

The impact of pre-analytical variable, type of anticoagulant and time delay, on the measurement of mean platelet volume

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Abstract. Introduction: Mean platelet volume (MPV), parameter provided by automated haematology analysers could become a useful biomarker to detect, in vivo, platelet activation. Unfortunately, MPV is affected by time, storage conditions and the nature of anticoagulants. Our study was designed to determinate the influence of anticoagulant and time delay from blood collection on MPV measurements. Materials and methods: Fasting venous blood samples were collected from 102 patients diagnosed with Ph-MPN (Philadelphia-negative myeloproliferative neoplasms) and from 57 healthy subjects into sterile tubes with K2EDTA and sodium citrate 3.2%. Blood cell counts including MPV were assessed through the impedance method on Sysmex XS 1000i at 30 minutes and 2 hours after blood collection. Results: In our study we found a significant difference between MPV values measured in blood collected into tubs with K2EDTA compared with those containing sodium citrate 3.2% at 30 minutes and 2 hours ($p < 0.001$). MPV from citrated samples revealed significantly smaller MPV. The values of MPV measured in tubs with K2EDTA at different times, 30 minutes and two hours, revealed a significant difference ($p < 0.001$). We did not notice this difference from tubs containing sodium citrate as anticoagulant. Conclusion: In our study we demonstrated that time and type of anticoagulants influence the MPV values, measured by impedance based method. Result from the present study show that storage of blood samples in EDTA based anticoagulant resulted in a progressive increase in the MPV and make EDTA not suitable as anticoagulant for the research on platelet function.

Key Words: platelet, mean platelet volume, anticoagulant, time delay.

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Introduction

Platelets are essential in primary haemostasis and a low or high platelet count is a significant risk factor for bleeding or thrombosis. Platelet volume indices, like mean platelet volume (MPV), platelet distribution width and platelet cell ratio, are a group of parameters which are derived from routine blood counts.

The MPV is the most attractive for research in clinical settings and is routinely measured by automated hematology analyzers using impedance or optical fluorescence method (Leader et al 2012). Platelet size, measured by MPV, has been proposed to become marker of platelet activity, as it was found to correlate with in vitro platelet activation (Riedl et al 2014).

It has been recently shown that MPV is highly correlated with cardiovascular events. Patients with acute myocardial infarction (AMI) have a higher MPV than those without AMI (Chu et al 2010; Klovaite et al 2011). A higher MPV is associated with cardiovascular risk factors like diabetes mellitus, dyslipidemia, hypertension, smoking and obesity. Also, MPV was found increased during acute non-lacunar ischemic stroke and venous thrombosis (Muscari et al 2008; Brækkan et al 2010).

Many studies recognized that MPV have potential for clinical utility especially in hematology (inherited macrothrombocytopenia) and in vascular medicine (Gohda et al 2007).

There are conflicting data on the relevance of MPV to distinguish between thrombocytosis from Ph-MPN and reactive thrombocytosis (Leader et al 2012). Song et al found an increased MPV on essential thrombocytemia (ET) than in reactive thrombocytosis (Song et al 2009), whereas others have found a lower MPV in myeloproliferative neoplasms (Sehayek et al 1988) or the same (Small & Bettigole 1981).

Classic Philadelphia-negative chronic myeloproliferative neoplasms (Ph-MPN), according to the World Health Organization (WHO) classification, include polycythemia vera (PV), essential thrombocytemia (ET) and primary myelofibrosis (PMF) and are characterized by an incidence of arterial and venous thrombosis (Falanga & Marchetti 2012).

Numerous platelet abnormalities, both quantitative and qualitative, have been identified in PV and ET patients, but not clearly linked to thrombotic events (Landolfi et al 2008).

The clinical utility of MPV is significantly limited by variability of measurement and lack of standardization. If the MPV is

to be reliably measured, the potential influence of anticoagulant on MPV must be controlled and standardizing the time delay between sampling and analysis (Machin & Briggs 2010). Our study aimed to demonstrate the influence of anticoagulants and time delay from blood collection on the measurement of the MPV.

Material and methods

This prospective study comprised two study groups. In the first group we determined MPV for all patients diagnosed with Ph-MPN, 36 PV, 54 ET, 12 PMF, taken in evidence by the Hematology Department of Emergency County Hospital Sibiu and were tested from June 2012 to June 2013. The average age of the patients included in our study was 62.7 ± 13.1 years, 50 patients (49%) were males and 52 (51%) were females. A second group comprised 57 healthy subjects, volunteers, without history of thrombotic or bleeding events, symptoms of acute infection or chronic inflammatory diseases with average age 54.7 ± 14.8 years, 22 males (38,6%) and 35 females (61,4%). Whole blood was drawn in the morning, after overnight fasting, into vacutainers containing di-potassium-ethylene-diamine-tetra-acetic-acid (K2EDTA) and sodium citrate 3.2% 105 M 9:1 v/v. (BD Vacutainers).

Full blood count (including platelet volume indices) was performed on Sysmex XS 1000i. Samples were analyzed at 30 minutes and 2 hour after blood collection. We store the samples at room temperature.

Sysmex XS 1000i performs blood cell counts according to impedance based methods with Hydro Dynamic Focusing . MPV is expressed in femtoliters (fl), and is calculated using the following equation: $MPV(fl) = [(plateletcrit \% / platelet\ number \times 10^3 / \mu L) \times 10000]$.

