Thrombosis in myeloproliferative neoplasms during cytoreductive and antithrombotic drug treatment

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Abstract
A state-of-the-art lecture titled “Myeloproliferative Neoplasm-associated Thrombosis” was presented at the ISTH congress in 2021. We summarize here the main points of the lecture with two purposes: to report the incidence rates of major thrombosis in polycythemia vera and essential thrombocythemia and to discuss to what extent cytoreductive therapy and antithrombotic drugs have reduced the incidence of these events. Unfortunately, the incidence rate of thrombosis remains high, ranging between 2 and 5/100 person-years. It is likely that new drugs such as interferon and ruxolitinib can be more efficacious given their cytoreductive and anti-inflammatory activities. Despite prophylaxis with vitamin K antagonists and direct oral anticoagulants after venous thrombosis in either common sites or splanchnic or cerebral sites, the incidence rate is still elevated, as high as 4 to 5/100 person-years. Future studies with new drugs or new strategies should consider thrombosis as the primary endpoint or surrogate biomarkers only if previously validated.

KEYWORDS
antithrombotic drugs, cytoreduction, epidemiology, myeloproliferative neoplasm, thrombosis

Essentials
- The risk of arterial and venous thrombosis is increased in myeloproliferative neoplasms.
- Hydroxyurea and thromboprophylaxis have a partially favorable risk-benefit profile.
- New formulations of interferon and JAK2 inhibitors will hopefully improve the thrombotic burden.
- New intervention trials should assess surrogate biomarkers of thrombosis with proven validation.

1 | INTRODUCTION

Classic Philadelphia-negative myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF), are characterized by uncontrolled clonal proliferation of multipotent bone marrow progenitors sustained by acquired mutations in the JAK2, CALR, and MPL genes.1 The expansion of the mutated clone triggers an inflammatory response that influences the development of associated vascular complications and disease progression into MF and acute leukemia.2 Novel insights into the pathogenesis of MPN-associated arterial and venous thrombosis and the complex interplay among blood cells, the endothelium, and
the hemostatic system are now available.³ Despite progress in diagnosis, prognosis, and therapy, vascular events remain a major unmet clinical need in these diseases.¹,⁴–⁶

This review will focus on the prevention and treatment of major arterial and venous thrombosis in PV and ET with the aim of reporting (1) quantitative estimates of major thrombosis incidence; (2) the incidence of thrombosis under treatment with cytoreductive drugs; and (3) the incidence of thrombosis under aspirin and oral anticoagulants.

2 | THROMBOSIS REMAINS A MAJOR PROBLEM IN CONTEMPORARY PATIENTS WITH MPNs

Thrombosis can be the event heralding the diagnosis of MPN in 20% of cases, with a persisting risk during the follow-up, where the incidence is highest in patients with PV (3.5/100 person-years) and slightly lower in patients with ET⁷ and primary myelofibrosis (PMF) (2.5/100 person-years).⁸ Particularly in ET, arterial events are more prevalent (70%) than venous thromboembolism (VTE), the latter encompassing deep vein thrombosis (DVT) of the legs or pulmonary embolism or thromboses in uncommon sites such as the splanchnic or cerebral veins.⁹

A recent population-based study carried out in Sweden recruiting 9429 patients with MPNs and 35,820 matched controls from 1987 to 2009, with follow-up to 2010, found that the hazard ratios (HRs) for arterial thrombosis among patients with MPNs compared with controls at 3 months, 1 year, and 5 years were 3.0, 2.0, and 1.5, respectively; the corresponding HRs for venous thrombosis were 9.7, 4.7, and 3.2. The incidence of thrombosis was significantly elevated across all age groups and similar among MPN subtypes.¹⁰

