The Impact of Mean Platelet Volume (MPV) and JAK-2 Mutation on Thrombosis in Chronic Myeloproliferative Diseases

Mesut Ayer¹ · İlhan Menken² · Mehmet Yamak² · Fatma Aylin Ayer² · Onur Kırkızlar³ · M. Burak Aktuğlu²

Received: 12 February 2016 / Accepted: 17 May 2016 / Published online: 24 May 2016
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Abstract Thrombosis and bleeding are the main complications of chronic myeloproliferative diseases. Mean platelet volume (MPV) is an important indicator of the platelet activation. The aim of the present study was to assess the interrelationships between MPV, JAK-2 gene mutation and thromboembolic events in patients with ET and PV. Patients with ET (n = 60) and PV (n = 46) were compared to the secondary erythrocytosis group (n = 19); and a control group of age and sex matched healthy volunteers (n = 52). Besides demographic, clinical and laboratory data; thrombotic and hemorrhagic events were recorded for each patient. Platelet counts, MPV and JAK2 mutations were studied; and their relation with thromboembolic events were investigated using SPSS program for statistical analysis. There was no significant difference between groups regarding age ($p = 0.188$). Mean platelet count was significantly higher in ET group than other groups ($p < 0.0001$). Mean platelet count in PV group was significantly higher than control ($p < 0.0001$) and secondary erythrocytosis groups ($p < 0.0001$). In the ET group, MPV values were significantly lower than the control group and PV group. In the ET group, those with thromboembolia had lower platelet counts. There was no relation between MPV and thromboembolic event rate in PV, ET and secondary erythrocytosis groups; while no event was recorded in the

control group. There was no relation between thromboembolic event rate and JAK 2 mutation. The association of JAK-2 mutation and high MPV especially in ET and PV groups does not contribute to the thromboembolic events.

Keywords Mean platelet volume · Chronic myeloproliferative neoplasm · Thromboembolism · JAK-2 mutation

Introduction

Chronic myeloproliferative diseases (CMPD) are clonal disorders that exhibit hyper proliferation defects without significant alterations in cellular differentiation and maturation. The term CMPD covers chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythosis (ET), and primary myelofibrosis, also known as myelofibrosis with myeloid metaplasia (MMM). Granulocytes, erythrocytes, and platelets are increased in CML, PV and ET, respectively. However, overlaps commonly occur in CMPD because myeloplastic cellular clones may differentiate into other myeloid cell series or even into B lymphocytes [1–4]. In PV and ET, the main complications are thrombosis and bleeding. The frequency of those complications is decreased by reducing the number of platelets [1].

Mean platelet volume (MPV) is an important indicator of platelet activation. MPV increases with an increase in thrombocyte production. Important alterations in volume parameters of thrombocytes may have prophylactic and diagnostic importance in thrombotic events and prothrombotic tendency [2]. Large thrombocytes are more active than small thrombocytes enzymatically and metabolically, and produce more thromboxane-A2 [3]. In

✉ Mesut Ayer
mesutayerdr@hotmail.com

¹ Department of Hematology, Haseki Training and Research Hospital, Millet Cd., Aksaray/Fatih, 34087 Istanbul, Turkey

² Department of Internal Medicine, Haseki Training and Research Hospital, Istanbul, Turkey

³ Department of Hematology, Medeniyet University Goztepe Training and Research Hospital, Istanbul, Turkey

the normal population, increments in the platelet volume had been reported in acute myocardial infarction (AMI), acute cerebral ischemia, and transient ischemic attacks [4].

During the last decade, with the discovery of Janus kinase 2 (JAK2) or exon 12 mutation in patients with CMPD, information on the pathogenesis of BCR/ABL-negative CMPD has increased; a disease algorithm has been developed and new options for treatment have emerged. This somatic mutation that arises after V617-F substitution in the JAK2 gene leads to an excessive sensitivity to growth factors in hemopoietic precursor cells in CMPD. It is not known whether the presence of this mutation has any effect on thrombosis in patients with CMPD.

The aim of the present study was to assess the interrelationships between MPV and JAK 2 gene mutation and thromboembolic events in patients with ET and PV, and to investigate whether MPV could be used as a marker of thromboembolic risk in CMPD.

Patients and Methods

One hundred six patients were diagnosed as having ET ($n = 60$) or PV ($n = 46$) in the hematology clinic of our hospital between 2004 and 2011 and were included in the study. The diagnostic criteria proposed by the World Health Organization in 2009 were used for the diagnosis of PV and ET [5]. These groups were compared with a secondary erythrocytosis group ($n = 19$), and an age- and sex-matched control group of healthy volunteers ($n = 52$). The diagnosis of secondary erythrocytosis was made in patients with isolated erythrocytosis with elevated hemoglobin and normal erythropoietin levels, and without other known disease (e.g., cyanotic heart disease, lung disease), JAK2 gene mutation, and BCR-ABL mutation.

