

CRP Levels, Thrombocyte Index and Coagulation Status with TEG Profile in ITP Patients

by Banundari Rachmawati

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Suryanto^{1*}, Banundari Racmawati², Imam Budiwiyo²

ABSTRACT

Examinations related to Immune Thrombocytopenia (ITP) cases have been widely demonstrated in diagnosing and monitoring hemostasis status. The increased MPV and PDW have a high sensitivity to the diagnosis of ITP and CRP is one of the causes of ITP. Monitoring of bleeding in ITP still uses conventional examination. Thromboelastography (TEG) is a global hemostasis assessment tool that assesses real-time overall hemostasis. TEG profiles especially MA and R-time can be used for case assessment of ITP patients. This research describes the correlation between CRP levels, platelet index and coagulation status with TEG profiles (MA and R-time) in ITP patients. This research used analytical observational with cross sectional approach. A sample of 32 respondents aged >18 years with a platelet count <150,000/mm³. Then the respondents measured EDTA blood samples for platelet index (MPV and PDW) and CRP levels, in the citrate blood sample 3.2% measured coagulation status (aPTT and PT) as well as examining the TEG profile, especially R-time and MA. Correlation test uses the Pearson test for data that is normally distributed, while the Spearman test is for data that are not normally distributed. There is a correlation between CRP levels, platelet counts, MPV and MA TEG ($p=0.002$, $p=0.000$, $p=0.001$), there is no correlation between PDW and MA TEG ($p=0.06$), there is a correlation between aPTT and R-time TEG ($p=0.001$) and there is no correlation between PT and R-time TEG ($p=0.667$). Overall, there is a correlation between conventional variables and the TEG profile in ITP patients.

Keywords: ITP, CRP, TEG.

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¹Department of Clinical Pathology,
Faculty of Medicine and Health Science,
Universitas Muhammadiyah Yogyakarta,
Indonesia;

²Department of Clinical Pathology,
Faculty of Medicine, Universitas
Diponegoro Semarang, Indonesia;

*Corresponding author:

Suryanto;
Department of Clinical Pathology,
Faculty of Medicine and Health Science,
Universitas Muhammadiyah Yogyakarta,
Indonesia;
suryapatklinik@umy.ac.id

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INTRODUCTION

Patients with Immune Thrombocytopenia (ITP) often present with severe thrombocytopenia or even with hemorrhagic events. Some cases are emergencies, where there is a risk of serious bleeding complications such as intracranial or gastrointestinal bleeding.¹ ITP can affect anyone regardless of gender, race, and age. Data from the Maryland Health Care Commission, the prevalence of ITP in the United States is 9.5 cases per 100,000 children aged 1-5 years, 7.3 cases per 100,000 children aged 6-10 years, and 4.1 cases per 100,000 children aged 11 - 14 years; while in Northern Europe the annual incidence is 2.68 cases per 100,000 people.² Yohmi et al² found that the clinical picture of ITP was more common in boys (1.9:1), the mean age was 4.78 years. Bleeding complications that occurred were petechiae (89%), epistaxis (18%), oral mucosal bleeding (12%), subconjunctival bleeding (8%), hematemesis/melena (6%), hematuria

(5%). The largest percentage of patients who can recover from ITP are children. For adults the percentage of cure is small. ITP is reported to be about 2 per 100,000 adults. The median age of diagnosis was 50 years. ITP is more common in women of childbearing age and pregnancy. In adults, the course is more chronic although spontaneous remission may also occur within months of initial diagnosis.³

The diagnosis of ITP within a few hours must be established with the help of a medical history, physical examination, routine blood examination and peripheral blood smear, but it is not sufficient to establish a definite diagnosis of ITP. So that in many cases doctors administer corticosteroids or intravenous immunoglobulin (IVIG) treatment with a feeling of uncertainty.⁴ Several case studies using the platelet Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) index have a fairly high sensitivity and specificity for ITP patients, but it is not clear on coagulation status such as activated Partial Thromboplastin Time

(aPTT) and Prothrombin Time (PT). The fact that MPV and PDW increase in ITP has been recognized since 1983, but is still rarely used in everyday clinical practice.¹

