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Combined score of C-reactive protein level and neutrophil-to-lymphocyte ratio: A novel marker in distinguishing children with exacerbated asthma

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Abstract

Background: Both C-reactive protein (CRP) level and neutrophil-to-lymphocyte ratio (NLR) are commonly elevated in patients with asthma. It is necessary to develop a novel marker, the combined score of CRP level and NLR (C-NLR score) based on cutoff points of CRP and NLR, and apply it in asthma diagnosis. The aim of this study was to explore whether C-NLR could distinguish children with exacerbated asthma.

Methods: Children suffering from exacerbated asthma were regarded as the asthmatic group (n = 86), which was divided into three groups: mild (n = 54), moderate (n = 17), and severe (n = 15). The control group consisted of children without any allergic disease and infection (n = 38). To compare CRP level and NLR between the asthmatic group and control group, a receiver-operating characteristic curve was constructed to determine area under the curve (AUC) and optimal cutoff point. Thereafter, the C-NLR score was classified as follows: C-NLR score of 2 with an elevated CRP level and high NLR, a C-NLR score of 1 with one of these abnormalities, and a C-NLR score of 0 with a normal CRP level and low NLR. The C-NLR score was then compared among different asthma groups.

Results: In the control group, the CRP level and NLR were 1.9 (0.5–2.6) mg/L and 1.01 (0.69–1.31), respectively. In the asthmatic group, the CRP level and NLR were 7.3 (3.2–14.2) mg/L and 3.08 (1.73–5.34), respectively, which were higher than those in the control group ($p < 0.001$ for CRP and $p < 0.001$ for NLR). The AUC of CRP was 0.86, and the optimal cutoff point was 3.6 mg/L. The AUC of NLR was 0.86, and the optimal cutoff point was 1.72. The AUC of the C-NLR score was 0.94, and the optimal cutoff point was 1.

Conclusions: C-NLR, a novel inflammatory marker, was applied here for the exacerbated asthma for the first time. Our study has shown C-NLR is a promising marker to distinguish children with exacerbated asthma from healthy children.

Keywords
C-reactive protein, neutrophil-to-lymphocyte ratio, exacerbated asthma

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Introduction

Asthma is a chronic inflammatory disease of multifactorial etiology that affects 300 million people worldwide and results in airway hyper-responsiveness and acute

bronchoconstriction.[1] It is well known that asthma often begins in childhood but can occur at any time throughout life.[2] Children experience more asthma attacks and emergency visits than adults do and uncontrolled asthma exacerbation in children results in significant costs to families and society.[3] About 57.9% of children with asthma experience asthma exacerbation more than once a year.[4]

As a chronic inflammatory disease, airway inflammation has been regarded as the most important pathological characteristic of asthma patients.[5] Sputum analysis is a remarkable technique to investigate the respiratory tract in patients with asthma.[6] However, collection of sputum samples is difficult and not feasible for children most of the time. Therefore, collection of blood samples from children is commonly applied.

Some inflammatory markers in blood such as white blood cell count (WBC), eosinophil count, and lymphocyte ratio perform well in asthma diagnosis.[7] The neutrophil-to-lymphocyte ratio (NLR) represents a cost-effective and easily available but non-specific marker of inflammation.[8] The NLR is higher in patients with asthma.[9,10] C-reactive protein (CRP) is an acute-phase protein synthesized by hepatocytes in response to pro-inflammatory cytokines during inflammatory/infectious processes,[11] and the level of CRP is also elevated in asthma.[12,13] Since both NLR and CRP are elevated in asthmatic patients, it is necessary to develop a novel marker, the combined score of CRP level and NLR (C-NLR score), which can take full advantage of meanings of both NLR and CRP in asthmatic patients. Therefore, in this article, we calculated C-NLR scores based on cutoff points of CRP and NLR to distinguish children with exacerbated asthma from healthy children.

Materials and methods

Study population

All patients (aged < 14 years) were from Wuxi Children's Hospital between January and November 2019. Children who were hospitalized because of exacerbated asthma were regarded as the asthmatic group. Based on the Global Initiative for Asthma guidelines,[14] children with exacerbated asthma were divided into mild, moderate, and severe groups according to clinical and or laboratory findings. Any patient with tumor, cardiovascular disease, or endocrine disease was excluded. The control group consisted of children without any allergic disease and infection. This retrospective study was approved by the Ethics Committee of Wuxi Children's Hospital Affiliated to Nanjing Medical University and was performed in accordance with the Declaration of Helsinki.

