Academic Journal of Medical Sciences
ISSN: 2708-2725
ORIGINAL ARTICLE

The Validity of Mean Platelet Volume in Diagnosis of Thrombocytopenia among Iraqi Children

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Funding information
Self-funded

Conflict of interest
None declared by author

Received *: October, 2023*
Published*: December, 2023*

ABSTRACT

Background:*Thrombocytopenia is a medical condition characterized by a reduction in platelet count to less than 150 × 109/L. It often due to reduced platelet production, sequestration in an enlarged organ, or accelerated destruction. Mean Platelet Volume (MPV) aids in identifying morphological abnormalities, predicting bone marrow metastasis, and identifying the underlying cause of thrombocytopenia.*

Objective: *To evaluate the importance of the mean platelet volume regarding its ability to discriminate between thrombocytopenia due to decreased platelet production or increased platelet destruction.*

Methods: *A cross-sectional study included 128 Thrombocytopenic children who were seen in Karbala Teaching Hospital for Children, Hematology\Oncology Clinic of Child Central Teaching Hospital and the Children Welfare Teaching Hospital during the period between April 2011 and December 2013. MPV value is determined using automated method. The sensitivity and specificity of MPV in both hypo and hyper production thrombocytopenia were compared at different cutoff values.*

Results: *Out of the 128 patients enrolled in the study, 60 patients had hyperproductive thrombocytopenia immune thrombocytopenic purpura and 68 patients had hypoproductive thrombocytopenia of them 35 had acute leukemia and 33 had aplastic anemia. Among the 60 patients with hyperproductive thrombocytopenia, 53 had high MPV, 6 had normal and only one patient had low MPV. In the hypoproductive group, 31/68 had normal MPV, 5/68 had high and 32/68 patients had low MPV*

Conclusion: *Mean platelet volume can now be part of the routine blood count, and it is a simple test available in all hospitals, and most of the health centers . It can reliably distinguish between ITP, AL, and AA, enabling pediatricians to eliminate the need for invasive bone marrow sampling and the expertise of a hematologist for interpretation. Hence, Greater emphasis should be placed on the platelet indices to ascertain and compare their reliability in diagnosing thrombocytopenia and other platelet diseases.*

Keywords: *Thrombocytopenia, Pathogenesis, Diagnosis, Mean Platelet Volume (MPV), Validity*

1. INTRODUCTION

Thrombocytopenia is a pathological condition of the body, characterized by a decrease in the number of platelets in the bloodstream lower than 150,000 / ml and accompanied by increased bleeding (1). There are different causes and factors associated with thrombocytopenia; decreased production due to congenital or acquired disorders; Hereditary diseases, Diseases that disrupt the production of platelets; most often associated with bone marrow problems, oncological diseases, reaction to chemical and radioactive elements, leukemia. And sometimes even against the background of alcohol and drug abuse. excessive utilization of platelets as in DIC, splenomegaly, Autoimmune thrombocytopenia and Drug-induced thrombocytopenia, some groups of drugs can destroy platelets or suppress their formation in the bone marrow, for example, long-term use of cytostatics (1–4). All symptoms of thrombocytopenia are associated with bleeding; frequent nasal bleeding, spontaneous appearance of bruises, appearance of purpura, gum bleeding, gastrointestinal tract bleeding, hematuria, profuse and prolonged bleeding during menstruation. Additionally, patients presented with weakness, fatigue, headache, dizziness, reduced physical activity, pale skin, bruises under the eyes, shortness of breath . However, Manifestations and severity of symptoms depend on the degree of thrombocytopenia (5). Severity of thrombocytopenia categorized into Mild in which platelets count ranged between 101-140 x109 /L. Most often, there are no obvious symptoms, and a low level of platelets is diagnosed during preventive examinations or treatment of concomitant diseases. Moderate degree (platelets count : 51-100 x109 /L . General symptoms of malaise, irregular and not heavy bleeding from injuries that are difficult to stop. And Severe degree where platelets count below 21-50 x109 /L and very severe form (platelets count below ≤ 20 x109 /L). In severe form of Thrombocytopenia , spontaneous and profuse bleeding in internal organs and inability to stop bleeding without inpatient treatment (6). Due to several reasons that can lower platelet count, a careful diagnosis and consultation with many specialists are needed. If thrombocytopenia is suspected, the patient is referred for a quantitative platelet count, hematological study, and blood coagulation system assessment. Antibody testing, liver and spleen ultrasounds, chronic bleeding searches, immunological tests, hepatitis and tumor markers. Sometimes bone

