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Risk factors of thrombosis in a cohort of 206 patients with BCR-ABL1 negative myeloproliferative neoplasms

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Thrombosis is the most common complication in BCR-ABL1 negative myeloproliferative neoplasms (MPN) that significantly impacts patients' mortality. Generally, there is an agreement on risk factors that possibly contribute to the increased risk of thrombosis, including age, history of thrombosis, JAK2V617F mutation, and cardiovascular risk factors. This study retrospectively investigates MPN-related and patient-related variables in relation to the thrombosis occurrence in MPN. Our analyses show that JAK2V617F-mutated patients are at a significantly increased risk of thrombosis within five years before the MPN diagnosis point with a hazard ratio (HR) of 15.49 (p=0.006). In multivariate analyses, independent risk factors for thrombotic complications during the follow-up are history of thrombosis (HR=2.23, p=0.019), age over 60 years at diagnosis (HR=1.56, p=0.037), the presence of JAK2V617F mutation (HR=3.01, p=0.002), and tobacco smoking (HR=1.75, p=0.01). Our results support the multifactorial mechanism of thrombosis in MPN patients, which demands individual and complex management.

Key words: myeloproliferative neoplasm, thrombosis, risk factor, JAK2V617F mutation, smoking

Cardiovascular diseases are currently the leading cause of mortality worldwide, accounting for 16% of the world's total deaths (https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (accessed June 16, 2021)). In an international meta-analysis, thromboembolism was responsible for each 4th death case worldwide [1]. Some inherited and acquired hematologic diseases are associated with an increased risk of thromboembolic complications. The group of BCR/ABL1-negative myeloproliferative neoplasms (MPN) is known to have an increased incidence of thrombotic complications. The most common MPN are polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), which are clonal myeloid disorders driven in almost 90% of cases by acquired molecular mutations (JAK2^{V617F}, CALR, and MPL mutations). In 10–15% of patients, no driver mutations are detectable, and these patients are considered triple-negative [2]. MPN group is characterized by excessive stimulation of the marrow hematopoietic precursors, leading to the uncontrolled production of mature blood cells [3]. The mortality rate due to thrombotic complications was reported to account for 41%, 26%, and 12% of deaths in PV, ET, and PMF patients, respectively [4–6]. Thrombosis can be the initial manifestation of MPN and may occur at typical sites (venous, arterial, and microcirculatory) or atypical venous sites (splanchnic and cerebral sinus veins), which is a hallmark of MPN [7, 8].

Age over 60 years and previous history of thrombotic events have been identified as the definite risk factors for thrombosis in MPN [9]. JAK2^{V617F} as the most common clonal mutation in MPN patients presents in up to 100% of PV, 72% of ET, and 86% of PMF patients [10]. The presence of JAK2^{V617F} mutation has been associated with an increased risk of thrombotic complications [11, 12]. Other risk factors that have been linked to increased risk of thrombosis include thrombophilia conditions and cardiovascular risk factors, especially hypertension, diabetes mellitus, and tobacco smoking [13, 14]. Identifying thrombotic risk factors is an important part of the treatment strategy in MPN patients.

This study aims to investigate the possible MPN-related and patient-related risk factors of thrombotic complications in patients with BCR/ABL1-negative myeloproliferative neoplasms.

Patients and methods

We retrospectively analyzed clinical and laboratory data of 206 patients (91 males and 115 females, median age: 57 (28–78) years) with BCR-ABL1 negative MPN according to the diagnostic criteria of the World Health Organization (WHO). Patients were treated at the Department of Clinical Hematology of the University Hospital Brno, Czech Republic, between the years 2003 and 2019 with a median follow-up of 4.8 (0.3–16.1) years. The local ethical committee approved this study; all patients signed informed consent.

Clinical and laboratory assessment was done for all patients at the time of MPN diagnosis and it included investigating the presence of cardiovascular risk factors (hypertension, hyperlipidemia, diabetes mellitus, tobacco smoking, and obesity), thrombophilia conditions (deficiency of protein C or S, hyperhomocysteinemia, FV Leiden mutation, PTG20210A mutation, presence of anti-phospholipid antibodies), and driver mutations ($JAK2^{V617F}$, CALR, MPL). Polymerase chain reaction (PCR) assays were used to detect $JAK2^{V617F}$, CALR, and MPL mutations. Follow-up of patients was conducted regularly with check-ups being performed every 3 or 6 months depending on patient's clinical state. The regular check-up consisted of physical examination, imaging studies if needed, and laboratory studies including complete blood count, coagulation indicators, and biochemistry profile.

