities are responsible for thrombotic complications. High blood counts, platelet alterations, presence of JAK2 mutation and possibly of other CHIP mutations such as TET2, DNMT3A, and ASXL1, procoagulant microparticles, NETs formation, endothelial activation and neo-angiogenesis are some of the parameters accounting for hypercoagulability in patients with myeloproliferative neoplasms. Bleeding complications emerge as a result of platelet exhaustion. They can be also linked to a functional deficiency of von Willebrand factor, when platelet counts rise above 1000G/L. The mainstay of management consists on preventing hemostatic complications, by antiplatelet and/or anticoagulant treatment and myelosuppressive agents in high-risk patients. Circumstances related to a high thrombohemorrhagic risk, such as pregnancy and the perioperative period, prompt for specific management with regards to anticoagulation and myelosuppression treatment type. In order to apply a patient-specific treatment strategy, there is a need for a risk score assessment tool encompassing clinical parameters and hemostasis biomarkers.

Keywords

myeloproliferative neoplasms, thrombosis, bleeding

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Introduction

Myeloproliferative neoplasms (MPNs) are clonal stem cell-derived diseases.¹ According to 2016 WHO classification, MPNs include the Philadelphia positive chronic myeloid leukemia (CML), and the Philadelphia-negative MPNs: polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), as well as chronic neutrophilic leukemia, chronic eosinophilic leukemia (not otherwise specified), and unclassifiable MPNs. The commonest Philadelphia -negative MPNs are PV, ET and PMF and for the sake of simplicity, will be the focus of this review.

Despite their genetic heterogeneity, MPNs share common disease features, including tendency towards myeloproliferation, splenomegaly, potential for progression to acute myeloid leukemia or myelofibrosis and thrombohemorrhagic events. It is well established that MPN patients bear a higher risk of arterial or venous thrombosis when compared to the general population.² Thrombotic complications also include microvascular events, which contribute to morbidity and mortality. Similarly,

MPN patients can present with a minor bleeding or suffer from a major internal organ hemorrhage.³

The aim of this review is to provide a comprehensive overview of the mechanisms leading to thrombosis or bleeding in Ph- myeloproliferative neoplasms as well as current practices for prevention and treatment of these complications. This

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review article highlights the mechanisms contributing to thrombosis or hemorrhage and rises unmet needs on the management of vascular complications in patients with MPNs.

Methods

Using a search strategy that included the terms for myeloproliferative neoplasms, thrombosis, and bleeding, two investigators (LP and IE) independently searched for published articles indexed in the PUBMED and Google Scholar databases from inception to November 2021. In addition, the references of the included studies were also manually reviewed to identify additional eligible studies. This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. To be eligible for inclusion into this review, each study needed to report at least one thrombotic or hemorrhagic mechanism of Philadelphia negative MPNs (PV, ET, or PMF). Then, the study needed to report the overall prevalence of thrombosis and/or bleeding at diagnosis of that cohort. The secondary outcomes of interest, including treatment of thrombosis and hemorrhage for each MPN subtype, were also collected for pooled analysis and there were part of the inclusion criteria. Both investigators evaluated all studies independently. If different decisions regarding the eligibility of a study were made, the study in question was jointly reviewed by the two investigators and the final determination was reached by consensus.

The Magnitude of the Problem: Thrombotic and Hemorrhagic Complications in Myeloproliferative Neoplasms

Vascular involvement in patients with MPNs can be manifested with various symptoms, such as headaches, atypical chest pain, visual disturbances (eg, amaurosis fugax, scotomata, and/or ophthalmic migraines), acral paresthesias, digital discoloration/ischemia,

Table 1. Pooled Prevalence Of Arterial and Venous Thrombosis and Major and Clinically Significant Hemorrhage in Patients with MPNs.⁶

	Arterial and venous thrombosis	Hemorrhage
PV	28.6%	6.9%
ET	20.7%	7.3%
PMF	9.5%	8.9%

PV: polycythemia vera

ET: essential thrombocytemia

PMF: primary myelofibrosis

Table 2. Pooled Prevalence of Venous, Arterial Thrombosis and of Bleeding Complications Per Localization.

Venous thrombosis	Arterial Thrombosis	Hemorrhage
<ul style="list-style-type: none"> • Pooled prevalence : 6.2% • Deep vein thrombosis : 3.4% • Splachnic vein thrombosis : 1.4% • Pulmonary embolism : 0.9% • Cerebral sinus thrombosis : 0.7% 	<ul style="list-style-type: none"> • Pooled prevalence: 16.2% • Cerebrovascular disease : 7.4% • Transient Ischemic Stroke : 3.5% • Coronary artery disease : 6.1% • Peripheral artery disease: 3.3% 	<ul style="list-style-type: none"> • Pooled prevalence : 6.2% • Mucocutaneous bleeding : 2.8% • Gastrointestinal bleeding :2.1% • Epistaxis : 1% • Postoperative bleeding: 1.1%

