Differences in Immune Responses between Children and Adults with COVID-19*

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Summary: Over 85 590 000 individuals have been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although there have been an increasing number of reports on coronavirus disease 2019 (COVID-19), it is unclear why infected children show milder symptoms than adults. A retrospective case study was performed at two designated hospitals for COVID-19. Patients (56 children and 63 adults) with confirmed SARS-CoV-2 infection and mild pneumonia were randomly enrolled in this study. The median age of the children was 7.0 years, and 51.79% of them were boys. The median age of the adults was 57 years, and 47.62% were men. The most common symptoms were fever, cough, sputum and diarrhoea. There were no significant differences in symptoms between children and adult patients. In terms of immunological indices on admission, adult patients displayed typical leukopenia and markedly higher levels of IL-2, IL-4, and IL-6 than child patients. The elevation of IL-2, IL-4 and IL-6 in adults induced more extensive lung injury. The effective and non-aggressive immune response successfully resisted SARS-CoV-2 invasion and maintained mild symptoms in child patients. The correlation of higher IL-2, IL-4, and IL-6 with the lung injury might be evidence that preventing excessive cytokine production can avoid further lung damage in these patients.

Key words: COVID-19; child; adult; immune response

The coronavirus disease 2019 (COVID-19) pandemic is rapidly spreading across the globe. Over 85 590 000 people have been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A large number of studies have reported that approximately 80% of infections are mild or asymptomatic, and 15% are severe, requiring ventilation^[1]. Fever, cough, and fatigue are the most common symptoms^[2]. However, 15% of COVID-19 patients rapidly develop severe pneumonia symptoms and complications, including acute respiratory distress syndrome (ARDS) and multiple organ failure^[3]. The fatality rate of COVID-19 is approximately 3.7% worldwide^[4]. Although children and adults share a number of common clinical manifestations, children appear to have mild symptoms and low mortality. However, the underlying viral or host characteristics that mediate this clinical difference

remain incompletely understood.

In this study, we aimed to compare the clinical and immunologic features of children and adults with mild pneumonia. These findings may reveal the relationship between the immune response and mild clinical symptoms in child patients and suggest a strategy for the development of new therapies for SARS-CoV-2 infection.

1 MATERIALS AND METHODS

1.1 Study Population and Design

A retrospective case study was performed at two designated hospitals for COVID-19 in Wuhan: Wuhan Children's Hospital and Union Hospital, which are affiliated to Tongji Medical College of Huazhong University of Science and Technology. All patients provided informed consent. This study was approved by the Ethics Committee of Wuhan Children's Hospital and Wuhan Union Hospital of Tongji Medical College of Huazhong University of Science and Technology, and written informed consent was waived due to the rapid emergence of this infectious disease.

We identified all hospitalized patients with SARS-

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CoV-2 infection between January 25, 2020 and March 8, 2020. Fifty-six children were diagnosed as having mild COVID-19 pneumonia according to the Chinese Clinical Guidelines for Children with COVID-19. In detail, mild pneumonia was indicated by any of the following criteria: children with or without fever who had respiratory symptoms such as cough and chest imaging indicating pneumonia but did not meet the criteria for severe pneumonia^[5]. Sixty-three hospitalized adults in the general ward of Wuhan Union Hospital were randomly selected. According to the Guidelines for Diagnosis and Management of COVID-19 (6th edition, in Chinese) released by the National Health Commission of China^[6], mild pneumonia was indicated by the following criteria: patients with symptoms such as fever and mild respiratory tract symptoms and pneumonia manifestations on imaging. None of the children or adult patients had comorbidities.

1.2 Laboratory and Radiological Procedures

Nasopharyngeal swabs were collected on admission for detection of SARS-CoV-2 RNA by reverse real-time polymerase chain reaction (RT-PCR) as previously described^[7]. To compare the immunological indicators with COVID-19 results, we examined peripheral blood mononuclear cells (PBMCs), including T lymphocytes, B lymphocytes, and natural killer (NK) cells. Flow cytometry was used to determine the percentages of T cells and their subsets (CD3⁺, CD4⁺, CD8⁺), B cells (CD19⁺), and NK cells (CD16⁺/56⁺) among PBMCs. Chest CT scans from patients at Wuhan Children's Hospital or Wuhan Union Hospital were read by two radiologists independently. The evaluators assessed the CT features by both axial CT images and multiplanar reconstruction images. The distribution of lesions was recorded according to the bronchopulmonary segment.

