Influence of blood count, cardiovascular risks, inherited thrombophilia, and JAK2 V617F burden allele on type of thrombosis in patients with Philadelphia chromosome negative myeloproliferative neoplasms

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Source / Izvornik: **Clinical Lymphoma Myeloma and Leukemia, 2019, 19, 53 - 63**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1016/j.clml.2018.08.020

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:559063

Title Page

The influence of blood count, cardiovascular risks, inherited thrombophilia and JAK2 V617F burden allele on the type of thrombosis in patients with Ph(-) myeloproliferative neoplasms

Conflict of Interest Page

Conflicts of interest: none

Microabstract

In this study we have demonstrated a significant difference in the risk factors for arterial and venous thrombosis in MPN patients, as well as between different subtypes of MPN, according to leukocyte and platelet count, V617F burden allele and cardiovascular risk factors.

Abstract

INTRODUCTION: Thrombosis is the most common complication in Ph(-) MPN patients.

PATIENTS AND METHODS: In a cohort of 258 Ph(-) MPN patients, the difference between patients with and without thrombosis was analysed according to genetic thrombophilia factors, JAK2 V617F status and burden allele, blood count, cardiovascular risk factors and age. Patients were also divided in PV, ET and PMF subgroups and by the type of thrombosis.

RESULTS: Analysis of cardiovascular risk factors regarding arterial thrombosis showed that PV patients with thrombosis had higher incidence of diabetes (p=0.030), ET patients had more often hypertension (p=0.003) and hyperlipidemia (p=0.005) while PMF patients had hyperlipidemia (p=0.046) and at least one cardiovascular risk factor (p=0.044). Moreover, leukocytes >18x10e9/L and V617F burden allele >25.7% were statistically significantly different in PV patients (p=0.019 and borderline significance p=0.055, respectively), while in ET patients leukocytes >9.2x10e9/L (p<0.001) and age at diagnosis >55 years were statistically significantly different (p=0.002). PMF patients with V617F burden allele ≤34.8% were more prone to thrombosis (p= 0.032). When comparing patients with and without venous thrombosis, cut-off value of V617F burden allele >90.4% was significant for PV patients with thrombosis (p=0.036), as was >56.7% for PMF patients with thrombosis (p=0.046). Platelets ≤536x10e9/L and age at diagnosis >54 years showed statistically significant difference for ET patients with thrombosis (p= 0.015 and p=0.041, respectively).

CONCLUSION: Based on our results, a new scoring system for thrombosis risk in PV could be made while PMF prognostic model may be expanded for better recognition of potential thrombotic risk factors.

Keywords

thrombotic risk factors; MPN patients; V617F mutation; cardiovascular risk factors; inherited thrombophilia

Introduction

Philadelphia chromosome negative myeloproliferative neoplasms (Ph(–)MPN) are a group of clonal hematopoietic diseases including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Besides Calreticulin and MPL mutations, the hallmark of MPN is the V617F JAK2 mutation that is positive in 95% of PV patients, 50% of ET patients and 50% of PMF patients [1]. MPNs are thought to be very rare diseases with an incidence of 0.5-2.2 for ET, 0.6-2.8 for PV and 0.3-1.9 for PMF per 100 000 [2].

Thrombosis is one of the most common complications of MPN which adds significantly to the increased morbidity and mortality rate of patients in all three subgroups of the disease [3]. Whether the patient with MPN is more prone to thrombosis or bleeding depends on the type of disease, mutation status, number and function of leukocytes, erythrocytes and platelets, and finally other cofactors which are partly still unknown. Incidence of thrombosis in MPN patients varies from 18.3 to 33.6 %, as was observed in different studies.[4,5]. Although some risk factors are very well known and are included in clinical evaluation, such as age >65 years and prior history of thrombosis [6,7], additional risk factors are still under investigation. Several studies looked into risk factors for thrombosis in MPN like leukocytosis [8,9,16], presence of cardiovascular risk factors [10], V617F burden allele [11], platelet count [12,13,16], hematocrit or hemoglobin level [12,13,16], or thrombophilia genetic factors [14,15] . However, no study analysed all of these factors on the same cohort of patients. Moreover, the question whether some of those risk factors contribute only to arterial or also venous thrombosis is still open.

The aim of this study was to describe risk factors for thrombosis in Ph(-) MPN patients, and furthermore analyse risk factors typical for each disease subgroup, and also for the type of thrombosis.

Patients and Methods

We conducted a prospective study evaluating 258 MPN patients diagnosed at the University Hospital Center Zagreb in the period of six years, between June 2011 and August 2017. All patients were diagnosed according to the 2008 World Health Organization (WHO) Classification. The patients that were diagnosed as having MPN in another hospital were re-evaluated at our Center, including testing for investigated mutations.

The following parameters were analysed: V617F JAK2 mutation, V617F burden allele, genetic thrombophilia factors FV Leiden and PT G20210A mutation, blood count, age at diagnosis, sex, cardiovascular risk factors, thrombotic events, timeframe of the first thrombotic event, and current therapy of MPN. All laboratory and genetic testings were done at the Department of Laboratory Diagnostics, University Hospital Center Zagreb. Clinical data were taken from medical documentation. The study was approved by Ethics Committee of University Hospital Center Zagreb. After patients gave their consent, 10 ml of EDTA blood was withdrawn and used for separation of granulocytes from whole blood by centrifugation in density gradient with Polymorphprep. Genomic DNA extraction was performed by using the standard salting out method [17].

Analysis of complete blood count, including leukocytes (WBC), erithrocytes (RBC), platelets (PLT) and hemoglobin (HGB) concentration was performed for all patients on routine Coulter DxH 800 instrument at the moment of inclusion of patients in the study. Testing for V617F JAK2 mutation was done by allele-specific PCR according to Baxter et al [1]. If positive result was obtained , V617F burden allele was quantified by real time PCR method established by Larsen et al [18]. Screening for FV Leiden and PT G20210A mutation was done by using RFLP-PCR method [19,20]. Information about thrombotic events in the past, previous and current hematologic therapy, and cardiovascular risk factors (CV) which included hypertension, diabetes mellitus, hyperlipidemia, smoking and alcohol usage was obtained from medical documentation. During follow up, all thrombotic events were recorded.

