

ORIGINAL ARTICLE

Retrospective study: Neutrophil-to-lymphocyte ratio for distinguishing pulmonary tuberculosis and community-acquired pneumonia in children

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Abstract. *Background and aim:* Tuberculosis and pneumonia remain among the top ten causes of death in children under five. Clinical manifestations of pulmonary TB in children are mostly nonspecific and resemble acute pneumonia. The paucibacillary nature of TB in children contributes to diagnostic challenges. The neutrophil-to-lymphocyte ratio (NLR) is a straightforward biomarker that connects the innate and adaptive immune systems. This study aims to analyze the effectiveness of the NLR in distinguishing pulmonary tuberculosis (TB) from community-acquired pneumonia (CAP) in children. *Methods:* This research is a retrospective case-control observational study based on medical record data of pediatric subjects with pulmonary TB or CAP treated at RSUD Dr. Soetomo. Characteristics and laboratory data were collected from medical records. *Results:* A total of 50 subjects with pulmonary TB and 50 subjects with CAP demonstrated a significantly lower NLR in the pulmonary TB group (median 1.31; 0.23–5.62) vs. the CAP group (median 3.98; 1.19–53.51), $p < 0.001$. The NLR differed significantly based on age. Pulmonary TB subjects aged <5 years old had a lower NLR (median 1.18; 0.23–5.00) compared to those >5 years old (median 2.89; 1.16–5.62) with $p < 0.001$. Community-acquired pneumonia subjects aged <5 years old also had a lower NLR (median 3.79; 1.19–10.69) vs. >5 years old (median 6.79; 1.37–53.51) with $p = 0.048$. The cut-off <2.06 demonstrated good performance for differentiating pulmonary TB from CAP with high sensitivity (92%) and good specificity (68%). *Conclusions:* The NLR is a useful biomarker to distinguish pulmonary TB from CAP in limited resource settings. (www.actabiomedica.it)

Key words: NLR, tuberculosis, community-acquired pneumonia

Introduction

Tuberculosis and pneumonia continue to be among the top ten causes of death for children under 5 years old. Tuberculosis is a communicable disease caused by *Mycobacterium tuberculosis* (MTB) and primarily affects the lungs. Approximately 10.6 million cases of tuberculosis (TB) were identified in 2021 and 11% were pediatric cases. Tuberculosis accounted for 70% of the deaths of children under five (1). Diagnosing

pulmonary TB in children can be challenging because its clinical symptoms are typically nonspecific and can mimic acute pneumonia. Moreover, the paucibacillary nature of TB in children makes it challenging to collect samples for microbiology testing. Delay in the diagnosis of pulmonary TB leads to poor prognosis for individuals, families, and eventually become an economic burden (2,3). Numerous studies have been conducted in search of biomarkers to differentiate pulmonary TB and community-acquired pneumonia (CAP), such as

C-reactive protein (CRP), Procalcitonin (PCT) and neutrophil-to-lymphocyte ratio (NLR). This ratio reflects the balance of the innate and adaptive immune systems. Neutrophils are the primary cell in innate immune system and recruited immediately to site of infection. A higher NLR indicates acute immune response corresponding with the clinical course of CAP. In contrast, the clinical course of pulmonary TB is more chronic. Several studies on NLR to differentiate pulmonary TB and CAP in adults or children showed significant results (4,5). This study aims to analyze the performance of NLR for differentiating pulmonary TB and CAP in children.

Methods

Study design

This was a retrospective case-control study conducted at RSUD Dr. Soetomo, Surabaya, Indonesia of medical record data of pediatric subjects with pulmonary TB or CAP from January 2022 to June 2024. The estimated minimum sample size calculated was 85. Inclusion criteria were subjects aged 1 month to 18 years old newly diagnosed with pulmonary TB or CAP with complete data in the medical records. The exclusion criteria included incomplete data, a history of malignancy, sepsis, congenital heart diseases, Systemic Lupus Erythematosus (SLE) and co-infection of pulmonary TB with CAP. A total of 100 subjects from two groups were included in this case-control study. While 50 subjects with pulmonary TB comprised the control group, 50 subjects with CAP formed the case group. The collection of characteristics and laboratory records was conducted accordingly.

