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Immune-related Factors Associated with Pneumonia in 127 Children with Coronavirus Disease 2019 in Wuhan

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ABBREVIATED TITLE

Immune features in Children with COVID-19

Abstract

Objective: Information regarding the association of immune-related factors with pneumonia in children with coronavirus disease 2019 (COVID-19) is scarce. This study aims to summarize the immune-related factors and their association with pneumonia in children with COVID-19.

Methods: Children with COVID-19 at Wuhan Children's Hospital from January 28 to March 12, 2020 were enrolled. Pneumonia due to causes other than COVID-19 were excluded. The clinical and laboratory information including routine blood tests, blood biochemistry, lymphocyte subsets, immunoglobulins, cytokines and inflammatory factors were analyzed retrospectively in 127 patients. Normal ranges and mean values of laboratory markers were applied as parameters for logistic regression analyses of their association with pneumonia.

Results: In non-intensive care unit patients, 48.8% and 22.4% of patients had increased levels of procalcitonin and hypersensitive C-reactive protein (hs-CRP) respectively. 12.6% and 18.1% of patients had decreased levels of

immunoglobulin (Ig) A and interleukin (IL)-10 respectively. Approximately 65.8% of patients had pneumonia. These patients had decreased levels of globulin (odds ratio [OR] 3.13, 95% confidence interval [CI] 1.41-6.93, *P*=0.005), IgA (OR 4.00, 95% CI 1.13-14.18, *P*=0.032), and increased levels of hs-CRP (OR 3.14, 95% CI 1.34-7.36, *P*=0.008), procalcitonin (OR 3.83, 95% CI 2.03-7.24, *P*<0.001), IL-10 (OR 7.0, 95% CI 1.59-30.80, *P*=0.010), and CD4+CD25+ T lymphocyte < 5.0 % (OR 1.93, 95% CI 1.04-3.61, *P*=0.038).

Conclusion: Decreased IgA and CD4+CD25+ T lymphocyte percentage, and increased hs-CRP, procalcitonin and IL-10 were associated with pneumonia, suggesting that the immune-related factors may participate in the pathogenesis of pneumonia in children with COVID-19.

1. INTRODUCTION

A cluster of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly spread throughout the world. On March 11, 2020; the World Health Organization (WHO) made the declaration that COVID-19 outbreak could be characterized as a pandemic[1]. Published studies have reported that children with SARS-CoV-2 infection have a milder clinical course than infected adults[2; 3].

Coronavirus infection can induce increased cytokines and accumulation of inflammatory cells in the lungs, which is associated with severe clinical outcomes[4]. In adult patients with COVID-19, the most severe cases demonstrated elevated levels of cytokines and lowered levels of regulatory T cells[5; 6]. Particularly, interleukin (IL)-2 receptor, IL-6, IL-8 and IL-10 were markedly

increased in patients with severe form of the disease and IL-6 was found to be a risk factor for mortality in adult patients with COVID-19[6; 7].

Although some studies have delineated the clinical features of children with COVID-19, information regarding immune-related laboratory findings in these patients has not been described yet[3; 8]. COVID-19 has a diverse presentation in children, ranging from asymptomatic infection, upper respiratory tract infection and pneumonia[3], but the factors associated with pneumonia remained unknown. Therefore, the present study aimed to summarize the immune-related factors and the factors associated with pneumonia of COVID-19 in children.

2. MATERIALS AND METHODS

Study Population

A total of 127 children with confirmed diagnosis of COVID-19 and hospitalized in the isolation wards of Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China from January 28, 2020, to March 12, 2020, were reviewed retrospectively. The inclusion criteria were 1) suspected patients positive for SARS-CoV-2 ribonucleic acid after analysis of throat or nasopharyngeal swab specimens through real-time reverse transcription polymerase chain reaction (RT-PCR) assay and 2) patients who underwent at least one of the following examinations: cytokine levels, lymphocyte subsets and immunoglobulin levels. Exclusion criterion was confirmed pneumonia caused by pathogens other than SARS-CoV-2. Wuhan Children's Hospital is the only hospital designated by the government as responsible for central treatment of pediatric patients with COVID-19 in Wuhan. All children enrolled in the study

were diagnosed based on the WHO interim guidelines[9] and National Recommendations for Diagnosis and Treatment of COVID-2019 (seventh edition)[10]. The present study was approved by the Medical Ethics Committee of Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, and was in agreement with the Declaration of Helsinki.

