# Neutrophil- Lymphocyte Ratio (NLR) and Platelet- Lymphocyte Ratio (PLR) as Inflammatory Markers in Preterm Birth

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**Abstract**
**Introduction**: Preterm births make up roughly 16–18% of all live births in Indonesia. One of the factors that contribute to preterm birth is inflammation. The study aimed to assess the role of platelet/lymphocyte ratio (PLR) and neutrophil/lymphocyte ratio (NLR) as inflammatory markers in preterm birth.
**Method**: This is a cross-sectional retrospective Study. Data were collected from medical records at Margono Soekarjo Hospital, January 2022-February 2023. The cohort comprised 150 participants with singleton pregnancies, ranging from 28 to less than 37 weeks of gestation, divided into three groups: preterm birth without preterm premature rupture of membranes (PPROM), preterm birth with PPROM, and threatened preterm labor (TPL). Multivariate ANOVA tests were employed for data analysis.
**Result**: There was a statistically significant difference NLR values , notably in preterm births without PPROM compared to other groups (p value < 0.005), whereas the only difference noted in PLR values was noted between preterm births with and without PPROM. Our finding differs from that of previous studies, which indicated higher NLR values in preterm births with PPROM.
**Conclusion**: NLR and PLR have the potential to be used as inflammatory markers indicative of heightened risk of preterm birth.

**Key word**: NLR, PLR, preterm birth

# Rasio Neutrofil-Limfosit (RNL) dan Rasio Platelet-Limfosit (RPL) sebagai Penanda Inflamasi pada Persalinan Prematur

**Abstrak**
**Pendahuluan**: Angka kejadian persalinan prematur di Indonesia mencakup 16-18% dari semua kelahiran hidup. Inflamasi atau infeksi dianggap sebagai salah satu penyebab persalinan prematur. Penelitian ini bertujuan untuk menilai peran rasio trombosit/limfosit (PLR) dan rasio neutrofil/limfosit (NLR) sebagai penanda inflamasi pada kelahiran premature.
**Metode**: Penelitian merupakan studi retrospektif cross-sectional. Data dikumpulkan dari rekam medis RSUD Margono Soekarjo periode Januari 2022 - Februari 2023. Didapatkan 150 pasien hamil tunggal dengan rentang usia kehamilan 28 hingga kurang dari 37 minggu yang terbagi dalam tiga kelompok: kelahiran prematur tanpa ketuban pecah dini (KPD), kelahiran prematur dengan KPD, dan ancaman persalinan prematur. Tes ANOVA multivariat digunakan untuk analisis data.
**Hasil**: Terdapat perbedaan nilai NLR yang signifikan secara statistik, terutama pada kelahiran prematur tanpa KPD dibandingkan dengan kelompok lain (nilai p <0,005), sedangkan perbedaan nilai PLR hanya terdapat pada kelahiran prematur dengan dan tanpa KPD. Temuan kami berbeda dengan penelitian sebelumnya yang menunjukkan nilai NLR lebih tinggi pada kelahiran prematur dengan KPD.
**Kesimpulan**: NLR dan PLR berpotensi digunakan sebagai penanda inflamasi yang mengindikasikan peningkatan risiko kelahiran prematur.

**Kata kunci**: NLR, PLR, persalinan prematur

## Introduction

Preterm birth is labor occurring before 37 completed weeks of gestations or with a birth weight between 500-2499 gr. World Health Organization (WHO) (2018) divided preterm birth into three categories, extremely preterm (birth before 28 completed weeks), very preterm (birth from 28-32 weeks), and moderate to late preterm (birth from 32-37 weeks). Preterm births are a major cause of perinatal and neonatal mortality and morbidity, with the high incidence rate around 8-10% in the United States and 16-18% in Indonesia of all live births.[1] According to the World Health Organization (WHO), an estimated 13.4 million babies were born prematurely in 2020, and there were approximately 900,000 children in 2019 who died from complications of preterm birth.[2]

The causes of preterm birth could vary, including stress, infection and inflammation both systemically and in the maternal genital tract, placental ischemia or vascular lesions that result in decidual bleeding, and excessive stretching of the uterus.[1] Based on the mode of delivery, preterm birth is divided into spontaneous preterm birth (75%) and preterm birth with medical/iatrogenic indications (25%).[1,3] Risk factors for spontaneous preterm birth include a history of previous preterm birth, short cervix, non-Hispanic black race, short interval between pregnancies, multiple pregnancies, and uterine abnormalities. Risk factors for preterm birth with medical/iatrogenic indications include preeclampsia, uncontrolled diabetes, fetal growth retardation, and placental abnormalities.[3]

