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Original Article

Changes and reference intervals of immature granulocytes in the peripheral blood of women according to pregnancy trimester

Ling-Ling Yu[1], Yi-Mei Jin[1], Mian-Mian Li[1], Xiao-Jian Chen[1], Xiao-Qun Zheng[1,2]

[1]Department of Laboratory Medicine, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, China; [2]College of Laboratory and Life Sciences, Wenzhou Medical University, Wenzhou 325035, China

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Abstract: Background: Immature granulocyte (IG) counts as a powerful clinical parameter can be obtained by new generations of hematology analyzers automatically. Establishment of IG reference intervals in healthy pregnant women is a prerequisite for clinically meaningful interpretation of the parameter. Methods: By using a Sysmex XE-5000 hematology analyzer, a large number of pregnant women comprising 1372 samples were analyzed to determine pregnancy trimester stratified reference intervals for IG, including their absolute values (#IG) and relative values (%IG). Results: Compared with healthy non-pregnant women, #IG and %IG in healthy pregnant women increased significantly ($P<0.05$). Although there was no difference in #IG and %IG of healthy pregnant women of different ages, these values were positively correlated with pregnancy trimester and there was significant difference among the first, second, and third trimester groups ($P<0.05$). According to a non-parametric statistical method, the reference intervals for #IG in the first, second, and third trimesters were $(0.003\text{-}0.091)\times10^9$/L, $(0.007\text{-}0.247)\times10^9$/L, and $(0.018\text{-}0.456)\times10^9$/L, respectively, while those for %IG were (0.04-0.92)%, (0.10-2.00)%, and (0.20-3.80)%, respectively. Conclusion: This study demonstrates IG counts in healthy pregnant women increase significantly during pregnancy, especially in the second and third trimesters. IG reference intervals according to pregnancy trimester have been established, and it may be used for evaluating health status of pregnant women and help obstetricians to make their clinical decisions.

Keywords: Automatic hematology analyzer, pregnant women, immature granulocytes, non-parametric statistical method, reference interval

Introduction

Immature granulocytes (IG) include metamyelocytes, myelocytes, and promyelocytes. Their appearance in the peripheral blood is an important indication of enhanced hematopoiesis in bone marrow, leakage in the blood-spinal cord barrier, or extramedullary hematopoiesis. Thus, they are important clinic hematologic parameters for diagnosis, prognosis and treatment of inflammation, hemopathy and infectious diseases [1, 2]. IG counts were traditionally assayed by manual smear examination in which the limitation of few observed leukocytes, differences among individuals, and different morphological criteria led to low test accuracy and poor reproducibility [3].

With the development of modern technology, a new hematology analyzer automatically was developed to assay IG counts through the differential and immature myeloid information channels and have been widely used in clinical application due to its simple, fast, precise and accurate features [4, 5]. Previous work has focused almost on IG in hospitalized patients and their potential role in predicting sepsis. Similarly, even 'normal' reference intervals for IG have largely been derived from and validated against hospitalized patients [6-8]. Although a large data set of more than 2400 samples from non-hospitalized outpatients were used to derive a variety of IG reference ranges stratified by age and sex [9], IG changes and reference intervals in pregnant women, a special population, have not been reported.

The aim of the present study was to observe the changes of IG counts in pregnant women

Table 1. Age distribution characteristics of the healthy pregnant and non-pregnant groups

	Pregnancy Group*				Non-pregnancy group*
	Total	First trimester#	Second trimester#	Third trimester#	
Number of cases	1,372	176	522	674	221
Age (years)					
Range	18-42	20-41	19-39	18-42	18-41
Average	28.29	28.33	28.59	28.05	27.91
Standard deviation	4.13	3.62	4.69	3.76	4.11
Median	28	28	28	27	28
25th percentile	25	25	25	25	26
75th percentile	31	31	31	30	30
Kolmogorov-Smirnov Z	4.343	1.679	2.632	3.029	1.247
P	0.000	0.007	0.000	0.000	0.089

Notes: *: compared by Wilcoxon test, $P = 0.615$; #: compared by Median test, $P = 0.128$.

and define their reference intervals by the Sysmex XE-5000 automated hematology analyzer. In this study, a large data set of more than 1300 samples from healthy pregnant women was used to derive a variety of IG reference intervals stratified by age and trimester. One great advantage is that, because of the distribution of the data set, nonparametric statistical method was used, which is recommended for estimating reference intervals [10]. These reference intervals then can be used as criteria for assessing the health status of pregnant women.

