Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Medical Hypotheses 143 (2020) 109842

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Reflection on lower rates of COVID-19 in children: Does childhood immunizations offer unexpected protection?

Jinglu Lyu[a,d,1], Tianyu Miao[b,1], Jiajia Dong[b], Ranran Cao[c], Yan Li[a,*], Qianming Chen[a]

[a] State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, China
[b] Department of Pulmonary and Critical Care Medicine, Vascular Surgery, West China Hospital of Sichuan University, Chengdu, Sichuan 610041, China
[c] Sichuan Provincial Centers for Disease Control and Prevention, Chengdu, Sichuan 610041, China
[d] Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing 210008, China

ARTICLE INFO

Keywords:
COVID-19
Mild symptom in children
Childhood immunization
Trained immunity
Immune fitness

ABSTRACT

The incidence of COVID-19 in children and teenagers is only about 2% in China. Children had mild symptoms and hardly infected other children or adults. It is worth considering that children are the most vulnerable to respiratory pathogens, but fatal SARS-like virus had not caused severe cases among them. According to the pathological studies of COVID-19 and SARS, a sharp decrease in T lymphocytes leads to the breakdown of the immune system. The cellular immune system of children differs from that of adults may be the keystone of atypical clinical manifestations or even covert infection. The frequent childhood vaccinations and repeated pathogens infections might be resulting in trained immunity of innate immune cells, immune fitness of adaptive immune cells or cross-protection of antibodies in the children. Therefore, due to lack of specific vaccine, some vaccines for tuberculosis, influenza and pneumonia may have certain application potential for the front-line health workers in the prevention and control of COVID-19. However, for high-risk susceptible populations, such as the elderly with basic diseases such as hypertension and diabetes, it is necessary to explore the remedial effect of the planned immune process on their immunity to achieve the trained immunity or immune fitness, so as to improve their own antiviral ability.

According to *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19)* (called 2019 Report) submitted on 28 February 2020, data on children (≤18 years) suggest that there is a relatively low attack rate in this age group (2.4% of all reported cases) [1]. Despite the low incidence of children, they also have milder presentations, severe clinical manifestations such as respiratory distress are rarely detected, even when pathological changes are moderate to severe [2,3]. This epidemiological feature is consistent with another outbreak of coronavirus-related disease, SARS, in 2003 [4]. We reviewed the existing literatures on COVID-19 and SARS and found that some studies have addressed this phenomenon. Determining the difference between how children and adults respond may be a new way to treat and prevent the disease.

Epidemiological characteristics

Humans are generally susceptible

The pathogen causing COVID-19 is a newly identified pathogen, bioinformatic analyses indicated that the virus had features typical of the coronavirus family and belonged to the β-coronavirus lineage [5], named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is mainly transmitted through respiratory droplets and close contact, although virus particles have been detected in patients' feces, lacrimal secretions, and aerosols, there is no clear evidence that these secretions are infectious. Currently, the main source of infection is confirmed patient, including asymptomatic infections.

In theory, humans have no pre-existing immunity to this newly identified pathogen. Everyone is assumed to be susceptible, although there are a variety of factors that can increase the risk of infection. Whether there is life-long neutralizing immunity after infection requires further study. There have been recent reports of “re-positive” cases of

* Corresponding author.
[1] Contributed to this paper.

https://doi.org/10.1016/j.mehy.2020.109842
Received 10 April 2020; Received in revised form 25 April 2020; Accepted 12 May 2020

nucleic acid testing in discharged patients, which may be related to "false positive" tests at discharge, rather than actual recurrence [6,7]. The latest follow-up study of SARS survivors found that specific IgG antibodies persisted for up to 12 years [8]. Other earlier observations have also reported maintenance ranging from 2 to 4 years of SARS-CoV specific antibodies [9,10].

Like MERS and SARS, it is a cross-species infectious disease. Since MERS did not occur on a large scale in China, we compared it with the epidemiological and pathological characteristics of SARS in the following content.

