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RESEARCH

NEUTROPHIL-LYMPHOCYTE COUNT RATIO IN BACTERIAL SEPSIS

(Rasio Neutrofil-Limfosit Pada Sepsis Bakterial)

Danny Luhulima[1-3], Marwito[2], Eva O[1]

ABSTRAK

Sepsis akibat infeksi bakteri merupakan masalah kegawatdaruratan medik yang serius sehingga memerlukan penanganan cepat dan tepat. Saat ini C-RP (C- reactive protein) dan PCT (procalcitonin) sering digunakan sebagai petanda sepsis bakterial. Sepsis adalah infeksi yang disertai inflamasi sistemik. Respons fisiologis terhadap inflamasi sistemik adalah peningkatan jumlah neutrofil dan penurunan jumlah limfosit, sehingga gabungan perbandingan neutrofil dan limfosit Neutrophil Lymphocyte Count Ratio (NLCR)) dapat digunakan sebagai petanda sepsis. Penelitian ini bertujuan untuk mengetahui kepekaan dan kekhasana dari uji NLCR di pasien sepsis akibat infeksi bakteri. Terdapat 70 pasien SIRS dengan rentang usia 14–70 tahun di RS Mitra Keluarga Bekasi Timur dan RS FK - UKI Jakarta masa waktu bulan Juli–September 2015. Penelitian ini merupakan studi observasional komparatif dan potong lintang. Hasil penelitian menunjukkan uji NLCR terhadap sepsis bakterial berdasarkan kurva ROC memiliki kepekaan 97,8% dan kekhasan 84,0% pada cut off ≥6,4 (AUC: 0,94, nilai p<0,05). Neutrophil lymphocyte count ratio dapat diandalkan sebagai petanda sepsis bakterial dengan uji kepekaan dan kekhasan yang baik.

Kata kunci: *Sepsis, neutrofil, limfosit*

ABSTRACT

Sepsis due to bacterial infection is a matter of very serious medical emergency and requires prompt and proper handling. Various parameters such as CRP (C-reactive protein) and PCT (procalcitonin) levels are used as a marker of sepsis due to bacterial infection. Sepsis is a condition of systemic inflammation with infection. Physiological response to systemic inflammation determines increased the level of neutrophil and reduction of lymphocyte count, therefore combining both of parameters could be a marker in predicting sepsis. The aim of this research was to determine the sensitivity and specificity of the NLCR test in patient with sepsis due to bacterial infection. This research was conducted by observing 70 SIRS patients aged 14–70 years, at Mitra Keluarga Bekasi Timur Hospital and Faculty of Medicine Universitas Kristen Indonesia (UKI) Hospital in July-September 2015. This research was an observational comparative study with cross-sectional design. Based on ROC curve showed that NLCR test has a sensitivity of 97.8% and specificity of 84.0%, with cut off ≥6.4 (AUC: 0.94, p value <0.05). In conclusion, the NLCR is an ideal and efficient marker to diagnose sepsis due to bacterial infection with good sensitivity and specificity.

Key words: Sepsis, neutrophil, lymphocyte

INTRODUCTION

Sepsis has been a problem in the medical world, often leading to death due to late diagnosis. Therefore, there should be a marker of sepsis that aims to detect sepsis as early as possible. The ideal sepsis diagnostic markers must be: very specific and sensitive; easy to use; fast and cheap; and directly proportional to severity.[1]

Culture is the gold standard, but takes a long time. As a result, it often causes a delay in diagnosis. Currently, there have been some ideal sepsis markers, such as Procalcitonin (PCT) and C-Reactive Protein (CRP), but they are still limited, especially in

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developing countries due to high cost of examination. Consequently, a good and cheap sepsis marker that is easy to use is still needed.[1]

Systemic Inflammation Response Syndrome (SIRS) is a collection of symptoms that can be triggered by ischemia, inflammatory processes, trauma, infection, or a combination of some of them, as a result, SIRS may not always be correlated with infection.[2] Infection is an inflammatory response due to microorganisms or microorganism invasion to tissues that are supposed to be sterile. Bacteremia is the discovery of bacteria in blood. Thus, sepsis can be considered as SIRS caused by microorganisms, such as bacteria, viruses and parasites. Of all these microorganisms, the most common cause of sepsis is bacteria.[2-3]

