Evaluation of Complete Blood Cell Count Parameters in the Diagnosis of Threatened Preterm Labor and Premature Rupture of Membranes

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Keywords
Preterm premature rupture of membranes · Threatened preterm labor · Complete blood cell count parameters · C-reactive protein · Preterm birth

Abstract
Purpose: The purpose of this study was to investigate a possible relationship between systemic inflammatory markers such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), lymphocyte monocyte ratio (LMR), threatened preterm labor (TPL), and preterm premature rupture of membranes (PPROM), using complete blood cell count (CBC) parameters. Method: The study was carried out prospectively with patients admitted to our university hospital due to preterm labor and at risk of PPROM. The cases were divided into three groups according to their pregnancy status. Group 1 consisted of 90 patients with PPROM between 24th and 36th gestational weeks; group 2, 115 patients diagnosed with TPL between 24th and 36th gestational weeks; group 3, 101 patients over 36 weeks of gestation (control) who were not in labor. In addition to the demographic data of the patients, CBC parameters (white blood cell (WBC), Hg, Hct, Plt, lymphocyte, monocyte, mean platelet volume, PLR, LMR, and NLR) and C-reactive protein (CRP) values were examined and compared in each of the three groups. Results: CRP and WBC values of group 1 (PPROM) were higher than group 2 (TPL) and group 3 (control) (p < 0.05 for both). Pearson correlation between the gestational age and PLR and NLR values was significant (r and p values for PLR and NLR, respectively, r = −0.18, p < 0.001; r = −0.23, p < 0.001). However, there was no significant difference between the groups regarding PLR, NLR, LMR, platelet, monocytes, and lymphocyte counts. Conclusions: Only WBC was a valuable parameter in predicting preterm labor and PPROM. Although there was no significant difference between the groups in PLR and NLR, a negative correlation was found with gestational age at delivery. CRP value is still a helpful parameter in PPROM and TPL prediction.

Introduction
Preterm birth (PTB) is a complication that is one of the leading causes of perinatal morbidity and mortality. The WHO defines PTB as any birth before 37 completed weeks of gestation, or fewer than 259 days since the first day of the woman’s last menstrual period [1]. Threatened preterm labor (TPL) is defined as at least 2 cm of cervical dilatation, effacement, and regular uterine contractions [2]. There are 15 million premature births and 1 million infant deaths on average per year worldwide. Although it...
varies from country to country, 11% of births worldwide are considered premature [3], and the risk of morbidity and mortality is higher in deliveries before 32 weeks of gestation [4]. The interaction of genetic, environmental, and lifestyle risk factors is mentioned in the etiology of PTB. Etiologies are associated with inflammation, decidual and vascular diseases, and uterine hyperactivation caused by low progesterone levels [5]. Premature rupture of membranes (PROM) is defined as leakage of amniotic fluid before labor begins. If this occurs before 37 weeks of gestation, it is defined as preterm premature rupture of membranes (PPROM). PPROM [6] and chorioamnionitis in 2–3.5% of pregnancies can cause serious maternal and fetal outcomes such as placental abruption, oligohydramnios, fetal pulmonary hypoplasia, fetal extremity contractures, umbilical cord prolapse, and stillbirth [7]. About 75% of perinatal deaths occur in preterm or PPROM infants. Therefore, accurately predicting preterm labor prevents unnecessary interventions (use of unnecessary tocolytics and corticosteroids) and reduces mortality, especially under 32 weeks [8, 9]. Cervical length measurement and fetal fibronectin levels are widely used to diagnose TPL in symptomatic pregnant women [10, 11].

Various methods can evaluate intrauterine infection—the systemic immune response to intrauterine infection results in the release of neutrophils and lymphocytes. An increase in these cells is indicative of subclinical infection [12]. Various scoring systems such as platelet-lymphocyte ratio (PLR) and neutrophil-lymphocyte ratio (NLR) are used to predict the prognosis of inflammatory diseases [13, 14]. Studies also report an increase in neutrophil counts, a decrease in lymphocyte counts, and an increase in NLR due to this situation in preterm labor [15].

Research has shown that using a single biomarker to predict TPL is still difficult [16]. It is thought that there may be changes in the parameters of the maternal serum in PPROM and the threatened preterm. This study examined whether CBC parameters and C-reactive protein (CRP) values during pregnancy can be used in the diagnosis of TPL and PPROM. This study obtained information about PPROM and TPL from routine CBC sampling without additional laboratory testing and cost.

