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Obstetrics; *Maternal Fetal Medicine and Perinatology*

Hemogram-Derived Inflammatory and Oxidative Stress-Related Indices in Second-Trimester Pregnancy Loss

Omer Faruk BAYRAKTAR[1], Ozlem YUKSEL AYBEK[1], Hakan GURASLAN[1]

Istanbul, Türkiye

ABSTRACT

OBJECTIVES: To investigate hemogram-derived inflammatory indices in women with spontaneous second-trimester pregnancy loss and to compare these parameters with those of the women who achieved term delivery.

STUDY DESIGN: This retrospective case-control study included women aged 18-35 years. There were two groups: women who experienced spontaneous second-trimester pregnancy loss between 14+0 and 23+6 gestational weeks (Group A), and women who achieved term delivery (≥37 weeks) (Group B). We excluded pregnancies terminated for medical or fetal indications, multiple gestations, chronic systemic or blood (hematological) diseases, active infection, and pregnancy complications. Complete blood count (CBC) parameters, which are laboratory measurements of different types of blood cells-such as white blood cells (WBCs), red blood cells, and platelets-were obtained during the second trimester. We calculated hemogram-derived indices, including the neutrophil-to-lymphocyte ratio (NLR), the ratio of neutrophils to lymphocytes) and the systemic immune-inflammation index (SII, calculated as platelet count multiplied by neutrophil count, then divided by lymphocyte count). Multivariable logistic regression models-statistical analyses that account for several influencing factors at once-were adjusted for maternal age, parity (number of previous pregnancies), previous abortion history, and gestational age at sampling. An additional adjustment for total white blood cell (WBC) count was done in hierarchical analyses. Receiver operating characteristic (ROC) curve analysis, a statistical method to evaluate how well a test distinguishes between groups, was performed to assess discriminative performance.

RESULTS: A total of 439 women were included-218 with spontaneous second-trimester pregnancy loss and 221 with term delivery. Women in the pregnancy loss group were older and more often had a history of previous abortion ($p<0.001$).WBC count, NLR, and SII values were significantly higher in the pregnancy loss group (all $p<0.001$). In a multivariable analysis adjusted for maternal age, parity (number of pregnancies), previous abortion history, and gestational age at sampling, both NLR and SII were significantly associated with second-trimester pregnancy loss. However, after additional adjustment for total WBC, these associations were reduced (attenuated). ROC (receiver operating characteristic) curve analysis, which assesses how well a test differentiates between groups, showed limited discriminative performance with area under the curve (AUC) values ranging from 0.61 to 0.63. SII had the highest AUC.

CONCLUSION: Hemogram-derived inflammatory indices, particularly NLR and SII, are elevated in women with spontaneous second-trimester pregnancy loss. These indices are associated with this outcome after adjustment for key clinical factors. However, their discriminative performance is modest. These parameters are readily available. They should be considered adjunctive markers rather than standalone predictive tools in this clinically heterogeneous setting.

Keywords: Inflammation; Lymphocytes; Neutrophils; Pregnancy; Second trimester

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Introduction

Second-trimester pregnancy loss, usually defined as loss between 14 and 24 weeks of gestation, represents a clinically distinct entity from first-trimester miscarriage (1,2). Although

[1] *Department of Obstetrics and Gynecology, Bagcilar Training and Research Hospital Dr. Sadik Ahmet Street 34200 Bagcilar Istanbul, Türkiye*

Address of Correspondence: *Omer Faruk Bayraktar*
Department of Obstetrics and Gynecology
Bagcilar Training and Research Hospital
Dr. Sadik Ahmet Street, 34200 Bagcilar
Istanbul, Türkiye
ofbayraktar@icloud.com

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ORCID IDs of the authors: *OFB: 0009-0002-2142-362X*
OYA: 0000-0003-0332-496X *HG: 0000-0003-3033-985X*

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less frequent, it is associated with considerable maternal morbidity and involves multiple mechanisms, including cervical insufficiency, placental dysfunction, subclinical infection, inflammatory activation, and maternal systemic factors (1,2). Understanding these mechanisms is key to improving outcomes.

