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Developing Localized Reference Intervals for Platelet Indices in South India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The Platelet Indices (PI) are part of the most frequently done tests in the hospital. They are considered as biomarkers in numerous systemic and metabolic diseases. But the real challenge has been to define the clinical cutoffs which enable management based on the above parameters. So we tried to develop Reference Intervals(RI) for PIs to enable them for clinical use.
Aim: The aim of the study was to develop RIs for the PIs from ostensibly healthy adult males from the local population.
Materials and Methods: 123 healthy adult males between the age 18 to 64, were chosen after screening and their blood samples collected and examined on Sysmex XN1000 cell counter for the PIs.

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Results: The mean values of Platelet Count (PC), Distribution width (PDW), Mean Platelet volume(MPV), Plateletcrit (PCT), Large cell Ratio(P-LCR), Immature Platelet Fraction(IPF) and Absolute Immature Platelet Number(AIPN) were $2.58x10^9$/L, 12.3%, 10.2fl, 0.281%, 27.17%,0.682%, and 1551.4 cells. PDW showed a strong positive correlation with MPV (r = 0.937) and P- LCR (r = 0.949). The PLT showed a very strong positive correlation with PCT (r = 0.901). The linear regression analysis of the rest of the PIs with PLT showed an 81% strong predictability for PCT.

Conclusion: The sex specific, instrument specific, localized RIs developed the study was compared to other published studies World over. It was also compared to an Indian and other South Indian studies. It also offers an opportunity for institutions using this cell counter to make use of the above RIs in clinical situations.

Keywords: Platelet Indices; Reference Intervals; IPF; Sysmex XN1000.

1. INTRODUCTION

Platelet Indices(PIs) are the most common patient values looked at, considering that, they are the part of the innumerable Complete Blood Counts(CBC) being done everyday. PIs are available at no extra cost, provide insights into platelet kinetics and are used in guiding therapy. They include Platelet Count(PC), Distribution width(PDW), Mean Platelet volume(MPV), Plateletcrit(PCT), Platelet Large cell Ratio(P-LCR), Immature Platelet Fraction(IPF) and Absolute Immature Platelet Number(AIPN). The study was planned to construct RIs for the above 7 PIs on Sysmex XN1000 counter, in a bid to raise localized, instrument and sex specific RIs.

PIs have been employed and are advocated as novel biomarkers in many acute and chronic conditions [1]. But their routine use is hindered by non-availability of localized, instrument and sex specific RI. As a result, we tried to develop RI for the 7 PIs to enable them for regular clinical use. Considering the sample size required for reference ranges and the fact that Sysmex XN 1000 uses florescent stain for IPF in the Reticulocyte mode, the whole process was deemed costly in a low resource setting. So as to bring down the cost, we employed the method advised elsewhere in developing RIs for the PIs [2]. The study was planned to develop RI for PIs from healthy adult males. The fact that gender specific RIs show difference with regard to PIs is evident in literature. The RIs so developed, was compared to those published recently in literature.

2. MATERIALS AND METHODS

The Institutional Ethics and Review Board had approved the study protocol (no:IEC/GMCTSR/128/2021). The sample size of 120 was based on the IFCC recommendation which stated that minimum 120 samples are required for reliable estimates of a parameter [3]. Ostensibly healthy, consenting males, aged 18-64 years who came for voluntary blood donation to Government Medical College Thrissur, from August to September 2021 in the Department of Transfusion Medicine, were interviewed. A detailed questionnaire was given to exclude preexisting kidney, coronary, lung, liver diseases, neoplasms, diabetes, hypertension, anemias, significant drug history, iron therapy, thyroid disease, antimetabolite drugs, smoking, alcohol, insulin, vaccination, and history of allergy. After screening 123 consenting male blood donors were found fit and was included in the study. The SOPs were followed for bleeding and collection of blood. 2ml blood was collected in K3 EDTA tubes, by careful venipuncture on the right arm in recumbent position, which were inverted gently 3 times for mixing and stored at 22^0 Celsius. The samples were analyzed in the Sysmex XN1000 (Sysmex Corporation, Kobe, Japan) cell counter, within 3 hours of collection, to minimise the effects of storage and for uniformity, in the reticulocyte mode, for PIs. The AIPN was a derived from the IPF, whereas the rest of the six PIs were obtained directly. The cell counter is under a EQAS form CMC Vellore and regularly does daily IQC at 3 levels. The results of the 123 samples employed in the study were recorded.