livedo reticularis and erythromelalgia which are manifestations of ischemic alterations in the micro-vasculature. Erythromelalgia manifests as a burning pain in the feet or hands, usually accompanied by erythema, pallor, or cyanosis. Superficial vein thrombosis (SVT), distal or proximal deep vein thrombosis (DVT), pulmonary embolism (PE) and venous thrombosis at unusual sites may be also related to MPNs. The prevalence of abdominal vein thrombosis ranges from 1% to 23% with a disease-specific prevalence for PV, ET and PMF estimated to be 10, 13, and 1%, respectively.⁴ Moreover, MPNs are the most frequent underlying cause in patients presenting with hepatic vein thrombosis (Budd–Chiari syndrome) and portal vein thrombosis which is not related to cirrhosis or a solid tumor. The prevalence of MPNs is around 41% in Budd–Chiari syndrome and 32% in non-cirrhotic portal vein thrombosis.⁵ Given all the above, routine screening for JAK2 mutations in the diagnostic workup is essential in patients with abdominal thrombosis, even in the absence of obvious myeloproliferative features on blood count analysis.

A recent study on 13,436 newly diagnosed MPN patients⁶ showed that thrombotic complications (either arterial or venous) are much more common than hemorrhagic complications (20% vs 6% respectively; $p < 0.05$). Patients develop more frequently arterial than venous thrombosis and the classical sites include ischemic stroke, coronary artery disease and deep venous thrombosis (DVT). The most common hemorrhagic sites include gastrointestinal, mucosal and cutaneous bleedings. Data are summarized in tables 1 and 2.

Thrombotic Complications

Mechanisms of thrombogenesis in MPNs.

Clinical Factors

As age increases, the risk of arterial thrombosis or VTE in patients with MPNs becomes more important.^{1–3} Personal history of arterial or venous thrombosis is an independent predictive factor for recurrent thrombosis. The interaction with age has a multiplicative effect on vascular risk. Recurrent episodes generally occur in the same localization (arterial or venous) as the first vascular event in both PV and ET.^{7–10}

Cardiovascular Risk Factors

The contribution of well-established cardiovascular risk factors (hypertension, smoking, hypercholesterolemia and diabetes)

has been assessed in studies enrolling MPN patients.^{11,12} Patients with MPNs and particularly those with ET, classified according to the IPSET-scoring system (that includes age, previous thrombosis, cardiovascular risk factors, and JAK2-V617F mutation) are at high thrombotic risk. Thrombotic risk is amplified by smoking, diabetes, arterial hypertension, and hypercholesterolemia.

Blood Cells

Red cells. In PV, high hematocrit results in increased blood viscosity and is correlated with an increase in thrombotic risk. The hematocrit cut-off for increased blood viscosity was set at 45%.¹³ Under low-shear conditions, high hematocrit has a more important effect on blood viscosity, causing a major disturbance to blood flow. At high shear rates, the rise in red cell mass displaces the platelets towards the vessel wall with the consequences of shear-induced platelet activation and increased platelet–platelet interactions secondary to thrombocytosis.^{14,15} In PV, it is suggested that RBC induce platelet aggregation by ADP release.¹⁴ Furthermore, RBC aggregation activates endothelial cells (ECs) by increasing shear stress on the vessel wall and facilitates platelet and WBC interaction with ECs.¹⁶

Platelets. Thrombocytosis can contribute to vascular events of ET or PV, as suggested by evidence that platelet count reduction lowers the risk of microcirculatory disturbances.^{17,18}

Paradoxically, marked thrombocytosis can favor hemorrhagic rather than thrombotic manifestations in ET patients,¹⁹ due to the possible occurrence of an acquired von Willebrand syndrome (avWBS), a phenomenon which will be analyzed further below.

Platelets play an important role in MPN-related thrombosis through various mechanisms. The importance of an elevated platelet count remains uncertain, with platelets > 1000 G/L/L carrying an hemorrhagic rather than a thrombotic risk. Thus, MPN patients often present with the coexistence of both thrombotic and hemorrhagic complications. The mechanisms that link impaired platelet function and bleeding will be analyzed further below.

It is suggested that two main mechanisms contribute to platelet-related thrombosis²⁰: 1) platelet activation linked with hyperactive JAK-2 dependent signaling on megakaryocytes,²¹ and on platelets,²² 2) platelet interaction with their microenvironment. Platelets interact a) with neutrophils through NET formation, intracellular protease release and elaboration of reactive oxygen species b) with monocytes which express tissue factor and secrete cytokines and c) with endothelial cells through vWF release as previously described.²⁰

Platelets in MPNs enhance thrombin generation through elevated surface tissue factor expression,²³ TF-bearing microparticle secretion,²⁴ and elevated phosphatidylserine expression. It has been shown that these patients present higher levels of platelet activation markers such as sCD40L and sP-selectin

that have been shown to be correlated with a higher thrombotic rate.²⁵

Leukocytes. The increase in white blood cell count is a risk factor for thrombosis in patients with PV and ET.^{9,26–29} In addition, qualitative abnormalities of polymorphonuclear leukocytes (PMNs), are present in MPNs as reflected by a significantly increased expression of the membrane integrin CD11b, leukocyte alkaline phosphatase and in the cellular content of elastase.³⁰