Data collection

The clinical, laboratory and radiological data of the patients were obtained from the electronic medical records. Clinical information included previous medical history and management data. Laboratory information on admission included routine blood tests, serum blood biochemistry, whole blood lymphocyte subsets (T cells, B cells and natural killer cells), serum immunoglobulins (IgA, IgG and IgM), serum complement proteins (C3 and C4), plasma cytokine profiles (IL-2, IL-4, IL-6, IL-10, tumor necrosis factor [TNF]-α, and interferon [IFN]-γ) and serum inflammatory factors (hypersensitive C-reactive protein [hs-CRP], ferritin, and procalcitonin). Radiological data of the patients were examined using chest computed tomography (Siemens medical system; Siemens, Germany). In addition, information regarding influenza A and influenza B, respiratory syncytial virus, adenovirus, cytomegalovirus, Epstein-Barr virus, mycoplasma, and bacteria was also collected.

The routine blood work was carried out using hemocytometer (XN-3000, Sysmex Corp., Kobe, Japan). Blood biochemistry was tested using an automatic biochemical analyzer (VITROS5600, Ortho Clinical Diagnostics, Raritan, NJ, USA). Lymphocyte subsets and cytokines were analyzed using a FACSCanto flow

cytometer (BD FACSCanto, BD Biosciences, Franklin Lakes, NJ, USA). Immunoglobulins and complement proteins were examined by immunofixation electrophoresis (BNII, Siemens, Germany) and hs-CRP was examined by nephelometry immunoassay (BNII, Siemens, Germany). Ferritin and procalcitonin were tested by chemiluminescent immunoassay (CL-6000i, Mindray, Shenzhen, China). The RT-PCR assay and diagnostic criteria of SARS-CoV-2 were in accordance with the protocol established by the WHO[11]. Serology of other pathogens were tested by enzyme-linked immunosorbent assay (URANUS95, Aikang, Shenzhen, China). Bacteria were detected from sputum culture.

Treatment Regime

The treatment regime for children with COVID-2019 was based on the National Recommendations for Diagnosis and Treatment of COVID-2019 (seventh edition)[10]. The primary antiviral regime included interferon-α2b nebulization, intravenous ribavirin, oral oseltamivir and arbidol (an anti-viral drug). Initially, intravenous methylprednisolone (1-2 mg/kg/day, for 3-5 days) was administered to patients with severe form of the disease. Subsequently, a dose-tapering course of oral prednisolone was prescribed in the following two weeks.

Statistical analysis

Continuous variables were expressed as medians and interquartile range (IQR) values. Categorical variables were presented as numbers and percentages. Continuous variables were compared using independent group *t*-tests when the data were distributed normally. Mann-Whitney test was used when the data were not normally distributed. Categorical variables were compared using chi-squared

test or Fisher's exact test when the data were limited. Univariate and multivariate analysis with stepwise logistic regression were performed to identify the association with pneumonia. All abnormal laboratory markers and age were used as candidate associated factors. The normal range and mean values of the markers were applied as parameters for the univariate model. Significant factors of univariate analysis were then tested in the multivariate model. All statistical analyses were performed using Version 23.0 of SPSS Software. Statistical significance was defined as a two-sided *P*-value of less than 0.05.

