

# Improving Early Identification of Microbial Etiology in Severe Pediatric Pneumonia by Using Inflammatory Profiles: A Cross-sectional Study

## Enflamatuvar Profiller Kullanılarak Ağır Çocukluk Çağı Pnömonisinde Mikrobiyal Etiyolojinin Erken Belirlenmesinin İyileştirilmesi: Kesitsel Bir Çalışma

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**Cite this article as:** Ngo QC, Tran MD, Nguyen NTT, Hoang ML, Duong LKT. Improving early identification of microbial etiology in severe pediatric pneumonia by using inflammatory profiles: a cross-sectional study. *J Curr Pediatr.* 2026;24(1):1-9



### Keywords

Severe pneumonia, inflammatory markers, neutrophil-to-lymphocyte ratio, early diagnosis, microbiology.

### Anahtar kelimeler

Ağır pnömoni, enflamatuvar belirteçler, nötrofil/lenfosit oranı (NLR), erken tanı, mikrobiyoloji

**Received/Geliş Tarihi** : 09.09.2025

**Accepted/Kabul Tarihi** : 11.11.2025

**Published Date/**

**Yayınlanma Tarihi** : 10.04.2026

**DOI:**10.4274/jcp.2025.55632

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### Abstract

**Introduction:** Pediatric pneumonia can result from bacterial or viral pathogens, presenting with overlapping clinical features, which makes accurate differentiation challenging. In developing countries, where access to microbiological diagnostics is limited, timely identification of the etiology is crucial to guide treatment. Basic inflammatory markers such as absolute neutrophil count (ANC), neutrophil-to-lymphocyte ratio (NLR) and C-reactive protein (CRP) may help distinguish bacterial from viral infections. This study evaluates the diagnostic value of routine inflammatory markers in severe pneumonia.

**Materials and Methods:** This cross-sectional study included 61 children aged 2 months to 5 years with severe pneumonia at Children's Hospital 1, Vietnam. Bacterial etiology was confirmed using real-time polymerase chain reaction from tracheal aspirates; viral pneumonia was identified by detecting respiratory viruses in the absence of bacteria. Clinical characteristics and inflammatory markers were collected. Comparative and receiver operating characteristic (ROC) curve analyses were performed to assess diagnostic performance.

**Results:** 51 children (83.6%) had bacterial pneumonia, and 10 (16.4%) had viral pneumonia. Bacterial pneumonia was more common with recurrent pneumonia and high fever ( $p < 0.05$ ). ANC ( $p < 0.005$ ), NLR ( $p = 0.0002$ ), and CRP ( $p = 0.01$ ) were significantly elevated in bacterial cases. Area Under the ROC Curve (AUROC) showed NLR had the highest discriminatory value (AUROC = 0.87). The optimal NLR cut-off value ( $\geq 0.8$ ) yielded 78.4% sensitivity and 90% specificity. Combining NLR and CRP with clinical symptoms increased specificity but reduced sensitivity. A moderate positive correlation was observed between NLR and CRP (Spearman's  $\rho = 0.514$ ,  $p < 0.0001$ ).

**Conclusion:** NLR and other inflammatory markers offer practical value in distinguishing severe bacterial pneumonia, particularly in resource-limited settings. Nevertheless, interpretation should always be guided by clinical context.



## Öz

**Giriş:** Gelişmekte olan ülkelerde mikrobiyolojik tanı olanaklarının sınırlı olduğu durumlarda, çocuklarda bakteriyel ve viral pnömoniye hızla ayırt etmek güçtür. Bu çalışmanın amacı, ağır pnömonisi olan çocuklarda mutlak nötrofil sayısı (MNS), nötrofil/lenfosit oranı (NLR) ve C-reaktif protein (CRP) gibi rutin inflamatuvar belirteçlerin etiyolojik ayırıcı tanıdaki değerini değerlendirmektir.

**Gereç ve Yöntem:** Bu kesitsel çalışma, Vietnam Çocuk Hastanesi 1’de ağır pnömoni tanısı ile izlenen 61 çocuğu (yaş: 2 ay–5 yaş) kapsamaktadır. Bakteriyel etiyoloji, trakeal aspiratlardan gerçek zamanlı polimeraz zincir reaksiyonu (rt-PCR) ile doğrulandı; viral pnömoni ise bakteriyel etken saptanmaksızın solunum virüslerinin gösterilmesiyle tanımlandı. Klinik özellikler ve inflamatuvar belirteçler kaydedildi. Gruplar arası karşılaştırmalar ve ROC eğrisi analizleri yapılarak tanısal performans değerlendirildi.