De Jager *et al.*[4] in a retrospective study stated that neutrophil lymphocyte count ratio (NLCR) was a simple and good examination in diagnosing sepsis due to bacterial infection compared to routine parameters, such as total leukocytes and CRP.[4] Similarly, Holub *et al.*[5] explained that NLCR diagnostic test on sepsis had a sensitivity of 91% and a specificity of 96% due to bacterial infection. Other studies also suggest that C-RP has a sensitivity of 98.5% and a specificity of 75.0%, while PCT has a sensitivity of 85.0% and a specificity of 91.0%.[5–7]

Therefore, it can be said that NLCR can be considered as a fast and cheap sepsis marker that has good sensitivity and specificity. Thus, this research aimed to investigate whether NLCR can be relied upon to diagnose sepsis due to bacterial infection in adults.

METHODS

This research was conducted at Mitra Keluarga Bekasi Timur Hospital and Faculty of Medicine, Universitas Kristen Indonesia (UKI) Hospital, Jakarta. Samples of the research were SIRS patients with certain criteria. Firstly, they had to be at the age of 14 to 70 years. Secondly, they had to have two or more clinical manifestation criteria of SIRS, such as body temperature of >38˚C or <36˚C, pulse of >90 beats/min, respiratory frequency of > 20 times/min or $PaCO_2$ of <32 mmHg, leukocyte count of >12,000/mm^3 or <4,000/mm^3, or immature cell formation of >10%. They also had to be sterile from steroids as well as beta-blockers or calcium channel blockers. They did not undergo cardiopulmonary resuscitation.

Sepsis, based on the American College of Chest Physicians/Society of Critical Care Medicine Consensus in 1992, is defined as an infection, involving two or more manifestations of SIRS.[3] Therefore, there were certain criteria for receiving bacterial sepsis samples. Firstly, the samples had to meet criteria of the SIRS sample required. Secondly, positive culture result had to indicate infectious bacteria or clinically had to support bacterial sepsis.

On the other hand, there were certain criteria for receiving non-bacterial sepsis samples of SIRS. Firstly, they had to meet SIRS criteria. Secondly, culture results had to show no bacterial growth. Thirdly, there was a definite diagnosis of non-bacterial infection.

NLCR is a neutrophil and lymphocyte count ratio obtained by the following formula:

$$\text{NLCR} = \frac{\text{neutrophil (rod, segment)} + \text{immature granule}}{\text{Lymphocytes}}$$

Culture could be considered to be positive if there were causative bacteria. Next, the percentages of neutrophils and lymphocytes were calculated based on manual differential on peripheral blood smear using Wright staining and then the results were analyzed using a hematoanalyzer since the hematoanalyzer instrument cannot excrete neutrophil stem, immature granule, or young granule cells.

