Clinical characteristics of 182 pediatric COVID-19 patients with different severities and allergic status

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Abstract

The pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has made widespread impact recently. We aim to investigate the clinical characteristics of COVID-19 children with different severities and allergic status. Pediatric COVID-19 patients tended to have a mild clinical course. Patients with pneumonia had higher proportion of fever and cough and increased inflammatory biomarkers than those without pneumonia. There was no difference between allergic and non-allergic COVID-19 children in aspects of incidence, clinical features, laboratory and immunological findings. Allergy was not a risk factor for developing and severity of SARS-CoV-2 infection and hardly influenced the disease course of COVID-19 in children.

1 INTRODUCTION

On December 12, 2019, 27 pneumonia cases of unknown cause emerged in Wuhan, Hubei, China.[1] The etiological agent was identified as a novel coronavirus and later renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV).[2-4] Community transmission is now evident, and it is clear that SARS-CoV-2 is a highly contagious virus.[5] Until 9 May 2020, the coronavirus disease 2019 (COVID-19) has wreaked havoc in 210 countries and territories, affected more than 3.8 million cases and 265,862 deaths around the world.[6] SARS-CoV-2 infection induces pneumonia, acute respiratory distress syndrome and death, particularly in vulnerable populations such as elderly adults and those with chronic medical conditions, such as cardiovascular diseases, diabetes, respiratory diseases, hypertension and malignancy.[7]Knowledge on SARS-CoV-2 infection in children is still yet to be fully developed and only limited studies on pediatric patients are currently available.[8-12]

According to the Chinese expert consensus on the diagnosis, treatment and prevention of SARS-CoV-2 infection in children (2nd Version), pediatric COVID-19 cases are classified to five clinical types with different severities: 1) asymptomatic infection; 2) acute upper respiratory infection (AURI); 3) mild pneumonia; 4) severe pneumonia; 5) critical pneumonia.[13] In contrast to infected adults, most infected children appear to have a milder clinical course.[8] Asymptomatic infections are not uncommon. Despite that the clinical features of COVID-19 pediatric patients have been established so far, the difference between children with pneumonia and without pneumonia (asymptomatic and AURI), in aspects of clinical features, laboratory

findings, immunological changes and outcomes, were not reported. In addition, the allergy status, and the information of the allergic diseases-related laboratory findings of these patients, have not been reported yet. Allergic diseases are common and with increasing prevalence in children.[14-16] Previous studies showed virus infection is one of the triggers for the exacerbation of asthma.[17] However, there was limited information about the association between asthma and coronaviruses infection, especially SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV).[18,19] Most animal models established for the research of SARS-CoV and MERS-CoV are also found less relevant to asthma.[20] On the other hand, atopic sensitization had no effect on the severity of viral pneumonia in children, as shown in a multi-center prospective study, but the history of allergic diseases such as atopic dermatitis, food allergy and drug allergy were associated with severe pneumonia.[21] In a previous study on 140 adult COVID-19 cases, allergic diseases and asthma showed much less prevalence compared to population levels, suggesting that allergy is not a predisposing factor for SARS-CoV-2 infection.[22]

This study aims to investigate the clinical and laboratory characteristics of hospitalized COVID-19 pediatric patients, and to reveal the relationship between SARS-CoV-2 infection, immune response and allergic status, with a special focus on disease severity and allergy in patients.

2 METHODS

2.1 Patients' enrollment and data collection

This retrospective study involved hospitalized children with SARS-CoV-2 infection, and they were admitted to the Wuhan Children's Hospital from January 28 to February 28, 2020, which was the only designated hospital for treating COVID-19 patients under 16 years in Wuhan. Children who contacted with confirmed or suspected COVID-19 cases have undergone confirmatory SARS-CoV-2 nucleic acid real-time reverse transcription polymerase chain reaction (RT-PCR) testing. All of the individuals enrolled in this study were tested positive. The clinical courses and outcomes were followed up until April 30. In consideration of the possible secondary literature and statistical studies that can be performed in the future,[23] it should be noted that part of our cases had been reported concisely in a previous correspondence paper about the main clinical, laboratory and radiological findings.[8] This study was approved by the institutional ethics board of the Wuhan Children's Hospital (Approval No. WHCH 2020003).

Data comprised of demographic information, clinical presentation, medical history and comorbidities, chest computed tomography (CT) images, laboratory results, treatments (medications and oxygen therapy) and outcomes were obtained from the medical records system and checked by two independent researchers. The duration of hospitalization, and the time of RT-PCR conversion (days from the first positive result to the first negative result of RT-PCR assays for SARS-CoV-2 nucleic acid), were also calculated. In particular, the information of previously diagnosed allergic diseases or related, including allergic rhinitis (AR), asthma, atopic dermatitis (AD), urticaria, and food/drug allergy, and known allergens were collected, and reconfirmed by telephone enquiries.

