RESEARCH ARTICLE

Establishment of complete blood count reference intervals for Chinese preschoolers

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Background: Reference intervals are critical for interpreting test results of a clinical laboratory. The aim of this study was to establish local reference intervals of complete blood count for healthy preschoolers in China.

Methods: Three thousand eight hundred and twenty-one blood specimens from children aged 4 months to 6 years were collected and analyzed. Complete blood counts were determined by Sysmex XT-4000i Automated Hematology Analyzer. The non-parametric 2.5th to 97.5th percentile reference ranges were calculated according to CLSI EP28-A3c guideline.

Results: Reference intervals for each blood cell parameter are determined as follows: total WBC $4.86\text{--}12.1 \times 10^9/\text{L}$ for males and $4.73\text{--}12.3 \times 10^9/\text{L}$ for females; RBC $4.13\text{--}5.32 \times 10^{12}/\text{L}$ for males and $4.08\text{--}5.24 \times 10^{12}/\text{L}$ for females; HGB 109–145 g/L for males and 111–143 g/L for females; HCT 33.1–41.2% for males and 33.3–41.1% for females; MCH 23.5–29.7 pg for males and 24.6–30.0 pg for females; MCHC 320–365 g/L for males and 321–362 g/L for females; MCV 71.4–85.1 fL for males and 73.8–86.9 fL for females; RDW-SD 33.5–41.9 fL for males and 33.5–41.0 fL for females; RDW-CV 12.0–15.2% for males and 11.8–14.5% for females; PLT $181\text{--}475 \times 10^9/\text{L}$ for males and $179\text{--}456 \times 10^9/\text{L}$ for females; PCT 0.18–0.44% for males and 0.18–0.43% for females; MPV 8.20–11.6 fL for males and 8.20–11.5 fL for females; PDW 8.40–14.4 fL for males and 8.40–14.0 fL for females; P-LCR 12.0–36.6% for males and 11.8–35.6% for females.

Conclusions: We established local complete blood count reference intervals for apparent healthy preschoolers in China. It is necessary to establishing region-specific reference intervals of complete blood count for preschoolers.

KEYWORDS

complete blood count, preschoolers, reference intervals

Abbreviations: HCT, hematocrit; HGB, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MPV, mean platelet volume; PCT, platelet-crit; PDW, platelet distribution width; P-LCR, platelet large cell ratio; PLT, platelets count; RBC, red blood cell count; RDW-CV, red blood cell distribution width of CV; RDW-SD, red blood cell distribution width of SD; WBC, white blood cell count.

Guo-Cheng Wang and Na Li contributed equally to this work.

1 | INTRODUCTION

Complete blood count is one of routine clinical laboratory tests in a hospital. As we all known, comparing test results with reference intervals is the first step of result analysis. Unfortunately, reference intervals being used in all most clinical laboratories in china, including ones

of complete blood count for preschoolers, are established originally based on American and/or European populations and may not fit for Chinese subjects. Consequently, a panel of blood cell reference intervals were established and are recommended by the National Health and Family Planning Commission of the People's Republic of China.¹ These reference intervals, however, are derived from and merely suitable for adult samples. Reference intervals of blood cell analysis for child, especially preschoolers, have been not clear until now in China. Shunyi is one of the typical suburbs of Beijing and lifestyle also dietary structure of preschoolers in this area have made a substantial change in the past few decades with the impetuous development of local economy. In this research, thus, we analyzed complete blood count results of apparently healthy preschoolers resident in Shunyi at least 5 years and aimed at establishing local reference intervals for commonly used hematological blood cell parameters according

to EP28-A3c guideline of the Clinical and Laboratory Standards Institute.²

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study was approved by the research ethics committee of Shunyi Maternal and Children's Hospital of Beijing Children's Hospital.