Thrombotic events were diagnosed by standard diagnostic procedures and they were divided into arterial and venous thrombosis. Arterial events included ischemic cerebrovascular insult (ICV), transient ischemic attack (TIA), acute myocardial infarction (AIM), major peripheral arterial events, splenic infarction and fetal growth restriction. Venous thrombotic events were deep venous thrombosis (DVT), pulmonary embolism (PE), splanchnic thrombosis, cerebral vein thrombosis, thrombosis of retina vein and superficial thrombophlebitis.

Statistical analysis was performed by Medcalc v.17.2-64 bit. Normality was checked by Kolmogorov-Smirnov test. If data were normally distributed they are presented as mean with standard deviation and compared by t-test for unpaired samples. If data were not normal, they are presented as median and interquartile range and Mann Whitney test was used for comparison between groups. For categorical variables, Fisher or Chi-square test was used to calculate the p value. Odds ratio was calculated for comparison of categorical data. In univariate analysis, value $p \leq 0.05$ is considered statistically significant. Multivariate logistic regression model was used for variables that were statistically significant in univariate analysis and results with $p \leq 0.1$ were considered statistically significant. For distinguishing cut-off values of quantitative risk factors between groups of patients, receiver-operating characteristic (ROC curve) was used followed by odds ratio calculation.

Results

We identified 258 patients with MPN diagnosis during the study period, including 134 ET (94 females and 40 males, median age 55, range 18-92), 70 PV (36 females, 34 males, median age 64, range 29-84 years) and 54 PMF patients (24 females, 30 males, median age 62, range 24-85 years).

Median time from diagnosis to end of this study was 67 months (range 15-463). The median follow-up of patients since the study started was 58 months (range 10-74).

Management of MPN patients included follow-up with no treatment, antiplatelet drugs and/or cytoreductive therapy, JAK-2 inhibitor, immunomodulatory therapy, venepuncture, or supportive RBC transfusions. Thirty-three MPN patients were therapy-free.

Out of 258 evaluated MPN patients, 79 (30.6%) patients developed thrombosis with a total of 109 thrombotic events; 72 (66.1%) arterial and 37 (33.9%) venous. Twenty (25.3%) patients had two or more thrombotic events and six (7.6%) developed both arterial and venous thrombosis. Thrombotic events were more common in PMF (35.2%) and PV (34.3%), than in ET patients (26.9%) (Table 1).

Regarding the analysed risk factors, MPN patients with thrombosis showed higher frequency of V617F JAK2 positivity (81% vs 68.2%, p=0.049), higher V617F JAK2 burden allele (30.4% vs 17.0%, p= <0.001), higher WBC count (10.5 vs 9.5 $x10^9$/L, p= 0.009), older age at the time of diagnosis (63 vs 57 years p=0.022), higher incidence of hypertension (73.4% vs 49.7%, p= < 0.001), hyperlipidemia (30.4% vs 11.2%, p= <0.001) and presence of least one CV risk factor (83.5% vs 60.9%, p= 0.001). (Table 1). The type of treatment did not have influence on the occurrence of thrombosis. In multivariate analysis, statistically significant difference remained only for hyperlipidemia (OR= 3.2, 95%CI= 1.3-7.6, p=0.011).

The same risk factors were found to be different with statistical significance when arterial thrombosis in MPN patients was analysed separately; V617F JAK2 positivity (84.7% vs 68.2%, p=0.021), higher V617F burden allele (29.1% vs 17.0%, p= <0.001), higher WBC count (10.6 vs 9.5 $x10^9$/L, p=0.001), older age at diagnosis (65 vs 57 years, p=0.008), hypertension and hyperlipidemia (76.3% vs 49.7%, p= <0.001 and 33.9% vs 11.2%, p= <0.001) and presence of at least one CV risk factor (89.8% vs 60.9%, p= <0.001) (Table 1). Multivariate analysis revealed statistical significance of hyperlipidemia and CV risk factors (OR=3.3, 95%CI =1.3-8.3, p=0.010 and OR=0.3, 95CI% =0.1-1.3, p=0.097).

On the other hand, analysis of risk factors for venous thrombosis in MPN patients showed statistically significant difference for higher V617F burden allele (38.1% vs 17.0%, p=0.003), but lower HGB level (133 vs 144 g/L, p= 0.049) and lower PLT count (447 vs 571 $x10^9$/L, p=0.011) (Table 1). In multivariate analysis, V617F burden allele and HGB level remained statistically significantly different (OR=1.0, 95CI%=1.0-1.1, p=0.002 and OR=1.0,95 CI%=0.9-1.0, p=0.064).

Polycythemia vera patients

The most frequent type of thrombosis in PV patients was arterial thrombosis (AMI, ICV, TIA, splenic infarction, occlusion of femoral arteries) that occurred in 19 (27.1%) patients, compared to venous events (DVT, PE, splanchnic thrombosis, cerebral venous thrombosis, peripheral thrombophlebitis) present in eight (11.4%) patients. Three patients (4.3%) had both types of thrombosis (combinations of DVT and ICV, cerebral venous thrombosis and fetal growth restriction, AMI and DVT). Seven (10.0%) patients had recurrent thrombosis with two or more thrombotic events. Eleven out of twenty-four patients (45.8%) developed thrombosis at the time of diagnosis, five patients (20.8%) had thrombosis before, and eight (33.3%) after diagnosis. Twelve patients (50.0%) developed thrombosis within one year of diagnosis.

In the group of PV patients, statistically significant risk parameter for thrombosis was diabetes (33.3% vs 10.9%, p=0.048). When analysed separately, diabetes (36.8% vs 10.9%, p=0.030) and presence of at least one CV risk factor (94.7% vs 69.6%, p=0.049) showed statistical significance for arterial thrombosis, while there was no statistically significant difference between patients with and without venous thrombosis (Table 2).

In multivariate analysis in PV patients with arterial thrombosis, diabetes remained statistically significant (OR=3.3, 95%CI= 0.9-12.7, p=0.072).

