

# Comparison of Neutrophils-lymphocytes Ratio and Procalcitonin Parameters in Sepsis Patient Treated in Intensive Care Unit Dr. Wahidin Hospital, Makassar, Indonesia

*by* Sitti Wahyuni

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# Journal of Medical Sciences

ISSN 1682-4474

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J. Med. Sci., 17 (1): 17-21  
January-March, 2017  
DOI: 10.3923/jms.2017.17.21

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## **35** **1** Comparison of Neutrophils-lymphocytes Ratio and Procalcitonin Parameters in Sepsis Patient Treated in Intensive Care Unit Dr. Wahidin Hospital, Makassar, Indonesia

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Neutrophils-lymphocytes ratio (NLR) **40** regarded as a cheap, simple and promising parameter to predict sepsis severity. This study was **19**ed to compare NLR and procalcitonin (PCT) parameters and its correlation with acute physiology and chronic health evaluation (APACHE) II scoring system in sepsis and severe patients. This **5** prospective study investigated 65 sepsis patients data in the medical record that been treated in Intensive Care Unit (ICU) in the last 2015 at Dr. Wahidin Sudirohusodo Hospital Makassar. Data collected including neutrophil, lymphocytes, PCT and the APACHE II score. Based on **5** clinical finding and the dependency to vasopressor, the patients were divi**5**d into sepsis and severe sepsis groups. The NLR, PCT and APACHE II scores in sepsis and severe sepsis were compared, the cut off point for NLR **4** and PCT were determined and the sensitivity and specificity were calculated. Procalcitonin level was significantly different in sepsis and severe sepsis groups ( $p < 0.001$ ), the difference was not seen for NLR. The Cut off Point (COP) to distinguish between sepsis and severe sepsis for NLR was 9.05 with sensitivity and specificity 59.6 and 50.0%, **32** respectively. For PCT it was 2.89 with sensitivity and specificity **31**2 and 75.0%, respectively. There was a significant positive correlation between APACHE II score with NLR ( $p = 0.036$ ) and with PCT ( $p = 0.027$ ). Procal**11**cinis superior than NLR in distinguishing sepsis and severe sepsis, however, since a positive correlation found between APACHE II score with NLR and with PCT, NLR can be used as parameter to predict the severity of sepsis in the hospital with limited laboratory facilities.

**Key words:** Neutrophil-lymphocyte ratio, procalcitonin, APACHE II, sepsis

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## INTRODUCTION

Sepsis is a Systemic Inflammatory Response Syndrome (SIRS) that accompanied the focus of infection that well-known (defined by positive culture for organisms that come from these places)<sup>1</sup>. The focus of infection in sepsis can occur in any organ, either respiratory tract or abdomen, brain and others<sup>2</sup>.

Sepsis mortality rates are still high both in developed and in developing countries. In Europe the incidence is around 90.4 cases per 100,000 population per year with mortality 28-50%<sup>3</sup>. In Indonesia the incidence of sepsis is not well dated but in some referral hospitals the prevalence ranged from 15-37.2% among intensive care patient with 37-80% of mortality. Hospital Dr. Cipto Mangunkusumo in Jakarta reported the prevalence of sepsis among intensive care patients in 2010 was 25% with a mortality rate 77.3%<sup>4</sup>.

The SIRS and sepsis have been studied intensively by using various parameters, assessment and scoring system for diagnosis, prognosis and to monitor the improve<sup>34</sup> or worsening of sepsis patients. Clinical conditions such as temperature, heart rate, blood pressure and respiration have been used in many investigations as<sup>17</sup> parameters with or without laboratory information such as leukocytes, c-reactive prot<sup>39</sup> (CRP), procalcitonin (PCT) and interleukin-6 (IL-6)<sup>5,6</sup>.