3. RESULTS

Clinical characteristics of children with COVID-19

A total of 127 children with COVID-19 were included in the present study. The median age of patients was 6 years (IQR: 1-9 years, range: 2 months to 15 years. An approximate 1.3:1.0 sex ratio (72 males and 55 females) was observed. Two pediatric patients transferred to the intensive care unit (ICU), as they met one of the following criteria[10]: (1) respiratory failure requiring mechanical ventilation, (2) septic shock, and (3) disease accompanied by other organ failure requiring ICU monitoring and treatment. The dynamic changes in cytokines and inflammatory factors were monitored during the hospitalization. Apart from two ICU patients, the remaining 125 non-ICU patients were discharged after recovery and negative conversion of SARS-CoV-2. Of the 125 non-ICU patients, most recovered with only interferon-α2b nebulization, and none required oxygen

therapy. Detailed information was displayed in Table 1 and 2. We compared the laboratory features among children stratified by age (median 6 years) and the results are showed in Table S1.

Laboratory features of non-ICU patients with COVID-19

Table 2 presents the laboratory results of non-ICU children with COVID-19 into three groups diagnosed as asymptomatic infection, upper respiratory tract infection and pneumonia. The laboratory results were recorded on the date of admission. In all the non-ICU patients, slightly increased levels of procalcitonin (48.8%) and hs-CRP (22.4%) were common abnormal inflammatory factors. Decreased levels of globulin were found in 28.0% of patients. The percentage of patients with levels of immunoglobulin below the lower limit of normal were 12.6% for IgA, 8.4% for IgG and 5.9% for IgM. Increase levels of IL-10 were found in 18.1% of patients. Decreased of lymphocyte counts were not common (0.8%) and reductions in CD4+/CD8+ T cells ratio (18.3%) and natural killer cells (23.1%) were observed among lymphocyte subsets. Decreased levels of IgA and increased levels of procalcitonin were more common in patients with pneumonia than asymptomatic patients.

Immune related characteristics of ICU patients with COVID-19

The patient who survived showed no B cells (0 cells/μL) but did not had an immunoglobulin result. The patient who could not survive showed hypogammaglobulinemia (IgG=1.52 g/L) with a normal count of B cells (820 cells/μL). The plasma concentration of cytokines IL-6, IL-10 and inflammatory factor ferritin markedly increased with the development of the disease (Figure 1). Other detailed information has been reported previously[3; 8].

Radiologic results and associated factors for abnormal chest radiologic findings

Nearly two thirds of the patients (65.8%) presented with pneumonia on chest radiologic findings. We used median age (6 years) and abnormal laboratory markers to evaluate their association with pneumonia in the chest radiologic findings. Univariate analysis showed that decreased levels of globulin, IgA, and CD4+CD25+ T lymphocyte percentage (<5.0 %), and increased levels of hs-CRP, procalcitonin and IL-10 (normal ranges were showed in Table 2) were associated with pneumonia in chest radiologic findings among patients with COVID-19 (Table 3). However, the multivariate analysis showed no associations. Some information was missing in some of the patients including chest radiologic findings, procalcitonin, ferritin, immunoglobulins, cytokines, and lymphocyte subsets due to the retrospective study design (Table S1).

4. DISCUSSION

The present retrospective study showed that children in Wuhan who were laboratory-confirmed COVID-19 experienced a mild clinical course. The present study identified several factors associated with pneumonia in children with COVID-19. Particularly, decreased levels of globulin, IgA, and CD4+CD25+ T lymphocyte percentage (<5.0 %) and increased concentration of hs-CRP, procalcitonin, and IL-10 were associated with the presentation of pneumonia in chest radiologic findings.

Pulmonary infection is the most common presentation of antibody deficiencies in children[12]. In the present study, 20/120 patients had low level of one immunoglobulin, and decreased IgA levels were associated with pneumonia. IgA has many functions, serving as a first-line barrier that protects the mucosal epithelium from pathogens[13]. In the present study, decreased IgA levels might have reduced the capability for antiviral defence in children with COVID-19.