Nutritional status

The nutritional status of children below 5 years old was assessed using the z-score of weight-for-height based on WHO child growth standards. Meanwhile, the nutritional status of children 5 years old and above was established using the Waterlow classification of stature-for-age and weight-for-age based on CDC growth charts.

Laboratory parameters and NLR

The laboratory data of leukocyte, neutrophil, and lymphocyte counts were collected from medical records. The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes.

Comorbidities

The comorbidities found in this study were asthma, bronchiectasis, central hypothyroidism, cerebral palsy, cleft lip and palate, chronic kidney disease, Down syndrome, epilepsy, Hirschprung's disease, interstitial lung disease, laryngomalacia, mucopolysaccharidosis, nephrotic syndrome, thalassemia, type 1 diabetes, and white matter disease. Every TB subject had been screened for HIV, and the results were negative.

Statistical analysis

All characteristic data (age, gender, nutritional status, and comorbidity) were summarized in descriptive statistics. The distribution of NLR was analyzed using the Kolmogorov-Smirnov test. All the data were abnormally distributed and thus further analyzed using the Mann-Whitney U-test. The cut-off was determined using the receiver operating characteristic (ROC) curve. Significant value was set at $p < 0.05$.

Results

Characteristics data from each group were shown on Table 1. Majority of the pulmonary TB group were female (58%) while the CAP group was predominantly male (58%). Overall gender distribution was even (50% male vs. 50% female). Most subjects were below 5 years old (78% in the pulmonary TB group and 58% in the CAP group). Majority of subjects had a normal nutritional status (50% in the pulmonary TB group and 58% in the CAP group). Overnutrition status was only found in the CAP group (12%). Comorbidity was noted in 32% of the pulmonary TB group and 54% of the CAP group. There were significant differences

Table 1. Characteristics of subjects

Characteristics	Pulmonary TB (n=50)		CAP (n=50)		Total (n=100)		<i>p</i>
	n	%	n	%	n	%	
Gender							0.11 ^a
• Male	21	42	29	58	50	50	
• Female	29	58	21	42	50	50	
Age (years)							0.032 ^a
• < 5	39	78	29	58	68	68	
• > 5	11	22	21	42	32	32	
Nutritional Status							0.023 ^b
• Severe malnutrition	6	12	5	10	11	11	
• Moderate malnutrition	19	38	10	20	29	29	
• Normal	25	50	29	58	54	54	
• Overnutrition	0	0	6	12	6	6	
Comorbidity							0.026 ^a
• No	34	68	23	46	57	57	
• Yes	16	32	27	54	43	43	

^a Chi-square Test ^b Fisher's Exact Test. Significant if $p < 0.05$. *Abbreviations:* TB: tuberculosis; CAP: community-acquired pneumonia.

Table 2. Laboratory Parameter

Laboratory parameter	Pulmonary TB (n=50)	CAP (n=50)	<i>P</i>
Leukocytes (/μl)	10,390 (5,690–32,520)	15,070 (2,490–30,680)	0.016
Neutrophils (/μl)	4,300 (1,820–22,850)	10,560 (1,690–27,290)	<0.001
Lymphocytes (/μl)	4,800 (1,280–13,000)	2,100 (510–8,770)	<0.001
NLR	1.31 (0.23–5.62)	3.98 (1.19–53.51)	<0.001

Significant if $p < 0.05$ with Mann-Whitney U-Test. *Abbreviations:* TB: tuberculosis; CAP: community-acquired pneumonia; NLR: neutrophil-to-lymphocyte ratio.

between 2 groups based on age, nutritional status, and comorbidity.

