

## Original Article

# Diagnostic performance of neutrophil CD64 in distinguishing between bacterial and mycoplasma community-acquired pneumonia in children

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**Abstract:** Objective: The goal of this study was to determine the diagnostic utility of CD64 on neutrophils (nCD64) in distinguishing bacterial community acquired pneumonia (CAP) from mycoplasma CAP in children, and to examine its performance at different age groups. Patients and Methods: Expression of nCD64, white blood cell count (WBC) and percentage of neutrophils were examined in healthy controls and children with bacterial or mycoplasma CAP. Area under receiver operating characteristic (ROC) curves were used to examine their diagnostic performance. Results: A total of 172 children with bacterial CAP, 64 children with mycoplasma CAP, and 131 healthy children were recruited. In all children and after stratification by age, expression of nCD64 was significantly increased in bacterial CAP compared to mycoplasma CAP and healthy controls. The area under ROC curve was 0.83 for nCD64 in discriminating bacterial CAP from mycoplasma CAP, which was significantly greater than that of WBC (0.73) or percentage of neutrophils (0.65). The nCD64 had the best performance for children of ages < 24 months, with the area under ROC curve of 0.90, a sensitivity of 97.6% and a specificity of 76.6%. Conclusions: Expression of nCD64 was significantly increased in bacterial CAP compared to mycoplasma CAP in children. nCD64 is a good diagnostic biomarker to discriminate bacterial CAP from mycoplasma CAP in children, particularly for those < 24 months old.

**Keywords:** CD64, WBC, pneumonia, bacterial, mycoplasma, diagnosis, children

## Introduction

Community-acquired pneumonia (CAP) refers to pulmonary parenchymal infections acquired outside of hospitals [1]. It is one of the most common infectious diseases and is a main cause of death in children [2, 3]. Timely and proper treatment is necessary to avoid progression to severe episodes, shorten hospital stay, reduce mortality, and alleviate healthcare burden. Early diagnosis and identification of etiology are the basis for timely and optimal treatment. Diagnosis of CAP is based on respiratory signs, symptoms, and chest x-rays. Bacteria, virus, and mycoplasma are major pathogens for the disease. Traditional blood tests such as white cell counts (WBC) and platelet counts are relatively insensitive and lack of the sensitivity to distinguish these pathogens. Sputum or blood cultures take a long time to identify pathogens. Finding novel biomarkers is needed to promptly identify the etiology of the disease.

CD64 is a high-affinity receptor for Fc domain of IgG [4]. Binding of CD64 to its ligand initiates the immune responses such as eliminating pathogenic microorganisms. In normal conditions, CD64 is expressed on the surface of antigen presenting cells such as monocytes, macrophages, and dendritic cells, while only a small amount of CD64 is expressed on the surface of neutrophils [5]. Expression of CD64 on neutrophils (nCD64) increases rapidly during a bacterial infection [6, 7]. Previous studies have demonstrated its diagnostic value in discriminating bacterial infections from virus infection [8, 9]. Many more studies reported that nCD64 is a very sensitive diagnostic marker for the identification of sepsis [10-18]. In addition, nCD64 was found to be a good prognostic marker for patients with sepsis [10, 19, 20].

Clinical signs, symptoms, and radiological findings of CAP caused by mycoplasma may not be differentiated from other causes of CAP in

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**Table 1.** Comparison of nCD64, WBC, and percentage of neutrophils among children with CAP and healthy controls

Variable	Bacterial CAP (n=172)	Mycoplasma CAP (n=64)	Healthy controls (n=131)	P value
nCD64	18.7 ± 75.5	3.1 ± 6.2	1.0 ± 1.1	< 0.0001
WBC (× 10 <sup>6</sup> /ml)	16.3 ± 8.3	10.3 ± 5.0	7.0 ± 1.6	< 0.0001
% Neutrophils	66.2 ± 18.9	55.8 ± 20.8	41.1 ± 11.2	< 0.0001

children [21]. The use of nCD64 to differentiate between bacterial and mycoplasma CAP in children has not been well addressed. It is unknown whether nCD64 has different diagnostic performance within varying age groups. The objective of this study was to determine the diagnostic utilities of CD64 in distinguishing bacterial CAP from mycoplasma CAP in children, and to examine its performance in different age groups. Expression of CD64 was compared between children with bacterial CAP and children with mycoplasma CAP. The area under receiver operating characteristic (ROC) curves were used to examine the diagnostic performance.