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.

For the statistical analysis we used the program Medcalc version 14.8.1. The quantitative variables were described by mean and standard deviation or median and 25-75 percentiles (distribution tested with the Kolmogorov-Smirnov test). The comparison of quantitative variables with normal distribution, between two groups, was made using the T test for independent variables. We evaluated the differences between two repeated measurements for a quantitative variable using the Wilcoxon test. We considered the differences statistical significant if $p < 0.05$.

Results

The results from the present study showed that storage of blood sample in anticoagulant based on EDTA resulted in a progressive increase in the MPV.

In our study we found a significant difference between MPV measured in blood collected in tubs with K2EDTA compared with those containing sodium citrate 3.2% at 30 minutes and 2 hours (table 1). The difference was observed in patients and healthy control groups. MPV from citrated samples revealed significantly smaller MPV.

Table 1. MPV from tubs with different anticoagulants (Ph-MPN patients group)

Time	MPV (citrate)	MPV K2EDTA	P
30 minutes	9.2 (8.6; 9.9)	10 (9.5; 10.6)	<0.001
2 hours	9.3 (8.8; 9.9)	10.3 (9.6; 10.9)	<0.001

The values of MPV measured in tubs with K2EDTA at different times, 30 minutes and two hours, revealed a significant difference. We did not notice this difference from tubs containing sodium citrate as anticoagulant (table 2).

Table 2. MPV at different times of blood collection (Ph-MPN patients group)

Time	30 minutes	2 hours	P
MPV (citrate)	9.2 (8.6; 9.9)	9.3 (8.8; 9.9)	0,4
MPV (K2EDTA)	10 (9.5; 10.6)	10.3(9.6; 10.9)	<0.001

The results did not show a difference of MPV values between patients group and control group from the tubs containing the same tip of anticoagulant and analyzed at the same time (table 3).

Table 3. Comparison of mean platelet volume values between patients and healthy controls

Variable	Patients group	Control group	P	
MPV (citrate)	30 minutes	9.3±1	9.3±0.8	0.8
	2 hours	9.4±0.9	9.4±0.9	0.8
MPV (K2EDTA)	30 minutes	10.0±0.9	10.1±0.8	0.9
	2 hours	10.3±0.9	10.4±0.9	0.2

Discussion

Our study was design to check if there is a difference between the MPV values issued from CBC (complete blood count) determination of blood collected in tubs using different anticoagulants (K2EDTA and sodium citrate) and at different processing times.

In our study we found an anticoagulant and time dependency of MPV values like others authors reported (Machin & Briggs 2010; Dastjerdi et al 2006; Diaz-Ricart et al 2010; Lancé et al 2010; Vinholt et al 2014). Values of MPV from K2EDTA tubs were significantly higher compared with those issued from sodium citrate tubs. While EDTA is perceived as an optimal anticoagulant for cell counting and white blood cell differential analysis, several studies point out that this anticoagulant is far from optimal for preservation of platelet ultrastructural and functional capabilities. Exposure to EDTA causes alterations in platelets that resemble those occurring during platelet activation, induce change in the phosphorylation patterns of platelet proteins (Diaz-Ricart et al 2010).

Regarding the time dependency of MPV values, in our study, the MPV values measured at 30 minutes and 2 hours from blood collection, revealed a significant difference only when blood was assessed from tubs with K2EDTA as anticoagulant. Lance et al recommend to process the sample within 120 minutes when platelet indices are performed (Lancé et al 2010) and

Dastjerdi *et al* conclude that MPV can be measured accurately if analysis is performed within 1 hour of sampling, using both anticoagulants based on EDTA and citrate (Dastjerdi *et al* 2006). The platelet parameters derived by the automated CBC are highly specific to the technologies from each type of analyzer (Latger-Cannard *et al* 2012).

Our results are in accordance with the observations of Machin & Briggs regarding measurement with impedance based method, when the MPV increase over the time as platelets swell in EDTA, with increase of 13.4% over 24 hours (Machin & Briggs 2010). Meanwhile MPV measured by an optical light scatter method decreases over the time, possibly as a result of the dilution of cytoplasmic contents leading to a decrease in light scatter (Diaz-Ricart *et al* 2010).

Different normal ranges for the MPV can result, influenced by factors such the anticoagulants used and the delay in time from sampling to analysis (Machin & Briggs 2010) and geographical areas and population structure (Demirin *et al* 2011; Cho *et al* 2012; Zhang & Huang 2014).

One limitation of our study might be the relatively small number of samples tested from healthy subjects compared with those of patients. Another potentially limiting factor of our study is that we tested samples only at two moments after blood collection (at 30 minutes and two hours) and we do not have information about the modification of MPV values between sampling and 30 minutes. The MPV values increases over time as platelets swell in anticoagulants, with increases of 7.9% within 30 minutes (Machin & Briggs 2010).

Conclusions

In our study we demonstrated that time and type of anticoagulants influence the MPV values, measured by impedance based method. Result from the present study show that storage of blood samples in EDTA based anticoagulant resulted in a progressive increase in the MPV and make EDTA not suitable as anticoagulant for the research on platelet function.

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