The severity of COVID-19 was also recorded according to the Chinese expert consensus on the diagnosis, treatment and prevention of SARS-CoV-2 infection in children (2nd Version).[13]Severe cases were identified when meeting one of the following criteria: (a) shortness of breath with increased respiratory rate (RR) except for the influence of fever and crying (RR [?] 60 breaths per minute for those younger than 2 months, RR [?] 50 breaths per minute for those aged between 2 and 12 months, RR [?] 40 breaths per minute for those aged between 1 and 5 years, and RR [?] 30 breaths per minute for those older than 5 years); (b) oxygen saturation [?] 92% at rest; (c) hypoxia with accessory respiration (groaning, flaring of nares, three concave sign), cyanosis, and intermittent apnea; (d) disturbance of consciousness with somnolence and convulsions; (e) food refusal or feeding difficulty, with signs of dehydration; (f) high-resolution CT showing bilateral or multi-lobe involvement, with rapid aggressiveness or pleural effusion. Critical type patients should meet one of the following conditions and admit to intensive care unit (ICU): (a) respiratory failure with mechanical ventilation required; (b) shock; (c) complications with other organ failures. Patients who only had mild symptoms without pneumonia changes in chest CT images were referred to as the acute upper respiratory

infection (AURI) type, and those who had COVID-19 pneumonia not meeting the above criteria of severe cases as the mild type. Individuals only positive for SARS-CoV-2, without any symptoms or changes in chest CT images were defined as asymptomatic (inapparent) infection.

2.2 Laboratory testing

Specimens of nasopharyngeal swabs from children younger than 2 years old and throat swabs from children 2 years or older were obtained for detection of SARS-CoV-2 nucleic acid using RT-PCR assay. The testing was performed in the clinical laboratory of the Wuhan Children's Hospital, and the detailed protocol had been described previously.[8]

Some routine laboratory results were collected from the clinical testing reports, including the complete blood count (CBC), and serum levels of biomarkers such as inflammatory indicators of C-reactive protein (CRP) and procalcitonin (PCT), the coagulation index of D-dimer, the myocardial injury marker of creatine kinase (CK)-MB, the liver function of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and the renal function of serum creatinine and blood urea nitrogen (BUN). Co-infections were indicated by available detection results of other pathogens, including serological measurements of mycoplasma pneumoniae (MP) and chlamydia pneumoniae (ChP), immunoglobulin M (IgM) against Epstein-Barr virus (EBV), cytomegalovirus (CMV) and adenovirus (AdV), and nucleic acid testing of influenza A and B viruses in specimens from pharyngeal swabs. Besides, immunological parameters were collected in detail, including total immunoglobulins (IgG, IgA, IgM and IgE), complements (C3 and C4), peripheral blood CD4+ and CD8+ T, B and NK lymphocyte subsets count, and concentration of serum cytokines including interleukin (IL)-2, 4, 6 and 10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ.

2.3 Statistical analyses

Continuous variables were described through median and interquartile ranges (IQR) values, and categorical variables were presented as frequencies and percentages (%). Two-tailed t test and Mann-Whitney U test were used, as appropriate, to compare the data from two groups. The rates of categorical parameters in two different groups were compared by chi-square test and Fisher's exact test, as appropriate. Spearman's correlation test was applied to calculation of the correlation coefficients between different variables. A test with p value of less than 0.05 was considered statistically significant. All statistical analyses were performed by GraphPad Prism 8 (GraphPad Software Company).

3 RESULTS

3.1 Demographics and clinical characteristics

A total of 182 hospitalized pediatric COVID-19 patients with confirmed RT-PCR assays and available data were included in this study, and the demographics and clinical features are summarized in Table 1. The median age was 6 years old, ranging from 3 days to 15 years. About the half (48.4%) were preschool children, and the majority (83.5%) were 10 years and younger. There were more boys who got infected, with the male-female ratio of nearly 2:1. Most of the children were infected through family members, such as parents and/or grandparents. Forty-three children had the history of allergic diseases, including AR, asthma, AD, food allergy, drug allergy and urticaria. Other pre-existing diseases included repetitive or annual pneumonia, frequent colds, adenoid hypertrophy, tonsillitis, etc. (Table 2).

Although the common symptoms in pediatric COVID-19 patients were fever (43.4%) and dry cough (44.5%), almost one third (30.2%) of these children were asymptomatic. In addition, gastrointestinal symptoms accounted for a perceptible proportion (11.0%), such as diarrhea, abdominal discomfort and vomiting. The most (97.8%) of pediatric infections were not severe. Besides the essential supportive care, inhalation of interferon-α was the most common treatment. Systemic antiviral agents, such as ganciclovir and arbidol, were much less used. In 39 (21.4%) cases, antibiotics such as cephalosporins and azithromycin were employed . Few severe/critical patients received intravenous immunoglobulin (1.6%), systemic glucocorticoids (1.6%), and supplemental oxygen (2.7%). Three critically ill patients were also received mechanical ventilation. Other therapies included budesonide inhalation, montelukast and traditional Chinese medicine. All the hospitalized

children with COVID-19 had recovered except one death. The median duration of hospitalization was 12 days, and that the time from first positive to first negative RT-PCR was 7 days (Table 1). Both the duration of hospitalization and the time of RT-PCR negative conversion were not significantly different between the different subgroups, such as allergic *vs* non-allergic patients, pneumonia *vs* no pneumonia, and younger *vs* older children (Figure 1).

