Platelet Indices as a Tool for Differentiation between Clonal Thrombocytosis and Reactive Thrombocytosis

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ABSTRACT

Objective: To determine the efficacy of platelet indices as a tool for differentiation between clonal thrombocytosis and reactive thrombocytosis.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Pathology, Combined Military Hospital, Lahore Pakistan, from Nov 2019 to Oct 2020.

Methodology: Fifty-six subjects were included in Reactive thrombocytosis group and 56 in Clonal thrombocytosis group. Fresh blood in EDTA anticoagulant was analyzed to determine complete blood counts and platelet parameters (Platelet distribution width, mean platelet volume, Platelet large cell ratio, Plateletcrit) using the automated hematological analyzer Sysmex KX-21.

Results: Mean age of the patients was 45.76 years with a range of 17-75 years. Assessing the cause of reactive thrombocytosis revealed that 20(35.7%) was infection and 16(28.5%) was iron deficiency anemia. In clonal thrombocytosis group 21(37.5%) were diagnosed with Chronic myeloid leukemia, 13(23.2%) with Essential thrombocythemia and 14(25%) with Polycythemia rubera vera. Platelet indices were compared in clonal thrombocytosis group and the reactive thrombocytosis group. Mean platelet volume, Platelet large cell ratio and Plateletcrit were significantly higher in the clonal thrombocytosis group (p=0.001). Difference in Platelet distribution width was not found to be statistically significant in the two groups (p=0.07)

Conclusion: Platelet indices were higher in the clonal thrombocytosis group. Along with platelet count, they can serve as an efficient and cost-effective method in differentiating between clonal and reactive thrombocytosis.

Keywords: Anemia, Clonal thrombocytosis, Infection, Mean platelet volume, Plateletcrit, Platelet distribution width, Reactive thrombocytosis.

INTRODUCTION

Thrombocytosis is described as an increase in platelet count. Generally, a platelet count greater than $450×10^9$/L is considered to be the working definition of thrombocytosis.[1] It usually comes across as an incidental laboratory finding when complete blood count is obtained for some unrelated reason. Thrombocytosis, when found creates an important diagnostic challenge for clinicians and pathologists alike.

The diagnostic workup for thrombocytosis starts with confirmation of presence of thrombocytosis by the evaluation of peripheral blood film. The next critical step in the diagnostic chain is to classify the condition into either primary/clonal or secondary/reactive thrombocytosis.[2] Various conditions contribute to the development of secondary (or reactive) thrombocytosis. These include iron deficiency anemia, hemolysis, inflammation and malignancy.[3] Other causes include fever, infection, hemorrhage, post-operative thrombocytosis amongst other causes included in secondary thrombocytosis group. No age, gender, or race demographic predilection exists for secondary thrombocytosis. Secondary thrombocytosis is driven by overproduction of thrombopoietin, interleukin-6, other cytokines, or catecholamines in inflammatory, infectious, or neoplastic conditions or in situations of stress.[4,5]

Primary (clonal) thrombocytosis may be a result of various neoplasms of myeloproliferative origin. These can be further categorized into chronic myeloid leukemia (CML), essential thrombocythemia (ET), polycythemia rubera vera(PRV).[6] Gene mutations in Janus kinase 2 (JAK2), myeloproliferative leukemia virus oncogene (MPL) and/or Calreticulin (CALR) are usually found in a pathophysiological role in cases of primary thrombocytosis, leading to unchecked prolife-rative and anti-apoptotic effects on hematopoetic stem cells.

The morphology of platelets and their profileration rate can be determined by automated

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complete blood count analysers. Platelet indices include plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW).[7] along with Platelet Large cell ratio (P-LCR). Variations in the platelet size, morphology and volume are determined by PDW. It is usually raised in case of platelet anisocytosis.[8] PCT is the volume occupied by platelets as a percentage and is calculated according to the formula PCT= platelet count×MPV/10,000. Mean platelet volume (MPV) is a measurement of average size of platelet cells in blood.[9]

Given the cost effectiveness of determination of platelet indices as compared to genetic testing, the aim of this study is to evaluate the effectiveness of platelet indices as a diagnostic tool in order to differentiate between clonal and reactive thrombocytosis.