Children have low incidence and low infectivity

At present, most of the reported cases in children are clustered cases caused by close contact, known as "second generation" infection. The sporadic features of the disease is particularly obvious in the areas outside of Wuhan [11]. Recently, as the epidemic in China has been gradually brought under control, especially after the release of some statistics of Hubei province, we can see more intuitively that the incidence of children is significantly lower than that of adults [12], newborns and infants are less susceptible than adolescents [2]. The incidence of COVID-19 in children is 2.4% according to 2019 Report, and it is worth noting that some cases of covert infection may not be detected [3,13]. Very few neonatal deaths had been reported [14].

The epidemiological investigations of SARS are also consistent with this conclusion. According to the statistics of 2003, the confirmed cases of children under 14 years old accounted for only 2.7% (as of May 4) of the total cases of SARS in Beijing municipality and 4.88% (as of April 27) in Guangdong province. 10 SARS children were admitted to a Hong Kong hospital in 2003, eight of whom had been in school before they were confirmed but had not spread the disease to other students [4]. Children and adolescents might be susceptible to SARS-CoV infection if they had close contact with confirmed patients, but the clinical course and outcome are more favorable in children younger than 12 years of age compared with adolescents and adults. Transmission of SARS from pediatric patients appears to be uncommon but is not impossible [15]. Only a few cases of child deaths or transmission of the disease to adults as a source of infection have been reported. These observations raise the question of whether children have natural resistance to these two coronaviruses.

Clinical manifestations

Most children have milder or atypical symptoms

COVID-19 was divided into four clinical subtypes according to the severity [16]: light type, normal type, severe type and critical type. Fever, dry cough, fatigue are the main symptoms. However, children with severe or critical illness may have moderate or low fever, or even no significant fever [17].

Compared with adult cases, children tend to have milder symptoms, shorter disease course and generally better prognosis. Fever is the main symptom in most children, the fever duration is 2–3 days, up to 8 days, most within 1 week. Some children came to see the doctor due to gastrointestinal symptoms. According to 2019 Report, a very small proportion of those aged under 19 years especially with some basic diseases, have developed severe (2.5%) or critical disease (0.2%). Children with fever account for a relatively large number of the cases, which is convenient for early identification and diagnosis and contribute to fewer severe cases. There are no death cases in children under 9 years of age [18]. As the coverage of the nucleic acid test became wider, more and more asymptomatic patients were diagnosed, and the majority of them were young people [19,20].

Lymphocyte function was not seriously impaired in childhood cases

Routine blood tests in most patients show typical signs of viral infection, with normal or reduced white blood cell counts and reduced lymphocyte counts in early stage. In severe cases, d-dimer increased and peripheral blood lymphocytes progressively decreased, and cytokines were apparently accumulated [21]. Compared with 120 healthy controls, the absolute value of lymphocytes (0.87 vs 2.13 $\times$ 10^9/L) and the percentage (19.5% vs 33.7%) were significantly reduced [22]. Another study showed that 25% and 63% of the patients had leukopenia and lymphocytopenia, respectively [11].

Most viruses induce cell-mediated immune responses as well as humoral immunity. The main cells involved in cellular immune response include CD4+ T cells and CD8+ T cells. The virus activates primary CD4+ T cells which are involved in cell-mediated immune response. CD8+ T cells kill virus-infected target cells mainly by direct contact [23]. However, the vast majority of COVID-19 patients showed reduced lymphocytes. The proportion of CD4+ T cells decrease in mild and severe patients was 52.90% and 95.24%, and CD8+ T cells decrease was 28.40% and 61.90%, respectively [24]. Further anatomical and pathological findings showed that the state of T lymphocytes was overactivated, and CD8+ T cells had a high concentration of cytotoxic particles, thus causing serious immune damage [25]. In addition, compared with the mild group, the increase of serum inflammatory factors in the severe group significantly increased the risk of further tissue damage caused by cytokine storms [11]. The progressive increases in IL-6 and C-reactive protein as warning indicators of critical illness in adults [16]. Single-cell RNA sequencing of the bronchoalveolar lavage fluid of COVID-19 patients showed the proportion of macrophages in severe patients was higher, with more SPP1+ and FABP4+ subsets rather than FCN1+ macrophages. While the proportion of CD8+ T cell and NK cells in mild patients was higher [26].