In a standardized way, thromboembolic events were recorded for all patients according to the site of thrombosis as arterial events (ATE), venous (VTE), or microvascular disturbance (MVD). ATEs were defined as myocardial infarction (STEMI and NSTEMI), unstable angina pectoris, ischemic stroke, transitory ischemic attack (TIA), and peripheral arterial occlusion. VTEs were deep vein thrombosis,

Table 1. Characteristics of patients with BCR-ABL1 negative myeloproliferative neoplasms and according to the time of thrombosis occurrence.

	All MPN patients (n=206) N (%)	Patients with thrombosis anytime (n=72) N (%)	Patients with thrombosis before MPN diagnosis (n=37) N (%)	Patients with thrombosis at/after MPN diagnosis (n=45) N (%)
Age (years)				
Median age (5^{th}–95^{th} PCTL)	57 (28–78)	62 (36–81)	64 (34–84)	58 (36–81)
>60 years	87 (42.2)	37 (51.4)	22 (59.5)	20 (44.4)
Sex				
Male	91 (44.2)	32 (44.4)	19 (51.4)	17 (37.8)
Female	115 (55.8)	40 (55.6)	18 (48.6)	28 (62.2)
MPN subtype				
PV	51 (24.7)	21 (29.2)	12 (32.4)	11 (24.4)
ET	119 (57.8)	43 (59.7)	23 (62.2)	28 (62.2)
PMF	36 (17.5)	8 (11.1)	2 (5.4)	6 (13.3)
Driver mutations				
$JAK2^{V617F}$	155 (75.2)	62 (86.1)	33 (89.2)	39 (86.6)
CALR	37 (18)	6 (8.3)	3 (8.1)	3 (6.7)
MPL	4 (1.9)	1 (1.4)	1 (2.7)	0 (0)
Triple-negative	10 (4.9)	3 (4.2)	0 (0)	3 (6.7)
Cardiovascular risk factors				
Hypertension	121 (58.7)	48 (66.7)	23 (62.2)	31 (68.9)
Obesity (BMI >30)	28 (13.6)	7 (9.7)	2 (5.4)	5 (11.1)
Hyperlipidemia	51 (24.7)	15 (20.8)	7 (18.9)	12 (26.7)
Diabetes mellitus	29 (14.1)	13 (18.1)	6 (16.2)	8 (17.8)
Tobacco smoking	57 (27.7)	24 (33.3)	14 (37.8)	13 28.9)
Thrombophilia states				
Any condition *	88 (42.7)	40 (55.6)	19 (51.4)	25 (55.6)
Previous history of thrombosis				
Yes	53 (25.7)	*NA*	*NA*	10 (22.2)
Thrombotic complications				
ATE	41 (20)	41 (56.9)	21 (56.8)	22 (48.9)
VTE	36 (17.5)	36 (50)	18 (48.6)	23 (51.1)
MVD	9 (4.4)	9 (12.5)	1 (2.7)	9 (20)

Note: * including deficiency of protein C or S, hyperhomocysteinemia, FV Leiden mutation, PTG20210A mutation, or presence of anti-phospholipid antibodies. Abbreviations: MPN-myeloproliferative neoplasms; PCTL-percentile; ET-essential thrombocythemia; PV-polycythemia vera; PMF-primary myelofibrosis; ATE-arterial thrombotic events; VTE-venous thrombotic events; MVD-microvascular disturbance; NA-not applicable

pulmonary thromboembolism, splanchnic vein thrombosis, and superficial thrombophlebitis. In all patients, thrombotic events were diagnosed based on clinical symptomatology and laboratory findings, confirmed by sonographic and radiographic evaluation (computed tomography, and magnetic resonance imaging). MVDs included erythromelalgia, ophthalmic, and neurological manifestations not compatible with the diagnosis of stroke or TIA. A total of 72 (35%) patients had thrombotic events; 41 (19.9%) patients had ATE, 36 (17.5%) patients had VTE, and 9 patients (4.4%) had MVD. The characteristics of the patients are summarized in Table 1. According to the time of occurrence, events were classified as thrombosis that occurred within 30 days before or after MPN diagnosis time (at diagnosis), before this period (before diagnosis), or after this period (after diagnosis or during the follow-up).

Statistical analysis. Categorical variables were summarized as absolute and relative frequencies; continuous variables as medians, 5th and 95th percentiles. The incidence rate of thrombosis was calculated as the number of thromboses divided by the number of years at risk. Years at risk were counted from 15 years of the patient's age. A comparison of two incidence rates was made using the binomial test.

