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Which Platelet Index and Anticoagulation Method Should We Use? MPV or MPV/PC Ratio as an Index? EDTA or Citrate as Anticoagulant?

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Abstract

Purpose: This study to compare the effectiveness of anticoagulation with ethylenediamine tetraacetic acid (EDTA) and citrate on MPV measurement.
Methods: EDTA and citrate-based anticoagulant blood samples from the same patients were read in the auto-analyzer at 0, 30, 60, and 120 minutes after sampling and the results were compared.
Results: A total of 54 patients, 29 of whom were women, over the age of 18 were included in the study. Baseline blood MPV values were found to be 0.524 fL greater in the EDTA-based group ($p<0.001$). When the difference between the time periods in the EDTA group was examined, it was observed that there was a significant increase in each deltaMPV value. When the difference between the time periods in the citrate-based group was examined, there was a significant difference at the 30th and 60th minutes ($p<0.001$), however the difference disappeared at the second hour ($p>0.05$). When the deltaMPV values of the EDTA and Citrate groups were compared, it was found that there was no difference at 30 and 60 minutes ($p=0.531$; $p=0.566$, respectively). In addition,it was found that there was no significant difference between deltaMPV/Platelet count ratios (deltaMPV/PC) in all time periods in the EDTA group ($p>0.05$) and there was a significant difference between all time periods in the citrate group ($p<0.001$).
Conclusions: Results in the first hour were similar in both anticoagulation groups. However, additional increases were observed in each half-hour period in both groups. When EDTA is used as an anticoagulant, MPV/PC ratio performs better than MPV.

Keywords: ***EDTA, Sodium citrate, MPV, MPV/PC ratio, platelet indices, anticoagulation method***

Introduction

Platelets play an important role in immune reactions, fibrosis, and normal hemostasis. Platelets are activated by classical agonists such as PAF, TXA2, ADP and inflammatory cytokines such as IL-1, IL-6, TNF-alpha and accumulate in the damaged area and initiate fibrosis and inflammation by releasing their contents [1]. Platelets play an important role in the inflammation cascade in many rheumatological diseases neutrophils and lymphocytes [2]. Mean platelet volume (MPV) is a measure of the mean size of platelets and is widely used as a platelet activation marker. Large platelets secrete more proinflammatory cytokines and prothrombotic factors than small platelets. Therefore, it is accepted that MPV can be used as an indicator of platelet activation and severity of inflammation [3]. It has been shown that MPV can be used as a biomarker to predict increased activity or disease severity in some diseases [4-6].

The blood samples should be anticoagulated in order to enable electronic cell counters to count blood cells. For this purpose sodium citrate or ethylenediamine tetraacetic acid (EDTA) is used, generally. In previous studies, it has been shown that EDTA causes swelling in the platelet by increasing intracellular cAMP and increasing the permeability of the plasma membrane, which results in an increase in MPV [7]. İt has been reported that the increase in MPV may reach up to 50% as the time elapses after the contact of blood with EDTA [8]. In a study conducted by Bath et al., it has been shown that MPV will increase as the time elapses until the blood is counted, when EDTA is used as an anticoagulant and they suggested that tests can be done more safely by using citrated blood [9]. In the literature, whether the MPV level is lower or higher in FMF patients compared to healthy controls is still controversial. Sakallı et al. in their study on FMF induced amyloidosis patients found a significant correlation between proteinuria and MPV [4]. However, in a similar study by Bakan et al., such a correlation was not found [10]. Hartmann et al. in their study on SLE patients, found that MPV was inversely proportional to disease activity and MPV and decreased when disease severity increased [11]. On the other hand, Uzkeser et al. in a similar study on SLE patients found that MPV increased with disease activity [12].

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It is known that varying results can be obtained due to the lack of a standardized method of MPV, technical differences in electronic cell counters, different anticoagulants used, and the variability of the time elapsed between the contact of the blood with the anticoagulant and it is put into the counter. In the year 2022, considering the factors such as the use of advanced devices that we use in the modern era and the prolongation of the time between the anticoagulant contact and the reading of the blood after the construction of large-scale hospitals, it is not known exactly how far we advanced in this regard. We conducted this study, considering that there is a need for an up-to-date study since there are conflicting results in the literature.