1.3 Data Collection

Clinical, radiological and laboratory characteristics were obtained from electronic medical records with data collection forms. The data were reviewed by two independent physicians (WANG and SUN).

1.4 Statistical Analysis

Categorical variables are presented as frequency rates and percentages. Categorical variables were compared using the χ^2 test or Fisher's exact test. We used SPSS version 22.0 software for all statistical analyses. A two-sided *P* value of less than 0.05 was considered statistically significant.

2 RESULTS

2.1 Clinical Characteristics

By March 20, 2020, 56 children who were admitted to Wuhan Children's Hospital and 63 adults who were admitted to Wuhan Union Hospital were enrolled in this study. These two hospitals were designated

centres for quarantine and treatment of COVID-19, and all the patients were confirmed. All patients with SARS-CoV-2 infection were randomly selected from hospital admissions. The median age of the children was 7.0 years (IQR 2.0–10.0), and 29 (51.79%) of them were boys. For the adults, the median age was 57 years (IQR, 43–65; range, 24–97 years), and 30 (47.62%) were men (table 1). Of the 56 children, the most common symptoms were fever (76.79%), cough (82.14%), sputum (41.07%) and diarrhoea (17.86%). Of the 63 adults, the most common symptoms were fever (40.19%), cough (35.51%), sputum (17.76%) and diarrhoea (6.54%) (table 1). There were no significant differences in symptoms between children and adult patients.

Table 1 Characteristics of symptoms and CT images in children and adults with COVID-19

Symptoms and CT images	<i>n</i> (%)		<i>P</i> value
	Children (<i>n</i> =56)	Adults (<i>n</i> =63)	
Symptoms			
Fever	43 (76.79)	43 (40.19)	
Cough	46 (82.14)	38 (35.51)	
Sputum	23 (41.07)	19 (17.76)	
Diarrhoea	10 (17.86)	7 (6.54)	
CT lesions in the lung			
Single lobe	27 (48.21)	4 (6.35)	<0.001
Multiple lobes	29 (51.79)	59 (93.65)	
Unilateral	30 (53.57)	6 (6.52)	<0.001
Bilateral	26 (46.43)	57 (90.48)	
Ground glass opacities	33 (58.93)	43 (68.25)	0.341
Pulmonary exudation	3 (5.36)	2 (3.17)	0.662
Fibrosis and nodules	9 (16.07)	21 (33.33)	0.036

2.2 Laboratory Findings Related to Immune Response

We collected laboratory markers from illness onset. Indicators such as white blood cells (WBC), lymphocyte subsets, cytokines, hypersensitive C-reactive protein (hsCRP), and procalcitonin (PCT) were compared between children and adults on admission (table 1). The immunological indices on admission are shown in table 2. On admission, 25 (39.6%) adult patients had leukopenia, while only 1 (39.6%) child had leukopenia. In particular, CD3⁺ T cells increased in children compared to adult patients (*P*<0.05). WBC, NK cells, CD8⁺ cells and B cells did not differ between children and adults in this study. More adults (49.21%) than children (26.29%) showed elevated serum levels of hsCRP (*P*<0.05). In contrast, the elevation of serum PCT was more common in children (57.14%) than in adults (9.52%) (*P*<0.05). In addition, compared to the children, who had normal cytokine levels, the adults displayed markedly higher levels of IL-2, IL-4, and IL-6. Interestingly, no differences in IL-10, TNF- α or IFN- γ were observed between two groups (table 2).

