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SHORT COMMUNICATION

Anticoagulant Choices Affect the Mean Platelet Volume Measurement by Impedance

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SUMMARY

Background: **Mean platelet volume (MPV) is a parameter that evaluates the platelet size. Clinical applications of MPV are limited because of its poor standardization in routine laboratories. This study analyzed the effect of anticoagulants on MPV measurements by impedance technology.**
Methods: **Blood from 36 healthy volunteers was collected in vacuum tubes filled with K2EDTA and sodium citrate, analyzed immediately (basal) and at 1, 2, and 3 hours after venipuncture.**
Results: **Comparisons between the anticoagulants demonstrated a significant difference ($p < 0.05$) after 1 hour of exposure with K2EDTA, causing a time-dependent increase on MPV measured. No significant changes in MPV were observed with sodium citrate with 3 hours of exposure ($p > 0.05$).**
Conclusions: **The use of sodium citrate is highly indicated for assessment of MPV when the measurement time after blood collection is estimated to be more than 1 hour.**
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INTRODUCTION

The mean platelet volume (MPV) is a parameter that evaluates platelet size and can be determined using an automated hematology analyzer, either by electrical impedance or by light scattering using optical technology for complete blood count (CBC) [1-5]. The MPV has an important role in predicting the regenerative character of thrombocytopenia, distinguishing between increased peripheral destruction and decreased bone marrow platelet production [1,6,7]. Several studies have shown the relationship of increased platelet size as a marker of platelet activation and its importance as a predictor of cardiovascular diseases and venous thromboembolism [3,4,8,9]. However, clinical applications of the MPV are limited because of its poor standardization in routine

laboratories [3,7,10,11]. Reference values provided by the manufacturers are according to technologies developed for each type of analyzer. Other pre-analytical factors such as the type of anticoagulant, storage time, and temperature or interference from cellular debris may cause variations in MPV values, which lead to underutilization of this clinical parameter [1-4,7,12].

Dipotassium ethylenediaminetetraacetic acid (K2EDTA) and sodium citrate are anticoagulants widely used in laboratory routines [13,14]. EDTA is the anticoagulant of choice for CBC, while citrate is mostly used in coagulation tests [13-16]. EDTA acts as a binder hexadentate that chelates calcium through the binding of four carboxylic acid groups and two amine groups, forming a stable EDTA-calcium complex [13,15]. Citrate also binds the ionized calcium present in blood to form a non-ionized calcium-citrate complex, but not as strongly as EDTA. Calcium ions participate in platelet activation and coagulation cascades [16,17]. The chelator agents, in irreversible (EDTA) or reversible (sodium citrate) form, prevent clot formation and, consequently, the blood is kept fluid [2,15]. However, platelet activation can occur even in the absence of calcium ions chelated by these anticoagulants. EDTA has been pointed out to modify platelet ultrastructure and activity more than citrate, causing changes in platelet shape and volume [5,12,13,16]. Replacement of EDTA by citrate as an anticoagulant has been appraised, but this issue is questionable [12,15,16].

The aim of this study was to evaluate the influence of K2EDTA and sodium citrate, and the time of storage from blood collection on MPV measurements, in samples analyzed under the same pre-analytical conditions using the ABX Pentra 80 hematology analyzer (Horiba Diagnostics).

MATERIALS AND METHODS

Samples were obtained from 36 healthy volunteers (24 women, 12 men; age range 20 - 45 years) with normal CBC values. The Ethics Committee of the Federal University of Paraná approved the study, and written informed consent was obtained from the subjects.

Venous blood specimens were collected using syringes and transferred first to a tube containing sodium citrate 0.105 M and then to another tube containing K2EDTA, both vacuum tubes were from BD Vacutainer (Franklin Lakes, NJ, USA). Tubes were filled with blood and mixed according to the manufacturer's recommendations. The ABX Pentra 80 performed the CBC measurements and the MPV according to impedance technology. All settings and configurations of the hematology analyzer followed the manufacturers' recommendations. Quality control (three levels) from Horiba (Montpellier, France) was used to ensure good performance.

The MPV was determined in samples with K2EDTA and sodium citrate immediately after venipuncture (up to 5 minutes, basal), and at 1, 2, and 3 hours. All samples were stored at room temperature (21 - 24°C). Time and temperature were stringently controlled during the study. All samples showed platelet counts in the reference range (112 - 444 x 10^9/L). All samples were mixed by inversion by automated homogenization for 5 minutes prior to analysis.

The MPV measurements showed normal distribution assessed by the Kolmogorov-Smirnov test. Comparisons were performed with Student's *t*-test (two-sided) and analysis of variance (ANOVA) using Statistics 10.0 (StatSoft) software. The criterion for statistical significance was set at the nominal probability (p) level < 0.05.

RESULTS

The comparison of the K2EDTA and sodium citrate effects on MPV at different times are shown in Figure 1. In the basal group, no significant differences ($p = 0.280$) were observed between the studied anticoagulants at values of MPV for exposed platelets. After 1 hour of storage, a significant difference was observed ($p < 0.001$). K2EDTA showed a continuous increase in MPV with 8.1%, 10.8%, and 12.6% of basal values at 1, 2, and 3 hours of exposure, respectively. Sodium citrate showed less than half of the K2EDTA variation, with 3.0%, 3.1%, and 4.8% compared to the basal value.