The study was not much different from that conducted by Ahmed, who obtained 100% sensitivity for PDW cut off 15 fL) and 100% specificity for MPV (cut off 13 fL) in ITP patients. This platelet index helps differentiate hyperdestructive thrombocytopenia and hypo-productive thrombocytopenia very easily and cost-effectively.⁵

Research by Eunyp Lee et al showed that the MPV and PDW values in ITP patients were higher than the healthy group ($p<0.001$). While the MPV of Essential Thrombocytemia (ET) patients to healthy people was lower and the PDW of ET was higher in healthy people. The correlation between MPV and PDW with platelet count in ITP patients showed a strong negative correlation, but the correlation between MPV and PDW with platelets in ET patients showed a weak negative correlation.⁶ In addition,

research conducted by Kapur et al states that C-Reactive Protein (CRP) can also be used to help establish the diagnosis of ITP, where CRP increases in ITP patients and correlates with platelet count, but after IVIG treatment, CRP levels decrease, normal platelet count and reduction in clinical bleeding severity.⁷ CRP is conclusively a serum factor that enhances IgG-mediated platelet phagocytosis.⁸ The same study on CRP, conducted by Jia-Qi Pan et al showed that CRP levels were significantly increased in ITP patients with confirmed anti-GPIIb/IIIa antibodies, which was able to predict the severity of clinical bleeding in ITP patients. A slower decline in CRP levels after IVIG treatment predicts slower platelet count recovery in ITP.⁹

A newer and more rapid examination of coagulation factor and platelet function disorders is an examination using Thromboelastography (TEG). TEG provides a global assessment of hemostatic function and can be performed in a short time. This method is able to overcome some of the limitations of conventional hemostasis testing.¹⁰ The TEG profile in the form of Maximal Amplitude (MA) shows clot strength related to the number and function of platelets and is slightly influenced by fibrinogen levels, so that a decreased MA value indicates thrombocytopenia or platelet dysfunction, as well as the Reaction time (R) value as clotting time or coagulation factor. shows the time period from the start of the examination to the beginning of fibrin formation.¹¹

TEG is used to examine the different phases of coagulation and fibrinolysis thereby providing precise information for detecting hemostatic disorders.¹² TEG examines the blood coagulation process including the interactions of its components (cellular and plasma) that affect the speed, structure and breakdown of clots. The graphical display of the TEG profile helps to quickly and qualitatively assess different coagulation states reflecting specific abnormalities of clot formation and fibrinolysis.¹³

The results of the examination of CRP levels and the assessment of conventional platelet function, namely the platelet index (MPV and PDW) can be associated

with newer platelet function tests using MA TEG in ITP patients. Meanwhile, the coagulation status, namely the aPTT and PT values can be associated with the R-time TEG value. The relationship between these components needs to be proven by research so that it can see more clearly the correlation between CRP levels, platelet counts and platelet index on MA TEG results in ITP patients as well as aPTT values and PT values on R-time values on TEG. Therefore, researchers are interested in conducting research on the correlation of CRP levels, platelet index (MPV and PDW) and coagulation status (aPTT and PT) with TEG profiles, namely MA and R-time in ITP patients.

METHOD

This study is an analytical observational study with a cross-sectional approach by examining the relationship between the independent variables (CRP, Platelet Count, MPV, PDW, aPTT and PT) on the dependent variable (MA and R-time). This research was conducted after obtaining an ethical clearance from the Health Research Ethics Commission of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta with number 227/EC-KEPK FKIK UMY/VIII/2020.

The population of this study were new and old patients diagnosed with ITP who underwent inpatient and outpatient treatment at PKU Muhammadiyah Hospital, Yogyakarta. The inclusion criteria of this study are inpatients and outpatients with a diagnosis of ITP, patients with an age range equal to or greater than 18 years, patients whose platelet count is less than 150,000/mm³, and patients who are willing to become respondents or who are willing to sign the informed consent. While the exclusion criteria were patients with pancytopenia and thrombocytopenia due to hematological malignancies and patients who had received platelet transfusions in the last 7 days.

