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Immune responses in children with secondary infection of mycoplasma pneumoniae after COVID-19: focus on eosinophils and IgE

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Abstract

Background The COVID-19 (*SARS-CoV-2*) epidemic has posed a major challenge to global public health, especially in children. Some children may experience secondary infection with *Mycoplasma pneumoniae* after *SARS-CoV-2* infection, which has attracted widespread attention. Studies have shown that eosinophils play an important role in respiratory tract infections and are involved in regulating immune responses and inflammatory processes. However, there is a lack of systematic research on the specific manifestations and mechanisms of eosinophils in secondary infection with *Mycoplasma pneumoniae* after *SARS-CoV-2* infection.

Objective This study aims to explore the characteristics of immune response in children with *SARS-CoV-2* infection and *Mycoplasma pneumoniae* infection, focusing on the changes in immune indicators such as eosinophils (EOS), immunoglobulin E (IgE), interleukin-6 (IL-6), C-reactive protein (CRP), and procalcitonin (PCT).

Methods This study is a retrospective observational study, and a total of pediatric patients who were treated in our hospital from January 2023 to December 2023 were included. The study group included children who were diagnosed with *SARS-CoV-2* infection and further infected with *Mycoplasma pneumoniae*, and the control group included children who were only infected with *SARS-CoV-2* and had no other pathogens. The clinical data of the two groups of patients, including absolute eosinophil value, IgE quantification, IL-6, CRP and PCT levels, were collected and analyzed, and statistical comparisons were performed.

Results A total of 134 children were included, including 79 in the study group and 55 in the control group. The absolute eosinophil value [0.17 (0.09, 0.31) vs. 0.09 (0.06, 0.23), $P<0.01$] and IgE level [59.28 (37.54, 256.88) vs. 22.00 (11.00, 113.10) $P<0.01$] of the children in the study group were significantly higher than those in the control group, while IL-6 [16.81(4.72,31.86) vs. 9.5(3,57.3), $P=0.602$], CRP [2.82(1.10,6.13) vs. 1.94(0.50,8.94), $P=0.528$] and PCT[0.12(0.08,0.20) vs. 0.12(0.10,0.24), $P=0.329$] were no significant difference between the two groups. Binary logistic regression analysis showed that the absolute value of eosinophils and IgE were independent risk factors for secondary infection of *Mycoplasma pneumoniae* after *SARS-CoV-2* infection.

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Conclusion This study shows that after *SARS-CoV-2* infection, the increase in eosinophils and the increase in related immune indicators IgE may be closely related to secondary infection with *Mycoplasma pneumoniae*. This study provides an important basis for understanding the immune response of children after *SARS-CoV-2* infection and its related clinical management, suggesting that clinicians should closely monitor the eosinophil count and IgE level of children after *SARS-CoV-2* infection, especially for children at risk of secondary infection, so as to take timely intervention measures to prevent secondary infection with *Mycoplasma pneumoniae* and improve the prognosis of children.

Keywords COVID-19 (*SARS-CoV-2*), *Mycoplasma pneumoniae*, Eosinophils, IgE, Interleukin-6 (IL-6), C-reactive protein (CRP), Procalcitonin (PCT), Secondary infection, Children

Introduction

Since the outbreak of the novel coronavirus *(SARS-CoV-2)* at the end of 2019, it has spread rapidly around the world, bringing unprecedented pressure to public health systems and medical resources [1]. Although most infected people show mild or no symptoms, some high-risk groups, including children, may develop more complex clinical manifestations after infection [2]. Studies in recent years have shown that [3] *SARS-CoV-2* infection can lead to a variety of respiratory complications, including but not limited to secondary infection with bacterial and atypical pathogens.