Essential thrombocythemia patients

In ET group, 29 (21.6%) patients had 32 arterial thrombotic events (AMI, ICV, TIA, embolization of digital arteries , occlusion of femoral arteries, thrombosis of the carotid artery, splenic infarction, and fetal growth restriction), 10 (7.5%) patients had 14 venous thrombotic events (DVT with or without PE, splanchnic thrombosis, superficial thrombophlebitis), while three patients (2.2%) had both arterial and venous thrombosis (thrombosis of an upper limb and retinal thrombosis, combination of splenic infarction and deep vein thrombosis or peripheral thrombophlebitis). Seven (19.4%) patients had two or more thrombotic events.

Sixteen out of 36 patients (44.4%) developed thrombosis at the time of diagnosis, 14 (38.9%) patients had thrombosis before diagnosis and four patients (16.7%) after. Eighteen patients (50.0%) developed thrombosis within one year of diagnosis.

Statistically significant difference between ET patients with and without thrombosis was found for the frequency of V617F JAK2 positivity (77.8% vs 57.1%, p=0.047), higher WBC count (10.2 vs 8.5 $x10^9$/L, p=0.010), older age at diagnosis (65 vs 52 years, p=0.008), hypertension (72.2% vs 40.8%, p= 0.003), hyperlipidemia (30.6% vs 9.2%, p = 0.005) and incidence of at least one CV risk factor (86.1% vs 55.1%, p=0.001) (Table 3). After multivariate analysis was performed, WBC count, hyperlipidemia and hypertension remained statistically significantly different (OR= 1.2, 95CI%= 1.0-1.5, p= 0.022, OR=4.0, 95CI%=0.9-18.7, p=0.076 and OR= 4.0, 95CI%= 1.0-15.8, p=0.051, respectively). Moreover, all patients with two or more thrombotic events were V617F JAK2 positive.

Regarding the presence of arterial thrombosis in patients with ET, patients with thrombosis had higher WBC count (10.6 vs 8.5 $x10^9$/L, p= <0.001), higher PLT count (700 vs 617 $x10^9$/L, p= 0.050), were of older age at diagnosis (65 vs 52 years, p=0.009), had hypertension, hyperlipidemia or at least one CV risk factor (65.5% vs 40.8%, p= 0.021, 31.0% vs 9.2%, p=0.006 and 82.8% vs 55.1%, p= 0.009, respectively) (Table 3). Multivariate analysis showed that statistically significant difference was obtained for WBC count and hyperlipidemia (OR = 1.2., 95% CI =1.1-1.4, p= 0.009 and OR= 3.0, 95CI%=0.9-9.7, p=0.072 respectively).

All ET patients with venous thrombosis had hypertension, which was also statistically significantly different from patients without thrombosis (100.0% vs 40.8%, p= <0.001), also that generated the presence of at least one CV risk factor (100.0 vs 55.1%, p=0.005), (Table 3). Multivariate analysis showed no statistically significant difference in the results.

Primary myelofibrosis patients

In the group of PMF patients, 19 patients had thrombosis (35.2%), 11 (20.4%) arterial (AIM, ICV, abdominal thrombosis, thrombosis of subclavian artery, TIA and splenic infarction) and eight (14.8%) venous (DVT, PE, abdominal thrombosis, thrombophlebitis). Six (11.1%) patients had two or more thrombotic events.

Three out of 19 patients (15.8%) developed thrombosis at the time of diagnosis, seven (36.8%) had thrombosis before diagnosis and nine (47.4%) after diagnosis. Altogether, nine (47.4%) patients developed thrombosis within one year of diagnosis.

Comparing PMF patients with and without thrombosis, we found no statistically significantly different results in any of the investigated parameters (Table 4).

Evaluation of PMF patients with and without arterial thrombosis demonstrated higher incidence of hyperlipidemia (36.4% vs 8.5%, p=0.046) and at least one CV risk factor (100.0% vs 65.7%, p=0.044) in patients with thrombosis (Table 4). After multivariate analysis none of the parameters remained statistically significant.

There was no difference observed in any of the investigated parameters for PMF patients with venous thrombosis (Table 4).

Cut-off values for quantitative parameters in patients with arterial thrombosis compared to patients without thrombosis

Comparison of MPN patients with and without arterial thrombosis revealed statistically significant difference in V617F burden allele >14.6 %, WBC count >9.25x 10^9/L and age at diagnosis > 55 years (p= 0.011, p=0.003 and p=0.006, respectively) (Table 5). In multivariate analysis, results for V617F burden allele, WBC count and age at diagnosis remained statistically significantly different (OR= 0.4, 95%CI = 0.2-0.9, p=0.016; OR= 0.5, 95%CI= 0.2-1.1, p=0.079; OR=0.4, 95%CI=0.2-0.9, p= 0.032, respectively).

In PV patients with arterial thrombosis, WBC count >18 x 10^9/L was statistically significantly different (p=0.019) while V617F burden allele >25.7% showed borderline significance (p=0.055), (Table 5). Multivariate analysis for PV patients showed that cut-off values for WBC count and V617F burden allele remained statistically significant (OR=5.5, 95%CI=0.9-33.3, p=0.063 and OR= 0.2, 95%CI= 0.0-1.1, p=0.069).

Compared to patients without thrombosis, ET patients with arterial thrombosis had statistically significantly different results for V617F burden allele >14.6%, WBC count >9.2 x 10^9/L, RBC count >5.48 x 10^{12}/L, HGB level >106 g/L, PLT count >632 x 10^9/L and age at diagnosis >55 years (p= 0.021, p<0.001, p=0.046, p=0.028, p=0.049 and p= 0.002, respectively), (Table 6). After multivariate analysis, WBC count and age at diagnosis remained statistically significantly different (OR=0.2, 95%CI= 0.0-0.6, p=0.012 and OR=0.06, 95%CI=0.1-0.7, p=0.010).

In the group of PMF patients with arterial thrombosis, only V617F burden allele ≤ 34.8% showed statistically significant difference with p= 0.032, (Table 5).

Cut-off values for quantitative parameters in patients with venous thrombosis compared to patients without thrombosis

In all MPN patients, only WBC count >21x 10^9/L showed statistically significant difference (p=0.036), (Table 6).

PV patients with V617F burden allele >90.4% and HGB level ≤159 g/L were more prone to venous thrombosis (p=0.036 and p= 0.041), (Table 6). In multivariate analysis, HGB level ≤159 g/L remained statistically significantly different (OR= 6.4, 95%CI=1.0-39.5, p=0.044).

