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Reference intervals of systemic immune-inflammation index, neutrophil-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, and platelet-to-lymphocyte ratio during normal pregnancy in China

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Abstract. – OBJECTIVE: To observe the changes in systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR) during normal pregnancy and establish appropriate reference intervals (RIs) for healthy pregnant women.

PATIENTS AND METHODS: This retrospective study was conducted from March 2018 to February 2019. Blood samples were collected from healthy pregnant and nonpregnant women. The complete blood count (CBC) parameters were measured, and SII, NLR, LMR, and PLR were calculated. RIs were established using the 2.5th and 97.5th percentile of the distribution. Besides, the differences in CBC parameters between three pregnant trimesters and maternal ages were also compared to assess their influences on each indicator.

RESULTS: SII and NLR in three pregnant trimesters increased in pregnant women, and the upper limit of SII and NLR in trimester 2 showed the highest value. On the contrary, LMR decreased in all three pregnant trimesters compared with nonpregnant women, and the values of LMR and PLR showed a gradual downward trend along with the trimesters. Besides, RIs of SII, NLR, LMR, and PLR during different trimesters in different age partitions showed that the values of SII, NLR, and PLR increased with age in a general trend, while LMR showed the opposite trend ($p < 0.05$).

CONCLUSIONS: The SII, NLR, LMR, and PLR showed dynamic changes during pregnant trimesters. RIs of SII, NLR, LMR, and PLR for healthy pregnant women according to pregnant trimesters and maternal age were established and validated in this study, which will promote the standardization of clinical application.

Key Words:
Reference intervals, Systemic immune-inflammation index, Neutrophil-to-lymphocyte ratio, Lymphocyte-to-monocyte ratio, Platelet-to-lymphocyte ratio, Pregnancy.

Introduction

Complete blood count (CBC) parameters have been regarded as rapid, simple, and cost-effective indicators of systemic inflammation and immune balance. The systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR) are all indices calculated from simple complete blood count. Owing to the significant potential values of these ratios reflecting inflammatory status, they are frequently introduced as markers of underlying inflammatory burden in various diseases, including coronavirus disease[1], cardiovascular disease[2], sepsis[3], irritable bowel syndrome[4], inflammatory bowel disease[5], rheumatic diseases[6], pulmonary diseases[7], malignancy[8], and other conditions.

Nevertheless, these ratios play an important role not only in the diseases mentioned above but also in various pregnancy complications. During human pregnancy, a semi-allogeneic fetus implants into the endometrium. Due to the sterile maternal immune reaction against the alloantigen on the fetus or trophoblast, maternal systemic inflammation may occur[9] and, finally, be reflected in these inflammatory indicators. Study[10] shows that a high SII value in early pregnancy may be an

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additional marker for predicting miscarriage and threatened abortion. Besides, SII may be used as an additional indicator for predicting adverse neonatal outcomes[11]. NLR has become a novel potential inflammatory biomarker associated with various adverse obstetric complications, such as gestational diabetes mellitus[12], preeclampsia[13], and intrahepatic cholestasis of pregnancy[14]. The importance of LMR has been emphasized so far and is associated with a higher risk of preterm delivery[15], and decreased LMR may be an unfavorable prognostic factor for clinical outcomes in patients with hyperglycemia during pregnancy[16]. In addition, research has shown that PLR levels are higher in patients with hyperemesis gravidarum than in healthy pregnant patients and that PLR arose with the increase of ketonuria[17]. Not only that, PLR in the first trimester of pregnancy has great predictive value for spontaneous preterm birth[18]. Furthermore, low PLR at delivery was correlated with low placental and birth weights[19]. In correlation analyses, there was a negative correlation between PLR with fetal nutritional status[20].

Considering the important role of the abovementioned inflammatory indicators during pregnancy, the values of SII, NLR, LMR, and PLR must be seriously considered. However, due to geographical location, genotype, lifestyle, ethnicity, and many other factors, the four indicators may differ among regions and ages[21]. Furthermore, in consideration of the continuous studies[21-23] on the RIs of normal healthy adults, the RIs for healthy pregnant women are rarely reported. Therefore, we urgently need to establish specific and reliable RIs for SII, NLR, LMR, and PLR during normal pregnancy.

Patients and Methods

Study Participants

We conducted this retrospective study at Women's Hospital, Zhejiang University School of Medicine. The study was approved by the hospital Ethics Committee, and an informed consent exemption was applied due to anonymous patient records. The study was performed from March 2018 to February 2019. Pregnant women who received routine prenatal care and delivered at Women's Hospital, Zhejiang University School of Medicine, were included in this study. Nonpregnant women were randomly selected as the healthy nonpregnant group for routine physical examinations during the same period as controls. Pregnant women were divided into three groups according to different trimesters of pregnancy: trimester 1 (≤12 weeks), trimester 2 (13-27^{+6} weeks), and trimester 3 (≥28 weeks). Inclusion criteria were as follows:

1) ≥18 years old.

2) The required data is complete.

In addition, pregnant women with any of the following conditions were excluded:

1) Pregnancy-associated diseases: gestational diabetes, preeclampsia, pregnancy-related hypertensive disorder, intrahepatic cholestasis of pregnancy.