In recent years, many studies have been conducted to explore the impact of co-infection between *Mycoplasma pneumoniae* and other respiratory viruses (such as *influenza virus*, *adenovirus*, etc.) on immune response [4]. Studies have shown that after *Mycoplasma pneumoniae* infection, the levels of eosinophils and specific cytokines in children significantly increase. However, when this immune response is too intense, it may lead to a "cytokine storm". Further aggravating the condition, in addition, *Mycoplasma pneumoniae* is also considered to possibly increase the risk of secondary infection by inducing a chronic inflammatory state [5]. Studies have shown that *SARS-CoV-2* infection may lead to severe and lasting reductions in eosinophils, worsening and increasing the risk of clinically relevant death [6]. In the context of *SARS-CoV-2* infection, there is evidence that eosinophils can represent possible blood biomarkers for diagnosis, prognosis and severity prediction of the disease [7].

Mycoplasma pneumoniae infection is also closely related to eosinophil-related immune responses. Eosinophils play an important role in regulating the immune system, clearing pathogens, and participating in allergic reactions [8]. Recent studies have shown that [6], eosinophils act as regulators of local immunity and/or remodeling/repair in health and disease (LIAR hypothesis), acting through multiple mechanisms, such as phagocytosis and cytotoxicity, promoting angiogenesis and releasing related molecules, secreting cytokines and chemokines to regulate immune responses, and interacting with epithelial cells and immune cells through communication and signal transduction. In addition, eosinophils play a key role in the differentiation and tissue homing of regulatory T cells (Tregs), mainly through TFG-β [9]. Eosinophils may actively participate in the physiological and pathological mechanisms of increased inflammation and tissue damage in diseases such as endogenous asthma, chronic rhinosinusitis with nasal polyps, eosinophilic gastroenteropathy and hypereosinophilic syndrome [10].

In bacterial infections, eosinophils promote Bordetella pertussis as bacterial targets, initiating an asthma-like process that exacerbates symptoms through chronic inflammation as the frequency of infection increases and promotes an anti-inflammatory microenvironment in which bacteria persist [11]. In the gastrointestinal tract, studies have shown that eosinophils play a key role in Helicobacter pylori infection and engage these cells to promote persistence [12].

During viral infection, eosinophils can participate in antiviral immune responses directly or indirectly by producing various soluble mediators. Eosinophils express Toll-like receptors involved in viral recognition [13], increase the expression of adhesion molecules such as L-selectin and CD11b, induce superoxide anion production, and promote survival after IFN-g activation [14], and release cationic proteins, ribonucleases, reactive oxygen species and NO and other molecules with antiviral activity, inhibiting viral replication [15, 16]. A study on dengue virus infection showed a significant correlation between hematological parameters and dengue infection, with a significant decrease in eosinophils, neutrophils, and platelets [17].

There are relatively few studies on the combined infection of *SARS-CoV-2* and *Mycoplasma pneumoniae*, and the existing literature mostly focuses on the immune response mechanism of single infection. The immune changes after *SARS-CoV-2* infection and its impact on the interaction with other pathogens need to be further explored. IgE, IL-6, CRP and PCT are important indicators widely used in clinical evaluation of infection and inflammatory response [18]. IgE is closely related to allergic reactions, and its level changes in respiratory infections may reflect the body's immune status; IL-6, as a proinflammatory factor, plays a key role in the

inflammatory process caused by viral and bacterial infections; CRP is an acute phase protein, and its elevated level often indicates an inflammatory response; PCT has important diagnostic value for bacterial infections. The analysis of these indicators in this study will help to fully understand the characteristics of the immune response in children with *SARS-CoV-2* and *Mycoplasma pneumoniae* infection.

Based on the above background, this article will focus on analyzing the absolute value of eosinophils, IgE, IL-6, CRP and PCT levels in children with concurrent *Mycoplasma pneumoniae* infection after *SARS-CoV-2* infection, and explore the impact of dual infection on the body's immune response, hoping to provide a new perspective for understanding the immune response after *SARS-CoV-2* infection. Through more in-depth analysis, we hope to reveal the immune characteristics of *SARS-CoV-2* infection in children and its relationship with secondary *Mycoplasma pneumoniae* infection, and provide guidance for future research and clinical practice.

