Investigation on Reference Intervals and Regional Differences of Platelet Indices in Healthy Chinese Han Adults

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Background: Reference intervals are important for interpretation of clinical laboratory tests. The platelet (PLT) indices such as the mean platelet volume (MPV) and platelet distribution width (PDW) are newer hematological parameters, which have been recently reported as clinically valuable biomarkers. However, there are not many studies that have estimated the reference intervals for these parameters in healthy Chinese Han adults. **Objective:** The objectives of this study were to establish reference values of PLT indices [including PLT count, MPV, PDW, platelet-large cell ratio (P-LCR), and plateletcrit (Pct)] for healthy Chinese Han adults. We also aimed to determine the region-based differences of PLT indices in China. **Methods:** A total of 4,642 volunteers with a mean age of 43 were recruited from six regions of China. PLT indices were performed on Sysmex XE-2100 hematology

analyzers, whose traceability was well verified. **Results:** There were significant region-based differences for all PLT indices. Reference people in Chengdu had the lowest mean PLT count and Pct, but the highest MPV, PDW, and P-LCR among the six regions. Therefore, we derived the reference intervals in Chinese Han population excluding Chengdu reference people for PLT indices as PLT count: $(127\text{--}341) \times 10^9/\text{L}$; MPV: $(9.20\text{--}13.30)$ fL; PDW: $9.90\text{--}19.00\%$; P-LCR: $18.10\text{--}52.00\%$; Pct: $16.00\text{--}41.00\%$. **Conclusions:** Region-specific reference intervals are essential as there were statistically significant region-related differences in the PLT parameters. The reference intervals established in this study differed from the existing reference values. Chengdu region may need proper specific reference ranges, which apply to their people, for all PLT parameters. J. Clin. Lab. Anal. 29:21–27, 2015. © 2014 Wiley Periodicals, Inc.

Key words: reference intervals; platelet indices; PLT count; MPV; PDW; P-LCR; Pct

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INTRODUCTION

Reference intervals are crucial decision-making tools aiding clinicians in diagnosis orientation and treatment decision. The clinical utility of newer hematological parameters such as the analyzer-derived PLT indices has been explored in the recent years (1). Mean platelet volume (MPV) and platelet distribution width (PDW) have been reported to be correlated with PLT function and may be more sensitive indices than PLT number as a marker of clinical interest in various disorders such as cardiovascular disease (2) and stroke (3). Platelet-large cell ratio (P-LCR), when analyzed along with the other PLT parameters, helps in the differential diagnosis of the thrombocytosis-related disease (4). Recent studies have explored the role of plateletcrit (Pct) as independent risk factors of spontaneous echo contrast in patients with mitral stenosis (5). Considering the role of the PLT indices in clinical practice, it is encouraging to establish the reference values for these newer parameters.

However, PLT parameters are influenced by various factors such as age, gender, ethnicity origin, altitude, and geographic locations (6–8) and hence it is important to define the specific reference values with regards to age, gender, ethnicity, and the region. According to this context, we aimed to establish reference values of PLT indices in China by studying healthy Chinese Han adults from voluntary blood donors recruited from six regions (Chengdu, Beijing, Guangzhou, Shanghai, Shenyang, and Xi'an represented Southwest, North, South, East, North-east, and Northwest China, respectively).

MATERIALS AND METHODS

This study was approved by the Ethics Committee of each study center. Written informed consents were obtained from all participants.

Participants

A total of 4,642 blood donor volunteers were included in this study. Among all subjects, there were 644 from Chengdu, 760 from Beijing, 945 from Guangzhou, 736 from Shanghai, 861 from Shenyang, and 696 from Xi'an. Demographic features of all participants were provided in Table 1.

