Original Article

Access this article online

Quick Response Code:

Website: www.ajts.org

DOI: 10.4103/ajts.AJTS_121_20

Reference interval of platelet counts and other platelet indices in apparently healthy blood donors in North India according to Clinical and Laboratory Standards Institute guidelines: Need to redefine the platelet count cutoffs for repeat plateletpheresis donation?

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Abstract:

BACKGROUND: In clinical practice, laboratory results are of great importance for the diagnosis and treatment. Reference intervals of different parameters aid health-care professionals in the interpretation of results. There are very few studies on reference intervals from India. This prospective study was conducted to determine the reference intervals for platelet count (PLT) and PLT indices; mean PLT volume (MPV), PLT distribution width (PDW), and PLT large cell ratio (P-LCR). These values can be obtained as a part of a routine complete blood count (CBC) and have diagnostic and prognostic significance in certain diseases. PLT count is an important criterion for the selection of donors for repeat plateletpheresis donation.

MATERIALS AND METHODS: Sixteen hundred and thirty-four first-time healthy volunteer plateletpheresis donors were enrolled for the study. CBC was done, values of PLT, MPV, PDW, and P-LCR were noted, and the results were analyzed. The 95% of the reference distribution was estimated using the 2.5th and 97.5th percentiles following Clinical and Laboratory Standards Institute guidelines. Adverse donor reactions, if any and quality parameters of single donor PLTs (SDP) were also studied.

RESULTS: Reference range values of PLT, MPV, PDW, and P-LCR were 137,825–355,175/μl, 8.1–13.9/fl, 9.1–22.5/fl, and 11.7%–52.9%, respectively, and compared well with other published studies from India. It was observed that reference values of PLT count obtained in the study were lower than reference values that are currently used in most laboratories (150,000–450,000/μl) in India.

CONCLUSION: Based on our results, we are of the opinion that the PLT count cutoffs for repeat plateletpheresis donation may need to be revised downwards for our country which would also mitigate the scarcity of apheresis donors.

Keywords:

Cutoff for repeat plateletpheresis, platelet count, platelet indices, plateletpheresis donors, reference interval

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Submitted: 29-07-2020
Revised: 24-10-2021
Accepted: 26-11-2021
Published: 12-11-2022

How to cite this article: Pabbi S, Tiwari AK, Aggarwal G, Sharma G, Marik A, Luthra AS, *et al.* Reference interval of platelet counts and other platelet indices in apparently healthy blood donors in North India according to Clinical and Laboratory Standards Institute guidelines: Need to redefine the platelet count cutoffs for repeat plateletpheresis donation? Asian J Transfus Sci 2022;16:245-50.

Introduction

In clinical practice, laboratory results are of great importance both for diagnosis and treatment. Reference intervals of different parameters tested in laboratory aid the health-care professionals in the interpretation of results. Many population-based studies on the reference intervals have been carried out in developed countries, whereas there are very few studies on reference intervals from India. It has been documented that ethnic and racial differences exist for most of the biochemical and hematological parameters, and therefore, Indian data may be different from the data published on the Caucasian populations. International Federation of Clinical Chemistry (IFCC) and Clinical and Laboratory Standards Institute (CLSI) recommend that each country should develop and establish the reference ranges for all laboratory parameters, especially commoner biochemical and hematological parameters that are frequently used in the diagnosis and clinical management of various diseases.[1,2] The reference interval is defined as the interval between and including two numbers, an upper and lower reference limit, which are estimated to enclose a specified percentage (usually 95%) of the values for a population from which the reference subjects have been drawn. For most analytes, the lower and upper reference limits are assumed to demarcate the estimated 2.5th and 97.5th percentiles of the underlying distribution of values, respectively.[2]

The reference intervals of hematological parameters such as platelet count (PLT) and PLT indices, which are the important biomarkers of PLT activation; mean PLT volume (MPV), PLT distribution width (PDW), and PLT large cell ratio (P-LCR) have not been studied extensively. These biomarkers have a significant role in the diagnosis, treatment, and prognosis of certain diseases and can be monitored with the routine complete blood count (CBC) without additional testing or cost.[3] Due to the paucity of studies on these baseline PLT indices from the region, this study was carried out to establish the reference intervals for PLT counts and PLT indices.

Material and Methods

Settings

This prospective study was carried on healthy voluntary apheresis donors in the department of transfusion medicine at a tertiary care hospital of North India. The study was performed over a period of 6 months from March 2019 to August 2019.