Materials and methods

General information

This study is a retrospective observational study, and a total of 134 pediatric patients who visited a hospital from January to December 2023 were included, including 79 in the study group and 55 in the control group. According to the results of the *SARS-COV-2* test, the patients were divided into two groups: the study group (patients infected with the *SARS-COV-2* and *Mycoplasma pneumoniae*) and the control group (patients infected with the *SARS-COV-2* only).

Inclusion criteria:

Children aged between 1 and 14 years old; confirmed with *SARS-COV-2* infection (positive by nucleic acid test); the study group also had a positive *Mycoplasma pneumoniae* test result, or was infected with *Mycoplasma pneumoniae* during hospitalization (≤ 20 days), and the test result was positive; the control group was only positive for the *SARS-COV-2*, and the test results of other pathogens were negative.

Exclusion criteria:

Combined with serious underlying diseases (such as congenital immunodeficiency, severe cardiopulmonary disease, etc.); patients treated with immunosuppressants or antibiotics; patients who had other major infections recently (within the past three months); patients with incomplete clinical data or insufficient follow-up information.

Methods

Clinical data of all included patients, including age, gender, medical history, etc., were collected. Blood samples were collected to detect the absolute value of eosinophils, IgE, IL-6, CRP and PCT levels. The specific steps are as follows:

When the patient was first diagnosed with COVID-19, blood samples were collected for detection of immune indexes and inflammatory indexes, and throat swabs or sputum were collected for respiratory pathogen nucleic acid detection and general bacterial culture. If suspected *Mycoplasma pneumoniae* infection symptoms appeared during hospitalization, samples were collected again for *Mycoplasma pneumoniae* detection, and the time between the two infections was less than 10 days.

Eosinophil count: A blood routine test was performed using a fully automatic blood analyzer, and the absolute value of eosinophils was recorded.

IgE quantification: The concentration of IgE in serum was determined by enzyme-linked immunosorbent assay (ELISA).

IL-6, CRP and PCT detection: Chemiluminescence and immunoturbidimetry were used to detect the levels of IL-6, CRP and PCT in serum, respectively.

Respiratory pathogen detection: Fluorescence quantitative PCR method was used to detect respiratory pathogen nucleic acids in throat swabs of children, including: *SARS-CoV-2*, influenza A virus, influenza B virus, adenovirus, respiratory syncytial virus, *Mycoplasma pneumoniae*, Chlamydia pneumoniae, and human metapneumovirus.

Instruments and reagents

Eosinophil count and CRP detection were performed using the Mindray 6800PLUS fully automatic blood analyzer and supporting reagents produced by Shenzhen Mindray Company. The absolute reference range of EOS is (0.02 ~ 0.5)×10^9/L.

IgE detection was performed using the Roche combas600 fully automatic chemiluminescence analyzer and supporting reagents. The IgE reference range is: within 1 year old: less than 15 IU/mL. 1 ~ 5 years old: 0 ~ 60 IU/mL. 6 ~ 9 years old: 0 ~ 90 IU/mL. 10 ~ 15 years old: 0 ~ 200 IU/mL.

IL-6 and PCT were detected using the Wantai Wan200 + fully automatic chemiluminescence immunoassay analyzer and supporting reagents. IL-6 reference range: 1 ~ 10 pg/mL; PCT reference range: 0.05 ~ 0.5 ng/mL.

SARS-CoV-2 and *Mycoplasma pneumoniae* nucleic acid detection, using fluorescent quantitative PCR method, Hongshi SLAN – 96PPCR amplifier, reagents using the *SARS-COV-2* 2019-nCoV nucleic acid detection kit produced by Sun Yat-sen University Daan Gene Co., Ltd., the kit uses TaqMan probe technology to amplify and detect specific gene sequences of the *SARS-COV-2*, with high sensitivity and specificity (the detection limit can reach hundreds of copies per milliliter or even lower,

and the specificity can usually reach more than 99%); other projects use the kit produced by Shanghai Zhijiang Biotechnology Co., Ltd., with a sensitivity of 500 copies/mL and a specificity greater than 95%.