Elevated plasma concentrations of myeloperoxidase and the above-mentioned markers demonstrate an ongoing *in vivo* PMN activation. Hyperexpression of PMN membrane adhesive molecules (integrins and selectins) increases adhesion of PMN to endothelial cells and platelets. The cell–cell interactions stimulate the expression of prothrombotic features by endothelial cells and platelets and induce PMN release of reactive oxygen species and intra-granular proteinases.^{31,32} The PMN-platelet interaction is coordinated by binding of platelet P-selectin (CD62P) to neutrophil PSGL-1 receptors.^{33,34}

The interplay between activated PMN and activated platelets generates PMN/platelet-mixed heterocomplexes, which are sensitive markers for platelet activation, and are increased in several pathological conditions associated with a propensity to thrombosis.^{35,36} However, PMNs from these patients also express high levels of integrin CD11b, which is a prominent site for platelet attachment. Circulating PMN/platelet aggregates were simultaneously measured with the levels of activated PMN and activated platelets³⁷ and the results suggested the formation of high percentage of circulating mixed PMN/platelet heterocomplexes in ET and PV. This interpretation was further supported by evidence that, in whole blood samples from the same subjects, the “*in vitro*” induced PMN activation (without platelet activation) resulted in a significant increase of PMN–platelet aggregates formation. In ET aspirin-receiving patients, the increment in CD11b and PMN/platelet aggregates was significantly lower compared to non-aspirin-treated ET patient.

MPNs Genetics and Hemostasis

JAK2 mutation. Mutations of Janus Kinase 2 (JAK2) have been linked to thromboembolic complications and alterations in hemostasis and cell adhesion *in vitro* and *in vivo*.³⁸

Most recently, murine models of MPNs have given insight into JAK-induced pathophysiology regarding thrombotic complications. Heterozygous JAK2V617F mutation is sufficient for the induction of spontaneous megakaryopoiesis and erythropoiesis. An increase of hypersensitive platelets is the cause of aspirin-sensitive, platelet-mediated microvascular ischemic and thrombotic complications in ET and early PV mimicking ET. In the homozygous state, myeloproliferation becomes more prominent, leading to thrombotic complications, in addition to platelet-mediated microvascular thrombotic syndrome of thrombocytopenia.³⁹ Moreover, high expression of mutated JAK2 was associated with both formation of unstable thrombi

and bleeding events in vivo.⁴⁰ JAK2-mutated platelets showed a reduced activation capacity and a moderate glycoprotein (GP) VI deficiency as well as a reduced platelet adhesion due to a decreased proportion of high-molecular-weight von Willebrand factor multimers. Consistently, JAK2-V617F-activated megakaryopoiesis resulted in hypersensitivity to thrombopoietin (THPO) stimulation, higher mobility of megakaryocytes, elevated pro-platelets release and increased thrombus formation due to enhanced platelet aggregation.⁴¹ These findings lead to the assumption that JAK2-mutations indicate an increased risk for thromboembolic events per se compared to non-JAK-mutated MPN cases,⁴² thus, aspirin administration is routinely used to prevent thrombotic complications.⁴³ Expression of mutated JAK2 in endothelial cells, a finding that could be confirmed in the human system, resulted in abnormalities in blood flow and alteration of blood coagulation.^{44–47}

As to red blood cell (RBC) biology, it has been shown that abnormal adhesion of JAK2-V617F-mutated red blood cells is induced through upregulation of adhesion molecules through Rap-Akt-signaling even in the absence of erythropoietin (EPO), a phenomenon pharmacologically inhibited.⁴⁸

Neutrophils harboring activating JAK2-mutations show a significant increase in neutrophil extracellular trap (NET) formation.⁴⁹

Likewise, murine JAK2-V617F-mutated neutrophils contributed to increased thrombus formation following experimental venous ligation. JAK-inhibitor treatment reduced the phenotypes in both model systems. On a large cohort of otherwise healthy individuals, those harboring JAK2-mutations presented significantly higher rates of thromboembolic events.⁵⁰

Consistently, increased expression of beta1-integrins was described in granulocytes of MPN patients harboring JAK2-mutations compared to age-matched healthy donor controls.⁵¹ Functional studies on both mouse models and primary human cells confirmed JAK activation of integrins by JAK2 in a Rap1-GTPase dependent manner.⁵²

Integrin activation may lead to accumulation of myeloid cells in the spleen. Pharmacologic inhibition of integrin function in mice resulted in improvement of splenomegaly and in reduced cell numbers in the spleen, suggesting a possible therapeutic target for high-risk patients.

CHIP mutations. Clonal hematopoiesis of Indeterminate Potential (CHIP) refers to a context in which somatic mutations are identified in individuals who do not yet meet criteria for a hematologic neoplasm.

