

Analysis of Neutrophil/Lymphocyte Ratio and Culture Results on Clinical Severity of Patients with CAP

Nurul Afiah¹, Irda Handayani¹, Nursin Abd. Kadir^{1,2}

¹ Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo, Makassar, Indonesia. E-mail: nurulafiahbarham@gmail.com

² Labuang Baji Regional Hospital, Makassar, Indonesia

ABSTRACT

Community-Acquired Pneumonia (CAP) is an acute infection with high morbidity and mortality, especially among toddlers and elders in Indonesia. Culture is the gold standard for infectious diseases, which requires a long time. Therefore, a rapid, inexpensive, easy-to-use marker such as NLR is needed. To analyze the relationship of NLR and culture results with the clinical severity of CAP. A retrospective study with a cross-sectional design was performed using secondary data from CAP patients at Dr. Wahidin Sudirohusodo Hospital from January 1st, 2018 to July 31st, 2021. The study conducted on 113 samples showed no significant differences between NLR and clinical severity of CAP (p-value of 0.071). However, the mean value of NLR in severe CAP was higher (6 ± 4.74) than in moderate CAP (2.58 ± 1.8) with no significant correlation (p-value 0.071). There was a significant difference in culture on the clinical severity of CAP (p-value 0.005). A positive correlation was found between culture and clinical severity of CAP (p-value 0.004) with weak correlation strength ($r=0.266$). NLR is an early detection marker of infection. Stimulation of growth hormone causes an increase in neutrophil count, apoptosis acceleration, and lymphocyte redistribution; therefore, increased neutrophils are common in severe clinical conditions. The insignificant relationship between NLR and clinical severity might be caused by the therapeutic intervention given. The ATS/IDSA guidelines stated that culture results were positive in 4-15%. The mean NLR value in severe CAP was slightly higher than that of moderate CAP, but no significant difference was found. There was a weak correlation between culture results and the clinical severity of CAP patients.

Keywords: NLR, culture, CAP

INTRODUCTION

Community-Acquired Pneumonia (CAP) or community pneumonia is an acute infection of the lung parenchyma (pneumonia) by various microorganisms (bacteria, viruses, fungi, or parasites), which remains a serious health problem worldwide.¹ Community-acquired pneumonia is a type of pneumonia, which is frequently found in developing countries like Indonesia and can occur in all age groups, especially toddlers and the elderly, causing high morbidity and mortality. In addition, CAP is the cause of prolonged patient stays in the hospital and requires greater treatment costs.²

Pneumonia is the single largest infectious cause of death in children worldwide. Pneumonia killed 740,180 children under the age of 5 in 2019, accounting for 14% of all deaths of children under 5 years old but 22% of all deaths in children aged 1 to 5 years. Pneumonia affects children and families everywhere, but deaths are highest in southern Asia and sub-Saharan Africa. In the USA in 2013, CAP incidence reported in adults >65 years ranged from

63 cases to 164.³ cases per 10,000 in adults >80 years. For the same period in 2013, CAP incidence ranged from 76 to 140 cases per 10,000 adults in patients >65 years in Europe.^{3,4} Pneumonia in Indonesia was included in the top 10 inpatient diseases in hospitals with the highest Crude Fatality Rate (CFR) (7.6%) in 2011.¹ Indonesia Health Profile Report in 2015 showed that pneumonia caused 15% (approximately 922,000) of under-five deaths. The DKI Jakarta Health Service estimated that 43,309 cases of pneumonia were reported in toddlers in 2019. In addition, there were around 284 cases of pneumonia in Dr. Wahidin Sudirohusodo Hospital, Makassar in 2020.¹

The most common causative bacteria of CAP are *Klebsiella pneumoniae* spp. *pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus hominis* spp. *hominis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. *Streptococcus pneumoniae* caused >90% of cases of pneumonia in adults globally. On the other hand, atypical pneumonia is due to fastidious organisms, such as *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Coxiella burnetii*, *Chlamydophila*