Materials and Methods

Study Design
This study was conducted prospectively between patients hospitalized with the diagnosis of PPROM and TPL between May 2021 and January 2022 and control group patients who were past the 36-week gestation period. Institutional Ethics Committee approval (2021 No. 3081) was obtained from our center, and the study was performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

Study Participants
The cases were divided into three groups according to their pregnancy status. Group 1 consisted of 90 patients with PPROM between 24th and 36th gestational weeks; group 2, 115 patients diagnosed with TPL between 24th and 36th gestational weeks; group 3, 101 patients over 36 weeks of gestation (control) who were not in labor. CBC parameters, hospitalization Hb, Hct, platelet, white blood cell (WBC) values, NLR, PLR, mean platelet volume (MPV), and CRP values were obtained in all groups. In addition, patient and infant data such as age, parity birth weight, gestational age at hospitalization, and gestational age at birth were also examined. These data were compared in each of the three groups.

Preterm births were defined as birth before 37 completed weeks of gestation [1]. TPL was defined as the presence of four or more regular uterine contractions and cervical changes (<3 cm dilatation), each lasting at least 30 s, with intact membranes over 30 min. PROM is a rupture (breaking open) of the membranes (amniotic sac) before labor begins. If PROM occurs before 37 weeks of pregnancy, it is called PPROM [17]. The diagnosis of PPROM was made either by visualization of significant amniotic fluid passing through the cervical canal during speculum examination or by the presence of a vaginal pH above 6 in the posterior fornix. An Amnisure test was applied in patients with diagnostic difficulties. The patients between the 24th and 34th gestational weeks were followed up after betamethasone administration. Patients with PPROM were given amoxicillin. When delivery was not required for fetal or maternal reasons, fetal heart rate, uterine contractions, and cervical changes were monitored until they occurred. All patients were discharged if labor did not progress until delivery. Gestational age was calculated according to the date of the last menstrual period and confirmed by ultrasonography.

All blood samples, including ethylenediaminetetraacetate sterile tubes, were collected from each patient immediately after hospitalization and before any medication was administered. CBC measurements were performed using the Mindray BC6200 Automated Blood Count Analyzer (Mindray Headquarters, Shenzhen, China). CRP levels were measured by the immunoturbidimetric method (Abbott Aero set 1600 Autoanalyser; Abbott Reagents, Wiesbaden, Germany). Exclusion Criteria: patient data were not included in the study in the presence of multiple pregnancies, malignant diseases that may affect maternal CBC parameters, rheumatoid arthritis, gestational diabetes mellitus, renal and hematological diseases, and chorioamnionitis.

Statistical Analysis
Descriptive statistics were given for the relevant variables in the study. Numerical variables were shown as mean and standard deviation, and categorical variables were shown as frequency and percentage. All the analyses were performed using the Jamovi project (2022). Jamovi (Version 2.3.2). The Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. ANOVA and Tukey post hoc test were used in the analysis of numerical variables. p < 0.05 was considered significant. CBC parameters between the three groups were compared, and the association of gestational age at delivery with CBC parameters was evaluated using a bivariate correlation test.
Results

Groups 1, 2, and 3 consisted of 90, 115, and 101 patients, respectively. The mean age of group 1 was 28.28 ± 5.67 years, lower than the other groups (p values p < 0.05 for group 2, p < 0.05 for group 3). There was a significant difference between hospitalization weeks, delivery weeks, and birth weights of the groups (p values <0.05 for all). The mean hospitalization week, delivery week, and birth weight in group 1 were significantly lower than group 2 and group 3 (p < 0.05 for all) (Table 1). There was no statistically significant difference between the groups’ parity, Hg, Hct, platelet, MPV, NLR, PLR, and LMR values (p > 0.05). But, NLR and PLR values were higher in the PPROM and TPL groups, than the controls. The mean WBC of group 3 was 9.90 ± 2.67, and this value was significantly higher in groups 1 and 2 (p < 0.05 for both). Group 1’s mean CRP was significantly higher than the other groups (p < 0.05) (Table 2). Pearson correlation between gestational age, PLR, and NLR values was significant (r and p values for PLR and NLR, respectively, r = −0.18, p < 0.001; r = −0.23, p < 0.001) (Fig. 1).
Discussion

Although various factors are involved in the pathogenesis of preterm labor and PPROM, infection and inflammation play an important role in the onset of preterm labor. The most accurate detection of infection occurs with amniocentesis, which is an invasive procedure [18]. Thus, there is a need to research easier diagnostic methods. Many studies have revealed that maternal and fetal inflammation triggers preterm labor and affects birth weight [19–21]. In the present study, higher CRP values were observed in group 1 (PPROM), and lower birth weights and gestational age were observed in group 2 (TPL) compared to the control group.