Materials and Methods

Study design and study population

This prospective study was conducted in a single center. The patients who admitted to Internal Medicine Department between April 15 and May 15, 2022 and over the age of 18 were included in the study. Patients with Wiskott-Aldrich syndrome, essential thrombocythemia, splenectomy, hereditary macrothrombocytopenia, hematological or solid organ malignancies, those using antiaggregant or anticoagulants, alcohol users, pregnant women, and patients receiving immunosuppressive therapy and chemotherapy were excluded from the study. A written informed consent was obtained from each patient for all diagnostic procedures. The study protocol was approved by the institutional Ethics Committee (Date: 05.04.2022/ No: E-10840098-772.02-2203). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data collection

Blood samples were collected from the patients who admitted to the internal medicine outpatient clinic and met conditions for the study and the results were recorded in digital media. Data including age, gender, height, weight, blood pressure, comorbidities such as hypertension, diabetes, heart failure or coronary artery disease, and complete blood count results were recorded. Blood samples of the patients (n=54) were collected from the brachial vein in the antecubital region and stored in two tubes, one with sodium citrate and one with EDTA, for blood count. To collect samples in a citrate tube, 0.5 ml of sodium citrate and 4.5 ml of blood were added and mixed gently by inverting the stoppered tube. Tripotassium ethylenediaminetetraacetic acid, also known as K3 EDTA, was used to prepare the tube with EDTA. The collected blood samples was stored at room temperature (20-22 0C). Sysmex XN-550 (Sysmex, Japan) autoanalyzer device was used for blood count. MPV was measured by impedance technology with Sysmex XN-550 hematological analyzer. The duraiton between the collection of the blood samples and reading on the automatic blood cell counter was 10 minutes, on average and this initial count was defined as the zeroth minute (basal blood). Then, at the next 30, 60 and 120 minutes, the same samples were read again in the auto-analyzer.

At the time of admission, complete blood count analysis including hemoglobin level, leukocyte count, platelet count (PC), absolute neutrophil count, absolute lymphocyte count, and MPV was performed for each patient. Relevant ratios for MPV/PC ratio, neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) were calculated and included in the analysis.

Statistical analysis

Statistical analyses were performed by using the SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency, where applicable. The student t-test was used to compare normally distributed variables between the groups and the Mann-Whitney U test was performed to compare non-normally distributed variables between the groups. Categorical variables were analyzed by using the chi-square test. Pearson correlation coefficient was used to analyze the correlation between the EDTA group and citrate group. A value of $p<0.05$ was considered statistically significant.

Results

After applying the exclusion and inclusion criteria, 54 volunteer patients over 18 years of age, 29 of whom were female, were included in the study. The mean age of thepatients was 43.1±13.9 years and the mean age of the male group (49.2±11.7) was significantly greater compared to the females (37.7±13.7) ($F=1.08$, $p= 0.002$). The distribution of the genders was similar ($\chi2=0.296$, $p=0.586$). The mean body mass index (BMI) was 26.6±4.7, and there was no difference between the genders in terms of BMI values ($F=2.86$, $p=0.073$). The rate of smokers was 16.7% (n=9). Systolic, diastolic and mean arterial blood pressures and comorbidities of the patients are shown in Table 1.

When basal blood (zero-minute blood) values were compared between the two groups, it was observed that WBC, neutrophil, lymphocyte, platelet, Hb, and Hct values were significantly higher in the EDTA group (**Table 2**). The MPV values obtained in the basal blood count were found to be 0.524 fLgreater in the EDTA group compared to the citrate group ($t=14.2$; $p<0.001$). When the deltaMPV values were compared according to the time intervals including 0-30, 30-60, 60-120 minutes, a significant increase was observed (deltaMPV30-0-EDTA: 0.285±0.2 fL; $t=9.7$; $p<0.001$, deltaMPV60-30-EDTA: 0.194±0.2 fL; $t=8.5$; $p<0.001$, and deltaMPV120-60-EDTA: 0.130±0.2 fL; $t=4.7$; $p<0.001$, respectively). When the deltaMPV values were compared according to the time intervals including 0-30, 0-60 and 0-120 minutes, again it was observed that deltaMPV values increased significantly (deltaMPV30-0-EDTA: 0.285±0.2 fL; $t=9.7$; $p<0.001$, deltaMPV60-0-EDTA: 0.480±0.2, fL; $t=16.9$; $p<0.001$, and deltaMPV-120-0-EDTA: 0.609±0.3 fL; $t=14.4$; $p<0.001$, respectively). (**Table 3**).