The research sample was calculated by the sample size formula for numerical-numeric correlation analysis. With a minimum correlation coefficient that is considered significant, 0.5, type one error 5%, one-way hypothesis, type two error 10%, thus the number of subjects required

is 32. This research was conducted in the Clinical Laboratory Installation of PKU Muhammadiyah Yogyakarta Hospital and Installation Clinical Laboratory RSUP dr. Sardjito Yogyakarta. The research time is from the beginning of July 2020 to the end of August 2020.

The subject's blood was taken by the clinical pathology laboratory analyst at PKU Muhammadiyah Hospital Yogyakarta using a vacutainer once divided into 100 L EDTA for whole blood, 1 mL of 3.2% citrate for conventional coagulation tests and 1 mL of 3.2% citrate for TEG. Each variable before determining the correlation test between the independent and dependent variables, then a normality test is carried out. The normality test in this study was based on the number of samples less than 50, so the test used was the Shapiro-Wilk test and the normal distribution ($p > 0.05$) was MPV, PDW, and MA data, while the data on platelet count, CRP, R- Time, aPTT and PT were not normally distributed ($p < 0.05$). Both normal data were tested using the Pearson test (correlation test for numerical data with normal distribution), while one of the data was not normally distributed, so the test carried out was the Spearman test (correlation test for numerical data not normally distributed).

RESULT

Respondents in this study were patients diagnosed by clinicians both outpatients and inpatients with a diagnosis of ITP based on a platelet count less than 150,000/mm³. This study obtained 32 respondents at PKU Muhammadiyah Hospital, Yogyakarta, both outpatient and inpatient.

Based on table 1, it can be seen that of the 32 respondents, male sex is 20 (62.5%) and female is 12 (37.5%), this ratio is more male than female, namely 2:1 and the average age of the respondents, this is 50.63 years. From the results of a complete blood count, the lowest platelet count was 29,000/mm³ and the highest was 134,000/mm³, the platelet index, namely the mean MPV value was 11.02 fL, close to the upper limit reference value (7.2 – 11.1 fL) and the mean PDW was 16.32 fL, increase from the reference value (9 – 13 fL). For CRP levels in this study, from 32 respondents, all respondents increased

Table 1. Characteristics of Respondents.

Characteristics	n(%)	Median	
		Mean±SD	(Min- Max)
Gender			
Male	20 (62.5)		
Female	12 (37.5)		
Age		50.63±17.74	
Platelet count			103,000 (29,000-134,000)
MPV		11.02±3.27	
PDW		16.32±4.08	
CRP			60.85 (8.1-322.1)
MA		55.05±15.94	
R-Time			8.10 (4.4-26.8)
aPTT			30.70 (23-61.7)
PT			16.55 (12.9-31.7)

to the lowest level of 8.1 mg/L and the highest level of 322.1 mg/L. This indicates that one of the causes of the decrease in platelets is inflammation or inflammation. The coagulation status in this study was examined for aPTT, the lowest value was 23.0 seconds still within the reference value range (24.8-34.4 seconds) and the highest value of 61.7 seconds indicated a mild elongation because it had not exceeded twice the reference value, for the lowest PT value is 12.9 seconds and the highest value is 31.7 seconds, the PT value increased slightly from the reference value (9.8-12.6 seconds) but has not shown a clinically significant meaning because the increase does not exceed two times reference value.

TEG examination on the R-time value is a reaction time that shows the time from the beginning of the examination to the beginning of the formation of fibrin, an average of 9.69 minutes is obtained,

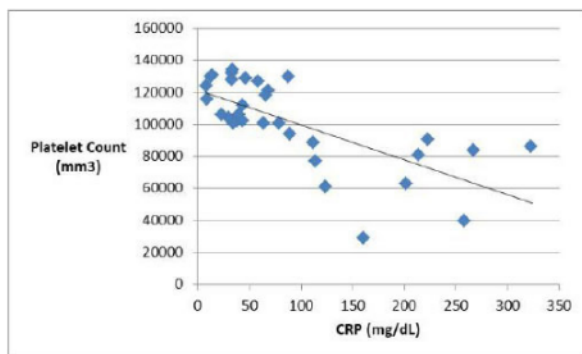


Figure 1. Correlation between CRP levels and platelet count ($r = -0.751$, $p = 0.001$ Spearman test correlation coefficient).