The impact of MPN- and patient-related variables on thrombotic complications was assessed using univariate logistic regression, which gives an odds ratio (OR) accompanied by a 95% confidence interval (CI). Time to event analyses was performed for thrombotic events both as an overall and separately in the specified time windows. The date of MPN diagnosis was set to time 0 and subtracted from all other dates (date of birth, date of thrombosis occurrence, and date of end of follow-up); thus, all time values are relative to the date of MPN diagnosis.

Extended Cox proportional hazard model for recurrent events was used to estimate thrombosis-free survival (TFS) and hazard ratios (HR). In the analysis of the recurrent events, an individual stays at risk for the event throughout the follow-up period, regardless of whether an event has occurred or not. Prentice, Williams, and Peterson's (PWP) total time model [15, 16] is stratified by event order and was selected because the PWP model assumes different hazard functions among the sequential events. The time window was also used as strata due to non-proportional hazard in individual time windows. The time scale used in the model was the time from MPN diagnosis. Univariate analyses were followed by a multivariate PWP total time model. A backward stepwise elimination procedure was applied for the final selection of factors included in the multivariate model to obtain adjusted hazard ratios. Both univariate and multivariate PWP models included patients' effects as a random component.

All tests were performed as two-sided at the significance level of alpha = 0.05. Analyses were done in R software and IBM SPSS Statistics.

Results

In the present study, 121 thromboembolic events were recorded. 53 (25.7%) patients had 65.3% (79/121) of thrombotic events before/at MPN diagnosis, while 29 (14.1%) patients had 34.7% (42/121) of events after MPN diagnosis. Out of all patients, only 10 (4.8%) patients had recurrent thrombotic events.

The annual incidence rate of any thrombosis anytime in the studied cohort was 1.26% per 100,000 person-year. Before MPN diagnosis, the incidence rate was 0.64% per patient-year, while after diagnosis was 3.37% per patient-year. According to the MPN subtype, 41.2% (21/51) of PV patients, 36.1% (43/119) of ET, and 22.2% (8/36) of PMF had at least one thrombotic event. The median age of patients who had any thrombosis anytime was significantly higher than the median age of thrombosis-free patients (62 vs. 54 years, p=0.002). The most common types for ATE in this study were myocardial infarction (STEMI/NSTEMI), stroke, and transient ischemic attack, respectively (37%, 30%, and 26%). The most common sites of VTE were deep, superficial, or splanchnic vein, respectively (42%, 30%, 17%). MVD occurred most commonly as erythromelalgia (33%), cephalgia, or visual disturbance (each 25%).

Table 2 shows the association between individual clinical and molecular variables with the occurrence of overall thrombotic events as well as ATE, VTE, and MVD. Age over 60 years at diagnosis was associated with a significantly increased probability of having arterial thrombotic complications (OR=2.02, p=0.047).

JAK2^{V617F} mutation was harbored by 100% (51/51) of PV, 70.6% (84/119) of ET, and 55.6% (20/36) of PMF patients. Out of 72 patients who had thrombotic events, 86.1% (62/72) of them were JAK2^{V617F}-mutated. The odds of thrombotic complications increased significantly in the presence of JAK2^{V617F} mutation. This association was significant for overall thrombotic events (OR=2.73, p=0.01) and especially for ATE (OR=2.78, p=0.044). Compared to JAK2^{V617F}, CALR mutation was associated with significantly decreased odds of overall thrombosis (OR=0.29, p=0.009). The presence of any thrombophilia risk condition was associated with an increased probability of overall thrombosis and ATE, respectively (OR=2.3, p=0.007, OR=2, p=0.046).

Complete blood counts (CBCs) were performed for all patients at diagnosis. Patients who had thrombotic complications only during the follow-up had significantly higher platelet count at diagnosis than patients without thrombosis (p<0.001), while no significant differences were observed for leukocyte, erythrocyte, hemoglobin, or hematocrit values (Table 3). Higher platelet count at diagnosis was responsible for increasing the probability of thrombotic complications during the follow-up (OR [95% CI]: 1.15 [1.02–1.31]; p=0.024).

The Cox model (Table 4) showed that age >60 years at diagnosis was associated with a higher risk of thrombosis

Table 2. Odds ratio of clinical and molecular variables in relation to thrombosis occurrence as overall thrombosis, ATE, VTE, and MVD.