marrow aspiration and testing are needed. The substance measures immature platelet cell quality and number. Genetic disorders are first investigated for childhood thrombocytopenia. Diagnosis of concomitant diseases is also carried out, because quite often thrombocytopenia can be a symptom of a certain diagnosis (7.8). Treatment of thrombocytopenia is carried out by a hematologist and pediatrician, and the general therapy depends on many factors: the degree of severity, concomitant diseases and the abundance of bleeding. First of all, treatment begins with identifying the cause that led to a decrease in the level of platelets in the blood. Corticosteroid like methyl prednisolone; immunoglobulins and ant-D in addition to stimulation of erythropoiesis are among the first line therapies. Splenectomy in some cases. If the patient has a critically low number of platelets, it is necessary to carry out hemotransfusion of platelet mass from a donor. Also, in the case of a sudden loss of a large amount of blood, a transfusion of erythrocytes and fresh frozen plasma is carried out (9–12). Platelets are anucleated cellular fragments that are generated by megakaryocytes in the bone marrow. A megakaryocyte is a recently discovered, large, polyploid cell seen in mammals. During the maturation process, the cytoplasm undergoes budding, resulting in the release of a significant quantity of platelets (13). Platelets provide procoagulant proteins and catalyze blood coagulation. Quantitative platelet abnormalities cause thrombocytosis, thrombocytopenia, and thrombocythemia. The number of platelets, platelet indices (MPV, PDV, PCT), and platelet histogram analyze the platelet component of the hemogram (14).

Mean platelet volume (MPV):

Certain automated hematology analyzers can check blood cell morphology. Instead of counting cells, these technologies may examine all blood cell types' size, nuclear/cytoplasmic ratio, cytoplasmic granularity, nucleus complexity, and internal cell density (15) Despite its importance in platelet morphology studies, MPV is underutilized for diagnostic purposes. The MPV helps identify platelet morphologic anomalies including clumping and large platelets. It also helps define thrombotic risk, predict cancer patients' bone marrow metastasis, prognosticate myelodysplasia patients, and identify the cause of thrombocytopenia (16.17).

MPV is calculated according to the following equation (18).

MPV (fl) = PCT (%) / Platelets' count ($x10^9$/L)) x 10^5

Immature platelets can be distinguished from mature platelets based on specific physical characteristics: The presence of a greater quantity of bigger immature platelets results in an elevated mean platelet volume (MPV). Recent advancements in technology have led to the development of methods that can accurately quantify the proportion of immature platelets by identifying them based on their reticulated cytoplasm. On the other hand, the MPV offers clear benefits: it is directly linked to the size of platelets and is regularly included in complete blood counts (CBCs) without any further charges. Hence, MPV, enabling the prompt identification of thrombocytopenia, without necessitating any supplementary tests. Multiple investigations have shown the clinical significance MPV and its ability to differentiate between reduced platelet generation and enhanced platelet destruction as the underlying causes of thrombocytopenia (17.19.20). However, MPV still underutilized in different clinical aspects due to different factors; using newer techniques rather than an existing one due to the continuous seeking for development of new techniques is one of these reasons of underutilization of MPV , another reason may be due to variability in the performance of different techniques in reporting MPV (17–20). Therefore, we tried to evaluate the importance of the mean platelet volume regarding its ability to discriminate between thrombocytopenia due to decreased platelet production or increased platelet destruction.