2.2 | Subjects selection

From July 28, 2015 to August 31, 2015, a total of 3821 apparently healthy preschoolers were selected in Shunyi District of Beijing as initial research subjects. The exclusion criteria were as follows: acute

TABLE 1 Reference intervals of complete blood count for all apparent healthy preschoolers^a

Parameters	Male	Female	All	P-value ^b
WBC ($\times 10^9/L$)	4.86 (4.78, 4.99) -12.1 (11.8, 12.4)	4.73 (4.60, 4.90) -12.3 (11.8, 12.7)	4.82 (4.74, 4.90) -12.1 (11.9, 12.5)	.619
RBC ($\times 10^{12}/L$)	4.13 (4.10, 4.17) -5.32 (5.28, 5.37)	4.08 (4.05, 4.11) -5.24 (5.20, 5.28)	4.11 (4.08, 4.13) -5.28 (5.26, 5.31)	<.001
HGB (g/L)	109 (108, 110) -145 (144, 146)	111 (109, 112) -143 (142, 144)	110 (109, 110) -144 (143, 145)	.641
HCT (%)	33.1 (32.8, 33.3) -41.2 (41.0, 41.5)	33.3 (33.1, 33.5) -41.1 (40.9, 41.3)	33.2 (33.0, 33.3) -41.1 (41.0, 41.3)	<.001
MCH (pg)	23.5 (22.9, 24.0) -29.7 (29.5, 29.8)	24.6 (24.3, 24.8) -30.0 (29.9, 30.2)	24.0 (23.6, 24.3) -29.9 (29.8, 30.0)	<.001
MCHC (g/L)	320 (317, 322) -365 (363, 367)	321 (318, 322) -362 (361, 363)	320 (319, 321) -363 (363, 364)	<.001
MCV (fL)	71.4 (70.9, 72.3) -85.1 (84.8, 85.4)	73.8 (73.1, 74.3) -86.9 (86.6, 87.2)	72.5 (72.1, 72.9) -86.3 (86.0, 86.6)	<.001
RDW-SD (fL)	33.5 (33.3, 33.6) -41.9 (41.2, 42.5)	33.5 (33.2, 33.6) -41.0 (40.9, 41.4)	33.5 (33.4, 33.6) -41.4 (41.0, 41.9)	.950
RDW-CV (%)	12.0 (11.9, 12.0) -15.2 (14.9, 16.2)	11.8 (11.7, 11.8) -14.5 (14.3, 14.8)	11.9 (11.8, 11.9) -14.9 (14.8, 15.1)	<.001
PLT ($\times 10^9/L$)	181 (175, 187) -475 (460, 483)	179 (164, 188) -456 (445, 466)	181 (175, 185) -464 (456, 473)	.103
PCT (%)	0.18 (0.17, 0.19) -0.44 (0.43, 0.45)	0.18 (0.17, 0.19) -0.43 (0.42, 0.44)	0.18 (0.17, 0.18) -0.44 (0.43, 0.44)	.105
MPV (fL)	8.20 (8.20, 8.30) -11.6 (11.5, 11.8)	8.20 (8.10, 8.30) -11.5 (11.4, 11.7)	8.20 (8.20, 8.30) -11.6 (11.5, 11.7)	.240
PDW (fL)	8.40 (8.40, 8.50) -14.4 (14.0, 14.6)	8.40 (8.30, 8.50) -14.0 (13.5, 14.4)	8.40 (8.40, 8.50) -14.2 (14.0, 14.5)	.070
P-LCR (%)	12.0 (11.6, 12.5) -36.6 (35.8, 37.3)	11.8 (11.5, 12.2) -35.6 (34.2, 36.7)	11.9 (11.6, 12.2) -36.1 (35.4, 36.8)	.151

^aReference intervals were calculated by a nonparametric method (2.5th-97.5th percentile), 90% confidence intervals were presented in parentheses.

^bDifferences between males and females were determined using Mann-Whitney U-test.

and/or chronic infectious diseases, trace elements or vitamin deficiency, myocarditis, allergic diseases, hematological diseases, congenital diseases, autoimmune diseases, height and/or weight out of corresponding ranges, any drugs and/or nutrients intake within 2 weeks, and inhabit time of children and/or mother <5 years. All participants are Han ethnicity and underwent an interview with a healthcare doctor by a life-health questionnaire and relevant physical examination. All participants provided informed consent.

2.3 | Laboratory analysis

After overnight fasting, all subjects were required to sit for at least 10 minutes before specimen collection. Two millilitre venous blood was taken into a vacuum vessel containing 3.6 mg ethylene diamine tetraacetic acid (EDTA) anticoagulant from the median cubital vein or jugular if necessary and then mixed immediately by inverting eight times. All blood specimens were analyzed within 2 hours in the

Sysmex XT-4000i Automatic Hematology Analyzer in line with standard operation procedure of our laboratory. Fourteen complete blood cell parameters including WBC, RBC, HGB, HCT, MCH, MCHC, MCV, RDW-SD, RDW-CV, PLT, PCT, MPV, PDW, P-LCR were tested and recorded.