All tests are performed with relevant quality control products and quality control is guaranteed, and all operations are strictly operated and used in accordance with the corresponding SOP documents.

Statistical methods

SPSS26.0 statistical software was used for data analysis. The measurement data that conformed to the normal distribution were expressed as mean ± standard deviation (SD), and the measurement data that did not conform to the normal distribution were expressed as quartile P_{50} (P_{25}, P_{75}). The independent sample t test or nonparametric test was used for inter-group comparison; categorical variables were expressed as frequency and percentage, and chi-square test was used for analysis; Spearman correlation analysis was used to analyze the correlation between the indicators; multivariate regression analysis was used to identify independent risk factors associated with secondary infection of *Mycoplasma pneumoniae*. $P < 0.05$ was considered statistically significant.

Results

General information

The basic demographic characteristics of the 134 children included in this study include 79 in the study group, aged 0.17 ~ 8.23 years old, with a BMI of 12.28 ~ 20.52, and 55 children in the control group, aged 0.08 ~ 13.56 years old, with a BMI of 12.71 ~ 24.72; There was no significant difference between the study group and the control group in terms of gender, age, BMI, family allergy history, and recent vaccination history. Therefore, the two groups of children had significant differences in physiological and immune system development, immune response, and infection risk. Comparability in all aspects ensures the credibility of the results. See Table 1 for specific statistical data.

According to the "Guidelines for Diagnosis and Treatment of Community-Acquired Pneumonia", the disease is classified as follows: Typical pneumonia includes "community-acquired pneumonia (non-severe)" and "segmental pneumonia". These two types of pneumonia differ in infection route and imaging manifestations. In principle, they have certain similarities with clinical treatment. They are mostly caused by common bacterial infections. Pulmonary imaging often shows pulmonary consolidation shadows with certain segmental distribution characteristics. They are combined into "typical pneumonia (non-severe)". SARS: "Acute pneumonia" is separately classified as "atypical pneumonia (non-severe)". This type of pneumonia may be caused by atypical pathogens such as mycoplasma and chlamydia. The onset is insidious and the symptoms are relatively atypical, such as dry cough, fever, etc., which is different from typical pneumonia in pathogens, clinical manifestations and treatment responses, so it is classified separately to highlight its characteristics and significance in research. Bronchiolitis-associated pneumonia: "alveobronchiolitis" is classified as a separate category because it mainly involves bronchioles and is often associated with viral infections. It has unique pathophysiology and clinical processes to emphasize its role in lower respiratory tract infections. Its special status and its difference from other types of pneumonia. The clinical diagnosis of the two groups of children is shown in Table 2.

Eosinophil and immune index levels

The absolute value of eosinophils and IgE levels in the study group were significantly higher than those in the control group. For IL-6, CRP and PCT, the existing data do not support significant differences between the study group and the control group. The specific data are detailed in Table 3 to facilitate further in-depth analysis and discussion.

In order to gain a deeper understanding of the role of EOS in secondary infection with *Mycoplasma pneumoniae* in COVID-19 patients, this study further explored the relationship between the absolute value of eosinophils (EOS) and multiple immune indicators (IgE, IL-6, CRP and procalcitonin PCT). correlation between. Correlation analysis showed that EOS had a significant positive correlation with IgE ($r = 0.216$, $P = 0.012$), a moderate negative correlation with CRP (r = -0.178, $P = 0.039$), and a slight negative correlation with PCT ($r = -0.092$, $P = 0.293$), and showed a slight positive correlation with IL-6 ($r = 0.054$, $P = 0.533$). The correlation

Table 1 Comparison of general data between the two groups $\bar{x}$±SD

item	*N*	male / female(%)	Age (year)	BMI	family allergy history *n*/%	recent vaccination *n*/%
Control group	55	29/26 (45.45%)	6.8 ± 3.5	18.7 ± 2.0	6/10.91	4/7.27
Study group	79	54/25 (68.35%)	6.5 ± 3.2	18.5 ± 2.1	9/11.39	6/7.59
X^2/t		2.52	-0.37	-0.22	0.01	0.05
P		0.11	0.61	0.59	0.92	0.82