### Patients and methods

All children with CAP were treated in our hospital during July 2015 to September 2017. Children with CAP were diagnosed based on with signs and symptoms of a lower respiratory tract infection and the presence of a new infiltrate on chest X-ray. Bacterial CAP was confirmed using sputum and/or blood culture and mycoplasma CAP was verified using antibody tests. Patients were excluded who received antibiotics before admission, or had chronic respiratory disease. Healthy children receiving routine examinations in our hospital were included as the controls. The study was approved by the Ethics Committee of Zhejiang Xiaoshan Hospital. Informed written consent was obtained from children's parents.

EDTA-anticoagulated whole blood was drawn within 48 hours after admission for routine hematology. Blood samples (50 µl) were stained with monoclonal anti-CD45 and anti-CD64 antibodies (Quantibrite™ CD64/CD45, BD Biosciences, San Jose, CA), and assessed by a FACSCalibur flow cytometer (Becton Dickinson, NY, USA) following manufacturer's instructions. WBC were examined by flow cytometry (Beckman Coulter, Pasadena, CA, USA) and percentage of neutrophils were calculated.

All data were analyzed using SAS version 9.3 (Cary, NC, USA). Quantitative data are presented as mean ± SD. Differences in nCD64, WBC and percentage of neutrophils among groups were determined by the non-parametrical Kruskal-Wallis test followed by

Dunn's post hoc tests. ROC curves were constructed to determine the diagnostic performance of the biomarkers. The best cut-off value was decided when the sum of sensitivity and specificity achieved the highest. A  $P < 0.05$  was considered to be statistically significant.

