Agreement between neutrophil/lymphocyte ratio and procalcitonin for determining severe community acquired pneumonia in children

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Abstract

Introduction: The use of inflammatory markers, such as procalcitonin, in assessing the severity of community acquired pneumonia (CAP) is limited by its relatively high cost. Currently, the Neutrophil/Lymphocyte ratio (NLR) is considered to have an important role in the inflammatory process.

Objectives: To evaluate the agreement between NLR and procalcitonin for determining severe CAP in children.

Method: This was a cross-sectional study carried out in children aged 2 months to 18 years with CAP in the paediatric unit at Sanglah Hospital, Denpasar, Bali, Indonesia from August 2019 – March 2021. Statistical analysis was performed using receiver operating characteristic (ROC) curve to find the cut-off point of NLR. Analysis was done to determine severe CAP based on cut-off points of procalcitonin and NLR. Cohen's Kappa analysis between NLR and procalcitonin was performed for determining severe CAP with significant p-value <0.05.

Results: There were 58 subjects analysed in this study. Analysis performed by ROC showed cut off point of NLR was 2.11 with sensitivity of 82.4% and specificity of 83.3% while procalcitonin had a cut-off point of 1 ng/ml with a sensitivity 85.3% and specificity 87.5% to predict severe CAP. The agreement analysis between NLR with cut-off point 2.11 and procalcitonin with cut-off point 1 ng/ml showed substantial agreement for determining severe CAP in children (k=0,62; p=0,001).

Conclusions: There was substantial agreement between NLR and procalcitonin for determining severe CAP in children.

(Key words: Neutrophil/lymphocyte ratio, Procalcitonin, Community acquired pneumonia, Children)

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Introduction

Community acquired pneumonia (CAP) is a common cause of morbidity and mortality in the paediatric population. In 2017, pneumonia accounted for 15% of all deaths in children under 5 years of age[1]. Pneumonia is characterized by coughing, shortness of breath, fever, fine wet crackles and smooth muscle infiltrates[2,3]. Several inflammatory biomarkers, such as C-reactive protein (CRP), proadrenomedullin and procalcitonin have good predictive value for assessing the severity and mortality of CAP[4]. In Indonesia, there are no specific biomarkers that are used routinely to predict the severity of CAP[5].

Procalcitonin levels have high sensitivity and specificity in assessing the severity of CAP[6]. A study reported that procalcitonin had a sensitivity of 92% and specificity of 73% in differentiating pneumonia caused by bacterial and viral infection and in that study bacterial infection was associated with severe CAP[7]. Procalcitonin as a biomarker of inflammation is relatively costly and is only available at a limited level. We need another biomarker with sensitivity and specificity close to procalcitonin that can be used to determine the severity of CAP quickly and less expensively.

The neutrophil/lymphocyte ratio (NLR) is a combination of neutrophils as active inflammatory components and lymphocytes as regulators and protective components in inflammatory reaction parameter. Compared with white blood cells, NLR can describe the balance between neutrophil and lymphocyte responses and has a better relationship to the severity of an inflammatory response. The NLR value at the beginning of admission was significantly higher in the non-survival CAP group[4]. The NLR is calculated from the ratio of the peripheral blood leucocyte count so that this examination is cheaper than the procalcitonin examination. NLR examination does not require

special reagents such as procalcitonin so it can be widely used.

Objectives
To evaluate the agreement between NLR and procalcitonin for determining severe CAP in children.

Method
This was a comparative analytical cross-sectional study conducted from August 2019 to March 2021 in the emergency ward of the Sanglah General Hospital, Bali, Indonesia. Consecutive sampling was done. The sample size was 45 subjects.

Inclusion criteria: Children 2 months to 18 years of age with diagnosed CAP based on clinical symptoms and chest X-ray examination.

Exclusion criteria: Children suspected or diagnosed to have malignancy, neurological disorders, immunological disorders, cardiovascular disease, severe protein energy malnutrition, or chronic lung disease and children who had received oral or intravenous antibiotic therapy for at least 3 days before being sampled.