The presenting symptoms of the patients, co morbidities, presence of splenomegaly (long axis of the spleen >13 cm with ultrasonography), history of hemorrhagic or thrombotic events, blood count values at the time of diagnosis, presence of JAK2 and BCR-ABL gene mutation, erythropoietin level and MPV were recorded. Risk factors that could contribute to thrombotic events such as hypertension, diabetes mellitus, smoking, and hyperlipidemia were investigated.

Complete blood counts including MPV were studied using a Symex XT 2000 hematology analyzer. MPV was accepted as low if less than 6 fL, and high if more than 10 fL.

In order to detect JAK2 gene, V617F point mutation were amplified using real-time polymerase chain reaction (PCR) with a LightMix kit. When adequate DNA is available, this method has 1 % sensitivity to detect JAK2 V617F mutant DNA.

In the present study, JAK2 V617F's 165 base pair and genomic cDNA target's 179 base pair fragments were

amplified using a specific primer and detected with Light Cycler Red 640 probes. The genotypes of selected nucleic acids (genomic DNA or cDNA) were determined with melting curves found at 61 °C for the mutant genotype and 57 °C for the wild-type genotype. An additional product composed of 116 base pairs was used to measure the DNA sample size (quantification). The program used consisted of 4 stages. The data (DNA quantification and melting curves) were analyzed using a LightCycler guide.

Statistical Method

Statistical Package for Social Sciences (SPSS) version 16.0 was used for statistical analysis. In descriptive statistics, mean, standard deviation, frequency and proportions were used. The Kruskal–Wallis test as used in the comparison of values between more than two groups, and the Mann–Whitney U test was used in the sub analysis. The Chi square test was used in the analysis of proportional data. Ninety-five percent confidence analysis was used in the tests.

Results

The demographic, clinical, and laboratory data of the groups are summarized in Table 1. There was no significant difference between the groups regarding age ($p = 0.188$). The percentage of male patients was significantly higher in the PV ($p = 0.031$) and secondary erythrocytosis groups ($p = 0.006$). In the secondary erythrocytosis group, the proportion of male patients was significantly higher than in the ET group ($p = 0.014$) (Table 1).

Thromboembolic events were detected in 21.6 % of patients (15.2 % of the PV and 21.05 % of secondary erythrocytosis groups). Of the 13 thromboembolic events recorded in the ET group, 8 were cerebral infarction, 3 were AMI, and 2 patients presented with portal vein thrombosis. In the PV group, cerebral infarction ($n = 3$), AMI ($n = 3$), and peripheral artery occlusion ($n = 1$) were reported as thromboembolic complications. Four thromboembolic events were recorded in the secondary erythrocytosis group, 1 cerebral infarction, 1 AMI, 1 deep venous thrombosis, and 1 peripheral artery occlusion; no events were recorded in the control group.

The mean platelet count was significantly higher in the ET group than in the other groups ($p < 0.0001$). The mean platelet count in the PV group was significantly higher compared with the controls ($p < 0.0001$) and secondary erythrocytosis groups ($p < 0.0001$) (Fig. 1).

MPV values in the ET group were found significantly lower than the control group ($p = 0.006$) and PV group ($p = 0.044$)

Table 1 Demographic and clinical data of the groups

	PV n = 46	ET n = 60	Control group n = 52	Secondary rythrocytosis n = 19	<i>p</i>
Age	58.87 ± 14.5 (23–86)	56.8 ± 15.7	58.5 ± 10.3	49.2 ± 19.7 (21–90)	0.118
Sex (male/female)	28/18	26/34	19/30	15/5	0.013
Smoker (%)	19	19		35	0.30
Thrombocyte (/mm ³)	577 ± 223 (172–1043)	795 ± 315 (219–1685)	249 ± 51	284 ± 111 (129–578)	<0.001
MPV (fl)	8.5 ± 1.1 (6.5–10.8)	8.4 ± 2.3 (5.4–24.0)	8.6 ± 1.0	8.4 ± 1.3 (6.2–11.6)	0.028
Thromboembolic event [n (%)]	6 (13)	15 (23)	–	5 (26)	0.167
JAK-2 mutation (%)	96	54	–	0	<0.001
Splenomegaly [n (%)]	72	32	–	44	0.001

Fig. 1 The distribution of thrombocyte counts between groups **Fig. 2** The distribution of MPV values in groups

and similar to the secondary erythrocytosis group (Fig. 2). Thirteen (22 %) patients had thromboembolic events. No relation was found between MPV and thromboembolic events in the ET group ($p = 0.483$) (Table 2).