Elevated production of cytokine has been continuously associated with pathogenesis and poor prognosis of respiratory viral infections[4]. Early studies have reported that marked elevation of IFN-γ, IL-1β, IL-6, and IL-12 and IL-8 were correlated with pulmonary inflammation in severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome[14]. Recent studies[5; 7] have

showed that higher plasma levels of IL-2, IL-6, IL-7, IL-10 and TNF-α were associated with severity and mortality of COVID-19 in adult patients. In the present study, plasma levels of IL-6 and IL-10 were the two elevated cytokines observed in pediatric patients with COVID-19. IL-10, a cytokine with anti-inflammatory properties, can be produced by many immune cells including macrophages, B cells, natural killer cells, and nearly all T cell subsets[15]. Although IL-10 is considered to protect tissues from damage during inflammatory reactions[16], overexpression of IL-10 reportedly increased the pathogen burden and exacerbation of infection[17]. A previous study reported that neutralization of IL-10 may lead to better control of tuberculosis[18]. In the present study, increased concentration of IL-10 was correlated with manifestation of pneumonia, suggesting that IL-10 may play an important role in the pathogenesis of COVID-19 in children.

Regulatory T cells, a subset of helper T cells, play a critical role in negative regulation of the activation and the proliferation of immune cells. They restrain the over-activation of inflammatory responses and prevent the development of immunopathology[19]. It has been documented that adult patients with COVID-19 have decreased levels of regulatory T cells which might result in the production of cytokine storm and might worsen the damaged tissue, especially in severe form of the disease[6]. In the present study, lower levels of regulatory T cells were in

coincident with pneumonia, suggesting an important role of dysregulated immune responses in COVID-19 pathogenesis. Increased levels of hs-CRP and procalcitonin were associated with pneumonia in COVID-19, supporting its inflammatory pathogenesis.

Reportedly, among adult patients with COVID-19, the majority of the patients had symptoms, comorbidities, and presentation of pneumonia[7; 20]. However, in the present study involving children with COVID-19, we found that nearly a quarter of the children manifested asymptomatic infection and one third of them showed normal lung imaging. Lymphocytopenia, an abnormal marker common in adult patients[20], was not common in children with COVID-19. These findings indicate that children with COVID-19 experienced a milder clinical course compared to adults, similar to the results observed in children with SARS[21; 22].

The present study has some limitations. Due to the retrospective study design, not all examinations were performed and monitored during hospitalization in all patients. Therefore, the risk factors predicting for pneumonia might be underestimated. All patients were diagnosed through RT-PCR using throat or nasopharyngeal specimens. The viremia of patients was not evaluated for technical reasons. The factors associated with pneumonia were limited by the sample size and there were only two ICU patients in this study.

In conclusion, children with COVID-19 in Wuhan experienced a mild clinical course. Increased concentrations of procalcitonin, hs-CRP, and IL-10 and decreased IgA level, and percentage of CD4+CD25+ T lymphocyte were associated with pneumonia in children with COVID-19. Our results suggest that immune-related factors may participate in the pathogenesis of pneumonia in children with COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests**.**

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TABLE 1 Clinical information of non-ICU patients.

Characteristics	**No. % (N=125)**
Comorbidities	13 (10.4)
Allergic rhinitis or asthma	6 (4.8)
Congenital heart diseases	2 (1.6)

Epilepsy	1 (0.8)
Growth retardation	1 (0.8)
Leukemia	1 (0.8)
Acute abdominal diseases	1 (0.8)
Traumatic intracranial hemorrhage	1 (0.8)
Co-infection	
Influenza virus A	1 (0.8)
Influenza virus B	2 (1.6)
Mycoplasma	39 (31.2)
Adenovirus	2 (1.6)
Respiratory syncytial virus	1 (0.8)
Cytomegalovirus	1 (0.8)
Epstein-Barr virus	1 (0.8)
Bacteria	2 (1.6)
Antiviral therapy	
Interferon-α2b	119 (95.2)

Ribavirin	9 (7.2)
Oseltamivir	12 (9.6)
Arbidol	4 (3.2)
Antibiotic therapy	48 (38.4)
Intravenous immune globulin	1 (0.8)
Oxygen therapy	0

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit.

