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Original Article

Utility of Immature Platelet Fraction as Diagnostic Biomarker for Isolated Thrombocytopenia

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ABSTRACT

Objective: To evaluate the utility of immature platelet fraction (IPF) as a marker in immune thrombocytopenia purpura (ITP) patients.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Department of Haematology, Chughtai Institute of Pathology, Lahore Pakistan, from Aug to Nov 2020.

Methodology: Sixty patients (Group-1) of immune thrombocytopenia purpura were selected. Sixty healthy individuals (Group-2) with normal blood count parameters were also included. A complete blood count with simultaneous evaluation of immature platelet fraction was performed for both groups on 3ml peripheral blood collected in K2EDTA. To check the reproducibility of results, each sample was run in duplicate.

Results: Group-1 had a mean platelet count of 43.77±32.82 x109 /L (range 2-98x109/L), a mean immature platelet fraction of 12.93±6.11% (range 3–25%) and a mean age 36.68±7.53 years, (range 22–56 years). Group-2 had a mean platelet count of 251.7±83.55 x109 /L (range 150-450 x109 /L), a mean immature platelet fraction of 3.78±1.86 %, (range 1-7.2%) and a mean age of 36.42±6.94 years, (range 22–55 years). ROC curve analysis yielded an immature platelet fraction value of 7.0% as the cut-off between immune thrombocytopenic purpura patients and healthy individuals.

Conclusion: Immature platelet fraction, though novel, is a useful marker to diagnose and monitor treatment response, diagnosis and management of immune thrombocytopenic purpura.

Keywords: Immature platelet fraction (IPF), Immune-mediated thrombocytopenic purpura (ITP), Reticulated platelets.

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INTRODUCTION

Thrombocytopenia is a commonly encountered entity, and its aetiology must be investigated to reach a conclusive management plan for the patient. Immune thrombocytopenia (ITP) is an acquired, immune-mediated disorder with a prolonged or transient fall in the platelet count.[1] Typically, all cases of isolated thrombocytopenia are investigated with due consideration to patient history, clinical presentation, blood counts and, in some cases- a bone marrow biopsy.[1,2] According to the guidelines of the American Society of Hematology (ASH) and the British Committee for Standards in Hematology (BCSH), performing a bone marrow examination is unnecessary. However, it is performed routinely in most setups in Pakistan. In resource-constrained setups, this, predictably, leads to unnecessary delays in diagnosis and patient management. Patients with bone marrow failure, such as aplastic anaemia, also present with cytopenias. In such situations, insight into the state of bone marrow thrombopoiesis provides valuable information regarding the cause of thrombocytopenia.[3,4]

Immature platelet fraction (IPF) is a tool that can provide this insight. Young platelets, also known as reticulated platelets, have a higher mRNA content, and their numbers directly correlate with megakaryocytic activity in the bone marrow. The higher number of reticulated platelets found in the circulation indicates increased thrombopoiesis. Patients with marrow failure and low megakaryocytic activity do not have the corresponding increase in the RNA content of new platelets.[5,6] A raised IPF implies peripheral utilization of platelets instead of defects in thrombopoiesis as the cause of isolated thrombocytopenia.[7,8]

Recently, immature platelet fraction has emerged as a reliable measure of thrombopoiesis in bone marrow. This study evaluated its utility as a marker in immune thrombocytopenic purpura. The fact that it is rapid and automated adds strength to its utility as a therapeutic monitoring and diagnosis tool.

METHODOLOGY

The comparative cross-sectional study was conducted at the Department of Haematology, Chughtai Institute of Pathology, Lahore Pakistan, from

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August to November 2020, after IERB approval. The sample size was calculated using, population SD of 8.62, test value of population mean of 16.39 and anticipated population mean of 7.69.[9]

Inclusion Criteria: Patients with the typical presentation of ITP and platelet count less than 100 x 109/L were included. Matched healthy individuals with normal blood count parameters were also included.

Exclusion Criteria: Patients with evidence of lymphoproliferative disorders, leukaemias, bone marrow failure syndromes and megaloblastic anaemia were excluded based on thorough medical history, physical examination and relevant investigations.