3.2 Radiological and laboratory findings

The chest CT results of all involved children were obtained on admission, and 130 (71.4%) had abnormal images, of which 57 (43.8%) appeared in both lungs, and the rest (56.2%) in unilateral lung. The common signs of pneumonia in chest CT scans were ground-glass opacities (GGO) and local patchy shadowing (Figure 2), with the incidence of 28.0% and 27.5%, respectively, whereas the pulmonary consolidation was much less (1.6%). It was worth noting that there were also 52 (28.6%) infected children without any changes in chest CT images. Thus, the concurrence of normal chest CT scan and no symptom contributed to 24 cases of asymptomatic infection, in the ratio of 13.2% (Table 1).

The median values of laboratory results were mostly within normal ranges, and the details were listed in Table 3 and 4. The rates of decreased count and percentage of lymphocytes were 3.9% and 34.6%, respectively; those of eosinophils were 29.5% and 18.8%, respectively. Other findings in differential of white blood cells included increased and decreased neutrophil percentage (27.4% and 24.0%), and increased monocyte percentage (33.3%). Although the levels of PCT and CK-MB elevated in almost the half (both 47.5%), they changed slightly and had no clinical significance. The majority (33/39, 84.6%) of identified possible co-infected pathogen was mycoplasma pneumoniae (MP), and the other tested pathogens were much less identified. With a further analysis of the subgroup of possible co-infection with MP, GGO in chest CT images was less found (21.1% vs 31.5%, $p = 0.025$), azithromycin was more used (12.1% vs 1.3%, $p = 0.01$), and decreased monocytes count was more common (26.1% vs 5.2%, $p = 0.005$). Possible MP co-infection had no influence on serum levels of inflammatory indicators, such as CRP, PCT and D-dimer. In addition, there was no difference in the prevalence of allergic diseases between children possibly co-infected with MP or not (Table S1 and S2).

3.3 Allergic and immune status of children with SARS-CoV-2 infection

As shown in Table 2, the most common allergic disease was allergic rhinitis (36, 83.7%), followed by atopic dermatitis, food allergy, asthma and urticaria. Only one child, a 13-year-old boy, was previously diagnosed with asthma. Ten (23.3%) children had self-reported allergy to drugs, all of which were penicillin. Among allergic children, 9 (20.9%) had more than one kind of allergic diseases. Other few reported allergens included dust mites, eggs and mangos, each in one child.

In comparison of COVID-19-related measurements between allergic and non-allergic children, no statistical difference was found in the demographics, clinical features and disease course (Table 1). Blood cell counts and biochemical results were mostly similar between two groups, except that eosinophils counts, and serum levels of PCT, D-dimer and AST were lower in allergic group ($p < 0.05$), but these measurements were all in normal ranges, without clinical relevance (Table 3).

Serum total IgE level of allergic children was higher than that of non-allergic individuals (median, 46.30 vs 28.75, unit of IU/ml, $p = 0.048$), and allergic children had greater percentage of increased IgE (42.4% vs 29.3%, $p = 0.155$) without statistical difference. In general, all of the tested immunological parameters were not different between these two groups ($p > 0.05$), including immunoglobulins, complements and cytokines (Table 4).

The lymphocyte subsets were not found significantly different between the allergic and non-allergic COVID-19 children (Table 4), as well as between patients with and without pneumonia (Table 6). The results of correlation analysis between immunological parameters were displayed in Figure 3. There appeared three clusters of correlations. The first one is lymphocyte, total T cells, CD4+ and CD8+ T cells as well as B cells and NK cells. This correlation was relatively strong in all patients including the allergic group

and pneumonia group. The second correlation was in total IgG, IgM, IgA, IgE, C3 and C4 levels. It was relatively stronger in pneumonia group whereas the correlations within this cluster decreased in allergy group. Immunoglobulin levels and lymphocyte subsets showed a negative correlation within all patients and pneumonia group. The third cluster was between the analyzed cytokines, which showed a stronger correlation between each other in pneumonia group. In addition, the duration of hospitalization was positively correlated with the time of RT-PCR conversion, but these two temporal indices had no significant correlation with immunological and inflammatory measurements, except for the slightly negative correlation between the duration of hospitalization and the level of IFN-γ in patients with pneumonia (r = -0.276) (Figure 4).

3.4 The severity of COVID-19 and comparison between pediatric patients with and without pneumonia

Fifty-four infected children did not develop pneumonia, including 24 asymptomatic infections and 30 cases with AURI, and they were compared to patients with mild pneumonia (Table 5 and 6). The prevalence of allergic diseases showed no difference between children without pneumonia and those with mild pneumonia (20.4% vs 25.0%, p = 0.504). When compared to asymptomatic/AURI types, COVID-19 pneumonia, though mild, occurred more in children with other past medical history (21.0% vs 7.4%, p = 0.046), manifested more as fever (50.0% vs 27.8%, p = 0.006) and cough (52.4% vs 29.6%, p = 0.005), and caused more increased level of PCT (57.9% vs 22.9%, p < 0.001), ALP (9.2% vs 0%, p = 0.035) and IL-10 (27.0% vs 0%, p < 0.001), and less decreased level of complement C3 (11.3% vs 28.8%, p = 0.006). The monocytes count, levels of PCT, AST and complement C4, and all the levels of tested cytokines (IL-2, 4, 6 and 10, TNF-α and IFN-γ) were higher, and the level of IgG was lower, in mild pneumonia group than those in non-pneumonia group (p < 0.05), though most of them were in normal ranges. Other clinical and laboratory parameters were not found different between the two groups.