2) Diseases with cardiovascular, liver, kidney, gastroenterology, pulmonary, hematology, endocrinology, autoimmune, neurology, infectivity, mental, hypersensitivity reaction, thyroid, acute or chronic inflammation, cancer, obesity [body mass index (BMI) ≥ 28 kg/m^2][24].

3) Recurrent abortion, polycystic ovary syndrome, reproduction system abnormality or malformation.

4) Fetal/neonatal related factors: multiple fetal gestations, preterm delivery, small for gestational age fetus, fetal growth restriction, fetal or neonatal anomaly, intrauterine fetal death.

Sample Collection

Peripheral venous blood samples (2 mL) were collected by vacuum blood collection tube with EDTA-K_2 [Becton Dickinson Company, (Franklin Lakes, NJ, USA)] according to a standard procedure of venous blood collection in three trimesters during pregnancy.

Laboratory Analysis

Hematological measurements were analyzed by the automated hematology analyzers XN-1000 [Sysmex Corporation, Kobe, Japan] in the hospital's clinical laboratory department, which performed internal quality controls every eight hours and calibrated the instrument every six months. To ensure accuracy, we also participated in external quality assessments organized by the national center for clinical laboratories and Zhejiang provincial center for clinical laboratories. All specimens were fully anticoagulated and tested within one hour after collection. During the whole trial process, internal quality control was conducted by Westgard multi-rule quality control method. The inter-and intra-assay coefficients of variation (CVs) of the three levels of quality controls were lower than the professional standard. All operations were carried out in strict accordance with the standard operating procedures of the in-

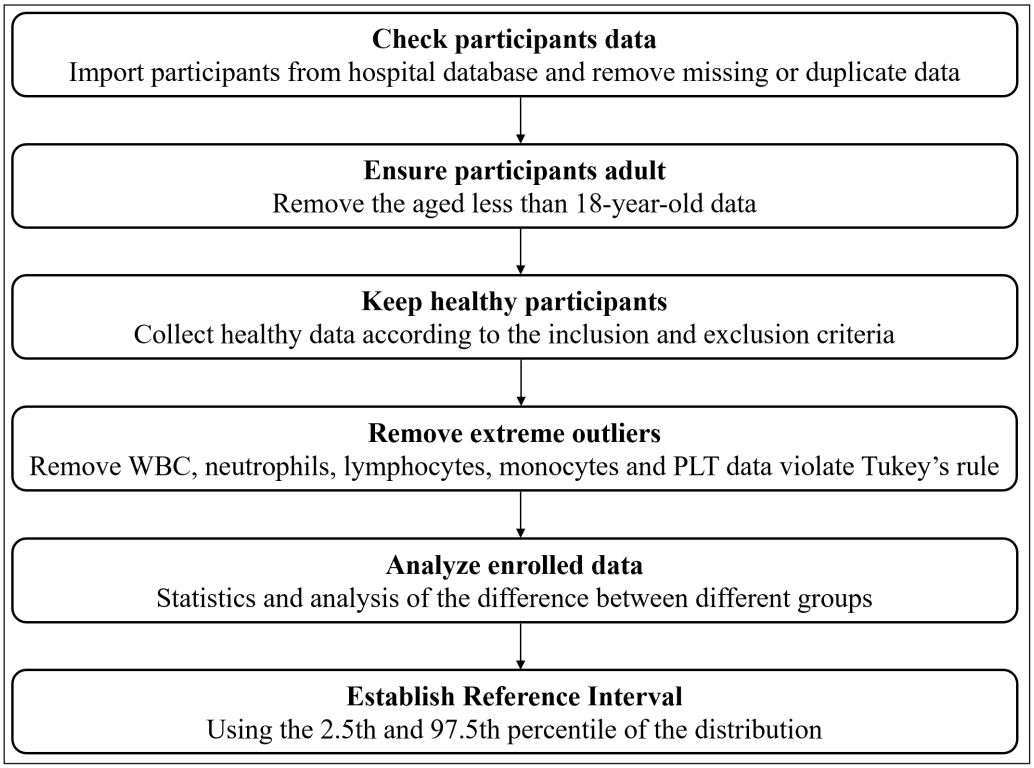

Figure 1. Flowchart presenting the steps of reference interval establishment.

strument, and the quality control met the requirements.

Hematological Analysis

CBC parameters included white blood cell (WBC), red blood cells (RBC), hemoglobin (Hb), platelet (PLT), neutrophils (Neu), lymphocytes (Lymph), monocytes (Mono), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), mean platelet volume (MPV), platelet crit (PCT), and platelet distribution width (PDW). In addition, another four parameters, which were SII, NLR, LMR, and PLR. They were calculated, according to the formulas reported in literature[25], as follows: SII = Platelet count x (Neutrophil count/Lymphocyte count); NLR = Neutrophil count/Lymphocyte count; LMR = Lymphocyte count/Monocyte count; PLR = Platelet count/Lymphocyte count. Besides we recorded pre-pregnancy BMI for each subject. The detailed procedures of the study are shown in Figure 1.

