

# Correlation between Interleukin-17 Levels with C- Reactive Protein and Neutrophil Lymphocyte Ratio in Sepsis

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Original Research Article

## Correlation between Interleukin-17 Levels with C-Reactive Protein and Neutrophil Lymphocyte Ratio in Sepsis

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### Abstract

**Background:** Sepsis is a condition in systemic infection associated with organ dysfunction. Interleukin-17 is a pro-inflammatory cytokine produced by Th-17 cells. C-reactive protein and neutrophil-lymphocyte ratio (NLR) have been widely used as markers of inflammation. The relationship between IL-17 as a proinflammatory cytokine with CRP and NLR has not been reported.

**Objective:** This study aims to prove the correlation between IL-17 with CRP and NLR in septic patients.

**Methods:** Analytical observational study with a cross-sectional approach was carried out on 40 septic patients in July–December 2020. IL-17 levels were obtained by the ELISA method while CRP levels were obtained using the immunoturbidimetric method. NLR was the result of dividing the absolute number of neutrophils and lymphocytes from the automatic hematology analyzer. Correlation between variables was performed using Spearman correlation test.

**Results:** The median levels of IL-17, CPR, and NLR were 363.55 (11.4-1695.80) pg/mL, 13.25 (0.43-53.87) mg/L, and 12.00 (2.26–48.5), respectively. The Spearman correlation test between IL-17 levels and CRP obtained  $p = 0.019$ ,  $r = 0.37$ , and NLR  $p = 0.425$ ,  $r = 0.13$ .

**Conclusion:** There is a weak positive correlation between IL-17 levels and CRP in septic patients. There is no correlation between IL-17 levels and NLR in septic patients.

**Keywords:** Interleukin-17; CRP; NLR; sepsis

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### INTRODUCTION

Sepsis is a collection of symptoms that often occurs in patients with systemic infections associated with organ dysfunction. The state of sepsis still has a sizable proportion of the critically ill patient population. Sepsis is defined as the presence or suspicion of infection associated with a systemic response, a life-threatening condition caused by dysregulation of the body's response to infection. There is no gold standard in the definition of sepsis, so the diagnosis of sepsis is based on a combination of physiology and laboratory abnormalities. The definition of sepsis itself changed at

international meetings in 1991, 2001, and, most recently, in 2016. The current recommendations and definitions of sepsis are based on organ dysfunction where a total SOFA score of 2 points is an indication of infection<sup>1</sup>.

The incidence of sepsis in the United States is about 300 cases per 100,000 population, 50% of whom undergo treatment in the Intensive Care Unit (ICU)<sup>2</sup>.

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**Table 1.** Subjects Characteristic Data

Parameter	n (%)	Mean±SD	Median (min;max)	p*
Gender				
Male	19 (47.5)			
Female	21 (52.5)			
Age (year)		54.18±13.25	56 (19;70)	0.316
BMI		23.24±4.82	22.8 (12.8;34.52)	0.244
Hemoglobin (g/dL)		11.01±2.97	11.5 (2.9;14.9)	0.006
Leukocyte (10 <sup>3</sup> /uL)		16.45±9.08	14.75 (2;38)	0.304
Platelets (10 <sup>3</sup> /uL)		287.42±166.5	277 (35;626)	0.186
Neutrophil absolute count (10 <sup>3</sup> /uL)		13.7±7.85	12.2 (1.34;32.97)	0.087
Lymphocyte absolute count (10 <sup>3</sup> /uL)		1.39±1.29	1.09 (0.08;5.48)	0.001
NLR		16.57±13.70	13.51 (2.26;46.5)	0.001
IL-17 (pg/mL)		549.60±489.84	337.95 (11.4;1695.80)	0.001
CRP (mg/L)		17.6±14.97	13.25 (0.43;53.87)	0.001
SOFA score		9.74±3.10	9 (4;17)	0.318
Comorbidity				
Diabetes mellitus	8 (20)			
Hypertension	5 (12.5)			
Positive culture	15 (37.5)			
Gram (+) bacteria	6 (40)			
Gram (-) bacteria	9 (60)			