Laboratory assays

Peripheral venous blood samples were collected in the morning and placed in EDTA anticoagulation tubes and coagulation-promoting tubes. Blood samples of asthmatic patients were drawn at the first or second day of hospitalization. Whole blood in anticoagulation tubes was analyzed in a Mindray BC-5100 Automatic Hematology Analyzer (Mindray Corp., Shenzhen, China). White blood cell count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), hemoglobin (HGB) level, platelet (PLT) count, and CRP level were measured directly. The neutrophil-to-lymphocyte ratio was calculated by dividing ANC by ALC. The platelet-to-lymphocyte ratio (PLR) was calculated by dividing PLT count by ALC. Blood samples in coagulation-promoting tubes were centrifuged at 3000 × *g* for 5 min, and serum samples were analyzed within 2 h by a *Beckman* AU5800 Automatic Analyzer (Beckman Coulter Inc., Brea, CA, USA) to detect serum total protein (TP) and albumin (ALB).

Calculation of C-NLR score

Based on cutoff points, CRP levels were classified as normal or elevated, and NLRs as low or high. The combined score of CRP level and NLR was classified as follows: 2, elevated CRP level and high NLR; 1, one of these abnormalities; and 0, normal CRP level and low NLR.

Statistical analysis

The distributions of continuous data were tested using the Kolmogorov–Smirnov test. Continuous data were presented as mean ± standard deviation (SD) when they were normally distributed; otherwise, they were presented as median (interquartile range). Categorical data were shown as numbers. Student's *t*-test and one way analysis of variance (ANOVA) were used for comparison of normally distributed data. The Mann–Whitney *U* test and Kruskal–Wallis test were used for comparison of non-normally distributed data. The chi-square test was used for comparison of categorical data. The receiver-operating characteristic curve was calculated to determine area under the curve (AUC) with 95% confidence interval (CI) and the optimal cutoff point. A two-tailed value of $p < 0.05$ was regarded as statistically significant. Statistical analyses were performed by SPSS version 20.0 (SPSS Inc., Chicago, USA).

Results

Comparison between control group and asthma group

We enrolled a total of 124 participants, including 86 children with exacerbated asthma and 38 control subjects (Table 1). There were no statistical differences between the asthmatic group and control group for sex and age ($p = 0.568$ and $p = 0.053$, respectively).

Table 1. Comparison between control group and exacerbated asthma group.

Parameter	Control group	Exacerbated asthma group	*p* value
N	38	86	
Male (*n*)	20	50	0.568*
Female (*n*)	18	36	
Age (yr)	7 (5–9)	6 (4–8)	0.053**
WBC (10^9/L)	6.91 ± 1.65	10.69 ± 3.60	< 0.001***
ANC (10^9/L)	2.71 (2.35–3.45)	6.77 (4.68–9.32)	< 0.001**
ALC (10^9/L)	2.90 (2.31–3.52)	2.17 (1.59–2.98)	< 0.001**
HGB (g/L)	130 ± 9	130 ± 11	0.967***
PLT (10^9/L)	303 ± 54	272 ± 80	0.013***
CRP (mg/L)	1.9 (0.5–2.6)	7.3 (3.2–14.2)	< 0.001**
TP (g/L)	69.9 ± 4.7	70.3 ± 5.5	0.714***
ALB (g/L)	44.0 ± 2.4	42.7 ± 3.9	0.027***
NLR	1.01 (0.69–1.31)	3.08 (1.73–5.34)	< 0.001**
PLR	99.8 (82.9–133.1)	122.76 (89.23–168.78)	0.03**

WBC: white blood cell count; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; HGB: hemoglobin; PLT: platelet; CRP: C-reactive protein; TP: total protein; ALB: albumin; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio.
* chi-square test, ** Mann–Whitney *U* test, *** Student's *t*-test.

The levels of WBC, ANC, CRP, NLR, and PLR were higher in the asthma group compared to the control group ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.031$, respectively). Absolute lymphocyte count, PLT, and ALB were lower in the asthma group compared to the control group ($p < 0.001$, $p = 0.013$, $p = 0.027$, respectively). There were no significant differences between the asthmatic group and control group for HGB and TP ($p = 0.967$ and $p = 0.714$ respectively).

Diagnostic value of CRP, NLR, and PLR

Diagnostic values of CRP, NLR, and PLR to distinguish children with exacerbated asthma from controls were presented in Figure 1 and Table 2. With an optimal cutoff point of 3.6 mg/L, CRP had sensitivity and specificity of 0.974 and 0.698, respectively, and AUC was 0.86 (95% CI: 0.80–0.92). With an optimal cutoff point of 1.72, sensitivity and specificity of NLR were 0.947 and 0.756, respectively, and AUC was 0.86 (95% CI: 0.80–0.93). For PLR, the optimal cutoff point was 144.8, and sensitivity and specificity were 0.921 and 0.361, respectively, and AUC of PLR was 0.62 (95% CI: 0.52–0.72), which was lower compared to CRP and PLR.