In the most extensive epidemiologic study on PV (i.e., the European Collaboration on Low-dose Aspirin [ECLAP] study), cardiovascular mortality accounted for 45% of all deaths; the incidence rate of cardiovascular death was 1.7/100 person-years, with a cumulative incidence of 4.5% over a median follow-up of 2.8 years (25th percentile, 1.9 years; 75th percentile, 3.8 years), mainly from coronary heart disease (15% of all deaths), congestive heart failure (8%), nonhemorrhagic stroke (8%), and pulmonary embolism (3.6%). The cumulative incidence of nonfatal thrombosis during the same follow-up period was 10.3%, with no difference between thromboses of arterial and venous vessels.¹¹ Of note, in recent studies dealing with contemporary patients with PV,⁶,¹² the incidence rate of major thrombosis after diagnosis was 2.62/100 person-years, a figure that is lower than that reported in the ECLAP cohort but is comparable to that found in the more recent randomized clinical trial CYTO-PV, where the incidence rate was 2.7/100 person-years.¹³ Therefore, because most studies in PV include patients with both remote and most recent diagnostic periods, the accuracy of reporting the incidence of events should be carefully taken into account by considering the time at which data were generated and which diagnostic criteria were adopted.

In prospective studies in ET, fatal and nonfatal incidence rates of thrombotic events ranged from 0.9 to 2.6/100 person-years. The incidence of arterial events was 2–3 times higher than that of venous events.¹,¹⁴ The epidemiology of thrombosis and bleeding in ET should be reinterpreted according to the 2008 and 2016 World Health Organization (WHO) diagnostic classification. The revised 2016 WHO classification system distinguishes “prefibrotic” from “overtly fibrotic” PMF; the former might mimic ET in its clinical presentation, and its clinical course is characterized by a similar incidence of vascular events but a different tendency to progress into overt MF and the blast phase.¹⁵,¹⁶ In the largest cohort of WHO-diagnosed patients reported to date (891 ET and 180 pre-PMF), the histories of major bleeding were relatively low (4% vs. 7%) and not significantly different between groups. Conversely, major bleeding occurred in only 6% of WHO-ET (median follow-up 6.2 years) but in 12% of pre-PMF patients (median follow-up 7 years) (p = 0.01), consistent with rates of 0.79 and 1.39/100 person-years, respectively (p = 0.04). This result provides persuasive evidence that the discrimination of pre-PMF from “true” ET is an effective tool for stratifying the risk for bleeding.¹⁷

In PMF, the prevalence of major thrombosis was assessed in 707 patients, followed-up in four European institutions. The overall incidence rate of cardiovascular death and nonfatal thrombotic complications was 2.23 events/100 person-years. No significant difference between nonfatal venous and arterial thrombosis was registered (0.76 and 0.86/100 person-years, respectively).¹⁸ In a more recent study,¹⁹ including 642 patients and 2568 matched controls, MF was independently associated with an increased risk of venous thromboembolism but not of arterial thromboembolism. The propensity score-adjusted HRs were 6.88 (95% confidence interval [CI] 2.02–23.45) for venous thromboembolism and 0.94 (0.49–1.77) for arterial thromboembolism. Venous thromboembolisms in atypical sites almost exclusively occurred in patients with myelofibrosis (four events of Budd-Chiari vs. none and two mesenteric vein thrombosis events vs. one) and were more likely to occur around the time of myelofibrosis.²

3 | ANTITHROMBOTIC EFFICACY OF CYTOREDUCTIVE THERAPY

The purpose of cytoreductive therapy is to obtain hematological responses because normalizing blood counts with phlebotomy and/or cytoreductive drugs is thought to be fundamental to reducing the incidence of both arterial and venous thrombosis. However, despite achieving similar hematological responses, it is likely that the various cytoreductive drugs administered both in the first and second lines do not have equal antithrombotic activity. In fact, for each of the three cytoreductive drugs currently used in clinical practice (hydroxyurea [HU], interferon [IFN], ruxolitinib [Ruxol]), additional antithrombotic properties are recognized. For instance, HU is thought to have minimal anti-inflammatory properties,¹⁹ whereas there is
evidence that IFN and Ruxo can normalize inflammatory markers, further mitigating thrombotic risk.\textsuperscript{20,21} Unfortunately, clinical trials comparing head-to-head standard HU with IFN or Ruxo did not provide solid evidence of the superiority of the latter in terms of thrombosis reduction. However, that the design of these studies envisaged hematological responses as primary endpoints, and the trials were not powered to directly evaluate a decrease in the risk of thrombosis. On the other hand, it has not yet been demonstrated that hematological response is a valid surrogate of thrombosis.\textsuperscript{22-24}

