

Diagnostic Accuracy of Immature Platelet Fraction (IPF) to Differentiate Between Thrombocytopenia due to Peripheral Destruction versus Bone Marrow Failure

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ABSTRACT

Objective: To analyse the predictive value of immature platelet fraction (IPF) as an independent diagnostic marker to differentiate between hyperdestructive and hypoproliferative thrombocytopenia.

Study Design: Cross-sectional observational study.

Place and Duration of the Study: Armed Forces Institute of Pathology Rawalpindi, from February to July 2022.

Methodology: A total of 164 samples were included in the study by non-probability consecutive sampling. Among these, 80 were obtained from normal individuals serving as control; 43 were obtained from patients having hyperdestructive thrombocytopenia (idiopathic thrombocytopenia, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation); and 41 were obtained from those hypoproliferative thrombocytopenia (acute leukaemia, aplastic anaemia, chemotherapy). Sysmex automated haematology analyzer, XN-3000 was used to determine the immature platelet fraction (IPF) of the patients. ROC curves analysis was done to ascertain area under curve.

Results: Immature platelet fraction (IPF %) was significantly higher in consumptive / hyperdestructive thrombocytopenia group i.e. median (IQR), 21% (14.4-26.2) as compared to 6.5% (4.6-8.9) in hypoproliferative thrombocytopenia, and 2.6% (1.3-4.1) in normal control group ($p < 0.001$). Cut-off value with the highest sensitivity and specificity for IPF vs. normal population was 7.95% with sensitivity of 97.7% and specificity of 86%.

Conclusion: Immature platelet fraction (IPF of 7.95%) possesses high diagnostic accuracy, sensitivity and specificity for differentiation between hyperdestructive vs. hypoproliferative thrombocytopenia. It can be used as a reliable marker to differentiate between the two entities.

Key Words: Immature platelet fraction, Thrombocytopenia, Bone marrow failure, Peripheral destruction.

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INTRODUCTION

Platelets are fragments of megakaryocytes released from bone marrow. Each megakaryocyte can give rise to 1000 – 5000 platelets.¹ Their production is regulated by thrombopoietin (TPO), 95% of which is secreted by liver. Platelets are small bodies with sizes varying around 0.5 – 3.0 μm .² With a life span of 10 days and normal range of 150 – 400 $\times 10^9/\text{L}$, they help to maintain hemostasis and prevent bleeding.

Decrease in platelet count (thrombocytopenia) can lead to a range of symptoms varying from petechiae (capillary bleed) and purpura (bruising) to excessive bleeding from mucocutaneous membranes.³ Thrombocytopenia is predominantly due to either decreased production from bone marrow or increased peripheral destruction. Other causes include abnormal distribution (hypersplenism), dilution factors (pregnancy) and drugs including antibiotics and chemical agents.⁴

Hypoproliferative causes encompass bone marrow failure conditions like acute leukaemia, aplastic anaemia, myelodysplastic neoplasm, bone marrow infiltration by secondary neoplasms, and patients on chemotherapy. On the other hand, hyperdestruction can be due to immune thrombocytopenic purpura (ITP), disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), infections like malaria, and dengue virus.

Following thrombocytopenia, bone marrow releases reticulated platelets which contain remnants of RNA. They are called

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immature platelet fraction (IPF) and are analogs of reticulocytes. As they age, their RNA content and size tend to decrease.⁵ Modern automated haematology analyzers like Sysmex XN – 3000 (Kobe, Japan) can be used to quantitate IPF.⁶ They calculate percentages (%) of immature platelets as compared to mature platelets and their absolute value. The machine works on the principle of flowcytometry to obtain IPF after staining RNA within immature platelets, thus representing bone marrow activity in response to thrombocytopenia.⁷

Studies have shown that IPF % can be near normal or slightly elevated in patients with bone marrow failure conditions. Whereas increased peripheral destruction can lead to massive increase in IPF %.⁸ The objective of this study was to evaluate the diagnostic accuracy of immature platelet fraction (IPF) to differentiate between the causes of thrombocytopenia occurring either due to peripheral destruction or bone marrow failure.