Study population

A total of 1634 healthy apheresis donors in the age group of 18–60 years were enrolled for the study. The prospective donors were registered at the front-desk of the blood bank, and the donors were given predonation counseling by a trained counselor. They were administered medical history questionnaire, and a brief physical examination was carried out in accordance with the Directorate General of Health Services (DGHS) guidelines.[4] The medical examination included weight, temperature, blood pressure, pulse rate, and hemoglobin. Predonation Hb was estimated by a photometer (Compo Lab TS, Fresenius, Germany) in finger-prick blood samples.

Inclusion criteria

1. PLT apheresis donors conforming to the selection criteria for blood donors mentioned in Drugs and Cosmetic Act, 1940 and criteria mentioned in Directorate General of Health Services (DGHS) Technical Manual, 2003 were included[4,5]
2. All consecutive apheresis donors from northern states (Rajasthan, Madhya Pradesh, Chhattisgarh, Jharkhand, Bihar, Uttar Pradesh, Uttaranchal, Himachal Pradesh, Punjab, and Haryana) and northern union territories (Ladakh, Jammu, Srinagar, Chandigarh, and Delhi) were included in the analysis.

Exclusion criteria

1. Donors who had taken analgesics (nonsteroidal anti-inflammatory drugs) in the last 3 days were deferred
2. Donors who had donated PLTs in the last 4 weeks were also excluded from not included in the study.

Sample collection and tests

The institutional standard operating procedures were followed for the sample collection and for conducting the tests. Complete hemogram was done on ethylenediaminetetraacetic acid (EDTA) samples using Sysmex XP 100 i (Sysmex Corporation, Kobe, Japan) analyzer within 2 h of sample collection. Values of PLT count, MPV, PDW, and P-LCR were noted, and the results were analyzed.

Apheresis procedure

The default setting of donor PLT count and PLT yield was set at 150,000/μl and 3.5 x 10^{11}/unit, respectively, in the apheresis equipment before commencing the procedure. Upon commencement, the CBC sample was obtained immediately after phlebotomy, from the in-line sample pouch. CBC value was usually available within 5–10 min of initiating the procedure. Thereafter, the donor PLT count "default value" in the apheresis device was "re-set" at the actual PLT count. If the "re-set count" was lower than 150,000/μl, the apheresis machine increased the "volume to be processed," proportionately and vice versa. In order to study and compare the donor safety and quality of single donor PLTs (SDP), the study participants were divided into two cohorts on the basis

of their PLT counts; cohort A (PLT counts >1,50,000) and cohort B (PLT counts <1,50,000). Quality control (QC) parameters of randomly selected 10% single donor PLT (SDP) products from donors in both cohorts were analyzed.

Quality assurance of various equipment used in the study

All equipment used in this study were calibrated. QC of Photometer (Compo Lab TS, Fresenius, Germany) was performed weekly using low, medium, and high standard controls. QC on Sysmex XP 100 i analyzer (Sysmex Corporation, Kobe Japan) was performed daily using three different controls.

Statistical analysis

All tests were performed using the SPSS software version 24 2016, March (Statistical Package for the Social Sciences; IBM Bangalore, India). The 95% of the reference distribution was estimated using the 2.5th and 97.5th percentiles following CLSI guidelines.[2]

Two proportion *Z*-test was used to compare the adverse donor reactions in two study cohorts. $P < 0.05$ was considered statistically significant.

Code of ethics and consent

The study was approved by the institutional review board and donors' informed written consent was obtained for the donation of apheresis PLTs. No additional consent was taken for the study, as no additional sample was drawn and no personal identifiers such as name, address, and UHID (Unique Hospital Identification) were used for the study. Anonymized donor data were used for the analysis of reference intervals, ADR and QC.

Results

The 95% of the reference distribution was estimated using the 2.5th and 97.5th percentiles. The reference range values of PLT, MPV, PDW, and P-LCR were found to be 137,825–355,175/µl, 8.1–13.9/fl, 9.1–22.5/fl, and 11.7%–52.9%, respectively, as shown in Table 1. Majority of the donors (1570) had PLT more than 150,000/µl, followed by 49 donors with PLT in the range of 137,825-150,000/µl, and finally, 15 donors with PLT in the range of 110,000–137,824/µl. It was observed that the lower and the higher limits of the reference values of PLT count obtained in the study were lower than the reference values that are currently used in most of the laboratories (150,000–450,000/µl) in India.[6] "*P*" value of ADR (citrate toxicity, hematoma formation, and vasovagal reactions) in donors in two study cohorts was found to be 0.928 which was statistically insignificant, as shown in Table 2. The percentage of donors who experienced ADR was found to be 5.98% and 6.25%, respectively, in Cohort A and Cohort B. The mean values of QC parameters (volume, PLT count, swirling, pH, RBC contamination, and WBC log reduction) of the products from donors in two cohorts were found to be similar and were conforming to the regulatory guidelines, as depicted in Table 3.[4]