Materials and methods

Subjects

After informed consents were collected and patients with underlying diseases (infections and fever during blood samples collection, medical history in heart, lung, liver, kidney and blood) were excluded, a total of 1,372 healthy pregnant women examined at the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University from Apr. 2014 to Mar. 2015 were enrolled into this study. Among them, 176 were in their first trimester (<12 weeks' gestation), 522 were in their second trimester (13-27 weeks' gestation), and 674 were in their third trimester (>28 weeks' gestation). A total of 221 healthy non-pregnant women who went to the hospital for a health examination during the same period were enrolled as a control group. This study was approved by the institutional review board of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University (Wenzhou, China).

Materials

The Sysmex XE-5000 automated hematology analyzer and its original supporting reagents were obtained from Sysmex Corporation (Kobe, Japan). Ethylenediaminetetraacetic acid (EDTA)-K_2 anticoagulant Vacutainer tubes were obtained from Becton, Dickinson and Company (Franklin lakes, New Jersey, U.S.A).

Methods

All peripheral venous blood samples were collected into EDTA-K_2 anticoagulant Vacutainer tubes. All IG count measurements were performed on the same Sysmex XE-5000 automated hematology analyzer. All samples were analyzed within 4 hours of collection and mixed thoroughly before analysis. Three levels of commercial quality control specimens (Sysmex e-Check) were run at least daily. Quality was further monitored via participation in a hematology external quality assessment program of Chinese National Center for Clinical Laboratories. All data were further analyzed by statistical software.

Treatment of extreme values

According to D/R rules in Clinical and Laboratory Standards Institute standard C28-A3 [11], the extreme value was determined to be homogeneous or non-homogenous. In detail, D = maximum value (or minimum value)-close to the maximum value (or minimum value), R = maximum value-minimum value. If D/R <1/3, the extreme value was kept and that individual should behomogeneous. Otherwise, the extreme value was removed. The approaching extreme value was processed by using the similar method.