pneumoniae, and *Chlamydia psittaci*, representing up to 22% of all cases. In immunocompromised patients with CAP, *Enterobacteriaceae spp.*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and extended-spectrum beta-lactamase positive are more frequent.⁴ Pneumonia has three types of transmission, such as aspiration, aerosol infection, and hematogenous spread. When bacteria are inhaled into the alveoli, macrophages will eliminate them with the help of surfactant proteins A and D, which serve as anti-bacterials. Bacteria are eliminated through the mucociliary system; however, if this defense system fails, alveolar macrophages initiate an inflammatory response to enhance the lower respiratory tract defense system. The inflammatory response triggers the release of inflammatory mediators such as IL and TNF, which cause fever. Chemokines such as IL-8 and granulocyte colony-stimulating factors can stimulate the release of neutrophils and peripheral leukocytes in the lungs, which increase purulent secretion. The release of inflammatory mediators causes alveolar capillary leakage, which causes tightness, crackles on auscultation, and infiltrates on radiological imaging.⁵

The diagnosis of CAP, however, still uses culture as the gold standard. Unfortunately, culture takes a long time, making it important to use other markers, which can be used quickly to predict possible causes of infection to assist clinicians in making decisions on the management of CAP patients as quickly as possible.

Various studies have been carried out previously to analyze the relationship between NLR and CAP such as research by Yullyantara *et al.*, which suggested that NLR was a risk factor for bacteremia infection, with an optimal cut-off value of 4.67 and odds ratio of 3.24 for the relationship between NLR and the incidence of bacterial infection. Another study by Nuhasna showed a cut-off Neutrophil/Lymphocyte Ratio (NLR) of 1.3 with a sensitivity of 80.5%, a specificity of 26.9%, a positive predictive value of NLR of 33%, and a negative predictive value of NLR of 74%.⁶ Another study by Elvira obtained an average NLR value of 9.9 and 39.5% of subjects had NLR <4.67, indicating that high NLR was associated with the mortality rate of CAP patients.⁷

Amir *et al.* investigated the usefulness of NLR as a biomarker of Bacterial Community-Acquired Pneumonia (BCAP) severity. The results were that 79 patients with BCAP had NLR increased in 68.4% of patients. The average NLR score was 8.27 ± 7 . NLR levels are higher in diabetics and are significantly associated with CRP and WBC. There is no

relationship between NLR, CURB-65 score, and BCAP results. However, NLR is higher in hypoxic patients and in those with bilateral infiltrates.⁸

Based on the background above, the authors were interested to perform a study to analyze the relationship between NLR and culture results on the clinical severity of new CAP patients to predict the incidence of CAP. It was expected that this would be a useful instrument for clinicians in the immediate management of CAP patients. In addition, there was still a limited number of studies on this topic, especially in Makassar.

METHODS

This study was a retrospective study performed in October 2021 with a cross-sectional design using secondary data from medical records of patients at the Dr. Wahidin Sudirohusodo Hospital from January 1 2018 to July 31, 2021. The study population was all inpatients diagnosed with CAP by clinicians and had met the inclusion criteria. Patients who did not have the results of routine blood tests or cultures and a history of infection caused by viruses were excluded.

Research permission was obtained from the Ethics Commission for Health Research, Faculty of Medicine, Hasanuddin University, and Dr. Wahidin Sudirohusodo Hospital, Makassar with number: 629/UN4.6.4.5.31/PP36/2021. Data were processed by statistical tests using Statistical Product and Service Solutions (SPSS) and presented as narrative analysis, tables, and graphs.

RESULTS AND DISCUSSIONS

The results showed that of the 113 subjects, there were 54 (47.8%) males and 59 (52.2%) females with an age ranging from 1 year to >65 years, with the most age being 1-11 years, which was found in 42 (37.2%) subjects. A total of 74 (65.5%) subjects had moderate clinical and 39 (34.5%) subjects had a severe clinical state. The specimen used for blood culture in this study consisted of 85 (75.2%) blood, 20 (17.7%) sputum, 6 (5.3%) bronchial washings, and 2 (1.8%) pleural fluid. The type of bacteria found in this study were Gram-negative bacteria in 30 (26.6%) subjects and Gram-positive bacteria in 19 (16.8%) subjects (Table 1).

Based on Table 2, Kolmogorov-Smirnov normality test results showed that data had normal distribution and the Independent T-test showed that there was no significant difference in NLR between moderate and severe CAP with a p-value of 0.071 (> 0.05). However, it was found that the mean NLR value

was higher (6 ± 4.74) in severe CAP compared to that of moderate CAP (2.58 ± 1.8).