	Overall thromboses		ATE anytime		VTE anytime		MVD anytime	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age at diagnosis								
>60 years†	1.78 (0.99–3.17)	0.052	2.02 (1.01–4.03)	0.047	1.46 (0.71–3.01)	0.301	1.1 (0.29–4.22)	0.891
Sex								
Male	Reference group							
Female	0.98 (0.55–1.75)	0.954	1.01 (0.51–2.02)	0.969	1.5 (0.71–3.16)	0.285	0.62 (0.16–2.38)	0.486
MPN subtype								
PV	Reference group							
ET	0.81 (0.41–1.58)	0.534	1.12 (0.51–2.47)	0.781	0.74 (0.32–1.67)	0.462	1.3 (0.25–6.67)	0.753
PMF	0.48 (0.15–1.51)	0.208	0.21 (0.04–1.03)	0.055	0.59 (0.19–1.86)	0.366	0.7 (0.06–8.03)	0.753
Driver mutations								
JAK2^{V617F}	2.73 (1.28–5.86)	0.01	2.78 (1.03–7.53)	0.044	1.8 (0.7–4.61)	0.221	2.72 (0.33–22.3)	0.351
CALR*	0.29 (0.11–0.74)	0.009	0.29 (0.09–1.01)	0.051	0.37 (0.11–1.28)	0.115	0.51 (0.06–4.21)	0.532
MPL*	0.5 (0.05–4.92)	0.552	1.1 (0.11–10.92)	0.934	1.39 (0.14–13.83)	0.779	0	NA
Triple-negative*	0.64 (0.16–2.58)	0.533	0.37 (0.05–2.99)	0.35	1.04 (0.21–5.16)	0.96	0	NA
Cardiovascular risk factors								
Hypertension	1.64 (0.91–2.99)	0.103	1.64 (0.8–3.4)	0.18	0.97 (0.47–2.01)	0.926	5.88 (0.72–47.89)	0.098
Obesity/BMI > 30	0.56 (0.22–1.41)	0.219	0.64 (0.21–1.98)	0.438	0.84 (0.27–2.64)	0.766	0.86 (0.1–7.42)	0.889
Hyperlipidemia	1.03 (0.48–2.24)	0.935	0.63 (0.23–1.76)	0.376	1.17 (0.44–3.13)	0.759	2.44 (0.39–15.12)	0.339
Diabetes mellitus	1.61 (0.73–3.57)	0.24	2.03 (0.84–4.86)	0.114	1.27 (0.48–3.39)	0.633	0.75 (0.09–6.23)	0.79
Tobacco smoking	1.49 (0.78–2.85)	0.227	1.4 (0.66–2.98)	0.381	1.45 (0.65–3.23)	0.368	0.42 (0.05–3.66)	0.431
Thrombophilia states								
Any condition ‡	2.26 (1.25–4.1)	**0.007**	2.04 (1.01–4.11)	**0.046**	1.48 (0.71–3.08)	0.291	2.73 (0.66–11.24)	0.164

Notes: † reference group is patients with age ≤60 years; * reference group is patients with JAK2^{V617F}; ‡ including deficiency of protein C or S, hyperhomocysteinemia, FV Leiden mutation, PTG20210A mutation, or presence of anti-phospholipid antibodies. Abbreviations: OR-odds ratio; ATE-arterial thrombotic events; VTE-venous thrombotic events; MVD-microvascular disturbance; MPN-myeloproliferative neoplasms; ET-essential thrombocythemia; PV-polycythemia vera; PMF-primary myelofibrosis

anytime (HR=1.69, p=0.007). Tobacco smoking was the only cardiovascular risk factor associated with an increased risk of thrombosis (HR=1.54, p=0.033). The presence of JAK2^{V617F} mutation was associated with significantly high risk for developing overall thrombosis (HR=2.39, p=0.002) as well as for ATE (HR [95% CI]: 2.39 [1.39–4.1], p=0.002) but not significantly for VTE (HR [95% CI]: 1.84 [0.85–3.98], p=0.122). Although it was not statistically significant, female sex, diagnosis with ET, and JAK2^{V617F} mutation increased the risk of recurrent thrombosis on average by 1.7, 2, and 3.2 folds, respectively (p=0.056, p=0.053, p=0.065). The presence of CALR mutation was associated with a significantly lower risk of overall thrombosis than the presence of JAK2^{V617F} mutation (HR=0.33, p=0.001). Similarly, the risk of thrombosis was significantly decreased in patients diagnosed with PMF (HR=0.45, p=0.014) compared to PV diagnosis.

The Kaplan-Meier curve of thrombosis-free survival (TFS) for MPN patients who harbor JAK2^{V617F} mutation (Figure 1) shows that the median TFS for mutated patients was 6 (1.7–13) years, whereas 13.9 (12.4-*NA*) years in negative patients. It was noticed that the curve of the JAK2^{V617F} mutated patients started to diverge from the negative patients five years before the MPN diagnosis.