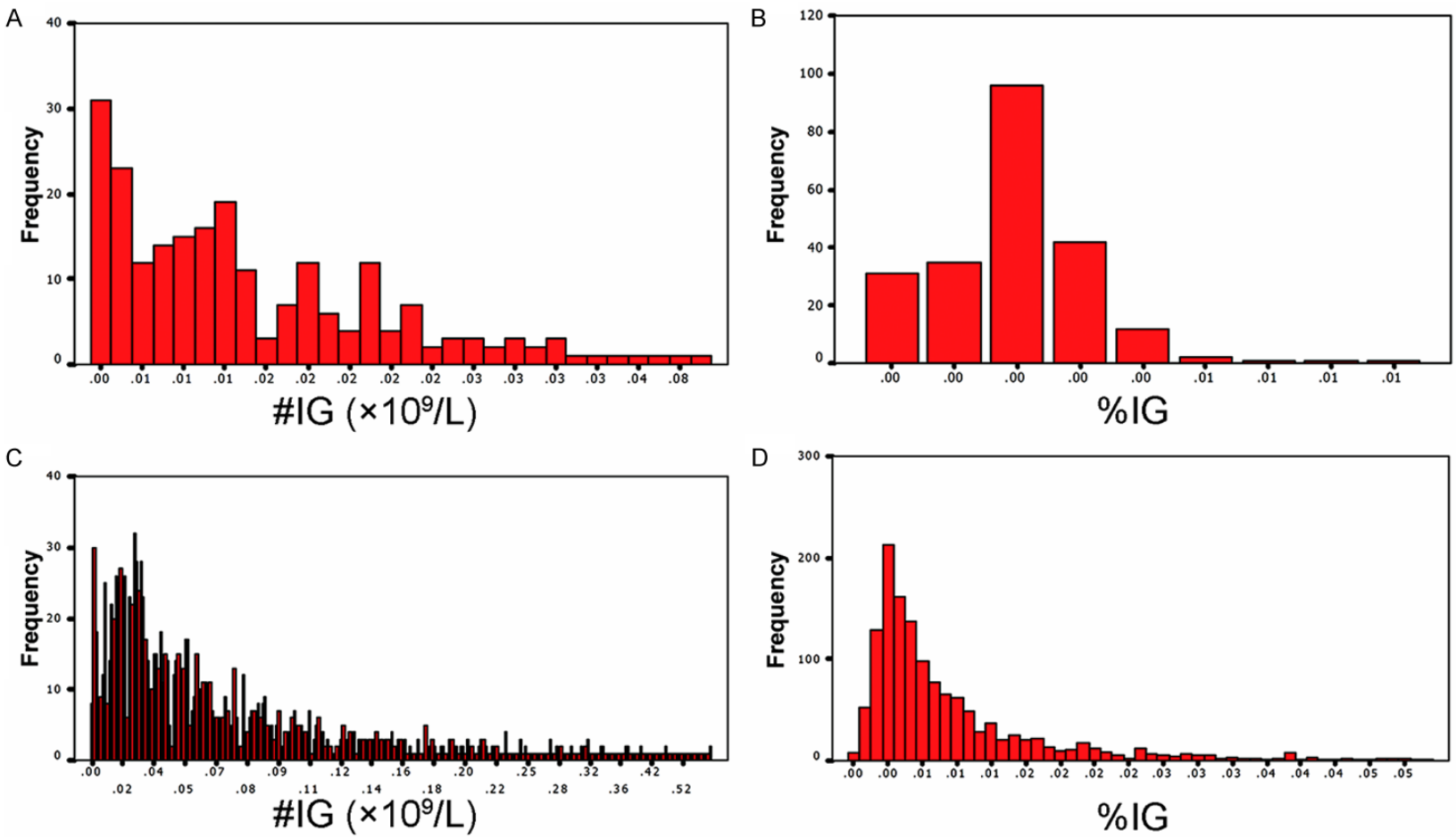

Figure 1. Distribution histograms for absolute immature granulocyte (#IG) and relative IG (%IG) concentrations in healthy pregnant and non-pregnant women. A. Histogram by #IG concentration data set in non-pregnant women (n = 221). B. Histogram by %IG concentrations data set in non-pregnant women (n = 221). C. Histogram by #IG concentration data set in pregnant women (n = 1,372). D. Histogram by %IG concentration data set in pregnant women (n = 1,372).

Table 2. Measurement of IG counts in healthy pregnant and non-pregnant women

Group	n	Median	
		#IG ($\times 10^9$/L)	%IG
Pregnancy	1,372	0.05	0.5
Non-pregnancy	221	0.011	0.2
Z		-19.23	-18.38
P		<0.05	<0.05

Notes: IG, immature granulocyte; #IG, absolute IG concentration; %IG, relative IG concentration.

Statistical analysis

Data were analyzed by SPSS13.0 software (Statistical Product and Service Solutions Corporation, Chicago, Illinois, U.S.A). Normal data distribution was verified by using a single sample Kolmogorov-Smirnov test (P<0.05, as a non-normal distribution). The central tendency of normal distribution data were represented as mean ± standard deviation ($\bar{x} \pm s$) and two independent sample t-tests were used for comparing the data between two groups, while ANOVA was used for multiple-group comparison. The central tendencies of the skewed distribution data were represented as the median, while the Wilcoxon test was used for two-group comparison and the median test was used for multiple-group comparisons. Statistical judgment was determined at $\alpha = 0.05$ level.