There was only one severe case and three critically ill cases, which were individually described in Table 7. Three of them were male and one was female. Patient 1 was a 13 years and 5 months old boy, who had a close contact with confirmed COVID-19 family members, his mother and grandparents. He was allergic to penicillin. He presented with chest congestion and polypnea. The patient recovered after oxygen supplement and antiviral treatment (inhalation of interferon-α). Patient 2 was a 13-month-old boy, who presented with vomiting, diarrhea and polypnea. He was complicated with septic shock, multiple organ dysfunction syndrome (MODS), kidney stone, hydronephrosis. He received mechanical ventilation and treatment with antiviral, antibiotics and intravenous immunoglobulin (IVIG) and his symptoms were improved. This patient had been elaborated in a Case Report in Chinese.[24] Patient 3 was a 10-month-old girl with intussusception, who had intestinal necrosis, septic shock and multiorgan failure, and died 4 weeks after admission. She was mentioned in a previous literature.[8] We included these patients here for the integrity of the data. Patient 4, an 8-year-old boy, got infected by his family members and was found in the COVID-19 screening during the hospitalization for treating acute lymphoblastic leukemia. On the 24th day of hospitalization, he was transferred to ICU due to respiratory failure and accepted mechanical ventilation (February 18); although with difficulty in weaning, he finally recovered It was evident that the levels of acute phase inflammatory indicators (CRP and PCT) and cytokines (IL-6 and IL-10) dramatically increased in three critically ill cases (Patient 2, 3 and 4).

3.5 Distinct characteristics of COVID-19 between younger and older children

Given the physiological and immunological differences of younger and older children, we divided the cases into two groups according to ages and compared the clinical features and laboratory results of them (Table S3 and S4). Children aged 10 years or older experienced more fatigue and had more GGO lesions in chest CT images (p < 0.05), but there remained more individuals to be further observed. The differences in several laboratory parameters were likely to be induced by their own changes with age, and the most results were in normal ranges without clinical significance. In addition, there was no difference in the prevalence of allergic diseases between the two age groups. Moreover, there was no difference in the proportion of children with possible co-infections, such as MP, between children <10 years and children [?]10 years.

4 DISCUSSION

In the present study involving 182 pediatric COVID-19 patients, we found that the most common symptoms were fever and dry cough, consistent with other reports.[25] A slightly male dominance was found in these patients, which was close to that reported in the USA[26] and China.[27] Most children were infected by family cluster, as observed previously[28], which was different from that of adults who were infected during social activities. A small proportion of pediatric patients were normal in CT images. As for those with abnormal CT images, ground glass opacity and patchy shadowing were the most common features, and consolidation was rare in pediatric patients when compared to adult patients.

The proportion of patients with allergies and asthma in the current study was 23.6% (43/182), which was similar to the general prevalence reported in China.[16] Age distribution, sex ratio, contact history, clinical symptoms, radiological alterations, severity, treatment and outcomes were not different between allergic and non-allergic patients. The proportion of COVID-19 children with allergic rhinitis was 19.8% (36/182), which was also close to the prevalence of AR recently reported (17.6%) in children of Wuhan.[29]In consideration of the increasing prevalence of AR in China,[30] it could be concluded that AR is not a predisposing factor for infection of SARS-CoV-2. Intranasal corticosteroids should be continued for COVID-19 children with AR, as stated in a position paper of the European Academy of Allergy and Clinical Immunology (EAACI).[31] There was no evidence to show that intranasal corticosteroids had any impact on the outcome and the virus shedding of children with AR.

There was only one asthma children in the current study, suggesting that asthma is not a predisposing factor for SARS-CoV-2 infection in children, the same as in adults.[22,32]This is probably because angiotensin-converting enzyme-2 (ACE2), the cellular receptor of SARS-CoV-2, is less expressed in airway epithelia of individuals with allergic asthma and rhinitis.[33] Another possible reason for rare asthma patients in COVID-19 is that most of asthmatic patients infected with SARS-CoV-2 were asymptomatic and thus could not be included in the study. Noticeably, this study involved 10 children (median age, 3.5 years old) with a history of recurrent wheezing, a common symptom in preschool children, which is mainly caused by bronchiolitis and asthma.[34] It is not easy to make a definite diagnosis of asthma in these children, although treatments such as bronchodilators or inhaled corticosteroids will relieve the symptom.[35]