Table 1. Characteristics of research subjects

Variable	Total n (%)
Gender	
Males	54 (47.8%)
Females	59 (52.2%)
Age	
1-11 years old	42 (37.2%)
12-25 years old	14 (12.4%)
25-55 years old	26 (23%)
56-65 years old	11 (9.7%)
> 65 years old	20 (17.7%)
Clinical severity	
Severe	39 (34.5%)
Moderate	74 (65.5%)
The specimen used for culture	
Blood	85 (75.2%)
Sputum	20 (17.7%)
Bronchoalveolar lavage	6 (5.3%)
Pleural fluid	2 (1.8%)
Culture results	
Positive result	49 (43.4%)
Negative result	64 (56.6%)
Type of bacteria	
Gram-positive	19 (16.8%)
Gram-negative	30 (26.6%)

Source: Secondary data, age group based on Department of Health (2009)

Table 2. Analysis of NLR on the clinical severity of CAP

Variable	NLR	p-value
Moderate	2.58 ± 1.8	0.071*
Severe	6 ± 4.74	

*Independent T-test ($p > 0.05$)

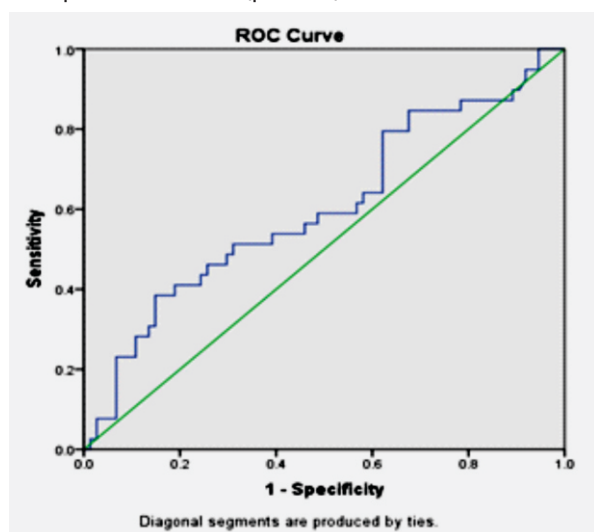


Figure 1. ROC curve of NLR on the clinical severity of CAP

Based on the Receiver Operating Characteristics (ROC) curve, a weak discrimination Area Under Curve (AUC) value of 0.602 (60.2%) with an NLR cut-off of 6.74 (41% sensitivity and 81.1% specificity) was obtained. The $\text{NLR} \geq 6.74$ was categorized as severe CAP and $\text{NLR} < 6.74$ was categorized as moderate CAP (Figure 1).

Based on Table 3, it was found that there was no significant difference in NLR values for the positive culture and negative culture groups with a p-value of 0.474 ($p > 0.005$).

Table 3. Analysis of NLR on culture

Variable	NLR Mean $\pm$ SD	p-value
Positive culture	4.58 ± 3.7	0.474*
Negative culture	4.87 ± 4.8	

Note: *Independent T-test ($p > 0.05$)

Based on Table 4, it was found that there was a significant difference in culture on CAP with moderate clinical and severe clinical state with a p-value of 0.005 (< 0.05).

Table 4. Analysis of culture to the clinical severity of CAP

Variable	Moderate n (%)	Severe n (%)	p-value
Negative culture	49 (66.22%)	15 (38.46%)	0.005*
Positive culture	25 (33.78%)	24 (61.54%)	

*Chi-Square test ($p > 0.05$)

Based on Table 5, it was found that there was a significant correlation between culture and CAP with a p-value of 0.004 (< 0.05) with a correlation strength of 0.266 (weak).