For ET patients, PLT count ≤536 x 10^9/L and age at diagnosis >54 years showed statistically significant difference (p= 0.015 and p=0.041), (Table 6). After multivariate analysis, both cut-off values for PLT count and age at diagnosis, remained statistically significant (OR=10.1, 95%CI=2.1-47.6, p=0.004 and OR=10.0, 95%CI= 1.8-56.6, p=0.009).

In the group of PMF patients, only V617F burden allele >56.7% was more often observed in patients with venous thrombosis (p=0.046), Table 6.

Discussion

Ph(-) MPN patients have higher incidence of thrombosis and bleeding and shorter life expectancy than healthy people of the same age [21]. Risk factors that contribute to thrombosis in MPN patients are partly known, and were mostly investigated in retrospective studies, although mostly for single MPN subgroup, or regarding just arterial or venous thrombosis. Here we present a prospective study on the risk factors for thrombosis in MPN patients with the median follow-up of 57.5 months. We investigated influence of several risk factors on thrombosis in MPN patients, including V617F driver status mutation and burden allele, inherited thrombophilia, blood count parameters and cardiovascular risk factors. Furthermore, we evaluated the difference in risk factors depending on the subtype of MPN and type of thrombosis due to the proven difference in their pathogenesis and the risk factors present in general population.

In our cohort of 258 patients, 79 (30.6%) patients had a total of 109 thrombotic events, 66.1% arterial and 33.9% venous, which is consistent with the studies published earlier [8,9,22]. Moreover, our results demonstrated that the incidence of thrombosis in ET and PV patients was similar to those previously reported [23,24]. Actually, 21.6% of our ET patients had arterial and 7.5% venous thrombosis, while 27.1% of PV patients had arterial and 11.4% venous thrombosis. On the other hand, our results for the PMF patients showed much higher incidence of thrombosis (35.2%) than those previously reported by Barbui et al [25] (7.2%). However, the cited study was performed on a larger number of patients but with shorter median of follow-up of 35.0 months, with only cardiovascular events during follow up included. Our results are also comparable to findings from a study by Buxhofer-Ausch et al [35] where thrombotic events were divided to those before diagnosis with the incidence of 15.5% for arterial and 6.4% with venous events and those after diagnosis with the incidence of 15.9% for non-fatal and 4.9% fatal thrombotic events. Higher incidence of thrombosis in our PMF patients could be result of the small number of patients, longer follow-up, or the fact that some of our patients were in prefibrotic phase of PMF when included in the study.

As reported before, the majority of thrombotic events in MPN patients occur around the time of diagnosis, and are often the first symptom related to the diagnosis [26]. Our results confirmed this thesis as we found the peak incidence of thrombosis to occur one year around the time of diagnosis and with almost Gaussian distribution of thrombotic events before and after diagnosis, even after a long follow-up.

Cardiovascular risk factors such are hypertension, diabetes mellitus, hyperlipidemia, smoking and alcohol usage were investigated in different studies with the conclusion that they mainly increase the risk of arterial thrombosis [27,28]. However, we found only diabetes to be a risk factor for arterial thrombosis in PV and hypertension and hyperlipidemia in ET. Interestingly, we noted that all ET patients with venous thrombosis had hypertension; however, this influence was lost in multivariate analysis. As for the PMF, available prognostic models do not include cardiovascular risk factors [29,30]. Correspondingly, we found only hyperlipidemia as a risk factor for arterial thrombosis in PMF patients, but only in univariate analysis. The key finding of this observation is that additional CV risk factors such are hyperlipidemia, hypertension and diabetes can enhance odds for thrombotic events in MPN patients. This could be of crucial clinical interest since majority of additional risk factors could be managed by the change of the patient's lifestyle who can thus potentially avoid thrombotic events.

Several groups of investigators were interested in incidence of inherited thrombophilia factors in MPN patients, but the results are inconclusive. Gisslinger et al [31] observed connection between PT G20210A mutation and venous thrombosis, while Trifa et al [15] obtained increased incidence of FV Leiden in patients with PV in arterial and venous thrombosis, although only in univariate analysis. Similarly, Schwarz et al [13] described FV Leiden as a risk factor for both venous and arterial thrombosis in all types of MPNs. In our study, incidence of heterozygosity for FV Leiden and PT G20210A mutation is similar as in healthy population [32] as well as in MPN patients with and without thrombosis. The role of molecular thrombophilia screening for FV Leiden and PT G20210A mutation in estimating the risk of thrombosis in MPN patients is still unclear and should be further investigated on a larger cohort of patients.

Regarding leukocytosis, the majority of investigators found connection between the number of leukocytes and incidence of thrombosis, but with different cut-off values for each subtype of MPN disease. For PV patients, Landolfi et al [28] found leukocyte count of $15x10^9$/L to be predictive for arterial thrombosis, while Carrobio et al [8] obtained the cut-off value of $8.7x10^9$/L as a predictive factor for both arterial and venous thrombosis in ET patients. For PMF patients, Barbui et al [25] reported borderline significance for WBC higher than $15x10^9$/L for all cardiovascular events. In our study, PV patients with arterial thrombosis had cut-off value for WBC >$18x10^9$/L, and ET patients >$9.2x10^9$/L both statistically significant (p=0.019 and <0.001, respectively). For PMF patients, no statistically significant difference for leukocyte number was observed. It seems that thrombogenic effect of leukocytes is more obvious in arterial thrombosis than venous, due to the activation of leukocytes, release of proteolytic enzymes and reactive oxygen species, that has effect on endothelial cells as well as formation of platelet-leukocyte aggregates which all contribute to the occurrence of arterial thrombi [33].

The influence of platelets on occurrence of thrombosis in MPN patients is still under investigation. Most studies report that extreme thrombocytosis reduces the risk for thrombosis in ET [34] and PMF [35]. Schwarz et al [13] confirmed this in a large study on Ph(-)MPN patients. We observed higher platelet count in ET patients with arterial thrombosis compared to ET patients without thrombosis, but this finding lost its significance in multivariate analysis. Moreover, the analysis of cut-off values demonstrated that PLT count in ET patients >$632x10^9$/L was significant for arterial thrombosis, while patients with PLT count ≤536 x 10^9/L were more prone to venous thrombosis. This finding clearly shows that same cells can contribute differently to occurrence of arterial and venous thrombosis.