In the citrated blood group, when the MPV values were compared according to the time intervals including 0-30, 30-60 and 60-120 minutes, it was found that there were significant differences between the baseline value and 30th and 60th minutes (deltaMPVCit-30-0: 0.320±0.3 fL $t=7.2$, $p<0.001$; deltaMPVCit-60-30: 0.130±0.3 fL, $t=2.9$, $p=0.005$ respectively), while there was no significant difference at the 2nd hour (deltaMPVCit-120- 60; -0.056±0.3 fL, $t=-1.4$, $p=0.172$) (**Table 3**).

When the deltaMPV values of the groups were compared, no significant differences were found at 30 and 60 minutes (respectively delta-deltaMPV30-0: -0,035 fL, t=-0,6, p=0,531; delta-deltaMPV60-0: 0,030 fL, t=0,6, p=0,566; delta-deltaMPV60-30: 0,065 fL, t=1,4, p=0,182), however we found that deltaMPV values were significantly higher in the EDTA group at 120 minutes (delta-deltaMPV120-60: 0,185 fL, t=3.9, p<0.001) (**Table 3**).

When the MPV/PC ratios were compared in the groups, no significant differences were found between the baseline, 30, 60, and 120 minutes in the EDTA group ($p>0.05$), while significant differences were found between the MPV/PC ratios at 30, 60 and 120 minutes in the citrate group ($p<0.001$) (**Table 3**).

There was no significant difference in MPV, delta MPV, and MPV/PC harvested with EDTA or Citrate between patients with or without diabetes, coronary artery disease, and hyperlipidemia ($p>0.05$). In addition, there was no significant differences of MPV values according to the presence of hypertension in both groups ($p>0.05$). It was found that, MPV/PC ratio at 30, 60 and 120 minutes in the EDTA group were higher in patients with hypertension ($U=231$, $p<0.01$; $U=222$, $p<0.05$; $U=214$, $p<0.05$ respectively), however no significant relationship was found in the citrate group ($p>0.05$).

Table 1: Demographic and clinical characteristics of patients

Age, year		**N**	**Percent**	**Mean**		**Value**	**p**
	total female male	54 29 25	% 	43,1±13,9 37,7±13,7 49,2±11,7	min=18; max=68 min= 18; max= 67 min= 25; max= 68	F=1,08	0,002
Gender	female male	29 25	53.7 46.3			$\chi 2$=0,296	0,59
BMI, kg/m^2	total female male	54 29 25	100 	26,6±4,7 25,5±4,9 27,8±4,2	min=15,4;max=40,3 	F=2,86	0,07
Systolic BP, mmHg		54		114,6±7,9	min=100; max=145		
Diastolic BP, mmHg		54		78,2±4,5	min=64; max= 95		
MAP, mmHg		54		90,4±5,0	min=79,3;max= 112		
Smoke	smoker	9	16.7				
	non-smoker	45	83.3				
Diabetes		10	18.5				
Hypertension		6	11.1				
CAD		2	3.7				
Hyperlipidemia		6	11.1				

χ^2=Pearson Chi-Square, BMI= Body mass index, Systolic BP= systolic blood pressure, Diastolic BP= diastolic blood pressure, MAP= mean arterial pressure, CAD= coronary artery disease.