CAP was defined as acquired pneumonia in the community which was not being treated in a hospital or other care setting within 14 days of the onset of symptoms. Severity of CAP was defined as CAP and severe CAP based on WHO 2014 criteria for pneumonia. NLR was defined as the ratio between the number of neutrophils and the number of lymphocytes on peripheral blood examination. Procalcitonin was obtained from blood serum using the electrochemiluminescence assay method. NLR and procalcitonin were obtained at the time of admission.

Ethical issues: This study was approved by the Research Ethics Committee of Faculty of Medicine, Udayana University/ Sanglah General Hospital, Denpasar, Bali, Indonesia (No. 2220/ UN14. 2.2. VII.14/LP/2019). Written informed consent was obtained from the parents of the participating children prior to conducting the study.

Statistical analysis: Continuous data are presented as mean and standard deviation if normally distributed or median and interquartile range if not normally distributed. Categorical variables are described in number and percentage. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software. Statistical analysis was performed using receiver operating characteristic (ROC) curve to find cut-off point of NLR and cut-off point of procalcitonin was > 1 ng/ml for determining severe CAP. Cohen's Kappa analysis between NLR and procalcitonin was performed to determine severe CAP with $p < 0.05$.

Results
From August 2019 to March 2021, 187 patients were admitted in Sanglah Hospital with a diagnosis of CAP and 129 of them were excluded. The study flow chart is shown in Figure 1.

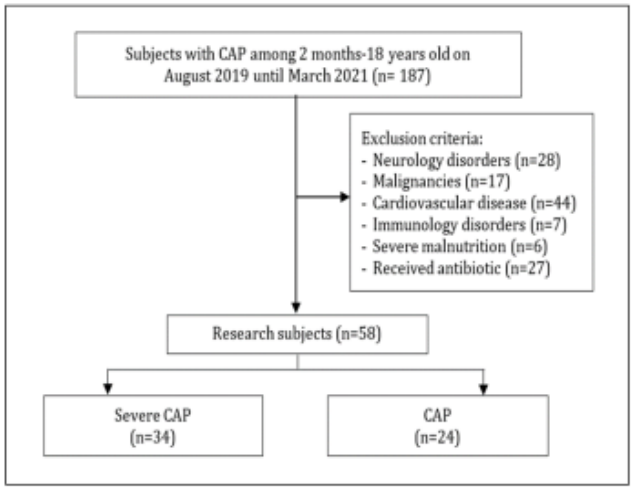

Figure 1: ***Study flow chart***

A total of 58 subjects was included in this study. The median age was 7 months (range 2 months to 12 years). Of the 58 subjects 65.5% were male, 79.3% had a birth weight >2500g, 50% were well nourished, 65.5% had a history of non-exclusive breastfeeding, 56.9% were not exposed to cigarette smoke, 58.6% had incomplete basic immunization and 94.9% had not received pneumococcal immunization. NLR and procalcitonin levels were higher in the severe CAP group. The characteristics of the research subjects are shown in Table 1.

Table 1: ***Characteristic of the research subjects***

Characteristic	**CAP *(n = 24)***	**Severe CAP *(n=34)***
Age: n (%)		
2-23 months	19 (79.1)	29 (85.3)
2-4 years	04 (16.7)	02 (05.9)
5-18 years	01 (04.2)	03 (08.8)
Gender: n (%)		
Male	19 (79.2)	19 (55.9)
Female	05 (20.8)	15 (44.1)
Birth weight: n (%)		
< 2500g (Low birth weight)	02 (08.3)	10 (29.4)
≥ 2500 g	22 (91.7)	24 (70.6)
Nutritional status: n (%)		
Underweight	06 (25.0)	19 (55.9)
Well nourished	17 (70.8)	12 (35.3)
Overweight	01 (04.2)	01 (02.9)
Obese	0 (0.0)	02 (05.9)
Exclusive breast feeding: n (%)		
Yes	12 (50.0)	08 (23.5)
No	12 (50.0)	26 (76.5)
Exposure of cigarette smoke: n (%)		
Yes	11 (45.8)	14 (41.2)
No	13 (54.2)	20 (58.8)
Basic immunization: n (%)		
Complete	13 (54.2)	11 (32.4)
Incomplete	11 (45.8)	23 (67.6)
Pneumococcal immunization: n (%)		
Yes	02 (08.3)	01 (02.9)
No	22 (91.7)	33 (97.1)
Laboratories: median (IQR)		
Neutrophil (10^3/µL)	5.9 (6, 7)	11.2 (8, 3)
Lymphocyte (10^3/µL)	5.1 (3, 3)	2.7 (3, 0)
NLR	1.6 (1, 3)	3.4 (2, 1)
Procalcitonin (ng/mL)	0.1 (0, 5)	3.9 (19, 6)