However, in most children patients, the white blood cell count and absolute number of lymphocytes in the peripheral blood were normal. In a few severe cases, the total number of white blood cells decreased, with decreased absolute number of T lymphocytes, and changes in T lymphocytes subsets, C-reactive protein was usually normal or temporarily increased [13]. In children with COVID-19 and SARS, the maintenance of T cell function may be related to thymic development. The thymus is the site of T cell development, and its absolute weight is the largest between 6 and 13 years old. More evidence is needed to confirm that differences in cellular immunity between children and adults are related to this phenomenon.

Children are frequently exposed to pathogens

Frequent childhood vaccinations and repeated pathogens infections have resulted in children who are exposed to various antigens.

China implements immunization policy

Immunization is considered one of the greatest public health achievements of the 20th century due to its important interventions for human health. The popularity of smallpox vaccine, *Mycobacterium tuberculosis* vaccine (Bacillus Calmette-Guérin, BCG), oral polio vaccine (OPV) and measles vaccine has reduced morbidity and mortality worldwide. Human smallpox has been eradicated and polio is also a disease to be overcome by immunization. China has officially become a polio-free country in 2000.

China's basic immunization policy mainly targets children (HepB, BCG, IPV, OPV, DTaP, DT, MR, MMR, JE, MPSV, HepA) to receive mandatory vaccines. These vaccines will prevent 12 diseases in total. Children should complete basic immunization at 6 years old and compensate the missing vaccines before 14 years of age [27].

Infectious diseases rank second in the global cause of death. Children and the elderly are the most susceptible groups to infectious

diseases, especially infectious diseases of the respiratory system. Common infectious diseases in children are divided into viral infections and bacterial infections, some of which have been covered by mandatory vaccines. Preventive vaccines have also been developed for influenza, hand-foot-mouth disease, and *Streptococcus pneumoniae* (*S.p*)-related respiratory diseases, although which are currently non-mandatory vaccines in China. With the gradual increase up-take rates, the prevention of these diseases will become increasingly apparent.

Mixed infection complicates the immune response

Mixed infection was more common in children's community acquired pneumonia (CAP) with a rate of 16.02%–34.63%. Viral infection is particularly important in the initial stage of infant CAP. Viral infection can directly invade bronchial epithelial cells, destroy the mucosal barrier, and cause immune disorders in the body and lungs. The detection rate of influenza virus in children's CAP is 4%–22%, and adenovirus-related pneumonia accounts for about 4%–10%. On this basis, it is easy to combine other pathogen infection, such as other virus, bacteria, fungi, mycoplasma. The infection rate of dual viruses was 19.1%–30.0%, like respiratory syncytial virus (RSV) and influenza viruses. The rate of virus-bacteria coinfection can reach 31%, which accounts for a high proportion in all age. *S.p* is the most common mixed infection bacteria. RSV+ *S.p* is the most common pattern of mixed infection. More than half of *mycoplasma pneumoniae*-related pneumonia has mixed infection.

In this state of mixed infection, the immune response tends to be more complicated. Take RSV infection as an example, N protein of RSV is one of the target antigens for cytotoxic T cells (CTL), whereas the G protein was not recognized and can at best represent a minor target antigen for CTL [28]. CD4+ and CD8+ T cells were productively infected by RSV in pediatric patients. Infection decreased IL-2 and IFN-γ production. The frequency of CD4+ RSV+ T cells positively correlated with disease severity [29]. IFN-γ and TNF produced by CD8+ T cells following RSV infection contributed to both the acceleration of virus clearance and the induction of immunopathology. The actions of a variety of cytokines, including IL-10 and IL-4, played a critical role in the regulation of CD8+ T cell effector activity [30]. The coinfection of RSV and *S.p* increased cytokine secretion and inflammatory cells infiltration in the airway. More importantly, when the G protein on RSV binds to *S.p*-related receptors, it can change the transcription level of *S.p*-related genes and significantly increase the virulence of *S.p*.