METHODOLOGY

The cross-sectional study was carried out at the Department of Pathology Combined Military Hospital Lahore, Pakistan over a timeframe of one year from November 2019 to October 2020. Permission from the ethical review board (Ltr no. 159/2020) was taken prior to conducting the study. Informed consent was taken from each patient. A sample size of 112 was calculated using OpenEpi sample size calculator taking mean MPV 9.8±2.0 fL in clonal thrombocytosis and 11.0±2.8 fL in reactive thrombocytosis group.[10]

Inclusion Criteria: Subjects of either gender, aged ≥12 years having platelet count >400 x10⁹/L were included.

Exclusion Criteria: Patients receiving growth factors such as thrombopoetin receptor agonists were excluded.

Fifty-six (56) patients were included in Reactive thrombocytosis group and 56 patients in Clonal thrombocytosis group based on history (fever, anemia, blood loss, thrombotic episodes, myocardial infarction, CVA, erythromylalgia), relevant physical examination (splenomegaly, hepatomegaly, lymphadenopathy) and laboratory investigations.

Patients fulfilling WHO criteria for Myeloproliferative neoplasms (including Chronic Myeloid Leukemia, Polycythemia Rubra Vera, Essential Thrombocythemia, Primary Myelofibrosis etc) were labeled as clonal thrombocytosis based primarily on findings of bone marrow examination, genetic studies and serum erythropoetin levels.[11]. Patients with history of fever, signs of localized or systemic infection, iron deficiency anemia, hemorrhage, chronic inflammatory diseases, malignancy, and history of surgery were included in reactive thrombocytosis group.

Five (05) milliliters of fresh blood in a tube containing EDTA anticoagulant was analyzed to determine complete blood counts (Total Leucocyte count (TLC), Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelet count, Differential leucocyte count (DLC) including neutrophils, lymphocytes, monocytes, eosinophils, basophils, myelocytes, metamyelocytes, blast cells) using automated hematological analyser Sysmex KX-21. Platelet parameters including Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large cell Ratio (P-LCR), Plateletcrit (PCT) were analysed using same hematological analyser which uses impedance counting as the principle for hematological analysis.

Statistical analysis was done using SPSS version 26.0. Mean and SD were calculated for quantitative variables and frequency and percentages for qualitative variables. Mean values of the complete blood picture and platelet indices were compared using independent samples t test between the two groups. Difference in the clinical parameters were analysed using Chi square test between the two groups. The *p* value of ≤0.05 was considered to be statistically significant. ROC (receiver operating characteristic) curve was used to assess the cut off value for sensitivity and specificity of platelet indices in differentiating reactive and clonal thrombocytosis.

RESULTS

Out of a total of 112 patients, 42(37.5%) were females and 70(62.5%) were males. Mean age of the patients was 45.76 years with a standard deviation of 16.74 years and a range of 17-75 years. Assessing the cause of reactive thrombocytosis revealed that in 20 (35.7%) cases it was infection and in 16(28.5%) it was iron deficiency anemia. In clonal thrombocytosis group, 21(37.5%) were diagnosed with chronic myeloid leukemia (CML), 13(23.2%) with essential thrombocythemia (ET) and 14(25%) with polycythemia rubera vera (PRV). There was a statistically significant difference in the mean value of TLC, Hb and platelet count between the two groups.

On analyzing differential leukocyte count, there was a statistically increase in monocytes, basophils and myelocytes in the clonal thrombocytosis group as compared to the reactive thrombocytosis group (*p*=<0.05).