2. METHODOLOGY

This was a cross-sectional study included Thrombocytopenic children who were seen in Karbala Teaching Hospital for Children, Hematology\Oncology Clinic of Child Central Teaching Hospital and the Children Welfare Teaching Hospital during the period between April 2011 and December 2013. Data collection was performed using a pre-constructed data collection form to gather data regarding baseline demographic variables of the patients (including the age, sex and date of referral), clinical data, current presentation, disease related variables (background of disease), bleeding symptoms (petechia , ecchymosis , purpura ,and mucous membrane bleeding) , constitutional symptoms, lymphadenopathy hepatosplenomegaly, and stigmata of congenital conditions) in addition to the results of the automated Complete Blood Count (CBC), including the White Blood Cell (WBC) count, Red Blood Cell (RBC) count, hemoglobin level, hematocrit level, mean corpuscular volume, mean

corpuscular hemoglobin level, RBC distribution width, platelet count, and mean platelet volume, as well as the results of the peripheral blood smear and bone marrow aspiration/biopsy. We enrolled a cohort of 128 patients with thrombocytopenia in our trial, ensuring that we got written agreement from their parents or legal guardians. The recruited patients were categorized into two groups based on the diagnosis of thrombocytopenia, defined as having platelet counts below 150,000/ml. The hyperproductive thrombocytopenic group comprised patients diagnosed with Idiopathic thrombocytopenic purpura (ITP). ITP was defined as the abrupt occurrence of a marked decrease in platelet count, without any other underlying diseases, as determined through a thorough examination of the patient's medical history, physical examination, complete blood count, peripheral blood smear, and bone marrow aspiration, in accordance with the Guidelines for the investigation and management of idiopathic thrombocytopenic purpura. Hypoproductive thrombocytopenia present in patients with acute leukemia or aplastic anemia (AA). They diagnosed by hematological, pathological, radiological, and sometimes chromosomal analysis (55). In acute leukemia cases, thrombocytopenia was caused by bone marrow infiltration or chemotherapy suppression. Cases with thrombocytopenia due to other causes or of unknown origin were excluded from the study. The sensitivity and specificity of mean platelet volume to make a diagnosis of ITP, and hypoproductive thrombocytopenia were calculated under various cut-off ranges, the first cut –off for MPV value is determined by the instrument at our institution (automated cell counter (CELL-DYN Ruby Software Version 2.0ML Analyzer S/N: 35863BG)) that ranged from 6.90 fL to10.6 fL, the second cut –off range is(7.2 fL-11.7fL) (21) and the last range is (8.5fL-12.8fL) (22)

A receiver operating characteristic (ROC) curve was generated by graphing the sensitivity against the 1-specificity for all possible choice thresholds. Sensitivity measures the likelihood of obtaining a positive result (in this case: MPV) when the patient actually has the specific condition of interest, which is thrombocytopenia caused by either increased platelet destruction or decreased platelet production. On the other hand, 1-specificity represents the corresponding probability of obtaining a positive result when the patient does not have the condition of interest, or in simpler terms, the probability of a false positive. The evaluation of a test's performance is determined by the area beneath the ROC curve. This region provides

the likelihood that a patient diagnosed with the disease (specifically ITP) will have a greater measurement value (specifically MPV) compared to individuals with other causes of thrombocytopenia (AA or AL). An ideal test that completely distinguishes between the two groups of patients would exhibit a line that aligns precisely with the left and top edges of the plot. In our study, we determined the sensitivity of mean platelet volume (MPV) in diagnosing immune thrombocytopenia (ITP) by calculating the ratio of true positive tests (ITP patients with high MPV) to the total number of ITP patients. Similarly, we calculated the specificity by determining the ratio of true negative tests (hypoproductive thrombocytopenic patients with normal or low MPV to the number of patients with autoimmune (AA) or acute leukemia (AL) thrombocytopenia. We also compared the different cut-off ranges of MPV mentioned above to assess their sensitivity and specificity in diagnosing the cause of thrombocytopenia. The statistical analyses were conducted using the SPSS 25 software program. Statistical significance was determined by a P. value below 0.05.