**Bulgular:** 51 çocukta (%83,6) bakteriyel, 10 çocukta (%16,4) viral pnömoni saptandı. Bakteriyel olgular rekürren pnömoni ve yüksek ateş ile daha sık ilişkiydi ( $p<0,05$ ). MNS ( $p<0,005$ ), NLR ( $p=0,0002$ ) ve CRP ( $p=0,01$ ) bakteriyel olgularda anlamlı düzeyde daha yüksekti. NLR, en iyi ayırıcı gücü gösterdi (AUROC=0,87). NLR  $\geq 0,8$  eşik değeri için duyarlılık %78,4 ve özgüllük %90,0 bulundu. NLR + CRP’nin klinik bulgularla birlikte kullanımı özgüllüğü artırırken duyarlılığı bir miktar azalttı. NLR ile CRP arasında orta düzeyde pozitif korelasyon izlendi (Spearman rho=0,514;  $p<0,0001$ ).

**Sonuç:** NLR başta olmak üzere temel inflamatuvar belirteçler, özellikle kaynakların kısıtlı olduğu ortamlarda ağır çocukluk çağı pnömonisinde bakteriyel-viral ayırımına pratik katkı sağlayabilir. Bununla birlikte, yorumlama her zaman klinik bağlam içinde yapılmalıdır.

## Introduction

Community-acquired pneumonia (CAP) remains a leading cause of death in children under five, responsible for nearly 2 million deaths annually, according to the World Health Organization (WHO) and the United Nations International Children’s Emergency Fund (UNICEF) (1). The primary causative agents are bacteria and viruses (2). In Vietnam, Tran Quang et al. (3) reported bacterial infections in 83.1% and viral infections in 50.5% of severe pneumonia cases.

Managing pediatric pneumonia in developing countries is challenging due to the high prevalence of severe cases, inconsistent diagnostic criteria, and limited access to microbiological tests needed to identify pathogens before initiating appropriate treatment (4). Notably, bacterial and viral pneumonia trigger distinct immune responses. Therefore, using accessible and affordable biomarkers that reflect these differences may aid in distinguishing between bacterial and viral etiologies, helping to reduce complications and mortality (5).

Numerous studies have attempted to distinguish clinical features and inflammatory markers to reduce reliance on microbiological testing, which is often unavailable in resource-limited settings. Moreover, pathogen culture results are time-consuming and thus unsuitable for managing severe pneumonia cases that require timely intervention. However, findings across studies remain inconsistent and continue to be a matter of debate. This article describes the clinical and paraclinical characteristics of severe bacterial versus viral pneumonia, while evaluating the diagnostic value of basic inflammatory markers in distinguishing between these two etiologies to improve the quality of pathogen identification in pediatric cases.

## Materials and Methods

### Study Design and Population

This cross-sectional descriptive study was conducted at a children’s hospital in southern Vietnam, from December 2022 to November 2023, enrolling children aged 2 months to 5 years diagnosed with severe CAP. Inclusion criteria required: i) cough or difficulty breathing accompanied by at least one of the following signs: age-specific tachypnea, abnormal lung examination findings, or chest indrawing combined with radiographic evidence of pulmonary infiltrates on chest X-ray (1); ii) classification as severe CAP based on criteria from the British Thoracic Society (6), defined respiratory rate greater than 70 breaths per minute, severe chest indrawing, cyanosis, intermittent apnea, grunting, inability to feed, or capillary refill time  $\geq 2$  seconds; iii) availability of C-reactive protein (CRP) along with routine blood test results obtained within 24 hours of admission; iv) specimen collection and testing for respiratory pathogens within 24 hours of admission. Exclusion criteria included: i) underlying cardiopulmonary diseases; ii) recent hospitalization within 30 days; iii) prior antibiotic or immunosuppressive therapy within 1 month before admission; iv) absence of parental consent.

### Data Collection Procedures

Children diagnosed with severe CAP were screened based on predefined inclusion and exclusion criteria. Eligible patients were enrolled consecutively, and demographic, clinical, and laboratory data were collected using a standardized case record form.

