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Platelet indices in Philadelphia-negative chronic myeloproliferative neoplasms

Indicii plachetari de volum în neoplasmele mieloproliferative cronice Philadelphia-negative

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Abstract

***Introduction**: Philadelphia-negative chronic myeloproliferative neoplasms (Ph-MPN): polycythemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF) are characterized by an increased rate of thrombosis complications partly due to platelets activation. Large platelets are more active, have an enhanced procoagulant function and have a pathogenic role in arterial and venous thrombosis. In our study we tried to establish if platelet volume indices (MPV, PDW, P-LCR) issued from automated complete blood count determination are significantly different in Ph-MPN patients in comparison to healthy subjects. **Materials and methods**: Blood cell counts including platelet volume indices were assessed for 102 Ph-MPN and 102 healthy subjects using the impedance method on Sysmex XS 1000i and glucose and lipid profile, were assessed on Architect c 8000. Assessement of JAK2V617 positivity was conducted with amplification refractory mutation system polymerase chain reaction (ARMS-PCR), in whole peripheral blood. **Results**: Platelet volume indices (PVI) measured with the impedance based method, did not show significant differences in Ph-MPN patients in comparison to healthy controls. We noticed a moderate correlation between these indices and the presence of JAK2V617F mutation. PVI were increased in the small subgroup of patients treated with anagrelide and decreased in patients treated with simvastatin, comparatively with untreated patients. **Conclusion**: In our study we did not find a significant difference between platelet volume indices from Ph-MPN patients and healthy subjects. Further studies are required to demonstrate correlations between platelet volume indices and JAK2 V617F mutation, treatment with anagrelide and statins, respectively.*

***Keywords**: chronic myeloproliferative neoplasms, platelet activation, platelet volume indices*

Rezumat

***Introducere**: Neoplasmele mieloproliferative cronice cromozom Philadelphia negative (Ph-MPN) -policitemia vera (PV), trombocitemia esenţială (TE) şi mielofibroza primară (MP) - sunt caracterizate de o rată mare de*

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complicaţii trombotice, parţial date de activarea trombocitară. Trombocitele mari sunt mai active, cu o funcţie procoagulantă crescută şi au rol patogenic demonstrat în tromboza arterială şi venoasă. În studiul nostru am încercat să stabilim dacă indicii trombocitari (MPV, PDW, P-LCR), furnizaţi de analizoarele de hematologie în hemoleucograma completă, sunt modificaţi la pacienţii diagnosticaţi cu Ph-MPN comparativ cu subiecţii sănătoşi. ***Material şi metodă****: Hemoleucograma, ce a inclus şi indicii trombocitari, a fost determinată la 102 pacienţi Ph-MPN şi la 102 subiecţi sănătoşi cu ajutorul analizorului de hematologie Sysmex XS 1000i (metoda impedanţei), iar testele biochimice (glucoza şi parametrii metabolismului lipidic), pe analizorul Architect c8000. Prezenţa mutaţiei JAK2 V617F, la pacienţii cu Ph-MPN, a fost evidenţiată prin tehnica ARMS-PCR.* ***Rezultate****: Indicii de volum plachetari (IVP) măsuraţi prin metoda impedanţei, nu au fost semnificativ modificaţi la pacienţii cu Ph-MPN comparativ cu cei ai subiecţilor sănătoşi. Totuşi, am observat o moderată corelaţie între valoarea acestor indici şi prezenţa mutaţiei JAK2V617F, fără însă a se atinge o diferenţă semnificativă din punct de vedere statistic. IVP au fost crescuţi la micul subgrup de pacienţi trataţi cu anagrelide şi scăzuţi la cei trataţi cu simvastatin, comparativ cu pacienţii care nu au primit aceste terapii.* ***Concluzii****: Folosind metoda impedanţei în determinarea indicilor trombocitari, în studiul nostru nu am demonstrat o diferenţă semnificativă între valorile indicilor trombocitari la pacienţii cu Ph- MPN comparativ cu subiecţii sănătoşi. Sunt necesare studii ulterioare pentru a demonstra o posibilă corelaţie între indicii trombocitari şi mutaţia JAK2V617F, respectiv cu tratamentul cu anagrelide şi statine.*