3. RESULTS

A total of 128 patients enrolled in this study with a mean age of 4.3 ± 3.2 (Range: 1 – 14) years. The distribution of patients according to the etiology showed that 60 patients had Hyperproductive (ITP) and 68 had Hypoproductive thrombocytopenia. On the other hand, in total, 35 (27.3%) patients had low MPV, 58 (45.4%) had high MPV and 35/128 (27.3%) had normal MPV. In ITP patients, only one (1.7%), had low MPV compared to 32/68 in AA/AL group. High MPV was significantly more frequent in IPT group compared to AA/Al group, where the rate was 88.3% vs. 7.4%, respectively. Normal MPV was much more frequent in AA/Al group than IPT group, 45.6% vs. 10%, P. value < 0.001, highly significant, (**Table 1**). The mean values of age, platelets count and MPV are demonstrated for both groups in addition to the total 128 patients. No significant difference was found in age of patients across the etiology of thrombocytopenia, (P. value > 0.05). The mean Platelet count was significantly lower in ITP compared to Hypoproductive group, it was 208 ± 20. Vs. 32.3 ± 22.6 (103/µL), respectively, (P. value = 0.003). Furthermore, the mean value of MPV was significantly higher in ITP than Hypoproductive group; 13.1± 2.2 (fl) vs. 7.0 ± 2.8 (fl), respectively, (P. value < 0.001) (**Table 2**). Further analysis was performed to assess the validity of MPV in prediction

of ITP, we used the Receiver Operating Characteristics (ROC) curve analysis which revealed that MPV was good predictor and sufficient enough to distinguish ITP from Hypoproductive thrombocytopenia. With an Area under the curve (AUC) of 0.949 at a cutoff value of MPV of 9.86 is the optimal cutoff value that produced the higher validity in prediction with a sensitivity , specificity and accuracy of 93.3%, 82.3% and 88.0%, respectively, in prediction of ITP. Also it was good predictor for Hypoproductive thrombocytopenia, it had a sensitivity of 82.3% and specificity of 93.3% with an accuracy of 88%, (**Figure 1 & Table 3**).

Table 1. Distribution of MPV category according to the etiology of thrombocytopenia

MPV category	ITP		AA/ AL		Total	
	No.	%	No.	%	No.	%
Low MPV	1	1.7	32	47.1	35	27.3
High	53	88.3	5	7.4	58	45.4
Normal	6	10.0	31	45.6	35	27.3
Total	60	100.0	68	100.0	128	100.0

Chi-Square = 85.6, P. value < 0.001 significant. ITP: immune thrombocytopenic purpura AA: Aplastic anemia , AL: Acute leukemia

Table 2. Comparison of mean values of age, Platelet count and MPV of the studied groups

Thrombocytopenia	Statistic	Age (years)	Platelet count (10^3/µL)	MPV (fl)
ITP (N=60)	Mean	4.5	20.8	13.1
	SD	3.4	20.0	2.2
	Range	1 - 13	1.76 - 96.0	6.8 - 17.0
Hypoproductive (N=68)	Mean	4.0	32.3	7.0
	SD	3.0	22.6	2.8
	Range	1 - 14	1.74 - 88.9	2.3 - 13.2
Total (N= 128)	Mean	4.3	27.0	9.9
	SD	3.2	22.1	3.9
	Range	1 - 14	1.74 - 96.0	2.3 - 17
P. value; ITP vs. Hypoproductive group		0.378 ns	0.003 sig	0.001 sig

MPV: Mean platelet volume, SD: standard deviation , sig: significant, ns: not signifciant

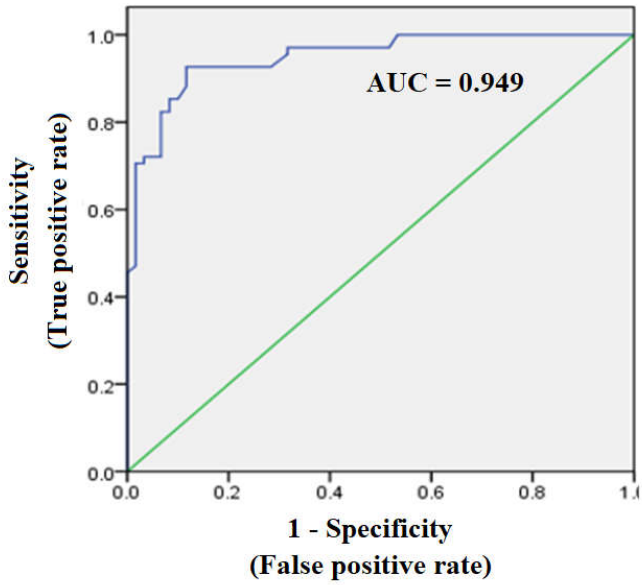

Figure 1. A diagram showing the Receiver Operating Characteristics (ROC) curve analysis for the validity of MPV to distinguish ITP in thrombocytopenic patients