Table 5. correlation between culture and clinical severity of CAP

Variable	n	r	p-value
Culture and clinical severity	113	0.266	0.004*

* Spearman correlation test ($p > 0.05$)

Table 1 shows that there was no significant difference in the gender distribution of research subjects, which consisted of 59 (52.2%) females and 54 (47.8%) males. The common age range of the subjects in this study was 1-11 years, which was found in 42 (37.2%) subjects. Pneumonia in Indonesia causes death in infants, toddlers, and the elderly. This study found that most bacteria isolated from patients' specimen was Gram-negative. The type of bacteria in this study was in line with a retrospective study by Nurhasanah, which suggested NLR as a predictor of bacterial infection, with Gram-negative

bacteria as the most common bacteria.^{6,9}

There was no significant difference between NLR with moderate and severe clinical degrees of CAP with a p-value of 0.071 (> 0.05) (Table 2). Figure 1 shows an AUC value of 0.602 (60.2%) with a cut-off NLR of 6.74 (sensitivity 41% and specificity 81.1%). The results of a cohort study by Moozadah *et al.* found that the normal NLR value was 1.70. The cut-off value for NLR in predicting CAP patients was 2.54 with a sensitivity of 83% and a specificity of 44% ($p < 0.05$). In patients who had a poor prognosis or were admitted to the ICU and/or died, an NLR cut-off of ≥ 10 was obtained. The NLR is a marker of infection that can be used as a predictor of bacterial infections. This occurs because delayed apoptosis and growth hormone are stimulated by stem cells resulting in an increase in neutrophils and acceleration of apoptosis accompanied by a redistribution of lymphocytes, thus increasing neutrophils number.¹⁰

Based on Table 3, it was found that there was a significant difference between NLR for culture and clinical severity of moderate and severe CAP with a p-value of 0.008 (> 0.05), which indicated that there were at least two groups that had different mean NLR values. Determination of the clinical degree of CAP used in this study was based on the CURB-65 prediction score for the classification of adult pneumonia: decreased awareness of urea > 20 mg/dL, respiratory rate ≥ 30 times/minute, blood pressure (systolic < 90 mmHg or diastolic ≤ 60 mmHg); each was given 1 score with a total score of 5, consisting of 0-1 low severity, 2 moderate severity, 3 or more indicated high severity. The clinical severity of pneumonia in children according to WHO is distinguished into mild and severe pneumonia. Mild pneumonia is characterized by coughing and shortness of breath. The indicator of shortness of breath in children aged 2-11 months is > 50 times/minute and in children aged 1-5 years is > 40 times/minute. Severe pneumonia is characterized by coughing and/or shortness of breath plus at least one of the convulsions, nostril breathing, subcostal chest wall indrawing, and chest X-ray showing pneumonia.^{11,12}

Based on Table 4, there was a significant difference in culture results between moderate and severe CAP with a p-value of 0.005 (< 0.05). In Table 5 it was found that there was a significant correlation between culture and clinical severity of CAP with a p-value of 0.004 (< 0.05) despite the weak correlation ($r = 0.266$). The American Thoracic Society/ Infectious Disease Society of America (ATS/IDSA) guidelines

state that blood cultures show positive results in 4-15% of CAP patients who are hospitalized.¹³ This is in line with a study by de Mangou *et al.*¹⁴ The microorganism(s) responsible for severe CAP were identified in 67% of cases. The most frequently isolated microorganisms were *Influenza* (21.9%), *S.pneumoniae* (12%), *Staphylococcus spp.* (10.8%), *Enterobacteriaceae* (9.8%), and *H.influenzae* (7.5%). A study by Suryawan about culture identification of the sputum specimens showed that 3.4% and 96.6% of the isolates were Gram-positive and Gram-negative, respectively. Isolated Gram-positive bacteria were *Staphylococcus aureus*, *Streptococcus parasanguinis*, and *Enterococcus faecalis* (1.1%, respectively). Meanwhile, isolated Gram-negative bacteria consisted of *Pseudomonas aeruginosa* (27.6%), *Klebsiella pneumoniae* (26.4%), *Acinetobacter baumannii* (20.7%), *Escherichia coli* (12.6%) and other Gram-negative bacteria such as *Proteus mirabilis* (3.4%), *Enterobacter cloacae* (2.3%), *Stenothropomonas malthophilia*, *Shigella spp.* and *Burkholderia cepasia* (1.1%), respectively. *Non-Enterobacteriaceae* were Gram-negative bacteria that caused most of the pneumonia cases (52.4%). Among all of the isolated bacteria, 56.3% were resistant to more than two classes of antibiotics or Multi-Drug Resistant (MDR) bacteria.^{14,15}

CONCLUSIONS AND SUGGESTIONS

Based on the results of this study, it was concluded that: The clinical mean NLR value was slightly higher in severe CAP compared to that of moderate CAP, although no significant difference was found; There was a weak correlation between culture results and the clinical severity of CAP patients

Further studies were needed to assess NLR in a cohort design with a larger number of samples to compare the control group in non-CAP infected patients with CAP patients to avoid the research bias found in this study.