Note: *N* is the number of people

Table 2 Comparison of clinical diagnosis in the two groups *n*(%)

		Study group (*n* = 79)	Control group (*n* = 55)
Upper respiratory tract infection			
	Acute tonsillitis	2(0.04)	6(0.12)
	Acute pharyngitis	1(0.02)	6(0.11)
Upper respiratory tract infection subtotal		3(0.06)	12(0.22)
Lower respiratory tract infection - non-severe			
	Bronchitis (including acute bronchitis)	6(0.12)	8(0.15)
	Typical pneumonia (non-severe)	12(0.24)	9(0.16)
	Atypical pneumonia (non-severe)	6(0.12)	1(0.02)
	Bronchiolitis-related pneumonia	4(0.08)	10(0.19)
Lower respiratory tract infection - non-severe subtotal		28(0.56)	28(0.51)
Severe and extrapulmonary injury			
Severe pneumonia and extrapulmonary injury subtotal		13(0.26)	7(0.13)
Other		2(0.04)	1(0.02)

Note: The main lesions of acute tonsillitis are concentrated in the palatine tonsils. The palatine tonsils are red and swollen, and there are yellow-white punctate exudates at the crypt openings, which may even fuse into pseudomembranes that are easy to wipe off. The pharyngeal pain is severe and often radiates to the ears. The systemic symptoms are severe, such as high fever and chills. Acute pharyngitis mainly affects the pharyngeal mucosa and submucosal tissues, and manifests as dryness, burning, and pain in the pharynx, which worsens when swallowing. The pain can radiate to the ears, and the systemic symptoms are relatively mild, such as low fever, headache, and loss of appetite

Table 3 Comparison of laboratory indicators in the two groups[P_{50}(P_{25}, P_{75})]

Index	Study group (*n* = 79)	Control group (*n* = 55)	Z	*P*
EOS(×109/L)	0.17(0.09,0.31)	0.09(0.06,0.23)	-3.003	0.003
IgE(IU/mL)	59.28(37.54,256.88)	22.00(11.00,113.10)	-4.481	< 0.01
IL-6(pg/mL)	16.81(4.72,31.86)	9.50(3.00,57.30)	-0.521	0.602
CRP(mg/L)	2.82(1.10,6.13)	1.94(0.50,8.94)	-0.631	0.528
PCT(ng/mL)	0.12(0.08,0.20)	0.12(0.10,0.24)	-0.976	0.329

analysis results between the absolute value of eosinophils and IgE, IL-6, CRP, and PCT are shown in Table 4.

In this study, we explored the potential immune mechanisms underlying *Mycoplasma pneumoniae* secondary infection after *SARS-COV-2* infection. Considering the possible immune system changes caused by COVID-19, we paid special attention to eosinophils (EOS), immunoglobulin E (IgE), C-reactive protein (CRP), procalcitonin (PCT), and interleukins − 6 (IL-6) and other immune markers to assess their association with the risk of secondary lung infection. By applying multivariable regression models, we controlled potential confounding factors such as age, gender, and underlying diseases to ensure the reliability of the results. Based on the considerations of reducing the risk of type I error, improving the candidate range of variables, statistical power, and clinical experience and theoretical basis, we selected variables with P values less than 0.2 in univariate analysis to be included in multivariate analysis. The results of binary logistic regression analysis showed that in the model of secondary infection with *Mycoplasma pneumoniae* (dependent variable), EOS had a significant impact on secondary infection with *Mycoplasma pneumoniae* (B = 2.636, Wald = 4.993, $P = 0.025$, Exp(B) = 13.961, 95% CI: 1.382–140.998), which means that for every unit change in EOS, the odds ratio of secondary infection with *Mycoplasma pneumoniae* is 13.961 times. IgE also has a significant effect on secondary infection with *Mycoplasma pneumoniae* (B = 0.003, Wald = 5.569, $P = 0.018$, Exp(B) = 1.003, 95%CI: 1.001–1.006), indicating that for every unit change in IgE, *Mycoplasma pneumoniae* II The odds ratio for secondary infection was 1.003 times. And