The most common clonal mutations include mutations in JAK2, DNMT3A, TET2, and ASXL1.

CHIP patients with JAK2 mutation bear 12.1 times more risk of coronary artery disease as compared to those with no mutation,⁵³ thought to be linked to an inflammatory response. Importantly, in addition to driver mutations, MPN patients may also have mutations in TET2, DNMT3A, and ASXL1,

the presence of which is have not yet be proven to add to thrombotic risk.

MPNs and Hypercoagulability

Thrombin generation and tissue factor expression. An increase in thrombin generation due to an acquired activated protein C (APC) resistance may also contribute to the hypercoagulable state of MPNs. Alteration of coagulation proteins induces an APC-resistant phenotype in these patients. By use of a thrombin generation assay, an APC-resistant phenotype has been shown in ET and PV patients, particularly in JAK2V617F carriers.⁵⁴ The JAK2 allele burden was correlated with higher thrombin generation.⁵⁵ Platelet contribution to the thrombophilic state of patients with myeloproliferative neoplasms remains uncertain. A study carried on 140 MPN patients (80 ET and 60 PV) and 72 healthy subjects, showed that MPN patients had significantly increased platelet thrombin generation potential compared to controls. This correlated with the extend of platelet activation. The cytoreductive therapy with hydroxyurea (HU) lowered platelet TG potential, thus affecting the prothrombotic phenotype.⁵⁶

Procoagulant microparticles. Platelets and vascular endothelial cells produce procoagulant microparticles, a phenomenon linked with the hypercoagulable state found in MPNs. The clinical significance of circulating MPs was assessed in a group of 179 patients with BCR/ABL-negative MPNs. An elevated count of platelet and erythrocyte MPs was found in MPN patients when compared with healthy controls. Procoagulation activity of MPs was as well significantly higher in patients compared to the control group. Presence of the JAK2V617F mutation was associated with a higher count of platelet MPs and a statistically significant correlation between platelet count and platelet microparticles was found⁵⁷ Furthermore, in blood cells from patients with PV or ET, the presence of the JAK2V617F mutation is linked to an hypercoagulable state with increased expression of platelet-associated tissue factor-bearing microparticles and formation of increased platelet/neutrophil aggregates.⁵⁸ The above suggest that MPs could be useful as a predictive biomarker of thrombotic risk in MPN patients.

Endothelial cell activation. Usually found in hematopoietic progenitors and stem cells, JAK2 mutation has also been described in endothelial cells (ECs) of MPN patients. A study applying transcriptomic assays showed that several genes and pathways involved in inflammation, cell adhesion and thrombotic events were over-represented in JAK2^{V617F} ECs and expression levels of von Willebrand factor and P-selectin (CD62P) proteins were increased. It was also found that leucocytes from MPN patients adhere more tightly to JAK2^{V617F} ECs. Study results show that JAK2^{V617F} ECs have a pro-inflammatory and pro-thrombotic phenotype and were functionally pro-adherent.⁵⁹

Guy et al⁶⁰ suggested that vascular endothelial cell expression of JAK2^{V617F} is sufficient to promote a pro-thrombotic

state due to increased P-selectin expression. They investigated the role of endothelial cells that express JAK2V617F in thrombus formation using an in vitro model of human endothelial cells overexpressing JAK2V617F and an in vivo model of mice with endothelial-specific JAK2V617F expression, providing evidence that JAK2V617F-expressing endothelial cells promote thrombosis through induction of endothelial P-selectin expression, reversible by HU. Probably, only a small fraction of endothelial cells harbor the mutation, therefore ex vivo experiments are needed to evaluate the clinical impact of the aforementioned findings.^{61,62}

Angiogenesis. TF-mediated angiogenesis in cancer is related to thrombosis.⁶³ Angiogenesis and JAK2V617F mutation are common in BCR-ABL1 negative MPNs. Cheng et al looked upon angiogenesis in newly diagnosed MPNs, finding that expression levels of p-JAK2, VEGF, HIF-1a and MVD were significantly increased and were related to the JAK2 V617FV burden. Ruxolitinib inhibited p-JAK2, VEGF, HIF-1a expression and suppressed blood vessels' formation in a chick embryo choriallantoic membrane model. These findings indicated that angiogenesis is related to JAK2V617F burden and ruxolitinib could decrease VEGF and HIF-1a expression in JAK2V617F 17F positive cells.⁶⁴

Neutrophil extracellular traps (NETs). It has been demonstrated that neutrophils from patients with MPNs are primed for NET formation, an effect blunted by pharmacological inhibition of JAK signaling. Mice with conditional knock-in of *JAK2*^{V617F}, have an increased propensity for NET formation and thrombosis. Inhibition of JAK-STAT signaling with ruxolitinib, abrogated NET formation and reduced thrombosis in a deep vein stenosis murine model. The study further showed that *JAK2*^{V617F}-driven NET formation and thrombosis in vivo is mediated by PAD, a protein required for NET formation. Finally, in a population study of over 10,000 individuals without a known myeloid disorder, *JAK2*^{V617F} positive clonal hematopoiesis was linked to NET formation and thrombosis and suggest that JAK2 inhibition may reduce thrombosis in MPNs through cell-intrinsic effects on neutrophil function.⁴⁹