Statistical Analysis

The statistical analyses were carried out with SPSS 20.0 software (IBM Corp., Armonk, NY, USA). The normality of the data was evaluated with the Kolmogorov-Smirnov test. The non-parametric Mann-Whitney U test was used to compare the differences in the two groups, and the Kruskal-Wallis H test was used for multiple groups. Categorical variables were expressed in the form of counts and % percentages. Data were displayed as medians, interquartile range (IQR), or 2.5^{th} and 97.5^{th} percentile. Extreme outliers were removed in this study according to Tukey's rule in CLSI C28-A3[26]. The graphs were generated by using Graph Pad Prism 5.0. $p < 0.05$ was considered statistically significant.

Results

Characteristics of Participants

A total of 4,811 pregnant women who met the criteria were enrolled from March 2018 to February 2019. Out of them, 250 women received routine prenatal care in trimester 1 (median age: 30 years), 4,643 in trimester 2 (median age: 30 years), and 4,668 in trimester 3 (median age: 30 years). There was no significant difference in age between three trimesters and healthy nonpregnant women (data are not shown). The characteristics

Table I. Basic sample characteristics of healthy pregnant women.

Groups	Categories	Subject tested
N	T1	250 (2.61%)
	T2	4,643 (48.56%)
	T3	4,668 (48.83%)
Age	T1	30.00 (27.75, 33.00)
	T2	30.00 (28.00, 33.00)
	T3	30.00 (28.00, 33.00)
BMI	<18.5	1,950 (20.40%)
	18.5-23.9	6,872 (71.88%)
	24.0-27.9	739 (7.72%)

Data are shown as number (proportion) or median interquartile range). T1: trimester 1; T2: trimester 2; T3: trimester 3; BMI: body mass index.

of inclusive pregnant women are shown in Table I.

Comparison of Hematological Parameters Between Healthy Pregnant and Nonpregnant Women

We compared the levels of hematological parameters of the participants between healthy pregnant and nonpregnant women. Results presented in Table II clearly showed the median values of WBC, Neu, Mono, MCV, MCH, MCHC, RDW, MPV, and PDW in the pregnant women group had significantly higher levels compared with nonpregnant women. In contrast, the levels of RBC, Hb, PLT, Lymph, HCT, and PCT decreased in pregnant women ($p < 0.05$).

Reference Intervals of SII, NLR, LMR, and PLR

The data of SII, NLR, LMR, and PLR showed skewed distribution according to Kolmogorov-Smirnov test (data are not shown) (Figure 2). To explore the impact of different trimesters during pregnancy on four parameters, the non-parametric Kruskal-Wallis H test was used to compare the difference among three pregnant trimesters. Results presented in Figure 3 show that SII and NLR in three trimesters were all increased in pregnant women, and the upper limit of SII and NLR in trimester 2 showed the highest value; while LMR decreased compared with nonpregnant women group and the values of LMR and PLR showed a gradual downward trend along with the trimesters. The detailed numerical representation is shown in Table III. To explore the influence of maternal age on SII, NLR, LMR, and PLR, the RIs of four indicators during different trimesters of pregnancy in different maternal age partitions were compared. Our result showed that the values of SII, NLR, and PLR showed a trend of increasing with age in general, while LMR shows the opposite trend ($p < 0.05$), see Figure 4 and Table IV.

Discussion

Table II. Comparison of hematological parameters between healthy pregnant and nonpregnant women.

Variables	Pregnancy	Nonpregnancy	Z	p
Total number	9,561	4,687		
WBC ($\times 10^9$/L)	8.9	5.7	-81.766	0.000
RBC ($\times 10^{12}$/L)	3.79	4.43	-65.891	0.000
Hb (g/L)	116	132	-68.432	0.000
PLT ($\times 10^9$/L)	194	233	-42.095	0.000
Neu ($\times 10^9$/L)	6.6	3.2	-89.896	0.000
Lymph ($\times 10^9$/L)	1.6	2.0	-49.190	0.000
Mono ($\times 10^9$/L)	0.6	0.4	-81.624	0.000
HCT	0.350	0.397	-72.790	0.000
MCV (fL)	92.2	89.3	-39.498	0.000
MCH (pg)	30.9	29.9	-37.102	0.000
MCHC (g/L)	335	333	-8.748	0.000
RDW (%)	13.3	12.5	-49.509	0.000
MPV (fL)	10.1	10.0	-3.080	0.002
PCT	0.19	0.23	-91.186	0.000
PDW (%)	12.8	12.3	-10.599	0.000

Data are shown as number or median. WBC, white blood cell; RBC, red blood cells; Hb, hemoglobin; PLT, platelet; Neu, neutrophils; Lymph, lymphocytes; Mono, monocytes; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, RBC distribution width; MPV, mean platelet volume; PCT, platelet crit; PDW, platelet distribution width.

Table III. Reference intervals for SII, NLR, LMR, and PLR during different trimesters of pregnancy.

Variable	Nonpregnancy	Trimester 1	Trimester 2	Trimester 3	χ^2	*p*
n	4,687	250	4,643	4,668		
SII	374 (153, 832)	754 (302, 1603)	868 (431, 1669)	718 (338, 1529)	6,539.618	0.000
NLR	1.60 (0.80, 3.36)	3.53 (1.76, 6.73)	4.36 (2.52, 7.55)	3.86 (2.12, 7.16)	8,641.479	0.000
LMR	5.25 (2.57, 10.00)	3.33 (1.82, 7.00)	3.00 (1.75, 5.67)	2.67 (1.50, 4.75)	6,231.826	0.000
PLR	118 (67, 206)	136 (73, 235)	126 (73, 215)	116 (64, 210)	262.313	0.000

Data are presented as number or median (2.5^{th} and 97.5^{th} percentile). SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

With increasing attention to maternal health management, CBC parameters are routinely used to monitor the health status during pregnancy worldwide since it is reliable, simple, fast, and cost-effective. During pregnancy, changes occur and can be observed in CBC. Some parameters decreased, such as Hb, RBC, and PLT, and others increased, like WBC, neutrophils, and monocytes.