For data with a skewed distribution, the non-parametric method was used to define the reference intervals according to Clinical and Laboratory Standards Institute standard C28-A3. The reference intervals were defined as those between and including the lower limit 2.5th and upper limit 97.5th percentiles, which were estimated to enclose the 95th percentiles of the IG values for healthy pregnant women.

Results

Baseline data analysis

As shown in **Table 1**, the results of the Kolmogorov-Smirnov test indicated that age distribution of all groups, except non-pregnancy group, consisted of skewed data. Accordingly, the Wilcoxon test was used for age comparisons between the pregnant and non-pregnant groups, while the median test was used for age comparisons among the first, second, and third trimester groups. There was no significant age difference between the pregnant and non-pregnant groups (P>0.05) or among the first, second, and third trimester groups (P>0.05).

Measurement of IG counts in healthy pregnant and non-pregnant women

The overall distribution of absolute IG (#IG) and relative IG (%IG) concentrations in healthy pregnant and non-pregnant women is depicted in **Figure 1**. All data sets were skewed, so the Wilcoxon test was used for #IG and %IG comparisons between the pregnant and non-pregnant groups. As shown in **Table 2**, there was a significant difference in #IG or %IG concentrations between pregnant and non-pregnant women (P<0.05).

Measurement of IG counts in healthy pregnant women of different ages

The overall distribution of the #IG and %IG concentrations in healthy pregnant women of different ages is depicted in **Figure 2**. All of the data sets were skewed distribution data, so the median test was used for #IG or %IG comparison among them. Median test results suggested that there was no significant difference in #IG or %IG concentrations among healthy pregnant women of different ages (P>0.05, **Table 3**).

Measurement of IG counts in women according to pregnancy trimester

The overall distribution of #IG and %IG concentrations in healthy pregnant women by trimester is depicted in **Figure 3**. All data sets were skewed, so the median test was used to compare the #IG with %IG. As shown in **Table 4**, #IG and %IG concentrations increased as gestation time increased, and there was a significant difference among the first, second, and third trimester groups (P<0.05) by the median test.

Reference intervals of IG counts in healthy pregnant women

Based on the results above, a statistical test of separate reference intervals by trimester would be of interest. **Table 5** shows the reference intervals of the peripheral venous blood IG counts by trimester by using the non-parametric statistical method. The lower and upper reference limits, which were estimated to enclose the 95th percentile values, were assumed to demarcate the estimated 2.5th and 97.5th per-