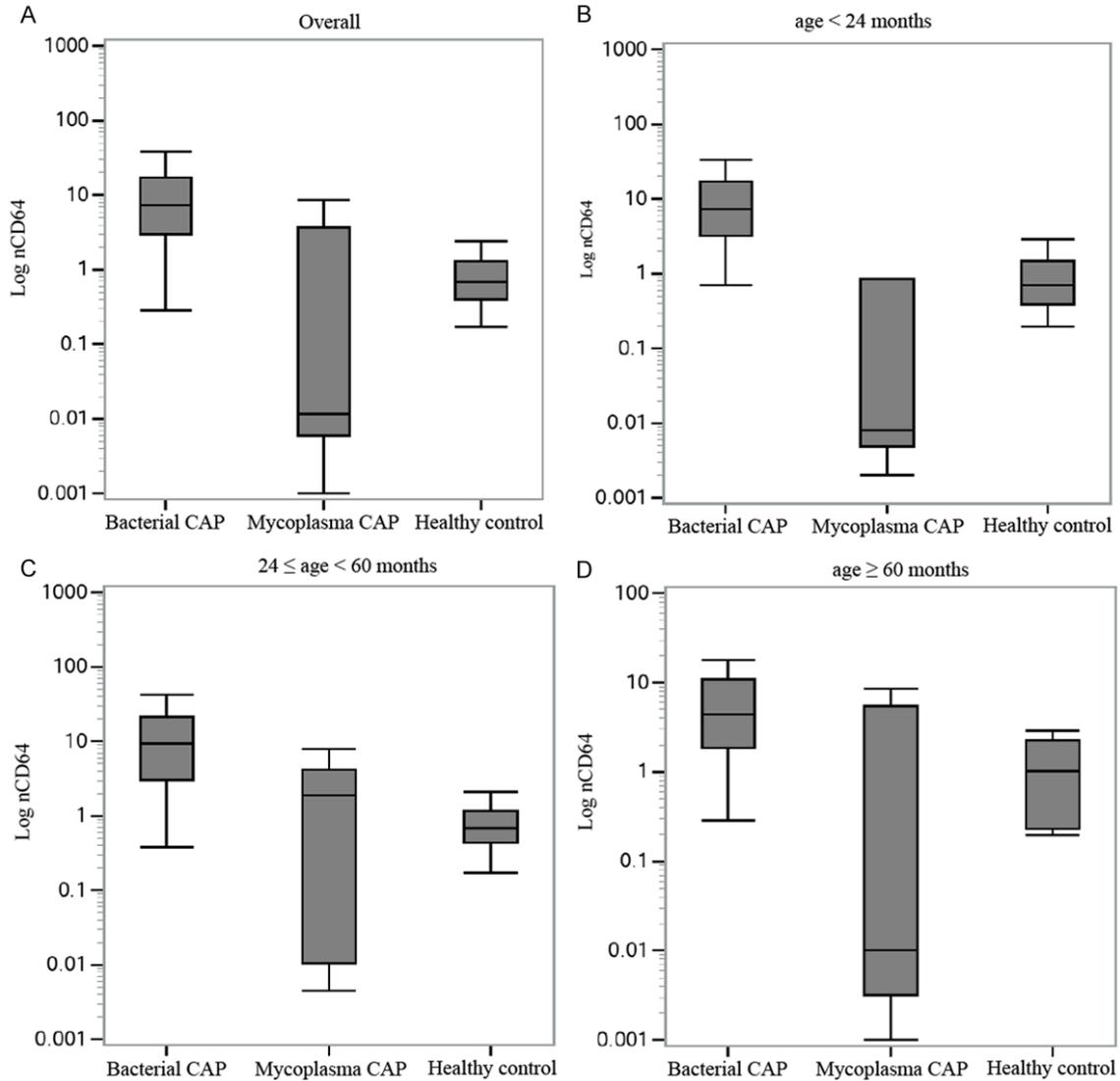
### Results

A total of 172 children with bacterial CAP, and 64 children with mycoplasma CAP were identified. In addition, 131 healthy children were recruited as the controls. Median ages were 24 (range, 1-196), 24 (range, 1-180) and 26 (range, 1-180) months for children with bacterial CAP, children with mycoplasma CAP and healthy controls, respectively. Expression of nCD64 was  $18.7 \pm 75.5$  for children with bacterial CAP, which was significantly higher than that ( $3.1 \pm 6.2$ ) in children with mycoplasma CAP ( $P < 0.0001$ ). In contrast, nCD64 expression in children with mycoplasma CAP was not significantly different from healthy control (Table 1). The boxplot distribution displays a higher expression of CD64 in children with bacterial CAP (Figure 1A). WBC and percentage of neutrophils were also significantly higher in children with bacterial CAP compared with mycoplasma CAP (Table 1).

Children were then stratified by age into three groups, < 24 months, ≤ 24 and < 60 months, and ≥ 60 months. Our results reveal that expression of nCD64 was significantly higher in bacterial CAP than mycoplasma in children at all three age groups (Table 2). The boxplot distribution displayed a higher expression of CD64 bacterial CAP at different age groups (Figure 1B-D).

Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic performance of nCD64 in distinguishing bacterial CAP from mycoplasma CAP in children. The area under ROC curves was 0.83 for the CD64, which was significantly greater than that of

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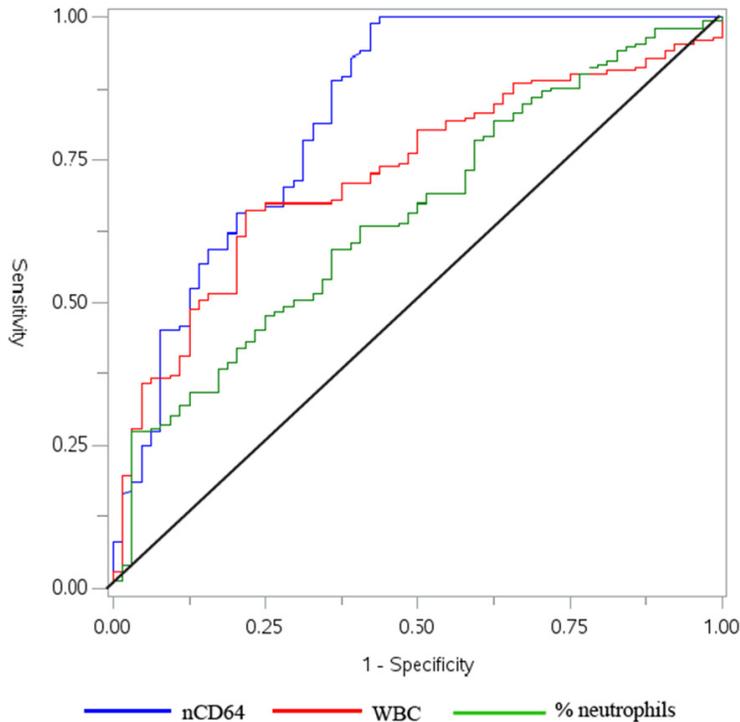
**Figure 1.** Boxplot presentation of CD64 among children with CAP and healthy controls. A. Expression of nCD64 in all children. B. Expression of nCD64 among children < 24 months old. C. Expression of nCD64 among children ≤ 24 and < 60 months old. D. Expression of nCD64 among children ≥ 60 months old.