Note: BMI: Body Mass Index. SD: Standard Deviation. NLR: Neutrophil Lymphocyte Ratio. CRP: C-Reactive Protein. SOFA: Sequential Organ Failure Assessment. \* Saphiro Wilk

**Table 2.** Correlation between IL-17 with CRP and NLR

Variable	IL-17 (pg/mL)	
	p	r <sup>s</sup>
CRP (mg/L)	0.019*	0.37
NLR	0.425	0.13

IL-17: Interleukin-17, CRP: C-Reactive Protein, NLR: Neutrophil Lymphocyte Ratio; <sup>s</sup>Spearman Rank correlation test; \*p < 0.05

The epidemiological trend of sepsis in Spain shows that the incidence of sepsis is increasing from 3.3 per 1,000 population in 2000 to 4.45 in 2013, with an increase in mortality from 6.34 to 7.89 per 1,000 population<sup>3</sup>. Research in Asia (2009) from 150 intensive care patients in 16 countries (including Indonesia) showed severe sepsis and septic shock accounted for 10.9% of treatment diagnoses intensive care, with a mortality rate of 44.5%. In Indonesia, data from Cipto Mangunkusumo Hospital (2012), sepsis and septic shock occurred in 27% of patients in the ICU, with a mortality rate of 47.8%. The pathogenesis of sepsis begins with the presence of infectious agents that enter a person's blood circulation causing a systemic inflammatory state, but the presence of pathogens in the systemic circulation is not always present in cases of sepsis. The presence of inflammatory mediators released systemically can induce sepsis while the cause of sepsis itself can be various infectious agents, including bacteria, fungi, parasites, and viruses<sup>4</sup>.

Interleukin-17 is a proinflammatory cytokine produced by T helper-17, Natural Killer (NK) cells, CD-8 T cells, neutrophils, and several other cells. IL-17 increases the production of chemokines so that it plays a significant role in the recruitment of monocytes and neutrophils to the site of inflammation. This shows the vital role of IL-17 as a proinflammatory cytokine that plays a role in infectious conditions. Neutrophil

lymphocyte ratio (NLR) has been widely used as a marker of the severity of the bacterial infection and to determine the prognosis of patients with infection. Recruitment of neutrophils to the site of infection will affect the value of NLR. Early studies in animal models induced sepsis showed that removing IL-17 was associated with a higher survival rate, but another study using mice with IL-17 receptor deficiency showed the opposite result<sup>5</sup>. Ali et al. reported that serum levels of IL-17 were associated with sepsis, where, in sepsis, IL-17 levels were 72 pg/mL compared to those without sepsis, namely 37 pg/mL with p = 0.0001. IL-17 at levels more than 45 pg/mL is reported to have a 28-day mortality risk in septic patients (p = 0.005), so it can be a predictor of mortality in sepsis<sup>6</sup>.

The state of sepsis is characterized by changes in acute phase protein levels. One of the acute phase proteins is C-reactive protein (CRP). Elevated levels of CRP are produced in response to tissue damage, infection, inflammation, and malignancy<sup>7</sup>. Yang et al. stated that the sensitivity and specificity of CRP in the diagnosis of sepsis were 90.7% and 49.7% while Pradhan et al. in their study using a CRP cut-off value of 50 mg/L obtained a sensitivity of 84.3% and a specificity of 46.15%<sup>8,9</sup>. Ranzani et al. showed that CRP is a prognostic factor for septic patients admitted to the ICU, where a CRP level of 20 mg/dl has a poor prognosis (p = 0.024)<sup>10</sup>.

Neutrophil lymphocyte ratio (NLR) is an examination that is easily obtained from routine hematological examinations. Recently increased NLR is promising as a diagnostic and prognostic marker in patients with sepsis. Research by Marik et al. showed the role of NLR in sepsis. NLR values > 10 had a sensitivity of 71% and specificity of 56% with a higher mean NLR value in positive bacterial cultures than in negative cultures<sup>11</sup>.

In sepsis, there is an increase in pro-inflammatory cytokines, one of which is IL-17 which plays a role in increasing natural, adaptive, and humoral immunity, in inflammation, an increase in acute-phase protein, namely CRP, and a response from leukocyte cells in the inflammatory process, so it is necessary to research the correlation of serum IL-17 levels with CRP levels and NLR values in septic patients.