Older age is an established thrombotic risk factor in both general population [36] and MPN patients [6,34,25]. For ET patients, IPSET scoring system considers age over 60 years as one of thrombotic risk factors [37]. The same age was also observed as a thrombotic risk factor for PMF patients [25], while slightly higher age of >65 years was in PV patients related to higher mortality [6]. Our results suggest even lower cut-off value for the age of MPN patients with risk of thrombosis. We found age >55 years to be a significant risk factor for arterial thrombosis in all MPN patients, as well as for a subgroup of ET patients. As for the risk of venous thrombosis, we found significant influence of age only in ET patients, with the cut-off value of >54 years. Based on this finding, we should emphasize the importance of age as a predictive factor for thrombosis. Overall, age is one of the parameters that should be included into scoring system for all MPN entities.

Driver mutation V617F JAK2 and V617F burden allele are important risk factors investigated since this mutation was discovered in 2005 [1]. Authors of IPSET scoring system also recognized the presence of V617F mutation as one of thrombosis contributing factors [37]. Although the presence of this mutation is much higher in PV than in ET and PMF patients, some studies showed that additional information can be obtained from quantifying V617F burden allele in each of the MPN subgroups [38,39]. For PV patients, Vannuchi et al [38] reported the value of burden allele >75% to be predictive for thrombosis. Our findings suggest different cut-off values for arterial and venous thrombosis in PV. Burden allele >25.7% showed borderline significance for arterial thrombosis in PV patients, while patients with venous thrombosis had burden allele >90.4%. Regarding the ET, Antonioli et al [39] found that burden allele >25% is related to an increased incidence of arterial thrombosis, while we found this relation for even lower values, i.e. >14,6%. In PMF patients, quantification of burden allele is mostly used to predict poor survival [40]. However, we observed for the first time that PMF patients had more often arterial thrombosis if their burden allele was ≤34.8% and venous thrombosis with burden allele >56.7%. These findings suggest different influence of burden allele on pathophysiology of arterial and venous thrombosis in MPN. Patients with arterial thrombosis, unlike those with venous thrombosis, had mostly lower burden allele in all MPN subgroups.

Conclusion

This study investigated influence of thrombotic risk factors on incidence of arterial and venous thrombosis in MPN patients. We found significant difference between risk factors causing arterial and venous thrombosis, specifically in leukocyte and platelet count, V617F burden allele and additional cardiovascular risk factors. Importantly, risk factors for arterial thrombosis could be at least partly preventable through the change of lifestyle, while risk factors for venous thrombosis depend mostly on the features of myeloproliferative disease. Furthermore, it should be highlighted that each subtype of MPN has its specific risk factors, implying different pathogenesis.

In conclusion, based on our results, an introduction of a new scoring system for thrombosis risk in PV could be made, while current prognostic model for PMF may be expanded for better recognition of potential thrombotic risk factors. However, the influence of certain risk factors on occurrence of thrombosis in MPN is still unclear and should be addressed on larger cohorts of patients.

Clinical Practice Points

Ph(-) MPN patients have higher mortality than healthy people due to their predisposition to thrombosis, bleeding or transformation of disease. Here we introduce new findings about differences in patients with and without thrombosis as well as differences in risk factors for arterial versus venous thrombosis. This knowledge may help to better recognize important risk factors that can influence thrombosis occurrence and give directions for new risk stratification models in PV and PMF patients.

Acknowledgments

None

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Table 1. Comparison of MPN patients with or without thrombosis regarding risk parameters.

All MPN patients, N=258										
Risk factor	**Patients with thrombosis N=79 (30.6%)**	**No thrombosis patients N=179 (69.4%)**	**OR (CI)**	**p value**	**Arterial thrombosis patients, N=59 (22.9%)**	**OR (CI)**	***p value**	**Venous thrombosis patients, N=26 (10.1%)**	**OR (CI)**	****p value**
FV Leiden mutation heterozygote, N (%)	4 (5.1)	7 (3.9)	1.3 (0.4-4.6)	0.741	2 (3.4)	0.9 (0.2-4.3)	1.0	2 (7.7)	2.0 (0.4-10.4)	0.319
FII G20210A heterozygote, N (%)	0 (0)	5 (2.8)	0.2 (0.0-3.7)	0.327	0 (0.0)	0.3 (0.0-4.9)	0.336	0 (0.)	0.6 (0.0-11.1)	1.0
V617F JAK2 mutation positive, N (%)	64 (81.0)	122 (68.2)	2.0 (1.0-3.8)	**0.049**	50 (84.7)	2.6 (1.2-5.6)	**0.021**	20 (76.9)	1.6 (0.6-4.1)	0.498
V617F JAK2 burden allele %, median (1.-3.quartile)	30.4 (15.9-59.7)	17.0 (8.4-32.3)		**<0.001**	29.1 (15.7-51.5)		**<0.001**	38.1 (19.2-84.8)		**0.003**
WBCx10^9/L, median (1.-3.quartile)	10.5 (8.3-14.7)	9.5 (7.2-12.1)		**0.009**	10.6 (9.0-14.7)		**0.001**	9.7 (6.4-14.9)		0.591
RBCx10^{12}/L, mean (± sd)	5.02 (±1.29)	5.09 (±1.12)		0.692	5.17 (± 1.28)		0.634	4.78 (±1.27)		0.204
HGB g/L, mean (± sd)	141 (±32)	144 (±26)		0.409	146 (±32)		0.666	133 (±30)		**0.049**
PLT x10^9/L, median (1.-3.quartile)	551 (376-685)	571 (437-720)		0.229	568 (404-734)		0.884	447 (349-586)		**0.011**
Age, years, median (range)	63 (23-92)	57 (18-90)		**0.022**	65 (55-74)		**0.008**	59 (51-65)		0.658
Male, N (%)	38 (48.1)	66 (36.9)	1.6 (0.9-2.7)	0.119	28 (47.5)	1.5 (0.9-2.8)	0.197	12 (46.2)	0.9 (0.4-1.8)	0.831
Smoking, N (%)	13 (16.5)	23 (12.8)	1.3 (0.6-2.8)	0.565	13 (22.0)	1.9 (0.9-4.1)	0.097	1 (3.8)	0.3 (0.0-2.1)	0.324
Alcoholism, N (%)	1 (1.3)	1 (0.6)	2.3 (0.1-37.0)	0.519	1 (1.7)	3.1 (0.2-49.8)	0.435	0 (0.0)	2.2 (0.1-56.6)	1.0
Hypertension, N (%)	58 (73.4)	89 (49.7)	2.8 (1.6-5.0)	**<0.001**	45 (76.3)	3.3 (1.7-6.3)	**<0.001**	18 (69.2)	2.3 (0.9-5.5)	0.099
Diabetes, N (%)	16 (20.3)	24 (13.4)	1.6 (0.8-3.3)	0.225	14 (23.7)	2.0 (1.0-4.2)	0.095	3 (11.5)	0.8 (0.2-3.0)	1.0
Hyperlipidemia, N (%)	24 (30.4)	20 (11.2)	3.5 (1.8-6.8)	**<0.001**	20 (33.9)	4.1 (2.0-8.3)	**<0.001**	5 (19.2)	1.5 (0.5-4.5)	0.330
At least one CV risk factor, N (%)	66 (83.5)	109 (60.9)	3.2 (1.7-6.3)	**0.001**	53 (89.8)	5.7 (2.3-13.9)	**<0.001**	18 (69.2)	1.4 (0.6-3.5)	0.547

