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Long-term biological variation estimates of 13 hematological parameters in healthy Chinese subjects

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Abstract

Background: The complete blood count (CBC) is a basic test routinely ordered by physicians as a part of initial diagnostic work-up on their patients. To ensure safe clinical application of the CBC, reliable biological variation (BV) data are needed to establish analytical performance specifications. Our aim was to define the BV of CBC parameters using a rigorous protocol that is compliant with the Biological Variation Data Critical Appraisal Checklist (BIVAC) provided by the European Federation of Clinical Chemistry and Laboratory Medicine.
Methods: Blood samples drawn from 41 healthy Chinese subjects (22 females and 19 males; 23–59 years of age) once monthly for 6 consecutive months were analyzed using an ABX Pentra 80 instrument. The instrument was precisely calibrated. All samples were analyzed in duplicate for 13 CBC parameters. The data were assessed for outliers, normality, and variance homogeneity prior to nested ANOVA. Gender-stratified within-subject (CV_I) and between-subject (CV_G) BV estimates were calculated.
Results: The number of remaining data for each subject was 442–484 after removing outliers. No significant differences existed between female/male CV_I estimates. Except for leukocytes, neutrophils, and lymphocytes, the mean values of 10 parameters differed significantly between genders, rendering partitioning of CV_G data between genders. No significant differences were detected between most BV estimates and recently published estimates representing a Europid population.
Conclusions: Most BV estimates in BIVAC-compliant studies are similar. The turnover time of blood cells and age distribution of participants should be considered in a CBC BV study. Our study will contribute to global BV estimates and future studies.

Keywords: biological variation; complete blood count; hematology analyzer; index of individuality; reference change value.

Introduction

The complete blood count (CBC) is a basic test routinely ordered by physicians as a part of an initial diagnostic work-up on their patients. To assure the safe and valid clinical interpretation of test results, objective analytical performance specifications (APSs) are needed in clinical laboratories [1]. Three models to establish APS have been identified by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), of which models 1 and 2 are recommended [2]. Although it has been proposed that the APS for hemoglobin (Hb), platelets (PLT), and neutrophil (Neu) leukocytes should be based on clinical outcomes (model 1) [3], no reliable clinical outcomes are available in practice and biological variation (BV) is the most reliable source of useful information in defining APS for CBC (model 2) [4].

Several studies involving the BV of the CBC have been included in the online 2014 BV database. The designs of these studies differed as follows: inclusion and exclusion criteria, duration of study, and type of statistical model. The observations reported in the previous studies showed a substantial difference in BV results. Specifically, there were differences in the within-subject BV (CV_I) of white blood cells (WBC; 9.4%–17.3%) [5–10] and the CV_I of PLT (6.6%–13.7%) [5, 6, 8, 10–12]. In the absence of an ideal

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standardized approach to estimate the BV, the EFLM Biological Variation Working Group (BVWG) was established and the Biological Variation Critical Appraisal Checklist (BIVAC) was proposed [13]. In addition, the BVs of CBC parameters were defined by the BVWG [14]; however, the study period was 10 weeks, which does not cover one turnover period for erythrocytes (approximately 4 months) [15–17]. This feature may impact the BV estimation of CBC parameters. Additionally, nearly all published studies involving BV of CBCs were based on Caucasian/White populations [6–8, 10–12, 18–21]. Indeed, only two studies have determined the BV among Asian populations, both of which were short-term studies (1 [22] or 3 days [23]). The duration of the studies was too short to generate reliable BV estimates. Therefore, a long-term study estimating BV is warranted.

The aim of this study was to estimate the components of long-term (6 months) BV, the index of individuality (II), reference change values (RCVs), APS, and the number of samples required to estimate the homeostatic set points (NHSPs) for 13 CBC parameters in a cohort of healthy Chinese subjects by applying a strictly designed protocol compliant with the BIVAC, as proposed by the EFLM BVWG.