SARS-CoV-2 has 16 antigenic components, which can cause different responses. The stimulating effect and the response duration of these components on CD4+ and CD8+ T cell subsets differ from one another. If SARS-CoV-2 co-infects with other pathogens, the immune disorders will be more difficult to correct as can be expected. Clinical data shows the prevention and treatment of coinfections in hospitalized COVID-19 patients, with 60–70% receiving antimicrobial treatment and 15% receiving anti-fungal treatment [31,32]. *Candida* was isolated from 4 airway specimens in a case report of patients with COVID-19 [33]. These *Candida* might come from the environment or the oral microenvironment [34]. Thus preventing invasive fungal infections should be an important part of treatment [35]. During the SARS epidemic in 2003, therapeutic regimen in Hong Kong recommended the use of second-generation cephalosporin plus macrolide antibiotics in the pediatric patients as the first step in response to coinfection [36].

Whether the relatively weak function or low expression of ACE2 receptor in children leads to the limited invasion pathway of the virus? Lower expression of ACE2 in children will limit virus invasion pathway, which will be responsible for avoiding large-scale outbreak in children. Previous studies have found that the expression of ACE2 receptor is inhibited in the infection of RSV, H5N1 and H7N9 avian influenza viruses [37–39]. On the other hand, ambroxol, which is selected as a potential ACE2 receptor binding agent by an artificial intelligence drug screen system, may block the entering pathways of SARS-CoV-2, which provides a valuable therapeutic approach for the prevention or reduction of acute lung injury (ARDS), or even kidney and heart injury caused by SARS-CoV-2 [40]. Ambroxol is a common drug in pediatric respiratory department due to the high incidence of respiratory tract diseases among children. Whether a lasting effect on ACE2 of ambroxol maintains and plays an immunoregulatory role faced with the invasion of SARS-CoV-2 is worth exploring.

Possible immune regulatory mechanisms

The high frequency of infection and vaccination in children, together with the special immune system in childhood, make the children's immune response to SARS-CoV-2 in a state of high immune clearance and low immune response. In addition, various immune adjuvants in vaccines can directly promote the body's non-specific and cellular immune function, make its innate immune trained, leading to immune fitness [41].

Childhood past infection history might contribute to trained immunity

The concept of trained immunity was first proposed in 2011 [42] and elaborated the characteristics of the innate immune system exhibiting immune memory or non-specific effects (NSE) [43]. G.B. Mackaness first proposed that bacterial infections may cause “cross-protection” of other pathogens. This classic form of cross-protection is mediated by lymphocytes, which release IFN-γ, thereby activating bystander macrophages, resulting in a temporarily enhanced innate immune status against secondary infections, and this cross-protection status will quickly disappear once the major pathogens have been eliminated [44]. Contrary to this, Berg et al. found that memory lymphocytes can also mediate longer-term cross-protection as a byproduct of adaptive immunity: CD8+ memory T cells can be activated by cytokines (IL-12 and IL-18) in early stages of infection in an antigen-independent manner, leading to the production of IFN-γ and enhanced response to subsequent infectious agents [45].

Memory T cells that are specific for unrelated pathogens might have roles in protective immunity and immunopathology caused by heterologous infectious agents [46]. Administration of BCG induces a trained phenotype in circulating monocytes with increased capacity on secreting proinflammatory mediators, leading to non-specific protection against unrelated pathogens. BCG vaccination also upregulates the production of proinflammatory cytokines, such as IL-1β and IL-6 by NK cells in response to the stimulation of *M. tuberculosis* and other pathogens. These characteristics of trained immunity indicate innate immune cells (NK, DC and macrophage) to be the alternative target for vaccination in order to generate more effective immune response. BCG, which can live on the skin for up to a few months, not only triggers the memory B and T cells characteristic of mycobacterium, but also stimulates the blood cells for a long time. BCG prevents about 60% of childhood TB cases, and may also enhance the immune to fight off 30% of all known pathogens (including viruses). The world health organization concluded that BCG appeared to reduce overall mortality of children.