3.1 | Phlebotomy

The identification of the optimal target hematocrit value to reduce blood viscosity and the risk of PV-associated vascular events has been a controversial topic following a post hoc analysis of two large, randomized clinical trials (Polycythemia Vera Study Group [PVSG]\textsuperscript{25} and European ECLAP\textsuperscript{11}) that reported no significant increase in major thromboses when the hematocrit was 45\%–50\% compared with <45\%. The controversy generated a large-scale, multicenter, prospective, randomized clinical trial (CYTO-PV\textsuperscript{13}), in which maintaining a hematocrit target of less than 45\% was shown to be associated with a fourfold lower risk of vascular events compared to a hematocrit level of 45\%–50\%. A recent consensus among experts established that a lower target hematocrit (40\%–42\%) can be appropriate in patients with persistent or recurrent symptoms of hyperviscosity, such as erythromelalgia, transient ocular attacks, headache, dizziness, and/or amaurosis fugax, at a target hematocrit of 45\% and when a benefit is documented.\textsuperscript{26}

3.2 | Hydroxyurea

There is a consensus among European LeukemiaNet\textsuperscript{26} and National Comprehensive Cancer Network\textsuperscript{27} experts concerning the use in high-risk patients with PV and ET of HU, which is currently the standard first-line drug in patients who need cytoreductive therapy. The recommendation on the use of HU in PV is not based on randomized clinical trials and is supported only by an old prospective observational study by the PVSG.\textsuperscript{28} Fifty-one patients were treated with HU, which, contrary to alkylating agents, was regarded as a nonmutagenic myelosuppressive drug. The PVSG found that this nonleukemogenic drug was associated with a lower incidence of total thrombosis compared with historical control patients managed with phlebotomy only. The effectiveness of this drug in PV was recently confirmed in a post hoc analysis of the ECLAP study,\textsuperscript{29} and a recent systematic review and metanalysis\textsuperscript{30} provided quantitative estimates of its efficacy in the real-world clinical practice of 2552 contemporary patients with PV recruited in 2008–2018. Analyzing 469 events, the estimates of thrombosis incidence rate appeared age-dependent, and in patients with median ages of 60, 70, and 80 years, the annual incidence rates under HU treatment were 1.6, 3.6, and 6.8/100 person-years, respectively (Figure 1A). Therefore, the residual incidence rate of thrombosis in HU-treated patients with PV remains high, and approximately threefold higher than that estimated in the general population, highlighting its limited effectiveness as an antithrombotic drug. More recent data suggest that HU exerts greater antithrombotic protection against arterial rather than venous thrombosis, and this is particularly shown in the prevention of recurrence (Figure 1B).\textsuperscript{31}