Materials and methods

Participants

Forty-three healthy volunteers (21 males and 22 females; age range, 23–59 years; median age, 43 years) were recruited for our study. The study protocol was approved by the Beijing Hospital Ethics Committee and all individuals gave written informed consent to participate. The exclusion criteria were as follows: surgery within 6 months; blood transfusion and donation 4 months before or during the current study; pregnant or anticipating pregnancy within 6 months; breastfeeding; excessive consumption of alcohol (average alcohol consumption >60 g ethanol/day for men and >40 g for women); tobacco abuse (average cigarette consumption ≥20 cigarettes/day); body mass index (BMI) >30.0 kg/m^2; and use of any medications. At the pre-study screening, all individuals were deemed healthy based on health questionnaires. A CBC and routine biochemistry parameters (fasting serum glucose, lipids, and liver and kidney function) were measured to exclude subtle blood cell abnormalities and chronic diseases. During the study, the subjects were requested to maintain normal lifestyle habits. All subjects were asked to fast, cease smoking cigarettes and consumption of alcohol, and refrain from heavy exercising from 8:00 p.m. on the day before blood was to be drawn.

Specimen collection

The blood samples were collected between 8:00 and 9:00 in the morning by one experienced phlebotomist after the participants rested 10–15 min in a sitting position. All samples were collected into K_3EDTA anti-coagulated 2-mL tubes (Greiner Bio-One Vacuette®; Kremsmunster, Austria) and analyzed within 2 h of collection. Samples were drawn from all subjects once monthly for 6 consecutive months. Using this protocol, the pre-analytical variance was considered to be negligible.

Analytical procedure

All specimens were analyzed in duplicate using one ABX Pentra 80 hematology analyzer (Horiba ABX SAS; Montpellier, France) in order to calculate the analytical variation. To minimize inter-batch analytical variation, these specimens were assayed using one lot of reagents throughout the entire study. Prior to first-batch specimen analysis, instrument calibration was performed using ABX Minocal (Horiba ABX SAS). Internal quality controls (IQC) were performed daily using ABX Difftrol Control (Horiba ABX SAS) with two different concentrations to provide consistent determination during the course of the study. Each assay was performed by one analyst.

Statistical analysis

The homogeneity of within-subject data was assessed using average of the duplicate results of each sample from one subject by Bartlett and Cochran tests, respectively. In heterogeneity cases, outlier data were excluded until homogeneity was achieved. The Dixon-Reed criterion was used to detect outliers between subjects. To confirm that all subjects were in a steady state, linear regression was performed on the mean group value during the entire study period for each parameter.

The Shapiro-Wilk test was used to verify the normality of between- and within-subject data. If the data were not normally distributed, the data were log-transformed prior to re-evaluation of normality.

According to the current recommendations of BIVAC [13], a nested ANOVA was used to provide an accurate estimate of the CV_I and between-subject biologic variation (CV_G). Analytical variation (CV_A) was calculated from the duplicate measurements of each sample. The 95% confidence intervals (CIs) for BV estimates were calculated as described by Roraas [24] and Burdick [25].

Female and male data were analyzed separately. The significance of differences in CV_I and CV_G was assessed in subgroups by estimating the overlap in the 95% CI. When the 95% CIs of CV_I of females and males overlapped, it was determined that no significant difference existed between female and male BV data, and CV_Is were reported for all subjects and these estimates were used in the application of the BV data. When the 95% CIs of the mean values of females and males did not overlap, the lower of the two CV_G estimates was used to calculate the APS.

CV_I and CV_G data were used to calculate the desirable APSs for imprecision (I_{APS}), bias (B_{APS}), total error (TE_{APS}), the IIs, the RCVs, and the NHSPs using the following equations [26, 27]:

$$I_{APS} = 0.50 \times CV_I \quad (1)$$

$$B_{APS} = 0.25 \times (CV_I^2 + CV_G^2)^{1/2} \quad (2)$$

$$TE_{APS} = (1.65 \times I_{APS}) + B_{APS} \quad (3)$$

$$II = (CV_I^2 + CV_A^2)/CV_G \quad (4)$$

$$RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2} \quad (5)$$

$$NHSP = (Z \times (CV_A^2 + CV_I^2)^{1/2}/D)^2, \quad (6)$$

where D is the allowed percentage deviation from the true homeostatic set point, CV_A denotes analytical variation, and Z is 1.96 for a p-value <0.05. We calculated NHSPs associated with 5%, 10%, and 20% deviations from the true homeostatic set points.

Data analysis was performed using SPSS (version 16.0; SPSS IBM, Chicago, IL, USA) and Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA).