Table 2: Baseline (0. min) Complete blood count analysis

		N	Mean ± SD	Min - max	T	p
WBC, (x10^6/L)	EDTA	54	6743,3±1539,6	Min=3510; max=10530	14,6	<0,001
	Citrate	54	6050,7±1363,1	Min=3370; max 9580		
Neutrophil, (x10^6/L)	EDTA	54	3806,3±1058,4	Min=1760; max 6710	16,7	<0,001
	Citrate	54	3392,2±951,6	Min=1650; max=6140		
Lymphocyte, (x10^6/L)	EDTA	54	2227,1±721,6	Min=990; max=4420	9,9	<0,001
	Citrate	54	2007,4±638,9	Min=890; max=4090		
Hgb, (g/dL)	EDTA	54	13,5±1,7	Min=9,2; max=17,0	28,2	<0,001
	Citrate	54	12,1±1,5	Min=8,3; max=15,1		
Hct, (%)	EDTA	54	40,5±4,7	Min=28,8; max=50,1	25,0	<0,001
	Citrate	54	36,7±4,2	Min=26,0; max=45,0		
PC (x10^9/L)	EDTA	54	244,5±58,8	Min=63,0; max=369,0	12,3	<0,001
	Citrate	54	185,5±41,6	Min=82,0; max=275,0		
MPV, (fL)	EDTA	54	10,2±0,8	Min=8,7; max=12,4	14,2	<0,001
	Citrate	54	9,6±0,8	Min=8,3; max=12,1		
MPV/PC	EDTA	54	0,045±0,02	Min=0,03; max=0,18	-3,4	<0,001
	Citrate	54	0,056±0,02	Min=0,03; max=0,15		
NLR	EDTA	54	1,87±0,8	Min=0,77; max=5,37	1,1	0,295
	Citrate	54	1,85±0,9	Min=0,74; max=5,45		
PLR	EDTA	54	0,12±0,05	Min=0,02; max=0,28	8,9	<0,001
	Citrate	54	0,10±0,04	Min=0,04; max=0,23		

SD: standard deviation; WBC: white blood cell; Hb: haemoglobin; Hct: haematocrit, PC: platelets count; MPV: mean platelet volume, MPV/PC: mean platelet volume to platelet count ratio, NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; EDTA; etilendiamin tetraasetik asit.

Table 3: Differences between time zones and anticoagulants

Paired-Samples T Test	Mean (fl)	SD ±	95% CI		t	Df	Sig (2-tailed)
			Lower	Upper			
deltaMPV-30-0-EDTA	,285	,22	,23	,34	9,7	53	,000
deltaMPV-60-0-EDTA	,480	,21	,42	,54	16,9	53	,000
deltaMPV-120-0-EDTA	,609	,31	,52	,69	14,4	53	,000
deltaMPV-60-30-EDTA	,194	,17	,15	,24	8,5	53	,000
deltaMPV-120-60-EDTA	,130	,20	,07	,19	4,7	53	,000
deltaMPV-30-0-Citrate	,320	,33	,23	,41	7,2	53	,000
deltaMPV-60-0-Citrate	,450	,35	,36	,54	9,6	53	,000
deltaMPV-120-0-Citrate	,394	,34	,30	,49	8,5	53	,000
deltaMPV-60-30-Citrate	,130	,33	,04	,22	2,9	53	,005
deltaMPV-120-60-Citrate	-,056	,30	-,14	,02	-1,4	53	,172

deltaMPV-0 (EDTA-Citrate)	,524	,27	,45	,60	14,2	53	,000
deltaMPV-30 (EDTA-Citrate)	,489	,37	,39	,59	9,6	53	,000
deltaMPV-60 (EDTA-Citrate)	,554	,31	,47	,64	13,1	53	,000
deltaMPV-120(EDTA-Citrate)	,739	,35	,64	,83	15,4	53	,000
delta-deltaMPV-30-0 (E-C)	-,035	,41	-,15	,08	-,60	53	,531
delta-deltaMPV-60-0 (E-C)	,030	,37	-,07	,13	,60	53	,556
delta-deltaMPV-60-30 (E-C)	,065	,35	-,03	,16	1,4	53	,182
delta-deltaMPV-120-0 (E-C)	,215	,44	,10	,33	3,6	53	,001
delta-deltaMPV-120-60 (E-C)	,185	,35	,09	,28	3,9	53	,000
deltaMPV/PC-30-0-EDTA	,004	,02	-,00019	,0077	2,0	53	,062
deltaMPV/PC-60-0-EDTA	,003	0,02	-0,0008	,0066	1,54	53	0,130
deltaMPV/PC-60-30-EDTA	,003	,01	-,001	,0066	1,5	53	,130
deltaMPV/PC-120-0-EDTA	,004	,02	-,001	,0083	1,8	53	,071
deltaMPV/PC-30-0-Citrate	,009	,01	,005	,0129	4,7	53	,000
deltaMPV/PC-60-0-Citrate	,019	,03	,011	,0265	4,8	53	,000
deltaMPV/PC-120-0-Citrate	,025	,03	,0162	,0331	5,8	53	,000

SD: standard deviation, MPV: mean platelet volume, MPV/PC: mean platelet volume to platelet count ratio, EDTA: Etilendiamintetraasetik asid, (E-C): EDTA-Citrate, PC: platelets count.