Cuvinte cheie*: neoplasme mieloproliferative cronice, activare trombocitara, indici trombocitari*

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Introduction

Myeloproliferative neoplasms are clonal hematopoietic stem cell disorders characterized by proliferation of one or more of the myeloid lineage (granulocytic, erythroid, megakaryocytic and mast cell [1]. Classic Philadelphia-negative chronic myeloproliferative neoplasms (Ph-MPN), according to the World Health Organization (WHO) classification, include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) and are characterized by an incidence of arterial and venous thrombosis, ranging from 12%-39% in PV and from 11%-25% in ET [1-3]. The plasma biomarkers of haemostatic system activation (thrombin-antithrombin complex, prothrombin fragments 1+2, and D-dimers) and vascular endothelium activation (thrombomodulin and von Willebrand factor/factor VIII) are present at increased levels in Ph-MPN [4]. The pathogenesis of acquired thrombophilia in Ph-MPN is complex and is related to the abnormalities of blood cells (platelets, erythrocytes and leucocytes) that acquired a prothrombotic phenotype, and to the inflammatory response of host vascular cells to the insult of cytokines and other mediators released by malignant cells [3]. Several studies showed an association between the JAK2V617F mutation, present in Ph-MPN, and the expression of cellular and soluble biomarkers of haemostatic system activation.

Numerous platelet abnormalities, both quantitative and qualitative, have been identified in PV and ET patients, but not clearly linked to thrombotic events [5]. Platelets circulate in an activated status in PV and ET patients with a direct, but varied participation in thrombin generation [6, 7]. Large platelets are more active and have enhanced procoagulant function. There is evidence supporting the idea that large platelets, probably younger and more reactive, have a pathogenic role in arterial and venous thrombosis [8, 9]. Platelet size, measured by mean platelet volume (MPV), has been proposed to become marker of platelet activity, as it was found to correlate with in vitro platelet activation [10]. Platelet volume indices (PVI), like mean platelet volume (MPV), platelet distribution width (PDW)

and platelet cell ratio (P-LCR), are a group of parameters which are derived from routine blood counts. The mean platelet volume (MPV) is the most attractive for research in clinical settings and is routinely measured by automated hematology analyzers using impedance or optical fluorescence method [11]. It has been recently shown that high MPV is correlated with cardiovascular events and cardiovascular risk factors like diabetes mellitus, dyslipidemia, hypertension, smoking and obesity [12, 13], with acute non-lacunar ischemic stroke and venous thrombosis [14, 15]. Many studies recognized that MPV have potential for clinical utility especially in hematology (inherited macrothrombocytopenia) and in vascular medicine [16], but this is significantly limited by variability of measurement and lack of standardization.

Our study aimed to establish if platelet indices (MPV, PDW, P-LCR) issued from automated complete blood count determination are significantly different in Ph-MPNs patients in comparison to healthy subjects and demonstrate a correlation between PVI and the prothrombotic status in patients diagnosed with chronic Ph-MPN.