Results

Main characteristics of participants

Thirty-seven subjects completed all six scheduled collections, four subjects completed five collections, and two subjects completed one collection. Thus, 41 of the 43 subjects (22 females and 19 males) were included in the study. The mean ages of the females and males were 44 years (age range, 23–59 years) and 38 years (age range, 23–59 years), respectively. There were no significant differences in age and BMI between males and females.

Outlier removal

Two hundred and forty-two fresh blood samples from 41 participants were collected and measured in duplicate. The number of replicates, samples, and subjects identified as outliers by Bartlett and Cochran tests is shown in Supplementary Table A. In total, 2.2% of obtained data were excluded from the final analysis. All subjects were in a steady state during the study ($p > 0.05$).

Comparison of BV data between the current study and BIVAC-compliant studies

The mean, CV_A, CV_I, and CV_G of all parameters are shown in Table 1. The CV_I of the CBC parameters displayed a constant overlap of 95% CIs between females and males; thus, there were no significant gender differences in CV_I estimates. Therefore, we reported the CV_I estimates for all subjects. With the exception of WBC, Neu, and lymphocytes (Lym), the mean values of other parameters in females and males differed significantly, rendering partitioning of the CV_G data between genders.

The 95% CIs for CV_I estimates of all parameters, except the mean cell hemoglobin concentration (MCHC) and basophil (Bas [$\times 10^9$/L]), overlapped with the BIVAC-compliant studies reported by Coskun et al. [14], which showed there were no significant differences in CV_I estimates between the two studies. Our 95% CIs for CV_G estimates of all parameters, except Bas ($\times 10^9$/L), overlapped with the CV_G estimates reported by Coskun et al. [14], which also showed that there were no significant differences in CV_G estimates between the two studies.

The 95% CIs of CV_I estimates for all parameters, except red blood cells (RBC), Hb, and mean cell hemoglobin (MCH), overlapped with the CV_I estimates of BIVAC-compliant studies reported by Buoro et al. [28–30] (medium-term BV), which showed there were no significant differences in CV_I between the two studies. Our 95% CIs of CV_G estimates for all parameters overlapped with those of Buoro et al. [28–30], which also showed that there were no significant differences in CV_G estimates between the two studies.

As shown in Table 1, the CVs of the IQC for all parameters, except the mean corpuscular volume (MCV) and MCHC, were higher than the CV_A based on duplicate tests.

Indices derived from BV

The APSs for hematological parameters are shown in Table 2. The CV_{APS} of all parameters, except MCV, MCH, and MCHC, and B_{APS} of all parameters, except MCHC, Lym ($\times 10^9$/L), and eosinophils ([Eos] $\times 10^9$/L), and the RCV of all parameters, except PLT, MCH, and MCHC, were higher than the corresponding values reported by Coskun et al. [14].

The IIs for nine of 13 parameters were <0.60, and for the remaining parameters IIs ranged from 0.63 to 1.50. The NHSP (within 5% of the actual value) was 2 in the erythrocyte group, but much higher in the leukocyte group (exceeding 100 for the Bas count). In the leukocyte group, particularly for Bas ($\times 10^9$/L), widening the target range to 20% still required measurement of seven samples to derive the estimates of homeostatic set points.

Discussion

In the experimental design of this study, several key elements of BV for the CBC, such as the number of subjects,

Table 1: Biological variation estimates for hematological parameters with 95% CIs, accompanied by the corresponding BV estimates in two BIVAC-compliant studies.

