Original Article

Differences in White Blood Cells, Neutrophil-to-Lymphocyte Ratio, Tumor Necrosis Factor-α based on Procalcitonin Level in Community-Acquired Pneumonia Patients

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Abstract

Background: Community-acquired pneumonia (CAP) is a respiratory infectious disease caused by bacteria, viruses, or fungi. Procalcitonin (PCT) levels will rise, especially in bacterial infection. PCT examination in CAP can help to confirm the diagnosis and anticipate complications. CAP is diagnosed by symptoms, vital signs, laboratory tests, and radiographic investigations. Inflammatory biomarkers are required for predicting causative microorganisms, guiding antibiotic therapy, and determining severity. The purpose of this study is to compare NLR, WBC, and TNF-α levels in CAP patients dependent on PCT level.

Methods: This was an analytical cross-sectional study involving 43 CAP patients admitted to Universitas Sebelas Maret (UNS) Hospital and Moewardi Regional General Hospital Surakarta between February and March 2023. Patients were separated into two groups based on PCT levels: those with levels ≤0.12 ng/mL and >0.12 ng/mL. All patients provided a blood sample for NLR, WBC, TNF-α, and PCT testing. The independent t-test and Mann-Whitney tests were performed for statistical analysis of two unpaired groups, and the Chi-square or Fisher exact test was utilized for ordinal categorical data. If the value of $P<0.05$, the result is statistically significant.

Results: NLR and TNF-α levels were higher in the PCT group >0.12 ng/mL and statistically significant with a value of $P=0.001$. WBC levels in the PCT group >0.12 ng/mL were higher, but the difference was not statistically significant ($P=0.096$).

Conclusion: The NLR value, WBC, and TNF-α levels were found to be higher in the group with PCT >0.12 ng/mL.

Keywords: community-acquired pneumonia, NLR, procalcitonin, TNF-α, WBC

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INTRODUCTION

Community-acquired pneumonia is a leading cause of death and a major public health concern worldwide. Pneumonia is a lung infection and inflammation caused by microorganisms such as bacteria, viruses, and fungi.[1] The presence of CAP is indicated by the presence of a pulmonary infiltrate shadow on a chest X-ray (CXR), as well as any symptoms such as cough, phlegm, fever, dyspnea, and chest pain.[2]

It can be difficult to diagnose CAP because viruses, fungi, and mycobacteria can all cause pneumonia, though bacteria are the most common culprits. Only antibiotics are required to treat bacterial CAP, not viral CAP. However, symptoms and vital signs are not specific for CAP diagnosis; thus, we used inflammatory biomarkers to differentiate bacterial CAP from viral and non-infectious respiratory diseases.[2]

In response to inflammatory stimuli, several cell types from different organs secrete procalcitonin (PCT). In 2005, the US Food and Drug Administration (FDA) approved PCT as a diagnostic tool for sepsis. A level of PCT above 0.1 ng/mL suggests a bacterial infection requiring antibiotic treatment, but a PCT concentration above 0.5 ng/mL indicates a risk of severe sepsis or septic shock.[1]

Procalcitonin has been proposed in recent years as a specific and early biomarker to identify

systemic bacterial infections.[3] An increase in PCT levels due to bacterial infection indicates immune activity in the overall body. Procalcitonin levels do not increase after viral infection. Studies have shown that procalcitonin and neutrophil-to-lymphocyte ratio (NLR) have comparable diagnostic accuracy in bacterial infections.[1]

White blood cells (WBC) are widely available inflammatory biomarkers of low to intermediate value for the prediction of microbial patterns and disease severity in CAP.[4] The WBC count is generally expected to rise in response to infection and has long been used by clinicians to help diagnose pneumonia, determine its etiology, and predict patient outcomes. An elevated WBC count is a characteristic finding in pneumococcal pneumonia.[5]

Neutrophil-to-Lymphocyte Ratio, which is an easily measurable index, is the ratio of absolute neutrophil count to absolute lymphocyte count. Under pathological stress, the number of neutrophils increases, whereas the number of lymphocytes decreases.[6] Research by Cil et al in 2022 showed that NLR was found to be significantly higher in the group with elevated procalcitonin in CAP.[1] Studies have shown that PCT and NLR have equivalent diagnostic accuracy in bacterial infections.[1]

Tumor necrosis factor-α (TNF-α) is an important proinflammatory cytokine in inflammatory and immune responses. Overproduction of TNF-α has been linked to lung damage in various diseases, as well as increased risk of sepsis.[7] The degree of lung damage is closely related to serum TNF-α levels.[8] In terms of PCT, increased TNF-α leads to patients presenting with elevated PCT.[7] Classification of CAP risk and assessment of prognosis remains difficult.[9] Limitations in detecting specific pathogens that cause CAP remain unresolved due to the large variety of pathogens and difficulty in obtaining reliably representative samples.[10]

Common biomarkers such as C-reactive protein (CRP) and PCT are commonly used to identify patients with CAP at high risk of death and to guide treatment with antibiotics, preferably in conjunction with a clinical risk score. However, these two biomarkers do not always perform well, highlighting the need for biomarker improvement.[9] The aim of this study was to see if there were differences in concentrations of leukocytes, NLR, and TNF or not, depending on the level of PCT in patients with CAP.