Lymphocytes play an important role in antiviral immunity and contribute to the cytokine storm responsible for pulmonary and systematic inflammation of COVID-19 patients. Lymphopenia was common in adult COVID-19 patients and the degree of lymphopenia was associated with the severity and outcome of the patients.[22,36] However, fewer patients with lymphopenia were observed in pediatric patients, as show in the current and previous studies.[10,11] This is consistent with and also the cause of the high prevalence of asymptomatic and mild courses in COVID-19 children. Interestingly, eosinopenia was also observed in a significant fraction (29.5%) of infected children, though the percentage was less than that of adults, as reported in a previous study that half of the adult COVID-19 patients had decreased count of eosinophils.[22] Given the positive correlation between lymphocyte counts and eosinophil counts, as we demonstrated previously, we speculate that decreased Th2 lymphocytes and cytokines such as IL-5 may be associated with eosinopenia. The ratios of patients with eosinopenia were not different between allergic and non-allergic patients, as well as between younger children and older children. On the other hand, the ratios of eosinophilia were similar between allergic and non-allergic COVID-19 children. Thus, eosinopenia could not be used as a diagnostic indicator of COVID-19 in children, in contrast to that suggested in adults.[22]

The median levels of total serum IgE in all patients, including allergic and non-allergic patients, were within the normal range. The ratios of patients with increased IgE levels were not different between allergic and non-allergic as well as younger and older children with COVID-19. Most of the allergic disease in these children was well-controlled AR in this study, since only few patients were still using intranasal corticosteroids during the COVID-19. It must be pointed out that no data about specific IgE, skin prick test or other provocation tests were available in these patients, which indicates that a few AR patients might be "misdiagnosed". Taken together, our results suggest that allergy has no obvious impact on the disease course of COVID-19 in children. Other immunoglobins such as total IgG, IgA, IgM, and complements C3 and C4 were all in

normal ranges, without difference between allergic and non-allergic patients, although higher ratio of patients with increased IgA, C3 and C4 were seen in older children, and both ratios of patients with increased and decreased IgG were observed in younger children.

As far as we know, there is no study focusing on the pediatric COVID-19 children with different severities. Considering that most children infected with SARS-CoV-2 were asymptomatic or presented as AURI and mild pneumonia, this is rational. In our cohort, only four children were categorized as severe or critical cases, including one death due to intussusception and secondary multiple-organ failure. The other three severe children were recovered.

Since the rarity of severe and critical pediatric COVID-19 cases, we compared the clinical and laboratory features of mild pneumonia cases with those patients without pneumonia. Clinically, mild pneumonia was associated with more patients with manifestations, especially fever and cough; in addition, allergy prevalence was not different between the two groups. In pediatric COVID-19 patients, most of these parameters were in the normal ranges, except for PCT and CK-MB, both were slightly elevated in nearly half of the cases, without clinical significance.

Most laboratory findings were comparable between the children with mild pneumonia and without pneumonia. Some biochemical and immunological parameters differed between the two groups, but all were in the normal ranges. These differences may reflect the inflammatory responses induced by pneumonia. The incidence of elevated PCT, ALP, IL-10 and decreased complement C3 in pneumonia was higher in mild pneumonia patients than those without pneumonia. This is a little different from adult COVID-19 patients, that inflammatory biomarkers CRP and PCT, coagulation indicator D-dimer, myocadiac injury indicator CK/CK-MB, and liver and renal function indicators ALT, AST, ALP and BUN in the blood were all elevated and were more prominent in severe and critical cases, as studies previously demonstrated.[22,37]

Peripheral lymphocyte subsets alteration has been reported in studies on adults COVID-19 patients. CD8+ cytotoxic T cells was an independent predictor for COVID-19 severity and treatment efficacy.[38] CD4+ and CD8+ T cells counts were also correlated with the disease severity and outcome.[39] So far, there were few studies focusing on the lymphocyte subsets in pediatric cases. In the present study, we found that in COVID-19 children, both the numbers and percentages of T cells, CD4+ T cells, CD8+ T cells, B cells and NK cells were mostly in the normal ranges, as well as the levels of cytokines involved in inflammation, immune regulation and antiviral immunity such as IL-2, IL-4, IL-6, IL-10, TNF-α and interferon-γ in the serum. The slight or unchanged immunological function may contribute to the clinical features of pediatric COVID-19 patients such as lower incidence, milder symptoms, shorter course of disease and fewer severe cases.[40] The median numbers of total lymphocytes and lymphocyte subsets were different between younger and older children, but still in the corresponding normal ranges of their ages. Older children had more changes in the lymphocyte subsets, including more cases with increased percentages of T cells and CD8+ T cells, and decreased numbers of B cells. Younger children were more frequently associated with increased CD4+ T cells numbers and percentages. Our results provide deep insight into the immunological features of COVID-19 children. Consistent with the clinical symptoms and laboratory findings, the lymphocyte subsets and cytokines were not different in allergic and non-allergic COVID-19 patients. Thus, allergy plays a negligible role in the incidence, disease course and outcomes of COVID-19 in children.