Latest research proposed that national differences in COVID-19 impact could be partially explained by the different national BCG childhood vaccination policies. According to their investigation, BCG vaccination reduced the number of national reported COVID-19 cases and outbreaks in countries without universal BCG vaccination are more critical [47]. This result is consistent with our hypothesis, and makes BCG vaccination a potential new tool in the fight against COVID-19.

Immune fitness tends to moderate the intensity of the immune response

Immune cells are able to clear the pathogen without causing a severe inflammatory response, resulting in covert infection or mild to moderate symptoms with short course, which is called immune fitness

[41].The severity of the infection is not necessarily related to the load of infection, but rather to host fitness, the balance between killing the pathogen and tolerance. The low incidence and mild symptoms of COVID-19 in children suggest that the immune system in children (including neonates and infants) is well developed and functional. In the past, the so-called immature infant immune system is actually that the infant immune system has not been exposed to the corresponding antigen and lacks immune memory, not that the immune function is low.

Multiple respiratory tract infections in childhood, among which a large proportion are covert infections, apply the host with opportunities of immune adaptation. At present, many cases of children with atypical symptoms or covert infections had been reported. This phenomenon did not only occur in children. After pathogen screening in close contacts and suspected contacts, a large number of confirmed patients were found with a high proportion of typical patients [3,11,12,19,48,49]. A covert infection, like the effect of vaccination, is the best clinical phenotype for immune fitness. The reason why the lymphocyte function of these patients maintains a moderate response and the immune homeostasis is maintained might be involved in immune fitness [41].

Children may have lots of memory T cells that target various antigens. The study on T cell subsets in children with SARS showed that the decrease of CD4+ and CD8+ T cells was not obvious, and confirmed that the decrease of CD4+ and CD8+ T cells was closely related to the degree of lung lesions. The absorption of inflammatory lesions in the lung in children was relatively mild. At the same time, with the assistance of T cells, B cells accelerated SARS-CoV-specific antibody production in children, leading to higher positive rate than adults.

BCG promotes the production of Th1 phenotype in CD4+ T cells, which secrete high levels of IFN-γ and have activity against intracellular pathogens. Again, it produces CD8+ T cell activation, which mediates the cytotoxic response. However, the TLR signal transduction stimulated by the components of *mycobacteria* induces B cells to secrete IL-10, and thus inhibits the activity of Th1 and Th17, which helps to suppress the autoimmune response.

The macrophages and especially dendritic cells, recognize and phagocytose pathogens, passing the information to the T cells. Immune cells secrete cytokines to induce T cells to differentiate into different functional subsets. Different viral infections cause different T cell responses. In HIV patients, the CD4+ cytotoxic T lymphocytes have a protection role on virus infection [50]. CD45RA+ memory T cells is highly enriched in dengue fever patients [51]. Lymphocytic choriomeningitis virus results in depletion of T follicular helper(Tfh) co-expressed by IL-10 and IL-21, which impaired humoral immunity and viral control [52]. Bacterial infection also stimulates a strong T cell response, such as *Mycobacterium tuberculosis*. Childhood vaccination might have the potential to vary subsets of immune cells and molecular markers, force the host to find a balance between killing pathogens and immune tolerance.

Cross-protection has been paid attention to for a long time

SARS-CoV-2 is 79% similar to SARS-CoV and 55% similar to Middle East respiratory syndrome coronavirus (MERS-CoV). By comparing the sequences of several coronavirus genomes, the homology of non-structural proteins and structural proteins in the coding region was 58% and 43%, respectively. SARS-CoV-specific antibodies were able to prevent SARS-CoV-2 from entering cells, indicating the presence of antibody cross-protection between the two viruses [53].