The 121 thrombotic events were classified into three groups according to the time of the event: i) thrombotic events that occurred within 5 years before MPN diagnosis confirmation

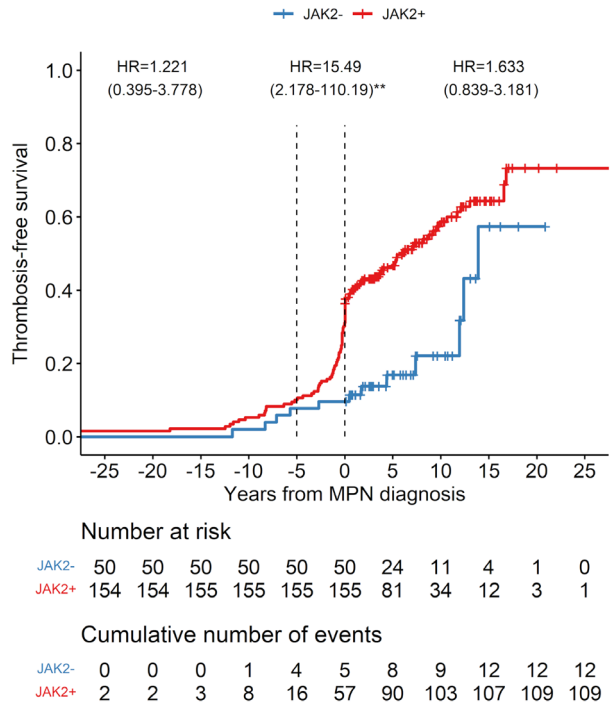

Figure 1. Thrombosis-free survival curves concerning the presence of JAK2^{V617F} mutation analyzed for the occurrence of thrombosis. **p=0.006

date were classified as (5 years before MPN diagnosis); ii) events that occurred before these 5 years as (in history); and iii) events that occurred after the date of MPN confirmation as (after MPN diagnosis or during the follow-up). Nearly half of all thrombotic events (56/121) had occurred within 5 years before MPN diagnosis with an overall incidence rate of 5.44% per patient-year, whereas incidence rates of events occurred in history (21/121) or during the follow-up (44/121) were 0.29% and 3.53% per patient-year, respectively. The difference in incidence rates between thrombotic events that occurred within 5 years before diagnosis and those that occurred during the follow-up was statistically significant (p=0.027). According to MPN subtypes, the incidence rate of thrombosis in the PMF group was significantly lower than the ET group within 5 years before diagnosis and during the follow-up (p=0.036, p=0.031).

The risk of the studied variables was re-evaluated using the Cox model in Table 5. As shown in Figure 1, within 5 years before MPN diagnosis, the presence of JAK2^{V617F} mutation has significantly increased the risk of thrombosis (HR=15.49, p=0.006). In contrast, the presence of CALR mutation has significantly decreased the risk compared to JAK2^{V617F} mutation (HR=0.09, p=0.015). In the same time window, tobacco smoking also increased the risk of thrombosis (HR=2.02, p=0.008). During follow-up, age over 60 years at diagnosis and female sex increased the risk of thrombotic complications during the follow-up by more than 2 folds (p=0.005, p=0.016).

In univariate analyses, a history of thrombotic events before or at MPN diagnosis increased the risk of thrombosis during the follow-up by 2 folds (HR [95% CI]: 2.08 [1.09–3.98]; p=0.027). This was proved in multivariate analyses (adjusted HR [95% CI]: 2.23 [1.14–4.37]; p=0.019).

Table 3. Evaluation of CBC results performed at diagnosis in relation to thrombosis during the follow-up.

CBC at MPN diagnosis	Patients with thrombosis during the follow-up		
	Yes (n=29)*	No (n=177)*	p-value
Leukocyte count (×10^9/l)	10.5 (6.6–15.3)	9.5 (6.52–17.54)	0.058
Erythrocyte count (×10^{12}/l)	4.89 (4.18–7.02)	4.88 (3.9–6.93)	0.531
Hemoglobin (g/l)	143 (129–175)	143 (112–180)	0.317
Hematocrit (%)	43 (38–53)	43 (34–55)	0.283
Platelet count (×10^9/l)	870 (497–1289)	725 (315–1298)	<0.001

Note: *values denote median and range between 5th and 95th percentile

Table 4. Evaluation of clinical and molecular variables in relation to the risk of thrombotic occurrence and recurrence.