CAP: community acquired pneumonia; IQR: interquartile range

Figure 2 shows the ROC curve with cut-off point. NLR >2.11 was used to determining severe CAP with 82.4% sensitivity and 83.3% specificity. In this study, the cut-off point for procalcitonin was determined >1ng/ml to predict severe CAP with 85.3% sensitivity and 87.5% specificity. Kappa analysis between NLR with a cut-off point >2.11 and procalcitonin >1ng/ml to determine severe CAP had a substantial agreement (k=0.62; p=0.001) as shown in Table 2.

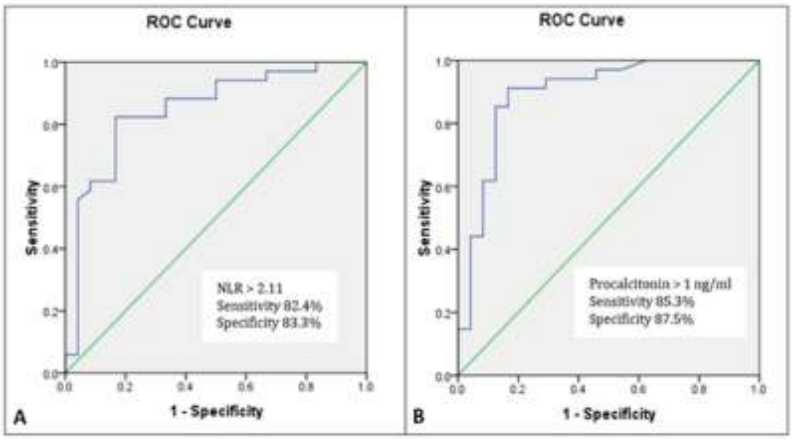

Figure 2: *(A) NLR ROC curve, (B) Procalcitonin ROC curve*

Table 2: ***Agreement between neutrophil/lymphocyte ratio and procalcitonin for determining severe community acquired pneumonia***

NLR	Procalcitonin		*Kappa*	P
	≤ 1 ng/ml	> 1 ng/ml		
≤ 2.11	20	5	0.62	0.001
> 2.11	6	27		

Discussion

This study showed that severe CAP with procalcitonin levels >1ng/ml had an AUC value of 89.5%, 85.3% sensitivity and 87.5% specificity while the NLR cut-off point >2.11 for determining severe CAP had an area under curve (AUC) value of 84.7%, a sensitivity of 82.4% and a specificity of 83.3%. Whether CAP was due to a bacterial or viral infection was difficult to distinguish based on clinical, laboratory or chest x-ray findings but most severe CAP was caused by bacterial infection[8]. Infection and damage to lung parenchyma tissue can cause stimulation of the immune system in the circulation. Lung parenchymal infection causes the release of proinflammatory cytokines such as interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF)-α. The release of these cytokines was in accordance with the damage to the lung parenchyma that occurred and correlated with the severity of the pneumonia[9,10].

The risk factors associated with CAP are divided into intrinsic and extrinsic factors. Intrinsic factors include gender, age, low birth weight, nutritional status, breastfeeding, and immunization status while extrinsic factors include air pollution and exposure to cigarette smoke[11]. In this study the proportion of CAP was more in males (65.5%). This was similar to a previous study which found that the incidence of CAP was more in males (58.8%) which may be caused by the diameter of the airways in male being smaller than in the female or due to differences in body resistance that are influenced by sex chromosomes[9,11].

Several studies have shown an increase in NLR associated with an increase in the inflammatory process that occurs in atherosclerosis, myocardial infarction, cystic fibrosis, and coronary artery disease. A study found that NLR had a correlation with the clinical condition of paediatric patients with cystic fibrosis; it was found that NLR >3 had poor lung condition[10,12]. NLR has been used as a biomarker of infection in various clinical cases including CAP. Previously used infection markers such as procalcitonin and CRP in assessing the severity of community pneumonia have high sensitivity and specificity but have limitations due to cost and accessibility[6,9].