In a latest long-term follow-up investigation to 2015, the serum specific IgG levels in health care workers participating in the treatment of infected SARS patients for up to 12 years [8]. At the late stage of the epidemic and after the outbreak of SARS, more and more cases of covert infection or asymptomatic infection with specific antibodies were reported [54,55].

Since the incidence of SARS in children was relatively lower, many studies at that time investigated the specific antibody levels of children and speculated that children's innate antibodies contributed to the natural resistance to SARS-CoV. Various surveys published between 2003 and 2005 on this controversial issue [56–59]. A group from Guangzhou held the opinion that the uninfected children had no specific antibodies. They confirmed the 1060 serum sample contained no virus particles by immunofluorescence prior to the serum specific antibody IgG and IgM detection, and only 2 cases of IgM and IgG antibody test result is positive respectively, they considered these few positive results for "false positive" of kits. This was at odds with a previously news report [60]. Researchers detected antibodies in serum collected from non-SARS children hospitalized for other illnesses in Beijing in 2001 and 2003 in order to exclude the possibility of covert infection in 2001. The positive rate was as high as 40% in both epidemic and non-epidemic year. The positive rate decreased gradually with age and down to zero in healthy adult control group, which led to the speculation that there were real differences between adults and children [58]. Another survey found that the antibody positive rate of healthy children and children was 2%, and the antibody positive rate of healthy adults and patients was only 0.2%, which also agreed that there was a significant difference between children and adults [56].

Prevention and control measures for COVID-19

In the current situation of virus outbreak, BCG vaccine, influenza vaccine and pneumonia vaccine may have certain application potential in the prevention and control of COVID-19. Emergency injections could be considered for front-line health workers. However, for high-risk susceptible populations, such as the elderly with basic diseases such as hypertension and diabetes, it is necessary to explore the remedial effect of the planned immune process on their immunity to achieve the immune fitness, so as to improve their own antiviral ability.

The implementation of programmed immunization based on trained immune and immune fitness may benefit the elderly with underlying diseases

We suggest a detailed immunization plan should be set for the elderly, after several years and multiple vaccines inoculated. Different pathogens have different attack effects on immune cells, so it is necessary to map the detailed immune cells against the planned vaccine, so as to achieve precise regulation of immune cells and inflammatory factors. Eleven T and B lymphocyte tests were used to analyze the changes of specific immune cells. Single cell RNA sequencing could demonstrated dynamically change the gene spectrum, host cell antiviral factors, and immune cell subsets that might cause cytokine storms. Finally, an appropriate immunization program was found that resulted in trained immune and immune fitness to protect the elderly population from lethal infection.

New insights can be gained from emergency immunization

Emergency immunization, also known as contingency vaccination (CV), is aiming at protecting vulnerable populations in case of an infectious disease begins to spread or there is a sign of epidemic. It follows the basic theories of vaccination and carry out emergency vaccination on specific populations in epidemic areas to achieve the purpose of preventing epidemic disease. For example, students and staffs must be equipped with contingency vaccination in case of the outbreak of measles in schools. Emergency vaccination is of special significance to curb the spread of infectious diseases.

Unfortunately, SARS-CoV-2-targeted vaccines have not yet been developed, but innovation proposals or hypothesis of new therapies, such as blocking agents of ACE2 receptor or ACE2 immunoadhesin strategy have been reported [61]. Here we assume that some provisional substitute candidates will work. Due to the same pathogenic mechanism [62], adjusted SARS-CoV-targeted vaccines can come in handy timelier. Vaccines for influenza may also make a difference in the

orientation of Th1/2 response. The S-glycoprotein of SARS-CoV-2 has certain similarities with the HA glycoprotein of several influenza viruses. It is not optimal, but the safety of commercial influenza vaccines has been confirmed [63]. Pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine may have some preventive effects on coinfection. Equipping confirmed COVID-19 patients with these vaccinations as emergent prophylaxis may prevent severe illness caused by secondary infection, in the meantime, it may mobilize the host's lymphocyte response to the opposite direction in response to SARS-CoV-2.