Laboratory data were shown in Table 2. The median of leukocytes was significantly lower in the pulmonary TB group (10,390/μl; 5,690–32,520/μl) compared to the CAP group (15,070/μl; 2,490–30,680/μl), with $p = 0.016$. Median of neutrophils was significantly lower in the pulmonary TBC group (4,300/μl; 1,820–22,850/μl) compared to the CAP group (10,560/μl; 1,690–27,290/μl), with $p < 0.001$. In contrast, the median of lymphocyte count was significantly higher in the pulmonary TB group (4,800/μl; 1,280–13,000/μl) compared to the CAP group (2,100/μl; 510–8,770/μl), with $p < 0.001$.

The NLR of the pulmonary TB group was significantly lower (3.98; 1.19–53.51) compared to the CAP group (1.31; 0.23–5.62), with $p < 0.001$.

Table 3 showed a significant difference of NLR based on age for both groups. Pulmonary TB subjects aged < 5 years old had a lower NLR with a median of 1.18 (0.23–5.00) compared to those > 5 years old with a median of 2.89 (1.16–5.62). This difference was statistically significant with $p < 0.001$. Subjects aged < 5 years old in the CAP group similarly had a lower NLR with a median of 3.79 (1.19–10.69), vs > 5 years old with a median of 6.79 (1.37–53.51). This difference was also statistically significant with $p = 0.048$.

Table 3. Neutrophil-to-lymphocyte ratio based on characteristics of both groups

Characteristics	NLR of pulmonary TB (n=50)	<i>p</i>	NLR of CAP (n=50)	<i>p</i>
Gender				
• Male	1.22 (0.23-5.00)	0.438 ^a	3.85 (1.37-53.51)	0.321 ^a
• Female	1.64 (0.29-5.62)		6.29 (1.19-16.91)	
Age (years)				
• <5	1.18 (0.23-5.00)	<0.001 ^a	3.79 (1.19-10.69)	0.048 ^a
• ≥5	2.89 (1.16-5.62)		6.79 (1.37-53.51)	
Nutritional Status				
• Severe malnutrition	0.93 (0.31-2.51)	0.404 ^b	5.23 (1.73-7.07)	0.892 ^b
• Moderate malnutrition	1.22 (0.23-4.38)		4.58 (1.37-13.08)	
• Normal	1.64 (0.32-5.62)		3.85 (1.19-53.51)	
• Overnutrition	0		2.87 (2.16-18.72)	
Comorbidity				
• No	1.19 (0.23-5.62)	0.382 ^a	3.79 (1.19-53.51)	0.719 ^a
• Yes	1.75 (0.29-5.00)		4.86 (1.37-16.91)	

^aMann-Whitney U-Test; ^bKruskal Wallis Test. Significant if $p < 0.05$. *Abbreviations:* NLR: neutrophil-to-lymphocyte ratio; TB: tuberculosis; CAP: community-acquired pneumonia.

Table 4. Neutrophil-to-lymphocyte ratio based on chest X-ray findings of pulmonary TB subjects

CXR finding of pulmonary TB (n=50)	NLR	<i>p</i>
Normal	0.61 (0.23-3.33)	0.083
Hilar adenopathy	0.52 (0.29-2.51)	
Infiltrates	1.33 (0.39-5.62)	
Consolidation	2.56 (0.72-3.25)	
Cavity	2.47 (0.57-4.38)	
Miliary	1.61 (1.27-1.78)	

Significant if $p < 0.05$ with Kruskal Wallis Test. *Abbreviations:* CXR: chest X-ray; TB: tuberculosis; NLR: neutrophil-to-lymphocyte ratio.

Table 4 demonstrated the difference in NLR based on chest X-ray (CXR) features of pulmonary TB subjects. Subjects with consolidation had the highest NLR, with a median of 2.56 (0.72-3.25), whereas subjects with hilar adenopathy had the lowest NLR, with a median of 0.52 (0.29-2.51). The difference in NLR based on CXR findings was not statistically significant, with $p = 0.083$.