**Figure 1** Incidence of thrombosis under hydroxyurea (HU) treatment in polycythemia vera (PV) (A: incidence rate; B: cumulative incidence) and in essential thrombocythemia (ET) (C: cumulative incidence)
In ET, HU was tested as an antithrombotic agent in three randomized clinical trials (Figure 1C). The first seminal trial demonstrated that patients who were treated with HU had a significant reduction in arterial events compared with those who were not. Two subsequent trials compared HU with anagrelide (ANA), which in those years had been shown to obtain significant hematomal responses in Phase 2 studies. In the UK-PT1 randomized clinical trial, it was shown that HU was superior to ANA in reducing arterial thromboses, particularly in JAK2-mutated patients, whereas ANA was more efficient in reducing venous thromboses. Subsequently, Gisslinger and colleagues failed to replicate these results in a randomized trial on patients with confirmed WHO-ET, in which ANA was not inferior to HU in reducing thrombosis. This result was attributed to the use of different ET diagnostic criteria, leading the PT1 investigators to include patients with pre-PMF in their study who had a distinct clinical and hematomal presentation compared to WHO-ET. Unfortunately, in the ANA arm of the UK-PT1 trial, an excess of MF evolution was shown that was subsequently confirmed in a large cohort of 3649 high-risk European patients with ET. Overall, the incidence rates of major thrombosis in the HU arms of these trials were in the range of 1.5–2.5/100 person-years, remarkably higher than those in the general population. As highlighted by Tefferi and Pardanani, these high estimates should lead to studies testing new strategies for decreasing the residual risk of thrombosis among patients with high-risk ET. For low-risk patients, a recent clinical trial showed that HU should not be administered to patients younger than age 60 years on the basis of extreme thrombocytosis, confirming the results of a prospective observational study by Ruggeri and colleagues. Therefore, in young ET patients with no thrombotic history and a platelet count <1500 × 10^9/L, a conservative therapeutic approach is recommended.

3.3 | Ruxolitinib

Currently, two JAK2 inhibitors are available for daily clinical use. Fedratinib, a JAK2/FLT3 inhibitor, has recently been approved for patients with MF. Ruxo is recommended in MF and as second-line treatment in patients with PV who become resistant or intolerant to HU or who are poor responders. The drug achieves hematomal responses and can maintain the target hematocrit level without phlebotomy. However, the evidence in favor of Ruxo for the prevention of cardiovascular events is uncertain and estimates regarding the incidence of these complications are scattered over a series of different studies. There are two meta-analyses exploring the role of Ruxo in relation to thrombosis. In the first, four randomized controlled trials including 663 patients with PV were considered. A thrombosis risk ratio of 0.56 was estimated for Ruxo versus the best available therapy, corresponding to incidence rates of 3.09 and 5.51/100 person-years, respectively. The difference was not statistically significant, and only a trend in favor of Ruxo was found (p = 0.09). In the second meta-analysis, the incidence rates of thrombosis were significantly lower among patients with PV or MF treated with Ruxo (risk ratio 0.45, 95% CI 0.23–0.88) than in controls. Although this result deserves consideration, it should be underscored that evidence for the effectiveness of Ruxo as an antithrombotic drug in MPNs has not yet been formally demonstrated in randomized clinical trials, even though its action as a cyto reducing and anti-inflammatory drug would indicate a very likely ability to reduce vascular events in these diseases.

3.4 | Pegylated interferon IFN-α

IFN-α was the first cytokine to be produced by the pharmaceutical industry and has been used to treat hematologic malignancies. The drug exerts direct antitumor effects by limiting the production of growth-promoting cytokines, stimulating apoptosis, inhibiting cellular proliferation, and increasing immunogenicity. Clinical studies have shown that IFN-α therapy, in addition to producing complete hematological responses, is also able to reduce the JAK2V617F allele burden because of its ability to exhaust the malignant stem cell pool, suggesting a disease-modifying potential. An antithrombotic action is likely because the drug can target both the excess circulating blood counts and the inflammatory status, which is constitutive in MPN and closely associated with the mechanism of thrombosis. Various clinical trials have been performed to demonstrate the efficacy and safety of IFN-α in patients with PV and ET. However, the small study samples, the use of different response criteria, and the relatively short follow-up periods have made it difficult to reach a clear and consistent appraisal of its antithrombotic properties. Thus, estimates of thrombosis risk in this setting are inconsistently reported. To summarize the efficacy and adverse event profile in the treatment of ET and PV, two systematic reviews and meta-analyses have been recently published (Figure 2). The first analyzed 44 studies including 1359 patients (730 ET, 629 PV) and reported that annualized incidence rates of thromboembolic events in patients treated with nonpegylated or pegylated IFN-α were low (i.e., 1.2 and 0.5/100 person-years for ET and PV patients, respectively). This finding is consistent with the recent PROUD-PV/CONTINUATION-PV studies that compared ropipinterferon alfa-2b with HU. Similar results regarding the estimates were found in the second meta-analysis, where the incidence rates of vascular events were 0.42/100 person-years for thrombosis and 0.01/100 person-years for hemorrhage.