***** for comparison of MPN patients with arterial thrombosis to MPN patients that had no thrombosis; ****** for comparison of MPN patients with venous thrombosis to MPN patients that had no thrombosis

Table 2. Comparison of PV patients with or without thrombosis regarding risk parameters.

PV patients, N=70										
Risk factor	**Patients with thrombosis,** N=24 (34.3%)	**No thrombosis patients,** N=46 (65.7%)	**OR (CI)**	**p value**	**Arterial thrombosis patients** N=19 (27.1%)	**OR (CI)**	***p value**	**Venous thrombosis patients,** N=8 (11.4%)	**OR (CI)**	****p value**
FV Leiden mutation heterozygote, N (%)	0 (0.0)	3 (6.5)	0.3 (0.0-5.1)	0.546	0 (0.0)	0.3 (0.0-6.5)	0.550	0 (0.0)	1.2 (0.3-5.3)	1.000
FII G20210A heterozygote, N (%)	0 (0.0)	1 (2.2)	0.6 (0.0-15.8)	1.000	0 (0.0)	0.8 (0.0-19.9)	1.000	0 (0.0)	1.8 (0.1-47.6)	1.000
V617F JAK2 mutation positive, N (%)	22 (91.7)	44 (95.7)	0.5 (0.1-3.8)	0.889	17 (89.5)	0.4 (0.0-3.0)	0.574	8 (100.0)	1.0 (0.0-21.7)	1.000
V617F JAK2 burden allele % median (1.-3.quartile)	51.2 (32.5-90.9)	37.6 (15.8-70.0)		0.089	55.1 (34.8-86.3)		0.082	41.5 (27.6-94.0)		0.348
WBCx10^9/L, median (1.-3.quartile)	11.4 (9.0-18.4)	10.9 (8.5-13.5)		0.276	12.2 (9.0-18.4)		0.223	11.4 (8.7-19.9)		0.636
RBCx10^{12}/L, mean (± sd)	6.28 (±1.05)	6.38 (±0.96)		0.692	6.34 (±1.14)		0.896	6.07 (±0.51)		0.412
HGB g/L, mean (± sd)	166 (±27)	172 (±18)		0.353	172 (±25)		0.115	156 (±27.7)		0.058
PLT x10^9/L, median (1.-3.quartile)	430 (375-594)	516 (367-674)		0.252	468 (377-594)		0.342	381 (373-576)		0.259
Age, years, median (range)	64 (29-81)	64 (31-84)		0.785	64 (34-81)		0.423	58 (29-69)		0.128
Male, N (%)	13 (54.2)	21 (45.7)	1.4 (0.5-3.8)	0.671	10 (52.6)	1.3 (0.5-3.9)	0.785	4 (50.0)	1.2 (0.3-5.3)	1.000
Smoking, N (%)	3 (12.5)	5 (11.1)	1.2 (0.3-5.4)	1.000	3 (15.8)	1.5 (0.3-7.2)	0.683	0 (0.0)	0.4 (0.0-8.8)	1.000
Alcoholism, N (%)	1 (4.2)	1 (2.2)	1.9 (0.1-31.3)	1.000	1 (5.3)	2.5 (0.1-42.2)	0.502	0 (0.0)	1.8 (0.1-47.6)	1.000
Hypertension, N (%)	19 (79.2)	30 (65.2)	2.0 (0.6-6.4)	0.280	17 (89.5)	4.5 (0.9-22.1)	0.067	4 (50.0)	0.5 (0.1-2.4)	0.450
Diabetes, N (%)	8 (33.3)	5 (10.9)	4.1 (1.2-14.4)	**0.048**	7 (36.8)	4.8 (1.3-17.8)	**0.030**	1 (12.5)	1.2 (0.1-11.6)	1.000
Hyperlipidemia, N (%)	8 (33.3)	8 (17.4)	2.4 (0.8-7.4)	0.227	7 (36.8)	2.8 (0.8-9.2)	0.112	1 (12.5)	0.7 (0.1-6.3)	1.000
At least one CV risk factor, N (%)	20 (83.3)	32 (69.6)	2.2 (0.6-7.6)	0.259	18 (94.7)	7.9 (1.0-64.9)	**0.049**	4 (50.0)	0.4 (0.1-2.0)	0.418

***** for comparison of PV patients with arterial thrombosis to PV patients that had no thrombosis; ****** for comparison of PV patients with venous thrombosis to PV patients that had no thrombosis

Table 3. Comparison of ET patients with or without thrombosis regarding risk parameters.