Dysregulated maternal immune responses and inflammatory activation have increasingly been recognized as important contributors to abnormal placentation and pregnancy loss (3,4). Notably, oxidative stress-caused by an imbalance between reactive oxygen species and antioxidant defenses-can damage the endothelium, induce apoptosis, and strengthen inflammatory pathways, all of which may result in placental dysfunction (3,4). Given the close link between inflammation and oxidative stress, systemic inflammatory markers may provide an indirect indication of underlying oxidative imbalance. However, direct biomarkers of oxidative stress are not always available in clinical practice, making reliance on these indirect measures more relevant.

In this context, complete blood count (CBC)-derived indices have emerged as inexpensive, accessible measures of systemic inflammatory activity (5). The neutrophil-to-lymphocyte ratio (NLR) and composite indices, such as the systemic immune-inflammation index (SII), combine several hematological factors to provide a broader reflection of the immune-inflammatory state (5-7). Several studies have found associations between high NLR and adverse pregnancy outcomes, including early and recurrent pregnancy loss (5,6), while SII has been suggested as a more comprehensive marker of inflammatory activation (7). Nevertheless, the utility of these indices may differ across various forms of miscarriage, and current literature remains inconsistent.

Despite accumulating interest, previous research has predominantly focused on first-trimester or missed miscarriage populations, leaving the evidence base for spontaneous second-trimester pregnancy loss relatively sparse (1,8). Moreover, it remains unclear whether hemogram-derived indices add prognostic value beyond total leukocyte counts, or if their discriminative performance is sufficient for clinical use in this context.

Therefore, the aim of this study was to evaluate hemogram-derived inflammatory indices, particularly NLR and SII, in women with spontaneous second-trimester pregnancy loss compared with those who delivered at term. Additionally, we assessed their independent associations using multivariable logistic regression models that included gestational age at sampling and evaluated their discriminative performance using receiver operating characteristic (ROC) curve analysis.

Material and Method

Study Design and Population: This study was designed as a retrospective case–control study and conducted at a tertiary referral center. Medical records of pregnant women followed at our institution were reviewed.

Patient Selection: Women aged 18-35 years were included and divided into two groups according to pregnancy outcome.

Group A consisted of women who experienced spontaneous pregnancy loss between 14+0 and 23+6 gestational weeks. We excluded pregnancies terminated for medical or fetal indications. Group B included women in the same age range who continued their pregnancies to ≥37 gestational weeks and delivered at term.

Women with multiple pregnancies, chronic systemic diseases (including hypertension, diabetes mellitus, and autoimmune disorders), hematological diseases, active infection, or pregnancy complications such as preeclampsia or HELLP syndrome were excluded. Only patients with complete hemogram data from the second trimester and adequate electronic medical records were included.

Data Collection: We retrieved data for patients managed between January 2017 and June 2025 from electronic medical records. Demographic and obstetric characteristics, such as maternal age, parity, and previous abortion history, were obtained.

All complete blood count parameters were obtained from routine clinical visits during the second trimester. Blood samples were collected at variable time points within the second trimester for both groups.

In the pregnancy loss group, we required at least a 2-week interval between CBC sampling and pregnancy loss. This minimized potential reverse-causation bias. Patients who did not meet this criterion were excluded.

We recorded gestational age at the time of CBC sampling. This information was incorporated into multivariable analyses to adjust for physiological hematological variation throughout the second trimester.

Hematological Parameters: Recorded complete blood count parameters included white blood cell (WBC) count, hemoglobin, neutrophil count, lymphocyte count, platelet count, red cell distribution width (RDW), and mean platelet volume (MPV). The neutrophil-to-lymphocyte ratio (NLR) was calculated as the absolute neutrophil count divided by the absolute lymphocyte count. The systemic immune-inflammation index (SII) was calculated as platelet count multiplied by neutrophil count and divided by lymphocyte count.