Procalcitonin (PCT) is a pro-hormone of calcitonin contained in the human body. In sepsis the increased levels blood PCT can be used as a sepsis biomarker<sup>7</sup>. Previous study done in Shahrekord hospital, Iran showed that sensitivity and specificity of using PCT as predictor of sepsis in infants suspected se<sup>30</sup> was 87.5 and 87.4%, respectively<sup>8</sup>. Another study done in children with sepsis and se<sup>10</sup> shock showed that the rise of PCT and IL-6 levels in 12 h after admission is related to the severity of disease<sup>9</sup>. Study done in Hasan Sadikin<sup>38</sup> spital Bandung, Indonesia reported the positive association<sup>18</sup> between neutrophil-lymphocyte ratio (NLR) and the score of Sequential Organ Failure Assessment (SOFA) in patient treated in Intensive Care Unit (ICU)<sup>29</sup>. Recent study reported from China concluded that PCT has a better diagnostic value than CRP and White Blood Count (WBC) when differentiate SIRS patient with and without infection. The report also stated that the optimal cutoff point to detect infection in SIRS<sup>11</sup> was 0.47  $\mu\text{g mL}^{-1}$ .

One of the physiological responses in the immune system against systemic inflammation is an increase in the number of neutrophils and the decrease of lymphocytes number. This is due to changes in the dynamics and regulation of apoptosis in a state of systemic inflammation when compared with non-inflammatory state<sup>5</sup>. Delay of neutrophil apoptosis will result in lengthening the function of neutrophils in the inflammatory process and prolong the process of metabolic toxicelaboration<sup>12</sup>. Conversely, increased apoptosis of lymphocytes will result in reducing the number of the effector

inflammation<sup>13</sup>. Toxic metabolic and inflammatory cytokines released by activated neutrophils can cause tissue damage and organ dysfunction. Moreover, apoptosis of lymphocytes may result in the suppression of adaptive immune responses where patient becomes susceptible to nosocomial infection and invasion of opportunistic microbes which is potentially trigger a further systemic inflammatory reaction<sup>12,13</sup>.

Lymphocytopenia and neutrophil-lymphocyte ratio (NLR) was reported<sup>25</sup> to be better in predict bacteremia compared to conventional infection markers such as leukocyte count, neutrophil count and CRP in emergency<sup>14</sup> patients<sup>6</sup>. Similarly, another study also stated that NLR is better than PCT as biomarker for bacteremia and severe sepsis in the emergency room<sup>14</sup>. In addition, neutrophils-lymphocytes ratio (NLR) was reported to be higher in SIRS patient compared with patients without SIRS<sup>10</sup>.

Clinical parameter combine with supporting (laboratory) parameter are needed to diagnose and to predict the outcome of SIRS and sepsis, however, the use of supporting parameters are still hampered by the constraints of the sensitivity, specificity, practicality and also financing. A simple, cheap and available parameter is needed when working in the hospital with limited facility.

In order to find suitable marker for sepsis and to predict the outcome of sepsis patient in limited facility setting, we conducted a study that was aimed to co<sup>3</sup>mpare NLR and PCT and to correlate both parameters with acute physiology and chronic health evaluation (APACHE) II scoring system, a severity of disease classification system<sup>5</sup>.

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## MATERIALS AND METHODS

**Location, variables and population:** The study was conducted in Dr. Wahidin Sudirohusodo Hospital by using the data of SIRS patients treated in intensive care units in the last 2015. The presence of neutrophils, lymphocytes, PCT, APACHE II score, age and patient therapy information<sup>3</sup> in the medical record file were used as inclusion criteria for the study. A total<sup>9</sup> number of 65 study subjects were included in the analysis. The patients were grouped into sepsis and severe sepsis based on the clinical signs and the dependence to vasopressor.

**Determination of cut off point:** The ROC curve was used to determine the Cut Off Point (COP) of procalcitonin and neutrophils-lymphocytes<sup>13</sup> among sepsis and severe sepsis. After COP had determined, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated.