	All thromboses (anytime)		Recurrent thrombosis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age at diagnosis				
>60 years †	1.69 (1.16–2.46)	0.007	1.53 (0.83–2.82)	0.173
Sex				
Male	Reference group			
Female	1.11 (0.78–1.58)	0.571	1.69 (0.99–2.89)	0.056
MPN subtype				
PV	Reference group			
ET	1.21 (0.81–1.81)	0.343	2.08 (0.99–4.39)	0.053
PMF	0.45 (0.24–0.85)	0.014	0	NA
Driver mutations				
JAK2^{V617F}	2.39 (1.39–4.1)	0.002	3.22 (0.93–11.13)	0.065
CALR*	0.33 (0.17–0.65)	0.001	0.21 (0.03–1.41)	0.107
MPL*	0.87 (0.18–4.27)	0.865	1.08 (0.76–1.53)	0.675
Triple-negative*	0.59 (0.24–1.47)	0.255	0	NA
Cardiovascular risk factors				
Hypertension	1.16 (0.8–1.67)	0.437	1.01 (0.57–1.78)	0.986
Obesity	0.74 (0.38–1.45)	0.385	1.17 (0.56–2.45)	0.681
Hyperlipidemia	0.91 (0.55–1.5)	0.71	0.9 (0.41–1.97)	0.797
Diabetes mellitus	1.03 (0.68–1.54)	0.902	0.63 (0.29–1.4)	0.258
Tobacco smoking	1.54 (1.04–2.29)	0.033	1.58 (0.87–2.88)	0.137
Thrombophilia states				
Any condition ‡	0.65 (0.3–1.42)	0.28	0.45 (0.19–1.07)	0.072

Notes: † reference group is patients with age ≤ 60 years; * reference group is patients with JAK2^{V617F}; ‡ including deficiency of protein C or S, hyperhomocysteinemia, FV Leiden mutation, PTG20210A mutation, or presence of anti-phospholipid antibodies. Abbreviations: HR-hazard ratio; ET-essential thrombocythemia; PV-polycythemia vera; PMF-primary myelofibrosis

Table 5. Evaluation of clinical and molecular variables in relation to the risk of thrombotic complications according to the time of occurrence.

	ALL thromboses (within 5 years before MPN diagnosis)		ALL thromboses (during the follow-up)	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age at diagnosis				
>60 years †	1.62 (0.98–2.7)	0.063	2.35 (1.29–4.27)	0.005
Sex				
Male	Reference group			
Female	0.6 (0.35–1.03)	0.062	2.3 (1.17–4.54)	0.016
MPN subtype				
PV	Reference group			
ET	1.13 (0.67–1.89)	0.655	1.23 (0.57–2.69)	0.598
PMF	0.42 (0.16–1.07)	0.068	0.43 (0.13–1.44)	0.173
Driver mutations				
JAK2^{V617F}	15.49 (2.18–110.19)	0.006	1.63 (0.84–3.18)	0.149
CALR*	0.09 (0.01–0.63)	0.015	0.46 (0.19–1.1)	0.081
MPL*	NA	NA	NA	NA
Triple-negative*	NA	NA	1.48 (0.55–3.95)	0.436
Cardiovascular risk factors				
Hypertension	1.08 (0.66–1.78)	0.753	1.62 (0.75–3.46)	0.217
Obesity	0.76 (0.29–1.96)	0.566	1.02 (0.35–2.94)	0.976
Hyperlipidemia	0.9 (0.46–1.77)	0.756	0.95 (0.44–2.03)	0.889
Diabetes mellitus	0.84 (0.43–1.64)	0.612	1.37 (0.7–2.68)	0.36
Tobacco smoking	2.02 (1.21–3.39)	0.008	1.09 (0.53–2.25)	0.809
Thrombophilia states				
Any condition ‡	0.65 (0.27–1.57)	0.336	0.59 (0.1–3.7)	0.0575

Notes: † reference group is patients with age ≤60 years; * reference group is patients with JAK2^{V617F}; ‡ including deficiency of protein C or S, hyperhomocysteinemia, FV Leiden mutation, PTG20210A mutation, or presence of anti-phospholipid antibodies. Abbreviations: HR-hazard ratio; ET-essential thrombocythemia; PV-polycythemia vera; PMF-primary myelofibrosis