2.4 | Quality assurance

All participant staffs had received one more training about operation procedures and relevant equipments for ensuring standardization and minimizing preanalytical variables. The imprecision, carry over, linearity of XT-4000i Automatic Hematology Analyzer were evaluated in accordance with guideline of International Council for Standardization in Hematology.^{3,4} Before and after every analytical run, commercial control samples at two levels (high and low) were measured. External quality assessment results from National Center for Clinical Laboratories of China were all satisfactory.

TABLE 2 Reference intervals of complete blood count for ≥7 months to 1 year old Preschoolers^a

Parameters	Male	Female	All	P-value ^b
WBC ($\times 10^9/L$)	5.30 (4.43, 5.81) -13.8 (12.9, 16.6)	5.28 (4.55, 6.00) -13.3 (12.6, 17.6)	5.30 (4.55, 5.81) -13.6 (12.8, 14.7)	.690
RBC ($\times 10^{12}/L$)	4.10 (3.97, 4.18) -5.33 (5.26, 5.75)	3.99 (3.65, 4.13) -5.17 (5.04, 5.66)	4.06 (3.97, 4.16) -5.32 (5.23, 5.57)	.003
HGB (g/L)	101 (84.0, 104) -133 (131, 134)	95.3 (89.0, 102) -137 (133, 137)	100 (89.0, 102) -134 (133, 137)	.436
HCT (%)	30.3 (28.7, 31.5) -40.9 (39.7, 42.7)	31.5 (30.4, 32.5) -40.0 (39.2, 41.0)	30.8 (30.3, 31.8) -40.5 (39.7, 42.0)	.397
MCH (pg)	19.7 (18.0, 20.8) -27.9 (27.8, 29.9)	19.9 (18.4, 21.8) -28.5 (28.1, 30.3)	19.8 (18.3, 20.9) -28.2 (28.0, 28.5)	.001
MCHC (g/L)	304 (286, 310) -351 (344, 355)	298 (283, 309) -348 (344, 351)	302 (293, 308) -349 (345, 353)	.193
MCV (fL)	62.8 (55.3, 66.9) -85.2 (84.4, 88.5)	66.5 (57.2, 71.4) -87.3 (85.9, 89.4)	63.7 (61.0, 66.9) -86.3 (85.2, 88.5)	.001
RDW-SD (fL)	34.5 (33.2, 34.8) -49.6 (48.6, 52.5)	31.6 (31.4, 33.8) -48.3 (44.0, 61.8)	33.6 (31.5, 34.6) -49.3 (45.6, 52.3)	.002
RDW-CV (%)	12.2 (12.0, 12.4) -21.4 (19.8, 23.9)	11.7 (11.3, 12.0) -19.2 (17.2, 24.7)	12.0 (11.7, 12.1) -21.1 (18.9, 21.6)	<.001
PLT ($\times 10^9/L$)	175 (143, 202) -490 (451, 573)	165 (138, 190) -462 (430, 556)	175 (148, 189) -487 (450, 538)	.922
PCT (%)	0.20 (0.16, 0.20) -0.52 (0.47, 0.57)	0.19 (0.14, 0.20) -0.49 (0.45, 0.58)	0.20 (0.17, 0.22) -0.51 (0.46, 0.53)	.667
MPV (fL)	9.10 (9.00, 9.20) -13.2 (12.3, 13.2)	9.02 (8.50, 9.30) -12.6 (12.0, 12.7)	9.10 (9.00, 9.30) -12.6 (12.3, 13.1)	.493
PDW (fL)	9.20 (8.60, 9.50) -17.9 (16.3, 20.4)	9.31 (8.00, 9.50) -16.8 (15.1, 20.3)	9.30 (9.10, 9.50) -17.3 (16.3, 20.3)	.595
P-LCR (%)	16.4 (15.2, 16.9) -46.1 (42.2, 47.8)	15.7 (11.3, 18.7) -44.2 (38.8, 45.0)	16.4 (15.2, 17.6) -44.3 (40.1, 46.6)	.626

^aReference intervals were calculated by a nonparametric method (2.5th-97.5th percentile), 90% confidence intervals were presented in parentheses.

^bDifferences between males and females were determined using Mann-Whitney U-test.