METHODOLOGY

This cross-sectional study was conducted at the Armed Forces Institute of Pathology Rawalpindi, over a period of 06 months from February to July 2022. WHO calculator was used to determine sample size which came out to be 164. Non-probability consecutive sampling was used to collect samples.

Among these, 80 samples had normal findings on blood complete picture (CP) so they were used as normal control. Eighty-four individuals had abnormal platelet count on CP. Clinical history, physical examination, peripheral blood film and bone marrow examination with trephine biopsy were used to label the diagnosis.

Forty-one patients were diagnosed with hypoproliferative thrombocytopenia. Diseases included acute lymphoblastic leukaemia, acute myeloid leukaemia, aplastic anaemia, myelodysplastic syndrome, and some patients on standard chemotherapeutic management as these conditions lead to bone marrow failure and decreased production of platelets. The exclusion criteria were the patients with anaemia and any solid organ tumours.

Forty-three patients belonged to hyperdestructive thrombocytopenia group. Patients included newly diagnosed cases of Idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, and disseminated intravascular coagulation. Patients having malaria and dengue were also included in this group as both lead to peripheral destruction of platelets.

Three ml of peripheral blood was collected in K3 EDTA (potassium - ethylene diamine tetra acetic acid) tube (Xinle sci & tech, Hebei, China). Analytes were evaluated via XN – 3000 automated haematology analyzer (Sysmex, Kobe, Japan). Samples were run within 30 minutes of procurement. Analyser revealed immature platelet fraction (IPF%) value at the same time along with the complete blood picture which also has platelet count (Plt), mean platelet volume (MPV), plateletcrit (PCT), platelet large cell ratio (P-LCR), platelet distribution width (PDW). All parameters were also recorded.

Data were statistically analysed using SPSS v 26. The descriptive statistics were obtained as mean and standard deviation for quantitative variables and frequency and percentages for qualitative variables. Kolmogorov-Smirnov test was applied to assess the distribution of data. Inferential statistics were recorded using to assess any significant difference between the study variables. Kruskal-Wallis test was applied for the comparison of medians between more than two groups. The receiver operating characteristics (ROC) curves were plotted for evaluating diagnostic accuracy of different parameters. A value of $p \leq 0.05$ was taken as statistically significant. Ethical approval and support letter were acquired from the Internal Review Board (IRB) dated 5th May 2021 before starting the research.

RESULTS

IPF %, platelet count and other complete blood picture parameters were recorded for all 164 patients including 80 normal individuals and 84 diseased patients, diagnosed on medical history, clinical examination and keeping bone marrow biopsy as gold standard. Among the diseased, 41 patients belonged to hypoproliferative and 43 were placed in hyperdestructive thrombocytopenia groups, respectively. The mean age was 27.4 ± 19.1 , 30 ± 24.2 , and 34 ± 25.2 years for healthy individuals, hypoproliferative and hyperdestructive group, respectively. There were 81 male and 83 female patients overall.

Median values were calculated for non-parametric data. Medians with 25th – 75th interquartile ranges (IQR) for white blood cells/total leucocyte count (WBC/TLC), haemoglobin levels (Hb), platelets (PLT), immature platelet fraction (IPF), platelet distribution width (PDW), mean platelet volume (MPV), platelet-large cell ratio (P-LCR) and Plateletcrit (PCT) are shown in Table I.

The IPF mean value of this study control group was $3.1 \pm 2.3\%$, whereas the median came out to be 2.6% (IQR 1.3 – 4.1). The comparison of IPF values showed that they were significantly higher in consumptive/ hyperdestructive thrombocytopenia group (mean 22.5 ± 11.1 and median 21%; IQR 14.4 – 26.2) as compared to hypoproliferative thrombocytopenia group (mean 6.7 ± 2.9 and median 6.5%; IQR 4.6 – 8.9). Despite having lower figures than hyperdestructive group, hypoproliferative figures were still higher than the normal control group (2.6%; IQR 1.3 – 4.1). Kruskal – Wallis test was used to ascertain the p-value for both groups, which was <0.001 . It was statistically significant, declaring IPF as a strong diagnostic marker.