Nasotracheal Aspiration (NTA) and Microbiological Analysis: Shortly after admission and before starting

antibiotics, NTA was performed under sterile conditions using a mucus extractor device (Global Medikit Limited, New Delhi, India) to collect lower respiratory tract secretions. Samples were sealed, placed in an icebox at 2–8 °C, and promptly transported to the International Research of Gene and Immunology Institute at Nam Khoa Biotek Laboratory (Ho Chi Minh City, Vietnam), which is certified under ISO 9001:2008 standards for real-time polymerase chain reaction (RT-PCR) testing. This procedure was carried out following the standardized protocol used at our institution to ensure specimen integrity and diagnostic accuracy.

**Sample Quality Assessment:** To ensure sample origin from the lower respiratory tract, all NTA specimens underwent Gram staining and microscopic evaluation. Specimens were considered high-quality if they contained fewer than 10 squamous epithelial cells (SECs) and more than 25 polymorphonuclear cells (PMNs) per low-power field ( $\times 100$  magnification), indicating minimal upper airway contamination and active inflammation (3).

**Microbial Identification:** The RT-PCR process included three main steps: i) sample homogenization in phosphate-buffered saline with N-Acetyl L-Cysteine; ii) nucleic acid extraction using the BIO-RAD CFX96 system and NKRNADNAprep-MAGBEAD reagents; and iii) amplification using specific primers and TaqMan probes (Thermo Fisher Scientific). The RT-PCR panel included 46 respiratory pathogens (29 bacterial and 17 viral agents). A full list of targeted pathogens is provided in Appendix 1.

A sample was considered positive for bacterial infection if RT-PCR detected one or more pathogenic bacterial species at a concentration exceeding  $10^5$  copies/mL, consistent with thresholds used for lower respiratory tract specimens. For viral pathogens, a result was considered positive when at least one respiratory virus was detected with a viral load of  $\geq 10^5$  copies/mL or a cycle threshold (Ct) value below 30, indicating active infection rather than asymptomatic carriage (3). Participants were categorized into either the bacterial severe CAP (BSCAP) group or the viral severe CAP (VSCAP) group. Assignment to the VSCAP required fulfillment of the following criteria: i) detection of at least one respiratory virus in NTA specimens (e.g., respiratory syncytial virus, rhinovirus); ii) absence of detectable bacterial DNA; iii) absence of clinical signs, symptoms, or radiographic evidence suggestive of empyema; iv) negative sputum culture results. Assignment to the BSCAP group required the following criteria: i) detection of one or more pathogenic bacteria in respiratory specimens by RT-PCR or culture (e.g., *Streptococcus pneumoniae*, *Haemophilus influenzae*);

ii) absence of detectable respiratory viruses in the same specimen. Mixed infections, in which both bacterial and viral pathogens were concurrently identified in the same sample by RT-PCR or culture, were excluded from the final analysis to ensure clear etiological differentiation.

These classifications were used as the reference standard for subsequent comparisons of clinical and inflammatory marker profiles. The detailed process of patient screening, eligibility assessment, exclusion, and final inclusion in the study cohort is illustrated in the flowchart provided in Appendix 2.

### *Statistical Analysis*

Statistical data were processed using standard medical statistical methods. Data analysis was conducted using Stata version 16.0. Qualitative variables were presented as frequencies and percentages, while quantitative variables were reported as mean  $\pm$  standard deviation or median (interquartile range), as appropriate. Group comparisons were performed with a 95% confidence interval. A p-value of  $< 0.05$  was considered statistically significant. The area under the receiver operating characteristic curve (AUROC) was used to evaluate the predictive ability of the basic inflammatory markers. Sensitivity, specificity, positive likelihood ratio (LR<sup>+</sup>) and negative likelihood ratio (LR<sup>-</sup>) were also calculated.

### *Ethics committee approval*

The authors affirm that all procedures and experiments conducted in this study complied with the ethical standards outlined in the Declaration of Helsinki (1975), as revised in 2008, as well as relevant national regulations. The study protocol was reviewed and approved by the Ethics Committee in Biomedical Research at Hội đồng Đạo Đức trong Nghiên cứu Y sinh học Bệnh viện Nhi Đồng 1 (approval number: 425/GCN-BVND1, date: 17.11.2022). Written informed consent was obtained from the parents or legal guardians of all participants. They were fully informed of the study's objectives, procedures, potential risks, and their right to withdraw from the study at any time without consequences.