2.5 | Statistical analysis

Data were pooled, transformed and analyzed with Microsoft Excel 2007, Statistical Package for Social Sciences 19.0 (IBM SPSS Incorporated, Armonk, NY, USA), and MedCalc 16.4.3 (MedCalc Software bvba, Ostend, Belgium). As represented in CLSI EP28-A3c guideline,² outliers were identified by one-third rule (suggested by Reed) for ratio D/R (proposed by Dixon), where D is the absolute difference between an extreme observation (large or small) and the next largest (or smallest) observation, and R is the range of all observations, including extremes. Normality test of all data was done by the Kolmogorov-Smirnov. Mann-Whitney *U*-test was utilized to identify significantly different groups for all the 14 parameters. Possible partitioning of three broad age groups: 4-6 months old, 7 months to 1 year old, and 2-6 years old was determined by visually inspecting distribution of reference values. And then, the Harris and

Boyd's test⁵ was applied for statistical evaluation. Reference values were combined and re-evaluated if the results of Harris and Boyd's test did not indicate partitioning. Differences were considered to be statistically significant when two-tailed *P* values were equal to .05 or less than.

3 | RESULTS

3.1 | Reference interval

In the end, 3814 children (1931 males and 1883 females) aged 4 months to 6 years were included to create our reference intervals of complete blood count. Data of seven children were identified and then eliminated as statistical outliers. All values of 14 parameters in this study were not in normal distribution after Kolmogorov-Smirnov testing. Therefore, nonparametric 2.5th-97.5th percentile reference

TABLE 3 Reference intervals for complete blood count for ≥2 years to 6 years old Preschoolers^a

Parameters	Male	Female	All	P-value ^b
WBC ($\times 10^9/L$)	4.83 (4.74, 4.94) -11.7 (11.3, 12.1)	4.72 (4.55, 4.86) -11.8 (11.4, 12.5)	4.78 (4.72, 4.87) -11.7 (11.5, 12.1)	.524
RBC ($\times 10^{12}/L$)	4.14 (4.10, 4.18) -5.30 (5.27, 5.36)	4.09 (4.07, 4.13) -5.25 (5.20, 5.30)	4.12 (4.08, 4.14) -5.28 (5.25, 5.31)	<.001
HGB (g/L)	115 (114, 116) -145 (144, 146)	115 (114, 116) -143 (142, 144)	115 (114, 116) -144 (144, 145)	.811
HCT (%)	33.5 (33.3, 33.8) -41.3 (41.0, 41.7)	33.6 (33.4, 33.7) -41.2 (40.9, 41.5)	33.5 (33.4, 33.7) -41.2 (41.0, 41.4)	.002
MCH (pg)	25.1 (24.9, 25.4) -29.8 (29.6, 30.0)	25.8 (25.4, 25.9) -30.1 (29.9, 30.3)	25.4 (25.2, 25.5) -29.9 (29.8, 30.1)	<.001
MCHC (g/L)	331 (330, 332) -366 (364, 367)	328 (327, 330) -363 (361, 364)	330 (329, 331) -364 (363, 365)	<.001
MCV (fL)	73.2 (72.9, 73.6) -85.1 (84.8, 85.6)	74.8 (74.6, 75.3) -86.9 (86.5, 87.2)	73.9 (73.3, 74.2) -86.3 (86.0, 86.6)	<.001
RDW-SD (fL)	33.5 (33.4, 33.7) -40.6 (40.5, 41.2)	33.5 (33.4, 33.7) -40.9 (40.4, 41.0)	33.5 (33.5, 33.6) -40.8 (40.5, 40.9)	.188
RDW-CV (%)	12.0 (11.9, 12.0) -14.5 (14.4, 14.7)	11.8 (11.7, 11.8) -14.1 (14.0, 14.3)	11.8 (11.8, 11.9) -14.4 (14.3, 14.5)	<.001
PLT ($\times 10^9/L$)	181 (175, 188) -468 (452, 480)	180 (166, 192) -449 (436, 460)	181 (175, 187) -458 (450, 467)	.046
PCT (%)	0.18 (0.17, 0.18) -0.42 (0.41, 0.43)	0.18 (0.16, 0.18) -0.41 (0.41, 0.42)	0.18 (0.17, 0.18) -0.42 (0.41, 0.42)	.066
MPV (fL)	8.20 (8.10, 8.30) -11.4 (11.2, 11.6)	8.20 (8.10, 8.20) -11.2 (11.1, 11.3)	8.20 (8.10, 8.20) -11.3 (11.2, 11.4)	.484
PDW (fL)	8.40 (8.30, 8.50) -13.6 (13.4, 14.1)	8.40 (8.30, 8.50) -13.4 (13.2, 13.7)	8.40 (8.30, 8.40) -13.6 (13.3, 13.8)	.089
P-LCR (%)	11.8 (11.3, 12.2) -34.5 (33.3, 35.8)	11.7 (11.3, 12.1) -32.8 (32.1, 33.9)	11.8 (11.4, 12.0) -33.7 (32.9, 34.5)	.301