In multivariate analyses, age >60 years represented an independent risk factor for thrombosis at MPN diagnosis or during the follow-up (adjusted HR [95% CI]: 1.56 [1.03–2.36], p=0.037). JAK2^{V617F} mutation retained its significance as an independent risk factor for thrombosis at MPN diagnosis or during the follow-up (adjusted HR [95% CI]: 3.01 [1.47–6.13], p=0.002). According to thrombosis type, the risk of ATE during the follow-up was increased in JAK2^{V617F}-mutated patients (adjusted HR [95% CI]: 4.12 [1.07–15.91], p=0.04), whereas for VTE this mutation lost statistical significance (adjusted HR [95% CI]: 1.89 [0.75–4.74], p=0.175). Tobacco smoking was the cardiovascular risk factor that retained significance as an independent risk factor for thrombotic complications at MPN diagnosis or during the follow-up (adjusted HR [95% CI]: 1.75 [1.14–2.68], p=0.01).

Discussion

In literature, thrombosis prevalence in MPN ranges from 7 to 33% during the follow-up [17–23], with available data showing thromboembolism being more frequent in PV than ET and the least in PMF patients [18, 22, 23]. In agreement with the literature, 14.1% of our patients had thrombotic complications during the follow-up. Also, the proportion of patients who had thrombosis was 41.1% in PV, 36.1% in ET, and 22.2% in PMF (Table 1). The incidence rate of fatal and nonfatal thrombosis per patient-year for our MPN patients was 1.26%, which is compatible with the incidence rates reported in the literature ranging between 0.74 and 10.9% [24]. Our results show that the annual incidence rate of thrombosis in our MPN patients is more than 10-times higher than the incidence rate of venous and arterial thrombosis reported in the European population ranging between 0.1% and 0.18% per 100,000 person-year [25].

Well-defined risk factors for thrombosis in MPN are age over 60 years and a history of thrombosis [9]. In the present study, both hazards have significantly increased the risk of overall thrombosis, especially during the follow-up, where the risk was more than 2-times higher than younger patients or those without a history of thrombosis. In multivariate analyses, age >60 years and the previous thrombotic events represented independent risk factors for thrombosis in MPN patients.

Cardiovascular risk factors, such as arterial hypertension, diabetes mellitus, and tobacco use, were reported to significantly increase the risk of thrombosis in MPN [14]. Some recent studies are devoted to cardiovascular risk factors [18, 20, 26]. Some studies have shown the value of individual risk factors such as diabetes mellitus [14], tobacco smoking [27], hyperlipidemia [28], and arterial hypertension [29]. Tobacco use was the only cardiovascular risk factor representing an independent risk factor for thrombosis in MPN patients at/after diagnosis. However, cardiovascular risk factors are generally accepted predisposing factors of thrombosis and should be considered in the prognostic models in MPN patients. Also, the treatment of patients should be individualized based on the presence of risk factors, including cardiovascular ones [30–32].

Since its discovery, JAK2^{V617F} mutation has been intensely studied [33]. The prognostic value of this mutation regarding the risk of thrombosis was demonstrated in several studies [11, 14, 26, 34, 35]. Although the mechanism of thrombotic complication in MPN is not entirely understood, the role of several contributors has been partially revealed. For instance, JAK2^{V617F} mutation may specifically affect the hemostatic system leading mutated patients to be more prone to thromboembolism through leukocytes and platelets activation, endothelial dysfunction, an increase of inflammatory cytokines, and alterations in the coagulation system [36–38]. Even without a proven diagnosis of MPN, JAK2^{V617F} mutation may present with arterial and venous thrombotic events. Thus, it was recommended to search for the presence of JAK2^{V617F} mutation in patients with thrombotic events, especially in splanchnic or cerebral vein thrombosis [8, 39]. JAK2^{V617F} mutation was the dominant driver mutation in our cohort, where its prevalence in each of the MPN subtypes was

in accordance with a recent meta-analysis [10]. Our study confirms the prognostic value of JAK2^{V617F} mutation as a risk factor of thrombotic complications in MPN. During the 5 years preceding the MPN diagnosis, JAK2^{V617F} mutation was the only disease-related variable that excessively increased the risk of thrombosis by 15 times more than JAK2^{V617F} negative patients (Table 5). Published studies reported that the highest incidence of thrombosis before MPN diagnosis was within two years before diagnosis [17, 21, 23]. However, we found that MPN patients developed the majority (56/77, i.e., 72.7%) of prior-to-diagnosis thrombotic events within the five years preceding MPN diagnosis in the presence of JAK2^{V617F} mutation. Moreover, studies had reported that JAK2^{V617F} mutation presents in healthy individuals [40, 41] and may present several years before the onset of MPN [42]. These data support the clinical implication of our study that the presence of JAK2^{V617F} mutation, even before the diagnosis of MPN, may carry a high risk of thrombotic complications in individuals, yet unconsidered to be MPN patients.