Table 3. Validity parameter of MPV in diagnosis of ITP and Hypoproductive thrombocytopenia at different cutoff values

Etiology of Thrombocytopenia	MPV cutoff value	S (%)	Sp. (%)	Accuracy (%)	PPV (%)	NPV (%)
Hyperproductive (ITP)	< 12.8	60.0	97.0	78.7	94.2	70.9
	< 11.7	81.6	94.1	88.1	89.8	79.7
	< 10.6	88.3	92.6	90.7	91.3	90.0
	< 9.86	93.3	82.3	88.0	82.3	93.3
Hypoproductive (AL or AA)	> 9.86	82.3	93.3	88.0	93.3	82.3
	> 8.5	66.1	98.3	82.4	97.8	71.9
	> 7.2	51.4	98.3	75.1	97.2	64.1
	> 6.9	47.0	98.3	72.9	96.9	62.1

MPV: mean platelet volume, S: sensitivity, Sp.: specificity, PPV: positive predictive value, NPV: Negative predictive value. AL: Acute leukemia, AA: Aplastic anemia

4. DISCUSSION

It is crucial to determine if thrombocytopenia is caused by a hypo or hyper production of platelts. Various diagnostic techniques are available to assist in distinguishing whether a patient's low platelet count is due to reduced production or increased destruction. Garg et al. (23) utilized the size of thrombocytes to establish a correlation between the proportion of megathrombocytes in a peripheral blood smear and the number of megakaryocytes in a bone marrow aspirate. This correlation indicates a state of hyperproduction. The diagnosis of ITP is established through the exclusion of other conditions that might lead to thrombocytopenia, including as systemic lupus erythematosus, malignancy, and disseminated intravascular coagulation (DIC). In order to achieve this objective, bone marrow aspiration is occasionally employed. Performing bone marrow sample is an invasive and costly treatment that is not essential as the initial diagnostic method. It is advised to save this technique for elderly patients or those with unusual characteristics (24–26). Hence, there is a requirement for a novel diagnostic method that is both non-invasive and economically efficient for the detection of thrombocytopenia. Automated hematology analyzers have enabled the recording of different platelet indices, including mean platelet volume (MPV), platelet distribution width (PDW), and platelet-large cell ratio (P-LCR), due to recent technological progress. Several reports have been published about platelet indices and platelet diseases. Nevertheless, they do not have clinical acceptance as standard indicators for thrombocytopenia (27). The test's accuracy is depending upon its ability to effectively distinguish between individuals with and without the specific ailment being tested for. The measurement of accuracy is determined by calculating the area under the ROC curve (AUC) which is a valuable tool for assessing the accuracy of laboratory tests. A laboratory test with a greater area under the ROC curve (AUC) is considered to be more reliable, as it is associated with a lower likelihood of misdiagnosis compared to a test with a smaller AUC. The findings of our study shown that the mean platelet volume (MPV) exhibited a highly favorable receiver operating characteristic (ROC) curve and a significantly large area under the curve (AUC) value of 0.949. Therefore, it serves as a reliable indicator for differentiating hyperproductive thrombocytopenia from hypoproductive thrombocytopenia.