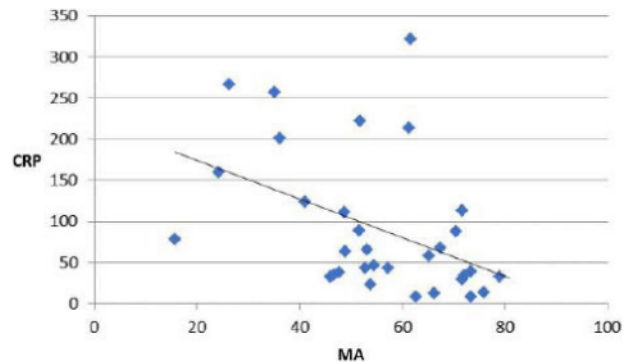


Figure 2. Correlation between CRP and MA levels ($r = -0.520$, $p = 0.002$, Spearman test correlation coefficient).

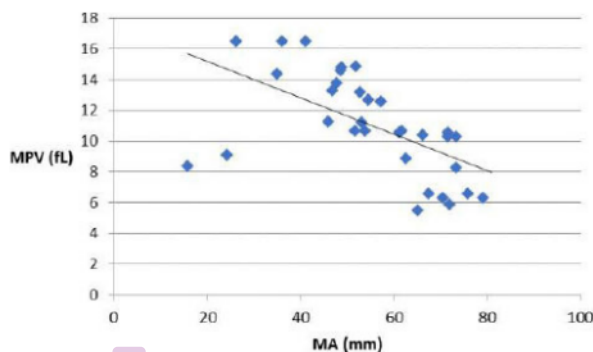


Figure 3. Correlation between MPV and MA levels ($r = -0.576$, $p = 0.001$, Person test correlation coefficient).

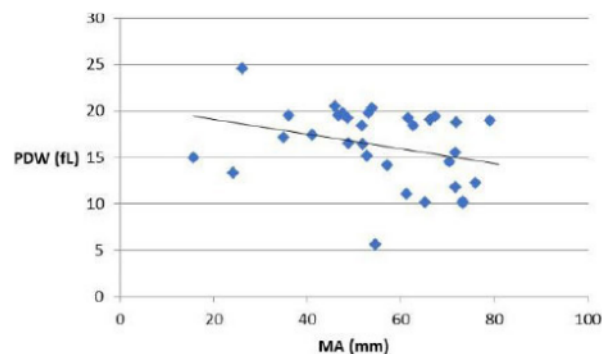


Figure 4. Correlation between PDW and MA levels ($r = -0.658$, $p = 0.086$ Person test correlation coefficient).

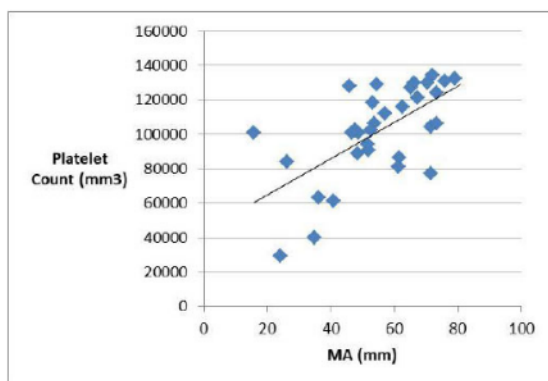


Figure 5. Correlation between Platelet Count levels and MA ($r = 0.382$, $p = 0.001$ Spearman test correlation coefficient).