Most studies provided little information regarding patient history of thrombosis or related drug treatments, such as aspirin or other anticoagulant drugs, which might influence the incidence of these events.

4 | ANTITHROMBOTIC EFFICACY OF ASPIRIN AND ORAL ANTI COAGULANTS

4.1 | Aspirin in primary prophylaxis

Low-dose aspirin (LDA) is recommended for primary thrombo prophylaxis in all patients with PV unless contraindicated owing to a history of major bleeding. In the ECLAP placebo-controlled clinical
Incidence rate of thrombosis per 100 person-years under Peg-INF-α treatment in polycythemia vera (PV) and essential thrombocythemia (ET)

**FIGURE 2** Incidence rate of thrombosis under pegylated interferon-α (Peg-INF-α) treatment in polycythemia vera (PV) and essential thrombocythemia (ET) trial, 100 mg of aspirin significantly reduced the risk of a combined endpoint for nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or cardiovascular death (relative risk 0.40, 95% CI 0.18–0.91, *p* = 0.03). Major bleeding episodes were not significantly more frequent than with placebo (relative risk 1.62, 95% CI 0.27–9.71).

The use of LDA in ET is based on indirect evidence and favorable results coming from the ECLAP study in PV, but the lack of randomized trials makes the risk-benefit profile of this drug in ET unclear.50 Thus, estimates of the safety/efficacy profile of LDA are based on retrospective observational studies, some of which have produced useful information for clinical practice. In a Spanish collaborative investigation,51 the incidence rates of arterial and venous thrombosis were evaluated in 300 low-risk patients with ET either treated with antiplatelet drugs as monotherapy (*n* = 198, total follow-up of 802 person-years) or observed only (*n* = 102, total follow-up of 848 person-years). The authors reported that the overall incidence rates of thrombotic events did not differ between these patient groups. However, two subgroups did worse with observation only: JAK2V617F-positive patients had an increased risk of venous thrombosis (incidence rate ratio [IRR]: 4.0; 95% CI 1.2–12.9; *p* = 0.02), and patients with cardiovascular risk factors had increased incidence rates of arterial thrombosis (IRR: 2.5; 95% CI 1.02–6.1; *p* = 0.05). An increase in the risk of major bleeding was observed in patients with platelet counts >1000 × 10⁹/L treated with antiplatelet therapy (IRR: 5.4; 95% CI 1.7–17.2; *p* = 0.004). The authors concluded that antiplatelet therapy reduces the incidence of venous thrombosis in JAK2-positive patients and the incidence of arterial thrombosis in patients with associated cardiovascular risk factors. In the remaining low-risk patients, observation may be an adequate option.51

Similar results were obtained in a multicenter cohort of 433 low-risk ET patients, where the benefit was not shown in CALR-mutated cases, in which a higher risk of bleeding complications was noticed.52 This finding suggests close monitoring for bleeding in patients with extreme thrombocytosis, which is very often associated with CALR mutation.53 This topic was recently delved into by European LeukemiaNet investigators,54 who provided key recommendations in patients with extreme thrombocytosis, including (1) careful observation for asymptomatic patients with classic low-risk, CALR-mutated ET without cardiovascular risk factors and (2) caution in the use of antiplatelet therapy for symptomatic patients at low risk with platelet counts of 1000–1500 × 10⁹ platelets per liter. In these patients, cytoreduction is an adequate option, especially if acquired von Willebrand disease is present. These investigators pointed out that unlike in JAK2 V617F-mutated patients with ET, thrombosis prevention is not the priority in most patients with ET and CALR mutations. In contrast, the correction of thrombocytosis, especially in patients with microvascular symptoms, may be considered, although new evidence to guide clinical practice is warranted.54