**Data analysis:** The patient data was analyzed by using Statistical Program and Service Solution (SPSS) 22. The difference of PCT and NLR in sepsis and severe sepsis was

calculated by Mann Whitney-U test, correlation between variables was calculated by Spearman's rank correlation. It was considered to be significant if  $p < 0.05$ .

**RESULTS**

A total of 65 patients mean age 42, ranged from 15-65 years old were included in the analysis. The mean, standard deviation and range of study variables presented in Table 1.

The patients were grouped into sepsis and severe sepsis and the mean of continuous variables between the groups was compared. In Table 2 it shown that the mean level of PCT was significantly different ( $p < 0.001$ ) between patient with sepsis and severe sepsis (2.2 versus 56.0). The same situation was not seen for neutrophils-lymphocytes ratio.

Area Under Curve (AUC) of NLR inspection with 0.587, interval from 0.386-0.787 and 95% significance obtained the cutoff point of 9.05 for NRL. Similarly, AUC inspection of procalcitonin 0.978, interval from 0.934-1.013 with 95% significance obtained 2.89 as the cut off point of PCT. Based on the cop of both parameters the sensitivity and specificity was calculated. In Table 3 can be seen that sensitivity and specificity of NLR was lower than NLR.

Correlation of APACHE II score with NLR and with PCT were calculated by using Spearman's correlation test and it found a significant positive correlation between APACHE II scores with NLR ( $R = 0.261$  and  $p = 0.036$ ) and with PCT ( $R = 0.275$  and  $p = 0.027$ ).

Table 1: Mean, standard deviation and range of sepsis patient treated in intensive care unit, Dr. Wahidin Sudirohusodo Hospitals in 2015

Parameters	Mean±SD	Range
Neutrophils-lymphocytes ratio	15.30±11.2	2.1-29.50
Procalcitonin level	49.30±59.9	0.5-201.0
APACHE II scores	18.74±7.26	7.0-36.00

Table 2: Comparison of parameters between sepsis and severe sepsis patients treated in Dr. Wahidin Sudirohusodo Hospital in 2015

Parameters	Sepsis stadium	N	Mean	SD	p-value
Neutrophils (%)	Sepsis	8	81.1	7.0	0.40
	Severe sepsis	57	81.6	13.0	
Lymphocytes (%)	Sepsis	8	9.1	4.6	0.32
	Severe sepsis	57	7.9	5.5	
Neutrophil-lymphocytes ratio (%)	Sepsis	8	12.5	9.4	0.43
	Severe sepsis	57	15.7	11.5	
Procalcitonin	Sepsis	8	2.2	1.7	<0.001*
	Severe sepsis	57	56.0	61.2	

\*p-value calculated by Mann Whitney-U test

Table 3: Comparison of sensitivity, specificity, positive, negative-predictive value and accuracy for predicting severe sepsis patients by using cut off point for the neutrophils-lymphocyte ratio and for procalcitonin levels

Variables	Cut off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Ratio neutrophils-lymphocyte	9.05	59.6	50.0	89.5	14.8	58.5
Procalcitonin	2.89	98.2	75.0	96.6	85.7	95.4

PPV: Positive predictive value, NPV: Negative predictive value

**DISCUSSION**

This study shown procalcitonin parameter still superior in distinguish between patient with sepsis and severe sepsis compared with neutrophils-lymphocytes ratio parameter. This result is different from previous studies which reported that NLR was superior than PCT<sup>14</sup>. One possibility to explain the difference of the result is the measurement time of neutrophils, lymphocyte and procalcitonin. Neutrophils are located in the circulation for 7-10 h before migrate to the network and can live there for several days. Neutrophils will increase in the circulation and inflamed tissue within a few hours after endotoxin is released by microbe<sup>16</sup>. The level of PCT increase quickly within 2 h after stimulation, peak after 12-48 h and slowly decreased in 48-72 h<sup>8</sup>. Another study also reported that NLR parameter is 10 times better than leukocytes, Absolute Neutrophil Count (ANC) and c-reactive protein (CRP) in predicting disease severity and outcome of the community-acquired pneumonia (CAP)<sup>6</sup>.