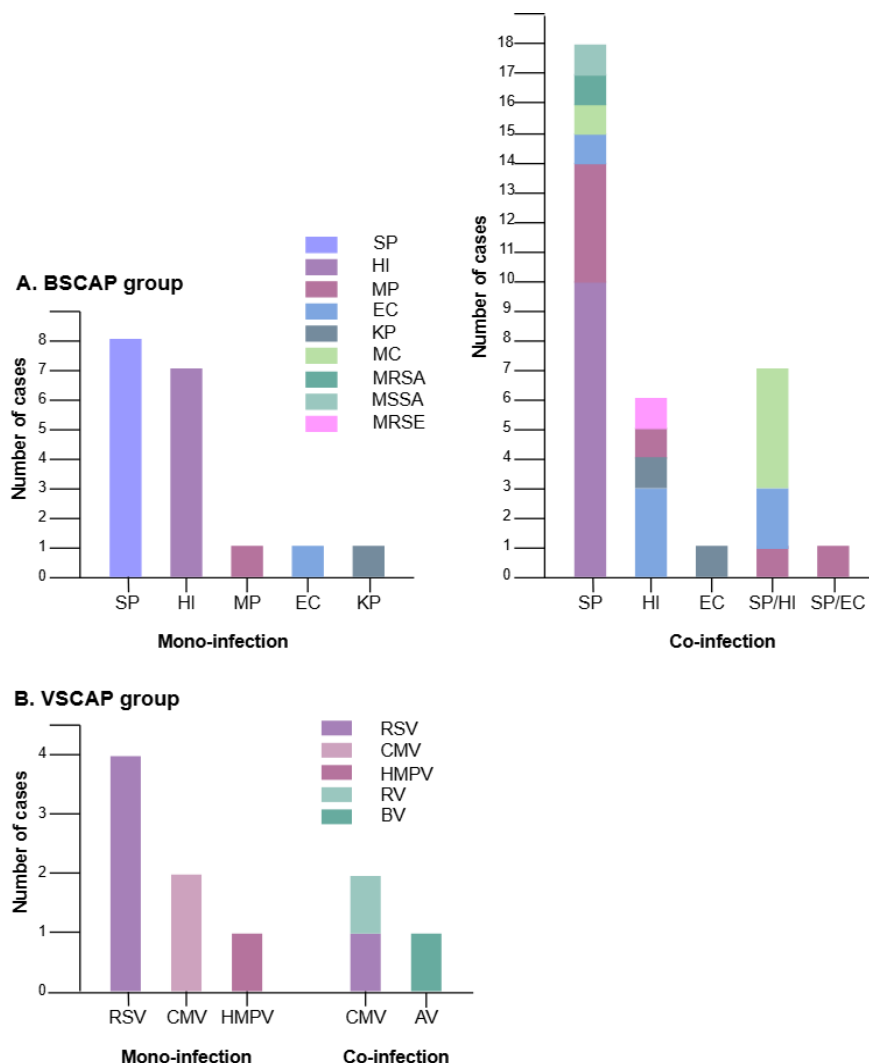
## **Results**

### *Microbial Profile and Infection Complexity*

61 children with severe CAP were included. RT-PCR identified 51 cases (83.6%) as bacterial and 10 cases (16.4%) as viral pneumonia. The distribution of cases by microbial etiology and infection complexity is shown in Figure 1. In the

BSCAP group (Figure 1A), 18 cases were classified as bacterial mono-infections and 33 as coinfections. *Streptococcus pneumoniae* (SP) and *Haemophilus influenzae* (HI) were the most common pathogens in mono-infections. The complexity of microbial etiology in severe CAP was particularly evident in bacterial coinfections involving SP, most notably SP–HI coinfection (10 cases). Notably, 8 patients presented with coinfections involving three distinct bacterial pathogens. A

wide range of other bacterial species were also detected in coinfection profiles, including *Moraxella catarrhalis* (MC), *Klebsiella pneumoniae* (KP), and *Staphylococcus aureus* (both MRSA and MSSA). In contrast, the VSCAP group showed a simpler pathogen profile, with 7 mono-infection cases – mostly respiratory syncytial virus (RSV), and only 3 cases involving viral coinfections (see Figure 1B).