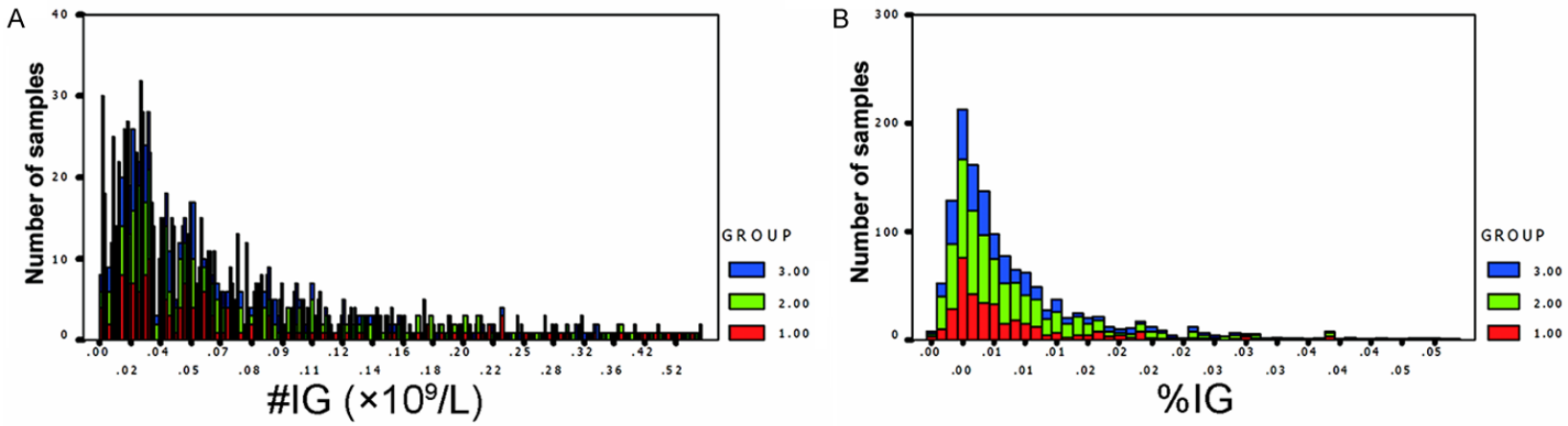

Figure 2. Age distribution histograms for the reference range data set (n = 1,372). A. Histogram by #IG concentrations data set in pregnant women of different ages. B. Histogram by %IG concentration data set in pregnant women of different ages. Red areas, ≤25 years pregnant women group (n = 373); green areas, 26-30 years group during pregnancy (n = 645); blue areas, ≥31 years group during pregnancy (n = 354), respectively.

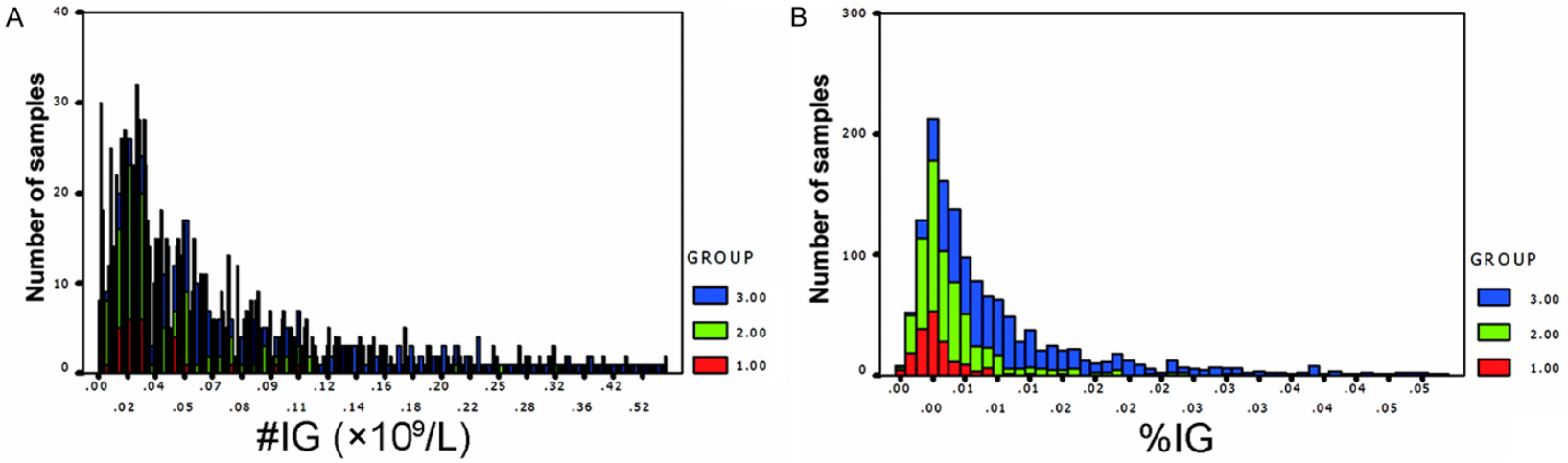

Figure 3. Trimester distribution histograms for the reference range data set (n = 1,372). A. Histogram by absolute immature granulocyte (#IG) concentrations in pregnant women by trimester. B. Histogram by relative IG (IG%) concentrations in pregnant women by trimester. Red areas, first trimester group (n = 176); green areas, second trimester group (n = 522); blue areas, third trimester group (n = 674).

Table 3. Measurement of IG counts in pregnant women of different age groups

Age Group	n	Median	
		#IG ($\times 10^9$/L)	%IG
≤25 years	373	0.048	0.5
26-30 years	645	0.051	0.5
≥31 years	354	0.05	0.5
P		0.595*	0.774*

Notes: IG, immature granulocyte; #IG, absolute IG concentration; %IG, relative IG concentration. **P*>0.05, compared by median test.