Anemia during pregnancy is the most common hematological problem doctors concerned about in the clinic. According to the statistics, anemia

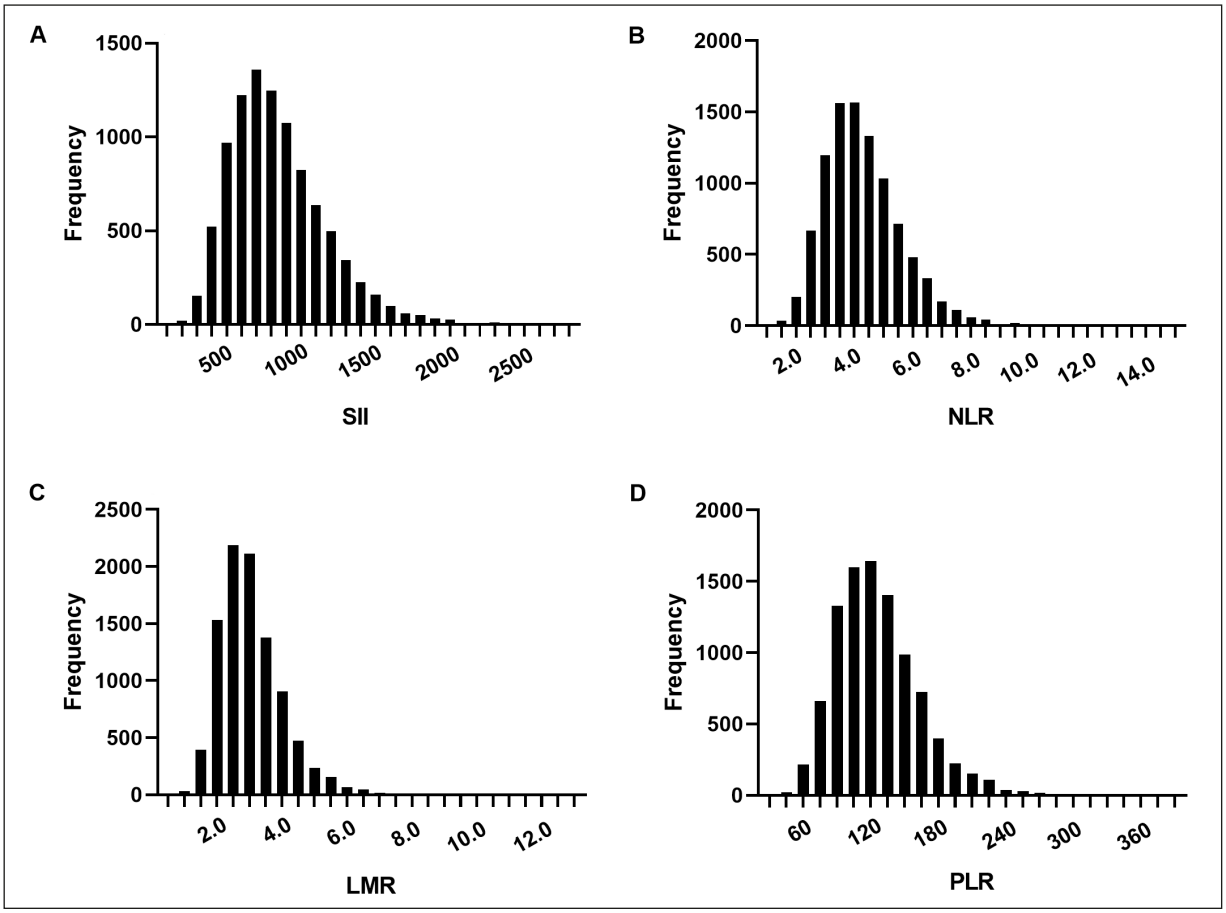

Figure 2. Distribution histogram of SII, NLR, LMR and PLR. **A**, The data of SII showed skewed distribution according to Kolmogorov-Smirnov test. **B**, The data of NLR showed skewed distribution according to Kolmogorov-Smirnov test. **C**, The data of LMR showed skewed distribution according to Kolmogorov-Smirnov test. **D**, The data of PLR showed skewed distribution according to Kolmogorov-Smirnov test. SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