Every subject underwent an interview with a physician using a life-health questionnaire and a physical examination form. Exclusion criteria included body mass index ≥ 28 , high blood pressure (systolic number ≥ 140 mmHg and/or diastolic number ≥ 90 mmHg), alcohol assumption > 30 g per day, tobacco use > 20 cigarettes per day, drugs intake within 2 weeks, surgery within 6 months, blood donation or transfusion within 4 months, preg-

TABLE 1. Demographics of the Reference Individuals

Region	<i>n</i>	Male/female	Mean age (age range, years)	<i>P</i> value
Chengdu	644	331/313	41.4 (20–75)	>0.05
Beijing	760	340/420	43.7 (20–79)	
Guangzhou	945	446/499	47.8 (20–79)	
Shanghai	736	292/444	44.0 (20–78)	
Shenyang	861	368/493	43.9 (20–79)	
Xi'an	696	359/337	38.4 (20–78)	
Total	4,642	2,136/2,506	43.2 (20–79)	

nancy, less than 1 year after childbirth, hypothyroidism and hyperthyroidism, diabetes, atherosclerosis and vascular disease, cardiopathy, chronic nephropathy, hepatobiliary disease, allergic diseases, hematological disease, myopathy, autoimmune disease, burns and muscle trauma, the presence of acute and chronic infection, plasma fasting glucose > 7.0 mmol/l, serum creatinine > 120 μ mol/l, serum creatine kinase > 400 U/l, positive hepatitis B surface antigen, positive antihepatitis C virus antibody, positive anti-immunodeficiency virus antibody, and positive urinalysis.

The institutional SOPs (standard operating procedures) were followed for the sample collection and for conducting the tests.

Methods

Blood samples

All samples were collected between 8:00 a.m. and 9:00 a.m. Three milliliter fasting whole blood was collected from the cubital vein with a BD (New York, USA) Vacutainer tube with EDTA-K2. Complete blood counts were all performed within 2 hr of the blood collection.

Instruments

XE-2100 hematology automatic analyzers from six laboratories (laboratories of Chengdu, Beijing, Guangzhou, Shanghai, Shenyang, Xi'an) were all purchased from Sysmex, Japan. Dedicated reagents and standard methodologies were used.

Quality control

Three levels of quality controls (e-CHECK for XE were purchased from Sysmex, Japan) were run every day and the analyzers were all maintained according to the manufacturer's instructions during the entire period of the study.

Performance verification

Data of the six analyzers (Sysmex XE-2100 from six laboratories of six regions in this study) were compared with the data from reference measurement procedures (established in Hematology Reference Laboratory of National Center for Clinical Laboratory, Beijing, China) by testing five fresh blood samples assigned target values of PLT count, which were performed three times during the entire study period. Biases were calculated using the mean values of twice tested by the three analyzers.

Statistical Analysis

Data were analyzed using SPSS 17.0 software (Chicago, IL). The point estimate of the mean and the median with an interval estimate of 2.5 percentile and 97.5 percentile were provided as the reference values. *ANOVA* and *Student–Newman–Keuls* were performed to detect the difference among groups. *P* values less than 0.05 were considered significant.

RESULTS

Reference Individuals

Among the 4,642 individuals, 2,136 (46.0%) were males and 2,506 (54.0%) were females. All of them were Han people, and the mean age was 43 (age range 20–79). Table 1 shows the demographic characters of the reference individuals, and the age–gender distribution of the reference individuals was shown in Fig. 1. A majority of them (83%) were in the 20–59 years age group.

Performance Verification

The calculated biases of six analyzers (Sysmex XE-2100 from six laboratories of six regions in this study) in comparison with the data from reference measurement proce-

Fig. 1. The age–gender distribution of the reference individuals.

dures were presented in Table 2. All biases matched the acceptable bias ranges.

Gender-Specific Reference Intervals and Means

The reference intervals were calculated based on the IFCC and the CLSI (10) guidelines. The study reference intervals (2.5th to 97.5th percentile) for the males and females are presented and compared with the existing reference values that are currently used in our laboratory (Table 3). Both the lower and the upper limits showed an increase with the PLT count but a decrease with Pct values in both males and females. While MPV, PDW, and P-LCR all showed a mild decrease in the lower limits but an increase in the upper limits for both genders.

Gender-based statistically significant differences in the means were observed for the mean PLT count and Pct values ($P < 0.05$). As shown in Table 4, the females had a higher mean PLT count, MPV, P-LCR, and Pct, whereas the males had a higher PDW than their counterparts.