In the PV group, thromboembolic events occurred in seven (15.2 %) patients; of which two were in patients with high MPV values and five were seen in patients with a normal MPV (Table 2). No relation was found between

MPV and thromboembolic events in the PV group ($p = 0.221$).

Thromboembolic events occurred in four (21.05 %) patients in the secondary erythrocytosis group. Although the MPV was normal in all patients who had thromboembolic events, no thromboembolic events occurred in patients with high MPV. MPV and thromboembolic events were not interrelated (Table 2).

Table 2 The percentage of patients with thromboembolism in the groups in terms of high or normal MPV

	Patients with thromboembolic events		Patients without thromboembolic events	
	High MPV	Normal MPV	High MPV	Normal MPV
ET group	3 (5 %)	10 (16.7 %)	7 (11.6 %)	40 (66.7 %)
PV group	2 (4.3 %)	5 (10.9 %)	4 (8.7 %)	35 (76.1 %)
Secondary erythrocytosis group	0 (0 %)	4 (21.1 %)	2 (10.5 %)	13 (68.4 %)

Fig. 3 The distribution of MPV values in groups

No patients had a thromboembolic event in the control group.

The groups were subdivided according to the presence of thromboembolism. Patients with thromboembolism had significantly lower platelet counts in the ET group ($p = 0.034$) (Table 1; Fig. 3) and similar MPV values to those who had no thromboembolism. In the PV and secondary erythrocytosis groups, there was no significant difference between those with and without thromboembolism in terms of MPV and platelet counts (Table 1).

JAK2 mutation and splenomegaly were significantly higher in the PV group than in the other groups. There was no statistically significant difference between patients with or without JAK2 mutation in the ET group regarding MPV and platelet count. When all patients were divided into two groups, those with MPV above or below 10 fL, there was no difference in terms of thromboembolic events and the presence of JAK2 mutation. No significant difference was found when this analysis was repeated for each group (Table 3).

When patients with or without JAK2 mutation were analyzed for MPV, MPV was found to be normal in 24 of 29 (82.8 %) patients with JAK2-positive ET and high in 5 (17.2 %) patients. In 26 of 31 (83.9 %) patients with JAK2-negative ET, MPV was normal and high in 5 patients (16.1 %). No significant relation was found between JAK2 mutation status (positive or negative) and MPV in the ET

Table 3 The overall relation of MPV (either less or more than 10 fL) with thromboembolism and the presence of JAK-2 mutation

		MPV		<i>p</i>
		<10 fL	>10 fL	
Thromboembolic event	Negative	90	12	0.47
	Positive	21	5	
JAK-2 mutation	Negative	11	5	0.317
	Positive	45	66	

group, neither was there any significant relation between thromboembolic events and MPV in patients with JAK2-positive ET ($p = 0.908$).

When all patients with PV were evaluated in terms of the relation between JAK2 status and MPV, no statistically significant difference was found between patients who were JAK2- positive with high MPV and those who were JAK2-positive with normal MPV ($p = 0.221$).

Discussion

The main complications of PV and ET are thrombosis and bleeding, which results from the qualitative thrombocyte impairment. In cases of reactive thrombocytosis where

similar numbers of thrombocytes are present, these complications usually do not occur. The incidence of these complications decreases with the reduction of thrombocyte counts.

The aim of the present study was to assess the interrelationships between MPV and thromboembolic events in patients with ET, PV, and secondary erythrocytosis, and to investigate whether MPV could be used as a predictor of thromboembolism risk. In addition, the association of MPV with JAK2 gene mutation and thromboembolic events was evaluated.

In general, arterial thrombosis occurs more frequently than venous thrombosis. In the 24 thromboembolic events of the present study, 21 were arterial and three were venous thromboses. It is reported in the literature that the rate of thrombosis is 11–25 % in ET and 11–39 % in PV. In the present study, the rate of thrombosis was found 21.6 % in ET, 15.9 % in PV, and 21.05 % in secondary erythrocytosis.

MPV is considered as an important indicator of platelet activation. Changes in platelet volume are considered as a physiologic variable that has hemostatic importance in platelet activation [4]. The degree of platelet activation is related to the number of thrombocytes, MPV, and the size of thrombocytes. Large thrombocytes are more active enzymatically and metabolically than small ones because they contain more granules and produce larger amounts of thromboxane-A₂ [3]. Likewise, increased MPV was also found associated with *in vitro* SDP and collagen-dependent aggregation [3]. In view of these data, high rates of MPV may be a risk factor for thromboembolic events in ET and PV, and may be used as a predictor of risk [2].

Several studies suggested that there was an association between MPV increase and acute cerebral ischemia and transient ischemic attacks [4]. Bath et al. [6] reported a positive correlation between MPV and the risk of stroke. The authors demonstrated that each 1 femtoliter increase in MPV corresponded to a 12 % increase in the odds ratio for stroke risk, independent of other risk factors. Nevertheless, in the study of O'Malley et al. [7] MPV values were compared between patients with and without previous ischemic stroke and no significant difference was found between them.