**Figure 1.** Distribution of pneumonia cases by etiology and infection complexity

(A) Bacterial severe community-acquired pneumonia (BSCAP) group: the bar graphs display the number of mono-infections and co-infections identified. *Streptococcus pneumoniae* (SP) and *Haemophilus influenzae* (HI) were the most common mono-infecting pathogens. Co-infections commonly involved combinations such as SP–HI, and SP/HI–MC (*Moraxella catarrhalis*), along with other pathogens including *Mycoplasma pneumoniae* (MP), *Klebsiella pneumoniae* (KP), *Methicillin-sensitive Staphylococcus aureus* (MSSA), *Methicillin-resistant Staphylococcus aureus* (MRSA), and *Staphylococcus epidermidis* (MRSE). (B) Viral severe community-acquired pneumonia (VSCAP) group: the figure shows the distribution of mono-viral and multi-viral infections. *Respiratory syncytial virus* (RSV), *Cytomegalovirus* (CMV), and *Human metapneumovirus* (HMPV) were frequently identified. Co-infections included CMV in combination with RSV and Rhinovirus (RV), as well as Adenovirus (AV) and Bocavirus (BV)

*Clinical Characteristics and Laboratory Markers associated with Microbial Etiology*

In Table 1, children with BSCAP were more likely to have a history of recurrent pneumonia (70.6% versus 20.0%,  $p < 0.005$ ), incomplete vaccination (58.9% versus 20.0%,  $p < 0.05$ ), and high fever (41.2% versus 10.0%,  $p < 0.05$ ). Wheezing was significantly more common in VSCAP (100% versus 64.7%,  $p < 0.05$ ). Other variables such as age, sex, prematurity, and nutritional status did not differ significantly between groups. In addition, children with BSCAP exhibited significantly higher levels of inflammatory markers compared to those with VSCAP. Median absolute neutrophil count (ANC) was 6.3 K/ $\mu$ L versus 3.2 K/ $\mu$ L ( $p < 0.005$ ), neutrophil-to-Lymphocyte Ratio (NLR) was 1.4 versus 0.5 ( $p = 0.0002$ ), and CRP was 10.2 mg/L versus 1.5 mg/L ( $p = 0.01$ ).

*Diagnostic Accuracy of Inflammatory Biomarkers and Impact of Combined Clinical Parameters*

AUROC analysis was performed to evaluate the diagnostic utility of ANC, NLR, and CRP in predicting bacterial etiology (Table 2 and Figure 2). NLR demonstrated the highest

discriminatory capacity with an AUROC of 0.87 (95% CI: 0.78–0.97,  $p < 0.0001$ ). ANC also performed well with an AUROC of 0.79 (95% CI: 0.65–0.93,  $p = 0.0015$ ), while CRP showed a moderate predictive value with an AUROC of 0.74 (95% CI: 0.58–0.90,  $p = 0.01$ ). However, the differences between the AUROCs were not statistically significant ( $p = 0.1427$ ).

Cut-off values were calculated using Youden’s Index to optimize sensitivity and specificity (Table 3). An NLR  $\geq 0.8$  yielded a sensitivity of 78.4% and specificity of 90%, corresponding to an LR<sup>+</sup> of 7.9 and an LR<sup>-</sup> of 0.2. In contrast, CRP  $\geq 7$  mg/L and ANC  $\geq 5.8$  K/ $\mu$ L showed lower sensitivity (56.9% and 60.5%, respectively) but similarly high specificity (both at 90%). When clinical features were incorporated into the diagnostic algorithm, the overall performance shifted. Combining NLR  $\geq 0.8$  and CRP  $\geq 7$  mg/L with the presence of high fever or a history of recurrent pneumonia slightly increased specificity to 93.3% and 94.4% respectively, while further reducing sensitivity. On the other hand, the correlation analysis between NLR and CRP revealed a Spearman’s rho of 0.514 ( $p < 0.0001$ ), suggesting a moderate positive correlation.