When all MPV means were statistically compared (using ANOVA), only K2EDTA showed a significant difference ($p < 0.001$), while sodium citrate did not ($p = 0.082$).

DISCUSSION

Ours results are in agreement with previous studies reporting that EDTA causes an increase in MPV values [2,6,18-20]. These results point out that the progressive increase in MPV occurs from the first hour up to a plateau [5,12,15]. Beyan and Beyan published a meta-analysis regarding the standardization of MPV measurements and reported that the venipuncture between 15 minutes and 2 hours was significantly different from the measurement times < 15 minutes and > 2 hours, describing a variation of up to 12.5%, which was in close agreement with our findings for K2EDTA [11].

Exposure to EDTA leads to alterations in platelets from disc to spherical shape; this is similar to those occurring during platelet activation, due to increases in intracellular cyclic AMP and alterations in plasma membrane permeability, inducing progressive cellular swelling and increases in apparent volume when the platelet is measured by the aperture-impedance method [5,6,19,20]. We demonstrated that sodium citrate interferes with MPV measurements with less intensity than K2EDTA after 1 hour of exposure (differences of 0.6 fL) and that this change in MPV is time-dependent [5,6,13,16,18, 20].

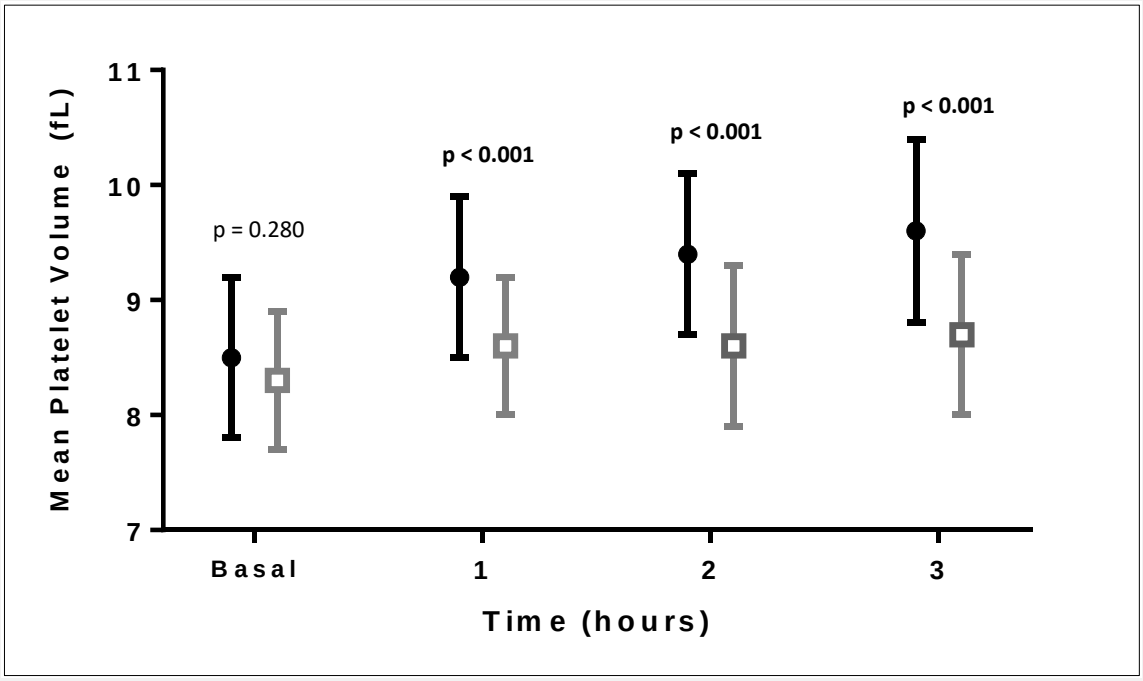

Figure 1. Mean platelet volume measurement at different times after exposure to anticoagulants.

The symbols are means (closed circles are K2EDTA and open squares are sodium citrate). Vertical bars are 1-standard deviation.
P, probability according to Student's *t*-test (two-sided). Significant p-values are in bold.
ANOVA for K2EDTA ($p < 0.001$) and for sodium citrate ($p = 0.082$).

The use of sodium citrate alone or in combination with other additives such as prostaglandins, ACD (acid-citrate-dextrose), or ACD/EDTA has been satisfactory in regards to the measurement of MPV [5,12,14,19]. A study using the hematology analyzer Cell-Dyn 4000 (Abbott Diagnostics) suggested that citrate is a suitable anticoagulant for routine CBC with normal ranges, not only in cases of thrombocytopenia induced by EDTA. The authors considered this possibility valid, whereas citrate was used with a corrected dilution. The citrate did not affect the quality of blood smears and could improve medical applications such as blood counts and coagulation tests [14].
We used K2EDTA as currently recommended by the International Council for Standardization in Hematology (ICSH) because the kind of salt could have influenced the results of MPV measurements [12,14-16].

CONCLUSION

In summary, we demonstrated that K2EDTA and sodium citrate showed no difference in MPV measurements only if the analysis was performed immediately after venipuncture. The blood-anticoagulant contact increases the MPV measurement significantly with K2EDTA. Sodium citrate showed minor effects on MPV measurement and should be recommended when the measurement time after blood collection is estimated to be more than 1 hour.

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Declaration of Interest:
The authors state that they have no conflict of interest.

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