New developments for the use of this drug for the primary prevention of thrombosis in MPNs may derive from recent knowledge on the pharmacodynamics of LDA. An accelerated renewal of platelet cyclooxygenase-1 has been documented in ET,55 thus making patients only partially protected. The ongoing ARES randomized Phase 2 trial will hopefully answer the question of whether in ET two or three doses of 100 mg aspirin daily is superior to the standard once-daily regimen in inhibiting platelet thromboxane (TX) A2 production without abolishing vascular prostacyclin biosynthesis.56

The first phase of the ARES trial conducted on 245 patients demonstrated that twice-per-day dosing significantly reduced serum TXB2 levels and TXA2-dependent platelet activation in vivo with respect to the once-daily regimen, whereas urinary prostacyclin metabolite, a surrogate marker for endothelial prostacyclin production and vascular safety, was not significantly reduced.57

### 4.2 | Vitamin K antagonists and direct oral anticoagulants

Several retrospective studies estimated the incidence rate of recurrent thrombosis in patients with MPN. The incidence rate of recurrent thrombosis was estimated to be 7.6/100 person-years, 3.4 on vitamin K antagonists (VKAs) and 9.4 off VKAs (*p* = 0.02).58 In a single-center study, the recurrent VTE incidence rate was 6.0/100 person-years, with more events off VKAs.59 In 206 patients with a well-characterized diagnosis of DVT of the legs and/or pulmonary embolism, the incidence rates of recurrent thrombosis were 5.3 and 12.8/100 person-years on VKAs and after discontinuation, respectively (*p* = 0.01). After stopping VKAs, the cumulative incidence of recurrence was 42.3% at 5 years of follow-up.60 Patients with thrombosis of hepatic or cerebral veins were more prone to recurrences.61,62 Despite a favorable effect of VKAs on the risk of recurrent thrombosis, an indirect comparison of MPN patients with VTE with non-MPN patients with VTE recruited in recent trials suggests a higher thrombotic potential in patients with MPN. In fact, the cumulative incidence of recurrent...
thrombosis at 1 year and 5 years of VKA treatment is 7.8% and 21%, respectively. This estimate is definitely higher than the cumulative incidence of 1.2% and 3.6% observed after 1 year and 5 years, respectively, of VKA treatment in non-MPN patients with unprovoked VTE. The higher thrombotic potential in high-risk patients with MPN in comparison with non-MPN patients may be due to combined mechanisms arising from the clonal proliferation of hematopoietic stem cells with a procoagulant phenotype, plasma hypercoagulable changes, the secretion of inflammatory cytokines, and endothelial dysfunction in response to prothrombotic insults, as a final result, anticoagulant action on a single pathogenetic pathway can be ineffective.

Moreover, recurrent thrombosis can circumvent the effect of cyto reduction, and a recent reappraisal of data from retrospective cohorts and clinical trials showed that the efficacy of hydroxyurea in preventing thrombosis is significant for arterial sites but doubtful for venous sites.

A major concern during anticoagulation is bleeding risk. In a recent meta-analysis on non-MPN patients receiving VKAs after an unprovoked VTE, the rate of major bleeding is 1.74/100 person-years (95% CI 1.34–2.20); it was pointed out that the incidence of major bleeding is significantly higher among patients older than 65 years or with a creatinine clearance less than 50 ml/min, a history of bleeding, concomitant use of antiplatelet therapy, or hemoglobin levels less than 100 g/L.