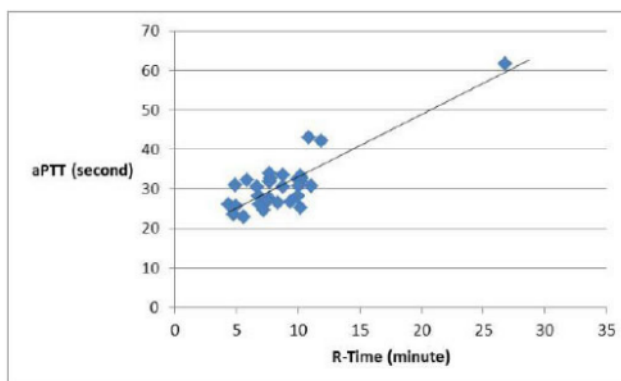


Figure 6. Korelasi antara kadar aPTT dengan R-time ($r = 0.585$, $p = 0.001$ koefisien korelasi uji Spearman).

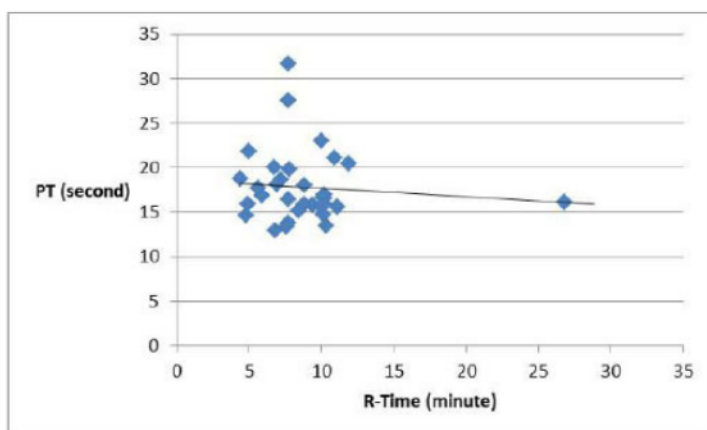


Figure 7. Correlation between PT levels and R-time ($r = -0.079$, $p = 0.667$ Spearman test correlation coefficient).

indicating that time is still within the reference value range (5-10 minutes) and the MA value is the clot strength associated with The number and function of platelets and their interaction with fibrin obtained an average value of 55.05 mm, this value is still within the reference value range (50-70 mm).

This study aims to determine whether thrombocytopenia is also caused by an increase in CRP levels. After examining the CRP of 32 respondents, it showed that the CRP levels of all respondents increased above the reference value (<5 mg/L), the lowest CRP level was 8.1 mg/L and the highest was 322.1 mg/L. The Spearman correlation test between CRP levels and platelet counts got $p=0.001$ ($p<0.05$)

and $r=-0.751$ indicating that there is a significant correlation between CRP levels and platelet counts on the strength of a strong negative correlation, which means that the higher the CRP level, the higher the CRP level. platelet count will decrease (see Figure 1).

Analysis of the correlation test data using the Spearman test because the data are not normally distributed and the value of $p = 0.002$ or $p < 0.05$ proves that there is a relationship between CRP and MA levels on the strength of the weak correlation and negative correlation ($r = -0.520$), which means the higher the correlation. CRP levels, the lower the MA value (see Figure 2).

The normality test showed that the

data were normally distributed $p>0.005$ for both MPV and MA data, and the results of the Person test showed that there was a significant relationship $p=0.001$ or $p<0.005$ with a strong negative correlation strength ($r = -0.576$), which means the higher the value MPV, the lower the MA value (see Figure 3).

Several case studies of the use of the platelet index in ITP cases have long been recognized but are still rarely used in daily clinical practice. In this case of ITP, the increased MPV value was associated with an increase in megakaryocytes due to increased platelet destruction, but the MA value decreased as the platelet count decreased.

The normality test of PDW data and MA TEG values showed that the data were normally distributed with $p>0.005$ and then Pearson's test was carried out showing that there was no significant relationship $p=0.086$ or $p>0.005$. The strength of the negative correlation is strong ($r = -0.658$), which means that the higher the PDW value, the lower the MA value (see Figure 4).

The PDW value as the platelet index value has a very significant meaning in the case of ITP. This PDW describes variations in platelet size or anisocytosis of platelets. PDW has an inverse relationship to platelet count but its biological effects and clinical significance are unclear. The presence of platelet destruction in ITP causes the platelet count to decrease and this can be seen from the MA value should decrease.