Material and methods

This prospective study comprised all patients (102) diagnosed with Ph-MPN, taken in evidence by the Hematology Department of Emergency County Hospital Sibiu since June 2012 to June 2013. The study was approved by the Ethical Committee of the Emergency County Hospital Sibiu and an informed written consent was obtained from patients and healthy controls. The diagnosis was established based on 2001 and 2008 WHO criteria for Ph-MPN [1, 17]. Some patients with PMF (n=6) were excluded because the repeated blood count did not provide the platelet volume indices. One hundred two healthy subjects, volunteers, without history of thrombotic or bleeding events, symptoms of acute infection, chronic inflammatory diseases, without anti-platelet agents or oral anticoagulants were included as controls. Whole blood was drown after overnight fasting, into vacutainers containing K2EDTA, for complete blood count (CBC) and vacutainers with clot activator for biochemistry tests. Assessment of JAK2V617 mutation was performed by an amplification refractory mutation system polymerase chain reaction (ARMS-PCR) described by Jones et al. [18]. Serum glucose and lipid profile parameters were performed on Architect c8000. CBC (including platelet volume indices) was performed on Sysmex XS 1000i, in the first hour after blood collection. MPV is expressed in femtoliters (fl), and is calculated from equation: MPV(fl) = [(plateletcrit % / platelet number $x10^3/\mu L$) x 10000]. The other PVI, PDW (fl) and P-LCR are calculated from the platelet size distribution curve. For platelet distribution width (PDW) the analyzer uses 3 discriminators, 2 moving between 2-6 fl and 12-30 fl, one fixed at 12 fl. The percent of platelets >12 fl was notified P-LCR.

For the statistical analysis we used the program SPSS version 21. Nominal variables were characterized by frequency and percents, and the quantitative variables, by mean and standard deviation, or median and 25-75 percentiles. We used the Kolmogorov-Smirnov test to analyze the distribution of quantitative variables. The comparison between two groups with normal distribution of quantitative variables was made using the Student T test or Mann-Whitney test and the correlation between two continuous variables with Pearson correlation or Spearman's rho. The frequent difference from one nominal variable between two groups was evaluated with chi-square test. We considered the differences statistical significant if $p<0.05$.

Results

The study population consisted of 102 Ph negative MPN patients (36 PV, 54 ET, 12 PMF) with slightly more female than male subjects, aged 20 to 81 years and sex-matched healthy control lot (n= 102), volunteers. The main characteristics of the study population were summarized in table I.

At the time of the enrolment almost all patients were receiving therapy: cytoreductive and anti-platelet treatment (n=40), anagrelide with aspirin (n=12), only aspirin (n=11), only anagrelide (n=14) or hydroxyurea (HU) (n=11). Thus, 50% of patients were receiving cytoreductive therapies. There were patients receiving statins (n=13) or resveratrol (n=15). Sixty-three patients were tested for JAK2V617F mutation, 40 of them were carriers (heterozygous n= 30 and homozygous n=10) and 23 patients were found negative for this mutation.

Concerning thrombotic events, 41 patients had one or more thrombotic events, arterial thrombosis (n=30) and venous thrombosis (n=11). We did not find significant differences of MPV, PDW and P-LCR values between the group of patients and healthy subjects, but the distribution of values was wider for patients, es-

Table I. General characteristics of the study population

		Ph-MPN Patients	Healthy Controls	*P*-value
Sex N(%)	Male	50(49%)	50(49%)	1
	Female	52(51%)	52(51%)	
Age (years)		62.7±13.1	56.1±15.4	0.001
White Blood Cells (10^3/μL)		7.3±4.2	6.5±1.4	0.07
Red Blood Cells (10^6/μL)		4.1±0.9	4.7±0.4	<0.001
Platelets (10^3/μL)		355±192.6	240±48.5	<0.001
Hemoglobin (g/dL)		13.6±2.3	14.3±1.2	0.01
Hematocrit (%)		40.6±6.8	41.7±3.3	0.1
MCV (fL)		98.8±13.6	88.2±3.9	<0.001
MPV (fL)		10.1±0.9	10.2±0.7	0.1
PDW (fL)		12.2±2.4	12±1.7	0.7
P-LCR (%)		25.9±6.8	27.2±6.3	0.1
PCT (%)		0.3±0.2	0.2±0.04	<0.001
Cholesterol (mg/dL)		170±38	194.3±35.3	<0.001
HDL- Cholesterol (mg/dL)		45.2±14.8	54.3±11.7	<0.001
LDL-Cholesterol (mg/dL)#		98.4±34	119.5±33.2	<0.001
Triglycerides (mg/dL)		107(46;380)	99(21;390)	0.04
Glucose (mg/dL)		99.6±19.8	95.5±12.4	0.07
Diabetes mellitus N(%)		19(18.8%)	9(10.5%)	0.6
Dyslipidemia N(%)		74(76.3%)	64(63.4%)	0.06
Overweight N(%)		21(20.6%)	7(12.3 %)	0.01

MCV – mean corpuscular volume; MPV – mean platelet volume; PDW – platelet distribution width; P-LCR – platelet large cell ratio; PCT – plateletcrit; # calculated by Friedewald equation

pecially for the P-LCR in ET subgroup. Patients with PMF showed a significantly lower MPV (p=0.05) and P-LCR (p=0.02).

