LARGE PLATELET FRACTION (LPF) - A NOVEL PARAMETER MEASURED BY YUMIZEN H2500 & ITS CORRELATION WITH IMMATURE PLATELET FRACTION (IPF) BY SYSMEX XN1000

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BACKGROUND

Platelet analysis and estimation is simple yet a complex process for various pathological conditions. Platelet activity can correlate with the platelet size and the platelet numbers^{1,2}. The platelet size can be assessed by various platelet indices and technological derivatives. Impedance technology is widely used however this has a limitation specially in thrombocytopenia cases with large or giant platelets, red cell fragmentation, when a platelet histogram cannot be drawn properly to derive an accurate platelet count or its indices. these conditions form the major chunk of platelet associated dilemmas in the laboratory set up. The indices could often help to distinguish hyper-destructive thrombocytopenia and hypo-productive thrombocytopenia very easily.

HORIBA Medical has developed a novel parameter called Large Platelet Fraction (LPF). Measurement is performed on an optical bench in hydrodynamic focusing flow cell, where for each cell is measured in size by impedance and optical extinction (Figure 1).

 \cdot Optical extinction is sensitive to cells refractive index.

index in linked to HGB contents

This property is used to determine separation between PLT and RBC.
Refractive index for PLT is lower than 1.40 but for RBCs refractive





Each cell is represented on two dimensional scattergram with size on X axis and extinction on Y axis. Optical extinction allows to better separate PLT from RBC even if size is similar (Figure 2).



Figure 2- Low LPF (left) Vs High LPF (right) from HORIBA Yumizen H 2500

METHODS

Total 328 patients venous blood samples were selected randomly using different criteria like MPV >11.5 fL, MPV <11.5 fL, schistocytes flag or MCV <60 fL. All the samples were processed in duplicate within 4 hours of phlebotomy using both haematology analysers in parallel for platelet determination. The analyser XN1000 uses laser flow cytometer based on florescent dye. The analyser Yumizen H2500 uses a combination of optical extinction and impedance technology. A number of parameters were available from both the analysers. After outlier exclusion, 272 samples were statistically analysed and LPF (available in Yumizen H2500) and IPF (available in XN 1000) were correlated. Manual slide review was performed following CLSI H20-2a guidelines.





Sysmex XN1000 uses Fluorescent Flow cytometry and a specific dye and measures Immature platelet fraction (IPF). Figure 3 Literatures suggest IPF indicates thrombopoietic activity and helps in differentiating underproduction, peripheral destruction and recovery process as well as platelet transfusion management in thrombocytopenia.^{3,4,5}

The aim of the study was to acquire data on YUMIZEN H2500 in PLT-O and compare with standard CBC mode and a predicate (Sysmex XN) in both PLT-F mode and standard CBC mode and to evaluate the analytical efficiency of a novel methodological solutions, used in haematology analyser Yumizen H2500, to measure the large platelet fraction (LPF) and to compare with a immature platelet fractions (IPF) measured by Sysmex XN 1000.



RESULTS

A good correlation was observed between the novel LPF by Yumizen H2500 & IPF by Sysmex XN 1000 with a correlation coefficient (r2) 0.933. The regression equation (y = 0.89x + 0) a good agreement between both parameter with intercept equal to 0 (95% CI - 0.192 to 0.192) and slope equal to 0.89 (95% CI 0.846 to 0.936) and an acceptable bias of -11.03% (95% CI -7.4 to -14.8) (Figure 4).



Figure 3 -Low IPF (left) Vs High IPF (right) from Sysmex XN 1000

DISCUSSION

Platelets are released into the blood from long pro-platelet extensions of megakaryocytes. Young platelets that are recently released by megakaryocytes are larger, denser and undergo remodeling in circulation, in part by shedding some of their surface components.

Macrothrombocytopenia may reflect a disturbance in the steps of platelet production, or hereditary disorders. The properties of large platelets may reflect unique attributes of platelets recently released from the marrow or pro-platelets produced under conditions of accelerated or abnormal production. 6 Counts of Large platelets with a volume >12 fl falls and smaller than 30fl fall in P-LCC category (normal platelet size varies between 7.5 fl to 10.5 fl), they are usually relatively young and more recently released from the bone marrow, while smaller platelets may be older and have been in circulation for a few days. The platelet large cell ratio is the number of cells falling above the 12 fl threshold divided by platelet count. A high P-LCR or PDW may indicates active thrombopoiesis. These parameters are derived from impedance platelet count and suffers from false count because of interference from giant platelets beyond 30fl, fragmented RBC & microcytes. Hence an alternative way of counting the large platelets will be more helpful and utmost clinical importance. Sysmex XN analyzers quantify these by a separate IPF parameter. Yumizen H2500 derived novel parameter LPF was found to be a valuable parameter which showed very good correlation and agreement with IPF indicating that IPF & LPF can be used interchangeably.

Figure 4 - Correlation LPF% Vs IPF % (Left) & Graph of difference (Right)

CONCLUSION

Large platelet counting could be an important challenge in specific haematology disorders. The novel parameter LPF can help laboratories in the critical decision making and is comparable to IPF. LPF can be a more appropriate nomenclature as both LPF and IPF gives the same information but using different methodology and can be used interchangeably.

References

1. Handtke, S, Thiele, T. Large and small platelets—(When) do they differ? J Thromb Haemost 2020; 18: 12561267. https://doi.org/10.1111/jth.14788.

2. Valeria V. Bodrova, Olga N. Shustova, Svetalana G. Khaspekova & Alexey V. Mazurov (2021) Platelet reticulated forms, size indexes, and functional activity. Interactions in healthy volunteers, Platelets, DOI: 10.1080/09537104.2021.1922659

3. Abe Y, Wada H, Tomatsu H, Sakaguchi A, Nishioka J, Yabu Y, Onishi K, Nakatani K, Morishita Y, Oguni S, Nobori T. A simple technique to determine thrombopoiesis level using immature platelet fraction (IPF). Thromb Res. 2006;118(4):463-9. doi: 10.1016/j. thromres.2005.09.007. Epub 2005 Oct 25. PMID: 16253312.

4. Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. Br J Haematol. 2004 Jul; 126(1):93-9. doi: 10.1111/j.1365-2141.2004.04987.x. PMID: 15198738.

5. Jeon, Kibum MD; Kim, Miyoung MD, PhD; Lee, Jiwon MD; Lee, Jee-Soo MD; Kim, Han-Sung MD, PhD; Lee, Young Kyung MD, PhD Immature platelet fraction: A useful marker for identifying the cause of thrombocytopenia and predicting platelet recovery, Medicine: February 2020 - Volume 99 - Issue 7 - p e19096 doi: 10.1097/MD.000000000000

6. Robier, Christoph. «Platelet morphology» Journal of Laboratory Medicine, vol. 44, no. 5, 2020, pp. 231-239. https://doi.org/10.1515/labmed-2020-0007