Table 2 The immune response in children and adults with COVID-19

Indices	n (%)		P value
	Children (n=56)	Adults (n=63)	
WBC			0.536
Normal	45 (80.36)	54 (85.71)	
High	3 (5.36)	4 (6.35)	
Low	8 (14.29)	5 (7.94)	
L*			<0.001
Normal	38 (67.86)	37 (58.73)	
High	17 (30.36)	1 (1.59)	
Low	1 (1.79)	25 (39.68)	
N			0.105
Normal	51 (91.07)	50 (79.37)	
High	1 (1.79)	7 (11.11)	
Low	4 (7.14)	6 (9.52)	
CD3 ⁺			0.002
Normal	27 (48.21)	48 (76.19)	
High	17 (30.36)	6 (9.52)	
Low	0 (0.00)	3 (4.76)	
CD3 ⁺ CD4 ⁺			0.227
Normal	20 (35.71)	43 (68.25)	
High	11 (19.64)	14 (22.22)	
Low	1 (1.79)	0 (0.00)	
CD3 ⁺ CD8 ⁺			0.338
Normal	29 (51.79)	46 (73.02)	
High	2 (3.57)	4 (6.35)	
Low	1 (1.79)	7 (11.11)	
NK cells			0.108
Normal	31 (55.36)	30 (47.62)	
Low	13 (23.21)	5 (7.94)	
B			0.358
Normal	35 (62.50)	30 (47.62)	
High	4 (7.14)	4 (6.35)	
Low	5 (8.93)	1 (1.59)	
CRP*			0.015
Normal	41 (73.21)	32 (50.79)	
High	15 (26.29)	31 (49.21)	
PCT*			<0.001
Normal	24 (42.86)	37 (58.73)	
High	32 (57.14)	6 (9.52)	
IL-2*			0.001
Normal	38 (67.86)	45 (71.43)	
High	0 (0.00)	13 (20.63)	
IL-4*			<0.001
Normal	38 (67.86)	36 (57.14)	
High	0 (0.00)	22 (34.92)	
IL-6*			<0.001
Normal	35 (62.50)	6 (9.52)	
High	3 (5.36)	52 (82.54)	
IL-10			0.365
Normal	29 (51.79)	38 (60.32)	
High	9 (16.07)	20 (31.75)	
TNF- α			0.154
Normal	36 (64.29)	58 (92.06)	
High	2 (3.57)	0 (0.00)	
IFN- γ			0.297
Normal	35 (62.50)	57 (90.48)	
High	3 (5.36)	1 (1.59)	

WBC: white blood cells; L: lymphocytes; N: neutrophils; NK: natural killer; CRP: C-reactive protein; PCT: procalcitonin; TNF- α : tumour necrosis factor- α ; IFN- γ : interferon- γ . * P <0.05

2.3 Radiologic Findings

Typical CT images of COVID-19 showed ground glass opacities (GGOs)^[8]. Table 1 shows the chest CT findings upon admission. CT images showed single-lobe involvement and unilateral infiltrates in the child patients. Adult patients displayed multiple lobe involvement and bilateral infiltrates. The lung lesions of adults were significantly more extensive than those of children on CT. Interestingly, there was no significant difference in GGOs or pulmonary exudation between children and adults. Adult patients clearly manifested fibrosis and nodules, which were associated with higher IL-6 levels. Further study showed that the elevation of IL-2, IL-4 and IL-6 was associated with an extensive distribution of CT lesions in adults (tables 3 and 4).

Table 3 The distribution of bilateral lung injury on CT images

Indicators in bilateral lung injury	n (%)		P value
	Children (n=56)	Adults (n=63)	
IL-2*			0.015
Normal	25 (83.33)	42 (73.68)	
High	0 (0.00)	12 (21.05)	
IL-4*			<0.001
Normal	25 (83.33)	34 (59.65)	
High	0 (0.00)	20 (35.08)	
IL-6*			<0.001
Normal	22 (73.33)	2 (3.51)	
High	3 (10.00)	52 (91.23)	
CRP			0.367
Normal	18 (60.00)	27 (47.37)	
High	12 (40.00)	30 (52.63)	
PCT*			0.021
Normal	19 (63.33)	36 (63.16)	
High	11 (36.67)	5 (8.77)	

CRP: C-reactive protein; PCT: procalcitonin. * P <0.05

Table 4 The distribution of fibrosis and nodules on CT images

Indicators in fibrosis and nodules	n (%)		P value
	Children (n=9)	Adults (n=21)	
IL-2			0.545
Normal	6 (66.67)	17 (80.95)	
High	0 (0.00)	4 (19.05)	
IL-4			0.284
Normal	6 (66.67)	15 (71.43)	
High	0 (0.00)	6 (28.57)	
IL-6			0.002
Normal	5 (55.56)	2 (9.52)	
High	1 (11.11)	19 (90.48)	
CRP			0.687
Normal	5 (55.56)	14 (66.67)	
High	4 (44.44)	7 (33.33)	
PCT*			0.005
Normal	3 (33.33)	13 (61.90)	
High	6 (66.67)	1 (4.76)	

CRP: C-reactive protein; PCT: procalcitonin. * P <0.05

3 DISCUSSION

In the present study, all adult COVID-19 patients with mild symptoms manifested lung damage on the basis of CT images. Only 69.4% of children showed positive CT findings, even though there were no significant differences in clinical signs, including fever, cough sputum, and diarrhoea. Regarding T cell immunity, lymphopenia appeared in adult patients with lower CD3⁺ and CD3⁺CD8⁺ T cell counts. Moreover, the cytokines IL-2, IL-4, and IL-6 were obviously elevated in adults. The immune response of the child patients displayed mild and regular resistance to SARS-CoV-2. Further information indicates that excessive IL-2, IL-4 and IL-6 levels are associated with extensive lesions on CT lung scan in adults.