Table: Mean value of blood cells in the Reactive Thrombocytosis and Clonal Thrombocytosis Group (n=112)

Parameter	Reactive Thrombocytosis n=56	Clonal Thrombocytosis n=56	p-value
Total leucocyte count×10⁹/L	11.68±5.0	86.2±112.7	0.001
Hemoglobin g/dL	10.39±1.9	12.7±3.8	0.001
Mean Corpuscular Volume fL	82.17±7.7	82.04±11.0	0.03
Mean Corpuscular Hemoglobin pg	26.84±3.2	27.10±5.5	0.005
Mean Corpuscular Hemoglobin Concentration g/dL	32.57±1.5	32.62±3.37	0.001
Platelet count×109/L	569.13±120.3	821.95±513.2	0.001
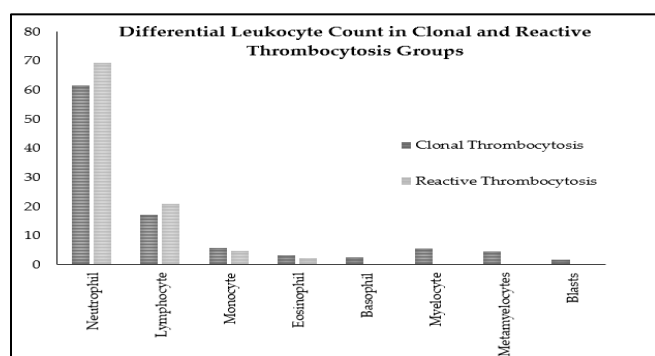
Figure-1: Differential Leukocyte count in Clonal and Reactive Thrombocytosis Groups (n=112)

Platelet indices were compared in clonal thrombocytosis group and the reactive thrombocytosis group. MPV, P-LCR and PCT were significantly higher in the clonal thrombocytosis group (*p*=0.001). The difference in PDW was not statistically significant in both the groups (*p*=0.07).
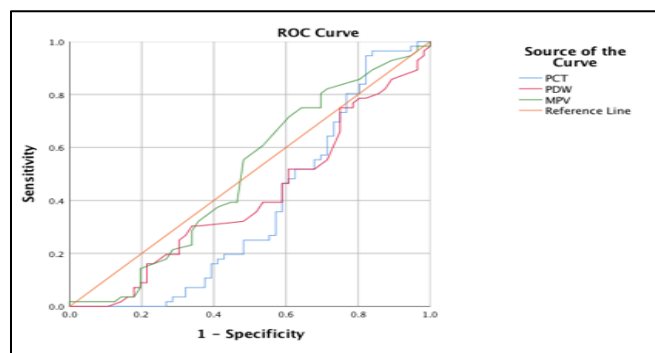
Area under the curve for PCT was 0.372, PDW was 0.41

Figure-2: ROC Curve showing Specificity And Sensitivity of MPV, PCT and PDW in clonal and Reactive Thrombocytosis (n=112)

Area under the curve for PCT was 0.372, PDW was 0.41 and MPV was 0.50.

DISCUSSION

Platelets are derived from bone marrow precursor cells called megakaryocytes. They have a diameter of 3-5 μm and a volume of 4.5–11 fL. Protoplatelets are inactivated forms of platelets in the bloodstream. These cells are anucleate and discoidal in shape. Platelet activation results in change in the morphology from discoid to dendritic shape. Prothrombotic molecules released from alpha and dense granules lead to aggregation and further recruitment of platelets.

Thrombocytosis is an increase in platelet count from its normal range (150-450x10⁹/L. A platelet count of >450x10⁹/L qualifies as thrombocytosis.

Establishing the differentiation between clonal and reactive causes of thrombocytosis has significant therapeutic implications. Clonal thrombocytosis has been linked with increased incidence of thromboembolic and bleeding tendency and complications according to a study by Andrew I. Schafer,[4] whereas secondary thrombocytosis usually does not show any such association. Cerebral, coronary and hepatic vessels are mostly involved. Thus, prophylaxis for prevention of thromboembolic events in high-risk patients falling in clonal thrombocytosis group is of utmost importance.