Figure 5 shows that the relationship between platelet count and MA value in the Spearman test obtained $p = 0.001$ or $p < 0.05$, which means that there is a relationship between platelet count and MA value. The strength of the correlation is indicated by the value of $r = 0.382$, indicating that the correlation is weak with a positive correlation, the higher the Platelet Count, the higher the MA (see Figure 5).

These results are consistent with several studies that on MA examination in ITP patients where the decreased MA is in line with the decreased platelet count and platelet dysfunction. A decreased MA indicates low clotting strength or hypocoagulation due to low platelet count and impaired platelet function.

Spearman correlation test between aPTT and R-time TEG showed a value of $p = 0.001$ or $p < 0.05$, meaning that there was a relationship between aPTT and R-time. The strength of the correlation is indicated by the value of $r = 0.585$, meaning that the correlation is quite strong with a positive correlation, the higher the aPTT, the higher the R-time (see Figure 6).

The aPTT value in this study is in accordance with the theory in ITP cases that the aPTT value is still within the reference value limit, which indicates that ITP patients do not have coagulation disorders, especially intrinsic coagulation factors, namely factors VIII, IX, XI and XII. The R-time value indicates the period of time from the start of the examination to the beginning of fibrin formation. This phase is prolonged if there is a deficiency of both intrinsic and extrinsic clotting factors.

The Spearman correlation test between the PT value and the R-time TEG value in ITP patients got a p value = 0.667 or $p > 0.05$, meaning that there was no correlation between PT and R-time. The strength of the correlation is indicated by the value of $r = -0.079$, indicating that the correlation is very weak with a negative correlation, the higher the PT, the lower the R-time (see Figure 7).

The PT value is not prolonged which indicates that in the case of ITP there is no case of disturbance of coagulation factors, especially extrinsic factors. But there is a negative correlation to the R-time value

in this correlation. The R-time value also describes the state of the role of coagulation factors in fibrin formation.

DISCUSSION

The TEG examination in this study was to determine the changes in viscoelasticity at the stage of clot formation and its resolution so as to make it different from other coagulation tests. TEG is an examination that analyzes the interaction between platelets (platelet count and function), fibrinogen and clotting factors so as to provide precise information to detect hemostatic disorders.^{14,15} The formation of fibrin-platelet bonds (blood clots), affects the magnitude of pin movement. The resulting electrical signal is converted into a cigar-shaped graphic display that describes the shear elasticity with respect to time. We did not check all the parameters for TEG examination, but we chose R-time and MA. The R-time indicates the period of time from the start of the sample examination until the formation of fibrin. This factor lengthens when there is a deficiency of clotting factors and shortens when there is hypercoagulation, this indicates the value of aPTT and PT.^{12,16,17} Maximum amplitude/MA indicates clot strength related to platelet count and its interaction with fibrin. MA value is influenced by platelet count and platelet function.^{11,12,15}

We examined CRP levels in this study, because thrombocytopenia in ITP patients can be caused by an infectious or inflammatory process. Several studies have described the role of C-reactive protein (CRP) as a major acute phase protein in humans and as a clinical marker of infection. CRP, a known ligand for FcR produced by the liver in response to inflammation due to various stimuli, has been shown to bind and activate Fc receptors (FcR) on monocytes and macrophages.⁷⁻⁹ CRP levels are useful as a clinical diagnostic tool for infection, and it is common knowledge that ITP is triggered by a viral infection that precedes the clinical picture of ITP by days to weeks.⁹ Therefore, we were interested in the role of CRP which interacts directly with antiplatelet IgG antibodies and serves as a novel pathogenic cofactor in IgG-mediated platelet destruction.^{7,8}