The optimal cutoff points were regarded as cutoff points to calculate the C-NLR score. Based on previous analyses in this study, CRP levels were classified as normal (< 3.6 mg/L) and elevated (≥ 3.6 mg/L); NLR was classified as low (< 1.72) and high (≥ 1.72). We then classified the C-NLR score as follows: 2, with elevated CRP level (≥ 3.6 mg/L) and high NLR (≥ 1.72); 1, with one of these abnormalities; and 0, with normal CRP level (< 3.6 mg/L) and low NLR (< 1.72).

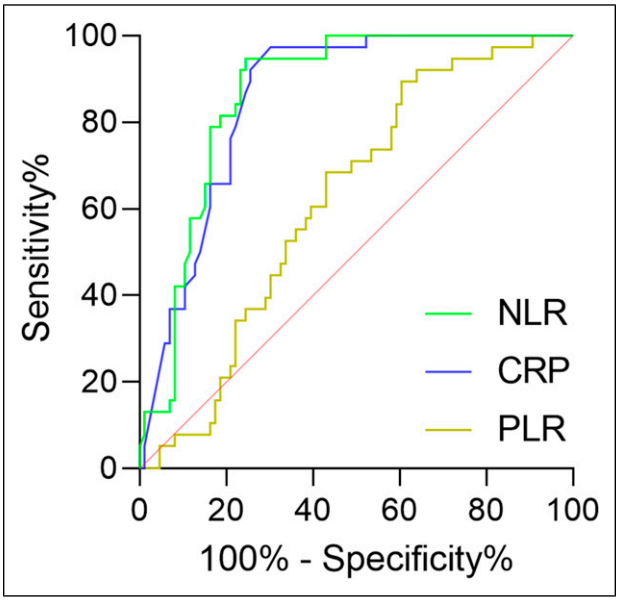

Figure 1. ROC curve of CRP, NLR, and PLR in diagnosis of children with exacerbated asthma. ROC: receiver-operating characteristic; CRP: C-reactive protein; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio.

Comparison among different exacerbated asthma groups

A total of 86 children with exacerbated asthma were divided into mild ($n = 54$), moderate ($n = 17$), and severe ($n = 15$) groups (Table 3). There were no statistical differences among groups with regard to sex and C-NLR score ($p = 0.096$ and $p = 0.337$, respectively).

Table 2. Results of diagnostic value of CRP, NLR and PLR.

Variable	Cutoff point	Sensitivity	Specificity	AUC (95% CI)
CRP	3.6	0.974	0.698	0.86 (0.80–0.92)
NLR	1.72	0.947	0.756	0.86 (0.80–0.93)
PLR	144.8	0.921	0.361	0.62 (0.52–0.72)
C-NLR score	1	0.907	0.921	0.94 (0.89–0.98)

CRP: C-reactive protein; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; C-NLR score: combined score of CRP level and NLR.

Table 3. Comparison among different exacerbated asthma groups.

Parameter	Mild group	Moderate group	Severe group	*p* value
N	54	17	15	
Male (*n*)	33	7	5	0.096*
Female (*n*)	21	10	10	
Age (yr)	6 (4–7)	7 (5–9)	4 (3–6)[b]	0.047**
WBC (10^9/L)	9.71 ± 3.15	12.09 ± 4.25[a]	12.61 ± 3.25[a]	0.004***
ANC (10^9/L)	6.03 (3.83–7.89)	8.67 (4.52–11.26)	9.29 (7.52–12.15)[a]	0.001**
ALC (10^9/L)	2.35 (1.75–3.13)	2.25 (2.03–3.15)	1.51 (1.00–1.69)[ab]	0.001**
HGB (g/L)	128 ± 10	138 ± 10[a]	129 ± 12[b]	0.006***
PLT (10^9/L)	276 ± 87	268 ± 81	264 ± 53	0.861***
CRP (mg/L)	7.4 (3.0–19.1)	4.7 (1.4–14.1)	7.9 (3.6–13.9)	0.345**
TP (g/L)	69.1 ± 4.7	70.5 ± 5.5	74.3 ± 6.7 a	0.004***
ALB (g/L)	42.1 ± 3.2	43.6 ± 4.5	44.1 ± 4.7	0.112***
NLR	2.64 (1.47–4.0)	3.09 (1.73–5.07)	7.20 (4.56–10.0)[ab]	<0.001**
PLR	121.8 (84.45–159.67)	103.77 (66.01–142.70)	171.76 (121.85–294.00)[ab]	0.005**
C-NLR score = 0	7	1	0	0.337*
C-NLR score = 1	19	8	4	
C-NLR score = 2	28	8	11	

WBC: white blood cell count; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; HGB: hemoglobin; PLT: platelet; CRP: C-reactive protein; TP: total protein; ALB: albumin; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; C-NLR score: combined score of CRP level and NLR.
[a] compare with mild group, $p < 0.05$; [b] compare with moderate group, $p < 0.05$; * chi-square test; ** Kruskal–Wallis test; *** one-way ANOVA.