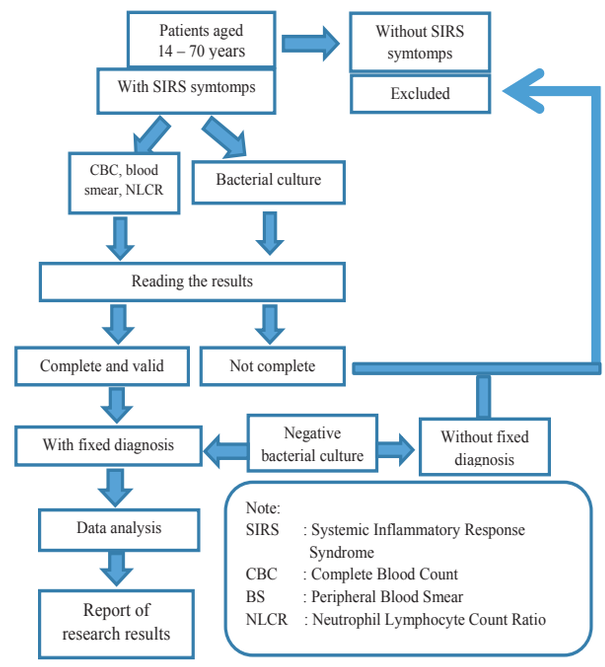


Figure 1. Research flow

The research flow can be seen in Figure 1. Data then were collected at two different hospitals, namely FK UKI Hospital Jakarta and Mitra Keluarga Bekasi Timur Hospital. Consequently, this research had to receive approval from local agencies first. The last, data

analysis was performed using Receiver Operator Curve (ROC) found on the IBM SPSS Statistic 22 application to determine the diagnostic test point in the form of a graph illustrating a bargain between sensitivity and specificity.

RESULTS AND DISCUSSION

There were 70 samples collected successfully. However, there were only 50 samples included in the criteria of bacterial sepsis, while only 14 samples met the inclusion criteria of non-bacterial sepsis. It means that there were 6 samples excluded.

Figure 1 illustrates the distribution of the samples' age. Most of the samples were in the age ranges of 26–31 years, 50–55 years and 62–67 years.

Figure 2 illustrates the distribution of the causative bacteria in the research samples. Of the 64 samples, there were 46 samples with positive bacterial cultures, namely *Klebsiella pneumonia* (17.39%), *Escherichia coli* (17.39%), *Pseudomonas aeruginosa* (10.87%), *Staphylococcus aureus* (10.87%) and *Streptococcus pneumonia* (6.52%). Meanwhile, there were 4 samples