Discussion

Reference ranges are important in practice of medicine for correct interpretation of laboratory results. Different studies have shown that reference ranges are influenced by genetic factors, age, gender, and environmental and social factors. CLSI guidelines recommend minimum of 120 reference participants for the determination of reference interval.[2] Our sample size being 1634 was reasonably bigger than 120 for calculating the reference intervals of different parameters. However, we could not study the gender differences as number of women donors was very few 52 (3.1%) in our study population and this gender discordance in blood donor population was in concordance with several Indian studies.[7,8]

Platelet counts

In this study, we observed that reference values of PLT count, 1.38–3.55 L/cumm were lower than the reference values which are used currently by most of the laboratories, 1.50–4.50 L/cumm. Sairam *et al*. observed gender differences and regionwise differences in the reference range of PLT count in the Indian population, as shown in Table 4.[9] The reference values of PLT count obtained in our study were comparable to the values observed by Sairam *et al*. for Delhi and those observed by Sundaram *et al*.[10] The variation in the reference intervals between different studies can be explained by environmental and other genetic factors.

Impact on the selection of repeat apheresis platelet donors

Donors can donate PLTs at a minimum interval of 48–72 h, not more than twice a week and not more than

Table 1: Reference interval of platelet count and platelet indices

Variables	Total	Mean	Reference interval	SDM	SEM
Platelet count (/µl)	1634	225,038	137,825-355,175	59,920	225,038
Mean platelet volume (fl)	1634	10.7	8.1-13.9	3.8	10.70
Platelet distribution width (fl)	1634	13.6	9.1-22.5	3.5	13.60
Platelet large cell ratio (%)	1634	29.2	11.7-52.9	11.5	29.20

SDM=Standard deviation of the mean, SEM=Standard error of the mean

Table 2: Adverse donor reactions in study cohorts A and B

Type of ADR	Cohort A Donors with counts >150,000 (*n*=1570)	Donors with counts <1,50,000 (*n*=64)	*P*
Citrate toxicity	42	3	0.928
Hematoma formation	14	1	
Vaso-vagal reactions	38	0	
Total number (%)	94 (5.98)	4 (6.25)	

ADR=Adverse donor reaction

Table 3: Quality control parameters of single donor platelet in study cohorts A and B

QC parameters (mean values)	SDP product from donors with platelet counts >150,000 (*n*=1570)	SDP product from donors with platelet counts <150,000 (*n*=49)
Volume (ml)	290	310
Platelet count (×1011)	3.2	3.0
Swirling	Present	Present
pH	>6	>6
RBC (ml)	0.4	0.4
WBC log reduction (%)	99	99

SDP=Single donor platelet, QC=Quality control, WBC=White blood cells, RBC=Red blood cells

Table 4: Comparison of reference interval of platelet count with other published studies

Author	Place	Year	Sample size	Platelet count (L/cu mm)
Sairam *et al*.[9]	Delhi	2014	1279	Male-1.3-3.6 and Female-1.0-3.8
Sairam *et al*.[9]	Ahmedabad	2014	812	Male-1.5-3.6 and Female-1.4-3.9
Sairam *et al*.[9]	Hyderabad	2014	4446	Male-1.5-3.8 and Female-1.6-4.4
Sairam *et al*.[9]	Chennai	2014	4128	Male-1.1-3.9 and Female-1.2-4.1
Sundaram *et al*.[10]	Chennai	2008	220	1.4-3.7
Present study	Gurugram	2019	1634	1.38-3.55

24 times a year. AABB standards and DGHS do not require a pre-PLT count for apheresis PLT collections. A predonation count is required only if the frequency of donation is within 4 weeks of last donation.[4,11] Therefore, for repeat donations, prospective apheresis donors are deferred at counts lower than 150,000/µl as of today. In the present study, 49 donors had PLT counts in the range of 138,000–150,000/µl. If the donors with PLT counts in the range of 138,000–150,000/µl were accepted, as suggested by reference range of the present study, it would have resulted in expansion of donor-pool by 3%. We, therefore, feel that the criteria for the selection of PLT apheresis donors, in case of repeat donation within 4 weeks need to be revised downwards from 150,000 to 138,000/µl for our population. This would help in reducing the deferral rates and add more plateletpheresis donors to the donor pool. This is further corroborated by the study conducted by Pujani *et al*. in which they concluded that the criteria for the preprocedure PLT count (150,000) needs to be lowered considering the Indian scenario where there is always shortage of apheresis PLT donors.[12] Developing country such as India is mainly dependent on replacement blood donations as compared to voluntary blood donations in developed countries. We also do not have active apheresis donor registries or database to fall back on, in times of need.