Table 4 Correlation analysis of the absolute values of eosinophils and IgE, IL-6, CRP, and PCT

		EOS	CRP	PCT	IL-6	IgE
EOS	r	1	−0.178*	-0.092	0.054	0.216*
	P	.	0.039	0.293	0.533	0.012
CRP	r	−0.178*	1	0.197*	0.279**	-0.06
	P	0.039	.	0.022	0.001	0.495
PCT	r	-0.092	0.197*	1	0.324**	-0.13
	P	0.293	0.022	.	0	0.135
IL-6	r	0.054	0.279**	0.324**	1	-0.032
	P	0.533	0.001	0	.	0.712
IgE	r	0.216*	-0.06	-0.13	-0.032	1
	P	0.012	0.495	0.135	0.712	.

Note: * At Level 0.05 (double tail), the correlation is significant.** At 0.01 (double tail) with significant correlation

Table 5 Multivariate regression analysis

	B	standard error	Wald	*P*	Exp(B)	The 95% confidence interval for the EXP (B)	
						lower limit	top limit
EOS	2.636	1.18	4.993	0.025	13.961	1.382	140.998
CR	-0.009	0.017	0.266	0.606	0.991	0.959	1.025
PCT	0.407	0.344	1.399	0.237	1.502	0.765	2.949
IL-6	-0.004	0.002	2.905	0.088	0.996	0.992	1.001
IgE	0.003	0.001	5.569	0.018	1.003	1.001	1.006

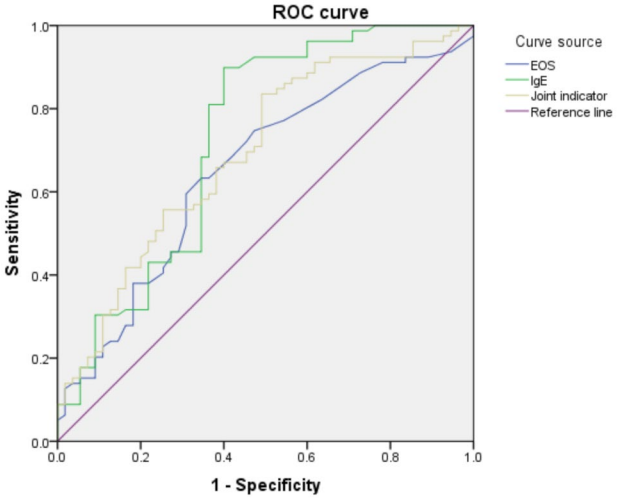

Fig. 1 Predictive efficacy of EOS, IgE and combined indicators for secondary infection

CRP (B = -0.009, Wald = 0.266, *P* = 0.606, Exp(B) = 0.991, 95%CI: 0.959–1.025), PCT (B = 0.407, Wald = 1.399, *P* = 0.237, Exp(B) = 1.502, 95%CI: 0.765–2.949) and IL-6 (B = -0.004, Wald = 2.905, *P* = 0.088, Exp(B) = 0.996, 95%CI: 0.992–1.001) on secondary infection with *Mycoplasma pneumoniae* The impact did not reach a significant level. The results of binary logistic regression analysis are shown in Table 5.