ET patients, N= 134										
Risk factor	**Patients with thrombosis,** N=36 (26.9%)	**No thrombosis patients,** N=98 (73.1%)	**OR (CI)**	**p value**	**Arterial thrombosis patients,** N=29 (21.6%)	**OR (CI)**	***p value**	**Venous thrombosis patients,** N=10 (7.5%)	**OR (CI)**	****p value**
FV Leiden mutation heterozygote, N (%)	2 (5.6%)	4 (4.1%)	1.4 (0.2-7.9)	0.659	1 (3.4)	0.8 (0.1-7.8)	1.000	1 (10.0)	2.6 (0.3-25.9)	0.391
FII G20210A heterozygote, N (%)	0 (0.0%)	3 (3.1%)	0.4 (0.0-7.4)	0.564	0 (0.0)	0.5 (0.0-9.2)	1.000	0 (0.0)	1.3 (0.1-26.9)	1.000
V617F JAK2 mutation positive, N (%)	28 (77.8)	56 (57.1%)	2.6 (1.1-6.3)	**0.047**	24 (82.8)	3.6 (1.3-10.2)	0.015	7 (70.0)	1.8 (0.4-7.1)	0.517
V617F JAK2 burden allele % median (1.-3.quartile)	19.3 (10.3-27.7)	16.7 (9.2-24.7)		0.253	19.8 (11.2-30.2)		0.140	18.7 (8.8-24.9)		0.948
WBCx10^9/L, median (1.-3.quartile)	10.2 (8.6-13.5)	8.5 (7.0-10.9)		**0.010**	10.6 (9.7-14.0)		**<0.001**	8.6 (5.9-10.4)		0.840
RBCx10^{12}/L, mean (± sd)	4.68 (±0.89)	4.68 (±0.66)		0.964	4.82 (±0.90)		0.500	4.54 (± 0.90)		0.517
HGB g/L, mean (± sd)	136 (±27)	136 (±19)		0.938	136 (±29)		0.949	135 (±20)		0.823
PLT x10^9/L, median (1.-3.quartile)	657 (543-850)	617 (526-745)		0.576	700 (571-910)		**0.050**	492 (444-707)		0.074
Age, years, median (range)	65 (23-92)	52 (18-90)		**0.008**	65 (23-92)		**0.009**	63 (38-82)		0.079
Male, N (%)	14 (38.9)	26 (26.5)	1.8 (0.8-3.9)	0.241	12 (41.4)	2.0 (0.8-4.6)	0.166	3 (30)	1.2 (0.3-4.9)	1.000
Smoking, N (%)	8 (22.2)	13 (13.3)	1.9 (0.7-5.0)	0.319	8 (27.6)	2.5 (0.9-6.8)	0.088	1 (10)	0.7 (0.1-6.2)	1.000
Alcoholism, N (%)	0 (0.0)	0 (0.0)	2.7 (0.1-138.5)	1.000	0 (0.0)	3.3 (0.1-171.9)	1.000	0 (0.0)	9.5 (0.2-502.7)	1.000
Hypertension, N (%)	26 (72.2)	40 (40.8)	3.8 (1.6-8.7)	**0.003**	19 (65.5)	2.8 (1.2-6.5)	**0.021**	10 (100.0)	30.3 (1.7-532.4)	**<0.001**
Diabetes, N (%)	6 (16.7)	10 (10.2)	1.8 (0.6-5.2)	0.470	6 (20.7)	2.3 (0.8-7.0)	0.198	1 (10.0)	1.0 (0.1-8.5)	1.000
Hyperlipidemia, N (%)	11 (30.6)	9 (9.2)	3.1 (1.2-8.1)	**0.005**	9 (31.0)	4.5 (1.6-12.6)	**0.006**	3 (30.0)	4.2 (0.9-19.3)	0.081
At least one CV risk factor, N (%)	31 (86.1)	54 (55.1)	5.1 (1.8-14.1)	**0.001**	24 (82.8)	3.9 (1.4-11.1)	**0.009**	10 (100.0)	17.1 (1.0-300.8)	**0.005**

***** for comparison of ET patients with arterial thrombosis to ET patients that had no thrombosis; ****** for comparison of ET patients with venous thrombosis to ET patients that had no thrombosis

Table 4. Comparison of PMF patients with or without thrombosis regarding risk parameters.

PMF patients, N= 54										
Risk factor	**Patients with thrombosis,** N=19 (35.2%)	**No thrombosis patients,** N=35 (64.8%)	**OR (CI)**	**p value**	**Arterial thrombosis patients,** N=11 (20.4%)	**OR (CI)**	***p value**	**Venous thrombosis patients,** N=8 (14.8%)	**OR (CI)**	***p value**
FV Leiden mutation heterozygote, N (%)	2 (10.5%)	0 (0.0)	10.1 (0.5-222.9)	0.119	1 (9.1)	10.1 (0.4-267.9)	0.239	1 (12.5)	14.2 (0.5-383.5)	0.186
FII G20210A heterozygote, N (%)	0 (0.0 %)	1 (2.9)	0.6 (0.0-15.2)	1.000	0 (0.0)	1.0 (0.0-26.3)	1.0	0 (0.0)	1.4 (0.1-36.2)	1.0
V617F JAK2 mutation positive, N (%)	14 (73.7%)	22 (62.9)	1.7 (0.5-5.7)	0.550	9 (81.8)	2.7 (0.5-14.2)	0.296	5 (62.5)	1.0 (0.2-4.8)	1.0
V617F JAK2 burden allele % median (1.-3.quartile)	35.1 (23.1-83.9)	36.5 (22.4-56.7)		0.795	29.3 (14.0-47.1)		0.361	80.5 (56.9-85.3)		0.070
WBCx10^9/L, median (1.-3.quartile)	9.5 (6.9-22.8)	9.0 (6.1-15.3)		0.556	9.4 (7.1-18.8)		0.690	10.3 (6.4-31.8)		0.596
RBCx10^{12}/L, mean (± sd)	4.16 (±1.11)	4.52 (±1.04)		0.246	4.32 (±0.97)		0.582	3.94 (±1.31)		0.186
HGB g/L, mean (± sd)	120 (±27)	129 (±27)		0.241	128 (±24)		0.871	109 (±29)		0.074
PLT x10^9/L, median (1.-3.quartile)	278 (198-562)	365 (209-597)		0.415	278 (204-547)		0.554	333 (137-556)		0.492
Age, years, median (range)	58 (30-81)	63 (24-85)		0.821	71 (30-81)		0.928	57 (44-79)		0.618
Male, N (%)	11 (57.9)	19 (54.3)	1.2 (0.4-3.6)	0.975	6 (54.5)	1.0 (0.3-3.9)	1.0	5 (62.5)	1.4 (0.3-6.8)	1.0
Smoking, N (%)	1 (5.3)	5 (14.3)	0.3 (0.0-3.1)	0.408	2 (18.2)	1.3 (0.2-8.1)	1.0	0 (0.0)	0.3 (0.0-6.5)	0.565
Alcoholism, N (%)	0 (0.0%)	0 (0.0)	1.8 (0.0-95.4)	1.000	0 (0.0)	3.1 (0.1-164.5)	1.0	0 (0.0)	4.2 (0.1-225.9)	1.0
Hypertension, N (%)	13 (68.4)	20 (57.1)	1.6 (0.5-5.3)	0.603	9 (81.8)	3.4 (0.6-18.0)	0.172	4 (50.0)	0.8 (0.2-3.5)	1.0
Diabetes, N (%)	2 (10.5)	9 (25.7)	0.3 (0.1-1.8)	0.292	1 (9.1)	0.3 (0.0-2.6)	0.410	1 (12.5)	0.4 (0.0-3.8)	0.656
Hyperlipidemia, N (%)	5 (26.3)	3 (8.5)	3.8 (0.8-18.2)	0.113	4 (36.4)	6.1 (1.1-33.6)	**0.046**	1 (12.5)	0.4 (0.0-3.8)	1.0
At least one CV risk factor, N (%)	15 (78.9)	23 (65.7)	2.0 (0.5-7.2)	0.365	11 (100.0)	12.2 (0.7-225.3)	**0.044**	4 (50.0)	0.5 (0.1-2.5)	0.443