For continuous data, age, WBC, ANC, LNC, HGB, TP, NLR, and PLR differed significantly among the groups ($p = 0.047$, $p = 0.001$, $p = 0.001$, $p = 0.004$, $p = 0.006$, $p = 0.004$, $p < 0.001$, and $p = 0.005$, respectively), while PLT, CRP, and ALB had no significant differences ($p = 0.861$, $p = 0.345$ and $p = 0.112$, respectively).

Diagnostic value of C-NLR score

The combined score of CRP level and NLR had an AUC of 0.94 (95% CI: 0.89–0.98) to distinguish children with exacerbated asthma from control subjects (Table 2, Figure 2). The optimal cutoff point was 1, and sensitivity and specificity were 0.907 and 0.921, respectively.

Discussion

Asthma is a heterogeneous disorder of the airways that involves chronic inflammation.[15] The current study showed that CRP level and NLR, two inflammatory markers in the blood, were higher in children with asthma compared to the control group, which was in agreement with previous studies.[9,12,13] This study also showed that PLR, another inflammatory marker that can diagnose inflammatory rheumatic diseases and predict clinical outcomes in rectal cancer,[16,17] was also higher in children with exacerbated asthma. Total protein is a marker reflecting nutritional status and is closely related to the immune system.[18] Albumin is the most abundant plasma protein reflecting the nutritional status and synthesis of serum; ALB is inhibited by malnutrition and inflammation.[19,20] The current study showed that the ALB level was lower in asthma group, revealing that exacerbated asthma may contribute to malnutrition in children.

Like the combined score of fibrinogen level and NLR (F-NLR score), or the Glasgow Prognostic Score derived from CRP level and ALB level,[21,22] we calculated the C-NLR score from the CRP level and NLR according to their

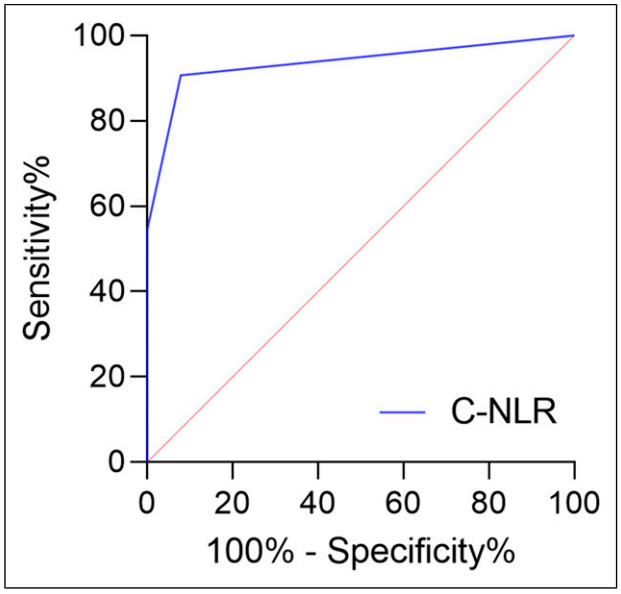

Figure 2. ROC curve of C-NLR score in diagnosis of children with exacerbated asthma. ROC: receiver-operating characteristic; C-NLR score: combined score of CRP level and NLR.

optimal cutoff points. We did not calculate a combined score of the CRP level and PLR due to lower diagnostic values of PLR compared to NLR. To our knowledge, the C-NLR score, a novel inflammatory marker, was applied here to exacerbated asthma for the first time. Our study showed that the diagnostic ability of the C-NLR score to differentiate children with exacerbated asthma from the control group was superior to CRP or NLR alone. Furthermore, for the C-NLR score, there was no significant difference among the exacerbated asthma groups according to severity, showing that the C-NLR score was not correlated with severity of exacerbated asthma.

This study inevitably had some limitations. First, the study was only conducted based on a single center and the sample size was small. Second, we did not enroll children with stable asthma,[23] which ignored the different effect of stable asthma on NLR compared to that of exacerbated asthma. Third, therapeutic measures involving inhaled or oral corticosteroids as maintenance treatment to influence the levels of routine blood parameters were not evaluated.

Conclusions

The combined score of CRP level and NLR determined by CRP level and NLR is a novel and promising marker to distinguish children with exacerbated asthma from children without any allergic disease and infection.

Authors' contributions

XMZ and YC designed the study. LZ recruited patients and enrolled controls. JZ performed laboratory tests. QL and RL contributed to the data. XMZ, QL and RP conducted statistical analyses. XMZ drafted the article, and YB revised the article. All authors read and approved the final article.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Wuxi Children's Hospital Affiliated to Nanjing Medical University.

Informed consent

Written informed consent was obtained from legally authorized representatives before the study.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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