METHODS

This cross-sectional analytical study was conducted from February to March 2023 at UNS Hospital and Dr. Moewardi Regional General Hospital in Surakarta, Indonesia. The Medical Research Ethics Committee No. 71/I/HREC/2023 issued by Dr Moewardi Regional General Hospital Surakarta approved this study.

This study included 43 CAP patients divided into two groups: those with normal PCT levels (PCT ≤1.2 ng/mL) and those with elevated PCT levels (PCT >1.2 ng/mL). The Indonesian Society of Respirology (ISR) or *Perhimpunan Dokter Paru Indonesia* (PDPI) diagnostic guideline for CAP is as follows: If a CXR shows air bronchial infiltration/appearance with some symptoms such as cough, change in sputum characteristics, temperature 38°C or history of fever, chest pain, difficulty in breathing, and on examination there are physical signs of bronchial breath sounds or rhonchi, WBC levels above 10,000 or below 4500.[11]

Once the number of subjects was achieved, PCT and TNF-α testing were performed simultaneously. The blood sample obtained was then centrifuged to obtain serum, which was then stored in a refrigerator at −80°C. TNF and PCT concentrations were determined using enzyme-linked immunosorbent assay (ELISA), while routine blood tests (WBC, neutrophils, and lymphocytes) were performed using Sysmex XN-1000. Human TNF-α ELISA Test Kit (DE75111) (Demeditec Diagnostics GmbH, Kiel, Germany) and Human Procalcitonin ELISA Test Kit (E-EL-H1492) (Elabscience Biotechnology Co., Ltd., Wuhan, China) were used for quantitative detection.

SPSS software version 22.0 was used to analyze data for this study. Categorical data are presented as frequency distribution (%), while numerical data are presented as median (min-max) and mean standard deviation (SD).

Statistical analysis using various tests. Independent t-tests were used to analyze numerical data from matched groups that met the assumption of normality but did not meet the assumption of normality or ordinal data using the Mann-Whitney test. Chi-squared test or Fisher Exact test was used to analyze unpaired categorical data. If the value of *P*<0.05, the analysis results are statistically significant.

RESULTS

According to the findings of this study, the mean age of the 43 CAP patients was 64.65±11.63, with a minimum age of 38 years and a maximum age of 83 years. The average PSI of the patients was 69.79, with a standard deviation of 12.60 (69.79±12.60), ranging from 48 to 100.

Table 1. Description of age and blood examination of patients

Parameter	Mean	SD	Min	Max
Age	64.65	11.63	38.00	86.00
PSI	69.79	12.60	48.00	100.00
Blood examination				
PCT (ng/mL)	0.87	1.16	0.01	4.62
WBC (cells/mm^3)	12071.40	5478.98	5030.00	32680.00
NLR	7.59	5.51	2.09	29.40
TNR-α (pg/mL)	14.85	15.42	2.76	70.30

Note: PSI=pneumonia severity index; PCT=procalcitonin; WBC=white blood cells; NLR=neutrophil-to-lymphocyte ratio; TNR-α=tumor necrosis factor-α

Patients were divided into 23 females (53.5%) and 20 males (46.5%). Tables 1 and 2 describe the patient demographics and laboratory results.

Table 2. Description of gender and comorbidity of patients

Parameter	n	%
Gender		
Male	20	46.5
Female	23	53.5
Comorbidity		
Asthma	2	4.7
Congestive heart failure	13	30.2
Chronic kidney disease	1	2.3
Diabetes mellitus	5	11.6
Hyperthyroid	1	2.3
Hypertension	8	18.6
Chronic obstructive pulmonary disease	7	16.3
None	6	14.0

The normal PCT group had a lower median WBC of 9000 cells/mm^3, with a minimum of 6300 cells/mm^3 and a maximum of 15700 cells/mm^3 than the elevated PCT group, which had a median WBC of 11855 cells/mm^3, with a minimum of 5030 cells/mm3 and a maximum of 32680 cells/mm3. The median NLR in the normal PCT group was lower, at 4.80 with a minimum of 2.09 and a maximum of 7.80 than in the elevated PCT group, at 7.60 with a minimum of 4.00 and a maximum of 29.40. The median TNF-α in the normal PCT group was 7.31 pg/mL, with a range of 2.76 pg/mL to 26.61 pg/mL, compared to 10.59 pg/mL in the elevated PCT group, with a range of 4.03 pg/mL to 70.30 pg/mL (Table 3).