TABLE 2 Demographic and laboratory results of non-ICU patients.

Characteristics		**No. % / Median (IQR)**				***P* value**
	Normal Range	**All patients (N=125)**	**Asymptomatic patients (n=21)**	**URTI patients (n=27)**	**Pneumonia patients (n=77)**	
Age, y						0.011
<1		20 (16.0)	0	1 (3.7)	19 (24.7)	
1-3		29 (23.2)	3 (14.3)	8 (29.6)	18 (23.4)	
4-6		22 (17.7)	2 (9.5)	5 (18.5)	15 (19.5)	

7-12		42 (33.6)	12 (57.1)	11 (40.7)	19 (24.7)	
13-17		12 (9.6)	4 (19.0)	2 (7.4)	6 (7.8)	
Sex						0.580
Male		71 (56.8)	12 (57.1)	13 (48.1)	46 (59.7)	
Female		54 (43.2)	9 (42.9)	14 (51.9)	31 (40.3)	
Blood biochemistry						
Albumin (g/L)*	39.0-53.0	44.9 (42.8-47.6)	46.3 (45.3-49.3)	45.9 (43.3-48.7)	44.4 (42.1-46.6)	0.004
Globulin (g/L)	20.0-40.0	23.3 (19.5-26.9)	22.5 (20.3-26.6)	23.6 (20.8-26.6)	23.0 (18.1-27.2)	0.843
Inflammatory related factors						
Hypersensitive C-reactive protein (mg/L)	0.0–3.0	0.8 (0.8-4.0)	0.8 (0.8-0.8)	0.9 (0.8-3.8)	0.8 (0.8-5.0)	0.110
Procalcitonin (ng/mL)*	0.00–0.05	0.05 (0.04-0.08)	0.05 (0.03-0.06)	0.05 (0.04-0.09)	0.06 (0.04-0.08)	0.035
Ferritin	27.0-375	68.6 (44.5-102.	69.4	68.6 (58.5-86.5	67.8 (40.9-126.	0.64

(ng/mL)	.0	9)	(32.1-98.5)	)	1)	3
Immunoglobulins[a]						
Immunoglobulin A (g/L)[*]		1.10 (0.41-1.68)	1.35 (1.14-1.80)	1.11 (0.57-1.73)	0.88 (0.28-1.54)	0.034
Immunoglobulin G (g/L)		8.87 (6.68-11.00)	9.51 (8.58-11.30)	9.22 (8.05-11.13)	8.22 (5.29-11.00)	0.097
Immunoglobulin M (g/L)		0.90 (0.62-1.27)	0.89 (0.63-1.40)	0.90 (0.62-1.31)	0.91 (0.60-1.28)	0.960
Complement proteins[b]						
C3 (g/L)		0.91 (0.83-1.09)	0.95 (0.85-1.15)	0.93 (0.85-1.14)	0.89 (0.82-1.06)	0.301
C4 (g/L)		0.21 (0.15-0.29)	0.17 (0.13-0.22)	0.22 (0.18-0.29)	0.22 (0.15-0.31)	0.164
Cytokines						
Interleukin-2 (pg/mL)	0.0–11.4	1.4 (1.2-1.8)	1.5 (1.2-1.8)	1.4 (1.2-2.4)	1.4 (1.2-1.7)	0.725
Interleukin-4 (pg/mL)	0.0-12.9	2.5 (2.1-3.3)	2.4 (2.1-3.3)	2.6 (2.3-3.6)	2.5 (2.1-3.3)	0.815