Several studies showed that JAK2^{V617F} mutation is associated with a higher risk of overall thrombosis [11, 34, 43]. According to the type of thrombosis, data exist linking this mutation to a significant risk of arterial but not venous thrombosis [44], and even proving it as an independent risk factor for ATE [14]. Consistently, our multivariate analyses showed that JAK2^{V617F} positive patients are at three times significantly higher risk of developing any thrombotic complications and more than 4 times higher risk of arterial but not venous thrombosis.

It was reported that CALR-mutated patients are at lower risk of thrombotic complications. CALR mutation has been described as a protective mutation against thrombosis [45, 46]. Consistent with these results, our univariate analyses showed a lower risk of developing thrombosis among CALR-mutated compared to JAK2^{V617F}-mutated patients (Tables 4 and 5). Our results would seem to support that the type of mutation affects the complications pattern, in which JAK2^{V617F} mutation plays a prothrombotic role [47].

Regarding the sex variable, diversity exists in literature, with some studies reported male patients at higher risk of thrombosis in MPN [14, 48], while other studies reported female patients at higher risk [28, 49]. We observed that female patients were at two times higher risk of thrombotic complications during the follow-up (Table 5). However, in multivariate analyses and after adjustment for age and the presence of JAK2^{V617F} mutation, cardiovascular risk factor, and thrombophilia conditions, the sex variable lost its significance. Moreover, female patients were at a slightly increased risk of event recurrence even though this was not significant from a statistical point of view. The reason why female patients were more likely to develop thrombotic complications in literature is unknown. However, in one study, authors pointed at the 46/1 haplotype as a sex-related factor leading to more prevalent JAK2^{V617F} mutation among women with splanchnic venous thrombosis [50]. Albeit a possible cause, in our study, the prevalence of JAK2^{V617F} mutation was similar between females and males in our cohort (78% vs. 83%). Another possible explanation is the effect of circulating sex hormones or the use of hormonal therapies in the form of oral contraceptives, which showed an association with an increased risk of venous thrombosis [51]. A limitation here is the lack of data on the use of hormonal therapy by our female patients.Elevated blood cell counts, especially leukocytosis and erythrocytosis, have an important impact on the prothrombotic state of MPN patients [14, 52]. Controversy exists in the literature regarding elevated platelet count with evidence for [53] and against [14] its importance as a risk factor for thrombosis. In the present study, a higher platelet count at diagnosis was responsible for a significant increase of thrombosis probability during the follow-up. None of the blood count parameters are included in thrombosis risk stratification models for MPN patients [9, 54, 55]. Our results indicate a prognostic value of platelet count at diagnosis.

Although thrombophilia states are associated with increased risk of thrombosis, they have not been used in IPSET-thrombosis score [54]. In our study, we found that the presence of any thrombophilia condition was associated with significantly increased odds of thrombosis, especially ATE. Although in multivariate analyses, thrombophilia states lost their significance, they should be taken into account when assessing the risk of thrombosis in MPN patients.

In conclusion, the mechanisms leading to thrombosis in MPN are complex. Several factors have been found to increase the risk of thrombosis, including MPN-related and patient-related risk factors. The present study highlights the importance of JAK2^{V617F} mutation as an MPN-related independent risk factor of thrombosis, especially arterial events, in BCR-ABL1 negative MPN patients. The risk of thrombosis that this mutation carries is high during the disease course and can be significantly increased within the five years preceding the diagnosis of MPN. Although routine testing for JAK2^{V617F} mutation is recommended in patients with unexplained splanchnic or cerebral vein thrombosis, our results indicate that testing for this mutation can also be useful in some patients with unexplained arterial thrombosis. Elevated platelet count at diagnosis may predict a higher risk of thrombotic complications during the follow-up. Based on evaluation of patient-related risk factors, the presence of previous thrombotic events and the older age at diagnosis, especially age >60 years, carry a significantly increased risk of thrombosis in MPN patients. Evaluating cardiovascular risk factors is important in the prognostic stratification of patients as tobacco smoking was herein proved to be an independent risk factor for thrombosis in MPN. Female MPN patients seem to have a higher risk of thrombosis during the disease course.

Our findings provide further evidence that thrombosis in BCR-ABL1 negative MPN is a multifactorial matter with a contribution of a wide range of risk factors. This complexity demands an individual approach based on the patient's risk

stratification, and the effort to eliminate conventional cardiovascular risk factors is one of the crucial points of therapy for these patients.

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