Table 4. Measurement of IG counts in healthy pregnant women by trimester

Group	n	Median	
		#IG ($\times 10^9$/L)	%IG
First trimester	176	0.023	0.3
Second trimester	522	0.032	0.4
Third trimester	674	0.086	0.9
P		0.000*	0.000*

Notes: IG, immature granulocyte; #IG, absolute IG concentration; %IG, relative IG concentration. **P*<0.05 by median test.

centiles of the underlying distribution of IG values in healthy pregnant women, respectively.

Discussion

To observe the changes of IG concentrations in maternal blood is of great significance for diagnosing diseases in pregnant women as well as assessing fetal growth and mental development. However, obstetrician's judgments of increased IG counts in pregnant women depend on the establishment of its reference interval, which is also the basis of clinical diagnosis and prognosis on a variety of diseases [12].

Change of IG in the peripheral blood during pregnancy is a rather complex physiological phenomenon. Results of this study showed that peripheral venous blood IG concentrations in the pregnant group were significantly higher than those of the non-pregnant group (**Table 2**). Considering that changes in peripheral blood IG counts are closely related with body organs and granulocytes are the most easily stimulated cells, this difference may be due to the special physiology of pregnancy. During pregnancy, in the fetal growth and development process, the mother's reproductive, endocrine, and metabolic systems undergo a series of changes that will affect her hematopoiesis function and lead to changes in blood components, especially higher IG counts. The mechanism of IG changes remains unclear, but it may be similar to the white blood cell release from the marginal pool after strenuous exercise.

As shown in **Table 4**, varying degrees of #IG and %IG changes are observed in the first, second, and third trimesters, and the increases in #IG and %IG concentrations are positively correlated with the gestational period. The change is because that pregnant women are in acute nonspecific inflammation state during pregnancy [13]. With a large number of mature granulocytes entering the peripheral blood, metamyelocytes and myelocytes are also released, resulting in the increase in maternal blood IG counts, especially in the second and third trimesters. This is a special physiological state, rather than a pathological elevation. In other IG-elevated diseases, such as hemopathy and infectious diseases, the IG counts may be increased even further. For example, patients with hemopathy have more than 10% IG counts increase and are often characterized by clinical manifestations [14-16]. Therefore, establishing IG reference intervals during pregnancy may help to classify increased values of IG counts, which is prerequisite for clinicians to determine whether it is a physiological or pathological increasing.

Data in this study showed that IG counts were significantly different among the different pregnancy stages. Thus, pregnancy stage should be considered when a reference interval is defined. For IG, separate reference intervals by trimester do appear to be clinically useful for diagnostic purposes. Again, there is physiological evidence to support this conclusion. However, the IG count in pregnant women of different ages did not differ significantly, suggesting that age need not be considered in establishment of a reference interval, a finding that differs from the previous report of an age-dependent IG reference range [9]. This difference may be due to sample volume and case selection, which can be solved by expanding the sample size in future studies. For various reasons, there may be different IG reference intervals in different laboratories, so each laboratory should establish specific reference intervals for specific populations as what have been done here.

Table 5. Reference intervals of IG counts in healthy pregnant women

Group	#IG (×10^9/L)		%IG	
	LL (2.5th percentile)	UL (97.5th percentile)	LL (2.5th percentile)	UL (97.5th percentile)
First trimester	0.003	0.091	0.04	0.92
Second trimester	0.007	0.247	0.10	2.00
Third trimester	0.018	0.456	0.20	3.80

Notes: IG, immature granulocyte; #IG, absolute IG concentration; %IG, relative IG concentration; LL, lower limit of reference intervals; UL, upper limit reference intervals.

By performing a comprehensive analysis of IG count changes in peripheral blood of healthy pregnant women, a laboratory reference interval specific for pregnant women is defined. The established reference interval can be used for evaluating the health status of pregnant women as a powerful hematologic parameter and help obstetricians make their clinical decisions.