Heparanases. Kogan et al⁶⁵ in an in vitro study, performed on two erythropoietin receptor positive cell lines, showed that heparanases' level and procoagulant activity were higher in JAK-2V617F-U87 cells compared to control. This effect was reversed using JAK-2 inhibitors (Ruxolitinib, VZ3) and hydroxyurea. Erythropoietin induced an increase while JAK-2 inhibitors a decrease of the levels of heparanases and of the procoagulant activity of U87 and MCF-7 parental cells. Overall, it seems that JAK-2 is involved in heparanase up-regulation via the EPO receptor, a finding potentially indicating a new thrombotic mechanism in JAK-2 positive ET patients.

A mechanistic approach and unresolved issues emerging. In order to understand MPN-related thrombosis, one should refer to Virchow's triad. Thrombogenesis requires the ensemble of

the three triad components (venous stasis, endothelial injury, hypercoagulability) which need to be present at the same time and location. Accordingly, isolated deregulation of one of the three components is not sufficient to induce thrombosis. An elevated hematocrit leading to hyperviscosity is not sufficient to induce an hypercoagulable state in polycythemia vera. This is shown by the fact that, in case of congenital or high altitude polycythemia, thrombosis is not routinely observed. Similarly, leukocytosis is, as suggested from the CYTO-PV study, an independent thrombotic risk factor. However, in Philadelphia + CML, the high leucocyte count is not related to a high thrombotic rate.

On the other hand, the presence of JAK 2 mutations is an independent risk factor for thrombosis even in patients with normal full blood counts. Of note, JAK 2 –positive ET patients, present a higher risk of thrombosis, compared to CALR positive, or triple negative patients.

In the authors' view, deregulation of the homeostatic mechanism in JAK2 + MPNs is closely related to the presence of JAK 2 mutations leading, as previously mentioned to a) a procoagulant phenotype of red blood cells through increased red cell adhesion molecules b) increased platelet aggregation and a high platelet MP-release c) increased NET formation d) endothelial cell activation e) angiogenesis f) heparanase up-regulation. It is suggested that treatment with ruxolitinib leads to a lower thrombotic risk linked to a lower JAK 2 allele burden leading to the assumption that diminishing the JAK2 load could serve as an anticoagulant prophylaxis on these patients.⁶⁶

Prevention and Treatment of Thromboembolic Complications in PV and ET

As a general principle, treatment for Ph - MPNs is aimed at preventing thrombohemorrhagic complications.

Polycythemia Vera

Patients with PV should be managed with low-dose aspirin and phlebotomy to maintain hematocrit at less than 45%.⁶⁷ Currently, the conventional model of risk stratification in PV which categorizes patients as "low-risk" or "high-risk" is recommended. The presence of either age above 60 and/or history of thrombosis defines a high-risk patient.^{14,68}

In PV, low-dose aspirin is indicated for all patients who do not have a contraindication, regardless of risk category. Phlebotomy is another cornerstone of treatment for PV and is used to maintain hematocrit at less than 45% in PV patients. The multicenter randomized clinical trial "CYTO-PV" showed a reduction in the primary end point of cardiovascular death and major thrombosis when hematocrit was maintained at a target of less than 45% as compared to a higher goal of 45–50% (HR in the high-hematocrit group = 3.91, P = 0.007). This study supported the use of phlebotomy with the addition

of cytoreduction, if needed, to maintain a hematocrit of less than 45% in PV patients in order to prevent thrombosis. Of note, that cytoreductive treatment with hydroxyurea was given to all patients with a high thrombotic risk (> 65 years old or prior thrombosis requiring myelosuppressive therapy), giving indirect evidence for the beneficial role of cytoreduction in reducing thrombotic risk. In PV, there are no contemporary randomized trials proving that cytoreductive therapy modifies thrombotic risk. Cytoreductive therapy is indicated if patients present poor tolerance to phlebotomy, symptomatic or progressive splenomegaly, severe symptoms, a platelet count greater than $1500 \times 10^9/L$ and/or progressive leukocytosis. Options for cytoreductive therapy include hydroxyurea or interferon-alpha. As previously seen, hydroxyurea has a clear role in inhibiting thrombotic risk, through various mechanisms as opposed to interferon – alpha, whose role in hemostasis is still unclear. Hence, not all cytoreductive therapy is equal in terms of managing thrombotic risk. Moreover, cardiovascular risk factors (ie, tobacco use, hypertension, hyperlipidemia, diabetes) factors must be aggressively managed.

Essential Thrombocythemia

The “revised IPSET-thrombosis” model was developed to assess vascular risk in ET patients. This model includes thrombotic history, age above 60, and JAK2V617F mutational status. Using such variables, there are four risk categories: “very low-risk” (no adverse features), “low-risk” (presence of JAK2V617F only), “intermediate-risk” (presence of age above 60 only), and “high-risk” (presence of thrombosis history or presence of both JAK2V617F and advanced age). This classification was then validated in a study with 585 ET patients.