^aReference intervals were calculated by a nonparametric method (2.5th-97.5th percentile), 90% confidence intervals were presented in parentheses.

^bDifferences between males and females were determined using Mann-Whitney *U*-test.

TABLE 4 Reference intervals of complete blood count for 4-6 months healthy Preschoolers

Parameters	Male ^a	Female ^a	All ^b	P-value ^c
WBC ($\times 10^9/L$)	4.96 (4.24, 5.76) -12.6 (12.0, 13.3)	5.48 (4.80, 6.17) -12.8 (12.0, 13.5)	5.60 (4.90, 6.21) -12.8 (11.9, 15.2)	.260
RBC ($\times 10^{12}/L$)	3.96 (3.83, 4.10) -5.39 (5.25, 5.52)	3.87 (3.77, 4.00) -5.27 (5.14, 5.38)	3.85 (3.68, 4.10) -5.34 (5.20, 5.61)	.070
HGB (g/L)	101 (97.6, 104) -135 (132, 138)	104 (101, 106) -133 (130, 136)	101 (97.0, 105) -134 (131, 138)	.494
HCT (%)	31.4 (30.6, 32, 2) -40.2 (39.3, 40.9)	32.0 (31.2, 32.7) -40.7 (39.8, 41.5)	30.9 (29.6, 32.7) -40.2 (39.4, 41.1)	.123
MCH (pg)	21.8 (21.1, 21.7) -28.6 (27.7, 29.4)	23.6 (23.3, 24.0) -28.4 (27.9, 28.8)	22.7 (18.7, 23.4) -28.5 (27.9, 29.1)	.001
MCHC (g/L)	309 (305, 313) -346 (342, 350)	311 (309, 314) -343 (340, 345)	311 (306, 313) -347 (341, 361)	.649
MCV (fL)	67.0 (64.5, 69.6) -86.5 (83.8, 89.1)	72.2 (71.0, 73, 4) -86.6 (85.4, 87.9)	69.5 (55.3, 72.0) -86.7 (84.6, 87.8)	.001
RDW-SD (fL)	32.1 (31.0, 33.2) -41.0 (40.0, 42.0)	32.7 (32.0, 33.4) -40.9 (40.1, 41.6)	32.6 (31.4, 33.2) -40.6 (39.8, 45.6)	.604
RDW-CV (%)	10.7 (9.63, 11.9) -16.0 (14.8, 17.1)	11.5 (11.2, 11.8) -14.5 (14.2, 14.7)	11.9 (11.5, 12.0) -15.5 (14.9, 20.9)	.003
PLT ($\times 10^9/L$)	144 (114, 175) -519 (473, 554)	159 (133, 191) -503 (469, 534)	165 (117, 206) -517 (493, 579)	.825
PCT (%)	0.16 (0.14, 0.20) -0.50 (0.47, 0.54)	0.19 (0.17, 0.22) -0.50 (0.47, 0.52)	0.22 (0.15, 0.24) -0.52 (0.49, 0.57)	.834
MPV (fL)	9.78 (8.48, 9.04) -12.0 (11.6, 12.3)	8.4 (8.09, 8.75) -12.3 (11.9, 12.7)	9.10 (8.60, 9.40) -12.7 (12.0, 13.2)	.755
PDW (fL)	7.97 (7.40, 8.64) -14.5 (13.7, 15.3)	7.11 (6.19, 8.11) -15.4 (14.3, 16.3)	9.38 (8.50, 9.70) -17.0 (15.0, 19.1)	.897
P-LCR (%)	14.0 (11.8, 16.4) -39.7 (36.6, 42.1)	11.9 (9.70, 14.5) -41.7 (38.6, 44.4)	16.5 (13.3, 19.0) -43.9 (39.5, 48.3)	.772

^aReference intervals were calculated by a robust method when the number of 4-6 months male (66) and female (70) preschoolers were <120, 90% confidence intervals were presented in parentheses.