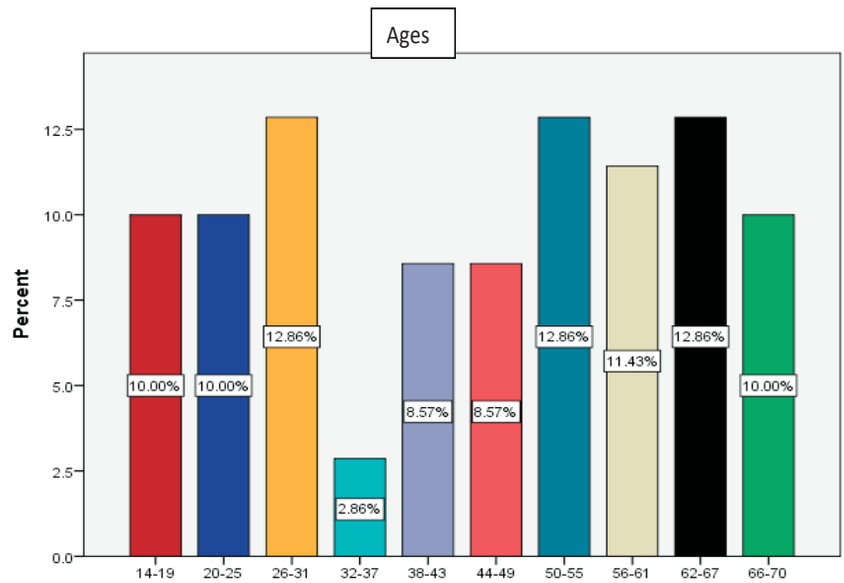


Figure 2. Distribution of the samples' age

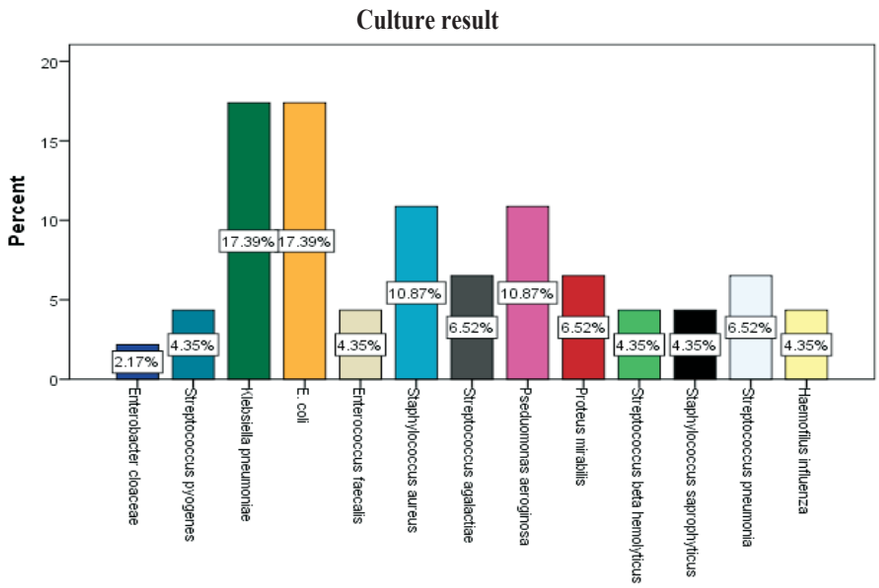


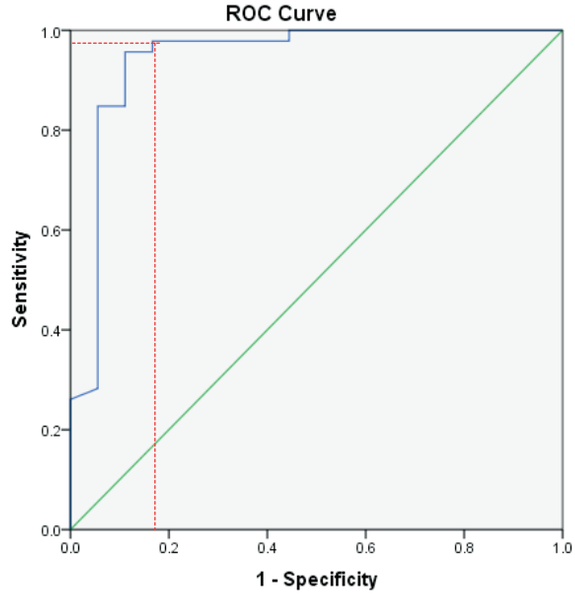
Figure 3. Culture results of the research samples

with negative culture results. Nevertheless, based on the data and clinical diagnosis, they could be included in bacterial sepsis. Thus, they were considered as inclusion samples.

Furthermore, the greatest *Klebsiella pneumonia* culture was found in the age range of 14–43 years with a percentage of 75%, whereas Escherichia coli were dominant in the age range of 41–70 years with a percentage as much as 75%. Both of these bacteria are Gram negative bacteria. This is in accordance with previous researches explaining that bacterial infection in sepsis is mostly due to Gram-negative bacteria with a percentage of 60% to 70%.[2]

However, there were 14 samples (20%) classified as negative controls, i.e. non-bacterial sepsis with certain diagnoses, such as Hemorrhagic Fever Dengue (DHF), Herpes Simplex Virus and Hepatitis B. There were also 6 samples excluded (8.57%) since given no bacterial culture examination or without unsupported clinical data.

The diagnostic test of sensitivity and specificity of NLCR in this research was performed based on the ROC curve. In graph 1, the best NLCR for patients with bacterial sepsis had a sensitivity of 97.8% and a specificity of 84.0% with an Area Under Curve (AUC) of 0.94 and a NLCR cut off of ≥6.4, $p<0.05$.



Graph 4. Curve of ROC NLCR in sepsis patients

Sepsis is an inflammatory response that is systemic due to infection (bacteria, viruses, parasites). Bacterial infection is the most common cause. Sepsis is also considered as one of the medical emergency problems, so it needs a quick, precise, and inexpensive diagnosis.[1–2]

There were 50 out of 70 research samples that matched the inclusion criteria of bacterial sepsis. Among 70 samples of sepsis research, 46 samples (65%) were obtained with positive culture result, while 4 patients (5.7%) with negative culture result. Nevertheless, the clinical data of those four patients supported the diagnosis of bacterial sepsis. Thus, they still were included in bacterial sepsis. On the other hand, there were 14 sepsis patients (20%) with non-bacterial infections. These data suggest that the greatest etiology of sepsis in this research was the presence of bacterial infection.