Sixty patients (Group-1) with the typical presentation of ITP and platelet count less than 100x109/L were selected. The sampling technique was non-probability consecutive. Platelet count was confirmed manually on microscopy of stained peripheral smears. About 60 matched healthy individuals (Group-2) with normal blood count parameters were also included. 3ml of anticoagulated peripheral blood (K2EDTA) was collected from each subject. A complete blood count and simultaneous evaluation of immature platelet fraction were performed for both groups. To check the reproducibility of results, each sample was run in duplicate. All samples were analyzed within 4 hours after collection. Immature platelet fraction was obtained by analyzing samples on Sysmex haematology analyzer XN 9000.

Statistical Package for Social Sciences (SPSS) version 20.0 was used for the data analysis. Mean IPF and standard deviation were calculated for both Groups (confidence interval 95%). Independent samples t-test was applied to evaluate differences between both groups. The *p*-value of ≤0.05 was considered statistically significant. ROC curve analysis was obtained to determine the cut-off values of immature platelet fractions between both groups.

RESULTS

A total of 60 immune thrombocytopenic purpura patients (30 males,30 females) were evaluated in Group-1. The mean platelet count in these patients was 43.77±32.82 x109/L (range 2-98 x109 /L), and the mean immature platelet fraction of 12.93±6.11 % (range 3–25%). The mean age of this Group was 36.68±7.53 years (range 22–56 years). For Group-2, samples were taken from 60 healthy individuals (30 males, 30 females). This population had a mean platelet count of 251.7±83.55x109/L (range 150-450x109 /L) and a mean immature platelet fraction of 3.78±1.86% (range 1-7.2%) (Table-I).

The platelet count of Group-1 was significantly lower than Group-2 (*p*-value<0.001). There was no significant difference in subject age between the two groups (*p*-value=0.841). It was noted that the immature platelet fraction values in Group-1 were higher than in Group-2. There was a significant difference between the two groups (*p*-value<0.001). ROC curve analysis yielded a cut-off value of immature platelet fraction (7.0%) for differentiating Group-1 from Group-2, with a sensitivity of 83.3% & a specificity of 96.7%(Table-II).

Table-I: Comparison of Characteristics Between Group-1 (Patients) and Group-2 (Healthy Controls) (n=60)

Baseline Characteristics	Group-1 (n=60)	Group-2 (n=60)	*p*-value
Age (years)	36.68±7.53	36.42±6.94	0.841
Platelet count (x109 /L)	43.77±32.82	251.7±83.55	<0.001
Immature platelet fraction (%)	12.93± 6.11	3.78±1.86	<0.001

Table-II: Performance of Immature Platelet fraction as a Biomarker in Preliminiary Diagnosis of Immune Thrombocytopenia Purpura (n=60)

Diagnostic Parameters	Values	95% CI
Sensitivity	83.33%	71.48-91.71%
Specificity	96.67%	88.47-99.59%
Positive Predictive Value	96.15%	86.43-98.99%
Negative Predictive Value	85.29%	76.68-91.1%

DISCUSSION

The diagnosis of ITP, though clinical, does require laboratory confirmation.[10,11] Isolated thrombocytopenia with an otherwise normal peripheral smear, no organomegaly and classic symptoms of ITP is adequate information to make the diagnosis.[12] Indications for bone marrow biopsy in suspect cases of ITP (as per BCSH guidelines) are age 60 years and above, evidence of atypical features or refractoriness to first-line therapy.[13] However, most setups rely on bone marrow biopsy to reach a sure diagnosis and rule out a hypoplastic marrow as the cause of thrombocytopenia.[14,15] Although IPF is an emerging parameter, local data regarding IPF analyses on new-generation Sysmex analyzers is limited.[16]

In 2016, a British study,[16] on 2336 subjects established an IPF reference range of 1.6-10.1% for healthy controls. In comparison, the literature review suggests a lower IPF in Asian populations. The reference range was 0.7–8.4% in a Chinese cohort,[17] and 1–7.3% in Korean population.[18] A study at the National Institute of Blood Disease and Bone Marrow Transplantation,[19] (NIBD), Karachi 2016, conducted on 231 subjects,

reported the upper limit of IPF for the normal Control Group as 7.69%. The reference range and mean IPF for the Control Group in our study is (1-7.2%) and is comparable to similar studies.