Figure 1 showed the AUC for leukocytes, neutrophils, lymphocytes, and NLR. The AUC of leukocyte was 64% with a confidence interval (CI) of

52.8%-75.3%. The neutrophil demonstrated an AUC of 76.2% (CI 66.5%-85.9%). The lymphocyte demonstrated the lowest AUC at 22.4%. The AUC of NLR was 87.9% with $p < 0.001$. Cut-off 2.06 had the best performance in differentiating pulmonary TB and CAP with a sensitivity of 92% and a specificity of 68%.

Discussion

In this study, majority of the pulmonary TB group consisted of females (58%), while the CAP group was predominantly male (58%). Previous studies have suggested that the gender predominancy in the pulmonary TB might be attributed to factors such as puberty, TB contact, exposure to cigarette smoke, and environmental influences (6,7). The observation of a predominance of females in the pulmonary TB group in this study is consistent with earlier research indicating that puberty can lead to immune dysregulation. Meanwhile, in CAP, males were more dominant, in line with prior studies. This trend was believed to be influenced by genetics, testosterone levels, high-risk behavior, and gender preferences. In the pediatric community, boys typically spend more time outdoors,

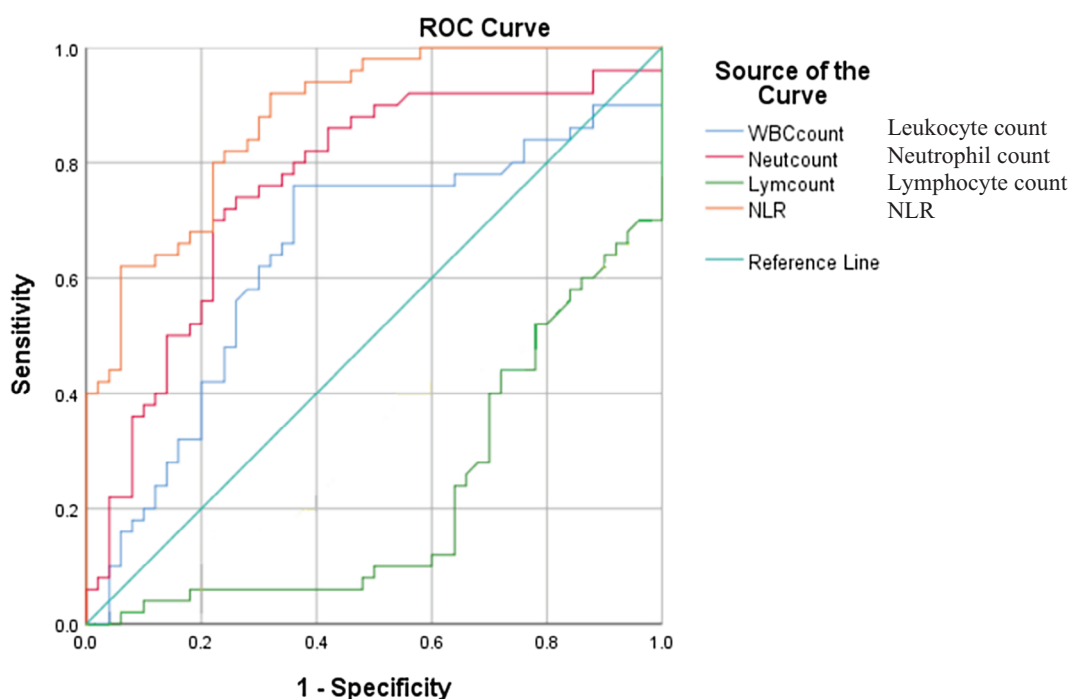


Figure 1. Area under the curve for laboratory parameters. *Abbreviations:* NLR: neutrophil-to-lymphocyte ratio.

increasing their risk of contracting pneumonia (8,9). The majority of the subjects were under 5 years old. This is supported by the other studies indicating that the immune response on this age group is still developing. The other possible cause was higher social interaction (e.g. childcare facilities) raising the likelihood of exposure (8,10,11). Normal nutritional status was prevalent in both groups. Prior studies have shown that severe malnutrition is a risk factor for TB (12,13). We obtained different results, which could be attributed to proportional height and weight leading to normal nutritional status. Conversely, the result for CAP group were consistent with previous study suggested that overnutrition could impact lung capacity, work of breathing, and potentially increase susceptibility to CAP (14,15). A significant difference in comorbidity was observed between both groups, with 32% in the pulmonary TB group and 54% in the CAP group. This difference was influenced by various demographic factors (age, socio-economic backgrounds, healthcare). The higher infection risk in CAP was thought to be a result of the comorbidity itself reducing immune response (16,17). In this study, we found that the

pulmonary TB group had lower leukocyte levels compared to the CAP group, highlighting a fundamental difference in the inflammatory response between two groups. Consistent with earlier studies, leukocytosis serves as a specific indicator of rapid response to acute infections such as CAP (18,19). We also demonstrated that the pulmonary TB group had lower neutrophil levels compared to the CAP group. Neutrophils are the main effector cell in acute infections like CAP. This finding aligns with previous research indicating that immune response in pneumonia is rapid and intense (20-22). On the other hand, higher lymphocyte levels in pulmonary TB indicate a more mature adaptation to chronic infection. Increased lymphocyte counts signify the development of adaptive immunity (23). The findings of this study align with a previous study that demonstrated the chronic nature of pulmonary TB infection. The lower NLR in the pulmonary TB group (1.31 vs 3.98 in the CAP group) explains the dynamics of immune response (24). A sole elevation of neutrophil will increase the NLR, a condition found in bacterial or fungal infection, acute stroke, myocardial infarct, atherosclerosis, severe trauma, cancer post-surgery

complication and injury (25). The finding in this study demonstrated a significant difference of NLR based on age. Subjects aged <5 years old had a lower NLR, which was in line with a previous study that stated that the immune response matures as age progresses (26,27). This reflects the immune response to repeated exposure or the accumulation of inflammatory factors as we age (28). A previous study stated that pulmonary TB with cavitation had a significantly higher NLR. This was in line with our study. During MTB infection, neutrophil products destroy lung tissue, resulting in a cavity. High bacillary burden in TB with cavitation will induce type-1 IFN, which causes overactivation of neutrophils, leading to increased NLR (29). In this study, NLR demonstrated the highest AUC (87.9%), compared to leukocytes, neutrophils, and lymphocytes to differentiate pulmonary TB and CAP. Cut-off of < 2.06 demonstrated the best performance with a sensitivity of 92% and a specificity of 68%. This cut-off was lower than a study in South Korea, which found that a cut-off 7 had a sensitivity of 91.1% and a specificity of 81.9% (4). Other study in Ethiopia indicated a cut-off ≥ 2.74 with a sensitivity of 74.0% and a specificity of 52.1% (5). A study in Colombia showed that cut-off of 5.25 had a sensitivity of 49.3% and a specificity of 36.1% (30). No study analyzed the performance of this biomarker in differentiating pulmonary TB and CAP in pediatric population. We concluded that NLR < 2.06 was indicative of a pulmonary TB infection.

Conclusion

This study showed that NLR in the pulmonary TB group was lower than that in the CAP group. A significant difference of NLR was observed based on age. The NLR is a useful biomarker to distinguish pulmonary TB from CAP in limited resource settings.

Ethic Approval: This study was approved as ethically appropriate by the Research and Ethics Scientific (1805/LOE/301.4.2/X/2024) released on October 24th, 2024, by the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

Authors' Contribution: Concept framework: WD, RAS, and MRA; methodology: WD, RAS, and MRA; initial draft preparation: WD; manuscript review and revision: RAS and MRA; supervision: RAS and MRA. All authors have reviewed and approved the final version of the manuscript.

Declaration on the Use of AI: None.

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