Ethical Approval: This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical ap-

proval was obtained from the Bagcılar Training and Research Hospital Non-Interventional Clinical Research Ethics Committee (Approval No: 2025/08/08/071; Date: 07 August 2025).

As this was a retrospective study based on fully anonymized patient records, and no identifiable personal data were accessed, the ethics committee waived the requirement for informed consent.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (version 26.0; IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro-Wilk test and are presented as median (interquartile range, IQR). Group comparisons were performed using the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. Multivariable logistic regression analyses were performed to evaluate the independent association of NLR and SII with second-trimester pregnancy loss.

Two hierarchical models were constructed for each inflammatory index:

• Model 1: Adjusted for maternal age, parity, previous abortion history, and gestational age at CBC sampling.
• Model 2: Model 1 + total WBC count.

This approach was used to assess potential biological overlap between total leukocyte burden and derived inflammatory indices.

Variance inflation factors (VIF) were calculated to evaluate multicollinearity among covariates.

Because SII was analyzed as a continuous variable, odds ratios were additionally recalculated per 100-unit increase to facilitate clinical interpretability.

Receiver operating characteristic (ROC) curve analysis was performed to assess discriminative performance. Area under the curve (AUC) values with 95% confidence intervals were calculated. Optimal cut-off values were determined using the Youden index, and corresponding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were reported.

Sensitivity analyses were conducted after excluding extreme hematological values defined as measurements below the 1st percentile or above the 99th percentile for WBC, NLR, and SII.

A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 439 pregnant women were included in the analysis: 218 with spontaneous second-trimester pregnancy loss and 221 with term delivery.

Baseline clinical and hematological characteristics are presented in Table I. Women in the pregnancy loss group were significantly older compared with controls (median 29 vs. 27 years, p<0.001). Gestational age at the time of CBC sampling differed between groups, with lower median gestational weeks in the loss group (14 vs. 18 weeks, p<0.001) (Table I).

Among hematological parameters, WBC, NLR, and SII values were significantly higher in women with second-trimester pregnancy loss (all p<0.001). PLR did not differ significantly between groups (p=0.082). RDW showed a borderline association (p=0.058), whereas MPV was not significantly different (p=0.988). Previous abortion history was more frequent in the loss group (35.3% vs. 18.1%, p<0.001) (Table I).

To account for potential physiological variation across the second trimester, gestational age at the CBC sampling was included as a covariate in all multivariable models.

In Model 1 (adjusted for age, parity, previous abortion history, and gestational age at CBC), higher NLR values remained independently associated with second-trimester pregnancy loss (OR 1.27, 95% CI 1.13-1.42, p<0.001) (Table II).

Table I: *Baseline clinical and hematological characteristics*

Variable	Term (n=221)	Loss (n=218)	p
Age (years)	27.00 (23.00-30.00)	29.00 (24.00-34.00)	<0.001
Gestational age at CBC (weeks)	18.00 (16.00-20.00)	14.00 (12.00-15.00)	<0.001
WBC (×10³/μL)	9.70 (8.40-11.30)	11.15 (8.70-14.50)	<0.001
NLR	3.92 (3.18-4.77)	4.67 (3.35-6.81)	<0.001
PLR	130.00 (106.84-160.83)	137.67 (107.13-176.61)	0.082
SII	914.36 (699.64-1178.45)	1142.61 (793.29-1808.98)	<0.001
RDW (%)	14.00 (13.30-15.30)	13.90 (13.10-14.90)	0.058
MPV (fL)	10.20 (9.30-11.10)	10.20 (9.50-10.90)	0.988
Parity ≥1	154 (69.7%)	159 (72.9%)	0.517
Previous abortion history	40 (18.1%)	77 (35.3%)	<0.001