Parameters	Sex	Mean (95% CI)	CV% (IQC)	This study			Coskun et al.			Buoro et al.		
				CV_A (95% CI), %	CV_I (95% CI), %	CV_G (95% CI), %	CV_A (95% CI), %	CV_I (95% CI), %	CV_G (95% CI), %	CV_A (95% CI), %	CV_I (95% CI), %	CV_G (95% CI), %
WBC, $\times 10^9$/L	All	6.03 (5.88–6.16)	1.84	1.48 (1.36–1.63)	11.61 (10.57–12.88)	23.05 (18.72–29.74)	1.49 (1.38–1.62)	NA	16.53 (12.83–22.08)	1.5 (1.3–1.7)	11.1 (9.62–13.18)	15.0 (10.9–22.5)
	M	6.08 (5.88–6.29)	NA	1.47 (1.30–1.69)	10.40 (9.06–12.19)	23.85 (17.79–35.59)	NA	7.96 (7.02–9.19)	NA	NA	NA	NA
	F	5.97 (5.78–6.15)	NA	1.50 (1.34–1.71)	12.56 (11.09–14.48)	22.96 (17.35–33.23)	NA	12.82 (11.51–14.46)	NA	NA	NA	NA
RBC, $\times 10^{12}$/L	All	4.49 (4.45–4.53)	1.79	0.70 (0.64–0.77)	3.23 (2.93–3.59)	8.95 (7.31–11.50)	0.66 (0.61–0.72)	2.77 (2.55–3.04)	NA	0.64 (0.56–0.74)	1.8 (1.5–2.1)	9.1 (6.9–13.1)
	M	4.81 (4.77–4.86)	NA	0.93 (0.82–1.07)	3.12 (2.71–3.67)	6.10 (4.52–9.13)	NA	NA	5.58 (4.01–9.40)	0.6 (0.5–0.75)	1.4 (1.1–1.9)	7.8 (5.2–14.9)
	F	4.22 (4.19–4.26)	NA	0.75 (0.67–0.85)	3.26 (2.87–3.77)	6.11 (4.62–8.84)	NA	NA	6.98 (5.15–10.65)	0.66 (0.56–0.80)	2.4 (1.9–3.0)	7.1 (5.0–12.1)
Hb, g/L	All	140.72 (139.30–142.15)	1.29	0.60 (0.55–0.66)	3.16 (2.87–3.51)	11.00 (9.00–14.11)	0.58 (0.53–0.63)	2.74 (2.52–3.00)	NA	0.52 (0.46–0.60)	2.0 (1.7–2.4)	8.2 (6.3–11.9)
	M	153.45 (152.13–154.77)	NA	0.60 (0.53–0.69)	2.86 (2.49–3.36)	5.94 (4.42–8.88)	NA	NA	5.81 (4.08–9.57)	0.44 (0.37–0.56)	1.3 (1.1–1.7)	6.3 (4.2–12.1)
	F	130.12 (128.70–131.54)	NA	0.59 (0.53–0.67)	3.46 (3.05–3.99)	8.47 (6.45–12.19)	NA	NA	6.57 (4.83–10.01)	0.59 (0.48–0.69)	2.3 (1.9–2.9)	6.8 (4.8–11.7)