Patients with thrombotic events had not statistically different values of platelet volume indices in comparison to those without thrombotic events. Considering the difference of these indices, in carriers of JAK2V617F mutation versus patients without this molecular mutation, we found differences that did not reach statistical signification.

Patients treated with anagrelide had significantly higher values (p<0.002) for PVI comparatively with patients without anagrelide treatment (table II). No influence of treatment with HU and aspirin was noted for MPV and P-LCR patients' parameters. Only PDW values were significantly lower in patients treated with cytoreductive and anti-platelet therapies comparative with patients without this treatment (p <0.05). The patients receiving statins had significantly lower PVI values than patients without this treatment (p <0.05). On the other hand, the treatment with resveratrol seems to have no influence on platelet indices.

In our study the MPV, PDW and P-LCR values did not correlate with age, body index, cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glucose. It was a significant correlation of MPV with platelet count (p=0.05), but this was not noticed after adjustment for Ph-MPN diagnosis (p=0.3).

Discussion

The study was designed to check if there is any difference of MPV and other PVI in Ph-MPN patients compared to healthy controls. Previous

Table II. Influence of different treatments on platelet volum indices in Philadelphia-negative chronic myeloproliferative neoplasms (MPN)

Treatment / PVI	Ph-MPN treated patients (Median, percentiles 25 ; 75)	Ph-MPN non treated patients (Median, percentiles 25 ; 75)	p
Anagrelide (No)	26	76	
MPV (fL)	10.5 (10.0; 11.5)	9.9 (9.3; 10.4)	0.001
PDW(fL)	12.4 (11.5; 15.3)	11.4 (10.2; 12.9)	0.002
P-LCR (%)	27.9 (25.3; 35.5)	25.0 (20.2; 27.9)	0.001
Aspirin (No)	63	39	
MPV (fL)	10.0 (9.5; 10.6)	10.3 (9.6; 10.8)	0.253
PDW(fL)	11.3 (10.5; 13.0)	12.5 (11.4; 13.8)	0.038
P-LCR (%)	25.4 (20.9; 29.8)	26.9 (21.5; 31.5)	0.159
Hydroxyurea (No)	51	51	
MPV (fL)	9.9 (9.3; 10.5)	10.2 (9.6; 10.8)	0.095
PDW(fL)	11.2 (10.1; 12.9)	12.4 (10.8; 13.7)	0.016
P-LCR (%)	25.0 (20.5; 28.1)	26.8 (21.7; 31.4)	0.052
Simvastatin (No)	13	89	
MPV (fL)	9.4 (8.9; 10.1)	10.1 (9.6; 10.8)	0.022
PDW(fL)	10.4 (9.3; 11.3)	12.0 (10.7; 13.4)	0.009
P-LCR (%)	20.6 (16.4; 25.3)	25.8 (22.2; 31.0)	0.008