Thrombosis-free survival from time of diagnosis to first thrombotic event after diagnosis was calculated, and patients were grouped according to conventional stratification (two-tiered), “IPSET-thrombosis” (three-tiered), and the “revised IPSET-thrombosis” (four-tiered) models. There was a significant difference in thrombosis-free survival between the “very low-risk” and “low-risk” groups ($P = 0.024$), as well as the “intermediate-risk” compared to the “high-risk” groups ($P = 0.03$).⁶⁹

This validated model may provide useful information in ET patients regarding thrombotic risk and can be used to direct treatment. Typically, cytoreductive therapy would be reserved for the high-risk group. Additionally, those falling into lower risk groupings, but with uncontrolled vascular symptoms (or bleeding due to thrombocytosis) would be candidates for cytoreduction. In 809 patients with high-risk ET randomized to low-dose aspirin plus either anagrelide or hydroxyurea, hydroxyurea plus aspirin reduced the composite end point of arterial and venous thrombosis as compared to anagrelide plus aspirin ($P < 0.01$).⁷⁰

In a subsequent randomized control trial (the “ANAHDRET” trial), 259 previously untreated high-risk

ET patients were treated with either anagrelide or hydroxyurea. Anagrelide was shown to be non-inferior to hydroxyurea after a 36-month observation period, and there was no significant difference between the groups in incidences of thromboses or bleeding events.⁷¹

Society guidelines differ. The NCCN includes hydroxyurea, interferon, and anagrelide as first-line choices; ELN guidelines offer anagrelide as a second-line option. As with PV, pegylated interferon is under evaluation in ET; again, detailed analysis regarding thrombosis risk reduction from phase 2 and phase 3 consortium studies are not yet published.

Ruxolitinib is not recommended in ET. In 110 ET patients who were resistant to hydroxyurea, a randomized control trial comparing ruxolitinib to best available therapy did not show any evidence of improvement in complete response within one year (46.6 vs 44.2%, $P = 0.40$).⁷²

In addition, the rates of thrombosis, hemorrhage, and hematologic transformation were not significantly different at two years. The observation that ruxolitinib was as good as best available therapy in patients refractory to hydroxyurea can be interpreted as evidence that ruxolitinib is a reasonable choice in such patients, if they are intolerant of other therapeutic options, given the high frequency of side effects of both anagrelide and interferon.

Platelet-lowering treatment should be considered at platelet counts greater than $1500 \times 10^9/L$ in order to reduce the risk of bleeding. Again, cardiovascular risk factors must be aggressively managed.

Though cytoreductive therapy remains the mainstay of therapy for extreme thrombocytosis, plateletpheresis may be offered as a temporizing measure in certain instances where rapid reduction in platelet count is required. In a case-based review of plateletpheresis in the management of extreme thrombocytosis in MPNs, plateletpheresis was successfully used in patients for the following indications: neurological symptoms due to transient thromboembolic episode and hyperthrombocytosis-related avWFS, as a prophylactic measure to reduce platelet counts below a particular range, as well as for symptomatic relief in context of an ischemic toe.⁷³

Though data on clinical utility of plateletpheresis is largely case report-based, this treatment modality may be an option for patients with extreme thrombocytosis complicated by a thrombohemorrhagic event, when rapid reduction is required prior to emergent surgery, or when cytoreductive agents are contraindicated. The decision to use plateletpheresis is discussed case by case, based on both individualized and clinical scenario audit. In asymptomatic ET patients, current guidelines do not specify a platelet count threshold at which apheresis should be performed. In patients with counts above $1500 \times 10^9/L$, plateletpheresis should be considered due to an increased risk of major hemorrhage related to an avWFS.⁷⁴

Antithrombotic Therapy

Anticoagulation therapy is indicated for those patients who develop venous thrombosis. The choice of anticoagulant and

appropriate duration of therapy, however, is unclear. The majority advise an indefinite anticoagulation given the intrinsic and persisting thrombophilic nature of MPNs, which may represent an ongoing/permanent risk factor for recurrence. Even hematologists who specialize in MPNs lack consensus in.⁷⁵

Overall, there is a tendency to prolong treatment with aspirin in those with arterial thrombosis, whereas in patients with venous thrombosis, there is a tendency for more prolonged treatment with a vitamin K antagonist (VKA) with or without aspirin.