Discussion

This study was designed to examine the changes in MPV values as the time elapsed between the contact of blood in hemogram tubes containing EDTA and citrate as anticoagulants and loading into the automatic blood count device. The basal, 30th, 60th and 120th minute MPV values of the blood samples in EDTA group were found to be significantly higher compared to the citrated blood (0.524 fL, t=14.2, p<0.001; 0.489 fL, t= 9.6, p<0.001; 0.554 fL, t=13.1, p<0.001; and 0.739 fL, t=15.4, p<0.001, respectively) (Table 3). As the time elapsed until the EDTA blood was read in the automatic blood count device, significant increases were observed in MPV values in every half an hour (deltaMPV30-0: 0.285 fL, p<0.001; deltaMPV60-30: 0.194 fL, p<0.001; and deltaMPV120-60: 0.130 fL, p<0.001) (Table 3). Similarly, significant increases were observed in MPV values in the 30th and 60th minutes until the citrated blood was read in the automatic blood count device, however the MPV values remained stable at the second hour and no significant change was observed (deltaMPV30-0: 0.320 fL, t=7.2, p <0.001; deltaMPV60-30: 0.130 fL, t=2.9, p=0,005; deltaMPV120-60: -0.056 fL, t=-1.4, p=0.172, respectively) (**Table 3**).

When the deltaMPV values at 0-30 and 30-60 minutes were compared between the groups, no significant differences were found (deltadeltaMPV0-30: -0.035 fL, t=-0.6, p=0.531; deltadeltaMPV60-30: 0.065 fL, t=1,4, p=0,182; deltadeltaMPV 60-0: 0,030 fL, t=0,60, p=0,556, respectively) (Table 3). However the difference between the groups were significant in terms of the deltaMPV values at 60-120 minutes (deltadeltaMPV60-120: 0.22 fL, t=3.6, p<0.001). If the blood samles will be loaded into an auto-analyzer in the first hour, EDTA or citrate-based anticoagulation may be preferred in studies related to MPV, since similar results will be obtained in both anticoagulation groups. However, since there are significant differences between the MPV values at 0, 30 and 60 minutes in both anticoagulation, we think that a standardization should be done in the studies. For this reason, we suggest that both control and case samples should be examined within a narrow time frame such as the first 30 minutes or the second 30 minutes.

When we searched Pubmed for MPV, we found that 2183 articles were written in the last five years. In a study by Dastjerdi et al., similar to this study, it was reported that the results were similar in both anticoagulation types if the collected blood samples were loaded into the autoanalyzer and the differences were not significant. However, they did not examined the differences for each half-hour period of the first hour [13]. In a study by Lancé et al., MPV was measured every half hour for four hours. Similar to our study, they emphasized that platelets swelled until the first 120 minutes in EDTA-based blood, whereas platelet swelling in citrate-based blood occured only in the first hour. However, in this study, it was not emphasized that the measurements should be made especially in the same half-hour time period of the first one-hour [14]. In the study conducted by Bath et al., it was reported that MPV was not affected when low concentrations of citrate were used, and therefore measurement with citrate anticoagulation was safe [7]. On the other hand, in our study, we observed that significant increases in MPV continued with citrated blood as the incubation period extended in the first hour. The differences between the half-hours in the first hour were even significant. In the study conducted by Mannuß et al., it was determined that the swelling in platelets continued for the first 3 hours in anticoagulation with EDTA. Again, in the same study, it was determined that the swelling in platelets was milder in citrated blood, but there was no change in anticoagulation with MgSO4 [15].

MPV value has been widely used in many studies to determine disease progression and disease activity, as well as to detect the need for intensive care earlier in some cases, and to predict mortality. However, the results of many studies are frequently contradictory. Khan et al., in their study found no change in MPV in patients with psoriasis [16]. Ghodsirad et al. in their study found that MPV can be used as a predictor of myocardial perfusion defect [17]. Dinçer et al. found that although NLR can be used as an indicator of subclinical inflammation in FMF patients, MPV and PLR cannot be used for this purpose [18]. In the study conducted by Akbaş in pediatric group migraine patients, it was found that PC number increased whereas MPV values decreased compared to the control group and they concluded that inflammation plays an important role in the pathophysiology of migraine [19]. In the study of Vélez-Páez et al., it was suggested that MPV and MPV/PC ratio could be used as a predictor of disease severity and mortality in patients with sepsis [6]. There are publications stating that measurement of MPV may be useful in cardiovascular disease risk assessment [20, 21], whereas disease in a study on patients with metabolic syndrome, it was emphasized that larger platelets are not associated with the incidence and severity of coronary artery disease [22]. In another study, MPV was found to be a predictor of early prognosis in ischemic stroke, whereas PC was found to be a predictor of early prognosis in hemorrhagic stroke [23].