**Table 2.** Comparison of levels of nCD64 among children with CAP and healthy controls at different age groups

Age groups (months)	Bacterial CAP	Mycoplasma CAP	Healthy controls	P value
< 24	13.6 ± 20.6 (n=83)	1.7 ± 4.5 (n=26)	1.1 ± 1.0 (n=48)	< 0.0001
24 ≤ and < 60	33.1 ± 31.7 (n=54)	4.5 ± 7.6 (n=24)	0.9 ± 0.8 (n=72)	< 0.0001
≥ 60	8.2 ± 9.9 (n=35)	3.3 ± 5.9 (n=14)	1.7 ± 2.4 (n=11)	< 0.0001

WBC (0.73) or percentage of neutrophils (0.65) (Figure 2). At the cutoff value of 0.40, the sen-

sitivity and the specificity for CD64 were 98.8% and 57.8%, respectively (Table 3).



**Figure 2.** Receiver operating characteristics (ROC) curves for nCD64, WBC and percentage of neutrophils in differentiating bacterial CAP from mycoplasma CAP.

The ROC curves for nCD64 to distinguish bacterial CAP from mycoplasma CAP were also constructed after stratification by age (**Figure 3**). These results found that nCD64 had the best performance in the age group of < 24 months. The area under ROC curves was 0.90. The sensitivity was 97.6% and the specificity was 76.9% at the cutoff value of 0.85 for this age group of children. In contrast, the areas under ROC curves were 0.78 and 0.78, respectively, for the age groups of ≤ 24 and < 60 months, and ≥ 60 months. At the cutoff value of 3.75 and 0.02, the sensitivity and specificity were 72.2% and 75.0%, 100% and 64.2%, respectively, for age groups of ≤ 24 and < 60 months, and ≥ 60 months (**Table 3, Figure 3A-C**).

The diagnostic performance of CD64 in discriminating children with CAP from healthy controls was also examined. Our results showed that the area under ROC curves was 0.78 for CD64, which was significantly worse than that of WBC (0.87), but not significantly different than the percentage of neutrophils (0.83) (**Figure 4**).

**Discussion**

Identification of the ideology of CAP in children assists timely and proper treatment for the diseases. This study determined the diagnostic value of nCD64 in distinguishing bacterial CAP from mycoplasma CAP in children. Our results revealed that expression of nCD64 was significantly increased in children with bacterial CAP compared to children with mycoplasma CAP. No significant difference was observed between children with mycoplasma CAP and healthy children. The nCD64 demonstrated its good diagnostic performance in discriminating bacterial CAP from mycoplasma CAP in children, particularly for children < 24 months old.