TABLE 2. Biases of the Six Analyzers Compared With the Reference Measurement Procedures

Region	Biases for PLT count (%)			Acceptable bias for PLT count ^a
	First time	Second time	Third time	
Blood sample number	5	5	5	
Chengdu	–3.8 ~ 6.9	–7.5 ~ 1.2	–6.8 ~ 6.6	<12.5%
Beijing	2.2 ~ 10.6	–5.0 ~ 8.6	0.5 ~ 9.8	
Guangzhou	–2.5 ~ 8.3	–7.5 ~ 2.5	–3.0 ~ –4.0	
Shanghai	2.9 ~ 4.1	–4.2 ~ 1.0	–3.1 ~ 4.7	
Shenyang	–0.4 ~ 6.5	–7.5 ~ 1.2	–4.7 ~ 5.3	
Xi'an	–2.1 ~ 4.6	–8.4 ~ 1.2	–8.5 ~ 4.7	

^aThe acceptable bias ranges for PLT count is the 1/2 allowable error as described in CLIA'88 (9).

TABLE 3. Study Reference Intervals and Existing Reference Intervals of the PLT Indices

Parameter	Gender	Study reference intervals	Existing reference intervals
PLT ($\times 10^9/l$)	Males	111–305	100–300
	Females	122–334	
	Together	115–323	
MPV (fl)	Males	9.24–13.22	9.4–12.6
	Females	9.29–13.21	
	Together	9.27–13.22	
PDW (%)	Males	9.21–18.37	9.8–16.2
	Females	9.09–18.29	
	Together	9.13–18.33	
P-LCR (%)	Males	18.60–50.59	19.1–47.0
	Females	18.50–50.81	
	Together	18.50–50.72	
Pct (%)	Males	12.87–35.27	16.0–38.0
	Females	15.43–36.23	
	Together	14.05–35.95	

TABLE 4. Gender-Specific Means and Standard Errors for PLT Indices

Parameter	Males (<i>n</i> = 2,136)	Females (<i>n</i> = 2,506)	<i>P</i> value
PLT ($\times 10^9/l$)	208 $\pm$ 50	228 $\pm$ 54	<0.05
MPV (fl)	11.23 $\pm$ 1.02	11.25 $\pm$ 1.00	>0.05
PDW (%)	13.79 $\pm$ 2.34	13.69 $\pm$ 2.35	>0.05
P-LCR (%)	34.60 $\pm$ 8.16	34.65 $\pm$ 8.24	>0.05
Pct (%)	24.07 $\pm$ 5.72	25.83 $\pm$ 5.31	<0.05

Data were expressed as mean $\pm$ SE.

Region-Specific Means and Reference Intervals

When analyzing the region-based differences of the PLT indices, we found an interesting phenomenon. Statistically significant region-based differences in the means were also observed for all the PLT parameters. As shown in Table 5, reference people in Chengdu had the lowest mean PLT

count and Pct but the highest MPV, PDW, and P-LCR among the six regions. Meanwhile, when compared with total (means of six regions, including Chengdu, Beijing, Guangzhou, Shanghai, Shenyang, Xi'an), the mean PLT count ($164 \times 10^9/l$ vs. $219 \times 10^9/l$) and Pct (21.86% vs. 25.00%) in Chengdu people were also lower while MPV (12.36 fl vs. 11.24 fl), PDW (16.26% vs. 13.73%), and P-LCR (43.55% vs. 34.61%) were higher.

Gender-Region Based Reference Intervals and Means

As the differences of means for all PLT indices in Chengdu from other regions, we compared the data of Chengdu with those of other five regions (data excluding Chengdu). As shown in Table 6, both the males and females in Chengdu had lower mean PLT count and Pct but higher MPV, PDW, and P-LCR in comparison with the means of other five regions in the same gender group.

The region-gender specific reference intervals in this study for the males and the females are presented and compared with the existing reference values in Table 7. In Chengdu, both the lower and upper limits showed a decrease with the PLT count and Pct values but an increase with the MPV, PDW, and P-LCR in both genders in comparison with the existing reference intervals. Surveying overall samples excluding that from Chengdu, both the lower and upper limits showed an increase with the PLT count and PDW in both males and females; while both the MPV and P-LCR showed a mild decrease in the lower limits and an increase in the upper limits, when compared with the existing reference intervals. In addition, an increase in the upper limits of Pct in both genders were observed, whereas the lower limit decreased in males and increased in females. However, when compared with overall regions excluding Chengdu, a decrease in both the lower and upper limits of the PLT count and Pct and an increase in limits of the MPV, PDW, and P-LCR were observed in both genders in Chengdu.