White blood count results on normal PCT and elevated PCT groups did not show a significant difference with a *P*-value of 0.096, indicating that normal and high PCT were not related to WBC parameters.

Table 3. Description of demographic characteristics and blood examination between normal PCT group and elevated PCT group

Parameters	Normal PCT group (PCT ≤0.12 ng/mL) (n=17)	Elevated PCT group (PCT >0.12 ng/mL) (n=26)	*P*
Age[a]	62.94±13.11	65.77±10.86	0.442
Gender (M/F)[c]	8/9	12/4	0.954
PSI[a]	68.88±12.73	70.38±12.73	0.707
Blood examination mean (min-max)			
WBC[b] (cells/mm^3)	9,000.00 (6,300.00–15,700.00)	11,855.00 (5,030.00–32,680.00)	0.096
NLR[b]	4.80 (2.09–7.80)	7.60 (4.00–29.40)	<0.001*
TNF-α[b] (pg/mL)	7.31 (2.76–26.61)	10.59 (4.03–70.30)	0.020*

Note: [a]Independent t-test (numerical data meets normality assumptions);
[b]Mann-Whitney test (numerical data does not meet the assumptions of normality or ordinal data);
[c]Chi square test/fisher exact test (ordinal categorical data),
*Significant if *P*<0.05; M=male; F=female

The NLR and TNF-α values differed significantly between the normal and elevated PCT groups, with values of *P*=0.001 and *P*=0.020, respectively, indicating that normal and high PCT were associated with NLR and TNF-α parameters. Table 3 gives a detailed description of the demographic characteristics and blood tests performed on the normal PCT and elevated PCT groups.

DISCUSSION

Pneumonia is an infection and inflammation of the lung parenchyma caused by microorganisms such as bacteria, viruses, and fungi.[1] Reliable prediction of CAP patients may significantly improve patient management, antibacterial therapy, and management intervention.[9] Although CRP levels rise in response to inflammation, CRP cannot distinguish between bacterial and non-bacterial inflammation.[1] On the other hand, elevated PCT levels caused by bacterial infections indicate the organism's overall immunological activity. PCT levels do not rise as a result of viral infections.[1]

Numerous studies have been conducted to investigate PCT's potential role as a prognostic biomarker. They discovered that as the severity of sepsis and organ dysfunction increases, so do PCT levels. Multiple studies have shown that PCT levels can predict pneumonia severity and, depending on the magnitude of the survival outcome. PCT has been proposed as a prognostic factor rather than a diagnostic factor in CAP patients. Christ-Crain et al discovered that as CAP severity was measured by PSI score, PCT levels gradually increased (*P*=0.001).[12]

In daily clinical practice, "old" infection markers like CRP, WBC count, and neutrophil count are still widely used. Even though newly introduced infection markers such as PCT, several cytokines, and markers such as endothelin-1, copeptin, and pro-adrenomedullin show promising results in risk assessment and prediction, implementation of these "new" infection markers is hindered by confirmation, cost, and accessibility. The physiological response of circulating leukocytes in various stressful situations is characterized by an increase in neutrophils and a decrease in lymphocytes.[13–15]

The findings of this study revealed that NLR and TNF-α values in the elevated PCT group were higher than those in the normal PCT group, and these differences were statistically significant with values of *P*=0.001 and *P*=0.020, respectively. This study also observed that WBC levels were higher in the elevated PCT group than in the normal PCT group, but the difference was not statistically significant (*P*=0.096).

The findings of this study are consistent with the findings of Cil et al, who studied 54 CAP patients and divided them into two groups based on PCT levels (normal and elevated). Cil et al proposed that patients with elevated PCT levels had a statistically significant high NLR.[1] The correlation test, however, did not show a relationship between PCT level and NLR; this could be due to a lack of differentiation between viral and bacterial infections, as NLR increases due to lymphopenia in viral infections while PCT does not.[1] There was no correlation test in our study.