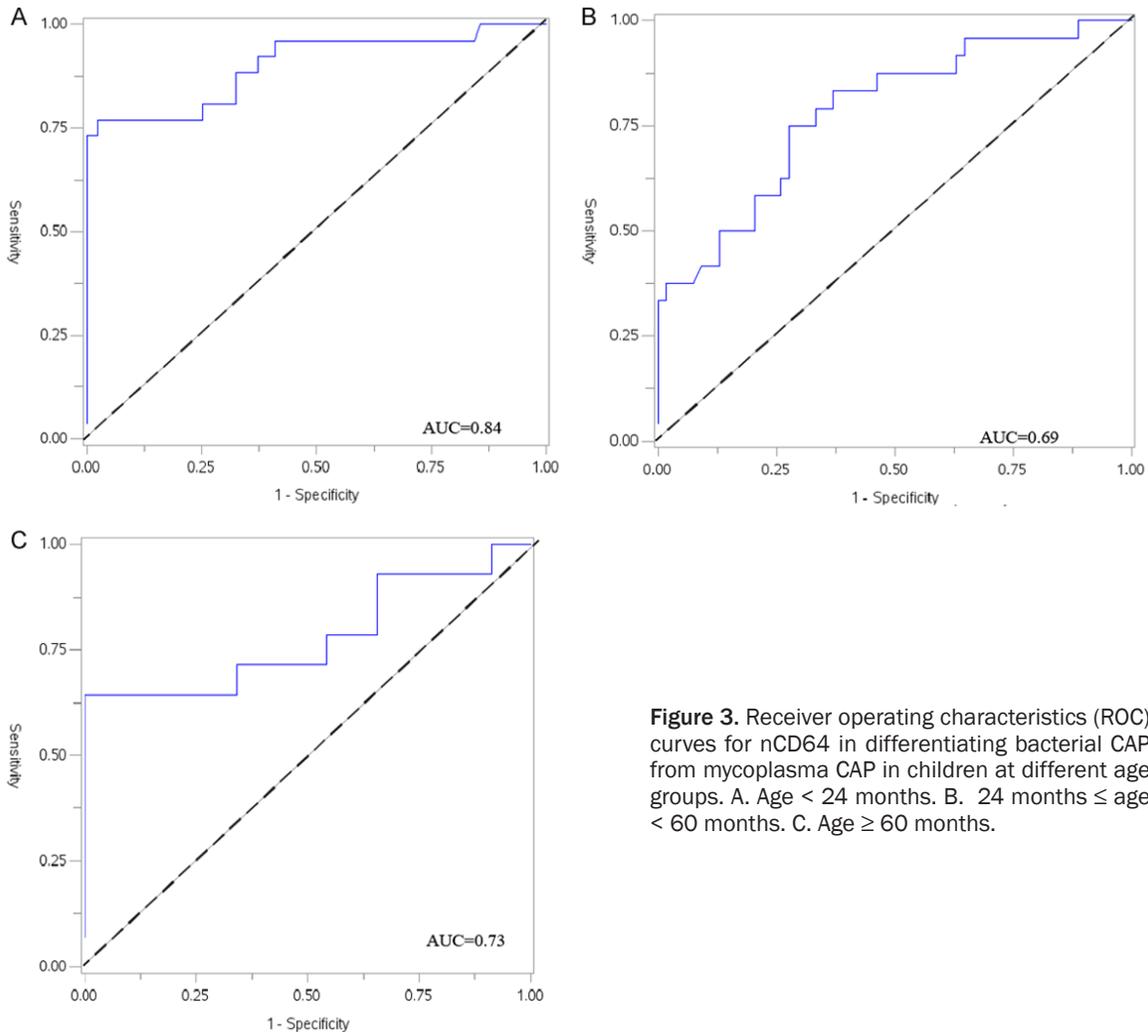
Differences in expression of nCD64 are the basis to differentiate children with bacterial CAP from children with mycoplasma CAP. A previous study showed that the expression of nCD64 was significantly higher in children with bacterial pneumonia compared to children with viral or mycoplasma pneumonia, or healthy controls [22]. Similar to their finding, the results of this study revealed that expression of nCD64 was significantly higher in children with bacterial CAP compared to children with mycoplasma CAP or healthy controls. Accordingly, nCD64 demonstrated good diagnostic performance in differentiating children with bacterial CAP from children with mycoplasma CAP. The area under ROC curve was 0.83 for nCD64, which was significantly greater than that of traditional hematological tests (WBC or percentage of neutrophils). Similarly, Zhong et al. reported that the area under the ROC curve was 0.878 for CD64 in diagnosing bacterial pneumonia in children with the sensitivity of 81.3% and specificity of 92.3% [22]. Previous studies also reported its diagnostic value in discriminating bacterial infections from viral infections [8, 9]. However, one study found that nCD64 was a poor marker to distinguish bacterial infection from viral infections with the area under the ROC curve of 0.64 [23].

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**Table 3.** Diagnostic performance of nCD64 in distinguishing bacterial CAP from mycoplasma CAP in children

	Area under ROC curve	95% CI	Cut-off value	Sensitivity (%)	Specificity (%)
All children	0.83	0.76-0.89	0.40	98.8	57.8
Age groups (months)					
< 24	0.90	0.82-0.98	0.85	97.6	76.9
24 ≤ and < 60	0.78	0.60-0.96	3.75	72.2	75.0
≥ 60	0.78	0.67-0.90	0.02	100.0	64.2

CI, confidence interval; ROC, receiver operating characteristics.