***** in the comparison of PMF patients with arterial thrombosis to PMF patients that had no thrombosis; ****** in the comparison of PMF patients with venous thrombosis to PMF patients that had no thrombosis

Table 5. Cut-off values for quantitative parameters in patients with arterial thrombosis compared to patients without thrombosis

Risk factor	All MPN patients			PV patients			ET patients			PMF patients		
	Cut-off value for patients with arterial thrombosis, N=59	OR (CI)	p value	Cut-off value for patients with arterial thrombosis, N=19	OR (CI)	p value	Cut-off value for patients with arterial thrombosis, N=29	OR (CI)	p value	Cut-off value for patients with arterial thrombosis, N=11	OR (CI)	p value
V617F JAK2 burden allele %	> 14.6	2.3 (1.3-4.3)	**0.011**	> 25.7	3.4 (1.0-11.9)	0.055	> 14.6	2.9 (1.2-6.8)	**0.021**	≤ 34.8	5.1 (1.2-21.4)	**0.032**
WBCx10^9/L	> 9.25	2.8 (1.4-5.3)	**0.003**	> 18	7.9 (1.4-45.1)	**0.019**	> 9.2	6.1 (2.1-17.4)	**<0.001**	> 6.5	5.2 (0.6-45.7)	0.141
RBCx10^{12}/L	> 4.43	0.9 (0.5-1.6)	0.768	≤ 5.45	3.8 (0.9-15.9)	0.108	> 5.48	4.0 (1.2-13.6)	**0.046**	≤ 2.98	7.6 (0.6-93.0)	0.138
HGB g/L	> 142	1.6 (0.9-2.9)	0.175	> 191	3.8 (1.0-14.5)	0.067	> 106	0.2 (0.1-0.7)	**0.028**	≤ 158	6.1 (0.3-114.9)	0.171
PLTx10^9/L	> 535	1.4 (0.7-2.5)	0.394	≤ 640	4.1 (0.8-20.2)	0.118	> 632	2.6 (1.1-6.3)	**0.049**	≤ 315	2.6 (0.6-10.7)	0.298
Age at diagnosis, years	> 55	2.6 (1.3-5.0)	**0.006**	> 59	3.2 (0.9-11.0)	0.093	> 55	4.6 (1.8-11.7)	**0.002**	> 68	3.0 (0.7-12.1)	0.153

Table 6. Cut-off values for quantitative parameters in patients with venous thrombosis compared to patients without thrombosis

Risk factor	All MPN patients			PV patients			ET patients			PMF patients		
	Cut-off value for patients with venous thrombosis N= 26	**OR (CI)**	**p value**	**Cut-off value for patients with venous thrombosis N=8**	**OR (CI)**	**p value**	**Cut-off value for patients with venous thrombosis N=10**	**OR (CI)**	**p value**	**Cut-off value for patients with venous thrombosis, N=8**	**OR (CI)**	**p value**
V617F JAK2 burden allele %	> 26.1	1.9 (0.8-4.3)	0.193	> 90.4	1.9 (0.4-9.3)	**0.036**	> 18.3	2.1 (0.5-7.9)	0.281	> 56.7	6.0 (1.1-32.1)	**0.046**
WBCx10^9/L	> 21	3.6 (1.2-11.5)	**0.036**	> 18	7.3 (0.9-62.2)	0.100	≤ 5.9	3.1 (0.8-15.1)	0.120	> 18.9	4.7 (0.8-27.3)	0.106
RBCx10^{12}/L	≤ 3.9	2.0 (0.8-5.1)	0.199	≤ 6.42	2.1 (0.4-11.6)	0.461	≤ 4.23	2.8 (0.7-10.8)	0.215	≤ 3.9	4.8 (1.0-24.3)	0.089
HGB g/L	≤ 154	1.3 (0.5-3.6)	0.804	≤ 159	5.3 (1.1-25.8)	**0.041**	≤ 101	7.9 (1.2-54.5)	0.067	≤ 140	7.0 (0.4-132.6)	0.165
PLTx10^9/L	≤ 462	1.1 (0.5-2.2)	0.967	≤ 385	4.2 (0.9-20.3)	0.100	≤ 536	5.6 (1.3-23.0)	**0.015**	≤ 218	2.9 (0.6-14.0)	0.217
Age at diagnosis, years	> 54	1.4 (0.6-3.6)	0.578	≤ 69	8.4 (0.5-154.5)	0.089	> 54	5.6 (1.1-27.6)	**0.041**	≤ 61	3.6 (0.6-20.2)	0.240