Values are presented as median (IQR) or n (%). CBC: Complete blood count. WBC: White blood cell. NLR: Neutrophil-to-lymphocyte ratio. RDW: Rd cell distribution width. MPV: Mean platelet volume

Table II. *Multivariable logistic regression analysis including NLR*

Model 1 Variable	**Model 1 OR**	**Model 1 95% CI**	**Model 1 p**
Age (per year)	1.08	1.01-1.14	0.014
Parity (≥1)	0.55	0.28-1.06	0.075
Previous abortion history	2.25	1.20-4.22	0.012
Gestational age at CBC (per week)	0.51	0.45-0.58	<0.001
NLR	1.27	1.13-1.42	<0.001
Model 2 Variable	**Model 2 OR**	**Model 2 95% CI**	**Model 2 p**
Age (per year)	1.09	1.03-1.16	0.006
Parity (≥1)	0.59	0.29-1.18	0.134
Previous abortion history	2.11	1.10-4.02	0.024
Gestational age at CBC (per week)	0.50	0.43-0.57	<0.001
WBC	1.25	1.13-1.39	<0.001
NLR	1.11	0.98-1.25	0.104

Model 1 (Clinical covariates + Gestational age at CBC: Complete blood count + NLR: Neutrophil-to-lymphocyte ratio), Model 2 (Model 1 + WBC: White blood cell)

In Model 2 (Model 1 + WBC), the association between NLR and pregnancy loss was attenuated and no longer statistically significant (OR 1.11, 95% CI 0.98-1.25, p=0.104), whereas WBC remained independently associated with the outcome (Table II).

Similarly, in Model 1, SII was independently associated with pregnancy loss (OR 1.00052, 95% CI 1.00021-1.00083, p=0.001). Because SII was analyzed as a continuous variable, the unit-based odds ratio appears numerically small. When recalculated per 100-unit increase to enhance interpretability, each 100-unit increment in SII was associated with an approximately 5.3% increase in the odds of second-trimester pregnancy loss (OR=1.053; Table III).

In Model 2 (Model 1 + WBC), the association between SII and pregnancy loss was attenuated and became statistically borderline (p = 0.067; Table III).

Variance inflation factor (VIF) analysis demonstrated no significant multicollinearity among covariates in either NLR or SII models, with all VIF values below 2.

Receiver operating characteristic (ROC) analysis demonstrated limited discriminative performance of hemogram-derived indices (Table IV, Figure 1).

Table III: *Multivariable logistic regression analysis including SII*

Model 1 Variable	**Model 1 OR**	**Model 1 95% CI**	**Model 1 p**
Age (per year)	1.08	1.02-1.15	0.011
Parity (≥1)	0.57	0.29-1.12	0.102
Previous abortion history	2.18	1.16-4.09	0.016
Gestational age at CBC (per week)	0.52	0.46-0.59	<0.001
SII (per unit)	1.00052	1.00021-1.00083	0.001
Model 2 Variable	**Model 2 OR**	**Model 2 95% CI**	**Model 2 p**
Age (per year)	1.09	1.03-1.16	0.005
Parity (≥1)	0.60	0.30-1.21	0.152
Previous abortion history	2.05	1.07-3.92	0.030
Gestational age at CBC (per week)	0.50	0.44-0.57	<0.001
WBC	1.24	1.12-1.38	<0.001
SII (per unit)	1.00027	0.99998-1.00056	0.067

Model 1 (Clinical covariates+Gestational age at CBC: Complete blood count+SII: Systemic immune-inflammation index*), Model 2 (Model 1+WBC: White blood cell)*

Table IV: *ROC analysis for hemogram-derived indices*

Marker	**AUC**	**95% CI**	**Optimal Cut-off (Youden)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
WBC	0.626	0.570-0.678	12.7	39.4	88.2	76.8	59.6
NLR	0.614	0.559-0.666	5.16	43.6	82.4	70.9	59.7
SII	0.633	0.578-0.686	1576	34.9	90.0	77.6	58.4