The reaction due to inflammation of the lung parenchyma causes the body's response to fight pathogens, in addition to stimulating the release of proinflammatory cytokines. The affected part of the lung will experience consolidation due to polymorphonuclear cell (PMN) infiltration and fibrin, erythrocytes, oedema fluid and bacteria can be found in the alveoli. The number of leucocytes will increase, especially PMN cells due to inflammation of the lung parenchyma. Leucocytosis will occur mainly in CAP caused by bacteria. Leucocytosis is a systemic inflammatory response to infection due to stimulation of proinflammatory cytokines and the presence of endotoxaemia. Neutrophils are a type of leucocyte whose number is increased in CAP. Neutrophils are released primarily to fight pathogens by phagocytosis at the site of inflammation. This results in excessive activation of the inflammatory cascade so that the release of proinflammatory cytokines and lung tissue damage that occurs is also excessive[9,13].

The physiological response of circulating leucocytes was characterized by an increase in the number of neutrophils and a decrease in the number of lymphocytes. Neutrophilia was caused by neutrophil demargination, delayed neutrophil apoptosis and stimulation of stem cells by growth factors. Re-distribution of lymphocytes and accelerated apoptosis lead to lymphopenia in the setting of infection. Lymphopenia indicates a bacteraemia in an infectious state and can be used as a marker of the severity of community pneumonia, especially in the acute phase[14].

As a normal physiological response to infection is a change in the status of leucocytes, especially neutrophils and lymphocytes, it is often found in children with CAP. This change in leucocyte status has been studied and generally used to distinguish the aetiology of pneumonia. In viral pneumonia, for example, blood tests will generally find leucocytes that are normal or slightly elevated with a predominance of lymphocytes. Different findings can be found in cases of bacterial pneumonia, which generally will experience a further increase in the number of leucocytes with a predominance of granulocytes[15]. This result was similar to a study by Bekdas M, *et al*[16] who found a significant difference in NLR values in the CAP group with a viral aetiology and the CAP group with a bacterial aetiology (0.6 vs 2.7; $p < 0.001$).

Research conducted by Kartal O, *et al*[10] showed that the mean NLR value in paediatric CAP patients was 3.48. The NLR cut-off point >2.54 can be used to predict severe CAP with a sensitivity value of 83% and a specificity of 44%. Katleya F, *et al*[14] found that the NLR cut-off point >1.335 increased the risk of worsening outcomes in paediatric CAP by 1.727 times with an AUC value of 0.658, sensitivity of 75% and specificity of 67.9%. In a previous study, it was showed that from 88 children with CAP (procalcitonin cut-off point 1 ng/mL) procalcitonin was found to be more sensitive and specific than CRP (20 mg/L cut-off point), IL-6 or leucocyte count in differentiating bacterial and viral infections[8]. Another study showed that the 0.5 ng/ml procalcitonin cut-off point had a sensitivity of 93% and a specificity of 74% as a marker of infection[17].

Until now there was no agreement on the value of the NLR that can be used to predict severe CAP in children. Similar to previous studies, the sensitivity and specificity of procalcitonin in diagnosing severe CAP were higher than that of NLR. In this study, the NLR cut-off point obtained was close to the cut-off point in the research conducted by Kartal O, *et al*[10], but was slightly higher than the cut-off point used in the study by Katleya F, *et al*[14]. The low NLR cut-off point in the study conducted by Katleya F, *et al*[14] occurs because the AUC value is 0.658, which is <0.7 so it cannot be used to predict severe CAP. In that study the number of samples in each group was low so that further research is needed with a larger sample size in order to describe the population.

In this study, an analysis was done to assess the agreement between procalcitonin and NLR and it was found that there was a substantial agreement (kappa=0.62) between NLR and procalcitonin for determining severe CAP. NLR values >2.11 were in agreement with procalcitonin values >1 ng/ml for determining severe CAP. Therefore, although the NLR has a lower sensitivity and specificity than procalcitonin, it can be used to determine severe CAP when procalcitonin is not available. In this study, there was no serial examination for NLR and procalcitonin and we did not include healthy children so we cannot state there were increase of NLR in CAP.

Conclusion

There was a substantial agreement between NLR and procalcitonin for determining severe CAP in children.

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