The conflicting results related to MPV in the literature has caused these results to be questioned. In most studies, it has been determined that MPV values increase more significantly when EDTA is used as an anticoagulant. Similarly, in our study, the increase in MPV values was found to be significantly higher in the EDTA group. However, we found that MPV was also significantly increased in the citrate group, although not as much as in the EDTA group. of both groups. When the deltaMPV values at 0-30, 0-60 and 30-60 minutes were compared, we found that there was no

significant difference between both groups (p>0.05). However, while a significant increase in MPV value continued in EDTA blood, it remained stable in citrated blood, after the first hour. As a result, deltaMPV values at the 2nd hour were found to be significantly higher in the EDTA group compared to the citrate group (p<0.001). Therefore, if the samples are loaded into the analyzer within the first hour, one of the two anticoagulated blood may be preferred. However, we concluded that whether the studies should be performed in the first or second half of the first hour should be standardized, in order to increase the power of the studies and to minimize the variability.

The relationship between PC and MPV/PC ratio and diseases has been investigated in many studies. Similar to previous studies, we also found an inverse correlation between MPV and PC in the measurements made with both anticoagulants (r= -0.35 for EDTA, p=0.009; r= -0.29 for citrate, p=0.031) [24]. Zhong et al., in their study on COVID-19 patients, concluded that MPV/PC ratio could be used as a useful marker predicting severe pneumonia [25]. In a study conducted on patients with glioblastoma multiforme, the MPV/PC ratio was found to be an independent predictor of survival without progression [26]. Lin et al., in their study, concluded that the MPV/PC ratio can be used as an independent risk factor associated with disease progression in various cancer types [27]. In our study, while there was no significant difference between deltaPC30, deltaPC60 and deltaPC120 in the EDTA group (p>0.05), a significant decrease in PC was observed in the citrate group as the time elapsed, resulting in a significant difference between deltaPC30, deltaPC60 and deltaPC120 (p<0.001). When we compared the MPV/PC ratio, there was no significant difference between the 30th, 60th and 120th minutes in the EDTA group (p>0.05), whereas MPV/PC at the 30th, 60th and 120th minutes differed significantly in the citrate group, due to the decrease in PC over time (p<0.001) (Table 3). We concluded that EDTA should be preferred as an anticoagulant, since there is no time limitation in studies related to MPV/PC ratio and there is no significant difference between time periods. Since the MPV/PC ratio obtained in EDTA blood did not change for 2 hours, we suggest that it may be preferred over MPV. Likewise, since there was no significant change in PC, we concluded that EDTA should be used as an anticoagulant without time constraints.

In the study conducted by Icli et al., it was shown that MPV increased in patients with familial hypercholesterolemia and it was independently associated with total cholesterol level [28]. However, in our study, no correlation was found between MPV or MPV/PC ratio and hyperlipidemic patients (F=1.16, p>0.05, F=21.2, p>0.05, respectively). The fact that this was a small-scale study or the normalization of total cholesterol values with the hypolipemic drugs used by the patients may have affected it. In the previous studies it was shown that both gestational diabetes and uncontrolled diabetes are positively correlated with MPV [29, 30]. However, in our study, no correlation was found between MPV, PC and MPV/PC ratio and diabetes in both anticoagulated groups (p>0.05). In a study conducted by Sansanayudh et al., it was shown that increased MPV is associated with coronary artery disease. However, we did not find such a relationship in our study (p>0.05) [20]. In addition, no correlation was found between the MPV values and hypertension, in both groups (p>0.05). On the other hand, although there was a significant correlation between MPV/PC ratio and hypertension in the EDTA group at 30th, 60th, and 120th minutes (U=231, p=0.017; U=222, p=0.032; U=214, p=0.048, respectively), no relationship was detected in the citrate group (p>0.05).