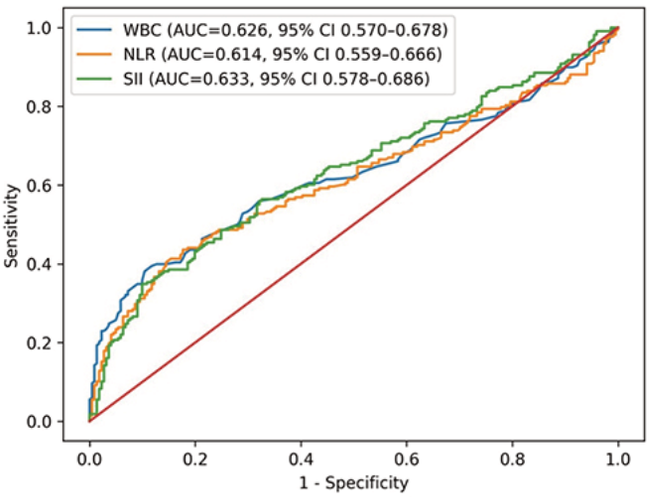

Figure 1: *Receiver operating characteristic (ROC) curves showing the predictive performance of WBC, NLR, and SII for second-trimester pregnancy loss.*

SII showed the highest area under the curve (AUC 0.633, 95% CI 0.578-0.686), followed by WBC (AUC 0.626, 95% CI 0.570-0.678) and NLR (AUC 0.614, 95% CI 0.559-0.666).

The optimal cut-off value for SII was 1576, yielding a sensitivity of 34.9% and specificity of 90.0%. Although specificity was relatively high, sensitivity and overall discriminative performance remained modest, limiting their clinical utility as standalone predictive markers (Table IV).

After exclusion of extreme hematological values (1st and 99th percentiles), 23 women (5.2%) were removed, and analyses were repeated in 416 participants.

In these analyses, increasing maternal age and a history of previous abortion were independently associated with second-trimester pregnancy loss, while gestational age showed a clear inverse relationship with risk (all $p<0.05$). NLR remained a significant independent predictor; however, its effect size was attenuated after adjustment for WBC.

Similarly, for SII, associations with key clinical variables were preserved. However, although initially significant, SII lost its independent predictive value after accounting for WBC.

Overall, the findings after exclusion of extreme values were consistent with the primary analysis, with some attenuation in effect sizes observed following adjustment for WBC.

Discussion

In this retrospective case–control study, we found that hemogram-derived inflammatory indices, particularly NLR and SII, were significantly elevated in women with spontaneous second-trimester pregnancy loss compared with those who delivered at term. After adjustment for maternal age, parity, previous abortion history, and gestational age at sampling, both indices remained independently associated with pregnancy loss. However, including total WBC in the model attenuated these associations, suggesting partial biological overlap between total leukocyte burden and derived inflammatory indices. These findings indicate that NLR and SII largely reflect heightened systemic inflammatory activity rather than entirely distinct biological pathways.

Previous studies have consistently demonstrated associations between hemogram-derived inflammatory markers and early or recurrent pregnancy loss (5,9-12). Our results corroborate the idea that systemic inflammatory activation can lead to bad pregnancy outcomes, which is what this literature says. Importantly, our work adds to these findings by looking at them in the context of spontaneous second-trimester pregnancy loss, which is a clinical condition that hasn't been studied as much.

Second-trimester pregnancy loss is a clinically diverse syndrome that can have many different causes, such as cervical insufficiency, subclinical infection, placental pathology, and other obstetric variables (8). Our study focused only on spontaneous losses, not medically necessary terminations. However, because the study was retrospective and the clinical data were poor, it was not possible to undertake a full phenotypic sub-classification. This variety may have affected inflammatory indicators, and the size of the observed relationships may partly be due to case-mixing. So, our results should be seen as showing the overall level of inflammation in spontaneous second-trimester losses, not specific impacts of a particular type of loss.