The findings of this study align with those of Bessman et al. (28), which stated that the mean platelet volume was increased in individuals with ITP. The presence of greater mean platelet volume (MPV), as indicated by studies conducted by Rajantie et al. (29) and Ken Kaito et al. (2005) (30), supports the diagnosis of immune thrombocytopenic purpura (ITP) and is related with an increase in the number of megakaryocytes. These findings are consistent with our own observations. Nevertheless, there is limited knowledge regarding MPV and thrombocytopenia, and the extent to which this platelet index serves as a reliable laboratory test for thrombocytopenia remains largely unexplored. The mean MPV was 7.03 fL in the group with bone marrow disease (AL and AA), and 13.11 fL in the group without bone marrow disease (ITP). The disparity in mean platelet volume (MPV) between the two groups, one with bone marrow involvement and the other without, was found to be statistically significant (P value <0.001). Additionally, the sensitivity and specificity scores of MPV, as determined by the receiver operating characteristic (ROC) curve with a cut-off of <10.9 fL, were highly satisfactory (88.3% sensitive and 92.6% specific). Thus, it serves as a reliable indicator for differentiating between these two causes of thrombocytopenia, particularly when considering higher threshold values. In such cases, the specificity is as high as 97% when MPV is less than 12.8FL. According to Altman and Bland (31), it is not always desirable to choose the point with the maximum sum of sensitivity and specificity. The primary purpose of any test used to assess thrombocytopenia is not only to have sufficient sensitivity to detect most hyperproductive conditions like ITP, but also to have enough specificity to effectively exclude hypoproductive cases such as acute leukemia and aplastic anemia. Table 6 demonstrates that selecting a threshold of >10.6fL will identify 88.3% of ITP instances while avoiding 92.6% of hypoproductive scenarios. However, setting the MPV cut-off at a value greater than 12.8fL will correctly identify 60% of the instances with ITP, while effectively rejecting 97% of the hypoproductive circumstances. This assessment can assist the clinician in evaluating thrombocytopenia conditions and facilitate decision-making on the necessity of bone marrow testing. While the bone marrow examination of our patients, including those with low MPV, confirmed the diagnosis of ITP, it is important to note that normal or increased numbers of megakaryocytes do not guarantee sufficient platelet production. In addition to increased destruction, decreased platelet counts in some ITP

patients may also be due to reduced platelet production. Low mean platelet volume (MPV) is not considered one of the markers of elevated platelet turnover. Furthermore, a significant number of children in our research who had ITP also experienced a viral prodrome. It is possible that the release of cytokines during the early stages of the viral infection led to the inhibition or reduction of platelet formation in certain youngsters. Based on our observations, research conducted by Bessman JD (28) has indicated that bone marrow containing megakaryocytes with greater ploidy levels generates platelets that are both larger and more diverse in nature. As the number of platelets increased, even though there were still mostly "young" platelets, the mean platelet volume (MPV) decreased. This indicates that both the stimulation of megakaryocytes and the age of the platelets can influence their size.

Platelet indices, when included in a report, offer significant clinical insights into the underlying causes of thrombocytopenia. Further investigation is required to determine the utility of platelet indices in conditions other than ITP, AA, and AL, which also result in thrombocytopenia. It is crucial to acknowledge certain constraints inherent in our investigation. Multiple factors can influence the MPV value. As an illustration:

Over time, there has been an observed rise in mean platelet volume (MPV) values in venous blood that has been treated with EDTA as an anticoagulant. This type of blood specimen is widely used for analyzing peripheral blood counts. Likewise, MPV can also be influenced by factors like temperature, osmolarity, and pH (32.33). Although automated cell counters are generally fairly reliable in assessing platelet count and mean platelet volume (MPV). However, it is important to note that there is still a chance of instrumental errors occurring at low platelet counts, which cannot be completely ruled out. Platelet indices cannot be measured in cases of severe thrombocytopenia and when red cell fragmentation is present. Therefore, their utility as a thrombocytopenia indicator is limited.

5. CONCLUSIONS

Mean platelet volume can now be part of the routine blood count, and it is a simple test available in all hospitals, and most of the health centers . It can reliably distinguish between ITP, AL, and AA, enabling pediatricians to eliminate the need for invasive bone marrow sampling and the expertise of a hematologist for interpretation. Hence, Greater emphasis should be placed on the platelet indices to ascertain and compare their reliability in diagnosing thrombocytopenia and other platelet diseases.

Ethical Approval:

All ethical issues were approved by the author. Data collection and patients enrollment were in accordance with Declaration of Helsinki of World Medical Association , 2013 for the ethical principles of researches involving human. Signed informed consent was obtained from each participant and data were kept confidentially.

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Citation:

Shibat S.H., Abdaljabar M.Q., Al-Ghabban W.S.. The Validity of Mean Platelet Volume in Diagnosis of Thrombocytopenia among Iraqi Children. AJMS 2023; 9 (4):142-55