This rate is consistent with the frequency found in patients with MPN whose median age is 60–65 years, in whom the incidence rate of major bleeding per 100 person-years is 0.9–2.4 on VKAs and 0.7–1.5 off VKAs, and as high as 2.8 when combining VKAs and aspirin.

Direct oral anticoagulants (DOACs; i.e., apixaban, dabigatran, edoxaban, and rivaroxaban) have been recently investigated in a large observational international study in 442 patients with MPN with nonvalvular atrial fibrillation and VTE. In patients with atrial fibrillation, after a follow-up of 1.7 years (IQR: 0.8–3.1), 10 major thrombotic events were reported, with an incidence rate of 2.1/100 person-years, whereas among 158 patients with VTE of the legs and/or pulmonary embolism, the incidence rate was 5.1/100 person-years, occurring predominantly in the venous districts. Interestingly, this incidence rate per 100 person-years of recurrence after VTE is comparable to the 5.3 value found in the previously reported series of MPN patients receiving VKAs after VTE of the legs and/or pulmonary embolism (Table 1).

In regard to the role of cytoreductive therapy, a recent pooled analysis on 1500 patients with MPN-related arterial (n = 935) or venous (n = 565) thromboses examined the role of HU in combination with aspirin or oral anticoagulants. Multivariate models adjusted for age and sex limited to patients with first arterial thrombosis confirmed that recurrent arterial thrombosis was prevented by antiplatelet agents (HR: 0.49, 95% CI 0.31–0.78, p = 0.003) and by HU (HR: 0.64, 95% CI 0.42–0.98, p = 0.04) and only partially by VKAs (HR: 0.53, 95% CI 0.27–1.04, p = 0.06); conversely, in patients with first venous thrombosis, venous recurrences were more prevented by VKAs (HR: 0.57, 95% CI 0.35–0.84) than by antiplatelet agents (HR: 0.71, 95% CI 0.41–1.24, p = 0.24) or HU (HR: 0.75, 95% CI 0.46–1.23, p = 0.26).62 Moreover, HU did not show a significant effect on the rate of recurrent thrombosis in 218 patients with splanchnic vein thrombosis (HR: 0.81, 95% CI 0.39–1.65, p = 0.56) after adjustment for age, sex, antiplatelet treatment, VKA treatment, and cytoreductive agents other than HU.

These findings were not confirmed in a recent systematic review of 1235 patients with MPN receiving antithrombotic treatment and HU. In 738 patients with VTE, the combination of cytoreduction and VKAs (n = 313) or DOACs (n = 63) was more effective in preventing recurrences than VKA alone (n = 106) (relative risk 0.51, 95% CI 0.23–1.14) or DOACs alone (n = 14) (relative risk 0.21, 95% CI 0.08–0.60). However, the results of this univariate analysis are weakened by the small number of patients analyzed in some treatment groups and by the heterogeneity of the sites of thrombosis, which have been reported to be associated with different effectiveness of cytoreductive treatment in preventing recurrences. Moreover, such results are not comparable with those of the cohort studies because no information is given about the incidence rate of recurrent thrombosis in the different treatment groups.

In summary, antiplatelet agents and HU are the drugs of choice in patients with MPN with a history of arterial thrombosis. In patients with VTE at common sites, the risk of recurrence is halved by VKAs or DOACs. The latter should be preferred over VKAs, given the advantages of the ease of administration and patient convenience, although new studies on the bleeding risk associated with these drugs are warranted. The benefit of HU after VTE in addition to oral anticoagulation remains uncertain, particularly in patients with splanchnic vein thrombosis.