Only a minority of children were with evidences of possible co-infection with other pathogens, most of which was MP, and few were EBV, CMV, adenovirus and influenza B virus. COVID-19 children with evidence of MP co-infection were associated with relatively lower monocyte counts and more azithromycin use, and also lower ratio of ground glass opacity in chest CT when compared to those without evidence of MP co-infection (Table S1 and S2). The severity and percentages of children with allergy were not different between children with or without evidence of MP co-infection. Thus, MP co-infection might play a minor role in the disease course of COVID-19 in children. It is noticeable that there were no patients with evidence of coinfection with rhinovirus (RV) and respiratory syncytial virus (RSV), which are more commonly found in children with AR and wheezing.[41] During the quarantine period, many tests performed in commercial institutions were stopped including RV and RSV detections, which resulted in no RV and RSV identified in this study.

In addition, the physicians tended not to find other pathogens if the children had been confirmed with SARS-CoV-2 infection by RT-PCR assays. We could not exclude the co-infection in COVID-19 children with other respiratory viruses, which may also play a role in the immunity against SARS-Cov-2.

Treatments of COVID-19 children during hospitalization were diverse and mostly empirical, without evidence of randomized controlled trials (RCTs). Almost all (97.8%) children were treated with inhalation of interferon-α, based on the studies and experiences of its usage in treating other respiratory infection and viral pneumonia.[42] Therefore, we could not know the exact clinical effects of interferon-α by comparing the outcomes of patients treated with or without interferon-α. There is no clinical evidence to address the effect of IFN-α use in COVID-19 children. Therefore, the treatment of interferon-α should be evaluated with well-designed RCTs in the future.

In the present cohort, almost all pediatric patients had good outcomes, except one death. This is consistent with previous report of the outcome in pediatric COVID-19 patients.[9-11] Both patients with mild and without pneumonia recovered well and were discharged from the hospital. Duration of hospitalization in this study was not different between patients with mild and without pneumonia, but shorter than that of adult patients.[37] This is due to the large proportion of patients without pneumonia, which could be discharged after a few days of medical surveillance in the hospital. In addition, the median time from first positive to first negative RT-PCR results of SARS-CoV-2 were 7 days in both patients with mild and without pneumonia. This is also shorter than the time needed for virus clearance in adult patients.[37,43] The cause of this difference is unclear, but it was reported that older age and presence of chest tightness were independent factors affecting negative conversion of the virus RNA.[43] It may also be associated with different immunological response between pediatric and adult patients. The negative conversion of RT-PCR (two consecutive negative results at least with a 24-hour interval) was one of the discharge criteria, which caused its positive correlation with the duration of hospitalization. Interestingly, in patients with pneumonia, the duration of hospitalization had mildly negative correlation with the level of IFN-γ, which indicated the possible antiviral roles of interferon. More importantly, most of children were treated with IFN-α, and its effect on the negative conversion of virus RNA in respiratory tract sample could not be excluded.

There are a few limitations of this study. Firstly, not all children in the designated hospital could be included in the present study; secondly, the allergic status of most children was according to medical history, but not with medical records or allergen testing; and the allergens responsible for the allergy were reported only in 30% of allergic patients. Thirdly, the dominance of asymptomatic patients and mild pneumonia prevented most patients from repetitive laboratory tests, thus the estimation of dynamic variations in blood cell counts and biochemical parameters was impossible.

In conclusion, pediatric COVID-19 patients tended to have mild clinical course, and severe cases were rare. Patients with mild pneumonia had higher proportion of fever and cough and increased inflammatory biomarkers than those without pneumonia, and the both were with favorable prognosis. Allergic and non-allergic COVID-19 children were not different in aspects of incidence, clinical characteristics, laboratory and immunological findings. Allergy is not a predisposing factor for SARS-CoV-2 infection and plays no role in the disease course of COVID-19 at least in children.

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AUTHOR CONTRIBUTIONS

Hui Du collected the clinical data, Xiang Dong organized and analyzed the data, and they prepared the manuscript. Jinjin Zhang was involved in the preparation of the manuscript. Yiyuan Cao interpreted the radiological images. Peiqi Huang, Hongwei Chen and Ying Li participated in the clinical work and data extraction. Guanghui Liu made some instruction in the study. Cezmi A. Akdis worked in the writing and critical review of the manuscript. Yadong Gao participated in the preparation of the manuscript. Xiaoxia

Lu and Yadong Gao designed the study and reviewed the manuscript.

REFERENCES

1. Wuhan Municipal Health Commission. Report of clustering pneumonia of unknown etiology in Wuhan City ,2019.

2. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020;382:727-733. https://doi.org/10.1056/NEJMoa2001017

3. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*2020;579:270-273. https://doi.org/10.1038/s41586-020-2012-7

4. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020;5:536-544. https://doi.org/10.1038/s41564-020-0695-z

5. World Health Organization (WHO). Responding to community spread of COVID-19 (Interim guidance) 7 March 2020. https://www.who.int/publications-detail/responding-to-community-spread-of-covid-19 Accessed April 23, 2020

6. World Health Organization (WHO). Coronavirus disease (COVID-2019) situation reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports Accessed April 17, 2020

7. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020. https://doi.org/10.1001/jama.2020.2648