2.1 Statistical Analysis

All tests were performed using SPSS (Statistical Product and Service Solutions), developed by IBM corporation version 16). The results were analyzed for normality, and the mean and reference ranges were derived statistically. The above 7 parameters were expressed as mean, standard deviation (SD), standard error of the

mean (SE), 95% confidence interval with special reference to minimum and Maximum values. Pearson's correlation analysis was used to find out the intercorrelation between the PIs. Linear regression analysis of the other 5 variables were computed with respect to IPF and AIPN. The objective of the study which was calculation of RIs was done using CLSI guidelines.

3. RESULTS

The youngest donor of the 123 males was 18 years while the oldest was 57 years old. The mean age was 29 years. The RIs deduced, after the analysis is depicted in the Table 1 for the seven PIs. The age showed a Standard deviation(SD) of 8 with 50% of the values lying between 23 and 35 years. The mean PLT was $2.58x10^9$/L with a range of 2.47 to $2.67x10^9$/L. The IPF ranged from 0.5 to 0.8% with a mean value of 0.6%. The PDW ranged from 11.9 to 12.6 fl with a mean value of 12.3 fl. The MPV ranged from 10 to 10.3 fl with a mean value of 10.2 fl. The PCT ranged from 0.27 to 0.29% with a mean value of 0.28%. The RI for P-LCR ranged from 25.8 to 28.3% with a mean value of 27.1%. The AIPN had a reference range from 1325 to 1777cells/mm3.

The study also looked at the intercorrelation between various PIs. The PLT showed a very strong positive correlation with PCT (r = 0.901) significant at 0.01 level. PDW showed a strong positive correlation with MPV(r = 0.937)and LCR (r = 0.949) (p value 0.01). MPV showed a strong positive correlation with P-LCR (r = 0.994) and PDW(r = 0.937)with a p value significance at 0.01. MPV and P-LCR showed a positive correlation with both IPF values (r = 0.580 to 0.464)(p value significance at 0.01 level).We also did linear regression for the predictive analysis of the PIs with respect to IPF and PLT. The analysis of PDW with respect to IPF showed 25% predictability while PLT and PCT showed a negative relationship. The regression analysis of the rest of the PIs with PLT showed an 81% strong predictability for PCT. All the other PIs showed a negative regression coefficient with respect to PLT.

Table 1. Biological reference intervals for platelet parameters

	Mean	SD	SEM	95% confidence interval	
				LB	UB
Age	29.537	8.803	0.794	27.981	31.093
PLT (10^9/L)	2.58	0.604	0.054	2.473	2.687
AIPN	1551.488	1281.512	115.55	1325.009	1777.967
IPF%	0.682	0.804	0.073	0.540	0.824
PDW(fl)	12.333	1.946	0.175	11.989	12.677
MPV(fl)	10.217	0.85	0.077	10.067	10.367
PCT(%)	0.281	0.065	0.006	0.270	0.292
LCR(%)	27.117	7.004	0.632	25.879	28.355

PLT-Platelet Count, AIPN-Absolute Immature Platelet number. IPF- immature Platelet fraction. PDW-Platelet distribution width. MPV-Mean Platelet volume. PCT-Plateletcrit P-LCR- Platelet large cell ratio

Table 2. Intercorrelation between Platelet Parameters

	Plt Cnt	IPF#	IPF%	PDW	MPV	PCT	LCR
Plt Cnt	1	-0.06748	-.394**	-.280**	-.251**	.901**	-.259**
AIPN	-0.06748	1	.822**	.619**	.587**	0.085594	.580**
IPF%	-.394**	.822**	1	.506**	.464**	-.267**	.464**
PDW	-.280**	.619**	.506**	1	.937**	0.069483	.949**
MPV	-.251**	.587**	.464**	.937**	1	0.124167	.994**
PCT	.901**	0.085594	-.267**	0.069483	0.124167	1	0.114213
LCR	-.259**	.580**	.464**	.949**	.994**	0.114213	1

PLT-Platelet Count, AIPN-Absolute Immature Platelet number. IPF- immature Platelet fraction. PDW-Platelet distribution width. MPV-Mean Platelet volume. PCT-Plateletcrit P-LCR- Platelet large cell ratio

*** Correlation is significant at the 0.01 level.*

Table 3. Comparison of platelet parameters with other studies done in XN-series over the last 10 years