The IPF values for the patient Group were predictably higher. This concurs with IPF values calculated for ITP patients in other studies. A study on 45 patients in France in 2020 reported the mean IPF value of ITP patients as 15%. The same study proposed a cut-off IPF of 13% to differentiate peripheral thrombocytopenia from central thrombocytopenia. The mean IPF value of ITP patients was 16.39% in a study at NIBD in 2016.[19] This study also concluded that all 62 ITP patients evaluated had an IPF greater than 7%. A study in Korea,[18] in 2010 reported the IPF cut-off value of 7.3% for differentiating ITP from aplastic anaemia. An inverse relationship exists between the platelet count and IPF. Other published data support this. The cut-off values of IPF for discriminating ITP and control Group in our study were computed as 7.0% by ROC curve analysis.

In our study, platelet counts improved in patients following first-line treatment for ITP. This accompanied a corresponding decrease in IPF. It was also noted that patients on treatment displayed lower IPF for similar platelet counts than those not on treatment/ newly diagnosed. These results are similar to studies conducted at the national/regional level.

IPF is a non-invasive, simple, rapid diagnostic parameter and can also be used to monitor treatment response in ITP. Many studies conducted to assess its reproducibility conclude that the results are reproducible in an EDTA sample for 24 hours. More importantly, the greater utility of IPF in the clinical setting will help change the routine bone marrow examination trend in these patients. It must be stressed here, however, that patients with decreased IPF in the presence of low platelet count mandate bone marrow examination. It is beyond the scope of this study to assess these observations, but the utility of IPF extends to many domains of haematology. This includes decisions on prophylactic platelet transfusions and hematologic recovery after chemotherapy, bone marrow transplant and monitoring/changing immunosuppressive therapy in ITP patients. An increase in IPF> 8% suggests that platelet count will increase 24-48 hours before platelet count recovery in dengue patients.[20]

CONCLUSION

Immature platelet fraction should be incorporated into the diagnostic workup and further therapeutic monitoring for a patient with isolated thrombocytopeni

Conflict of Interest: None.

Author's Contribution

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

ZRK: & AI: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

NAM: Study design, drafting the manuscript, data interpretation, critical review, 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.

REFERENCES

1. Sakuragi M, Hayashi S, Maruyama M, Kabutomori O, Kiyokawa T, Nagamine, et al. Clinical significance of IPF% or RP% measurement in distinguishing primary immune thrombocytopenia from aplastic thrombocytopenic disorders. Int J Hematol 2015; 101(4): 369-375. https://doi.org/10.1007/s12185-015-1741-0
2. Li J, Li Y, Ouyang J, Zhang F, Liang C, Ye Z, et al. Immature platelet fraction related parameters in the differential diagnosis of thrombocytopenia. Platelets 2020; 31(6): 771-776. https://doi.org/10.1080/09537104.2019.1678118
3. Jeon K, Kim M, Lee J, Lee JS, Kim HS, Kang HJ, et al. Immature platelet fraction: A useful marker for identifying the cause of thrombocytopenia and predicting platelet recovery. Medicine (Baltimore) 2020; 99(7): e19096. https://doi: 10.1097/MD.0000000000019096.
4. Khodadi E, Asnafi AA, Shahrabi S, Shahjahani M, Saki N. Bone marrow niche in immune thrombocytopenia: a focus on megakaryopoiesis. Ann Hematol 2016; 95(11): 1765-1776. https://doi: 10.1007/s00277-016-2703-1.
5. Doobaree IU, Nandigam R, Bennett D, Newland A, Provan D. Thromboembolism in adults with primary immune thrombocytopenia: a systematic literature review and meta-analysis. Eur J Haematol 2016; 97(4): 321-30. https://doi: 10.1111/ejh.12777.
6. Melboucy-Belkhir S, Khellaf M, Augier A, Boubaya M, Levy V, Le Guenno G, et al. Risk factors associated with intracranial hemorrhage in adults with immune thrombocytopenia: A study of 27 cases. Am J Hematol 2016; 91(12): E499-E501. https;//doi: 10.1002/ajh.24529.
7. Nishimoto T, Kuwana M. CD4+CD25+Foxp3+ regulatory T cells in the pathophysiology of immune thrombocytopenia. Semin Hematol 2013; 50 Suppl1: S43-49. https://doi: 10.1053/j.seminhematol.2013.03.018.
8. Adly AA, Ragab IA, Ismail EA, Farahat MM. Evaluation of the immature platelet fraction in the diagnosis and prognosis of childhood immune thrombocytopenia. Platelets 2015;26(7):645-50. https://doi: 10.3109/09537104.2014.969220.
9. Terrell DR, Beebe LA, Vesely SK, Neas BR, Segal JB, George JN, et al. The incidence of immune thrombocytopenic purpura in children and adults: A critical review of published reports. Am J Hematol 2010; 85(3): 174-180. https://doi: 10.1002/ajh.21616.
10. Tana MM, Zhao X, Bradshaw A, Moon MS, Page S, Turner T, et al. Factors associated with the platelet count in patients with chronic hepatitis C. Thromb Res 2015; 135(5): 823-828. https://doi: 10.1016/j.thromres.2015.02.010.

11. Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. Br J Haematol 2004; 126(1): 93-99. https://doi: 10.1111/j.1365-2141.2004.04987.x.
12. Ali U, Knight G, Gibbs R, Tsitsikas DA. Reference intervals for absolute and percentage immature platelet fraction using the Sysmex XN-10 automated haematology analyser in a UK population. Scand J Clin Lab Invest 2017; 77(8): 658-664. https://doi: 10.1080/00365513.2017.1394488.
13. Van De Wyngaert Z, Fournier E, Bera E, Carrette M, Soenen V, Gauthier J, et al. Immature platelet fraction (IPF): A reliable tool to predict peripheral thrombocytopenia. Curr Res Transl Med 2020; 68(1): 37-42. https://doi: 10.1016/j.retram.2019.04.002.
14. Purohit A, Aggarwal M, Singh PK, Mahapatra M, Seth T. Re-evaluation of need for bone marrow examination in patients with isolated thrombocytopenia. Indian J Hematol Blood Transfus 2016; 32(2): 193-196. https://doi: 10.1007/s12288-015-0533-2.
15. Kickler TS, Oguni S, Borowitz MJ. A clinical evaluation of high fluorescent platelet fraction percentage in thrombocytopenia. Am J Clin Pathol 2006; 125(2): 282-287.https://doi: 10.1309/50H8-JYHN-9JWC-KAM7.
16. Briggs C, Longair I, Kumar P, Singh D, Machin SJ. Performance evaluation of the Sysmex haematology XN modular system. J Clin Pathol 2012; 65(11):1024-1030. https://doi:10.1136/jclinpath-2012-200930.
17. Ko YJ, Kim H, Hur M, Choi SG, Moon HW, Yun YM, et al. Establishment of reference interval for immature platelet fraction. Int J Lab Hematol 2013; 35(5) :528-533. https://doi: 10.1111/ijlh.12049.
18. Cho YG, Lee JH, Kim DS, Lee HS, Choi SI. Clinical usefulness of the simple technique to diagnose thrombocytopenia using immature platelet fraction. Korean J Lab Med 2007; 27(1): 1-6. https://doi: 10.3343/kjlm.2007.27.1.1.
19. Naz A, Mukry SN, Shaikh MR, Bukhari AR, Shamsi TS. Importance of immature platelet fraction as predictor of immune thrombocytopenic purpura. Pak J Med Sci 2016; 32(3): 575-579. https://doi: 10.12669/pjms.323.9456.
20. Amrutha BS, Adarsh E, SreeKrishna Y, Apoorva Naidu SN. Immature platelet fraction in children infected with dengue fever. Int J Contemp Pediatr 2018; 6(1): 5-8. https://doi.org/10.18203/2349-3291.ijcp20185151.