**Table 1. Clinical and laboratory comparison between severe bacterial and viral CAP**

Characteristics	Bacterial (n=51)	Virus (n=10)	p-value
Age <24 months, n (%)	44 (86.3)	8 (80.0)	>0.05 <sup>†</sup>
Underlying conditions, n (%)	18 (35.3)	4 (40.0)	>0.05 <sup>†</sup>
Incomplete vaccination, n (%)	30 (58.9)	2 (20.0)	<0.05 <sup>†</sup>
History of pneumonia, n (%)	36 (70.6)	2 (20.0)	<0.005 <sup>†</sup>
High fever, n (%)	21 (41.2)	1 (10.0)	<0.05 <sup>†</sup>
Wheezing, n (%)	33 (64.7)	10 (100.0)	<0.05 <sup>†</sup>
WBC (K/ $\mu$ L), median (min, max)	11.3 (8.3, 17.1)	11.9 (9.2, 16.4)	>0.05 <sup>‡</sup>
ANC (K/ $\mu$ L), median (min, max)	6.3 (3.6, 10.8)	3.2 (1.5, 4.9)	<0.005 <sup>‡</sup>
ALC (K/ $\mu$ L), median (min, max)	4.2 (2.8, 6.4)	6.4 (4.1, 8.8)	>0.05 <sup>‡</sup>
NLR, median (min, max)	1.4 (0.9, 2.3)	0.5 (0.4, 0.7)	0.0002 <sup>‡</sup>
CRP (mg/L), median (min, max)	10.2 (3.4, 31.4)	1.5 (1.0, 4.6)	0.01 <sup>‡</sup>

CAP: Community-acquired pneumonia, WBC: White blood cell count, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein,  $\mu$ L: Microliter, mg: Milligram, L: Liter, <sup>†</sup>Fisher’s exact test, <sup>‡</sup>Mann-Whitney U test

**Table 2. AUROCs of basic inflammatory markers**

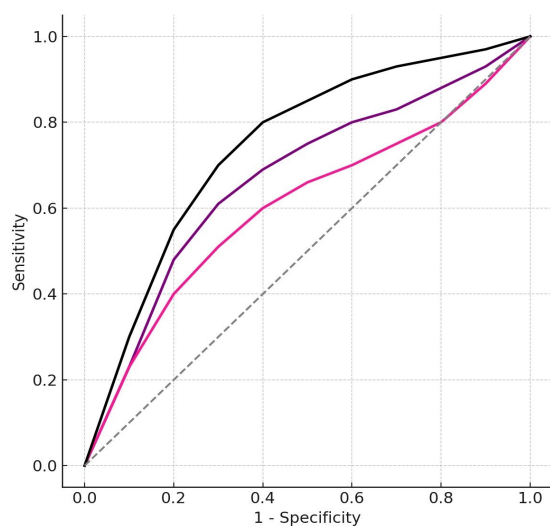
Inflammatory markers	AUROC	95% confidence interval	p-value
CRP	0.74	0.58-0.9	0.01
ANC	0.79	0.65-0.93	0.0015
NLR	0.87	0.78-0.97	<0.0001

AUROC: Area under the receiver operating characteristic curve, ANC: Absolute neutrophil count, NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein

**Table 3. Optimal cut-offs and diagnostic metrics of inflammatory markers**

Inflammatory marker	Sensitivity (%)	Specificity (%)	LR <sup>+</sup>	LR <sup>-</sup>
CRP ≥ 7 (mg/L)	56.9	90	5.7	0.4
NLR ≥ 0.8	78.4	90	7.9	0.2
ANC ≥ 5.8 (K/ $\mu$ L)	60.5	90	6.1	0.4
CRP ≥ 7 (mg/L) + NLR ≥ 0.8 + high fever	55.5	93.3	8.8	0.2
CRP ≥ 7 (mg/L) + NLR ≥ 0.8 + history of pneumonia	57.3	94.4	8.5	0.3

LR<sup>+</sup>: Positive likelihood ratio, LR<sup>-</sup>: Negative likelihood ratio, CRP: C-reactive protein, NLR: Neutrophil-to-Lymphocyte ratio, ANC: Absolute neutrophil count, mg: Milligram, L: Liter,  $\mu$ L: Microliter

**Figure 2.** Receiver operating characteristic (ROC) curve of basic inflammatory

The neutrophil-to-lymphocyte ratio (NLR) (black) showed the highest discriminative performance (AUC = 0.8725), followed by neutrophils (purple; AUC = 0.7922) and C-reactive protein (CRP) (pink; AUC = 0.7412). The dashed diagonal line indicates no discrimination.