8. Lu X, Zhang L, Du H, et al. SARS-CoV-2 Infection in Children. *N Engl J Med* 2020. https://doi.org/10.1056/NEJMc2005073

9. Cai J, Xu J, Lin D, et al. A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features. *Clin Infect Dis* 2020. https://doi.org/10.1093/cid/ciaa198

10. Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding.*Nat Med* 2020. https://doi.org/10.1038/s41591-020-0817-4

11. Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study.*Lancet Infect Dis* 2020. https://doi.org/10.1016/S1473-3099(20)30198-5

12. Dong X, Cao YY, Lu XX, et al. Eleven faces of coronavirus disease 2019. *Allergy* 2020. https://doi.org/10.1111/all.14289

13. Jiang Y, Lu XX, Jin RM, et al. [Expert consensus on the diagnosis, treatment and prevention of SARS-CoV-2 infection in children (2nd Version)]. *Chin J Appl Clin Pediatr* 2020;35. https://doi.org/10.3760/cma.j.issn.2095-428X.2020.02.012

14. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ J* 2014;7:12. https://doi.org/10.1186/1939-4551-7-12

15. Akinbami LJ, Simon AE, Schoendorf KC. Trends in allergy prevalence among children aged 0-17 years by asthma status, United States, 2001-2013. *J Asthma* 2016;53:356-362. https://doi.org/10.3109/02770903.2015.1126848

16. Wong G, Li J, Bao YX, et al. Pediatric allergy and immunology in China. *Pediatr Allergy Immunol* 2018;29:127-132. https://doi.org/10.1111/pai.12819

17. Gilles S, Blume C, Wimmer M, et al. Pollen exposure weakens innate defense against respiratory viruses. *Allergy* 2019. https://doi.org/10.1111/all.14047

18. Papadopoulos NG, Christodoulou I, Rohde G, et al. Viruses and bacteria in acute asthma exacerbations–a GA²LEN-DARE systematic review. *Allergy* 2011;66:458-468. https://doi.org/10.1111/j.1398-9995.2010.02505.x

19. Greenberg SB. Update on Human Rhinovirus and Coronavirus Infections. *Semin Respir Crit Care Med* 2016;37:555-571. https://doi.org/10.1055/s-0036-1584797

20. Han M, Rajput C, Ishikawa T, Jarman CR, Lee J, Hershenson MB. Small Animal Models of Respiratory Viral Infection Related to Asthma. *Viruses* 2018;10. https://doi.org/10.3390/v10120682

21. Erdem SB, Can D, Girit S, et al. Does atopy affect the course of viral pneumonia. *Allergol Immunopathol (Madr)* 2018;46:119-126. https://doi.org/10.1016/j.aller.2017.04.003

22. Zhang JJ, Dong X, Cao YY, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020. https://doi.org/10.1111/all.14238

23. Bauchner H, Golub RM, Zylke J. Editorial Concern-Possible Reporting of the Same Patients With COVID-19 in Different Reports. *JAMA*2020. https://doi.org/10.1001/jama.2020.3980

24. Chen F, Liu ZS, Zhang FR, et al. [First case of severe childhood novel coronavirus pneumonia in China]. *Zhonghua Er Ke Za Zhi*2020;58:179-182. https://doi.org/10.3760/cma.j.issn.0578-1310.2020.03.003

25. Zheng F, Liao C, Fan Q, et al. Clinical Characteristics of Children with Coronavirus Disease 2019 in Hubei, China. *Curr Med Sci*2020;40:1-6. https://doi.org/10.1007/s11596-020-2172-6

26. CDC COVID-19 Response Team. Coronavirus Disease 2019 in Children - United States, February 12-April 2, 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:422-426. https://doi.org/10.15585/mmwr.mm6914e4

27. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 Among Children in China. *Pediatrics* 2020. https://doi.org/10.1542/peds.2020-0702

28. Hong H, Wang Y, Chung HT, Chen CJ. Clinical characteristics of novel coronavirus disease 2019 (COVID-19) in newborns, infants and children. *Pediatr Neonatol* 2020. https://doi.org/10.1016/j.pedneo.2020.03.001

29. Lei Y, Yang H, Zhen L. Obesity is a risk factor for allergic rhinitis in children of Wuhan (China). *Asia Pac Allergy*2016;6:101-104. https://doi.org/10.5415/apallergy.2016.6.2.101

30. Zhang Y, Zhang L. Increasing Prevalence of Allergic Rhinitis in China. *Allergy Asthma Immunol Res* 2019;11:156-169. https://doi.org/10.4168/aair.2019.11.2.156

31. Bousquet J, Akdis C, Jutel M, et al. Intranasal corticosteroids in allergic rhinitis in COVID-19 infected patients: An ARIA-EAACI statement. *Allergy* 2020. https://doi.org/10.1111/all.14302

32. Li X, Xu S, Yu M, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020. https://doi.org/10.1016/j.jaci.2020.04.006

33. Jackson DJ, Busse WW, Bacharier LB, et al. Association of Respiratory Allergy, Asthma and Expression of the SARS-CoV-2 Receptor, ACE2. *J Allergy Clin Immunol* 2020. https://doi.org/10.1016/j.jaci.2020.04.009

34. Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. *Lancet* 2014;383:1593-1604. https://doi.org/10.1016/S0140-6736(14)60615-2

35. Bush A, Saglani S. Medical algorithm: Diagnosis and treatment of preschool asthma. *Allergy* 2020. https://doi.org/10.1111/all.14172

36. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020. https://doi.org/10.1016/S0140-6736(20)30566-3

37. Cai Q, Huang D, Ou P, et al. COVID-19 in a designated infectious diseases hospital outside Hubei Province, China. *Allergy* 2020. https://doi.org/10.1111/all.14309

38. Wang F, Nie J, Wang H, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis*2020. https://doi.org/10.1093/infdis/jiaa150

39. Liu Z, Long W, Tu M, et al. Lymphocyte subset (CD4+, CD8+) counts reflect the severity of infection and predict the clinical outcomes in patients with COVID-19. *J Infect* 2020. https://doi.org/10.1016/j.jinf.2020.03.054

40. Duan YN, Zhu YQ, Tang LL, Qin J. CT features of novel coronavirus pneumonia (COVID-19) in children. *Eur Radiol* 2020. https://doi.org/10.1007/s00330-020-06860-3

41. Turunen R, Koistinen A, Vuorinen T, et al. The first wheezing episode: respiratory virus etiology, atopic characteristics, and illness severity. *Pediatr Allergy Immunol* 2014;25:796-803. https://doi.org/10.1111/pai.12318

42. Shen K, Yang Y, Wang T, et al. Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts' consensus statement. *World J Pediatr* 2020. https://doi.org/10.1007/s12519-020-00343-7

43. Hu X, Xing Y, Jia J, et al. Factors associated with negative conversion of viral RNA in patients hospitalized with COVID-19.*Sci Total Environ* 2020;728:138812. https://doi.org/10.1016/j.scitotenv.2020.138812

Figure legends

Figure 1 , The comparisons in the duration of hospitalization and the time of negative conversion of RT-PCR assays for SARS-CoV-2 nucleic acid, respectively, between the following COVID-19 subgroups: (A, B), allergic and non-allergic patients; (C, D), no pneumonia and mild pneumonia patients; (E, F), patients aged $<$ 10 years and [?] 10 years. In each graph, the dots denote each of numerical values, the middle long lines denote medians, and bilateral short lines denote interquartile ranges. In particular, the black dots denote the numerical values of three severe/critical cases, and the hollow black dot denotes that of one dead case. Details of these four severe or critically ill patients were presented in Table 7. All of the comparisons were not significantly different ($p > 0.05$). RT-PCR, real-time reverse transcription polymerase chain reaction. (+), positive.

Figure 2 , Typical chest CT images of pediatric COVID-19 patients. (A) a 11-month-old non-allergic female, with chest CT showing ground-glass opacity in left lower lobe; (B) a 1-year-old non-allergic male, with chest CT showing bilateral ground-glass opacities and subpleural nodular consolidation; (C) a 4-year-old male with a history of allergic rhinitis, and his chest CT showed patchy opacities in left lower lobe; (D) a 3-year-old male with a history of allergic rhinitis and recurrent wheezing, and his chest CT showed patchy opacity distributed around the bronchovascular bundle in right lower lobe.

Figure 3 , Heatmap of correlation matrix between duration of hospitalization (DH), duration of RT-PCR conversion (DP, time from first positive to first negative RT-PCR results), blood cell counts and immunological parameters of pediatric patients with COVID-19 in (A) total cases (n = 182), (B) allergic cases (n = 43), and (C) mild pneumonia cases (n = 124), respectively. Analyzed variables included numbers of immune cells, serum levels of immunoglobulins (Igs), complements and cytokines: NEU (neutrophils count, x10^9/L), EOS (eosinophils count, x10^9/L), LYM (lymphocytes count, x10^9/L), T (T cells count, /μl), CD4+ and CD 8+ T (CD4+ and CD8+ T cells count, /μl), B (B cells count, /μl), NK (natural killer cells count, /μl), IgG, IgA and IgM (concentration, g/L), IgE (concentration, IU/ml), C3 and C4 (complements C3 and C4 concentration, g/L), IL-2, 4, 6 and 10 (interleukins concentration, pg/ml), TNF-α (tumor necrosis factor-α concentration, pg/ml), and IFN-γ (interferon-γ, pg/ml). The values of Spearman's correlation coefficients are shown in each cell of the graph. Red and blue backgrounds stand for positive and negative correlations, respectively, and the shades of color reflect the strength of correlation.

Figure 4 , Correlations between the duration of hospitalization and the time of RT-PCR conversion in pediatric COVID-19 patients: (A) all patients, (B) allergic patients, (C) patients with pneumonia, (D) the significantly negative correlation between the duration of hospitalization and the level of IFN-γin patients with pneumonia. Spearman's correlation test was used, with a calculation of correlation coefficient (r). The red dots denote the numerical values of three severe/critical cases, and the hollow red dot denotes that of one dead case. RT-PCR, real-time reverse transcription polymerase chain reaction. (+), positive; (-), negative.