TABLE 5. Region-Specific Means and Standard Errors for PLT Indices

Region	PLT ($\times 10^9/l$)	MPV (fl)	PDW (%)	P-LCR (%)	Pct (%)
Chengdu	164 $\pm$ 53	12.36 $\pm$ 1.34	16.26 $\pm$ 3.61	43.55 $\pm$ 10.67	21.86 $\pm$ 4.99
Beijing	244 $\pm$ 52*	10.92 $\pm$ 0.85*	12.81 $\pm$ 1.86*	32.05 $\pm$ 7.25*	26.53 $\pm$ 5.15*
Guangzhou	245 $\pm$ 54*	10.30 $\pm$ 0.80*	11.83 $\pm$ 1.63*	26.88 $\pm$ 6.62*	25.13 $\pm$ 5.05*
Shanghai	220 $\pm$ 53*	11.05 $\pm$ 1.05*	13.19 $\pm$ 2.25*	32.57 $\pm$ 8.16*	Not available
Shenyang	225 $\pm$ 50*	11.19 $\pm$ 0.86*	13.31 $\pm$ 1.89*	34.41 $\pm$ 7.28*	26.48 $\pm$ 7.15*
Xi'an	215 $\pm$ 56*	11.63 $\pm$ 1.16*	14.99 $\pm$ 2.84*	38.21 $\pm$ 9.33*	Not available
Total	219 $\pm$ 53*	11.24 $\pm$ 1.01*	13.73 $\pm$ 2.35*	34.61 $\pm$ 8.22*	25.00 $\pm$ 5.59*

Data were expressed as mean $\pm$ SE.

**P* < 0.05 versus Chengdu.

TABLE 6. Region-Gender Specific Means and Standard Errors for PLT Indices

Parameter	Males (<i>n</i> = 2,136)		Females (<i>n</i> = 2,506)	
	Chengdu	Total (except for Chengdu)	Chengdu	Total (except for Chengdu)
PLT ($\times 10^9/l$)	157 $\pm$ 48*	219 $\pm$ 51	171 $\pm$ 57**	240 $\pm$ 55
MPV (fl)	12.35 $\pm$ 1.30*	10.98 $\pm$ 1.04	12.37 $\pm$ 1.38**	10.97 $\pm$ 1.03
PDW (%)	16.33 $\pm$ 3.53*	13.22 $\pm$ 2.33	16.19 $\pm$ 3.71**	13.05 $\pm$ 2.31
P-LCR (%)	43.47 $\pm$ 10.34*	32.57 $\pm$ 8.49	43.64 $\pm$ 11.02**	32.39 $\pm$ 8.57
Pct (%)	20.90 $\pm$ 4.41*	25.00 $\pm$ 6.36	22.90 $\pm$ 5.37**	26.80 $\pm$ 5.31

Data were expressed as mean $\pm$ SE.

* $P < 0.05$ versus total (except for Chengdu) in males; ** $P < 0.05$ versus total (except for Chengdu) in females.

Effect of Age on Region-Specific Means

To understand the effect of age on the PLT parameters, graphs were plotted between the mean value and age in Chengdu and other five regions (Fig. 2A–E). In all age groups (20–29, 30–39, 40–49, 50–59, 60–69, 70–79 years), reference people in Chengdu had a lower mean value of PLT count and Pct but a higher MPV, PDW, and P-LCR than their counterparts. Chengdu people showed a gradual rise in the mean PLT count and Pct values with age, and a highest value in (40–49) age group for the mean MPV, PDW, and P-LCR. Means of all these PLT parameters in other five regions showed a mild decreasing trend. There were no significant differences among all the age groups for these PLT indices in both Chengdu and Total (except for Chengdu). However, statistically significant differences for all PLT parameters between Chengdu and overall regions excluding Chengdu in each age group were observed ($P < 0.05$).

DISCUSSION

By testing fresh blood samples assigned target values, all six hematology analyzers employed in this study were well verified for their traceability. Therefore, the reference ranges established in this study were analyzer-independent.