Limitations

The limitations of our study include the small sample size, the limitation of the study to 120 minutes, and not being able to verify MPV values with different measurement methods.

Conclusions

If the blood is loaded into automatic blood cell autoanalyzers in the first hour, since there is no difference between the use of citrate or EDTA as an anticoagulant, any of them can be preferred. However, since the increase in MPV values differed significantly between each 30-minute time frame of the first hour, in both groups, we suggest that the measurements should be performed in the same half-hour time frames. We concluded that EDTA should be preferred as an anticoagulant because there is no time limitation and no significant difference between time periods in studies related to MPV/PC ratio. We concluded that it would be more appropriate to prefer MPV/PC ratio to MPV values, since there was no difference even between each 30-minute time frame for the first 2 hours.

Declarations

Ethics approval and consent to participate

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Istanbul Medipol University Clinical Research and Ethic Committee (Date: 05.04.2022/ No: E-10840098-772.02-2203) and with the 1964 Helsinki Declaration.

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Conflict of interests

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Authors' contributions

TK; Design, data collection, methodology, data entry, calculation, formal analysis and writing. IB; Design, data collection, methodology, data entry, calculation, formal analysis. All authors have read and approved the final manuscript.

References

1. Linke B, Schreiber Y, Picard-Willems B, Slattery P, Nüsing RM, Harder S, et al. Activated Platelets Induce an Anti-Inflammatory Response of Monocytes/Macrophages through Cross-Regulation of PGE(2) and Cytokines. Mediators of inflammation. 2017;2017:1463216.
2. Kucuk A, Uslu AU, Ugan Y, Bagcaci S, Karahan AY, Akarmut A, et al. Neutrophil-to-lymphocyte ratio is involved in the severity of ankylosing spondylitis. Bratislavske lekarske listy. 2015;116(12):722-5.
3. Gorial FI. Diagnostic value of mean Platelets volume in ankylosing spondylitis as a predictor of disease activity. International Journal of Medical Research & Health Sciences. 2018;7(8):40-45.
4. Sakallı H, Kal O. Mean platelet volume as a potential predictor of proteinuria and amyloidosis in familial Mediterranean fever. Clinical rheumatology. 2013 Aug;32(8):1185-90.
5. Nageen S, Shah R, Sharif S, Jamgochian M, Waqas N, Rao B. Platelet Count, Mean Platelet Volume, and Red Cell Distribution Width as Markers for Psoriasis Severity. Journal of drugs in dermatology: JDD. 2022 Feb 1;21(2):156-61.
6. Vélez-Páez JL, Legua P, Vélez-Páez P, Irigoyen E, Andrade H, Jara A, et al. Mean platelet volume and mean