	Clinical Reference Range XN-series provided by the manufacturer 2011 [6]		**Ali U et al., United Kingdom population 2017 [7]**		**Pelt van JL et al., Dutch population 2022 [8]**	**South Indian population (XN-1000) 2022 [4]**		**Presesnt Study 2022 South India**
PIs	Male	Female	Male	Female	Male F	Male	Female	Male
Sample size	415	794	791	1565	18484	1185	698	123
PDW (fl)	9.8-15.2	9.6-15.2	9.3-17	9.3-17.3	10-17.4	9-16.4	9.1-16.6	11.98-12.67
MPV (fl)	9.1-12	9.2-12.1	9.1-13	9.2-12.8	9.3-12.7	9-12.3	9-12.6	10.06-10.36
PCT (%)	0.19-0.36	.19-0.40	0.16-0.35	0.18-0.37	0.2-0.4	0.15-0.36	0.14-0.41	0.27-0.29
P-LCR (%)	19.5-41.9	19.6-42.6	17.6-47	17.8-47.8	19.3-47.1	16-42.1	16.6-43	25.87-28.35
IPF (%)	0.9-5.4	1 – 4.8	Not Done		1.2 -8.9	Not Done		0.54-0.82
PLT x10^9/L	1.68-3.92	1.98-4.17	Not Done		1.67-3.77	Not Done		2.47-2.68

PLT-Platelet Count, AIPN-Absolute Immature Platelet number, IPF- immature Platelet fraction, PDW-Platelet distribution width, MPV-Mean Platelet volume, PCT-Plateletcrit P-LCR- Platelet large cell ratio.

Source: Table adapted with permission from Gnanadeepam et al [4].

Table 4. Comparison with a south Indian study

Sample size	**South Indian population (XN-1000) 2022 [4]**		**Current study Kerala population 2022**	
	1185		**123**	
	Median	**IQR**	**Median**	**IQR**
PDW(fl)	11.30	2.1	12.2	2.6
MPV(fl)	10.10	1.07	10.2	1.1
PCT (%)	0.26	0.06	0.27	0.08
P-LCR(%)	25.70	8.5	26.9	9.6
IPF(%)	Not Done		0.5	0.4

PLT-Platelet Count, AIPN-Absolute Immature Platelet number, IPF- immature Platelet fraction, PDW-Platelet distribution width, MPV-Mean Platelet volume. PCT-Plateletcrit P-LCR-

Platelet MPV – mean Platelet volume,large cell ratio

4. DISCUSSION

The platelet indices are affected by age, ethnicity, sex, hormonal status, intake of OCPs and drugs, the nature of anticoagulant used, time lag before testing, ambient temperature, cell counter technology, in addition to other pre analytical factors. But all this has not prevented PIs from being widely used in various systemic diseases as biomarkers for prognostication in various inflammatory and neoplastic diseases. This prompted us to develop and standardize RI for PIs for use in everyday clinical situations. That this attempt was done on Sysmex XN1000 should add to the utility, as the cell counter is widely employed and the fact that there are several studies [2] on the same topic on other earlier Sysmex analyzers like XE-5000, KX-21, XE-2100, which makes it comparable worldwide. The study attempts to define RIs in an apparently healthy male, adult, local, population in Thrissur, Kerala, and maximum was care taken to minimize variability in pre and post analytical phases. The RI published by the manufacturer (in this case, Sysmex) was developed in a population residing abroad (Kochi, Japan) and their suitability to South Indian population had to be verified. One of the major challenges we faced, being a low resource setting is, to get IPF done for every patient as the test involved more cost compared to a CBC. In order rationalize the cost, we followed the IFCC and CLSI recommendation which enabled us to choose a minimum sample size of 120 [3]. The literature review showed conflicting evidence regarding influence of age on PIs, with majority concluding that gender, was more of a significant variable than age, influencing RI ranges [4]. The heterogeneity of the sample to be studied while including females and rarity of female blood donors prompted us to construct separate RIs.

The ultimate test of a recently developed RI is its comparability to the known reference ranges [4,5]. Though RI for PIs has been developed on every cell counter in the market, it is not prudent to compare across platforms. The authors compared the results with RIs developed only on XN series over a period of 10 years [6-8] (Table 2). On comparison we found that RIs in the present study, has a smaller range compared to three other studies done on XN1000 series. (Table 2). The closest study geographically and technologically was of Gnanadeepam et al. [4], which was done on South Indian population. IPF was not done in their study. The sample size was the least in our study. Kunal et al had done a study on RIs for hematological parameters on the Indian population, in study from North India, which was available for camparison [5].The RI for PLT was highest in our case with a mean of 2.58 $x10^9$/L. Sachdeva et al reported a RI of 2.47 – 2.54 $x10^9$/L for the PLT which was carried out in a North Indian population on a Sysmex platform [9].One of the important deviations in our study, from the standard RIs mentioned elsewhere was the IPF% in the local population. Our RI of 0.5% - 0.8% did not compare well with Sysmex RI and other XN series studies except that by Gnanadeepam et al. Our upper limit of the IPF (0.8%) was well below the upper limit of 5.4%, which was reported in males from South India [4]. Our maximum value was 5.9%. IPF has role in prediction of platelet recovery following Stem cell transplant and was noted that IPF greater than 7.0% on Day 8 after Stem Cell Transplant predicted platelet recovery within 4 days (PPV 79%, sensitivity 76%) [10].