Impact on selection of first-time apheresis platelet donors

Many blood centers, arbitrarily, use 150,000/µl as the basis for donor acceptance, even in first-time donors. This arbitrary cutoff, although not supported by regulations or guidelines is probably based on reference range currently in use by most of the laboratories (150,000–450,000/µl). Donors with PLT count less than 150,000/µl are deferred. Low PLT count has been documented as the most common cause for donor deferral in many studies.[12-14] Pujani *et al*. reported as high as 43.5% donor deferrals due to low PLT count.[12]

Donor safety

It has been observed in different studies that the PLT count recovers to baseline by 5–7 days.[15,16] Few studies have also documented that repeat plateletpheresis is safe and can be done without any detrimental effects on cell counts of donors.[17,18] Adverse events such as citrate toxicity (hypokalcemia, numbness, tingling, nausea, and vomiting), hematoma formation, and vasovagal reactions were similar in donors with counts lower than 150,000/µl. Percentage of donors who experienced ADR in the present study was similar to the findings of the study conducted by Dogra *et al*. in which they showed that 5.86% apheresis donors had ADR.[19] In the present study, the donors could not be followed up for PLT counts postprocedure. No case of delayed donor reaction was reported during the study period. This apparent safety data of donors also firms up the argument that donors with count higher than 138,000/µl can be safely accepted for plateletpheresis donations. All SDP products which were submitted to QC checks conformed to the prescribed standards.[4]

Platelet indices

PLT indices are influenced by the various factors such as ethnicity, gender, age, and the measurement technique of the analyzer used. 'The reference intervals of PLT variables from other published studies[21-25] are shown in Table 5. Many studies have shown the clinical importance of PLT indices in making diagnosis and treatment, especially as they can be monitored with the routine CBC without the need of any extra test and without adding cost to the patient.[3] Studies have reported that majority of hematological parameters including PLT indices are underutilized in the patient clinical management.[26] The different

Table 5: Comparison of platelet indices from other published studies with the present study

Author	Place	Year	Sample size	PDW (fl)	MPV (fl)	P-LCR (%)
Subhashree *et al*.[20]	India	2012	500	8.9-16.4	8-13.5	
Sachdev *et al*.[21]	India	2014	945	8.3-25	8.6-15.5	11.9-66.9
Abass *et al*.[22]	Sudan	2015	300	8.2-11.6	8.3-15.9	
Maluf *et al*.[23]	Egypt	2015	580	9.6-15.3	8.9-11.8	15.6-39.5
Boshnak[24]	Egypt	2017	380	10-16.9	9.3-12.2	20-42.3
Stella and Ebirien-Agana[25]	Nigeria	2017	300	9.4-16.8	8.2-12	
Present study	India	2020	1634	9.1-22.5	8.1-13.9	11.7-52.9

PDW=Platelet distribution width, MPV=Mean platelet volume, P-LCR=Platelet large cell ratio

PLT indices assist in differentiating immune-mediated thrombocytopenia (immunethrombocytopenic Purpura; ITP) from nonimmune-mediated thrombocytopenia due to hypoplastic bone marrow alleviating the need of invasive bone-marrow biopsy procedures.[27,28]

PDW represents variation in PLT size (anisocytosis) and heterogeneity in PLT morphology. This helps in differentiating different causes of thrombocytopenia. PDW is increased in immune-mediated causes of thrombocytopenia as compared to the nonimmune causes.[29,30] The reference interval of PDW in our study was 9.1–22.5 fl which was comparable to the one found by Sachdev *et al*.[21] MPV is the measurement of average size of PLTs in blood. It is an important marker of PLT activation. Higher values of MPV have been found to be associated with higher incidence of arterial occlusive disease.[27,30] The reference interval of MPV in our study was 8.1–13.9 fl which compared well with other studies and closely resembled the values observed by Subhashree *et al*.[20] PLC-R indicates the percentage of circulating PLTs larger than 12 fl. It aids in differential diagnosis of conditions associated with abnormal PLT counts.[30] The reference interval found in our study was 11.7%–52.9% which was closest to the reference range found by Sachdev *et al*.[21] The reference intervals of PLT indices should therefore be defined for all the laboratories for the correct interpretation of results.

Conclusion

The reference interval of PLT count (and PLT indices) in the Indian population is different (lower PLT counts) from the published Caucasian population data. On the basis of the results of the present study, we are of the opinion that the PLT count cutoffs for repeat plateletpheresis donation may be revised downward for our country, which would also mitigate the scarcity of apheresis donors.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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