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# INFLAMMATORY MARKERS AND MORTALITY IN PATIENTS WITH GRAM NEGATIVE BACTEREMIA

Recai ACİ [1]*, Adem KESKİN [2], Murat ARI [3]

[1]Department of Biochemistry, Samsun Education and Research Hospital, Samsun University, Samsun, Turkey recaiaci35@gmail.com (https://orcid.org/0000-0002-3332-6619)
[2]Adnan Menderes University Faculty of Medicine, Department of Biochemistry, Aydın, Türkiye
ademkeskin78@gmail.com (https://orcid.org/0000-0003-1921-2583)
[3]Adnan Menderes University Söke Vocational School of Health Services, Aydın, Türkiye
muratari60@gmail.com, (https://orcid.org/0000-0002-1504-7050)

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*Corresponding author

**Abstract**

**Background/aim:**In our study, we aimed to determine the relationship between C-reactive protein (CRP), neutrophils, lymphocytes, platelets, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), length of hospitalization and mortality in patients with gram negative bacteremia in blood cultures.
**Materials and methods** :The files of patients who were hospitalized in our hospital between January 2022 and May 2022 and for whom CRP and CBC (complete blood count) tests were requested simultaneously with blood culture were retrospectively analyzed. The study included 50 patients between the ages of 31 and 88 who were diagnosed with gram (-) sepsis.
**Results :**There was no significant difference between the groups in terms of age, CRP, neutrophil, lymphocyte, platelet and NLR levels. The length of hospitalization in the ex group was higher than the length of hospitalization in the discharge group. In addition, the PLR rate in the ex group was lower than the PLR rate in the discharge group. CRP levels were positively correlated with NLR levels in patients with gram negative bacterial sepsis.
**Conclusion** :In this study, it was found that NLR and CRP values were not statistically significant in determining the prognosis of patients diagnosed with Gram negative bacteremia. However, due to the high mortality rate and hospitalization duration of these patients, it was concluded that follow-up and treatment of these patients is important together with some markers. These findings emphasize that the correct approach in the treatment of patients diagnosed with Gram-negative bacteremia should be planned by considering the symptoms.
**Keywords : Bacteremia, sepsis, C-reactive protein, NLR, PLR, Mortality**

## 1. Introduction

Bloodstream infections are associated with high morbidity and early diagnosis and treatment with mortality are clinical pictures in which mortality rates can be significantly reduced when treated. Bacteremia is the detection of the presence of live bacteria in the bloodstream. Bacteria can enter the sterile bloodstream and cause a serious complication of infections, such as pneumonia or meningitis. They can also occur after surgery, especially in mucosal interventions such as in the gastrointestinal tract, or due to foreign bodies such as catheters. The diagnosis is made by gold standard tests such as blood cultures.(Wakabayashi & Iwata, 2021)·(Sligl et al., 2006)

Blood culture is a reliable test to detect the presence of infectious agents in the bloodstream, but it requires 24-48 hours to obtain results. Therefore, early diagnosis and follow-up of infections requires the use of other biomarkers that can help in the time until blood culture results are available.(Demir-Çuha & Hazırolan, 2020) CRP (C-Reactive Protein) was first discovered by Tilet and Francis in 1930. This protein was found to increase in infections such as pneumococcal pneumonia.(Pathak & Agrawal, 2019)

CRP (C-Reactive Protein) is a calcium-dependent plasma protein secreted from hepatocytes and increases in response to immune response. Normally, CRP level is less than 10 mg/L in healthy people. However, in case of

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disease, the level starts to rise in the first 6-8 hours and reaches a peak of 350-400 mg/L approximately 48 hours later. CRP levels can increase for many reasons. Therefore, it cannot be used alone to support the diagnosis and should be evaluated together with other biomarkers.(Pathak & Agrawal, 2019) Neutrophilia (high neutrophil count) and lymphocytopenia (low lymphocyte count) are well-known markers of severe bacterial infections. Zahorec and colleagues investigated the neutrophil/lymphocyte count ratio (NLR), an easily measurable parameter indicating the severity of systemic inflammation and sepsis, in 90 oncology patients. In this study, NLR was reported to be a useful parameter for predicting bacteremia. (Chen et al., 2021)Although different threshold values have been determined in different studies, neutrophil/lymphocyte count ratio (NLR) is thought to be a useful biomarker to predict sepsis prognosis. However, an ideal cut-off value is still controversial. A meta-analysis revealed that a high NLR value at baseline indicates unfavorable prognoses in patients with sepsis and may be a useful prognostic factor for sepsis.(Huang et al., 2020)

In our study, we aimed to reveal the relationship between C-reactive protein, neutrophils, lymphocytes, platelets, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), length of hospitalization and mortality in patients with gram negative bacteremia in blood cultures.

## 2. Material & Method

The files of patients who were hospitalized in our hospital between January 2022 and May 2022 and for whom CRP and CBC (complete blood count) tests were requested simultaneously with blood culture were retrospectively analyzed. The study included 50 patients between the ages of 31 and 88 who were diagnosed with gram (-) sepsis. Blood cultures were monitored in BacT/ALERT 3D (bioMérieux, France) automated blood culture system. CRP levels were measured on the Roche Cobas 6000 modular system (Roche Diagnostics, Germany). Complete blood count analysis was performed on a UniCel DxH 800 hematology analyzer (Beckman Coulter, USA).

### Statistical analysis

SPSS for Windows 22.0 program was used for statistical analysis. Continuous variables obtained from the study were presented as mean±standard deviation (X±SD), categorical variables were presented as n (percentage frequency) and P values below 0.05 were consideredstatistically significant. Chi-square test was used for the comparison of categorical variables. Independent sample t test was used for the comparison of continuous variables.

## 3.Results

The study included 50 patients between 31 and 88 years of age who were diagnosed with gram (-) sepsis. The mean age of these patients was 70.84±12.09 years. Of these patients, 19 (38.00%) were female and 31 (62.00%) were male. Among the patients included in the study,37 (74.00%) died and 13 (26.00%) were discharged with cure. Descriptive data of the patientswho died and were discharged are presented in Table 1.

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**Table 1.** Descriptive information of ex and discharged patient groups

| Parameter | Ex groupn=37 | Discharge group n=13 |
|---|---|---|
| **Age** | 70.24±12.47 | 72.54±11.24 |
| **Gendern (%)** **Male** | 21 (56.76) | 10 (76.92) |
| **Woman** | 16 (43.24) | 3 (23.08) |
| **Length of hospitalization days** | 39.73±24.17 | 23.92±8.45 |
| **CRP (mg/L )** | 219.92±117.59 | 200.49±94.55 |
| **Neutrophils ($10^9$ /L)** | 10.75±6.37 | 11.82±7.12 |
| **Lymphocytes ($10^9$ /L)** | 1.14±1.18 | 0.92±0.69 |
| **Platelets ($10^9$ /L)** | 183.03±118.75 | 233.31±147.44 |
| **NLR** | 15.15±14.31 | 23.84±29.59 |
| **PLR** | 227.33±139.60 | 423.83±483.40 |

No significant difference was found between the groups in terms of age, CRP, neutrophil,lymphocyte, platelet and NLR levels (Table 1).

The length of hospitalization in the ex group was higher than the length of hospitalization inthe discharged group (p=0.026). In addition, the PLR rate in the ex group was lower than thePLR rate in the discharged group (p=0.029) (Table 1).

Correlation analysis was performed to see the relationship between CRP levels of the groupsand other parameters. The significant results of the correlation analysis are given in Table 2.

**Table 2.** Correlation of CRP levels with other parameters

| Parameter | | CRP |
|---|---|---|
| **Length of hospitalization** | **Cc** | 0.116 |
| | **p** | >0.05 |
| **Neutrophil** | **Cc** | 0.181 |
| | **p** | >0.05 |
| **Lymphocyte** | **Cc** | -0.205 |
| | **p** | >0.05 |
| **Platelet** | **Cc** | -0.051 |
| | **p** | >0.05 |
| **NLR** | **Cc** | 0.308 |
| | **p** | 0.029 |

| | | |
|---|---|---|
| **PLR** | **Cc** | 0.232 |
| | **p** | >0.05 |

**Cc= Correlation coefficient**

CRP levels were positively correlated with NLR levels in patients with gram negativebacterial sepsis (Table 2).

## 4.Discussion

Improper or delayed treatment of bacteremia can lead to sepsis, a serious clinical condition, especially in intensive care units, in patients receiving immunosuppressive therapy or in oncology and transplant patients. Sepsis due to bacteremia can progress rapidly and carries a high risk of death. Delayed treatment or inappropriate antibiotic therapy increases sepsis mortality. Hospitalizations and treatment costs increase due to irregular responses to microorganisms in the blood, and are increasing every year. Despite advances in medicine, sepsis mortality remains high. Blood culture is the most reliable and accepted method to determine the cause of infection caused by bacteremia. Classical methods require at least 48-72 hours to determine the species and antibiotic susceptibilities of microorganisms grown after blood culture. However, it is known that time is very important in the treatment of sepsis. Therefore, rapid diagnostic tests have started to be developed. These tests are used to rapidly identify the causative organism and initiate appropriate treatment in patients with sepsis.

Although the causative agents of bacteremia vary according to countries and clinics, studies conducted in Turkey show that among gram-positive agents, CNS, Staphylococcus aureus and enterococci spp. are the most common. Among gram-negative agents, Acinetobacter, Klebsiella, Escherichia coli, Pseudomonas, Enterobacter and Proteus spp. are the most frequently reported.

In a study of 297 patients with bacteremia, gram-negative bacteria were predominantly isolated, with E. coli and Klebsiella spp being the most common agents. S. aureus was the most common agent isolated from gram-positive bacteria.(Hattori et al., 2014) In a study by C. Leli et al. on 586 cases of bacteremia, E. coli, Klebsiella spp. and P. aeruginosa were the most common gram-negative bacteria infections, while S. aureus and Enterococcus faecalis were the most common gram-positive bacteria infections.(Leli et al., 2015) In the study by H. Liu et al. 147 out of 648 blood culture positive cases fulfilling SIRS (Systemic Inflammatory Response Syndrome) criteria were analyzed. In these cases, gram-negative bacteria, especially E. coli and Klebsiella spp. were the most common. Among gram-positive bacteria, S. aureus and CNS were most frequently isolated. Baseline procalcitonin and CRP levels were also among the factors analyzed.(Liu et al., 2017) In a study conducted in Portugal with 189 bacteremic patients followed up in a burn unit, the most frequently isolated gram-negative bacteria were Pseudomonas spp. and Acinetobacter spp. and gram-positive bacteria were CNS.(Cabral et al., 2019)

CRP is frequently used together with procalcitonin in the prediction of bacterial infections.(Simon et al., 2004) In a study of 166 bacteremic patients followed up in the intensive care unit, no significant difference was found in CRP levels in infections due to gram-negative and gram-positive pathogens.(Brodská et al., 2013) In a

retrospective study by H. Liu et al., similarity in CRP levels was found between infections due to gram-negative and gram-positive bacteria in bacteremic cases.(Liu et al., 2017) In the study by B. Bilgili et al. 124 bacteremic sepsis patients were examined and it was found that the median value of CRP was significantly higher in infections associated with gram-negative pathogens.(Kocazeybek et al., 2003) In another study conducted by Li S. et al. on bacteremic sepsis patients, it was found that CRP levels were significantly higher in patients with gram negative bacterial infections.(Li et al., 2016) In another study, 259 bacteremic patients diagnosed with sepsis, severe sepsis and septic shock in the intensive care unit were examined and it was found that CRP levels were significantly higher in bacteremic cases with gram-negative agents compared to gram-positive infections. In addition, gram-negative bacteria were found to cause septic shock at a higher rate in this study. This result was associated with the development of a stronger inflammatory response by gram-negative bacteria in sepsis.(Abe et al., 2010) In addition to studies showing that CRP is prognostic, there are also studies emphasizing that it has no prognostic value. In a study conducted by Silvestre et al. on 158 patients, CRP monitoring was not a good marker in determining the diagnosis and prognosis of sepsis.(Silvestre et al., 2009) A study by Prieto and colleagues showed that CRP levels correlated with the APACHE (Acute Physiology and Chronic Health Evaluation Score) score in intensive care unit patients and that CRP levels can be used as a prognostic marker to predict the development of organ failure and mortality.(Prieto et al., 2008)

In our study, no significant difference was found between the groups in terms of CRP levels. In addition, in our study, although the crp level detected in the Ex group was higher than the surviving group, this difference was not statistically significant. Correlation analysis was performed to see the relationship between CRP levels of the groups and other parameters. It was found that CRP levels were positively correlated with NLR levels in patients with Gram negative bacterial sepsis.

There are studies showing that lymphopenia and NLO seen in complete blood count are associated with bacteremia.(Djordjevic et al., 2018) Numerous studies have shown a direct correlation between high NLR and mortality in different patient populations. Neutropenia has been shown to be associated with an increased risk of mortality. This may be due to increased peripheral utilization of neutrophils, damage to neutrophils by bacterial products or suppression of bone marrow by inflammatory mediators. The relationship between lymphopenia and bacteremia is based on the presence of a large number of TNF-α binding receptors on the lymphocyte surface and the knowledge that lymphocytes are driven to apoptosis as a result of stimulation of these receptors.(Djordjevic et al., 2018) In a study by Zahorec in 2001, it was reported that lymphopenia was associated with the severity of sepsis in septic patients in intensive care units. In this study, a comparison was made between patients undergoing major surgery and patients with sepsis and it was found that the NLO value was higher in the sepsis patient group.(Zahorec, 2001) A study by Wyllie and colleagues suggested that lymphopenia could be used to predict bacteremia. In a later study, they found that NLO value was more significant than CRP level or lymphopenia alone.(Wyllie et al., 2004) In our study, neutrophil count was also higher in the excluded group but not statistically significant. There was no statistically significant difference between the two groups in terms of lymphocyte count. Similarly, Silvestre et al. examined the relationship between WBC count and prognosis in sepsis patients and found no significant difference.(Silvestre et al., 2009) Salciccioli et al. conducted a study on 5056 bacteremic patients

evaluating intensive care treatment uptake. According to the results of the study, there was no significant difference in NLO between surviving patients and patients who died.(Salciccioli et al., 2015) In our study, no significant difference was found when NLR was compared between exitus and survivors. In their study, Hwang et al. analyzed the NLR levels of patients They examined NLR levels in five different groups by dividing them into five different groups from the lowest to the highest, respectively. In this study, low NLR level was found to be caused by low neutrophil levels and neutropenia led to bacterial infection and septic shock and high mortality rates.(Hwang et al., 2017) In our study, similar to the study by Hwang et al., NLR was lower in the excluded group compared to the surviving group, but this was not statistically significant. In the study conducted by Zahorec et al. NLR in sepsis patients hospitalized in intensive care units association with mortality was found to be significant.(Zahorec, 2001)

In our study, the PLR rate in the ex group was lower than the PLR rate in the discharged group (p=0.029). In addition, in the correlation analysis, it was found that CRP levels were positively correlated with NLR levels in patients with Gram-negative bacterial sepsis.

The mortality rate of bacteremia ranges between 14-37% and is particularly high in critically ill patients.(Grumaz et al., 2016) The source of infection is an important factor determining the prognosis in patients with sepsis. Studies show that sepsis originating from the urinary system has the lowest mortality rate of 30%. However, in cases of sepsis of gastrointestinal or pulmonary origin, mortality rates are reported to be as high as 50% to 55%. In the study conducted by Mohan et al. in 2015, the overall mortality rate was 53%, 33.3% in sepsis, 33.3% in severe sepsis 55.7% and 65.7% in septic shock.(Artero et al., 2010) In our study, the duration of hospitalization in the ex group was higher than the duration of hospitalization in the discharged group (p=0.026). In addition, the PLR rate in the ex group was lower than the PLR rate in the discharged group (p=0.029).

**Conclusion**

The aim of this study was to investigate the relationship between the presence of growth in blood cultures and CRP, NLO, length of hospitalization and mortality in patients with gram negative bacteremia. To summarize our study, no significant difference was found between the groups in terms of age, CRP, neutrophil, lymphocyte, platelet and NLR levels in intensive care patients with gram negative bacteremia. The length of hospitalization in the ex group was higher than the length of hospitalization in the discharge group (p=0.026). In addition, the PLR rate in the ex group was lower than the PLR rate in the discharged group.CRP levels were positively correlated with NLR levels in patients with Gram negative bacterial sepsis.

**References**

Abe, R., Oda, S., Sadahiro, T., Nakamura, M., Hirayama, Y., Tateishi, Y., Shinozaki, K., & Hirasawa, H. (2010). Gram-negative bacteremia induces greater magnitude of inflammatory response than Gram-positive bacteremia. *Critical Care*, *14*(2), 1–7.

Artero, A., Zaragoza, R., Camarena, J. J., Sancho, S., González, R., & Nogueira, J. M. (2010). Prognostic factors of mortality in patients with community-acquired bloodstream infection with severe sepsis and septic shock.

*Journal of Critical Care*, *25*(2), 276–281.

Brodská, H., Malíčková, K., Adámková, V., Benáková, H., Šťastná, M. M., & Zima, T. (2013). Significantly higher procalcitonin levels could differentiate Gram-negative sepsis from Gram-positive and fungal sepsis. *Clinical and Experimental Medicine*, *13*, 165–170.

Cabral, L., Afreixo, V., Meireles, R., Vaz, M., Frade, J.-G., Chaves, C., Caetano, M., Almeida, L., & Paiva, J.-A. (2019). Evaluation of procalcitonin accuracy for the distinction between Gram-negative and Gram-positive bacterial sepsis in burn patients. *Journal of Burn Care & Research*, *40*(1), 112–119.

Chen, L., Wu, X., Qin, H., & Zhu, H. (2021). The PCT to albumin ratio predicts mortality in patients with acute kidney injury caused by abdominal infection-evoked sepsis. *Frontiers in Nutrition*, *8*, 584461.

Demir-Çuha, M., & Hazırolan, G. (2020). Hacettepe Üniversitesi Tıp Fakültesi Hastanesi'nde 2017-2019 Yılları Arasında Kan Kültürlerinden İzole Edilen Anaerop Bakteriler: Üç Yıllık Bir Değerlendirme. *Klimik Journal/Klimik Dergisi*, *33*(3).

Djordjevic, D., Rondovic, G., Surbatovic, M., Stanojevic, I., Udovicic, I., Andjelic, T., Zeba, S., Milosavljevic, S., Stankovic, N., & Abazovic, D. (2018). Neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and mean platelet volume-to-platelet count ratio as biomarkers in critically ill and injured patients: which ratio to choose to predict outcome and nature of bacte. *Mediators of Inflammation*, *2018*.

Grumaz, S., Stevens, P., Grumaz, C., Decker, S. O., Weigand, M. A., Hofer, S., Brenner, T., von Haeseler, A., & Sohn, K. (2016). Next-generation sequencing diagnostics of bacteremia in septic patients. *Genome Medicine*, *8*(1), 1–13.

Hattori, T., Nishiyama, H., Kato, H., Ikegami, S., Nagayama, M., Asami, S., Usami, M., Suzuki, M., Murakami, I., & Minoshima, M. (2014). Clinical value of procalcitonin for patients with suspected bloodstream infection. *American Journal of Clinical Pathology*, *141*(1), 43–51.

Huang, Z., Fu, Z., Huang, W., & Huang, K. (2020). Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. *The American Journal of Emergency Medicine*, *38*(3), 641–647.

Hwang, S. Y., Shin, T. G., Jo, I. J., Jeon, K., Suh, G. Y., Lee, T. R., Yoon, H., Cha, W. C., & Sim, M. S. (2017). Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. *The American Journal of Emergency Medicine*, *35*(2), 234–239.

Kocazeybek, B., Küçükoğlu, S., & Öner, Y. A. (2003). Procalcitonin and C-reactive protein in infective endocarditis: correlation with etiology and prognosis. *Chemotherapy*, *49*(1–2), 76–84.

Leli, C., Ferranti, M., Moretti, A., Dhahab, A., Salim, Z., Cenci, E., & Mencacci, A. (2015). Procalcitonin levels in gram-positive, gram-negative, and fungal bloodstream infections. *Disease Markers*, *2015*.

Li, S., Rong, H., Guo, Q., Chen, Y., Zhang, G., & Yang, J. (2016). Serum procalcitonin levels distinguish Gram-

negative bacterial sepsis from Gram-positive bacterial and fungal sepsis. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, *21*.

Liu, H. H., Zhang, M. W., Guo, J. B., Li, J., & Su, L. (2017). Procalcitonin and C-reactive protein in early diagnosis of sepsis caused by either Gram-negative or Gram-positive bacteria. *Irish Journal of Medical Science (1971-)*, *186*, 207–212.

Pathak, A., & Agrawal, A. (2019). Evolution of C-reactive protein. *Frontiers in Immunology*, *10*, 943.

Prieto, M. F., Kilstein, J., Bagilet, D., & Pezzotto, S. M. (2008). C-reactive protein as a marker of mortality in intensive care unit. *Medicina Intensiva*, *32*(9), 424–430.

Salciccioli, J. D., Marshall, D. C., Pimentel, M. A. F., Santos, M. D., Pollard, T., Celi, L. A., & Shalhoub, J. (2015). The association between the neutrophil-to-lymphocyte ratio and mortality in critical illness: an observational cohort study. *Critical Care*, *19*(1), 1–8.

Silvestre, J., Povoa, P., Coelho, L., Almeida, E., Moreira, P., Fernandes, A., Mealha, R., & Sabino, H. (2009). Is C-reactive protein a good prognostic marker in septic patients? *Intensive Care Medicine*, *35*, 909–913.

Simon, L., Gauvin, F., Amre, D. K., Saint-Louis, P., & Lacroix, J. (2004). Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clinical Infectious Diseases*, *39*(2), 206–217.

Sligl, W., Taylor, G., & Brindley, P. G. (2006). Five years of nosocomial Gram-negative bacteremia in a general intensive care unit: epidemiology, antimicrobial susceptibility patterns, and outcomes. *International Journal of Infectious Diseases*, *10*(4), 320–325.

Wakabayashi, T., & Iwata, H. (2021). Outcome, diagnosis, and microbiological profile comparison of community- and hospital-acquired bacteremia: A retrospective cohort study. *Journal of General and Family Medicine*, *22*(6), 327–333.

Wyllie, D. H., Bowler, I., & Peto, T. E. A. al. (2004). Relation between lymphopenia and bacteraemia in UK adults with medical emergencies. *Journal of Clinical Pathology*, *57*(9), 950–955.

Zahorec, R. (2001). Ratio of neutrophil to lymphocyte counts-rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratislavske Lekarske Listy*, *102*(1), 5–14.