Disclosure of conflict of interest

None.

Address correspondence to: Xiao-Qun Zheng, Department of Laboratory Medicine, The Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, China; College of Laboratory and Life Sciences, Wenzhou Medical University, Wenzhou 325035, China. E-mail: zhengxiaoqundd@163.com

References

[1] van der Geest PJ, Mohseni M, Brouwer R, van der Hoven B, Steyerberg EW and Groeneveld AB. Immature granulocytes predict microbial infection and its adverse sequelae in the intensive care unit. J Crit Care 2014; 29: 523-527.

[2] Nierhaus A, Klatte S, Linssen J, Eismann NM, Wichmann D, Hedke J, Braune SA and Kluge S. Revisiting the white blood cell count: immature granulocytes count as a diagnostic marker to discriminate between SIRS and sepsis--a prospective, observational study. BMC Immunol 2013; 14: 8.

[3] Nigro KG, O'Riordan M, Molloy EJ, Walsh MC and Sandhaus LM. Performance of an automated immature granulocyte count as a predictor of neonatal sepsis. Am J Clin Pathol 2005; 123: 618-624.

[4] Baranovskaia IB, Zentsova OA, Samokhina OF, Demidchenko GA and Ten FP. [The new indicators of total blood test in clinical practice]. Klin Lab Diagn 2013; 19-23.

[5] Newman TB, Draper D, Puopolo KM, Wi S and Escobar GJ. Combining immature and total neutrophil counts to predict early onset sepsis in term and late preterm newborns: use of the I/T2. Pediatr Infect Dis J 2014; 33: 798-802.

[6] Soni S, Wadhwa N, Kumar R and Faridi MM. Use of immature-to-total-neutrophil ratio in early neonatal sepsis. Pediatr Infect Dis J 2012; 31: 1101-1102; author reply 1102.

[7] Sauneuf B, Bouffard C, Cornet E, Daubin C, Desmeulles I, Masson R, Seguin A, Valette X, Terzi N, Parienti JJ and du Cheyron D. Immature/total granulocyte ratio: A promising tool to assess the severity and the outcome of post-cardiac arrest syndrome. Resuscitation 2014; 85: 1115-1119.

[8] Ha SO, Park SH, Park SH, Park JS, Huh JW, Lim CM, Koh Y, Hong SB and Jang S. Fraction of immature granulocytes reflects severity but not mortality in sepsis. Scand J Clin Lab Invest 2015; 75: 36-43.

[9] Roehrl MH, Lantz D, Sylvester C and Wang JY. Age-dependent reference ranges for automated assessment of immature granulocytes and clinical significance in an outpatient setting. Arch Pathol Lab Med 2011; 135: 471-477.

[10] PS H and AJ P. Reference Intervals: A user's guide. Washington DC: AACC Press; 2006.

[11] CLSI. C28-A3c: Defining, establishing, and verifying reference intervals in the clinical laboratory. USA: Wayne, PA; 2010.

[12] M B, GM F, G M and J T. Reference values for immature granulocytes in healthy blood donors generated on the Sysmex XE-2100 automated hematology analyser. Sysmex J Int 2004; 14: 5-7.

[13] Gou W. Gestational Hypertension Disease. Beijing: People's Medical Publishing House; 2011.

[14] Murphy K and Weiner J. Use of leukocyte counts in evaluation of early-onset neonatal sepsis. Pediatr Infect Dis J 2012; 31: 16-19.

[15] Cimenti C, Erwa W, Herkner KR, Kasper DC, Muller W and Resch B. The predictive value of immature granulocyte count and immature myeloid information in the diagnosis of neonatal sepsis. Clin Chem Lab Med 2012; 50: 1429-1432.

[16] Farias MG, Garcia MP and Cr C. Evaluation of the immature myeloid information (IMI) by Sysmex XE 210003 hematology analyzer in the identification of blasts of myeloid lineage. Clin Chem Lab Med 2012; 50: 1861-1864.