The immune response of leukocytes to the physiological stress such as tissue damage, severe trauma, major surgery and sepsis are characterized by an increase number of neutrophils and decrease of lymphocyte. Stress inflammation characterized by the ratio of neutrophil percentage and lymphocytes percentage in the blood and it was known as the Neutrophil Lymphocyte Stress Factor (NLSF). At the physiological condition the percentage of neutrophil/lymphocyte is less than 5. In pathological conditions due to severe infection or systemic inflammation, NLR will be increased, therefore some center use NLR for clinical evaluation of patients with systemic inflammation. Neutrophilia a phenomenon that occurs in systemic inflammation caused by demargination neutrophils, delayed neutrophil apoptosis and stimulating stem cells by growing factors (G-CSF)<sup>5</sup>. Delay neutrophil apoptosis process will result in lengthening the function of neutrophils in the inflammatory process and prolong the toxic metabolic elaboration. On the contrary, the increase lymphocyte apoptosis resulting in the decrease of inflammatory effectors and cause immunosuppression<sup>12,13</sup>.

Procalcitonin level in patient with severe sepsis was significantly higher than in patient with sepsis (56.0±61.5 versus 2.2±1.7) and we found PCT cut off point 2.89 has sensitivity and specificity 98.2 and 75.0%, respectively in predicting patient with severe sepsis. This cop is slightly higher with study reported from surgical intensive

care unit that stated procalcitonin  $>2 \mu\text{g mL}^{-1}$  was the most sensitive and specific value in diagnosing sepsis (87 and 80%, respectively)<sup>17</sup>. For NLR parameter, we did not find any difference between patient with sepsis and severe sepsis. From ROC analysis we found cut off point 9.05 can discriminate patient with sepsis and severe sepsis. However, the sensitivity and specificity were not strong (59.6 and 50.0%, respectively). Study from Netherlands reported that NLR  $>10$  has 77.2% sensitivity and 63% specificity for predicting bacteremia<sup>6</sup>. Interestingly, another study reported that NLR with cut off  $>7$  can be used as a prognostic marker in patients with bacteremia sepsis and in that study the NLR  $>7$  was an independent risk factor for mortality in sepsis patients with bacteremia<sup>18</sup>.

Acute physiology and chronic health evaluation (APACHE) II scoring system is designed to measure the severity of disease in patients admitted to the intensive care unit. The APACHE II score has been shown to be good in predict the outcome of patients admitted in the intensive care unit<sup>19</sup>. Here, we found a positive correlation between APACHE II score with NLR and with PCT levels. Although the correlation we found is weak,  $R = 0.275$  for PCT and  $R = 0.261$  for NLR but our study showed that both PCT and NLR are in line with APACHE II score when predict the severity of sepsis patient.

### CONCLUSION

Overall, this study concluded PCT superior than NLR in distinguish the degree of severity of sepsis. However, in clinical setting with limited laboratory facility the NLR can be used as predictor of sepsis severity since this parameter shown in our study to have a positive association with APACHE II scoring system.

### SIGNIFICANT STATEMENTS

- In limited facility, a cheap, simple and promising parameter to predict sepsis severity is needed<sup>2</sup>
- In modern facilities, procalcitonin (PCT) and acute physiology and chronic health evaluation (APACHE) II scoring system have been widely used
- This study compared neutrophils-leucocytes ratio (NLR), the most available parameter and PCT level in sepsis and severe sepsis patients and its correlation with APACHE II scores by using data from sepsis patients. The Cut Off Point (COP) for NLR and PCT in distinguishing sepsis and severe sepsis also determined
- We concluded NLR less sensitive than PCT in distinguishing sepsis and severe sepsis. However, since a positive correlation found between APACHE II score with NLR, the NLR COP 9.05 can be used to predict the severity of sepsis in limited laboratory facilities hospital

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