REFERENCES

1. Perhimpunan Dokter Paru Indonesia (PDPI). 2020. Pers release PDPI outbreak pneumonia di Tiongkok. 2020; 3-1.
2. Cataudela E, Giraffa CM, Marca SD, Pulvirenti A, Alaimo S, *et al.* Neutrophil to lymphocyte ratio: An emerging marker predicting prognosis in elder adults with community-acquired pneumoniae. JAGS 2017 The American Geriatrics Society, 2017; 5-1.
3. World Health Organization (WHO). Overview:

- Epidemiologi pneumonia pada anak. 2019. Available from: <https://www.who.int/news-room/fact-sheets/detail/pneumonia> (accessed Feb 3, 2023).
4. Tejada S, Romero A, Rello J. Community-acquired pneumonia in adults: What's new focusing on epidemiology, microorganisms, and diagnosis?. *Erciyes Med J*, 2018; 40(4): 177-82.
5. McCance KL, Huether SE. Pathophysiology the biologic basis for disease in adults and children. Eds. Julia L. Rogers, 9th Ed., Canada, Elsevier, 2018; 1186-1188.
6. Saputra YI, Gustawan W, Dwilingga MG, Arhana BNP, *et al*. Neutrophil to Lymphocyte Count Ratio (NLCR) as a risk factor for bloodstream infection in pediatric ward Sanglah Hospital Denpasar. *Sari Pediatri*, 2019; 20(6): 359-354.
7. Sari EF, Rumende CM, Harimurti K, Elvira D, *et al*. Correlation of Leukocytes concentration and neutrophil-lymphocyte ratio with serum interleukin- 17 level in geriatric subjects suffering community pneumonia. Padang, Andalas University, 2016; 4-1
8. Amir T, Toujani S, Khaled SB, Slim A, Hedhli A, *et al*. The neutrophil-lymphocyte count ratio in patients with community-acquired pneumonia. *European Respiratory Journal*, 2018; 6(52): PA2620.
9. Nurhasanah Lubis, Ayodhia Pitaloka Pasaribu, Bugis Mardina Lubis. Rasio neutrofil limfosit sebagai prediktor infeksi bakteri pada anak. Medan, Ilmu Kesehatan Anak USU, 2019; 29-24.
10. Moosazadeh M, Maleki I, Alizadeh R, Kheradmand M, Shamshirian A, *et al*. Normal values of neutrophil to lymphocyte ratio, lymphocyte to monocyte ratio and platelet to lymphocyte ratio among Iranian population: Results of Tabari cohort. *Caspian Journal Internal Medicine*, Mazandaran University: Iran, 2019; 10(3): 325-320.
11. Riqqoh AN, Dwiputri LA. Hubungan prokalsitonin dan rasio neutrofil limfosit dengan angka mortalitas pasien pneumonia anak di PICU RSUP. Haji Adam Malik Medan tahun 2018-2019. *USU, Medan. Saripediatri*, 2021; 23(6): 23-19.
12. World Health Organization (WHO). Pneumonia pada anak. 2019. Available from: <https://www.who.int/news-room/fact-sheets/detail/pneumonia> (accessed Octo 1, 2021).
13. Mendel LA, Wuderink RG, Anzueto A, Bartlett JG, Campbell GD, *et al*. Infectious disease society of America/American thoracic society consensus guidelines on the management of community-acquired pneumonia in adults. *Clinical Infectious Disease (CID)*, 2017; 44: 527-72.
14. de Mangou A, Combe A, Coolen-Allou N, Miltgen G, Traversier N, *et al*. Severe community-acquired pneumonia in Reunion Island: Epidemiological, clinical, and microbiological characteristics, 2016–2018. United States. *PLos One*, 2022; 17(4): e0267184.
15. Suryawan K, Iswari IS, Tarini A, Fatmawati D, Budayanti S, *et al*. Bacterial and antibiotics sensitivity patterns among pneumonia patients at Sanglah Hospital Denpasar, Bali. *International Journal of Medical Reviews and Case Reports (Article in Press)*. Denpasar, 2020; 3-4.