HCT, %	All	0.417 (0.412–0.420)	1.73	1.11 (1.02–1.22)	3.12 (2.82–3.48)	10.22 (8.36–13.12)	0.63 (0.59–0.69)	2.82 (2.59–3.09)	NA	0.60 (0.53–0.70)	2.4 (2.1–2.9)	7.4 (5.6–10.7)
	M	0.452 (0.449–0.456)	NA	1.17 (1.03–1.35)	2.82 (2.43–3.34)	5.25 (3.88–7.88)	NA	NA	5.46 (3.87–9.07)	0.58 (0.48–0.73)	2.2 (1.8–2.9)	5.7 (3.8–11.1)
	F	0.386 (0.382–0.390)	NA	1.04 (0.93–1.18)	3.40 (2.98–3.94)	7.62 (5.79–10.98)	NA	NA	5.51 (4.03–8.41)	0.62 (0.53–0.76)	2.6 (2.1–3.2)	6.2 (4.3–10.7)
PLT, $\times 10^9$/L	All	223.83 (218.89–228.77)	2.76	2.74 (2.51–3.01)	6.74 (6.08–7.53)	23.71 (19.39–30.42)	1.80 (1.67–1.96)	7.22 (6.63–7.91)	NA	1.09 (0.97–1.27)	7.22 (6.27–8.52)	15.4 (11.5–22.7)
	M	211.57 (204.17–218.98)	NA	3.08 (2.72–3.56)	6.76 (5.78–8.05)	25.54 (19.20–37.91)	NA	NA	7.23 (4.93–12.42)	NA	NA	NA
	F	233.76 (227.35–240.18)	NA	2.49 (2.22–2.83)	6.71 (5.87–7.80)	21.96 (16.80–31.51)	NA	NA	17.42 (12.90–26.71)	NA	NA	NA
MCV, fL	All	92.71 (92.32–93.10)	0.65	0.74 (0.68–0.81)	0.69 (0.58–0.81)	4.61 (3.77–5.93)	0.18 (0.16–0.19)	0.72 (0.66–0.79)	3.96 (3.15–5.33)	0.19 (0.17–0.22)	0.9 (0.8–1.1)	3.9 (3.0–5.6)
	M	94.05 (93.65–94.44)	NA	0.76 (0.67–0.88)	0.50 (0.30–0.68)	3.13 (2.36–4.64)	NA	NA	NA	0.17 (0.14–0.21)	0.9 (0.7–1.1)	3.2 (2.2–6.3)
	F	91.55 (90.93–92.16)	NA	0.73 (0.65–0.83)	0.83 (0.68–1.01)	5.41 (4.13–7.82)	NA	NA	NA	0.21 (0.18–0.25)	1.0 (0.8–1.2)	4.2 (3.0–7.2)
MCH, pg	All	31.51 (31.39–31.64)	0.93	0.70 (0.64–0.77)	0.69 (0.58–0.81)	4.94 (4.03–6.37)	0.78 (0.73–0.85)	0.75 (0.65–0.86)		0.77 (0.68–0.89)	0.3 (0.0–0.5)	4.7 (3. 6–6.9)
	M	31.93 (31.79–32.07)	NA	0.73 (0.64–0.84)	0.69 (0.53–0.87)	3.30 (2.48–4.90)	NA	NA	2.9 (2.22–5.15)	0.72 (0.60–0.91)	0.3 (0.0–0.6)	3.5 (2.3–6.7)