The addition of prophylactic aspirin in patients receiving anticoagulant treatment, is in the authors' view justified when a high risk of arterial thrombosis is present, such as in patients with a previous history of arterial thrombosis. In ET patients, an accelerated platelet turnover may lead to an increased thromboxan production and thus, to aspirin resistance. In this case a twice-daily schedule should be proposed along with a close follow-up for any hemorrhagic signs.⁷⁶

DOACs, with fixed dosage, larger therapeutic window, less drug-drug interferences and no required routine monitoring, may be more convenient, efficient and safe for use in MPN patients,⁷⁷ however further prospective randomized control studies are required. A retrospective chart review study on 53 patients compared the incidence of recurrent thromboembolic events on DOAC and VKA therapy and stated that both treatment options have a comparable thrombotic and hemorrhagic profile, although the study suggests that bleeding events may occur sooner in patients treated with DOACs.⁷⁸

Recent data derived from a multicentric study of 135 patients have demonstrated the efficacy of DOACs to prevent venous thrombosis in MPN patients with AF or VTE. Of note, on these very high-risk patients, low-dose DOACs was related to arterial thrombotic events and less bleeding events. APICAT, is an ongoing international multicenter prospective interventional study, which has the aim to assess the efficacy of DOACs in prevention of recurrent thrombotic episodes in patients with active cancer, including myeloproliferative syndromes.

Abdominal vein thrombosis, which includes hepatic vein occlusion, extrahepatic portal vein occlusion, and mesenteric vein thrombosis, is strongly associated with MPNs. Indefinite anticoagulation is typically indicated in these patients, based on consensus recommendations. The general consensus for treatment includes low molecular weight heparin initiation followed by indefinite oral anticoagulation. Usually, these patients require joint management with hepatology practitioners, for monitoring and management of esophageal varices and portal hypertension. In addition, in patients with thrombocytosis, erythrocythemia, and/or leukocytosis, cytoreductive therapy should be used to normalize blood cell counts.

Management of Thrombotic Risk in Pregnant Women with MPNs

Classic MPNs often present in patients of childbearing age, the majority of cases reported in essential thrombocythemia.⁷⁹ Calculated incidence of MPN pregnancies is 3.2/100,000 maternities per year.⁸⁰

Apart from the prothrombotic state related to pregnancy, a decrease in flow occurs in the inferior vena cava and left iliac vein due to compression by the gravid uterus. Overall, pregnancy in patients with ET and PV is associated with a high thromboembolic risk and several other complications such as recurrent abortions, intrauterine death, premature delivery, fetal growth retardation and preeclampsia. According to a study by Griesshammer et al,⁸¹ success rate in 793 ET pregnancies was 68.5%. Spontaneous abortion is the most frequent complication with 26.5% (rate in normal pregnancy: 11%) followed by stillbirth with 4.8% (rate in normal pregnancy: 0.43%). Women with ET have more than a 10-fold higher risk of stillbirth compared to the general population. Maternal complications are relatively low with 1.8% major thrombotic and 2.4% major bleeding events. In PV, maternal morbidity and stillbirth are significantly increased compared to ET.

A high-risk pregnancy is defined by the presence of⁸²:

1. A history of thrombosis or severe hemorrhage.
2. Previous pregnancy complications like spontaneous abortion, intrauterine death or stillbirth, pre-eclampsia necessitating preterm delivery at less than 37 weeks, or development of any such complication during the index pregnancy.
3. Platelet count rising to $1500 \times 10^9/l$ or uncontrolled hematocrit rising to $> 45-50\%$.
4. Pregnancy in PV patient.

As already suggested,⁸¹ during the preconception phase, a wash-out period of possible teratogenic drugs (hydroxyurea, busulphan, anagrelide) and a careful risk assessment (thrombophilia screen, family and personal history of thrombosis or hemorrhage) are needed. A joint management by a hematologist and an obstetrician is necessary.

Regarding normal risk pregnancy, it is advised that:

1. target hematocrit should be kept in the middle of normal range for gestation.
2. platelet count should be kept under 1000G/l.
3. aspirin at 50-100 mg/day.
4. a switch to LMWH one to two weeks prior delivery until 6 weeks postpartum.

Pregnant women with MPN who have a history of thrombotic events are on long term anticoagulant. Therefore, in case of high risk-pregnancy due to previous venous thrombotic event(s), women on oral anticoagulants should be switched to LMWH treatment throughout pregnancy, post-partum and breast-feeding. Epidural anesthesia must be carefully administered, especially if platelet count is uncontrolled at time of delivery. A programmed delivery is a safe option, in order to minimize the risk of bleeding complications. In case of previous history of arterial events, low-dose aspirin treatment should be maintained until up to 1-2 weeks prior delivery, where a switch to LMWH prevention should be considered.

After delivery and in the absence of bleeding complications, aspirin should be restarted the soonest possible. The importance of general preventive measures, such as adequate hydration, mobilization and use of compression socks should be discussed with all patients, as well as control of vascular risk factors (ex gestational diabetes, hyperlipidemia, weight gain, hypertension).

Prevention with both aspirin and LMWH should be prescribed throughout pregnancy and during six weeks postpartum, in case of a high risk pregnancy due to previous pregnancy complications or uncontrolled platelet and/or hematocrit counts as mentioned above, with the same restrictions during delivery. The same strategy is suggested in case of an abnormal artery uterine Doppler, if revealing of bilateral notching. Interferon-alpha could be a safe option, being a large molecule that does not cross the placenta, but not recommended during breastfeeding.⁸¹ During postpartum, target hematocrit should be kept in the middle of normal range and platelets below 1000 G/l.