Similarly, Guntur[2] stated that Gram negative bacteria was the largest etiology of sepsis, about 60–70%.[2] This condition was caused by lipopolysaccharide (LPS) of Gram negative bacterial endotoxin that can directly activate cellular and humoral immune system, resulting in the development of septicemia symptoms. Lipopolysaccharide of Gram-negative bacterial endotoxin also played a role in stimulating release of proinflammatory mediators responsible for sepsis.[2]

In addition, results of the NLCR diagnostic test in this research also indicated that the best cut off of NLCR was ≥6.4 with a sensitivity of 97.8% and a specificity of 84.0%. Like this result, a previous research on bacterial infection conducted by Holub *et al.*[5] revealed that the cut-off of NLCR was ≥6.2 with a sensitivity of 91.0% and a specificity of 96.0%. Similarly, another previous research on bacterial infection in sepsis conducted by Okashah *et al.*[8] showed that the cut-off of NLCR was ≥6.2 with a sensitivity of 88.0% and a specificity of 75.0%.[8] However, the different cut-off of NLCR between this research and the two previous researches might be due to differences in sample characteristics, location and sample total.

The increased number of neutrophils in sepsis patients is due to proinflammatory cytokines, such as IL-6, IL-1 and TNF-α produced by macrophages.[1,9] On the other hand, the decreased number of lymphocytes in bacterial sepsis is caused by increased secretion of the hormone glucocorticoids that suppress the production of lymphocytes in the Lymphocytopenia gland.[10] Another theory suggests that the mechanisms responsible for the lymphocytopenia process in sepsis involve marginalitation and redistribution processes of lymphocytes in the lymphatic system as well as the acceleration of apoptotic process.[4] In sepsis, the apoptotic process has occurred since the onset of sepsis, when bacteria or products stimulate macrophages to release proapoptotic substances, such as TNF-α, Nitrite Oxide (NO) and glucocorticoids. This condition then will suppress the production of lymphocytes.

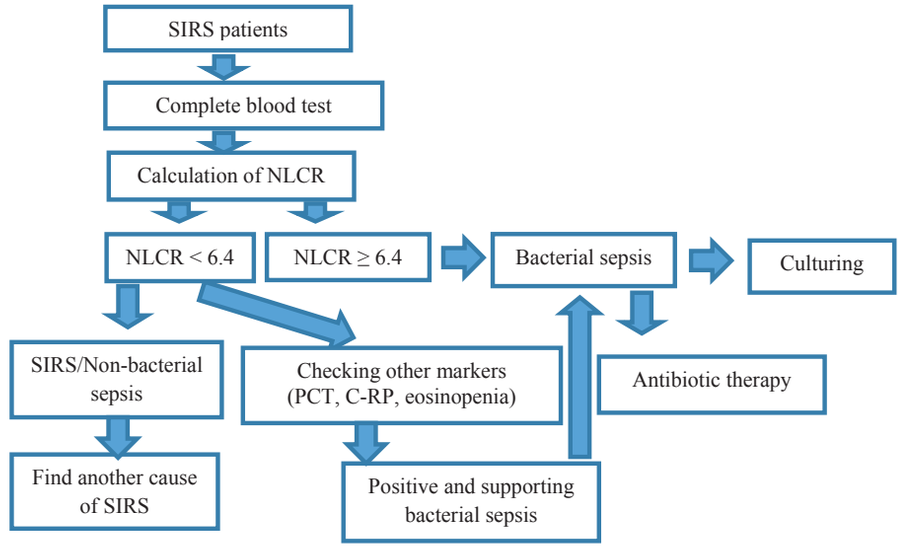


Figure 5. The flow of the use of NLCR use in bacterial sepsis

Along with disease progression in sepsis, there will be accumulation of apoptotic lymphocyte products that act an anti-inflammatory stimulus.[11]

