Preanalytical Factors Affecting the Mean Platelet Volume: a Review

Katalin S Zsóri[1,2], Judit M Mahalek[3], Mohammad A Mokarrami[4], Zoltán Csiki[5] and Amir H Shemirani[2,6,*]

[1]Central Pharmacy, Erzsébet Hospital, Sátoraljaújhely; [2]LabPharm Kft, Sátoraljaújhely; [3]Central Intensive Care Unit, Medical Center, Hungarian Defense Forces, Budapest; [4]Department of Surgery, Bács-Kiskun Hospital, Kalocsa; [5]Department of medicine, Debrecen university, Debrecen; [6]MTA-DE Vascular Biology, Thrombosis and Hemostasis Research Group, Hungarian Academy of Sciences, Debrecen, Hungary

Abstract: Mean platelet volume (MPV) as an indicator of platelet activation is a subject of many studies. Since the introduction of automated hematology analyzers, many authors described mean platelet volume as a marker for different pathologic conditions. It is also known that numerous preanalytical conditions could affect MPV results. Specimens are usually tested several hours after blood collection. There are some specific hematology analyzers which are in use by the majority of hematology laboratories. This review demonstrates some important aspects related to MPV measurement in routine laboratory.

Keywords: Mean platelet volume, Preanalytical variables, Hematology analyzer, Anticoagulation, Blood.

INTRODUCTION

Mean platelet volume (MPV) has been discussed as an indicator of platelet function: larger platelets are more reactive and related to a shortened bleeding time. It is established that the platelet volume is correlated with platelet activation and function [1]. Small platelets have lower functional abilities than larger ones [2]. Changes of MPV values have been shown in different patient groups [3-5]. MPV is universally available, an easy test to perform and economic in routine laboratories. Besides these attractive properties, there are some drawbacks concerning MPV measurements. We try to review some of the preanalyticalvariabilities related to platelet volume enumeration.

METHODS FOR MPV MEASUREMENT

Different analyzers have been used for MPV measurement and every finding specifically deserves mention, because the method and type of instrumentation is one of the co-variables to influence the results. Platelet volume has been measured by three different techniques in routine laboratories [6]. The electrical impedance method is based on the Coulter principle. When a dilute suspension of cells pass through a small aperture, each cell briefly changes the resistance of the electrical current between two electrodes on both sides of the aperture. Electrical impedance is proportional to the volume of the particle traversing the aperture, and so this method can estimate cell sizing and counting. A major disadvantage of the impedance method is that cell size analysis cannot discriminate large platelets from other similarly sized particles, such as small or fragmented red cells [7].

In optical method, a diluted blood specimen passes through the sensing zone, which a beam of laser light is focused. Scattered light is detected at a specific angle (one-dimensional) or two specific angles (two-dimensional), and converted to an electric impulse. The number of impulses generated is proportional to the number and volume of cells [8].

The third and new possibility to measure platelet count and platelet volume is optical fluorescence, which has been introduced recently on the Sysmexanalyzers. A polymethine dye is used in this technology to stain platelets and simultaneous counting of fluorescent platelets. The optical fluorescence count is more reliable at low platelet count and more appropriate for making clinical decisions, even for platelet transfusion [9].

It has been shown that for values in the normal range, three different methods demonstrated 20-25% variation in the measurement [6]. Another study calculated up to 40% difference between MPV measurements by impedance and optical methods [10].

ANTICOAGULANTS

Different anticoagulants and reagents is in use to minimize preanalytical activation *in vitro*, but most of them are hard to apply for routine laboratory because of impracticality [11]. It has been shown that standard

*Address correspondence to this author at the MTA-DE Vascular Biology, Thrombosis and Hemostasis Research Group, Hungarian Academy of Sciences, Debrecen, Hungary; Tel: +36306856608;
Fax: +3647525334; E-mail: shemirani1@gmail.com