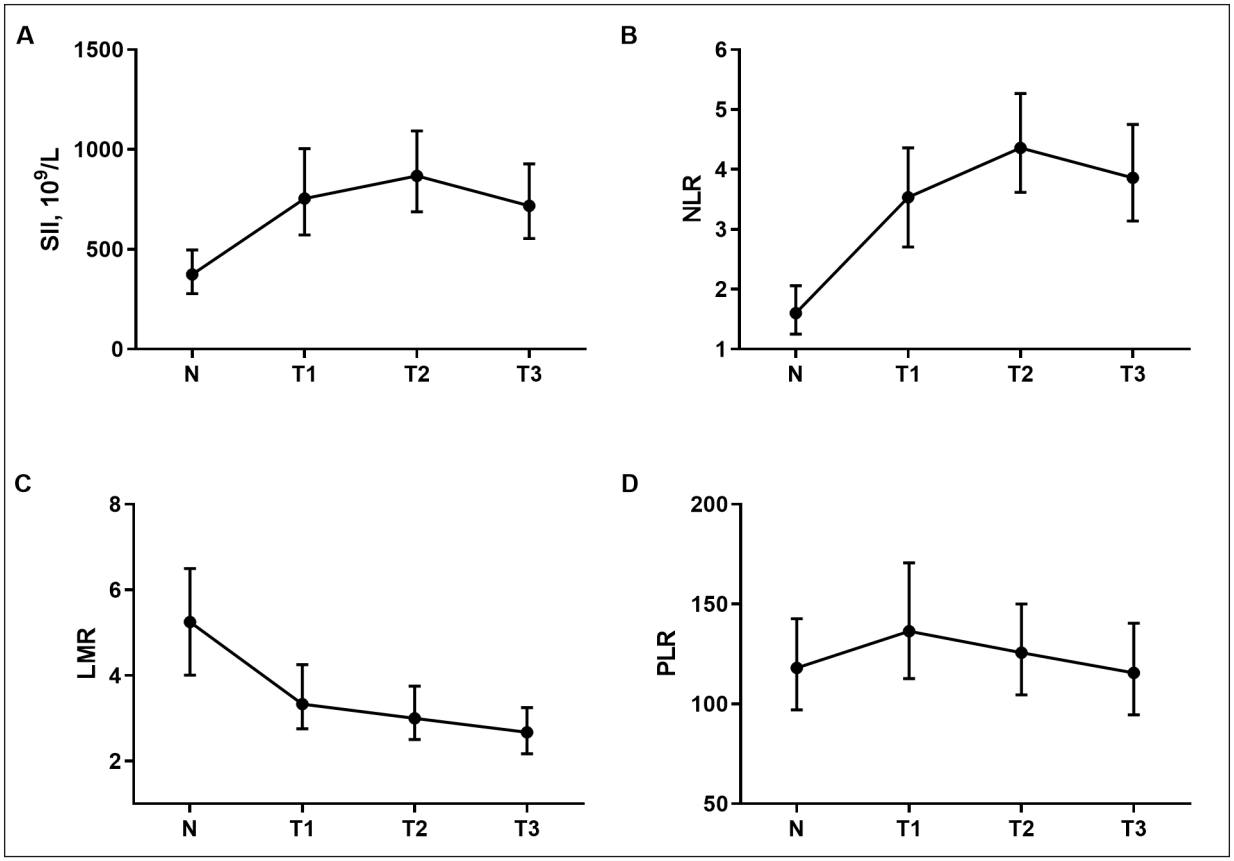

Figure 3. SII (**A**), NLR (**B**), LMR (**C**) and PLR (**D**) during different trimesters of pregnancy. The dots reflect the median, and the bars reflect the 25th and 75th percentiles. N: normal nonpregnant women; T1: trimester 1; T2: trimester 2; T3: trimester 3; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

affects approximately 40% of pregnant women worldwide[27]. What's more serious is that anemia in pregnancy has been associated with higher maternal and perinatal death rates, preterm birth, preeclampsia, low birth weight, small-for-gestational-age and cesarean delivery[28]. Our study found that RBC and Hb declined during pregnancy compared with nonpregnant women, similar to

Table IV. Reference intervals for SII, NLR, LMR, and PLR during different trimesters of pregnancy in different age partitions.

Variable	Nonpregnancy	Trimester 1	Trimester 2	Trimester 3	χ^2	*p*
SII	T1	737 (256, 2,012)	740 (337, 1,561)	866 (716, 1,142)[a]	3.837	0.147
	T2	867 (424, 1,653)	851 (436, 1,625)	903 (440, 1,858)	11.785	0.003
	T3	719 (334, 1,509)	703 (330, 1,519)	773 (355, 1,585)	14.469	0.001
NLR	T1	3.47 (1.55, 8.05)	3.43 (1.80, 6.47)	4.08 (3.26, 5.08)[a]	5.598	0.061
	T2	4.33 (2.44, 7.50)	4.32 (2.54, 7.33)	4.57 (2.56, 8.36)	19.511	0.000
	T3	3.81 (2.11, 7.02)	3.82 (2.10, 7.08)	4.06 (2.19, 7.57)	19.056	0.000
LMR	T1	3.45 (2.00, 7.33)	3.50 (2.06, 6.73)	2.75 (2.17, 3.32)[a]	14.889	0.001
	T2	3.00 (1.76, 5.67)	3.00 (1.75, 5.67)	2.87 (1.67, 5.50)	26.983	0.000
	T3	2.67 (1.50, 4.69)	2.67 (1.50, 5.00)	2.50 (1.38, 4.65)	15.175	0.001
PLR	T1	1323 (75, 238)	137 (69, 224)	155 (131, 215)[a]	8.551	0.014
	T2	123 (71, 210)	125 (74, 211)	134 (75, 234)	47.170	0.000
	T3	114 (63, 206)	114 (64, 200)	122 (67, 230)	44.614	0.000

Data are presented as median (2.5th and 97.5th percentile); [a]: data are presented as median (interquartile range) due to small sample size of this group. T1: trimester 1; T2: trimester 2; T3: trimester 3; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

previous studies[28,29]. During pregnancy, estrogen and progesterone secreted by the placenta lead the kidney to release renin, thus causing sodium retention[30]. The Hb and RBC levels changed may be related to the above-mentioned hormonal changes, thereby increasing plasma dilution[29]. In addition, research exhibited that plasma volume could increase by 25%-80% of pre-pregnancy volumes physiologically from trimester 2 to trimester 3 of pregnancy[29]. These changes are all for meeting the demands of new vascular bed and preventing blood loss during delivery[31].