PIs for eg, MPV can be modified by ethnicity, age, cigarette smoking, alcoholism, and sedentary/ physically active life style. The RI for MPV from our study correlated with the Median and the IQR value obtained in the South Indian study [4] (Table 3). A high MPV correlates with poorer prognosis in carcinoma pancreas. Many systemic inflammatory diseases show correlation between MPV value and degree of inflammation. MPV less than 8fl is a marker of decreased platelet production whereas a value greater than 13 suggests increased destruction. PDW is a marker of platelet anisocytosis. There is a suggestion that it varies between 10 to 18% in health. Budak et al, reports that PDW reference levels varies from 8.3 and 56.6%. Such a wide range is exceptional in literature while the present study reports a range (11.9–12. 6%).This value is comparable to Gnanadeepam et al. [4] et al and both the values had a comparable IQR of 2. A high PDW has been reported in perforated appendicitis, and in sickle cell anemia. As reported in the present study, MPV showed a strong positive correlation with PDW in health, whereas this correlation is lost in disease conditions like pre term labour [1]. The PCT, a measure of platelet mass varied from 0.27 – 0.29% in the study, was comparable to Gnanadeepam [4] et al, and both had an IQR of under one. A marker of platelet activation, P-LCR indicates the percentage of platelets more than 12 fl. The median value of this variable reported in the study (mean value 27.1%) and the IQR was comparable to the south Indian study [4] though both the series showed high

IQR, and marked variation in the data. It is to be noted that P- LCR also had a low predictability in linear regression and showed positive correlation with MPV and PDW (p value 0.01). The quantity of freshly released platelets, or absolute immature platelet number (AIPN)derived as IPF% x platelet count, whose range was reported as 2.4 - 20.7 × 10^9 /L by Taha et al. [11].

The PCT showed an excellent positive correlation with PLT and a negative correlation with IPF, AIPN, PDW, MPV, and P-LCR. The latter, P-LCR also showed positive correlation with MPV (r = 0.949 at p = 0.01) and PDW (r = 0.994 at p = 0.01). This was similar to the study by Sachdeva et al, who also reported positive correlation between MPV and PDW (r = 0.937 at p = 0.01), as in our case. Another similarity between both studies were the negative correlations observed between IPF% on comparison with PLT (r = -0.394) and PCT(r = -0.267).A higher r^2x100 value, on linear regression convey a proportionate change in a dependent, with regard to the independent variable. The data (Table 2) shows that predictability of PIs with respect to one another is low. Present study shows a good predictability of the PCT (r^2*100 = 81%) value when compared with the independent variable, PLT. None of the other parameters has good predictability compared with this index. Vani et al advocates PCT in addition to PLT to determine the requirement of transfusion and is also a useful tool for detecting quanitative platelet disorders [12-14].

On comparison, the RIs in the present study have a very tight range. The IPF reported in the study, is lower compared to other studies reviewed. That the ranges were comparable to Gnadeepam et al tells us two important things. It stresses the importance of developing localized, region and analyzer specific RIs for PIs. As mentioned, most of our donors fell between 23 and 35 years (95% CI 27.9 - 31.0) which avoided extremes of age as well as females, might have created an homogenous population from which we sampled, leading on to coherence in our reference ranges compared to the ranges we reviewed in literature. The IPF value which was lesser compared to all other studies prompts us to exclude the possibility of a lower value prevalent in the region and Kerala state and thereby affirm the importance of localized RIs. We also declare the importance to look at PCT as well as PLT in reaching clinical decisions as advocated by Vani et al. [12].

5. CONCLUSION

There has been a proliferation of articles in the last 10 years on PIs, probably due to improvement in cell counter technology, enabling the diagnostic and prognostic use of these parameters in numerous diseases. But their outright use has been marred by variability across platforms and lack of standardization. We tried to develop cost effective, analyzer, region, and sex specific parameters to enable them for clinical use. We report a set of RIs for the platelet Indices which is comparable to other south Indian studies but have smaller ranges. Our reported RIs have a very tight range and IPF% from our study have lower value compared to all other studies from India and all over the world. We expect to use the above RIs to used in real clinical situations to compare diagnostic and prognostic significance.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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