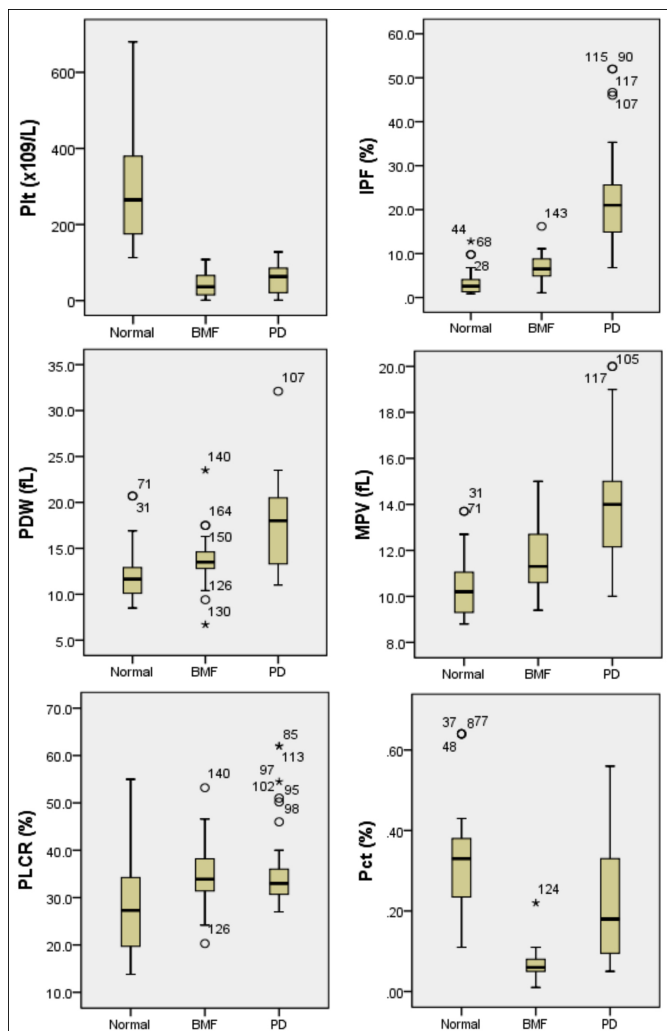
Median values of PDW were highest in hyperdestructive group (18 fL; IQR 13.2 – 21.0) as compared to hypoproliferative group (13.5 fL; IQR 12.5 – 14.8) which were again higher than normal control group (11.6 fL; IQR 10.5 – 12.9). Similarly, MPV values showed higher median in hyperdestructive group (14 fL; IQR 12.0 – 15.0) in comparison to hypoproliferative (11.3 fL; IQR 10.6 – 12.8) and normal individuals (10.2 fL; IQR 9.3 – 11.1). P – LCR and PCT figures were not statistically significant.

Graphical comparison among platelet indices among three groups is shown in Figure 1.

Table I: Comparison of demographics and clinical findings among groups.

Parameters	Healthy control group*	Hypoproliferative thrombocytopenia group*	Hyperdestructive / consumptive thrombocytopenia group*
Patients (N)	80	41	43
Age (years)	27.4 ± 19.1	30 ± 24.2	34 ± 25.2
TLC (x10 ⁹ /L)	8.6 (6.6 -11.4)	3.2 (1.8 -12.5)	7.6 (6.2 - 8.7)
Haemoglobin (g/dL)	12.5 (11.6 -14.8)	8.7 (7.8 -10.1)	11.3 (10.1 -12.4)
Platelets (x10 ⁹ /L)	265 (169 - 385)	36(15 - 70)	63 (20 - 87)
IPF (%)	2.6 (1.3 - 4.1)	6.5 (4.6 - 8.9)	21 (14.4 - 26.2)
PDW (fL)	11.6 (10.5 -12.9)	13.5(12.5 -14.8)	18 (13.2 -21.0)
MPV (fL)	10.2 (9.3 -11.1)	11.3(10.6 -12.8)	14 (12.0 -15.0)
P- LCR (%)	0.33 (0.22- 0.38)	0.06 (0.04 - 0.08)	0.18 (0.09 -0.33)
PCT (%)	27.3 (19.7 - 34.3)	33.9 (31.0 - 38.2)	33 (30.0 -36.0)

*Values are ± standard deviations or medians (25th - 75th interquartile ranges) for WBC/TLC, Hb, PLT, IPF, PDW, MPV, P-LCR and PCT.

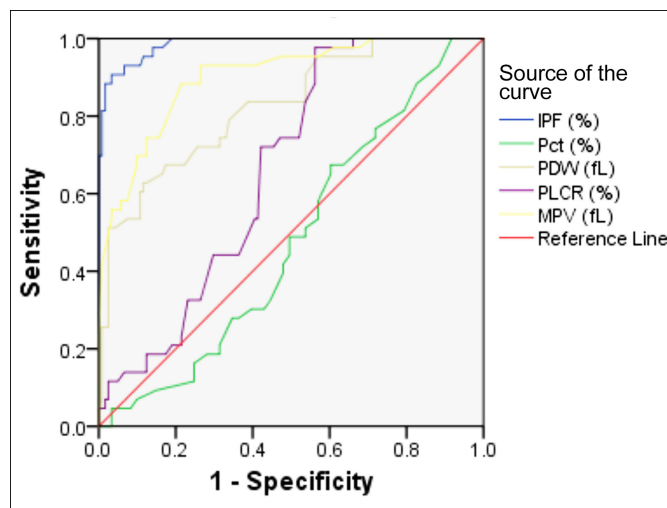
**Figure 1: Boxplot comparison among platelet indices among three groups; PLT, IPF, PDW, MPV, P-LCR and PCT.**

The correlation between platelet count and different parameters was determined for three platelet groups; <40 x10⁹/L, 40 - 90 x10⁹/L, and >90 x10⁹/L.⁹ For IPF, values came out to be 12.7±8.2%, 14.9±12.5%, and 5.5±8.5%, respectively. The values for PDW were 14.5±3.2%, 16.9±4.1%, and 12.6±3.5%, respectively. Similarly, figures for MPV for three platelet groups were 12.4±1.5%, 13.3±2.6%, and 10.6±1.8%, respectively. Values for P-LCR were also determined which were 34.4±5.6%, 36.9±10.9%, and 28.3±9.3%, respectively. These

results emphasised that with lower platelet counts, the values of different platelet parameters including IPF, tend to rise towards higher side. As the platelet count improves, the figures tend to take decreasing trend and normalise predicting recovery.

Receiver operating characteristics (ROC) curves and area under the curve (AUC) were ascertained for assessment of differences in sensitivities and specificities of IPF and other platelet indices in the target groups. Peripheral destruction/hyperdestructive group analysis showed that area under the curve was maximum for IPF (0.98), which highlights its discriminatory ability to differentiate between the two study groups. The second highest indicator was MPV (0.90), followed by PDW (0.82) and P-LCR (0.66). PCT showed the lowest AUC (0.48), so it is not a suitable indicator. Cut-off values with highest sensitivity and specificity were also ascertained. For IPF, it was 7.95% with sensitivity of 97.7% and specificity of 86%.

ROC curves for different platelet indices are shown in Figure 2.

**Figure 2: ROC curves for different platelet indices PLT, IPF, PDW, MPV, P-LCR and PCT.**

DISCUSSION

Although, bone marrow aspiration with trephine biopsy is the gold standard for the assessment of patients presenting with thrombocytopenia, however, platelet indices, especially IPF holds significant importance in determining the cause over

the rest of the indices. Therefore, it holds strong significance in routine haematology.

This analysis outcomes were coherent with those of previous studies. The IPF reference value as determined by Ali *et al.* in their study was 1.6 – 10.1% with median 4.4% (11),⁹ while median of this study control group was 2.6% (IQR 1.3 – 4.1). Sachdev *et al.* demonstrated the reference interval for IPF in their study to be 0.3 – 8.1% (mean 2.1%).¹⁰ Whereas for this study, the control group mean was $3.1 \pm 2.3\%$.

In a study conducted by Adly *et al.*, it was demonstrated that IPF can be significantly higher in patients having ITP as compared to those having decreased production.¹¹ Similarly, Strauss *et al.* reported IPF as a parameter for distinguishing thrombocytopenia in pediatric acute lymphoblastic leukaemia from ITP, established that ALL patients due to bone marrow failure have reduced IPF levels in comparison to patients having ITP.¹² Same was demonstrated in this study as the hypoproliferative population consisted of patients having acute leukaemia (ALL/AML), AA, MDS, cancers and those on chemotherapy, all having reduced IPF and other platelet parameter levels as compared to peripheral destruction group.