The objective of this study was to establish reference values for PLT indices (including PLT count, MPV, PDW, P-LCR, Pct), which may serve as standards for interpretation of laboratory results in China. Under strict exclusion criteria, a total of 4,642 reference individuals were recruited from Southwest China (Chengdu), North China (Beijing), South China (Guangzhou), East China (Shanghai), Northeast China (Shenyang), and Northwest China (Xi'an), and all of them were Han adults.

In comparison with the existing reference intervals for all PLT indices (PLT count, MPV, PDW, P-LCR, and Pct), the study reference values calculated with the data

TABLE 7. Region-Gender Based Reference Intervals for PLT Indices Compared With Existing Reference Intervals

Parameter	Gender	Study reference intervals		Existing reference intervals
		Chengdu	Total (except for Chengdu)	
PLT ($\times 10^9/l$)	Males	68–254	122–322	100–300
	Females	67–304	138–354	
	Together	67–280	127–341	
MPV (fl)	Males	9.94–14.60	9.30–13.35	9.4–12.6
	Females	9.80–14.90	9.20–13.30	
	Together	9.84–14.70	9.20–13.30	
PDW (%)	Males	10.60–23.77	10.00–19.40	9.8–16.2
	Females	10.60–23.50	9.80–19.00	
	Together	10.60–23.56	9.90–19.00	
P-LCR (%)	Males	23.50–60.22	18.45–51.75	19.1–47.0
	Females	22.90–62.09	18.00–52.00	
	Together	23.16–61.02	18.10–52.00	
Pct (%)	Males	13.00–29.58	15.00–45.00	16.0–38.0
	Females	13.03–34.98	17.00–39.28	
	Together	13.00–33.58	16.00–41.00

Fig. 2. PLT indices with age in Chengdu and total regions excluding Chengdu. (A) The mean PLT count with age; (B) the mean MPV with age; (C) the mean PDW with age; (D) the mean P-LCR with age; (E) the mean Pct with age.

from above six regions showed obvious differences. This suggested that the existing reference intervals for PLT parameters could not apply to our population. And it is necessary and important to develop new reference intervals for PLT indices, especially the newer parameters such as MPV and PDW.

In this study, gender-based statistically significant differences were found for the mean PLT count. In males, the mean platelet count was $208 \times 10^9/L$ and in females $228 \times$

$10^9/L$. The same results were observed in African (11, 12) and North American (13) studies. The reasons for these differences according to gender are still unclear. However, considering the clinically practical significance and convenience, the reference ranges regardless of gender were recommended.

Besides, region-based differences for all PLT parameters were also statistically significant. Among six study centers, we observed a lowest mean PLT count and Pct

but a highest MPV, PDW, and P-LCR of reference people in Chengdu, while the mean values of these parameters in other five regions did not show so obvious differences. This was in agreement with the observations that were made by other Chinese researchers (14). We therefore calculated the reference intervals for all PLT parameters with only five regions excluding Chengdu, with both genders together, both the lower and upper limits showed an increase with the PLT count but a decrease with the Pct; while MPV, PDW, and P-LCR all showed a mild decrease in the lower limits and an increase in the upper limits, when compared with the existing reference intervals. This highlighted the necessity and importance of deriving new and region-specific reference ranges for PLT indices.

We also calculated the reference values for Chengdu only, and both the limits of these values in both genders showed obvious differences from either the above study reference intervals (calculated without data of Chengdu) or the existing reference intervals. Reference people in Chengdu had lower PLT count and Pct but higher MPV, PDW, and P-LCR than overall people excluding Chengdu people, which suggested that PLT indices of Chengdu people was characterized by low PLT concentration but high volume. This suggested that the study reference intervals for PLT indices, calculated by data without Chengdu, might not be suitable to apply to the interpretation of laboratory tests sometimes in Chengdu region. Chengdu people may need their own reference ranges for PLT indices. This observation merits further studies and we are focused on it.

CONCLUSIONS

Region-specific reference intervals are essential as there were region-related statistically significant differences in the PLT parameters. The study reference intervals for PLT indices in our population excluding Chengdu reference people differed from the existing reference values and are established as PLT count: $(127-341) \times 10^9/L$; MPV: $(9.20-13.30)$ fl; PDW: $9.90-19.00\%$; P-LCR: $18.10-52.00\%$; Pct: $16.00-41.00\%$. Chengdu region may need proper reference ranges, which apply to their people, for PLT indices.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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