Table 1 (continued)

Parameters	Sex	Mean (95% CI)	CV% (IQC)	This study			Coskun et al.			Buoro et al.		
				CV_A (95% CI), %	CV_I (95% CI), %	CV_G (95% CI), %	CV_A (95% CI), %	CV_I (95% CI), %	CV_G (95% CI), %	CV_A (95% CI), %	CV_I (95% CI), %	CV_G (95% CI), %
	F	31.13 (30.95–31.32)	NA	0.67 (0.59–0.77)	0.69 (0.55–0.85)	4.69 (3.56–6.86)	NA	NA	6.11 (4.55–9.31)	0.81 (0.68–0.99)	0.2 (0.0–0.6)	5.6 (3.9–9.9)
MCHC, g/L	All	337.86 (337.39–338.33)	1.11	1.02 (0.94–1.12)	0.54 (0.34–0.70)	1.07 (0.85–1.40)	0.79 (0.73–0.86)	0.97 (0.87–1.09)	1.59 (1.13–2.69)	0.77 (0.68–0.89)	0.8 (0.6–1.0)	1.8 (1.3–2.6)
	M	339.30 (338.67–339.90)	NA	1.13 (1.00–1.30)	0.32 (0.00–0.64)	0.77 (0.53–1.20)	NA	NA	NA	0.74 (0.61–0.93)	0.8 (0.5–1.1)	0.9 (0.6 –2.0)
	F	336.66 (336.00–337.32)	NA	0.92 (0.82–1.05)	0.67 (0.47–0.86)	1.18 (0.87–1.73)	NA	NA	NA	0.8 (0.67–0.96)	0.9 (0.6–1.2)	2.1 (1.4–3.6)
Neu, $\times 10^9$/L	All	3.22 (3.13–3.32)	2.51	1.70 (1.56–1.87)	15.43 (14.03–17.13)	28.62 (23.20–36.97)	1.88 (1.74–2.05)	NA	NA	1.9 (1.7–2.2)	14.6 (12.7–17.2)	24.1 (17.9–35.4)
	M	3.18 (3.05–3.31)	NA	1.72 (1.52–1.99)	14.21 (12.37–16.69)	27.68 (20.53–41.44)	NA	11.60 (10.20–13.44)	16.68 (11.80–28.39)	NA	NA	NA
	F	3.26 (3.13–3.39)	NA	1.94 (1.73–2.21)	16.32 (14.39–18.85)	29.96 (22.63–43.36)	NA	20.09 (18.05–22.65)	27.81 (20.17–42.79)	NA	NA	NA
Lym, $\times 10^9$/L	All	2.11 (2.06–2.15)	4.5	2.12 (1.95–2.33)	11.08 (10.07–12.31)	21.71 (17.62–28.02)	2.40 (2.21–2.61)	9.81 (9.00–10.77)		2.7 (2.4–3.1)	11.0 (9.5–13.0)	23.0 (17.2–34.2)
	M	2.14 (2.05–2.22)	NA	2.09 (1.84–2.41)	10.77 (9.36–12.67)	25.77 (19.23–38.43)	NA	NA	23.66 (17.1–39.9)	NA	NA	NA
	F	2.08 (2.03–2.14)	NA	2.15 (1.92–2.45)	11.31 (9.96–13.08)	18.06 (13.56–26.24)	NA	NA	20.36 (15.17–31.62)	NA	NA	NA
Mono, $\times 10^9$/L	All	0.44 (0.42–0.45)	9.19	7.26 (6.67–7.97)	15.40 (13.85–17.25)	27.69 (22.40–35.83)	4.95 (4.58–5.39)	NA	NA	3.9 (3.5–4.5)	13.4 (11.6–15.9)	22.2 (11.5–32.7)
	M	0.47 (0.45–0.49)	NA	6.71 (5.93–7.74)	14.24 (12.19–16.92)	24.44 (17.99–36.78)	NA	11.07 (9.67–12.88)	17.01 (12.34–29.47)	NA	NA	NA
	F	0.41 (0.39–0.42)	NA	7.79 (6.95–8.86)	15.57 (13.49–18.22)	29.65 (22.39–42.92)	NA	15.33 (13.68–17.40)	29.78 (22.53–46.92)	NA	NA	NA
Eos, $\times 10^9$/L	All	0.17 (0.16–0.18)	16.25	8.67 (7.94–9.55)	16.15 (14.41–18.24)	54.65 (44.67–70.17)	11.01 (10.07–12.13)	NA	70.5 (59.4–100.6)	9.5 (8.3–11.1)	15.6 (13.0–19.1)	61.5 (45.7–90.5)
	M	0.18 (0.17–0.20)	NA	8.23 (7.22–9.57)	13.58 (11.33–16.51)	48.43 (36.34–71.96)	NA	10.11 (7.77–12.26)	NA	NA	NA	NA
	F	0.16 (0.15–0.17)	NA	9.05 (8.06–10.32)	14.58 (12.42–17.29)	60.77 (46.56–87.11)	NA	14.83 (12.42–17.62)	NA	NA	NA	NA
Bas, $\times 10^9$/L	All	0.035 (0.034–0.037)	21.28	18.57 (16.99–20.48)	18.85 (15.93–22.07)	43.71 (35.43–56.48)	16.65 (15.24–18.35)	11.36 (7.66–13.24)	22.10 (16.82–29.93	12.2 (10.7–14.1)	12.8 (10.1–16.1)	33.9 (25.5–49.5)
	M	0.040 (0.037–0.043)	NA	17.82 (15.65–20.70)	19.26 (15.09–24.19)	42.32 (32.34–65.55)	NA	NA	NA	NA	NA	NA
	F	0.032 (0.030–0.034)	NA	18.94 (16.82–21.67)	17.87 (13.87–22.30)	42.02 (31.73–60.82)	NA	NA	NA	NA	NA	NA

NA, not available; IQC, internal quality control.

Table 2: The APS for hematological parameters based on biological variation, accompanied by the corresponding BV estimates in BIVAC-compliant study.