Interleukin-6 (pg/mL)	0.0–20.9	4.0 (2.9-6.7)	3.6 (2.6-5.0)	3.9 (2.6-7.7)	4.1 (2.9-9.2)	0.278
Interleukin-10 (pg/mL)	0.0-5.9	3.9 (3.2-4.9)	3.4 (3.0-4.4)	3.8(3.1-4.5)	4.3 (3.4-6.1)	0.127
Tumor necrosis factor-α (pg/mL)	0.0-5.5	1.6 (1.1-2.2)	1.5 (1.0-2.1)	1.5 (1.1-2.7)	1.7 (1.3-2.2)	0.695
Interferon-γ (pg/mL)	0.0-17.3	2.2 (2.0-4.4)	2.7 (1.9-4.4)	2.8 (2.4-4.3)	3.1 (2.0-4.5)	0.676
Lymphocyte subsets						
CD4+ T cells (cells/μL)	345-2350	1232 (790-1978)	1124 (849-1474)	1187 (819-1610)	1341 (769-2109)	0.570
CD8+ T cells (cells/μL)	314-2080	963 (713-1281)	982 (675-1244)	952 (683-1244)	959 (723-1290)	0.913
B cells (cells/μL)	240-1317	611 (380-1069)	529 (465-690)	610 (368-837)	665 (291-1437)	0.815
Natural killer cells (cells/μL)	210-1514	328 (202-546)	259 (167-531)	399 (194-572)	342 (222-546)	0.480
CD4+/CD8+ T cells ratio	0.96-2.05	1.28 (1.03-1.71)	1.30 (1.00-1.52)	1.24 (1.00-1.77)	1.32 (1.03-1.73)	0.824

Regulatory (CD4+CD25+) T cells (%)	0.7-3.7	4.4 (3.8-5.5)	5.1 (4.1-6.8)	4.5 (4.1-5.7)	4.1 (3.6-5.2)	0.097

[a]Normal range: 1) IgA: <1 year, 0.11-1.06 g/L; 1-6 years, 0.28-1.08 g/L; 7-12 years, 0.33-1.78 g/L; 13-17 years, 0.59-3.90 g/L. 2) IgG: <1 year, 3.58-10.69 g/L; 1-6 years, 4.00-10.39 g/L; 7-12 years, 5.96-13.64 g/L; 13-17 years, 7.00-16.50 g/L. 3) IgM: <1 year, 0.33-1.26 g/L; 1-6 years, 0.42-1.73 g/L; 7-12 years, 0.52-2.42 g/L; 13-17 years, 0.56-3.45 g/L. [b]Normal range: 1) C3: <1 year, 0.70-1.12 g/L; 1-17 years, 0.80-1.26 g/L. 2) C4: <1 year, 0.10-0.38 g/L; 1-17 years, 0.10-0.40 g/L. [*]P<0.05 compared between pneumonia patients and asymptomatic patients.

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; IQR, interquartile range; URTI, upper respiratory tract infection.

TABLE 3 Univariate analysis of factors associated with pneumonia.

Characteristics	**OR**	**95% CI**	***P* value**
Globulin <20.0 g/L	3.13	1.41-6.93	0.005
Immunoglobulin A < lower normal limit[a]	4.00	1.13-14.18	0.032
Hypersensitive C-reactive protein >3.0 mg/L	3.14	1.34-7.36	0.008
Procalcitonin >0.05 ng/mL	3.83	2.03-7.24	<0.001
Interleukin-10 >5.9 pg/mL	7.00	1.59-30.80	0.010
CD4+CD25+ T lymphocyte percentage <5.0 %	1.93	1.04-3.61	0.038

[a]Normal range: <1 year, 0.11-1.06 g/L; 1-6 years, 0.28-1.08 g/L; 7-12 years, 0.33-1.78 g/L; 13-17 years, 0.59-3.90 g/L.

Abbreviations: COVID-19, coronavirus disease 2019; OR, odds ratio; CI, confidence interval.

FIGURE 1 Dynamic changes in cytokines and ferritin in children with COVID-19 admitted to the ICU. Figure 1 shows temporal changes in the serum ferritin (a), plasma concentrations of interleukin-4 (b), interleukin-6 (c), interleukin-10 (d), tumor necrosis factor-α (e) and interferon-γ (f) in two patients with COVID-19 admitted to the ICU (one non-survivor and one survivor). The solid blue lines show the upper normal limit of each parameter. The upper normal limit of interleukin-4 was not reached. Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit.