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Original Research Article

TO CORRELATE THESE INDICES WITH MORPHOLOGICAL FEATURES OF PLATELETS ON A STAINEDPERIPHERAL SMEAR

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Abstract

In evaluating the mechanism of thrombocytopenia, it is necessary to know which pathogenic mechanism is the more dominant one, decreased production or increased destruction, since treatment is vastly different for the two entities.

Materials and methods

The study was Dept-Pathology, VTSM Peripheral Cancer Centre (Branch Of Kidwai Cancer Institute ,Bangalore

Results

Several quantitative platelet parameters are now available on hematology analyzers. The following are directly measured; Platelet count (Impedance, Optical/ Fluorescence) and Immature platelet fraction and the remaining

Conclusions

Patients with thrombocytopenia due to peripheral platelet destruction have a significantly higher IPF than patients with thrombocytopenia due to decreasedplatelet production

Keywords: Platelet, Immature

Introduction

Thrombocytopenia results from one or more of three processes: (1) decreased bone marrow production, (2) sequestration, usually in an enlarged spleen, and/or (3) increased platelet destruction. In evaluating the mechanism of thrombocytopenia, it is necessary to know which pathogenic mechanism is the more dominant one, decreased production or increased destruction, since treatment is vastly different for the two entities. For this purpose, bone marrow aspiration is often used. A bone marrow examination provides information about the degree of thrombopoiesis by judging the adequacy of megakaryocytes but it is subject to sampling errors, delays and subjectiveinterpretation.

The concept of 'young' platelets or reticulated platelets has been around for over 40 years and for several years after its discovery in 1969, has been a topic of intense research, especially as evidence of it being a good indicator of thrombopoiesis mounted.Up until the late 1990's, the only way to quantify them was by fluorescence flow cytometry, which had a large repertoire of drawbacks. With the advent of automated hematology analysers, automated methods of reticulated platelet estimation also came into being, in the forefront of which is the Immature Platelet Fraction or IPF. In the recent past, there has been a lot of research done on the utility

of IPF in the differentialdiagnosis of thrombocytopenia and it is proving to be an inexpensive, non-invasive and reliable alternative to a bone marrow examination. It is also gaining importance as a marker of platelet recovery in patients with thrombocytopenia due to chemotherapy orpost hematopoietic stem cell transplant. This knowledge is being used to curtailunnecessary prophylactic platelet transfusions in these patients.

In addition to IPF, hematology analysers also provide an array of platelet parameters like Mean Platelet Volume (MPV), Plateletcrit (PCT), Platelet Large Cell Ratio (P- LCR) and Platelet Distribution Width (PDW), most of which are not yet approved for routine clinical use as there is limited data available on them.

Most studies relating to IPF have been carried out in the West and in Japan. There is nostudy from India that has researched the utility of Immature Platelet Fraction in the diagnosis of thrombocytopenia or its predictive value in platelet recovery in patients with a recovering marrow. The study of platelet morphology under the light microscope in cases of thrombocytopenia has also been a neglected area in the recent years and there have been no studies that have looked at platelet morphology in relation these newer platelet parameters.This study will be the first of its kind from India to look at the utility of all the novel platelet parameters in conjunction with platelet morphology on smear in the diagnosis of thrombocytopenia and also the first from India to study patterns of IPF in patients with a recovering marrow.

MATERIALS AND METHODS

The study was Dept-Pathology,VTSM Peripheral Cancer Centre (Branch Of Kidwai Cancer Institute ,Bangalore

Inclusion criteria:

- All patients presenting for the first time to the Clinical Hematology OPD in ourhospital with an initial platelet count of less than 100000/µL were enrolled in the study irrespective of whether or not they had had previous treatment for their condition from another hospital. This stand was taken since our institutionis a tertiary referral care center.

Exclusion criteria:

- Patients who had presented to the Hematology OPD for similar or other hematological complaints prior to the start of the study period.
- Cases in which peripheral smear slides were not available for review.

RESULTS

Several quantitative platelet parameters are now available on hematology analyzers. The following are directly measured; Platelet count (Impedance, Optical/ Fluorescence) and Immature platelet fraction and the remaining i.e. Mean platelet volume, Platelet distribution width, Plateletcrit and Platelet large cell ratio are derived from other primary modalities. We have determined reference intervals for these usingthe normal population that we studied for adults and children. Since all parameters had a non-parametric distribution, reference intervals were calculated using the 5^{th} and95^{th} percentiles (Table 1).

Table 1: The normal reference intervals obtained for our population on SysmexXN9000 and Mindray BC6800.

PARAMETER	SYSMEX	MINDRAY
	Median (5^{th} – 95^{th} percentile)	Median (5^{th} - 95^{th} percentile)
MPV (fL)	10.6 (9.5 - 12.1)	9.5 (8.3-11.2)
IPF (%)	2.5 (1.1 - 6.1)	4 (1.6-8.9)
PDW (fL)	12.6 (10.4-16.3)	15.9 (15.4-16.5)

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PCT (%)	0.3 (0.22-0.42)	0.28 (0.20-0.39)
P-LCR (%)	30.1 (20.7-42.5)	23 (14.9-35.4)

Since there is a high prevalence of constitutional macrothrombocytopenia in individuals from North Eastern India who come to our institution, we calculated a separate set of reference intervals for them using data from 131 blood donors comingfrom these areas (Table 2).

Table 2: Reference intervals obtained for North East Indian population on Sysmex XN 9000.

Parameter (5 - 95 percentile)	Reference intervals
MPV (fL)	10.3 – 14.9
IPF (%)	2.1 - 34.8
PDW (fL)	12.0 - 24.1
PCT (%)	0.19 - 0.37
P-LCR (%)	27.8 - 64.2

Table 3: Reference intervals obtained for children on Sysmex XN9000.

Parameter (5 - 95 percentile)	Reference intervals
MPV (fL)	8.6 – 11.3
IPF (%)	0.4 – 3.1
PDW (fL)	7.9 – 14.8
PCT (%)	0.22 – 0.47
P-LCR (%)	13.4 - 36

The difference between adult and child populations was probably due to the smallersample size used in the reference interval calculation for children (N=27) when compared to the sample size of 248 in the reference interval calculation of the adultpopulation.

As a part of the standardization process, we performed Altman-Bland agreementstatistics on the IPF data obtained from analysis on the Sysmex and Mindray platforms.
Given the fact that clinical laboratories have different workflows based on where samples are sourced from and the need for add-on testing on samples already in thelaboratory, stability of the measured parameter is an important consideration. We evaluated the stability of platelet parameters by timed repetitive analysis of samplesstored at different temperature conditions up to 24 hours.

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Table 4. Time (in hours) within which samples have to be analysed on Sysmex and Mindray analysers to get reliable results when stored at two different temperatures.

	SYSMEX (IN HOURS)		MINDRAY (IN HOURS)	
PARAMETER	22-24°C	2-8°C	22-24°C	2-8°C
IPF (%)	48	48	8	4
MPV (fL)	24	4	24	4
PLT F (x 10^9/L)	32	48	Not provided	Not provided
PLT I (x 10^9/L)	48	48	48	48
PLT O (x 10^9/L)	48	48	48	48
P-LCR (%)	32	0	32	0
PCT (%)	48	24	48	24
PDW (fL)	24	4	48	48

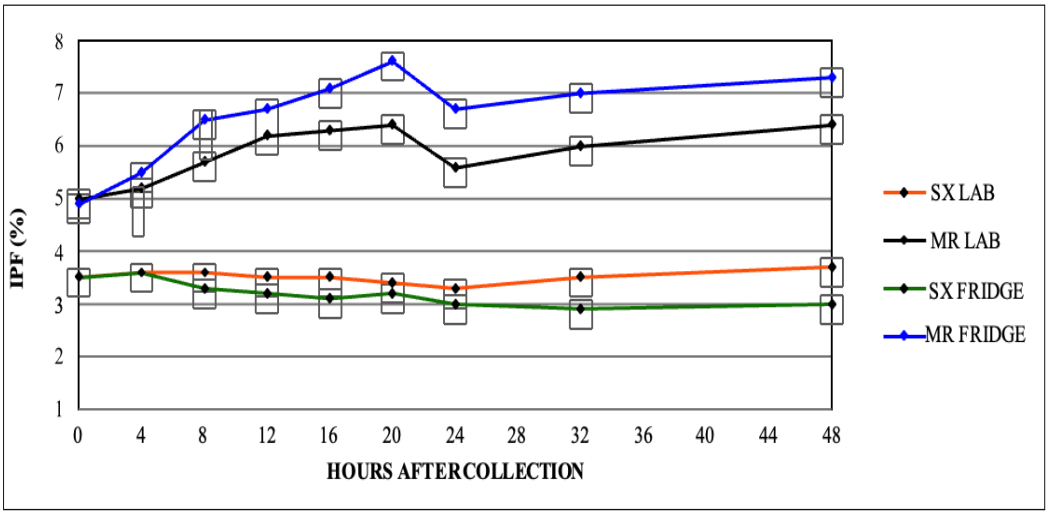

Figure 1. Serial IPF measurements over time in Sysmex (SX) and Mindray (MR) analysers in samples stored at 22-24°C (LAB) and 2-8°C (FRIDGE) temperatures.

The IPF in the Sysmex analyser was stable up to 48 hours at both the storage conditions (Table 4) whereas the IPF obtained in the Mindray analyser was stable onlyup to 8 hours when stored at 22-24°C and up to 4 hours when stored at 2-8°C (indicated by red arrows in figure 1), beyond which, there was a significant rise in IPF% above the true value.

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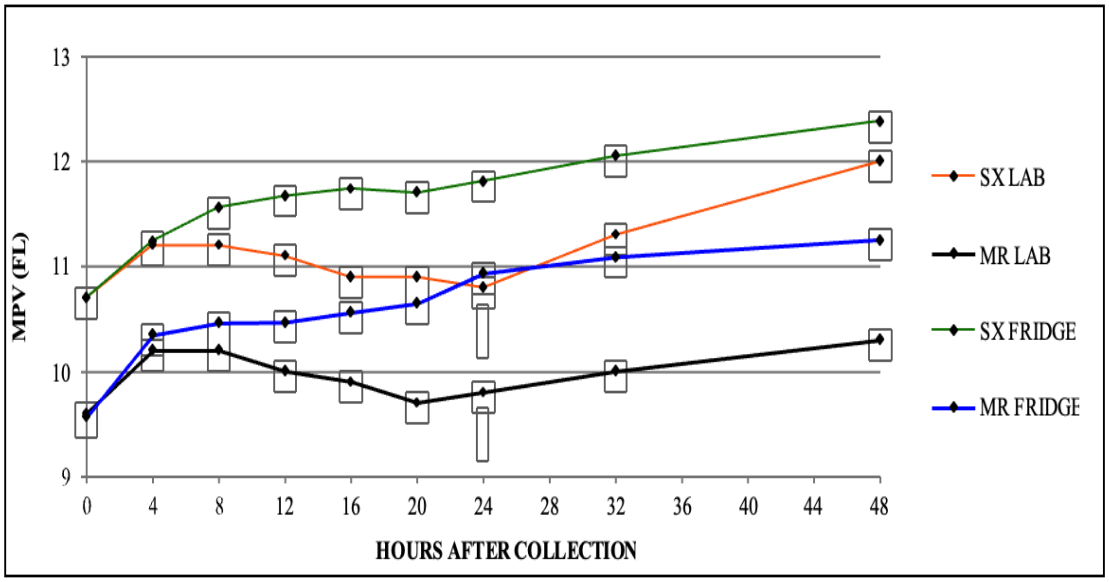

Figure 2. Serial MPV measurements over time in Sysmex (SX) and Mindray (MR) analysers in samples stored at 22-24°C (LAB) and 2-8°C (FRIDGE) temperatures.

The MPV in both analyzers was unstable, with 24 hours of storage at 22-24°C beingthe latest by which a sample could be analysed to get accurate results on the Sysmexanalyser (indicated by red arrows in figure 2). Beyond the stable time periods, there was a steady rise in MPV with time.Due to the lack of stability of IPF and MPV on Mindray, only Sysmex XN9000 wasused for further study purpose.

DISCUSSION

Age specific reference intervals for the following parameters: MPV, IPF, PDW, P- LCR and PCT from 248 healthy adults and 27 healthy children on both Sysmex XN 9000 and Mindray BC 6800 platforms were determined. The data was tested for normality of distribution. Since none of these parameters had a normal distribution weused 5th and 95th percentiles as reference ranges. This lack of a normal distribution forplatelet parameters has been reported previously and hence authors have used different methods for reference range calculation like Mean □ 2SD, 25th and 75th percentiles or minimum and maximum (4,5). Our reference interval of IPF in adultswas 1.1 – 6.1% and 1.6 – 8.9% in the Sysmex and Mindray analysers respectively.
The values we obtained for the Sysmex analyser were in keeping with the reference ranges obtained in studies by Briggs *et al*. and Seo *et al*. (6,7) but differ from thosequoted in other studies like the one by Jung *et al*. which gives a lower cut-off of 3.2% as the upper limit of normal (5). This difference could be due to differences in the statistical tools used to derive the reference ranges.

Our reference interval for IPF in children was much narrower and lower than that for adults and was taken as 0.4 – 3.1%. Seo *et al*. had reported a similar finding of an IPFnadir occurring between the ages of 2-6 years followed by increasing IPF to reach adult levels beyond 18 years of age (6). This phenomenon could be due to difference in platelet dynamics in children or could also be due to the smaller sample size that we had for the reference range calculation in children.

It has been observed that individuals from North Eastern states of India, Bangladeshand Nepal

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have a high incidence of a phenomenon called constitutional ethnic macrothrombocytopenia which is an asymptomatic condition wherein, the individual has giant platelets in the peripheral blood and thrombocytopenia (8). It has also been documented that these individuals have a high IPF (Median 25.5%) (9). Sinceour institution has a significant proportion of its patient population coming from thesestates, we calculated reference ranges separately for IPF from 131 healthy individuals from North Eastern India. We obtained a median IPF of 10.4% and a reference interval of 2.1 – 34.8% (5^{th} and 95^{th} percentile).

Bland Altman agreement statistics performed between the two analysers showed that majority of the data points fell within the 95% confidence intervals. The Intra-class correlation coefficient was found to be 0.92 (95% CI: 0.81-0.97) which suggested thatthere was a good agreement between the two analysers. There have been no previous studies that have looked at the agreement between these two analysers.

Previous literature so far has given contradicting evidence on stability of platelet parameters on Sysmex analysers. Studies by Briggs *et al.* on Sysmex XE series had shown that IPF was stable up to 48 hours of storage (7). However, certain other studies have quoted much lesser stability times varying between 3 – 24 hours with decreasing stability on storage at refrigerated conditions (10,11,12). The Sysmex XN analyser has been reported to have better stability of up to 48 hours with storage at room temperature or refrigerated conditions (13,14). The stability of these parameters on Mindray BC6800 has not been studied so far. In our study, we found that IPF on Sysmex XN was stable up to 48 hours when stored at either room or refrigerated temperatures which was in keeping with previous studies. However, IPF on Mindray was stable only up to 8 hours on storage at room temperature and up to 4 hours on storage in refrigerated conditions beyond which there was a statistically significant rising trend in IPF values. MPV was equally unstable in both analysers with increasing values being obtained beyond 4 hours of storage at refrigerated temperatures. Platelet counts were stable up to 48 hours in both analysers. This lack ofstability in IPF on Mindray could be due to the different fluorescent dye and effect of analytic reagents and methods in the chamber that is used in this analyser.