This study obtained an average CRP level of 92.8 mg/L, this indicates that infection/inflammation is the cause of thrombocytopenia, and the mean MA is 55.05 mm. The results of the correlation between CRP levels and MA in ITP patients, we previously did the correlation between CRP levels and platelet counts. Our results have a correlation with a strong negative correlation. As done by Kapur K et al there is a correlation between CRP levels and platelet counts in ITP patients with a weak negative correlation.⁷ Then we did with MA TEG. The results showed that there was a correlation between CRP levels and MA TEG results in the case of ITP patients with a fairly strong negative correlation. Elevated levels of CRP indicate a sign of an inflammatory process or tissue inflammation. One of the causes of ITP is inflammation which is characterized by increased levels of CRP. Increased levels of CRP in serum will increase platelet phagocytosis by macrophages mediated by IgG.^{8,9} MA shows clot strength related to the number and function of platelets and is slightly influenced by fibrinogen levels, so if the MA value decreases, it indicates thrombocytopenia or platelet dysfunction.^{11,12,15} There is a fairly strong negative correlation in the relationship between CRP levels and MA, this increase in CRP levels greatly affects MA values. The MA value will decrease according to the platelet count and platelet dysfunction.

The average MPV is 11.02 fL and PDW is 16.32 fL with an average MA of 55.05 mm. The results of this study there is a relationship between MPV and MA TEG, a fairly strong negative correlation and there is no relationship between PDW and MA TEG a weak negative correlation in the case of ITP patients. The correlation between PDW and MA is not in accordance with the statistical analysis hypothesis in this study. The strength of the platelet index correlation shows a negative correlation, which means that the higher the MPV and PDW platelet index values, the lower the MA value. Increased MPV is associated with an increased number of megakaryocytes.¹⁸ The same thing was also explained by Islam et al that the increased PDW and MPV were due to platelet destruction which resulted in high megakaryocytes in plasma.¹⁹ The

higher MPV level indicates the number of large platelets which is a sign of increased platelet turnover. While PDW indicates the level of anisocytosis of platelets.⁶ MA shows clot strength related to platelet count and function and is slightly affected by fibrinogen levels, so a decreased MA value indicates⁸ thrombocytopenia or platelet dysfunction. A low MA indicates a low platelet count, in line with the study of Kaito K et al that there is no correlation between the platelet index and the platelet count in ITP patients.¹³

The correlation between coagulation status and TEG R-time in this study showed that the mean aPTT of 31.15 seconds was still within the reference value and the average PT was 17.77 seconds while the average R-time value of 8.80 minutes also showed that it was still within the reference value, indicating that in the case of patients ITP that the coagulation status and R-time value are still within normal limits. The R-time (Reaction Time) shows the time period from the start of the examination to the beginning of fibrin formation. This phase will be prolonged if there is a deficiency of clotting factors or anticoagulant drugs and shortened if there is a hypercoagulable state indicating aPTT and PT values.^{12,16,17}

aPTT coagulation status is an examination of coagulation factors for intrinsic factors consisting of factors VIII, IX, XI and XII. The results of the study of the correlation between aPTT coagulation status and R-time there is a correlation with a fairly strong positive correlation. While the correlation between PT coagulation status and R-time was not correlated with a very weak negative correlation. In the Wua L study, in cases of thrombocytopenia there was no difference in TEG onset (R-time) with coagulation status. The lack of prolongation of the TEG time (R-time) with the coagulation status indicates that the activity of the coagulation factors prior to the initial platelet-fibrin interaction is not impaired. This component differs from the hypocoagulation state, being characteristically influenced by the consumption of clotting factors and anticoagulation.²⁰

CONCLUSION

There is a correlation between CRP levels and MA TEG in the case of ITP patients. There is a moderate correlation between MPV values and MA TEG in the case of ITP patients. There is no correlation between the PDW value and the MA TEG value in the case of ITP patients. There is a weak correlation between the platelet count and the MA TEG value in the case of ITP patients. There is a moderate correlation between the aPPT value and the R-time TEG value in the case of ITP patients. There is no correlation between the PT value and the R-time TEG value in the case of ITP patients.

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CONFLICT OF INTEREST

None to declare.

FUNDING

None.

ETHICAL STATEMENT

This study protocol was confirmed by the local institutional review board (letter no.227/EC-KEPK FKIK UMY/VIII/2020).

AUTHOR CONTRIBUTION

Study conceptualization, design, definition of intellectual content, literature search, data acquisition and analysis, S; statistical analysis, BR, IB; preparation, editing, and review of the manuscript was done by all authors. All authors serve as guarantors for this study.

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