Several pieces of research have shown that platelet counts during pregnancy are lower than those in nonpregnant women. About 6%-11% of healthy pregnant women have experienced decreased platelet counts (<150 x 10^9/L) during pregnancy[32,33]. Our study showed a significant decrease in platelet counts during pregnancy, consistent with other findings[32-34]. The significant decrease in platelet count may be related to various physiological changes during pregnancy, such as hemodilution, increased consumption caused by splenomegaly, and increased platelet circulation in the placenta[34].

Pregnancy is often described as a regulated inflammatory state[35], characterized by an increasing total number of circulating WBC[36], mainly due to the expansion of neutrophils and monocytes[37]. Study[38] showed that the reference upper limit of WBC in pregnancy increased by 36%, primarily driven by the increase in neutrophils (55% higher than the reference upper limit). In addition, the upper limit of lymphocytes decreased by 36%, while monocytes increased by 38%[38]. In our study, WBC increased significantly during

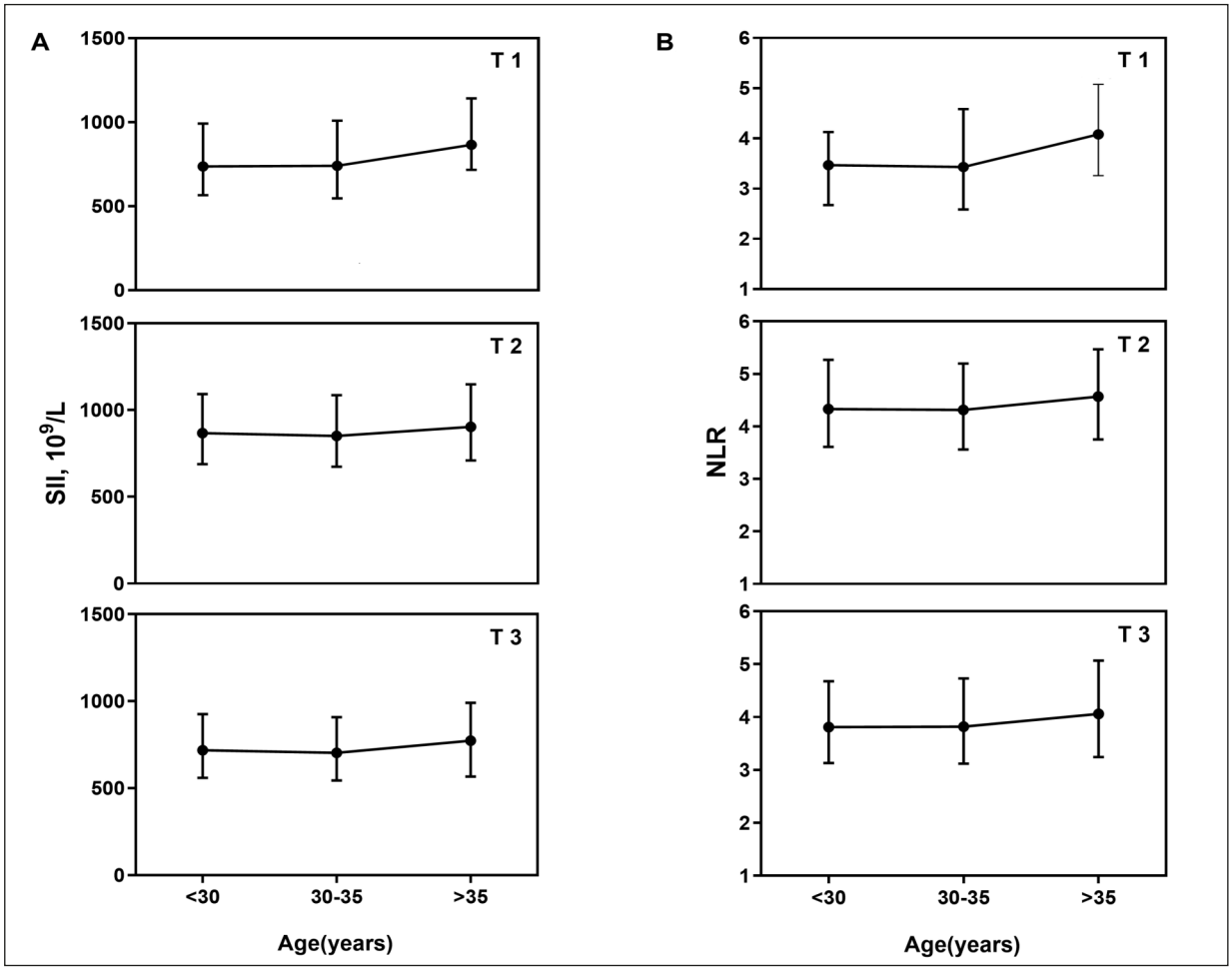

Figure 4. SII (**A**), NLR (**B**), LMR (**C**) and PLR (**D**) during different trimesters of pregnancy in different age partitions. The dots reflect the median, and the bars reflect the 25th and 75th percentiles. T1: trimester 1; T2: trimester 2; T3: trimester 3; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