^bReference intervals were calculated by a nonparametric method (2.5th-97.5th percentile), 90% confidence intervals were presented in parentheses.

^cDifferences between males and females were determined using Mann-Whitney *U*-test.

intervals⁶ were calculated and presented in Tables 1, 2 and 3, respectively. In Table 4, a robust method was used according to CLSI EP28-A3c guideline when the number of 4-6 months male (66) and female (70) preschoolers were <120.

3.2 | Age- and sex-related reference intervals

Continuous three levels (2.5th, 50th, 97.5th percentile) of four mainly hematologic analytes WBC, RBC, HGB, and PLT was plotted in Figure 1. As shown, WBC and PLT parameters, especially, represented visually two variations at 6 months and 2 years. Reference intervals of other 12 items also showed slightly significant differences when all preschoolers were divided into three groups: 4-6 months age group; 7 months to 1 year age group; 2-6 years old age group. Sex-related differences appeared only in MCH, MCV,

and RDW-CV parameters (all $P < .01$) in 4-6 months old group. RBC, RDW-SD (both $P < .01$) and HCT, MCHC, PLT (all $P < .05$) more parameters started to show significant gender differences after 6 months and 2 years old.

3.3 | CBC reference intervals comparison among different regions

Reference intervals comparison with other region⁷⁻¹⁰ of four key blood variables WBC, RBC, HGB, and PLT in present study are plotted in Figure 2. The 2.5th percentile levels of WBC count in present study was highest one among four regions, the 97.5th also one of the highest. The reference intervals of our study for RBC, HGB did not show visible difference comparing with others except United States, which showed the highest concentrations.

FIGURE 1 Continuous reference intervals of WBC, RBC, HGB, and PLT. Continuous three concentrations of four mainly hematology analytes WBC, RBC, HGB, and PLT are lined according to Age. Upper, median, and lower continuous curves denote the 97.5th, 50th, and 2.5th percentile, respectively

FIGURE 2 Reference intervals comparison of hematology analytes WBC, RBC, HGB, and PLT. The ordinate axes denote concentrations of four mainly hematology analytes and primary axes (left) show for WBC, RBC, subsidiary axes (right) show for HGB, PLT. Xicheng is a urban district of Beijing; Heilongjiang and Mongolia are two northern provinces in China. (A) comparison of 2.5th percentile reference intervals; (B) comparison of 97.5th percentile reference intervals

4 | DISCUSSION

The concept of reference values usually reported now as population-based reference intervals mostly encompassing 95% of apparent healthy reference subjects was introduced in 1969 by Grasbeck and Saris.¹¹ As we all known, reference intervals of complete blood count are absolutely necessary for evaluating medical condition and diagnosing diseases. In this present research, we established reference intervals of fourteen complete blood count parameters for preschoolers aged 4 months to 6 years in our region located rural-urban fringe zone of Beijing, China.

All the 14 parameters concentrations had non-Gaussian distributions which were the same as the test results analyzed by Zhang et al.¹² and Qiao et al.¹³, but not consistent with Du et al.⁸. These differences presumably are due to the size of the survey area, the number and selection of subjects, hereditary differences, aging, and also

inspection systems. Age and sex are obviously two critical influences when we aim to establish reference intervals. Physical development (height and weight) change with each passing month before 6 years.¹⁴ We also found that almost all parameters of complete blood cell count showed differences in relation to age and sex. For example, sex-related differences appeared only in MCH, MCV and RDW-CV parameters (all $P < .01$) in 4-6 months old group. RBC, RDW-SD (both $P < .01$) and HCT, MCHC, PLT (all $P < .05$) more parameters started to show significant gender differences after 6 months and 2 years old, which may be regarded as onset of age- and sex-specific dynamics concluded by Zierk et al.¹⁵.

Region is another critical influence when some reference intervals developed, which probably related to ethnicity, diet, lifestyle, living and economic conditions. There are some significant differences in 2.5th and 97.5th reference intervals among regions, visually showed in Figure 2. The 2.5th percentile reference interval of WBC count in

present study was the highest one among four regions, the 97.5th interval also one of the highest. This probably because our region locate in rural-urban fringe zone, whose living condition and medical care is not the best. The RBC, HGB reference intervals in present study did not show visible difference comparing with others except United States, which showed the highest levels. This probably related to higher living standards of American people.

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