It was reported that MPV increased in acute MI [8, 9]. Martin et al. [10] defined MPV as an independent risk factor for the recurrence of vascular events and death after acute myocardial infarction (AMI). It was also suggested that a high MPV value was an independent risk factor for AMI [8]. In addition, increased MPV was reported to be associated with unfavorable prognosis in patients with AMI [10, 11], stroke [12], and increased restenosis after coronary angioplasty [13]. This association is similar to better

known risk factors such as increased fibrinogen, blood viscosity, and the number of leukocytes.

In the study of Senaran et al. [14] MPV values were reported higher in patients with AMI and unstable angina pectoris than in control subjects. We detected no relationship between thromboembolic events and MPV.

JAK2 is a structurally-active tyrosine kinase, and when it is expressed together with erythropoietin receptor (EpoR), thrombopoietin receptor (MPL) or granulocyte colony-stimulating factor receptor (G-CSFR), it activates the JAK-STAT signal transduction system [15]. The JAK2 V617F mutation occurs through the substitution of valine for phenylalanine in codon 617 in pseudo kinase domain (JH2) of JAK2. JAK2 V617F is a somatic mutation in hematopoietic progenitors (exon 14 1849G → T) (165,167) that leads to constant activation of the JAK-STAT signal pathway [16].

Insight into the genetic basis of these diseases significantly increased after the JAK2 V617F mutation was described in patients with CMPD in 2005 [17]. In 2006, patients with JAK2 V617F-negative PV, JAK2 exon 12 mutation, and thrombopoietin receptor mutation (MPLW515L) were discovered [18]. It was demonstrated that each of the three mutations discovered in mice experiments induced the activation of JAK-STAT signaling and the development of myeloproliferative disorder phenotypes [19]. As expected, JAK2 mutations do not occur in patients with secondary erythrocytosis and secondary thrombocytosis [20]. JAK2 V617F mutation can be detected in around 90–95 % of patients with PV, 50–70 % of those with ET, and 40–50 % of patients with PMF.

For PV, the expected rate of JAK2 V617F mutation (exon 14-acquired somatic mutations) is ~95 %. JAK2 exon 12 mutation was shown in patients with JAK2 V617F-negative PV. The incidence of mutation in ET and PMF is not high enough for diagnosis and is supplementary to histologic characteristics [18].

Baxter et al. [17] reported the rate of JAK2 gene mutation as 97, 57, and 50 % in patients with PV, ET, and PMF, respectively. Levin et al. [21] reported the corresponding rates as 74, 32, and 35 %. Jones et al. [22] found that JAK2 mutation was found in none of 160 healthy control patients and 4 patients with secondary erythrocytosis, whereas the rates were 81, 41, and 43 % in patients with PV, ET and PMF, respectively.

Tefferi et al. [23] found JAK2 mutation in 92 % of patients with PV [23]; no significant difference was found between homozygote and heterozygote patients in terms of the number of platelets and leukocytes and the incidence of thrombosis at the time of diagnosis. In the present study, no distinction was made between homozygote and heterozygote patients in the evaluation of mutations. Vannucchi et al. [24] demonstrated a significant relation between

JAK2 mutation in patients with ET and the development of thromboembolic events, but they could not find the same relation in patients with mutation-positive PV. The results of Larsen et al. are compatible with Vannucchi et al. [25]. The differences between the studies could have originated from the different methods used to detect mutations. In our patient cohort, JAK2 mutation was found positive in all (100 %) patients with PV and in 29 (48.3 %) patients with ET, whereas it was negative in all (100 %) patients with secondary erythrocytosis.

In some studies, increased risk of thrombotic complications was found associated with JAK2 gene mutation in CMPD [26]. There are also studies that found no relation between them [27].

In the present study, no significant relation was found between thromboembolic events and JAK2 positivity in patients with ET ($p = 0.073$). There was no significant relation between thromboembolic events and MPV value in patients with JAK2 mutation-positive ET ($p = 0.908$). No statistically significant difference was found between patients who were JAK2 positive with high MPV and those who were JAK2 positive with normal MPV in the PV group ($p = 0.221$).

Conclusion

In conclusion, it is suggested that MPV does not vary between patients and controls and that MPV cannot be used as a risk factor in patient groups (ET, PV, and secondary erythrocytosis). Moreover, the association of JAK2 mutation and high MPV, especially in patients with ET and PV, does not contribute to thromboembolic events. Given the necessity for a larger overall sample size and increased number of patients in our subgroups, further and larger studies on this issue are required for full elucidation.

Compliance with Ethical Standards

Conflict of Interest None

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