Figure continued

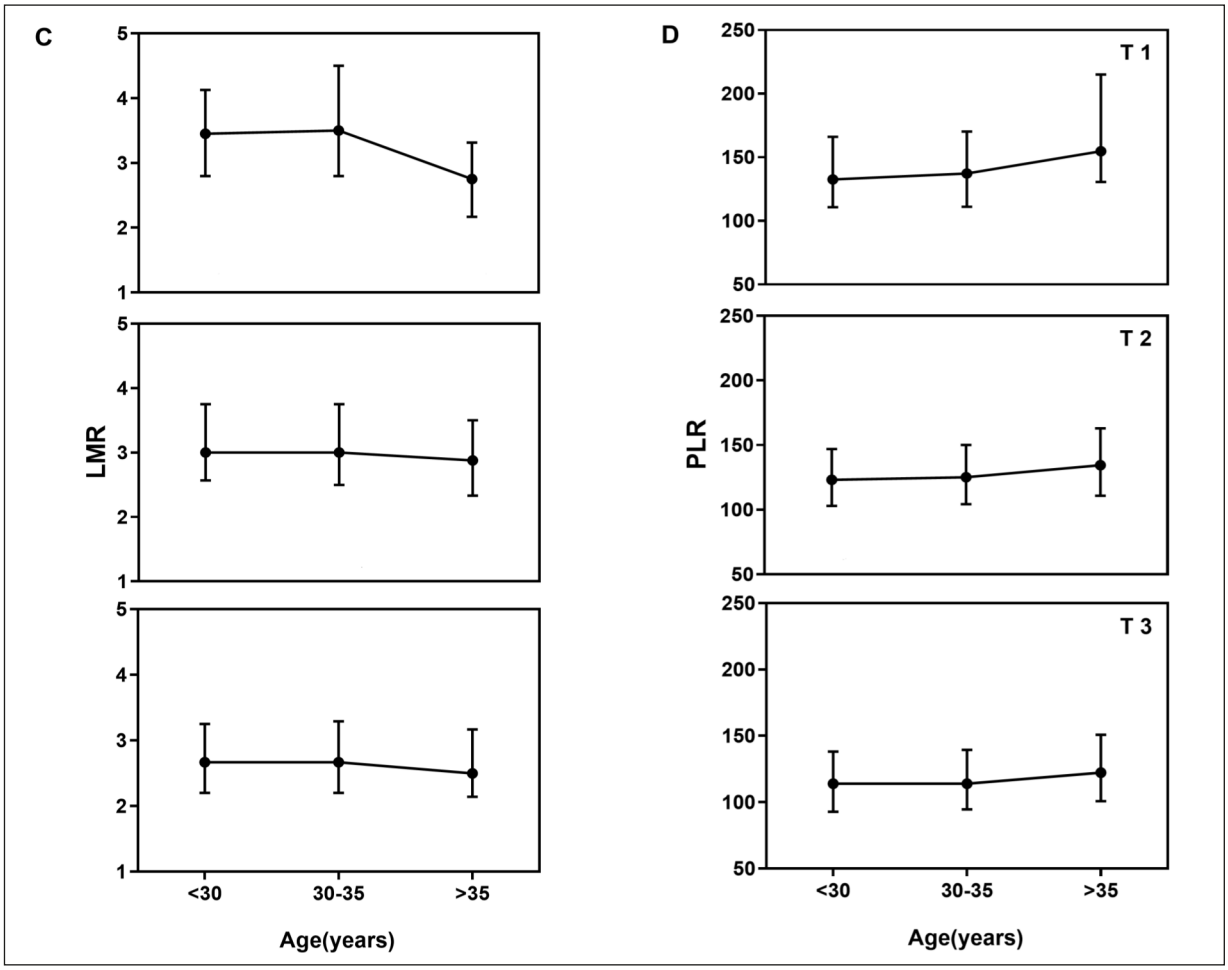

Figure 4. ***(Continued).*** SII (**A**), NLR (**B**), LMR (**C**) and PLR (**D**) during different trimesters of pregnancy in different age partitions. The dots reflect the median, and the bars reflect the 25th and 75th percentiles. T1: trimester 1; T2: trimester 2; T3: trimester 3; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

pregnancy, which is consistent with previous findings[31,39], and the median of WBC in healthy nonpregnant females was 5.7×10^9/L, while the value of pregnancy women far exceeds this figure, with a median of 8.9×10^9/L. Furthermore, the rise of WBC was primarily due to the expansion of neutrophils, and the neutrophils of pregnant women in our study were more than twice that of the nonpregnant group (6.6×10^9/L *vs.* 3.2×10^9/L). The reasons for this phenomenon of the increase in WBC and neutrophils accompanied by the decrease of lymphocytes may be as follows: pregnancy is regarded as a physiological inflammatory process, and the maternal immune system undergoes significant changes during pregnancy. These changes are essential for maintaining pregnancy and preventing fetal semi-allogeneic rejection[40]. Moreover, neutrophils and monocytes are phagocytes that can induce inflammatory activation and enhance resistance to extracellular infections. Additionally, the total lymphocyte count continues to decrease during pregnancy, mainly due to the reduction of circulating cytotoxic lymphocytes capable of directly recognizing and targeting fetal antigens[41]. As mentioned above, changes in maternal peripheral blood indicate that normal pregnancy is associated with a continuous systemic inflammatory response. One of the earliest pregnancy-related changes in peripheral blood is increased WBC count, especially neutrophils.