Parameters	This study											Coskun et al.		
	I_{APS}, %	B_{APS}, %	TE_{APS}, %	RCV, %	II	No (5%)	No (10%)	No (20%)	I_{APS}, %	B_{APS}, %	TE_{APS}, %	II	No (5%)	No (10%)	No (20%)
WBC, $\times 10^9$/L	5.81	6.45	16.03	32.44	0.51	22	6	2	3.98	4.59	22.40	0.48	11	3	1
RBC, $\times 10^{12}$/L	1.62	1.73	4.39	9.16	0.54	2	1	1	1.39	1.56	7.89	0.50	2	1	1
Hb, g/L	1.58	1.68	4.29	8.92	0.54	2	1	1	1.37	1.61	7.76	0.47	2	1	1
HCT, %	1.56	1.53	4.10	9.18	0.63	2	1	1	1.41	1.34	8.00	0.62	2	1	1
PLT, $\times 10^9$/L	3.37	5.74	11.30	20.17	0.33	9	3	1	3.61	2.55	20.60	1.00	9	3	1
MCV, fL	0.35	0.80	1.37	2.80	0.32	1	1	1	0.36	1.01	2.06	0.18	1	1	1
MCH, pg	0.35	0.84	1.41	2.72	0.30	1	1	1	0.38	0.75	3.00	0.26	1	1	1
MCHC, g/L	0.27	0.24	0.68	3.20	1.50	1	1	1	0.49	0.47	3.47	0.61	1	1	1
Neu, $\times 10^9$/L	7.72	8.13	20.86	43.03	0.54	38	10	3	5.80	5.08	32.50	0.70	22	6	2
Lym, $\times 10^9$/L	5.54	6.09	15.23	31.27	0.52	20	5	2	4.91	6.65	28.00	0.48	16	4	1
Mono, $\times 10^9$/L	7.70	7.22	19.93	47.19	0.70	45	12	3	5.54	5.07	33.20	0.65	23	6	2
Eos, $\times 10^9$/L	8.08	14.25	27.57	50.81	0.34	52	13	4	5.06	17.80	41.40	0.14	35	9	3
Bas, $\times 10^9$/L	9.43	11.51	27.06	73.35	0.63	108	27	7	5.68	6.21	55.80	0.51	63	16	4

I_{APS}, the desirable APSs for imprecision; B_{APS}, the desirable APSs for bias; TE_{APS}, the desirable APSs for total error.

number of samples, number of replicates, and the feasibility of conducting studies, were taken into account, as follows: (1) The number of subjects was between 20 and 40 in nearly all previously published studies involving BV for the CBC [6, 8, 10, 11, 18, 20, 23, 31, 32]. Recently, Braga et al. [4] concluded that a minimum of 10 subjects is sufficient to obtain a good BV estimate for each identified subgroup. Therefore, we enrolled 19 males and 22 females to participate in the study. (2) Based on the review by Braga et al. [4], the study duration must be neither too short (a few days) nor too long (years) to eliminate influence by additional causes. Based on the turnover time for CBC parameters (i.e. RBC ~4 months), a study period of 6 months was used in our study. (3) The number of samples from each individual was 5–12 and the number of measurements for each sample was 1–2 in previously published reports [6, 8, 10, 11, 18, 20, 23, 31, 32]. Considering that the CV_A was calculated with all replicates for the same subject, the number of measurements for each sample was two in our study. According to the model of the power estimated by Roraas et al. [24], at least six samples are required for each individual when the power is defined as >80% with a ratio of CV_A to CV_I < 2 and 41 subjects. In our study following the aforementioned design, the power of all parameters was 1.00, except MCHC, with a power of 0.91 (Supplementary Table B), which indicates that the power of our study design was higher.

Calibration and quality control are essential to ensure the accuracy of hematology analyzer. The Clinical Laboratory Improvement Amendments (CLIA §493.1255 Standard) [33] and National Health Standard of China (WS/T 347—2011) [34] require (re)calibration at least once every 6 months and when there is major preventive maintenance or replacement of critical parts, or when control materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits. In our study, we performed one calibration prior to first-batch specimen analysis due to the duration time being no more than 6 months, no major preventive maintenance or repair service, and no analytical run considered out of control.

In previous BV studies [7, 12, 20, 21, 23, 31, 32, 35], CV_A was derived from the laboratory IQC data generated by use of commercial sample materials. Fraser et al. [8] and Braga and Panteghini [4] suggested that CV_A is estimated as the average variance between duplicate measurements of the analyte. Roraas et al. [24] and Braga et al. [36] proposed that CV_A derived from the laboratory IQC data is not necessarily transferable to clinical samples. In our study, the CVs from the IQC of 12 CBC parameters were greater than the CVs from duplicate measurements of clinical samples, except the MCV, which indicates that the derived

parameters (such as II, RCV, and NHSP) would be overestimated. Therefore, we support the use of a duplicate analysis to calculate CV_A.