According to the findings of Rhee et al on the relationship between CAP elimination and pneumonia burden, there was a link between NLR and pneumonia burden. Leukocytes respond to physiological stressors such as tissue damage, severe trauma, major surgery, and sepsis by increasing neutrophils and decreasing lymphocytes.[2]

The ratio of neutrophils to lymphocytes in the blood, known as the neutrophil-lymphocyte stress factor (NLSF), determines stress-induced inflammation. The NLR is lower than under normal circumstances. Since NLR increases in pathological conditions caused by severe infection or systemic inflammation, some centers use it for clinical evaluation of patients with systemic inflammation. Neutrophil leucocytosis is a phenomenon caused by neutrophil degranulation, delayed neutrophil apoptosis, and growth factor stimulation (G-CSF) of stem cells during systemic inflammation. Increased lymphocyte apoptosis, on the other hand, results in

decreased inflammation and immunosuppression.[2,16]

The accuracy of NLR in diagnosing sepsis, pneumonia, and sepsis has received much attention. Several studies have shown that NLR is at least a modest predictor of sepsis, with an AUROC ranging 0.7-0.77. Compared with other biomarkers such as CRP and PCT, NLR shows a strong association and comparable effectiveness in identifying bacterial sepsis at the point of urgent care setting.[17] Both CRP and PCT appear to be more accurate than NLR in detecting sepsis in critically ill patients.

However, there is little evidence that it can be used to determine the underlying bacterial cause. Ratageri et al stated that NLR was inferior to WBC in distinguishing viral and bacterial pneumonia.[17] Only two pediatric studies have examined the ability of NLR to differentiate between bacterial and viral pneumonia, and both concluded that it was ineffective. One plausible explanation is that NLR, regardless of the microbiological cause, affects the physiological stress of patients when they are critically ill.[17]

The WBC count is used to determine the type and severity of pneumonia. Previous research has linked a low or normal WBC count during pneumonia admission to a poor prognosis. WBC levels in the elevated PCT group were higher than in the normal PCT group in this study, but the difference was not statistically significant. The median WBC in patients with elevated PCT levels was 11,855 cells/mm^3. These findings contradicted the findings of Cil et al, who discovered significant differences in WBC based on normal and elevated PCT levels. The elevated PCT group had a median WBC of 15,400 cells/mm^3. For bacterial pneumonia, the WBC cut-off was 16,400 cells/mm^3.[1]

The WBC count in the blood is a common method for clinical infection diagnosis. It is easy to use and produces results quickly. The WBC count is used to determine the type and severity of pneumonia. Previous research has linked a low or normal WBC count during pneumonia admission to a poor prognosis. WBC counts and neutrophil percentages are thought to be indicators of an infection-related inflammatory response.[18] When PCT or WBC levels skyrocketed, the likelihood of bacterial infection skyrocketed. It can guide the clinical early use of broad-spectrum antibiotics for treatment, such as cephalosporins or aminoglycosides, and achieve optimal therapeutic effects.[19]

In patients with CAP, the pathogen and severity of the disease determine the choice of specific treatment as well as the need for hospitalization or transfer to the intensive care unit.[10] Paats et al reported that 658 patients hospitalized with CABG were at higher risk. CRP, PCT, TNF-α, and IL-6 in people with sepsis than in people with CAP of unknown cause but not considered secondary to bacteria. Depending on the cause, different cytokine profiles and biomarkers were found atypical pneumonia had low PCT levels, *S.* pneumoniae had high PCT levels, and *Legionella pneumophila* had low PCT levels. C-reactive protein and TNF-α were higher.[20]

Kruger et al studied PCT in 1,337 CAP inpatients and outpatients, including 472 patients with identified pathogens.[21] The authors obtained that PCT, CRP, and leukocyte concentrations were significantly higher in typical bacterial pneumonia than in atypical bacterial or viral pneumonia. Based on these results, clinicians will need to use a combination of biomarkers such as PCT, WBC, and TNF-α to help them predict the microorganisms causing CAP.

LIMITATIONS

There were several limitations of this study: there were no microbial culture data, so the cause of the CAP is not definitively known. Apart from that, the clinical symptoms of each research subject were not included, so differences in clinical symptoms of CAP could not be described between the two groups.

CONCLUSION

This research confirms that the elevated PCT

group had higher NLR value, WBC, and PCT levels than the normal PCT group. An increase in PCT levels can be used as a predictor of worsening CAP and indicates bacterial infection. Based on the results of our research, we suggest that PCT, WBC, TNF-α, and NLR can be used as a guide for clinicians in assessing the prognosis, causes, and initial management of CAP. However, we still recommend that further research be carried out by including patient specimen culture results to confirm the definitive cause of CAP.

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CONFLICT OF INTEREST

This study has no conflict of interest.

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