Various inflammatory markers, including CBC parameters, have been identified as predictive factors in various diseases [22]. Since labor is also an inflammatory response, the role of inflammatory markers such as CRP or CBC parameters has been implicated in term and preterm labor [23]. Analysis of inflammatory markers in addition to CBC parameters may prove to be more informative. NLR is characterized by increased neutrophils and decreased lymphocyte count as a physiological immune response in various stress situations. Based on this information, some researchers have suggested using NLR as an additional infection marker [24].

Kim et al. [25] stated that the success of NLR in predicting preterm labor is second only to cervical length. Gezer et al. [26] reported that a high NLR value at the time of admission is an independent risk factor for preterm labor in women with TPL between 34 and 37 weeks of gestation. In a similar study, Ozel et al. [27] found high NLR values in PPROM cases in preterm pregnancies. In our study, although NLR values were higher in the PPROM and TPL groups, there was no statistically significant difference.

Lymphopenia, which indicates a decrease in cell-mediated immunity, has been indicated in many studies. Redistribution and apoptosis of lymphocytes are responsible for lymphopenia incidence [28, 29]. But, in our study, lymphocyte values were not statistically significantly different.

Although high leukocyte and CRP values have been used as a marker of intrauterine infection for years [30], research has shown that the diagnostic sensitivity and specificity of these values is low compared to amniotic cultures [31]. In the present study, CRP values in group 1 (PPROM) were found to be significantly higher when
compared to group 2 (TPL) and the control group. Although WBC values were found to be significantly higher in group 1 and group 2 compared to the control group, no significant difference was found in the 3 groups in terms of monocyte and lymphocyte counts.

Platelet activation is mentioned in cases of infection and malignancy. Ekin et al. [32] reported an increase in platelet counts with a decrease in MPV levels in patients with PPROM compared to control patients. A relationship between PLR values and PPROM formation and adverse maternal and newborn outcomes were observed in a study [33]. In the present study, no difference was found between the groups in terms of MPV and platelet and PLR values, and no correlation was found with TPL and PPROM.

Although there was no significant difference in CBC parameters, Cha et al. [34] found that a higher LMR in the second trimester was associated with a lower gestational age at birth. In the present study, in line with the literature, a negative Pearson correlation was found between PLR and NLR and gestational age at birth. As the gestational age decreases, there is an increase in PLR and NLR values (Fig. 1).

The main findings of our study are as follows:
1. CRP and WBC values of group 1 (PPROM) were found to be higher than both group 2 (TPL) and group 3 (healthy control patients).
2. There was no significant difference between the groups in terms of PLR, NLR, LMR, platelet, and monocyte and lymphocyte count. But, PLR and NLR values of groups 1 and 2 were higher than controls.
3. A negative Pearson correlation was found between PLR and NLR and gestational age at labor.

**Limitations of the Study**
The small sample size, missing data, and low response rate are other limitations of this study. Thus, the generalizability of our findings may be limited. A larger sample size and a better response rate could result in more representative results.

**Conclusions**
As a result, only WBC was found to be a valuable parameter in predicting preterm labor and PPROM, as the CBC test is a cheap and routinely applied test. Although there was no significant difference between the groups in values such as PLR, NLR, and LMR, a negative correlation was found with gestational age at labor. CRP value is still a useful parameter in PPROM and TPL prediction. It is thought that the combined use of these methods with cervical length measurement would be safer.

**Statement of Ethics**
All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Consent form was obtained from all patients in the study (Committee approval Necmettin Erbakan University, Meram Faculty of Medicine, approval no: 05.02. 2021 No. 3081). The authors declare that they are responsible for the article’s scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents, and approval of the final version of the article.

**Conflict of Interest Statement**
None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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**Author Contributions**
Jule Eriç Horasanlı contributed to initial ideas/concepts of the study, plan, tests preparation, and data analyses, as well as writing up the paper. Ramazan Bülbül and Jule Eriç Horasanlı discussed the study plan, full data collection, references, and writing review. Elifşena Canan Alp contributed to data analyses.

**Data Availability Statement**
Due to technical and repository reasons, the data are available only upon request and by writing from the corresponding author.

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