Management of Thrombotic Risk in Surgical Patients with MPNs

MPN patients are considered high-risk surgical candidates due to the increased risk of thrombohemorrhagic complications during the perioperative period. A multicenter retrospective analysis, aiming to estimate thrombosis and hemorrhage after 311 surgical procedures in patients with PV and ET, showed that there was variability with regards to the use of perioperative prophylaxis (54.3% received subcutaneous heparin and 15.4% received antiplatelet therapy). During the 3-month follow-up period, there were 12 arterial thrombotic events, 12 venous thrombotic events, 23 major, 7 minor hemorrhages, and 5 deaths.⁸² There was no correlation between bleeding episodes, type of diagnosis, use of antithrombotic prophylaxis, or type of surgery. Further investigations are required for optimal management of these patients. The risk assessed with models such as Caprini score helps to identify patients considered as high risk requiring mainly prolonged LMWH thromboprophylaxis.⁸³

Historical data from post-operative complications in polycythemia vera point out that blood counts should be brought to target values before any elective surgery is undertaken.

Bleeding

Hemorrhagic complications in Philadelphia negative MPNs are linked to several factors:

Due to the existing high shear stress in the microvasculature, platelets spontaneously activate, secrete their products, form aggregates mediated by von Willebrand factor (vWF) that transiently plug the microcirculation, disaggregate, and then recirculate as exhausted defective platelets with secondary storage pool disease. At platelet counts above $1000 \times 10^9/L$, the phenotype changes into an overt spontaneous bleeding tendency as a result of a functional vWF deficiency that is caused by

proteolysis of large vWF multimers. This is consistent with an acquired type 2 von Willebrand syndrome, reversible by reduction of the platelet count to normal. All of the above lead to a prolonged bleeding time, usually ranging from 6 to 10 s in MPNs patients.

In ET, platelets present an acquired form of storage pool deficiency, with an abnormal granule content and secretion,⁸⁴ as observed by the flow cytometric mepacrine uptake-release test.

In PV and ET platelets show a reduced expression or function of glycoprotein. As GpIb is correlated to platelet life span and acts as a receptor for vWillebrand factor, these findings may explain the reduced platelet survival time and the acquired vWillebrand disease of patients with ET. A reduction in Gp IIb–IIIa-associated receptors is also present and as a consequence, fibrinogen binding is found decreased. Platelet dysfunction in ET and PV is typically characterized by a missing second-wave in the aggregation response to adrenaline, an increased adenosine diphosphate aggregation threshold as well as a reduced ATP secretion during collagen-induced aggregation but a usually normal arachidonic acid and ristocetin-induced aggregation.⁸⁵

However, some patients are found to have spontaneous aggregation and platelet hyperaggregability. Patients with ET have a decreased number of α -adrenergic receptors and this defect may be associated with the functional loss of epinephrine responsiveness. There is a decreased platelet recovery and a reduced platelet survival time in patients with MPNs attributed to the enhanced removal from the spleen and their damage due to a constant “low-grade” thrombosis.

With regards to MF, there are several possible causes of bleeding. Progressive thrombocytopenia secondary to bone marrow failure, and functional abnormalities of platelets are common findings in MF patients. In MPNs patients, clot fragility may be observed due to a mechanical effect of the high platelet count, or to the inhibition of fibrin polymerization by GP1b.⁸⁶

Massive splenomegaly and splanchnic vein thromboses are commonly found in MF and can lead to portal hypertension and development of gastric and/or esophageal varices. Lastly, an important cause of bleeding in MPN patients is the use of antiplatelets and/or anticoagulants with an iatrogenic impact.

Unmet Needs

Several unresolved issues emerge and need further evaluation:

1. Which is the optimal choice between anticoagulants or antiplatelets for primary prevention.
2. The cost/benefit assessment of biological evaluation and monitoring of antithrombotic treatment.
3. An evidence-based modification of antithrombotic treatment in case of clinical or biological resistance.
4. The clinical and biological assessment of the efficacy of hemostatic strategies in case of hemorrhage in patients with MPNs.

Conclusions

Patients with MPNs are at increased risk for both arterial and venous thrombosis as compared to the general population. These include microvascular and macrovascular events which lead to increased morbidity and mortality. In addition to the traditional risk factors of older age (age above 60) and history of prior thrombosis, many factors, including mutational status, contribute to this pro-thrombotic state found in MPNs. MPNs also induce a chronic inflammatory state which leads to an increased cardiovascular mortality in these patients. Treatment is based on thrombotic risk assessment. Treatment strategies are aimed at preventing thrombohemorrhagic complications and include aspirin, phlebotomy, cytoreductive and anticoagulation therapy. Further research will hopefully identify more precise and personalized surrogates/ biomarkers of thrombotic risk and throw light on the contribution of novel therapies, such as JAK inhibitors or interferons, to reduce thrombotic risk.

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