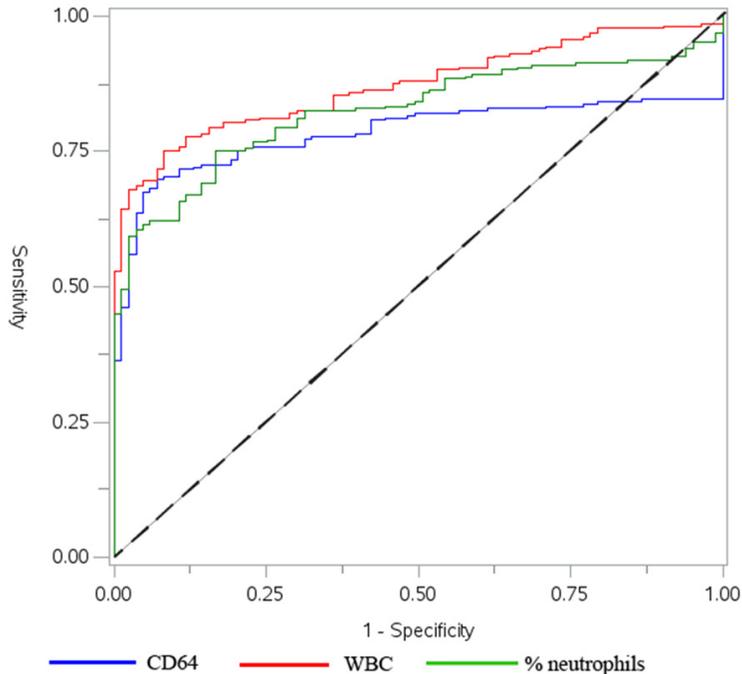


**Figure 3.** Receiver operating characteristics (ROC) curves for nCD64 in differentiating bacterial CAP from mycoplasma CAP in children at different age groups. A. Age < 24 months. B. 24 months ≤ age < 60 months. C. Age ≥ 60 months.

In addition, many previous studies have shown that nCD64 demonstrated its potential value in the diagnosis of sepsis [10-14, 24]. nCD64 was superior to traditional blood tests and CRP for the early diagnosis of systemic infections in adult patients admitted to intensive care unit [25], and bacterial infections during surgery

[26]. Its expression had the same diagnostic accuracy as CRP in diagnosing pneumonia in patients hospitalized with AECOPD [27]. nCD64 was also better than PCT for identifying patients who required treatment with antibiotics [28]. It served as a good prognostic marker for patients with CAP [29] and for patients with ventilator-

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**Figure 4.** Receiver operating characteristics (ROC) curves for nCD64, WBC, and percentage of neutrophils in differentiating children having CAP from healthy children.

associated pneumonia-induced sepsis [19]. All these previous findings supported its potential clinical utility for patients with infection.

To the best of our knowledge, this study is the first to examine diagnostic performance of CD64 in discriminating bacterial CAP from mycoplasma CAP in children at different age groups. Our data indicated that CD64 had the best performance in distinguishing bacterial CAP from mycoplasma CAP for children < 24 months old. The area under the ROC curve was improved to 0.90 for this group of children. At the cutoff value of 0.85, the sensitivity and specificity were 97.6% and 76.9%, respectively. Previous studies have reported the role of nCD64 in diagnosing sepsis and predicting survival in infants [10, 20]. Furthermore, nCD64 has several other advantages in becoming a diagnostic marker for in this age group of children. Although CAP in very young children is less common [30], children of < 24 months old have a higher risk of death from CAP due to an underdeveloped immune system. It is tough to obtain sputum from infants for culture tests. The flow cytometry method needs a minimal volume of blood to measure nCD64. The assay

method is accurate and fast. These advantages further support the potential clinical usage of nCD64 in diagnosing etiology of CAP in this age group of children.

This study revealed that expression of nCD64 was not significantly different between children with mycoplasma CAP and healthy controls. Similarly, a previous study demonstrated no significant difference in the expression of nCD64 among children with viral, mycoplasma pneumonia and healthy controls [22]. In contrast, it was shown to be significantly elevated in adult with mycoplasma pneumonia compared to healthy persons in another study [23]. Due to non-significant difference in expression of CD64 between children with mycoplasma CAP and healthy controls, these results revealed

that nCD64 had fair diagnostic performance in distinguishing children with bacterial or mycoplasma CAP from the healthy controls.

A limitation of this study is the relatively limited sample size. The retrospective study design may bring the bias in sample selection. Only children with CAP treated as inpatients were included in this study. In addition, children with CAP caused by other pathogens were not included in this study.

### Conclusions

Expression of nCD64 is significantly elevated in children with bacterial CAP compared with children having mycoplasma CAP. nCD64 has good diagnostic performance in distinguishing bacterial CAP from mycoplasma CAP, particularly for children of  $\leq 24$  months old.

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**Disclosure of conflict of interest**

None.

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