Globally, prematurity is the leading cause of death in children aged under five years.[2] The consequences of preterm birth include respiratory distress syndrome, necrotizing enterocolitis, neonatal sepsis, neurodevelopmental defects, cerebral palsy, and neonatal death. Despite decades of research, high rates of preterm birth and infant mortality occur in developing countries with low resources. A study that examined the relationship between prematurity and neonatal mortality in Indonesia reported that the highest frequency of neonatal deaths was in West Java. Given the high rate of neonatal mortality due to premature birth, prevention is needed in cases of preterm birth to reduce the number of deaths.[4] The incidence of prematurity is closely related to the incidence of preterm premature rupture of membrane (PPROM) because the majority of pregnant women with preterm birth have a history of PPROM.[5]

Although the pathophysiological mechanism of preterm birth and preterm premature rupture of the membrane has not been clearly defined, inflammation is said to play an important role in the rupture of the membranes and triggering the process of preterm birth. The role of inflammation in preterm birth and preterm with PROM has been evaluated in many studies, and a significant association has been reported. Many inflammatory markers have recently been evaluated for their ability to detect infection in preterm birth and premature rupture of membranes.[6]

The neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) are widely available, effective, and simple markers of inflammation that can be calculated from a simple blood count. Both parameters tend to change systemically in diseases with an inflammatory process as a pathophysiological basis. Various studies investigated these markers as a predictive and prognostic parameter in cardiovascular, gastrointestinal disease, and malignancy.[6,7] In obstetrics, NLR was reported to be increased in patients with many conditions, such as gestational diabetes, preeclampsia, and pregnancy-associated intrahepatic cholestasis. Pregnancy involves many physiological changes, including the immune system, so the NLR and PLR values require separate normal values.[6,8] In a study

involving 11,415 population, the mean NLR was 3.5±1.2 and PLR 118.1±42.0 in the third trimester of pregnancy, higher than the normal value in non-pregnant women.[8] Based on the explanation above, both NLR and PLR are known to have a relationship with cases of preterm birth and premature rupture of membranes. Therefore, researchers are interested in examining whether NLR and PLR can be candidates for inflammatory biomarkers in the incidence of premature rupture of membranes and preterm birth.

## Method

This study is a cross-sectional retrospective study by taking the data from the medical records of patients at Prof. dr. Margono Soekarjo Purwokerto Hospital from January 2022 to February 2023. The study involved patients with a diagnosis of singleton pregnancy at 28 to <37 weeks of gestation who experienced threatened preterm labor (TPL), preterm birth without PPROM, and preterm birth with PPROM. The sample is determined using the total sampling technique. Data are excluded if there is a diagnosis of inflammatory disease in pregnancy such as preeclampsia or gestational diabetes, bleeding due to placenta previa, known acute or chronic infectious diseases, liver disease, heart disease or malignancy, history of cervical surgery or undergoing invasive procedures such as amniocentesis, congenital fetal abnormalities, IUFD, and patients with incomplete laboratory data.

The variables used in this study are maternal age, gestational age, and laboratory parameters, including leukocyte, neutrophil, lymphocyte, platelet, neutrophil/lymphocyte ratio (NLR), and platelet/lymphocyte ratio (PLR) values. The NLR is calculated by dividing the neutrophil count by the absolute lymphocyte count, while the PLR is calculated by dividing the platelet count by the absolute lymphocyte count. The data was obtained from the patient's medical records at Prof. Dr. Margono Soekarjo Hospital.

All data were analyzed using SPSS 27.0. The Kolmogorov-Smirnov test was performed to assess whether the data is normally distributed. Multivariate analysis with ANOVA or Kruskal-Wallis test was performed to compare the numerical variables distributed between groups. Data on each variable is displayed as the mean ± standard deviation, with a p-value determines whether there is a significant relationship between the groups (p-value <0.05).

## Results

The population of patients with preterm birth at Prof. dr. Margono Soekarjo hospital from January 2022 to February 2023 who met the inclusion and exclusion criteria was a total of 151 cases, divided into 37 patients with threatened preterm labor, 60 patients with preterm birth with PPROM, and 53 patients with preterm birth without PPROM. An overview of the demographic variables and clinical criteria of the patients in this study is shown in Table 1.

The characteristics of the patients in this study do not show any differences in maternal age, gestational age, mean lymphocyte count, and platelet count between the three groups (p-value >0.05). Leukocyte values were significantly lower in the threatened preterm labor group than in preterm birth with PPROM and preterm birth without PPROM (p-value <0.05). There was a significant difference in the NLR values in the three groups, with the highest average value obtained in preterm birth without PPROM compared to the preterm birth with PPROM and threatened preterm labor group (6.40 ± 2.4 vs. 5.35 ± 2.6 vs. 5.03 ± 2.01, p-value <0.05). PLR values were significantly different between groups of preterm birth with PPROM and preterm birth without PPROM (p-value 0.027).