Moreover, increased corticosteroids during stress have an immunosuppressive effect on the lymphecircular system by suppressing the function and the number of lymphocytes, ultimately decreasing the number of lymphocytes.[12] Thus, the increased number of neutrophils and the decreased number of lymphocytes can lead to an increase in the absolute ratio of neutrophils and lymphocytes compared with patients without systemic inflammatory reactions.[8]

The increased number of neutrophils in sepsis, furthermore, also adversely affects the patients since neutrophils previously suspected to be a mechanism for eradicating pathogenic germs are also likely to result in wider tissue damage due to an increase in excessive oxidant production together with an increase in proinflammatory mediators, such as TNF-α, IL- 1 and IL-6. This condition will also suppress other leucocyte series products.[13]

A previous research on patients who died of sepsis and multiple organ failure showed that sepsis could trigger a significant reduction in the number of lymphocytes through apoptosis. The reduced number of lymphocytes may be beneficial for the survival of those patients through a down-regulating mechanism of excessive inflammatory responses since lymphocytes also play a role in producing proinflammatory cytokines and activating macrophages. Besides, the reduced number of lymphocytes is also disadvantageous as impairing the ability of the immune system to fight pathogens.[14]

Another previous research conducted at Tianjin Medical University General Hospital from 2007 to 2008 stated that the percentage of $CD3^+$ and $CD4^+$ T lymphocyte counts as well as the ratio of $CD4^+$ and $CD8^+$ T lymphocyte in peripheral blood of sepsis patients were lower than in non-sepsis patients. This suggests that in septic patients, immunologic impairment occurs. The percentage of $CD4^+$ T lymphocytes in peripheral blood can illustrate the severity of the disease and can effectively predict a patient's prognosis of sepsis. The lower the percentage of $CD4^+$ T lymphocytes in peripheral blood is, the more severe the sepsis is and the worse the prognosis.[15]

In addition, a research on lymphocytes ($CD4^+$) in patients with sepsis conducted by Lestari *et al.* explained that there was a decrease in the number of lymphocytes ($p<0.001$). This decrease may be caused by an unbalanced Th2-dominated immune response suppressing Th1 activity, resulting in excessive suppression of immune responses that may affect the prognosis of sepsis patients. Neutrophils will increase in the early stages of the inflammatory response, usually with lymphocytopenia. This condition may be due to suppressing on innate immunity as supported by the data that $CD4^+$ will decrease during bacterial sepsis.[16]

Similarly, a research on 425 patients conducted by Ljungström *et al.*[17] from University of Gothenburg in Sweden revealed that NLCR was reliable as a biomarker for bacteremia by comparing PCT levels with NLCR in bacterial sepsis.[17] As a result, to use NLCR properly in hospitals, the following algorithm of sepsis diagnosis using NLCR was recommended.

If the patients had met the criteria of SIRS, they would have been recommended to immediately have a complete blood test together with manual differential. Next, NLCR was calculated. If the NLCR calculated was ≥6.4, the diagnosis of bacterial sepsis could have been enforced. Consequently, the use of antibiotics could be used rationally. At the same time, culturing was performed to reveal the causative bacteria. On the other hand, if NLCR calculated was <6.4, the possibility of bacterial infection could have been excluded. However, since the sensitivity and specificity generated still had not reached the perfect value, other bacterial sepsis markers, such as PCT, C-RP, or eosinopenia needed to be checked. If the markers increased, they still could be diagnosed as bacterial sepsis. But, if all markers did not lead to a bacterial infection, it was necessary to find another cause of SIRS in addition to bacterial infection. Nevertheless, since this research used adult samples with an age range of 14–74 years, NLCR as a marker of sepsis due to bacterial infections still needs to be studied further in children, infants and neonates.

CONCLUSION AND SUGGESTION

It can be concluded that neutrophil-lymphocyte count ratio can be considered as a reliable marker of sepsis in adults with a high sensitivity and specificity. However, NLCR needs to be studied further, especially in infants and children.

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