Naz *et al.* reported importance of IPF as predictor of ITP, expounded that IPF% value by Sysmex haematology analyzer can predict ITP.¹³ Their mean IPF% for ITP patients was 16.39% while for non-ITP patients, it was 7.69% being statistically significant with p-value of <0.001 . Hyperdestructive thrombocytopenia group with mean $22.5 \pm 11.1\%$ and hypoproliferative group with mean $6.7 \pm 2.9\%$ in the present study. Similar was the case with sensitivity and specificity. Therefore, the present conclusion was consistent with their results in determining IPF as a better predictor of ITP.

ROC analysis in this research has proven that hyperdestructive patient group has much higher AUC, sensitivity and specificity for IPF in comparison to bone marrow failure group. The ROC results were consistent with those of studies done in the past. Jeon *et al.*, concluded in their study that AUC was highest for IPF (0.93) with 95.5% sensitivity and 73.5% specificity, followed by PDW (0.87), P-LCR (0.82) and MPV (0.78).¹⁴ Whereas in the present study, AUC for IPF was higher (0.98) with sensitivity of 92% and specificity of 86%, favouring this hypothesis.

Van De Wyngaert *et al.* reported IPF as a reliable tool to predict peripheral thrombocytopenia, and concluded that on Sysmex haematology analyzer, IPF% of $>13\%$ is predictive of peripheral destruction of platelet, especially in cases which only have thrombocytopenia and rest CBC is within normal limits.¹⁵ In this study, with ROC curve analysis, value was 7.95%; with sensitivity of 92%; specificity of 86%. Therefore, this measurement could help the authors narrow down their differential and avoid bone marrow aspirations.

IPF, in addition to its role in differentiating the cause of thrombocytopenia, can also be a predictor of bone marrow recovery. Zucker *et al.* demonstrated that IPF, being non-invasive parameter, can be used as to monitor patients' response and recovery undergoing chemotherapy to acute leukaemias (ALL or AML).¹⁶ Results were consistent with the present study.

One of the related significance of IPF is in patients undergoing haematopoietic stem cell transplant. Sakuragi *et al.* examined percentages of IPF in patients of allogeneic stem cell transplant and declared it significant in predicting recovery in these patients.¹⁷ This adds up to the prime importance of IPF as discussed earlier. Although this study provides an excellent relationship between different non-invasive markers including IPF in diagnosis of the causes of low platelets counts, a more comprehensive, multicentric study at the national level is suggested to validate these markers for future use.

CONCLUSION

Among the patients presenting with thrombocytopenia, IPF has the best discriminatory power to determine the cause. Although, bone marrow examination remains the gold standard investigation being a non-invasive parameter, simple and easy to perform, less time consuming and high diagnostic accuracy, IPF holds vital significance in narrowing down the differentials.

ETHICAL APPROVAL:

Ethical approval and support letter were acquired from the Institutional Review Board (IRB) dated 5th May 2021 before starting the research.

PATIENTS' CONSENT:

Informed consent were obtained from patients to publish the data concerning this case.

COMPETING INTEREST:

There are no competing interest to disclose.

AUTHORS' CONTRIBUTION:

MBA: Conceptualised the study, collected and analysed the data, and drafted the manuscript.

FA: Conceived the idea and reviewed the manuscript.

AM: Revised it critically for important intellectual content.

NR: Did data acquisition, its interpretation, and manuscript writing.

NAR: Analysed the data and drafted the manuscript.

UBK: Worked on interpretation and intellectual content.

All of the study participants agreed to be responsible for all aspects of the project, ensuring that any issues about the work's accuracy or integrity are thoroughly examined and addressed.