The most common cause of reactive thrombocytosis in our study population was infection (35.7%) followed by iron deficiency anemia (28.5%). A study by Khalid Nafih and co-workers showed infection (27%) as the leading cause of thrombocytosis, followed by malignancies of non hematological type, iron deficiency anemia, hemolysis, postoperative thrombocytosis and vascular defects to name a few.[12]. According to Syed Naveen and colleagues, causes of thrombocytosis included infections (44.9%), rebound thrombocytosis, tissue injury, myeloproliferative neoplasms, iron deficiency anemia, malignancy, post splenectomy, etc.[13]

Previous studies on the subject showed male predominance in the frequency of thrombocytosis in adults as well as in children.[14,15] This correlates with our study as well showing 62.5% males and 37.5% females from a total sample of 112 subjects.

Findings of our study showed an increased platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT) and platelet large cell ratio (P-LCR) in the clonal thrombocytosis group as compared to the reactive thrombocytosis group. Our study showed the mean PDW value was 12.14±2.6 fL in the

clonal thrombocytosis group and 11.25±1.6 in the reactive thrombocytosis group which was a statistically insignificant difference (*p*=0.07). AUC for PCT was 0.372, PDW was 0.41 and MPV was 0.50. Tanzeel and colleagues revealed that mean PDW was significantly higher in the clonal thrombocytosis group as compared to reactive thrombocytosis group and the AUC was significantly higher for MPV (0.989) and PDW (0.981) as compared to the other study.[16] A study done by Sangeetha *et al.* showed a significantly raised PDW value in thrombocytosis group as compared to the normal control group. Rachita *et al.* revealed contradictory results and suggested that with the increasing number of platelets there was a decreasing trend in MPV, P-LCR and PDW.[17] Similar results were portrayed by Sabramaniam in his study increasing platelet counts were associated with decrease in MPV (*p*=0.001).[14]

A study conducted by Tafazzoli *et al.* concluded that platelet count of patients with primary thrombocytosis was greater than $1000×10^9/L$ as compared to secondary thrombocytosis and a higher mean platelet volume (MPV) was seen in primary thrombocytosis group (*p*=0.007).[18] In our study the platelet count was $569.13±120.3×10^9/L$ in the reactive thrombocytosis group and 821.95±513.2 in the clonal thrombocytosis group. In a study carried out by Khaleel *et al.* in Iraq, findings in accordance with our study were revealed. They concluded that MPV, PCT and PDW were appreciably raised in the primary thrombocytosis group as compared to the secondary thrombocytosis group.[19]

In our study the value of PDW was not significantly different between the two groups. This was in contrast to the study conducted by Golajapu *et al.* who concluded that MPV, PDW and PCT values were significantly higher in the clonal group as compared to the reactive thrombocytosis group.[20] Nafih and co-workers also suggested that MPV and PDW both were raised in the reactive thrombocytosis group.[12] Increase in the MPV may lead to a series of thrombotic complications which are more commonly linked to primary thrombocytosis group.

The reason behind this is that the large platelets are more enzymatically and metabo-lically active, leading to greater thrombotic activity.[21] MPV and PDW both have a direct relationship and they usually change in the same direction.[22] The diag-nostic role of PDW in differentiating clonal and reactive thrombocytosis group is some what contra-dictory and was not found to be of any significance in our study.

CONCLUSION

Platelet indices; MPV, PCT and P-LCR are higher in the primary (or clonal) thrombocytosis group. Along with platelet counts, they can serve as an efficient and cost-effective method in differentiating clonal and reactive thrombocytosis group.

Secondary Thrombocytosis occurs mostly as a result of infection or inflammation, anemia and hemorrhage along with certain other causes. Clonal (or primary) thrombocytosis is generally part of a myeloproliferative process and is usually confirmed with bone marrow examination and genetic testing. This study highlights the importance of platelet indices as a sensitive predictive marker to distinguish between clonal and reactive thrombocytosis, thereby reducing the need or urgency of invasive or expensive procedures.

Conflict Of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

AH: & NUDK: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

SN: & HMR: Critical review, concept, drafting the manuscript, approval of the final version to be published.

SM: & AM: Data acquisition, data analysis, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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