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phlebotomy does not activate platelets significantly with different anticoagulants [12]. Salts of EDTA (dipotassium or tripotassium EDTA, disodium EDTA) with around 1.5 mg/ml of blood generally used for routine hematology testing and Na_3-citrate (0.109 mol/l or 0.129 mol/l) generally used for routine coagulation testing [13]. These anticoagulants have been used and studied for their roles in stabilization of platelet parameters [11, 14-24]. Thompson *et al* found K_3EDTA reliable anticoagulant for MPV evaluation after two hours of blood drowning, and Na-citrate proved as unreliable [14]. A different research group by using blood samples containing K_3EDTA or Na_2EDTA and impedance method demonstrated significant continuing to increase MPV over seven hours after phlebotomy [15]. Blood samples anticoagulated with citrate revealed the same trend. Macey *et al* showed platelet volume measured by K_3EDTA anticoagulated blood and optical method was reliable two hours postvenesection [11]. Another study by the impedance method revealed that both EDTA and citrate are reliable anticoagulants after one hour of sampling [16]. On the contrary, McShine *et al* observed a significant increase of MPV in K_3EDTA samples and decreased in Na_3citrated samples by impedance method after one hour of blood incubation at ambient temperature, which became stable after approximately four hours [20]. The same pattern of results was also obtained using optical methods, however, higher MPV results were observed for EDTA samples.

Cord blood has been stored in EDTA at ambient temperature for four hours, and measured by impedance method presented no significant change of MPV results compare to time zero [17]. They demonstrated that MPV was significantly increased after 6 hours of venesection at room temperature and it was in accordance with markedly elevated CD62P as a marker of platelet activation. Lancé and co-workers concluded that best time to measure MPV from EDTA and sodium-citrate anticoagulated samples is 120 and 60 minutes after venipuncture, respectively [18]. Diaz-Ricard *et al.* demonstrated that MPV in EDTA anticoagulated blood did not significantly increased compared to baseline during the first three hours of blood drawing by optical method [19]. They measured samples by impedance method and depicted a reference range for each anticoagulant.

K_3EDTA is recommended by the International Council for Standardization in Hematology as the anticoagulant of choice for complete blood count [25]. Several studies have been published on the stability of MPV results against various conditions. Lance *et al*. demonstrated that platelets swell in the first two hours of phlebotomy in EDTA and in the first hour in citrate by impedance method [18]. They also concluded that platelet size stored in citrate is significantly smaller compared to those stored in EDTA. But some researches show MPV may be measured in sodium citrate with a better accuracy and reproducibility than in EDTA. Furthermore, such measurements are not influenced by incubation time, unlike for EDTA [22]. In blood collected in K_3EDTA, there was an increase in MPV in the first two hours, but sodium citrate proved unreliable for the measurement of MPV [14]. Trowbridge *et al*. found EDTA an unreliable anticoagulant to measure platelet volume [10]. On the contrary, Reardon *et al* showed MPV to be stabilized by sodium citrate and prostaglandin E1 together by the impedance method but not optical method [24]. In another study, MPV measured by fluorescent flow cytometry method and significantly increased in EDTA after three hours incubation at room temperature [26]. According to their results, false higher MPV can be overcome by sample storage at 4°C.

DISCUSSION AND CONCLUSION

Pre-analytical variables, such as storage time and temperature, type of anticoagulant and methodology of measurement, affect the evaluation of MPV. Even different concentrations of a specific anticoagulant result in various platelet swelling [22]. Artificial platelet swelling and shape change after addition of anticoagulant to the sample is known for a while [27]. The effect of EDTA on platelet structure is not completely understood. However, it is partly explained by modifications of both the membranes lining the canalicular system and those forming the platelet cell wall by exposure of platelets to EDTA [28]. Besides, phosphorylation patterns of platelet proteins change by exposure to EDTA [19].

As an MPV increases over time by exposure to some anticoagulants, this elevation was shown to be proportional to the delay in time between blood sampling and laboratory analysis. For reliable MPV measurement, researchers must carefully control the possible influence of anticoagulant on the MPV, either standardizing the incubation time between phlebotomy and analysis or using an alternative anticoagulant or additive.

Platelet aggregation by any means causes incorrect MPV measurement. Although EDTA is used in the

everyday hematological laboratory, it induces pseudothrombocytopenia [29], and heparin or citrate usage instead of EDTA did not overcome *in vitro* platelet aggregation [30]. Alternative anticoagulant has been suggested to inhibit pseudothrombocytopenia [20, 31-33].

Difference between results from different authors may come from a number of individuals included in the study, different method of comparison, or use of different physical principles to measure MPV. Neither impedance method, nor optical method takes into account the shape change of platelets [34]. As a result, these methods do not agree with each other.

In conclusion, until the standardization of MPV measurements for routine practical use, thorough description of MPV assessment is necessary *i.e*., technique, type and concentration of anticoagulant, storage time and temperature [35]. In this way, we may increase use of MPV as an effective diagnostic and prognostic factor in different pathologic disorders.

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