platelet volume to platelet count ratio as predictors of severity and mortality in sepsis. PloS one. 2022;17(1):e0262356.
7. Bath PM. The routine measurement of platelet size using sodium citrate alone as the anticoagulant. Thrombosis and haemostasis. 1993 Oct 18;70(4):687-90.
8. TI G. Automated hematology instrumentation. 2013; 2013.
9. Bath P, Butterworth RJ. Platelet size: measurement, physiology and vascular disease. Blood coagulation & fibrinolysis: an international journal in haemostasis and thrombosis. 1996;7 2:157-61.
10. Bakan A, Oral A, Alışır Ecder S, Şaşak Kuzgun G, Elçioğlu Ö C, Demirci R, et al. Assessment of Mean Platelet Volume in Patients with AA Amyloidosis and AA Amyloidosis Secondary to Familial Mediterranean Fever: A Retrospective Chart - Review Study. Medical science monitor: international medical journal of experimental and clinical research. 2019 May 24;25:3854-59.
11. Hartmann LT, Alegretti AP, Machado A, Martins EF, da Silva Chakr RM, Gasparin AA, et al. Assessment of Mean Platelet Volume in Patients with Systemic Lupus Erythematosus. The open rheumatology journal. 2018;12:129-38.
12. Uzkeser H, Keskin H, Haliloglu S, Cayir Y, Karaaslan Y, Kosar A, et al. Is mean platelet volume related to disease activity in systemic lupus erythematosus? International journal of clinical practice. 2021 Nov;75(11):e14676.
13. Dastjerdi MS, Emami T, Najafian A, Amini M. Mean platelet volume measurement, EDTA or citrate? Hematology (Amsterdam, Netherlands). 2006 2006/10/01;11(5-6):317-19.
14. Lancé MD, van Oerle R, Henskens YM, Marcus MA. Do we need time adjusted mean platelet volume measurements? Laboratory hematology: official publication of the International Society for Laboratory Hematology. 2010 Sep;16(3):28-31.
15. Mannuß S, Schuff-Werner P, Dreißiger K, Kohlschein P. Magnesium Sulfate as an Alternative In Vitro Anticoagulant for the Measurement of Platelet Parameters? American journal of clinical pathology. 2016 Jun;145(6):806-14.
16. Khan B, Yaqoob N, Ahmed N, Anwar A, Tahir M, Sadiq Malik S. Mean Platelet Volume in Patients of Psoriasis in a Tertiary Care Hospital. Pakistan Journal of Medical and Health Sciences. 2021 10/30;15:3122-25.
17. Mohammadali G, Maryam A, Toktam Hosseinnezhad A, Toktam A, Elinaz H, Mahasti A, et al. Prognostic Value of Mean Platelet Volume (MPV) to Predict Perfusion Defects in Myocardial SPECT in Diabetic and Non-Diabetic Patients. BMC Cardiovascular Disorders. 2022 2022/04/13.
18. Dinçer ABK, Gülöksüz EGA, Sezer S, Yılmaz R, Turgay TM, Ateş A, et al. Neutrophil/lymphocyte ratio but not platelet/lymphocyte ratio and mean platelet volume can be an indicator of subclinical inflammation in patients with Familial Mediterranean Fever. The Egyptian Rheumatologist. 2022;44(3):215-18.
19. Akbaş Y. Mean platelet volume and other hematological parameters in pediatric migraine. Acta Haematologica Polonica. 2022;53(1):53-57.
20. Sansanayudh N, Anothaisintawee T, Muntham D, McEvoy M, Attia J, Thakkinstian A. Mean platelet volume and coronary artery disease: a systematic review and meta-analysis. International journal of cardiology. 2014 Aug 20;175(3):433-40.
21. Pafili K, Penlioglou T, Mikhailidis DP, Papanas N. Mean platelet volume and coronary artery disease. Current opinion in cardiology. 2019 Jul;34(4):390-98.
22. Nardin M, Verdoia M, Barbieri L, De Luca G. Impact of metabolic syndrome on mean platelet volume and its relationship with coronary artery disease. Platelets. 2019;30(5):615-23.
23. Mayda-Domaç F, Misirli H, Yilmaz M. Prognostic role of mean platelet volume and platelet count in ischemic and hemorrhagic stroke. Journal of stroke and cerebrovascular diseases: the official journal of National Stroke Association. 2010 Jan;19(1):66-72.
24. Vasse M, Masure A, Lenormand B. Mean platelet volume is highly correlated to platelet count. Thrombosis research. 2012 Sep;130(3):559-60.
25. Zhong Q, Peng J. Mean platelet volume/platelet count ratio predicts severe pneumonia of COVID-19. Journal of clinical laboratory analysis. 2021 Jan;35(1):e23607.
26. Wach J, Apallas S, Schneider M, Weller J, Schuss P, Vatter H, et al. Mean Platelet Volume/Platelet Count Ratio and Risk of Progression in Glioblastoma. Frontiers in oncology. 2021;11:695316.
27. Lin YC, Jan HC, Ou HY, Ou CH, Hu CY. Low Preoperative Mean Platelet Volume/Platelet Count Ratio Indicates Worse Prognosis in Non-Metastatic Renal Cell Carcinoma. Journal of clinical medicine. 2021 Aug 19;10(16).
28. Icli A, Aksoy F, Nar G, Kaymaz H, Alpay MF, Nar R, et al. Increased Mean Platelet Volume in Familial Hypercholesterolemia. Angiology. 2016 Feb;67(2):146-50.
29. Zhou Z, Chen H, Sun M, Ju H. Mean Platelet Volume and Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis. Journal of diabetes research. 2018;2018:1985026.
30. Razak MKA, Akif AM, Nakeeb N, Rasheed JI. The relationship between mean platelet volume and albuminuria in patients with type 2 diabetes mellitus. Diabetes & metabolic syndrome. 2019 Jul-Aug;13(4):2633-39.