One of the objectives of the study was to study IPF in different patient groups, primarily to see if this parameter was different in patients who had a peripheral platelet destruction over those that had a bone marrow production problem. We also hypothesized that the trends of IPF during bone marrow recovery in patients who had undergone stem cell transplant for various hematological conditions could help to predict those who would display platelet recovery.

CONCLUSIONS

Patients with thrombocytopenia due to peripheral platelet destruction have a significantly higher IPF than patients with thrombocytopenia due to decreased platelet production. A subgroup of individuals from the North Eastern states of India have an asymptomatic form of thrombocytopenia called ethnic macrothrombocytopeniacharacterized by the presence of large blue platelets in peripheral smear and thehighest IPF among the patient groups studied.

REFERENCES

1. Brewer DB. Max Schultze (1865), G. Bizzozero (1882) and the discovery of theplatelet. Br J Haematol. 2006 May;133(3):251–8.
2. Machlus KR, Italiano JE. The incredible journey: From megakaryocyte development to platelet formation. J Cell Biol. 2013 Jun 10;201(6):785–96.
3. Kaushansky K. The molecular mechanisms that control thrombopoiesis. J ClinInvest. 2005 Dec 1;115(12):3339–47

4. Ko YJ, Hur M, Kim H, Choi SG, Moon H-W, Yun Y-M. Reference interval for immature platelet fraction on Sysmex XN hematology analyzer: a comparison study with Sysmex XE-2100. Clin Chem Lab Med CCLM [Internet]. 2015 Jan 1[cited 2016 Jul 25];53(7). Available from: http://www.degruyter.com/view/j/cclm.2015.53.issue-7/cclm-2014-0839/cclm- 2014-0839.xml
5. Jung H, Jeon H-K, Kim H-J, Kim S-H. Immature Platelet Fraction:Establishment of a Reference Interval and Diagnostic Measure for Thrombocytopenia. Korean J Lab Med. 2010;30(5):451.
6. Seo A, Yuan D, Daniels S, Yuan S, Gallagher M, Wong ECC. Reference intervals for immature platelet fraction and immature platelet count. Int J Lab Hematol. 2015 Feb;37(1):e1–2.
7. Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immatureplatelet fraction (IPF) in peripheral thrombocytopenia. Br J Haematol. 2004 Jul;126(1):93–9
8. Sukesh CN DD. Asymptomatic constitutional macrothrombovytopenia amongwest bengal blood donors. Am J Med. 2002;112(9):742–743.
9. Bhat R, Pai S. Immature platelet fraction: a significant platelet parameter in asymptomatic constitutional macrothrombocytopenia. Int J Lab Hematol. 2016Jun 1;38(3):e45–7
10. Dadu T, Sehgal K, Joshi M, Khodaiji S. Evaluation of the immature plateletfraction as an indicator of platelet recovery in dengue patients. Int J Lab Hematol. 2014 Oct;36(5):499–504.
11. Osei-Bimpong A. The effect of storage on the clinical utility of the immatureplatelet fraction. Hematology. 2009 Apr;14(2):118–21
12. Yamaoka G, Kubota Y, Nomura T, Inage T, Arai T, Kitanaka A, et al. The immature platelet fraction is a useful marker for predicting the timing of plateletrecovery in patients with cancer after chemotherapy and hematopoietic stem celltransplantation: PREDICTION OF PLATELET RECOVERY BY IPF. Int J LabHematol. 2010 Dec;32(6p1):e208–16.
13. Briggs C, Longair I, Kumar P, Singh D, Machin SJ. Performance evaluation ofthe Sysmex haematology XN modular system. J Clin Pathol. 2012 Nov 1;65(11):1024–30.
14. van der Linden N, Klinkenberg LJJ, Meex SJR, Beckers EAM, de Wit NCJ,Prinzen L. Immature platelet fraction measured on the Sysmex XN hemocytometer predicts thrombopoietic recovery after autologous stem celltransplantation. Eur J Haematol. 2014 Aug;93(2):150–6