## Discussion

This study highlights the microbial complexity in pediatric severe CAP, with a predominance of bacterial etiology confirmed by RT-PCR. Among the 61 children enrolled, 83.6% had bacterial pneumonia, reinforcing the high burden of bacterial pathogens. SP and HI were the most frequent organisms, consistent with global epidemiological data on pediatric CAP. (3,7). The frequent occurrence of SP–HI coinfections and the presence of triple-pathogen infections in 8 cases underscore the polymicrobial nature of BSCAP in children.

These findings align with previous reports suggesting that mono-infection is relatively uncommon in hospitalized pneumonia and that co-pathogen interactions may contribute to increased disease severity (3,8,9). The identification of

multiple pathogens in the BSCAP emphasizes the need for diagnostic approaches capable of detecting co-infections, as treatment regimens may need to be adjusted accordingly, particularly in resource-limited settings where microbiological diagnostics are not routinely available. Moreover, the study highlights the value of molecular diagnostics such as RT-PCR in informing targeted treatment strategies.

A history of recurrent pneumonia is a notable risk factor for BSCAP in children. Although multiple microbial causes have been described, data on specific pathogens in recurrent cases remain limited. A 13-year study of 1,395 hospitalized children with severe CAP found bacterial pathogens in 70% of cases, suggesting that recurrent episodes may impair mucosal defenses and mucociliary clearance, facilitating chronic bacterial colonization and secondary infections (9). Clinically, high fever is more indicative of BSCAP, while wheezing is more typical of VSCAP. Studies have shown that bacterial pneumonia is often suspected in the absence of wheezing, whereas wheezing is a hallmark of viral infections. Moreover, high fever was significantly more common in bacterial cases, supporting the use of these symptoms to guide early etiological suspicion in severe pediatric CAP (9).

In many developing countries, accurately identifying the microbial etiology of pediatric pneumonia remains a significant challenge due to limited access to specific diagnostic tests (7). This often results in empirical antibiotic use, leading to inappropriate prescriptions and excessive use, thereby contributing to the growing problem of antimicrobial resistance. In this study, all children (100%) received antibiotic treatment prior to microbiological confirmation. The primary aim of our research was to evaluate the utility of accessible inflammatory biomarkers, namely CRP, ANC, and NLR, to differentiate between viral and bacterial severe CAP. These markers are readily available in primary care settings and resource-limited areas. Our findings indicate that children with BSCAP had significantly higher ANC, NLR, and CRP levels compared to those with viral pneumonia.

Neutrophils play a critical role in the early immune response to infection, particularly against bacterial pathogens (10,11). In our study, ANC was significantly higher in children with BSCAP (AUROC = 0.79; 95% CI: 0.65–0.93;  $p = 0.0015$ ). This aligns with findings by Elemraid et al. (12), who reported higher neutrophil counts in bacterial compared to viral pneumonia, with an AUROC of 0.859 and a cut-off of 10,000 cells/mm<sup>3</sup> yielding 88.1% specificity. Although our identified cut-off (5,800 cells/ $\mu$ L) was lower, it achieved a comparable specificity of 90%. This difference may reflect immunological dynamics in severe infections, such as enhanced tissue recruitment, toxin-induced bone marrow suppression, or immune modulation (13). Thus, while ANC is a useful diagnostic marker, lower thresholds may be more appropriate in severe CAP.

CRP, a liver-derived acute-phase reactant, is commonly used to assess systemic inflammation. CRP has been reported as an independent predictor of bacterial pneumonia (14). In this study, CRP demonstrated moderate diagnostic utility for BSCAP with an AUROC of 0.74 (95% CI: 0.58–0.90). A CRP cut-off of  $\geq 7$  mg/L yielded 56.9% sensitivity and 90% specificity (LR<sup>+</sup> 5.7; LR<sup>-</sup> 0.4). Esposito et al. (15,16) reported varying CRP performance across studies, with mean CRP values ranging from 21.3–32.2 mg/L in bacterial cases and AUROCs between 0.58 and 0.63. While these studies confirm CRP's diagnostic relevance, an optimal threshold remains context-dependent. The lower cut-off observed in our cohort may reflect early-phase infections or immune dysregulation in severe cases, where pro-inflammatory signaling and hepatic CRP synthesis are suppressed (17). This suggests that lower CRP thresholds should be considered when evaluating children with severe pneumonia.