The previously published BV data were compared with the data listed in the online 2014 BV database. We reviewed the 15 articles cited in the BV database following the standards in the BIVAC and found the studies had a low-quality score. Twelve articles [6–11, 37–42] received a D score because the instruments used were obsolete or the methods are no longer used routinely. The estimates from these studies were considered unsuitable for application in clinical practice. Two articles [23, 32] were scored as a C due to the absence of outlier removal, normality assessments, and variance homogeneity examination, which affect the accuracy of BV estimates (over- or under-estimation). One article [12] was scored as a C for lack of CIs around estimates of CV_I and inappropriate methods of outlier analyses. Therefore, we used PubMed and Google Scholar to retrieve relevant literature without any time limits, using the following keywords: biological variation (variability); hematology; complete blood cell; leukocytes/WBC; erythrocytes/RBC; hemoglobin/Hb/Hgb; hematocrit/HCT/Htc; and platelet/PLT. As unique inclusion/exclusion criteria, the recruited studies should have an explicit aim of the experimental assessment of CBC BV components. Nineteen articles were recruited [6–12, 14, 23, 28–30, 32, 37–42]. These articles were reviewed by the quality indicators (QIs) included in the BIVAC. Four articles [14, 28–30] were considered to be grade A, which met the QIs of the BIVAC and were reliable estimations. Therefore, we used the BV estimation of these studies as the comparators.

Our study showed that the CV_Is for RBC, Hb, and HCT were higher than those reported in two studies by Coskun et al. [14] and Buoro et al. [28–30] (Supplementary Figure A). This finding can be explained by the different duration of the studies. The duration of our study was 6 months and longer than the two studies. Additionally, the CV_Is of 10 weeks' duration by Coskun et al. [14] were also higher than those of 5 weeks' duration by Buoro et al. [28–30], which followed the same trend. Within our study period of 6 months, we covered one turnover period for erythrocytes, and the CV_Is for the erythrocyte group tests might be more reliable. Although the 95% CIs of CV_I between genders were overlapped for all CBC parameters in the current study, the CV_Is for WBC, Neu ($\times 10^9$/L), Mono ($\times 10^9$/L), and Eos ($\times 10^9$/L) in the females were higher than those in the males. Significant differences in the CV_I estimates between genders for leukocytes, Mono, Neu, and Eos were also evident in the Coskun et al.'s study [14]. This finding can be explained by the cyclic variations in WBC and the WBC sub-populations during the female menstrual cycle [43, 44]. When the BVs of the WBC subpopulations for females are estimated, the influence of the female menstrual cycle should be considered.

The CV_Gs for PLT in the male group of the present study were higher than the CV_Gs reported by Coskun et al. [14]. Based on the findings of Biino et al. [45], the PLT count was stable for males in the 18–49-year age range, whereas the PLT count was significantly decreased in subjects >50 years of age. The age range in the Coskun et al.'s study [14] and the present study was 20–36 and 23–59 years, respectively. The difference in age range might account for the difference in CV_Gs. If we calculated the CV_G using data from males <36 years of age, the CV_G for PLT decreased from 25.54% to 13.66%. Therefore, the age distribution of participants might significantly influence the CV_G estimates.

The II has been used to investigate the utility of conventional population-based reference intervals (RIs). The IIs of nine of 13 CBC parameters were <0.6, which suggests that conventional RIs are of very limited utility. In these cases, the RCV is a better parameter for monitoring of longitudinal changes in test results in an individual [46]. The IIs of two of nine parameters <0.60 in our study are not in agreement with those reported by Coskun et al. [14]. Additionally, the II of MCHC was >1.4, which makes RI a useful tool for test interpretation. Such is not the case for II of MCHC in the Coskun et al.'s study [14]. Additional research is warranted to further clarify the differences.

Conclusions

In conclusion, the present study has provided an estimate of the BV and the main indices of healthy Chinese adults following an experimental protocol with high power and a rigorous statistical approach complying with the BIVAC. With some exceptions, the 95% CIs of BV in our study for CBC parameters overlapped with the 95% CIs of the recently published BIVAC-compliant BV studies of a Europid population, which showed that no differences exist between the BVs of the two populations. The BV data for erythrocyte parameters (RBC, Hb, and HCT) and PLT in this study, however, were higher than those in the BIVAC-compliant studies published because of the differences associated with the study period and age distribution. Our study will provide reference and aid for the global BV database by EFLM and future studies on this subject.

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