With the in-depth study on inflammatory markers, there is growing interest in research aimed at better understanding the disease status or predicting the prognosis of patients with simple blood inflammatory markers[21]. SII, NLR, LMR, and PLR

are new biomarkers of systemic inflammation, closely related to immune response, and can avoid the difference in hematopoietic function between individuals. A growing number of studies[10-17,19,20] show that these inflammatory indicators are related to pregnancy complications, and these markers have been widely used in clinical practice. However, they will lose clinical value without appropriate RIs of SII, NLR, LMR, and PLR in clinical application. Therefore, we established appropriate RIs according to trimesters and maternal age, which can provide an essential basis for the diagnosis, progression, and prognosis of clinical pregnancy-related diseases.

SII and NLR are ratio indexes calculated by inflammatory activators (neutrophils/platelets) and inflammatory regulators (lymphocytes), which are considered effective indicators of systemic inflammation and immune balance[9]. In our study, SII and NLR in three trimesters were all increased in the pregnant women group, and the values of SII and NLR reached their highest in trimester 2. Dockree et al[38] used highly sensitive, modern statistical techniques through a total of 80,637 results from pregnant women to define reference intervals for all WBC subtypes, with a subgroup analysis monthly during pregnancy. The result showed little variation between 8-40 weeks in total WBC or any cell subtype, such as neutrophils and lymphocytes. However, the value of neutrophils in trimester 2 was noticeably higher than in trimester 1 and 3. At the same time, lymphocyte data was stable throughout pregnancy, accompanied by increased monocytes with advancing gestation simultaneously, resulting in the increased values of SII and NLR in trimester 2 and a gradual downward trend along with the trimester of LMR, which was consistent with our conclusions. Ushida et al[34] conducted a multicenter retrospective study analyzing clinical data on platelet counts in women by selecting 28,073 pregnant women and 28,073 non-pregnant women after 1:1 matching based on three factors (age, weight, and height) for analysis. After statistical analysis of data, it was found that the platelet counts in trimester 1 were significantly lower than that in the non-pregnant state. Furthermore, the platelet counts declined throughout pregnancy until postnatal day 1. However, lymphocyte data were stable throughout pregnancy, and monocytes increased marginally with gestational age[38]. Therefore, PLR in pregnancy showed a significant downward trend with increased gestational weeks. This is also consistent with our results.

Besides, to explore the influence of maternal age on SII, NLR, LMR, and PLR, RIs for four indicators during different trimesters of pregnancy in different age partitions were analyzed. Our result showed that the values of SII, NLR, and PLR increased with age in a general trend, while LMR shows the opposite trend ($p < 0.05$). It is consistent with previous studies[21,23] on normal adult female. Fei et al[42] point out that the value of SII, NLR, and PLR increased with age in female groups between 18 and 50 years old. Wang et al[21] stated in their article that the RIs upper limit of LMR and PLR in females vary with age, which was related to the exuberance of sex hormones represented by estrogen and progesterone. These sex hormones could increase the aggregation of monocytes and promote the formation and release of PLT precursors.

To the best of our knowledge, the present study appears to be the first to report RIs of SII, NLR, LMR, and PLR in healthy pregnant women. Although our study has established the RIs of four indicators based on big data of 9,561, some limitations of our study must be considered.

Limitations

First, we have a small number of CBC data in trimester 1, especially the CBC data of women with advanced maternal age. The main reason is that most of the early prenatal examinations of pregnant women are completed in community hospitals. Second, this study lacks abundant data to eliminate the influencing factors of these indicators, such as BMI, at each trimester during pregnancy. Consequently, we will conduct further research and collect more data in the future. Third, the analysis was performed on a single center, which may make the results unrepresentative.

Conclusions

We have established the reference intervals of SII, NLR, LMR, and PLR for healthy Chinese pregnant women of different trimesters and maternal ages. These results may help assess pregnant women's health status, help predict disease progression in various clinical practices, and help better regulate the application of SII, NLR, LMR, and PLR in clinical practice. Besides, pregnant trimesters and maternal age were proposed to be taken into consideration when these parameters were applied in clinical.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Data Availability
The data are available from the corresponding author upon reasonable request.

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Authors' Contribution
All authors contributed to data collection, analysis, and participated in drafting or revising articles.

Ethics Approval
This research was reviewed and approved by the Ethics Committee of Women's Hospital, Zhejiang University School of Medicine (with the approval number: IRB-20220218-R).

Informed Consent
The Ethics Committee of Women's Hospital, Zhejiang University School of Medicine approved the informed consent exemption due to anonymous patient records.

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