### TABLE 1  Incidence rate of recurrent thrombosis and bleeding in MPN patients with DVT at common sites or with splanchnic vein thrombosis treated with VKAs or DOACs

| Treatment | Patients (N) | IR of recurrent thrombosis /100 person-years (95% CI) | IR of bleedings /100 person-years (95% CI) |
|-----------|-------------|-----------------------------------------------------|------------------------------------------|
| VKAs60    | DVT of legs ± PE (206) | 5.3 (3.2–8.4) | 2.4 (1.1–4.5) |
| DOACs67   | DVT of legs ± PE (158) | 4.5 (2.9–6.8) | 2.7 (1.4–5.2) |
| VKAs62    | SVT (139) | 3.9 (2.4–5.8) | 2.0 (1.1–3.5) |
| DOACs67   | SVT (51) | 3.2 (1.2–8.6) | 0.8 (0.1–5.5) |

Abbreviations: CI, confidence interval; DOAC, direct oral anticoagulant; DVT, deep vein thrombosis; IR, incidence rate; PE, pulmonary embolism; SVT, splanchnic vein thrombosis; VKA, vitamin K antagonist.
There are few randomized clinical trials that have thrombosis reduction as their primary endpoint. Most studies reporting vascular events in PV and ET are observational and almost always retrospective; therefore, they suffer from important limitations and often have reporting standards inadequate for a synthesis assessment, as required by systematic reviews and meta-analyses. Consequently, pooled estimates of vascular events related to both cytoreductive drugs and antithrombotics (aspirin and oral anticoagulants) are heterogeneous and include wide confidence intervals. As shown in the present review, the residual risk of thrombosis in contemporary patients with PV and ET remains substantially elevated, despite the correct use of therapeutic strategies suggested by the current guidelines. Unfortunately, the new drugs tested for registration in randomized clinical trials use biomarkers as surrogate primary endpoints instead of thrombosis. However, endpoints such as hematological response have never been formally validated as surrogates for vascular events; therefore, upcoming studies must aim to explore new biomarkers that are easily measurable in clinical practice and formally validated to be considered real surrogates of arterial or venous thrombosis.

Some abstracts describing several biomarkers of thrombotic predisposition in patients with MPN were presented at the ISTH 2021 Congress.

Smirnova and colleagues investigated 173 patients with MPN and 68 healthy controls. Endothelial activation and damage to the plasma soluble marker von Willebrand factor activity and antigen levels were increased in patients with MPN. A subgroup of patients with MPN (n = 49) was investigated by Korsakova and colleagues in comparison with the same control group. Von Willebrand factor activity and antigen levels were increased in patients with MPN (especially in those with ET). Endothelial function was also estimated with an EndoPAT 2000 apparatus by the noninvasive peripheral arterial tonometry method based on endothelial-dependent vasodilatation registration by digital plethysmography probes. Vasomotor endothelial dilatation was recorded in approximately one third of MPN patients, with a higher incidence in patients with PV.

Silina and colleagues assessed thrombin generation by calibrated automated thrombography with or without thrombomodulin (TM) in 18 patients with PV on aspirin and 20 patients with PV on cytoreductive therapy in combination with antiplatelet agents. The following parameters were evaluated: endogenous thrombin potential and peak thrombin. The sensitivity of endogenous thrombin potential and peak thrombin for TM were calculated as the percentage decrease in these parameters after addition to the TM assay. Decreasing these parameters indicates a dysfunction of the anticoagulant protein C system and is a potential risk factor for thrombotic complications. Sensitivity to TM was significantly reduced in patients of both groups in comparison with 43 healthy controls. The parameters of sensitivity to TM in patients on cytoreduction and antiplatelet agents were significantly lower than those in patients on aspirin. In a separate abstract, the authors found in the same study groups that ristocetin cofactor activity and von Willebrand factor levels were significantly increased in patients on cytoreductive and antiplatelet therapy, showing more pronounced endothelial dysfunction in this group.

Overall, such findings confirm that patients at high risk for thrombosis who need cytoreduction show a higher thrombotic potential because of blood coagulation activation and endothelial dysfunction.

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T. Barbui conceptualized and led the work and wrote and revised the paper. A. Carobbio wrote the paper and prepared the figures. V. De Stefano wrote the paper and supervised the literature search. All authors revised and approved the final version of the manuscript.

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