The induction of inflammatory cytokines is tightly controlled to prevent tissue damage and maintain proper immune homeostasis. Laboratory evidence from children with mild clinical disease showed that the T cell response against SARS-CoV-2 is important for the recognition and killing of viruses, particularly in the lungs of infected individuals. Moreover, cytokines and specific antibodies to SARS-CoV-2 exhibited normal levels in children. These results illustrate that the immune response was not excessive, with no cytokine storm, in child patients with COVID-19. In contrast to the children, there was evidently abnormal T cell inhibition and excessive cytokine production in adults with COVID-19. An increase in cytokine levels was observed in the adult patients. Similar to the results of a study in hospitalized patients, high levels of proinflammatory cytokines, including IL-2, IL-7, IL-10 and TNF- α , were detected in patients with severe infection as a major factor in the pathogenesis of COVID-19^[4]. Compared to the child patients, the adult patients in our present study displayed a remarkable elevation of IL-2, IL-4 and IL-6 levels. In particular, IL-6 increased in 82.54% of adult patients. IL-6 not only acts as an immune regulator to defend against viruses but also plays an important role in balancing the lung environment. Previous studies reported that IL-6 and transforming growth factor (TGF)- β participate in the pathogenesis of lung diseases, such as ARDS^[9], lung fibrosis, and chronic obstructive pulmonary disease. These results indicated a disordered immune response in the adults, although their clinical condition was mild.

Although their symptoms were mild, both children and adult patients manifested GGOs, fibrosis and nodules, and pulmonary exudation. Surprisingly, GGOs and pulmonary exudation presented in both groups consistently, despite the patterns of fibrosis and nodules, which were more frequent in adults. Moreover, the lung lesions were significantly expanded in adults compared to children. In addition, we found that abundant IL-2, IL-4, and IL-6 levels were correlated with extensive

lesions in the lung; moreover, IL-6 was associated with fibrosis and nodules. Furthermore, elevation of PCT was correlated with lung injury, including the distribution and pattern of fibrosis and nodules, in child patients. A higher hsCRP value in adult patients was not related to lung damage. The hsCRP can increase in many clinical conditions, such as cardiovascular diseases and trauma, in the acute phase. As lung biopsy specimens were not available, the relationship between radiological and histopathological findings remains to be investigated^[8].

In conclusion, the gap of age does not significantly affect the symptoms of mild pneumonia in children and adults with COVID-19. However, the distinct characteristics of the immune response, especially proinflammatory factors inducing lung injury, might be the most important factor in the development of COVID-19. This might be evidence that immune treatment to inhibit excessive cytokine production could reduce lung damage in these patients.

Conflict of Interest Statement

The authors have no conflict of interest.

REFERENCES

- 1 Huang C, Wang Y, Li X, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, 2020,395(10223):497-506
- 2 Chan JF, Yuan S, Kok KH, *et al.* A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet*, 2020,395(10223):514-523
- 3 Guan WJ, Ni ZY, Hu Y, *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*, 2020,382(18):1708-1720
- 4 Chen G, Wu D, Guo W, *et al.* Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*, 2020,130(5):2620-2629
- 5 Shen KL, Yang YH, Wang TY. Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts' consensus statement. *World J Pediatr*, 2020,16(3):223-231
- 6 New coronavirus pneumonia prevention and control program (6th ed.) (in Chinese). 2020(<http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfb1bc54639af227f922bf6b817.pdf>).
- 7 Wu P, Hao X, Lau E, *et al.* Real-time tentative assessment of the epidemiological characteristics of novel coronavirus infections in Wuhan, China, as at 22 January 2020. *Euro Surveill*, 2020,25(3):2000044
- 8 Shi H, Han X, Jiang N, *et al.* Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis*, 2020, 20(4):425-434
- 9 Pittet JF, Griffiths MJ, Geiser T, *et al.* TGF-beta is a critical mediator of acute lung injury. *J Clin Invest*, 2001,107(12):1537-1544

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