## MATERIALS AND METHODS

This research is an analytic observational study with a cross-sectional approach which was conducted in the ICU of Dr. Kariadi Hospital and Diponegoro National Hospital Semarang from July to December 2020. The research subjects were patients with sepsis criteria (SOFA score 2) with an age range of 18–70 years. Subjects were taken by consecutive sampling that met the inclusion and exclusion criteria. Patients with a history of liver disease and chronic renal failure were not included in this study.

The research variables consisted of levels of IL-17, CRP, and NLR. Examination of IL-17 levels measured using the sandwich enzyme-linked immunoassay (ELISA) principle from Elabscience® USA, was carried out at the GAKI Laboratory of Faculty of Medicine, Universitas Diponegoro. Sample allow to clot for 1 hour before centrifugation, 100  $\mu$ L serum used for this assay. From the examination of CRP levels using the immunoturbidimetric method, the calculation of the NLR value was done manually by dividing the absolute neutrophil count by the absolute lymphocyte count obtained from the automatic hematology analyzer. Based on the normality test using the Saphiro Wilk test, IL-17, CRP, and NLR data were not normally distributed after data transformation. Then, Spearman Rank correlation test was performed. The p-value is considered significant if  $p < 0.05$ . Ethical clearance was obtained from the Health Research Ethics Committee of Dr. Kariadi General Hospital, Semarang No. 484/EC/KEPK/2020.

## RESULTS

In this study, forty patients with sepsis criteria according to SOFA criteria were included in this study, consisting of 19 (47.5%) males and 21 (52.5%) females. The mean age of the patients was  $54.18 \pm 13.25$  years with a normal mean body mass index ( $23.24 \pm 4.82$ ). The mean hemoglobin level is 11.5 g/dL, the mean leukocyte count is  $16.45 \times 10^3/\mu\text{L}$ , and the mean platelet count is  $287.42 \times 10^3/\mu\text{L}$ . The results of blood cultures from 40 subjects obtained positive blood cultures in 15 subjects (37.5%), whereas the results of gram-positive bacteria were 6 subjects (40%) and gram-negative 9 subjects (60%). The characteristics of the data are presented in Table 1.

The normality test using Saphiro Wilk found that the three variables were not normally distributed, so the Spearman Rank correlation test was carried out. The correlation between IL-17 levels with CRP and NLR is presented in Table 2.

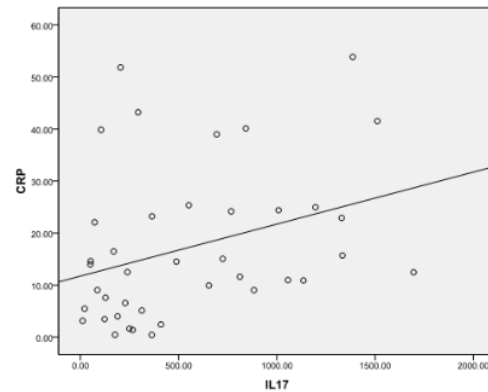


Figure 1. Correlation between IL-17 and CRP in Sepsis

There is a weak positive correlation between levels of IL-17 and CRP ( $p=0.019$ ,  $r=0.37$ ). The data distribution of IL-17 and CRP levels is shown in Figure 1. The correlation between IL-17 and NLR levels showed that the results were not statistically significant.

## DISCUSSION

The results showed that there was a positive correlation between IL-17 levels and CRP levels. C-reactive protein is a marker of inflammation routinely examined in clinical practice. The increase in CRP levels illustrates the increase in IL-17 levels in this study, which indicates an increase in proinflammatory cytokines. This is in accordance with the study of Akin et al. who reported a positive correlation between IL-17 and CRP ( $p = 0.014$ ,  $r = 0.225$ ) in patients with polymicrobial sepsis<sup>12</sup>. Comparable results were reported by the study of Zou et al. in patients with chronic obstructive pulmonary disease (COPD) and by Al-Saadani et al. in rheumatoid arthritis patients where there was a positive correlation between IL-17 levels and CRP ( $p < 0.001$ ,  $r = 0.506$ , and  $p < 0.001$ ,  $r = 0.696$ )<sup>13,14</sup>. Interleukin-17 is a cytokine produced by several cells, namely Th17 cells, T cells, NK cells, group 3 innate lymphoid cells (ILC3s), CD8+ cells (Tc17), microglia, and mast cells. The general function of IL-17 is to stimulate the production of inflammatory molecules, chemokines, and antimicrobial peptides (AMPs) such as  $\alpha$ -defensin, calgranulin, and lipocalin-2. IL-17 also functions in maintaining mucosal barrier integrity and induces acute-phase proteins. In sepsis, IL-17 stimulates various immune cells (neutrophils, lymphocytes, macrophages, monocytes, and B cells) to enhance the role of natural, adaptive, and humoral immunity. IL-17 signals play a protective role against infections caused by bacteria, fungi, viruses, and parasites through the body's defense



and release of cytokines, but IL-17 can also induce pathological immune responses and the occurrence of organ failures such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), hepatic dysfunction, immunity, and cardiomyocytes<sup>15</sup>. The results of the observation of IL-17 in this study indicate pathological immune responses, that showed a high level of IL-17. An experimental study by Marchesi et al. showed that sepsis caused by *Klebsiella pneumoniae* increased the production of IL-17 ( $p = 0.002$ ), and the highest level was reached at 48 hours after infection<sup>16</sup>. Ogiku et al. reported that mice with IL-17 deficiency showed a significant increase in mortality associated with bacteremia at 12 hours after the cecal ligation and puncture (CLP) procedure. This shows a partial protective role of IL-17 in sepsis, resulting in the positive control mice having a higher survival rate, compared to model mice that were knocked out of the IL-17 gene<sup>17</sup>. CRP is an acute-phase protein that can be used as a marker of inflammation. Li et al.'s study reported higher CRP levels in septic patients than in non-septic patients ( $p < 0.05$ ). Patients with poor clinical outcomes (non-survivors) also had higher CRP levels ( $p = 0.047$ )<sup>11</sup>.

The results of this study showed that there was no correlation between IL-17 levels and NLR. This is not following previous studies where the NLR value can be used as a marker for sepsis although, in this study, a high NLR value ( $> 5$ ) was not associated with IL-17 levels<sup>18</sup>. The results of this study are similar to those of Sag et al. which showed that there was no correlation between IL-17 levels and NLR in rheumatoid arthritis patients ( $p > 0.05$ ,  $r = 0.135$ )<sup>19</sup>. In sepsis, there can be a failure of recruitment and migration of neutrophils to the site of infection, which is related to the severity of the disease. Interleukin-17 plays a role in the migration and microbicidal activity of neutrophils, and the mobilization of T lymphocytes<sup>20</sup>. Brunialti et al. show profound lymphopenia and decreased CD4+T cell counts in septic patients, and increased percentage of IL-17-producing T helper lymphocytes in the peripheral blood of septic patients<sup>21</sup>. The increase in NLR indicates the presence of an inflammation that, in addition to sepsis, can occur in other inflammatory conditions. The comorbidity of the patients can affect the value of NLR.

Positive blood culture results in study subjects were obtained in 37.5% of patients, which is almost similar to the study by Liestiadi et al. where positive culture results were obtained in 32.6% of patients, and the prevalence of gram-negative bacteria was higher than gram-positive<sup>22</sup>. In the study, the results showed that there were no differences in the levels of IL-17, CRP, and NLR in the subjects between the cultures of gram-positive bacteria and gram-negative bacteria.

The limitation of this study was that it did not take into account comorbid factors from the patient that could affect IL-17, CRP, and NLR levels. Patients on immunosuppressant therapy and the presence of hematological abnormalities were not excluded from the study as it could affect the results of complete blood counts.

## CONCLUSION

In summary, there was a weak correlation between IL-17 levels and CRP in the septic patients. Potential biases of the study could not be avoided like small sample size, selection bias and variables in the clinical data of septic patients.

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