studies showed that PVI and especially MPV have potential prognostic and diagnosis value especially in cardiology, stroke, peripheral arterial diseases and unprovoked deep vein thrombosis [12-15]. Large population studies have shown that MPV values are correlated to the geographical area and population structure [9, 19-21] which emphasis the idea that MPV has a genetic determination [22]. There are conflicting data on the relevance of MPV values in determining the cause of thrombocytosis. A number of studies have shown that MPV is significantly higher in Ph-MPN than in reactive thrombocytosis (RT), whereas others have found it to be lower in Ph-MPN than in RT [11]. In our study we did not find an inverse correlation between MPV values and platelet count that Lance et al. found [23]. The other platelet volume indices, PDW and P-LCR have been less studied in comparison to MPV [11, 24]. In our study MPV and the others PVI, assessed through the impedance method, did not reveal a significant difference between values of patients and healthy controls. Regarding the value of MPV in neoplasms, there is conflicting data, while some have found a significant higher MPV in ET, using an optical method [25], while others communicated a lower value of MPV in solid neoplasms [26]. First time, Latger-Canard et al. have demonstrated that MPV values are tightly correlated to measurement methods and the automated hematology analyzers based on the impedance method underestimate it because they have a specific threshold and have an inability to recognize very large platelets [27]. The blood film has shown increased platelet anisocytosis and a large number of giant platelets in ET [28]. The peripheral blood film may show abnormal platelets (giant, bizarre shape) and circulating megakaryocyte fragments in PMF [29]. Because of this platelet anisocytosis, in our study, 6 patients with PMF were excluded due to the fact that analyzer did not provide results for PVI. The rest of the group of patients with PMF showed lower values for MPV and P-LCR compared with healthy subjects. Platelet volume indices values distribution, especially P-LCR, was wider in the Ph-MPN patients group than in healthy controls, being much more obvious in ET.

Almost all patients were treated with anti-platelet therapy (aspirin), cytoreductive therapy (HU), anagrelide, alone or in association. Shah et al. showed in their study that aspirin did not influence MPV values [30], corresponding to what we found in our study. We noticed also lower PDW in patients receiving aspirin. Cytoreductive therapy with HU affects in a significant way the prothrombotic phenotype in patients with Ph-MPN. The reactive platelets from PV and ET are significantly down-regulated by cytoreductive therapy [3]. The immature platelet fraction, tightly linked to the presence of the JAK2V617F mutation, is very sensitive to myelosuppressive treatment and lowers the thrombotic risk in these patients [31]. Thus, cytoreductive therapies could influence the platelet indices due to their effect on the platelet turnover reduction. In our study the treatment with anagrelide increased the PVI, but HU did not influence the MPV or P-LCR (only PDW was significantly lower in HU treated patients). Bellucci et al. also observed an increase in platelet volume in patients treated with anagrelide [32]. Coban et al. found that treatment with rosuvastatin decreased in a significant way the MPV values [33]. In our study we found similar results in a subgroup of 13 patients who underwent treatment with simvastatin.

In our study only 63 patients of 102 have been tested for detection of the JAK2V617F mutation and 40 patients were carriers of it. There are studies which have demonstrated that the rate of thrombotic events is higher in carriers of this mutation [34], with increases in platelet expression of tissue factor, platelets/PMN interactions, PMN expression of CD 14 and latency-associated

peptide (LAP) and plasma levels of thrombomodulin [35].

Limitation: For an important number of our patients we did not have any information about the JAK2V617F mutation. We demonstrated the influence of statins and anagrelide on the PVI values only in small subgroups of patients.

Conclusions

Platelet volume indices measured with the impedance based method, did not show significant differences in Ph-MPN patients in comparison to healthy controls. PVI were increased in patients treated with anagrelide comparatively with untreated patients. We noticed a moderate correlation between these indices and the presence of JAK2F617F mutation. Due to the small number of subjects in our group, further studies are required to demonstrate a correlation between platelet volume indices and JAK2 mutation, anagrelide and statins treatments, respectively.

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Disclosure

The authors state that they have no conflict of interest.

Abbreviation

ARMS - PCR – amplification refractory mutation system polymerase chain reaction
CBC - complete blood count
ET – essential thrombocythemia
HU – hydroxyurea
JAK2 – Janus tyrosine kinase 2
MPN – myeloproliferative neoplasms
MPV – mean platelet volume
PCT – plateletcrit
PDW – platelet distribution width
P-LCR – platelet cell ratio
PMF – primary myelofibrosis
PV – polycythemia vera
PVI – platelet volume indices

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