In recent years, the neutrophil-to-lymphocyte ratio (NLR), calculated from the absolute neutrophil and lymphocyte counts, has emerged as a promising biomarker for diagnosing bacterial infections such as sepsis and pneumonia. Previous studies reported AUROC values between 0.70 and 0.78, indicating moderate diagnostic accuracy, comparable to CRP and procalcitonin in emergency settings (18,19). In our study, NLR demonstrated the highest AUROC of 0.87 (95% CI: 0.78–0.97), with a cut-off value of  $\geq 0.8$  yielding a sensitivity of 78.4% and specificity of 90%. However, the difference between markers was not statistically significant ( $p > 0.05$ ). In the early stages of severe pneumonia, particularly in rapidly progressing cases, NLR rises much earlier and is more sensitive than ANC and CRP levels (20). Lymphopenia and neutrophilia represent a physiological response to systemic inflammation, especially bacterial

infections. Lymphopenia results from increased apoptosis, redistribution of lymphocytes into the reticuloendothelial system, and migration into lymphoid tissues. In contrast, neutrophilia occurs due to bone marrow stimulation by inflammatory cytokines. Therefore, NLR effectively integrates two opposing immune responses to systemic inflammation. Recent studies indicate that NLR has a stronger prognostic value than traditional inflammatory markers, including total leukocyte count, ANC, and CRP in CAP (9,21). However, a study published in *Scientific Reports* concluded that NLR alone is not a reliable marker for distinguishing bacterial from viral CAP (22). Thus, integrating clinical features and additional markers remains essential for improving diagnostic accuracy. A more accurate diagnostic approach involves combining NLR, lymphocyte-monocyte ratio (LMR), CRP levels, and clinical characteristics (23). According to Elemraid et al. (12), combining CRP, NLR, and ANC achieved an AUROC of 0.894, with 75.7% sensitivity and 89.4% specificity. Similarly, another study reported that adding clinical symptoms to CRP and NLR increased the AUROC to 0.897 (24). Research also shows that integrating serum amyloid A and CRP with clinical signs, such as wheezing or absence of fever, improves specificity but lowers sensitivity (14). These findings align with our study, where combining NLR and CRP with clinical features slightly increased specificity (93.3%–94.4%) at the cost of reduced sensitivity.

### *Study Limitations*

This study has several limitations. First, the sample size was relatively small and limited to a single tertiary pediatric hospital, which may restrict the generalizability of the findings to other settings, particularly in rural or primary care contexts. Second, although RT-PCR was employed as the gold standard for etiological diagnosis, it may not fully distinguish between active infection and colonization, especially in cases of co-infection. Third, the exclusion of mixed bacterial-viral infections may limit insights into the full clinical spectrum of severe CAP in children. Fourth, inflammatory markers such as CRP, ANC, and NLR may be influenced by factors beyond infection, including underlying conditions or host immune responses, which were not fully accounted for in this study. Future studies should include larger, multicenter cohorts to enhance external validity. Incorporating quantitative viral and bacterial load data and additional biomarkers such as procalcitonin, serum amyloid A (SAA), and cytokine profiles may provide a more comprehensive understanding of host-pathogen interactions. Furthermore, machine learning models integrating clinical, laboratory, and imaging data

may improve diagnostic accuracy and support antibiotic stewardship, particularly in low-resource settings.

### Conclusion

Basic inflammatory markers such as NLR, ANC, and CRP may offer valuable preliminary indications of severe bacterial pneumonia, particularly in resource-limited settings. However, due to their inherently modest sensitivity when used in isolation, relying solely on these markers may lead to diagnostic inaccuracies. Therefore, to enhance diagnostic precision, especially in distinguishing bacterial from viral etiologies, it is essential to incorporate a combination of clinical features, such as fever patterns or a history of recurrent infections, along with other complementary inflammatory biomarkers.

### Ethics

**Ethics Committee Approval:** The study protocol was reviewed and approved by the Ethics Committee in Biomedical Research at Children's Hospital 1 (approval number: 425/GCN-BVND1, date: 17.11.2022).

**Acknowledgements:** We thank Can Tho University of Medicine and Pharmacy for institutional support and the leadership and medical staff of Children's Hospital 1 for their valuable assistance during the study.

### Footnotes

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

**Appendix 1-2 Link:** <https://d2v96fxpocvxx.cloudfront.net/37eae217-e8b5-4f55-976f-35df98003e83/content-images/e001bccd-38de-4200-83ba-8fa443cc6cd3.pdf>

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