Timing is another crucial factor to consider. All CBC measurements were taken during the second trimester and adjusted for gestational age; hematological parameters naturally change during this time. Also, inflammatory activation may not be a cause of pregnancy loss, but rather an early sign of the disease process that leads to it. So, the higher levels of NLR and SII observed are probably due to associations rather than causes.

From a pathophysiological standpoint, the association between hemogram-derived indices and oxidative stress is indirect. These indices should be considered surrogate markers of the inflammatory–oxidative balance rather than direct measures of oxidative stress. Oxidative stress contributes to placental dysfunction by impairing spiral artery remodeling, inducing endothelial injury, increasing apoptotic activity, and amplifying inflammatory signaling (3,4). In this context, hemogram-derived indices may provide a pragmatic reflection of systemic inflammatory-oxidative interactions in clinical practice (5).

Even though these connections were present, NLR and SII didn't do a very good job of distinguishing them. ROC analysis showed that the AUC values were low (0.61-0.63) and that

sensitivity was also poor, even though specificity was higher. So, these indices shouldn't be thought of as stand-alone instruments for making predictions; instead, they should be seen as additional indicators that could help with clinical evaluation.

In conclusion, our results demonstrate that inflammatory indices derived from hemograms are associated with spontaneous second-trimester pregnancy loss and indicate greater systemic inflammatory activity across a wide range of clinical settings. To better understand temporal correlations and possible causal pathways, we require future prospective studies that include longitudinal measures, thorough phenotypic classification, and direct oxidative stress biomarkers.

Conclusion

In conclusion, women who lose their second-trimester pregnancy spontaneously have higher levels of inflammatory indices in their hemograms, especially NLR and SII. These levels stay high even after accounting for important clinical factors. However, the fact that the effect weakens when adjusting for total leukocyte count suggests some biological overlap with the overall inflammatory load.

These indices are easy to obtain and cheap, but they don't do a good job distinguishing between things; they shouldn't be used on their own to make predictions. Instead, they may be additional markers of systemic inflammatory activation across a wide range of clinical settings.

To better understand the links between time and the mechanisms underlying them, we need more prospective studies that examine changes over time, classify phenotypes in detail, and use direct oxidative stress biomarkers.

Strengths and Limitations: The strengths of this study include a relatively large sample size, the inclusion of exclusively spontaneous second-trimester pregnancy losses, and standardized assessment of hemogram parameters within the second trimester. Gestational age at the time of sampling was incorporated into multivariable models to account for physiological hematological variation. In addition, separate regression models with and without adjustment for total WBC count enabled evaluation of potential biological overlap and reduced the risk of overadjustment. Multicollinearity diagnostics demonstrated acceptable variance inflation factors, and sensitivity analyses excluding extreme hematological values confirmed the robustness of the findings.

Several limitations should be acknowledged. First, the retrospective case-control design precludes causal inference, and the findings should be interpreted as associative. Second, second-trimester pregnancy loss is a clinically heterogeneous condition, and detailed phenotypic sub-classification was not feasible due to incomplete documentation, potentially leading to case-mixing. Third, although gestational age was adjusted for, hematological measurements in the loss group may have been obtained closer to the clinical event, potentially confounding the analysis. Fourth, important confounders such as body mass index, smoking, assisted reproductive techniques, cervical length, thrombophilia, and infection markers were not consistently available, leaving the possibility of residual confounding. Finally, the modest discriminative performance observed in ROC analysis limits the standalone clinical utility of these indices.

Declarations

Ethics approval and consent to participate: This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Bagcılar Training and Research Hospital (Approval No: 2025/08/08/071; Date: 07 August 2025). As this was a retrospective study based on fully anonymized patient records, and no identifiable personal data were accessed, the ethics committee waived the requirement for informed consent.

Availability of data and materials: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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OYA: Data analysis, validation, writing, review, and editing.

HG: Supervision, project administration, writing, review and editing, and final approval of the manuscript.

All authors read and approved the final manuscript.

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