REFERENCES

1. Hoffbrand V, Higgs DR, Keeling DM, Mehta AB, editors. *Postgraduate Haematol John Wiley Sons* 2016; Jan 19.

2. Bain BJ, Bates I, Laffan MA, Lewis SM. Dacie and Lewis practical haematology: Expert consult: Online and print. *Elsevier Health Sci* 2016; Dec 22.
3. Kohli R, Chaturvedi S. Epidemiology and clinical manifestations of immune thrombocytopenia. *Hämostaseologie* 2019; **39(03)**:238-49. doi: 10.1055/s-0039-1683416.
4. Bain BJ. Hoffbrand AV, Steensma DP. Hoffbrand's Essential Haematology, 8th Ed. Oxford: Wiley Blackwell; 2020.
5. Ferreira FL, Colella MP, Medina SS, Costa-Lima C, Fiusa MM, Costa LN, Orsi FA, Annichino-Bizzacchi JM, Fertrin KY, Gilberti MF, Ozelo MC. Evaluation of the immature platelet fraction contribute to the differential diagnosis of hereditary, immune and other acquired thrombocytopenias. *Sci Rep* 2017; **7(1)**:1-8. doi: 10.1038/s41598-017-03668-y.
6. Saigo K, Sakota Y, Masuda Y, Matsunaga K, Takenokuchi M, Nishimura K, Sugimoto T, Sakurai K, Hashimoto M, Yanai T, Hayakawa A. Automatic detection of immature platelets for decision making regarding platelet transfusion indications for pediatric patients. *Transfusion Apheresis Sci* 2008; **38(2)**:127-32. doi: 10.1016/j.transci.2008.01.003.
7. Buttarello M, Mezzapelle G, Freguglia F, Plebani M. Reticulated platelets and immature platelet fraction: Clinical applications and method limitations. *Int J Lab Hematol* 2020; **42(4)**:363-70. doi: 10.1111/ijlh.13177.
8. Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. *Bri J Haematol* 2004; **126(1)**:93-9. doi: 10.1111/j.1365-2141.2004.04987.x.
9. 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. *Scandinavian J Clin Lab Inv* 2017; **77(8)**:658-64. doi: 10.1080/00365513.2017.1394488.
10. Sachdev R, Tiwari AK, Goel S, Raina V, Sethi M. Establishing biological reference intervals for novel platelet parameters (immature platelet fraction, high immature platelet fraction, platelet distribution width, platelet large cell ratio, platelet-X, plateletcrit, and platelet distribution width) and their correlations among each other. *Indian J Pathol Microbiol* 2014; **57(2)**:231. doi: 10.4103/0377-4929.134676.
11. 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. doi: 10.12669/pjms.323.9456.
12. Strauss G, Vollert C, von Stackelberg A, Weimann A, Gaedicke G, Schulze H. Immature platelet count: A simple parameter for distinguishing thrombocytopenia in pediatric acute lymphocytic leukemia from immune thrombocytopenia. *Pediatr Blood Cancer* 2011; **57(4)**:641-7. doi: 10.1002/pbc.22907.
13. 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. doi: 10.12669/pjms.323.9456.
14. Jeon K, Kim M, Lee J, Lee JS, Kim HS, Kang HJ, Lee YK. Immature platelet fraction: A useful marker for identifying the cause of thrombocytopenia and predicting platelet recovery. *Medicine* 2020; **99(7)**. doi: 10.1097/MD.00000000019096.
15. Van De Wyngaert Z, Fournier E, Bera E, Carrette M, Soenen V, Gauthier J, Preudhomme C, Boyer T. Immature platelet fraction (IPF): A reliable tool to predict peripheral thrombocytopenia. *Curr Res Transl Med* 2020; **68(1)**:37-42. doi: 10.1016/j.retram.2019.04.002.
16. Zucker ML, Murphy CA, Rachel JM, Martinez GA, Abhyankar S, McGuirk JP, Reid KJ, Plapp FV. Immature platelet fraction as a predictor of platelet recovery following hematopoietic progenitor cell transplantation. *Lab Hematol* 2006; **12(3)**:125-30. doi: 10.1532/LH96.06012.
17. Sakuragi M, Hayashi S, Maruyama M, Kiyokawa T, Nagamine K, Fujita J, et al. Immature platelet fraction (IPF) as a predictive value for thrombopoietic recovery after allogeneic stem cell transplantation. *Int J Hematol* 2018; **107**:320-6. doi: 10.1007/s12185-017-2344-8.

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