**Table 1 Overview of Clinical Characteristics in Patients with Threatened Preterm Labor (TPL), Preterm Birth with Preterm Premature Rupture of Membranes (Preterm PROM), and Preterm Birth without Preterm Premature Rupture of Membranes (Preterm Birth) at Prof. dr. Margono Soekarjo Hospital**

| Variable | TPL (n=37) | Preterm PROM (n=60) | Preterm Birth (n= 53) | P1 | P2 | P3 |
|---|---|---|---|---|---|---|
| Maternal age | 26.98±6.3 | 28.15 ± 6.9 | 26.38 ± 7.50 | 0.638 | 0.906 | 0.450 |
| Gestational age | 33.27±2.6 | 33.68±2.5 | 33.27 ± 1.95 | 0.652 | 0.901 | 0.722 |
| Leucocyte | 10961.08 ± 2609.43 | 12921 ±4376.8 | 13472.36 ±3683.6 | 0.037 | 0.006 | 0.017 |
| Neutrophil | 76.72 ± 6.40 | 75.83±6.9 | 79.65±6.6 | 0.800 | 0.106 | 0.008 |
| Lymphocyte | 16.98 ± 5.20 | 16.65±5.9 | 14.25±5.1 | 0.956 | 0.056 | 0.057 |
| Platelet | 269405 ±61486.4 | 273350 ±71842.9 | 285962.26 ±77312.7 | 0.351 | 0.281 | 0.557 |
| Absolute lymphocyte | 1816.08 ± 622.68 | 1992.85 ±562.6 | 1830.26 ±550.02 | 0.771 | 0.022 | 0.022 |
| NLR | 5.03 ± 2.01 | 5.35±2.6 | 6.40±2.4 | 0.006 | 0.006 | 0.007 |
| PLR | 161.78 ± 58.70 | 145.73±48.6 | 168.39±63.7 | 0.580 | 0.080 | 0.027 |

[a] Data are presented as means ± standard deviation, significant determined at *p-value* <0,05 (Anova test and Post Hoc Duncan test)
P1 : p-value between TPL and preterm birth with PPROM
P2 : p-value between TPL and preterm birth without PPROM
P3 : p-value between preterm birth with PPROM and preterm birth without PPROM

## Discussion

The study results show that leukocyte values in patients in the preterm birth without PPROM group were lower than those in the preterm birth with PPROM and threatened preterm labor group and showed a statistically significant difference (13472.36 ± 3683.6 vs. 12921 ± 4376.8 vs. 10961.08 ± 2609.43; p-value <0.005). Evaluating leukocyte values in the mother is indeed one of the markers proposed to assess the presence of infection in preterm labor.[5] However, from several current studies, leukocytes are not considered a useful marker for determining infection or inflammation in pregnant women due to the wide normal range of leukocyte levels in pregnancy, especially in the third trimester. In addition, several studies regarding acute chorioamnionitis, both clinically and histologically/subclinically, and neonatal infection as a delivery outcome show that maternal blood leukocyte levels are a weak diagnostic indicator for this condition.[6-8] Moreover, a systemic review and meta-analysis conducted by Sabogal et al. showed that leukocytosis has low sensitivity (51%) and specificity (65%) for assessing histological chorioamnionitis as a factor of inflammation in pregnant women.[8]

The results of the neutrophil examination showed a higher average in preterm birth without PPROM patients compared to the preterm birth with PPROM group with statistically significant results (p-value 0.008). In contrast, the results of the lymphocyte examination did not show any statistically significant differences between the three groups, however, the average lymphocyte value was lower in the group with preterm birth without PPROM. Higher neutrophil levels and low lymphocyte levels characterize

an acute inflammatory condition. Kim A. et al. stated that there was a significant relationship between neutrophil levels and lymphocyte levels with inflammatory or inflammatory events in the placenta. In conditions of acute inflammation, proinflammatory cytokines and chemokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNFα), granulocyte colony-stimulating factor (G-CSF), prostaglandins, and leukotrienes will cause an increase in neutrophils as a non-specific body defense system. On the other hand, lymphopenia, or decreased lymphocyte levels will occur in acute infection due to impaired antigen presentation, activated negative co-stimulatory signals, and the production of immunosuppressive factors; all of which can contribute to reduced lymphocyte levels. In addition, the activation of TNF can cause apoptosis in lymphocytes.[9]