Further ROC curve analysis was performed on EOS and IgE, and the AUC of the absolute value of eosinophils (EOS) was 0.653, indicating that the absolute value of eosinophils has a certain ability to differentiate between secondary infection and non-infection with *Mycoplasma pneumoniae* after *SARS-COV-2* infection., but not very strong. The P value is 0.003, indicating that this discriminating ability is statistically significant. The AUC of immunoglobulin IgE (IgE) is 0.728, which is the highest, indicating that immunoglobulin IgE has good discriminating ability in distinguishing secondary infection and non-infection with *Mycoplasma pneumoniae* after *SARS-COV-2* infection, and is the strongest single predictor. The AUC of the predicted probability is 0.693, which is between the two, indicating that the model has moderate discriminating ability. The model combines multiple predictors to improve the discriminating ability. The ROC analysis results are shown in Fig. 1; Table 6.

Discussion

This study aims to explore the immune response characteristics of children with *Mycoplasma pneumoniae* infection after *SARS-COV-2* infection, with special attention to the changes in eosinophils (EOS), IgE and other inflammatory factors (such as IL-6, CRP and PCT). The results showed that the absolute value of eosinophils and IgE levels of children in the study group were significantly higher than those in the control group, but no significant differences were found in IL-6, CRP and PCT. This is consistent with the literature [19] are different. A study in adults showed that COVID-19 - EOS counts often decrease in 19 patients, and compared with patients without decreased EOS counts, these patients are usually older, have more underlying medical conditions, and are at a higher risk of developing acute respiratory distress syndrome (ARDS) [20, 21]. Studies have shown that more than 71% of COVID-19 patients have decreased EOS counts, and the levels of inflammatory factors such as IL-1, IL-8 and tumor necrosis factor α (TNFα) in severe patients are significantly higher than those in mild patients. In addition, the EOS count of many patients who died dropped to 0, and during hospitalization, more than half of the patients who died had

Table 6 Predictive efficacy of EOS, IgE and combined indicators for secondary infection

variable quantity	AUC	standard error a	The asymptotic significance b	sensitivity	specificity	cutoff value	Asymptotic 95% confidence interval	
							lower limit	top limit
EOS	0.653	0.048	0.003	0.633	0.655	0.135	0.558	0.748
IgE	0.728	0.047	0	0.899	0.600	28.16	0.635	0.821
Joint indicators	0.693	0.046	0	0.835	0.509		0.602	0.784

always had a lower EOS count than those who survived, which also suggests that the number of EOS may become an important indicator of the severity of a patient's condition [22, 23]. The exact mechanism by which eosinophilia is associated with COVID-19 is unknown, but eosinophilia may result in reduced eosinophil production and/or release through the bone marrow, increased marginalization of the vasculature, and increased migration into somatic tissues and in the periphery Reduced survival in circulation, etc [24, 25]. In addition, increased hormone secretion in severe patients under acute lung injury will also inhibit the release of EOS. Although eosinophilia is frequently observed in COVID-19 hospitalized patients and is associated with patient prognosis [26], but recent data indicate that hospitalizations for COVID-19 did not rise significantly in asthma patients receiving eosinophil-depleting biologic treatments [27–30], this finding challenges the traditional role of eosinophils in severe cases, and the performance of eosinophils in respiratory infections in children whose immune systems are not yet fully developed Probably more complicated.

The immune pattern of COVID-19 includes lymphopenia, lymphocyte activation and dysfunction, increased production of cytokines, especially IL-1b, IL-6 and IL-10, increased IgG antibodies and elevated C-reactive protein (CRP) levels. IL-6, as a pro-inflammatory cytokine, plays an important role in both acute and chronic inflammation. Eosinophils participate in the homeostasis of the immune system and can mediate antigen sensitization of B cells and promote the production of antigen-specific IgM, thereby enhancing humoral immunity [31] and regulate the balance of intestinal flora [32]. Increased IgE may point to a more active allergic immune response, especially when multiple infections are present at the same time. This study found a moderate positive correlation between eosinophils and IgE, which further supports their functional connection in similar immune processes, especially in allergic reactions. The strong positive correlation between CRP and IL-6 may be reflected in the strong inflammatory response caused by *SARS-CoV-2* and *Mycoplasma pneumoniae* infection. The strong positive correlation between PCT and IL-6 suggests that these two indicators may increase synchronously in the case of *SARS-CoV-2* infection and combined *Mycoplasma pneumoniae* infection. Although a positive correlation was also shown between CRP and PCT, this correlation was not statistically significant, which may mean that the changes in these two indicators were not synchronized in the samples of this study. The negative correlation between eosinophils and CRP may indicate that in the case of *SARS-CoV-2* infection and combined *Mycoplasma pneumoniae* infection, the increase in eosinophils is not accompanied by an increase in CRP, which may be related to different types of inflammatory responses.