Moreover, this study found statistically significant differences in the mean NLR values between the three groups, with the highest mean in the preterm birth without PPROM group (6.40 ± 2.4 vs. 5.35 ± 2.6 vs. 5.03 ± 2.01, p-value <0.005). These results are different from previous studies, which stated that the neutrophil value would increase, and the lymphocyte value would decrease in conditions of preterm labor accompanied by premature rupture of membranes.[6] NLR is a marker of systemic inflammation, which is obtained by dividing the neutrophil count by the lymphocyte count from a hematological examination. NLR is considered one of the biomarkers that is easy to obtain, and its examination does not require invasive procedures.[10] The study found that there was an increase in NLR in various conditions of pregnancy, such as preeclampsia, gestational diabetes, and intrahepatic cholestasis.[11-13] NLR is considered one of the parameters or markers of inflammation or as a predictor of bacterial infection. Increased NLR can be used as a fairly accurate indicator for diagnosing chorioamnionitis and predicting the occurrence of preterm labor.[9] A study by Akgun et al. demonstrated that increased NLR is associated with preterm birth and low birth weight. This study predicts that the increase in NLR will be influenced by the hyperinflammatory state of the mother, which causes fetal growth restriction and initiation of preterm labor.[14]

PLR is also one of the inflammation markers currently being studied massively. PLR is used as a prognostic parameter in patients with cardiovascular disease and malignancy. In pregnancy, PLR has also been studied in patients with preeclampsia, gestational diabetes, and preterm PROM.[12, 15] Toprak et al. stated that there was a significant PLR value in patients with preterm PROM regardless of the amount of amniotic fluid or the duration of the rupture of membranes. PLR with a value of >117.14 is related to the rate of preterm PROM.[6] Previous research shows a relationship between PLR and the incidence of preterm labor. A meta-analysis by Peng et al. stated that PLR serum levels were significantly greater than patients with term labor. PLR has potential value as a biomarker for diagnosing and assessing the condition of women who experience spontaneous preterm labor.[16]

In this study, an increase in PLR values was found in the preterm birth with PPROM and preterm birth without PPROM group with values of 145.73 ± 48.6 and 168.39 ± 63.7 (p-value 0.027). However, there was no significant relationship between PLR in patients with threatened preterm labor. This indicates an inflammatory condition that occurs in patients with preterm birth, whether accompanied by PPROM or without. However, in contrast to other previous studies, PLR results were higher in patients with preterm labor without PPROM compared to the group with preterm PPROM.[10] Therefore, it is necessary to reconsider the existence of other factors that influence the occurrence of inflammation and cause an increase in NLR

and PLR values in patients with preterm birth in this study.

Several factors to consider include the presence of inflammation or infection that is not diagnosed and not ruled out, which causes different results from other studies. Histological chorioamnionitis is an intrauterine inflammatory condition characterized by granulocyte infiltration into the choriodecidual tissue, as well as fetal tissues such as the chorioamnionitis membrane, amniotic fluid, and umbilical cord. This condition can be diagnosed by examining the placenta after delivery, even if there are no symptoms during pregnancy. The incidence of histological chorioamnionitis is found in 50% of cases of preterm labor.[17] Histopathological examination of the placenta is necessary to confirm the occurrence of chorioamnionitis because only one-third of all patients have clinical symptoms, meaning that two-third of patients have chorioamnionitis without visible clinical symptoms. Non-invasive prenatal diagnosis has been widely studied as an alternative, including by examining the neutrophil count, NLR, IL-10, and IL-6.[8] In this study, postnatal placenta examination was not performed to confirm infection or inflammation of the placenta, so the incidence of histological chorioamnionitis could not be ascertained.

The retrospective form of research is also one of the limitations of this study, where the process of diagnosing preterm PROM and other accompanying infectious diseases cannot be standardized. The data obtained does not entirely include examinations other than a complete blood count, for example, a urinalysis examination to detect the possibility that a patient has an acute tract infection. Changes in the anatomy and physiology of the urinary tract during pregnancy contribute to an increased risk of infection. Asymptomatic bacteriuria is a bacterial infection that requires drug therapy in pregnancy, with a prevalence reaching 2-10% of infections in pregnancy. Early detection and management of asymptomatic bacteriuria can prevent complications, including preterm labor, preterm PROM, and low birth weight babies.[18] In this study, not all patients underwent urinalysis examination, so the possibility of undiagnosed infection could not be ruled out. Undiagnosed infectious conditions can be one of the factors that must be considered causing research results that are different from previous studies. This study shows a relationship between NLR values and the incidence of preterm labor. This result implies that NLR can be considered as one of the markers that can indicate a greater risk of preterm birth.

## Conclusion

The research conducted at Prof. dr. Margono Soekarjo Hospital found that the average difference in NLR values was significantly higher in patients with preterm birth without PPROM than in preterm birth with PPROM and threatened preterm labor. However, there are differences in results from previous studies where NLR values were higher in the group with preterm birth without PPROM compared to the preterm birth with the PPROM group. The use of NLR is more related to the risk of preterm labor. Further research is needed to screen infectious or inflammatory factors that occur and assess the causal relationship between NLR and the incidence of preterm labor and premature rupture of membranes to obtain significant results.

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