Through multivariable logistic regression analysis, this study confirmed the association between EOS and IgE as independent risk factors and secondary infection with *Mycoplasma pneumoniae* after *SARS-CoV-2* infection. This finding underscores the importance of continued attention to these immune indicators to allow early identification of children at risk for secondary infections. At the same time, although CRP, PCT, and IL-6 play important roles in inflammatory responses, they failed to demonstrate a significant association with the risk of secondary infection in this study, which may be due to the complex nature of these indicators in the context of specific infections. Caused by sex. ROC curve analysis results show that IgE has a good predictive ability in distinguishing secondary infection with *Mycoplasma pneumoniae* after *SARS-CoV-2* infection, while eosinophils have a certain distinguishing ability, but it is relatively weak. This provides a reference for clinical practice, suggesting that when assessing the risk of secondary infection in children, priority should be paid to changes in IgE levels. In addition, the model integrating multiple indicators showed moderate discriminatory ability, indicating that combining multiple immune indicators can improve the prediction accuracy of secondary infection risk. Especially in areas with limited resources, simple testing of eosinophil counts and IgE levels can be prioritized as a means to initially assess the risk of secondary infection in children after COVID-19. At the same time, comprehensive judgment should be made based on clinical symptoms and signs, which is reasonable. Provide the basis for allocating medical resources and timely treatment.

This study has limitations. Since this study adopted a retrospective observation design, the sample size was relatively limited. Future studies need to expand the sample size and conduct prospective studies to verify the general applicability of these results. At the same time, in-depth exploration of the specific mechanism of action of eosinophils in different types of infections will provide more theoretical basis for understanding the immune response to respiratory infections in children, and thus help develop more effective prevention and treatment strategies.

Conclusions

This study revealed the characteristics of the immune response of children infected with *SARS-CoV-2* and concurrently with *Mycoplasma pneumoniae*. Eosinophils may play an important role in the immune response after *SARS-CoV-2* infection, especially in the context of secondary infection. The increase in eosinophils and IgE may indicate that children face a higher risk of secondary infection after *SARS-CoV-2* infection. Therefore,

the monitoring of these immune indicators should be strengthened clinically to timely identify and intervene in the risk of secondary infection in susceptible children.

Abbreviations

EOS	Eosinophils
IgE	Immunoglobulin E
IL-6	Interleukin-6
CRP	C-Reactive Protein
PCT	Procalcitonin
APCs	Antigen-Presenting Cells
NO	nitric oxide
SARS	Severe Acute Respiratory Syndrome (in this study, referring to the acute pneumonia caused by SARS-CoV-2 and classified as 'atypical pneumonia (non-severe)' for research purposes

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Author contributions
Pingping Wang wrote the original draft. Jin Yao and Yaqiong Li revised the manuscript. Zhanjun Zhang supervised the investigation. Ruiling Zhang and Shouting Lu performed the data analysis. Meixia Sun and Xiaorong Huang translated the draft. All authors read and approved the final manuscript.

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Data availability
All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Luoyang Maternal and Child Health Hospital (LWOPN 2024020801.0). It was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The need for informed consent to participate was waived by the ethics committee that approved the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Authors'contributions
Pingping Wang wrote the original draft. Jin Yao and Yaqiong Li revised the manuscript. Zhanjun Zhang supervised the investigation. Ruiling Zhang and Shouting Lu performed the data analysis. Meixia Sun and Xiaorong Huang translated the draft. All authors read and approved the final manuscript.
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