Researchers in four countries (the Netherlands, Australia, Germany, and the United Kingdom) will soon begin clinical trials of vaccines for SARS-CoV-2 using unconventional methods. They will test whether a century-old BCG can broadly improve the body's immune system, making it better able to fight SARS-CoV-2, or prevent its symptoms from getting worse. The study will be administered to people at high risk, including doctors and nurses, and to older adults [64].

Acknowledgements

This study was supported by National Natural Science Foundation of China (grant No.: 81771085, 81991502, 81700036 and 81991500), Key Projects of Sichuan Provincial Department of Science and Technology (20SYSX0286), Special Funds for Prevention and Control of COVID-19 of Sichuan University (2020scunCoV10009).

Declaration of Competing Interest

There is no conflict of interest of this manuscript.

References

[1] who-china-joint-mission-on-covid-19-final-report.pdf. https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf.
[2] H. Yu, J. Shao, Y. Guo et al., Data-driven discovery of clinical routes for severity detection in COVID-19 pediatric cases, medRxiv (2020), p. 2020.2003.2009.20032219.
[3] A. Tang, W. Xu, M. Shen et al., A retrospective study of the clinical characteristics of COVID-19 infection in 26 children, medRxiv (2020), p. 2020.2003.2008.20029710.
[4] Ng PC, Leung CW, Chiu WK, Wong SF, Hon EKL. SARS in newborns and children. Biol Neonate 2004;85:293–8.
[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China. N Engl J Med 2019;382(2020):727–33.
[6] Lan L, Xu D, Ye G, et al. Positive RT-PCR test results in patients recovered from COVID-19. JAMA 2020.
[7] Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L, Clinical Characteristics on 25 Discharged Patients with COVID-19 Virus Returning, medRxiv (2020), p. 2020.2003.2006.20031377.
[8] Guo X, Guo Z, Duan C et al., Long-Term Persistence of IgG Antibodies in SARS-CoV Infected Healthcare Workers, medRxiv (2020), p. 2020.2002.2012.20021386.
[9] Wang H-J, Zhang L-L, Tan W-J, et al. Consecutive five-year follow-up analysis of specific IgG antibody of 22 cases of SARS patients after recovery. Bing du xue bao = Chin J Virol 2010;26:295–7.
[10] Yin C-H, Wang C, Wen Y, et al. Prospective 2-year clinical study of patients with positive IgG-antibodies after recovering from severe acute respiratory syndrome. Zhongguo wei zhong bing ji jiu yi xue = Chinese critical care medicine = Zhongguo weizhongbing jijiuyixue 2005;17:740–2.
[11] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
[12] Liu W, Zhang Q, Chen J, et al. Detection of Covid-19 in Children in Early January 2020 in Wuhan, China. N Engl J Med 2020. https://doi.org/10.1056/NEJMc2003717.
[13] Chen Z-M, Fu J-F, Shu Q, et al. Diagnosis and treatment recommendations for pediatric respiratory infection caused by the 2019 novel coronavirus. World J Pediatr 2020. https://doi.org/10.1007/s12519-12020-00345-12515.
[14] Zhu H, Wang L, Fang C, et al. Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. Transl Pediatrics 2020;9:51–60.
[15] Stockman LJ, Massoudi MS, Helfand R, et al. Severe acute respiratory syndrome in children. Pediatr Infect Dis J 2007;26:68–74.
[16] National recommendations for diagnosis and treatment of pneumonia caused by 2019-nCoV (7th trial version, in Chinese) http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf.
[17] Guan W-J, Ni Z-Y, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020. https://doi.org/10.1056/NEJMoa2002032.
[18] 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.
[19] Hu Z, Song C, Xu C et al., Clinical Characteristics of 24 Asymptomatic Infections with COVID-19 Screened among Close Contacts in Nanjing, China, medRxiv (2020), p. 2020.2002.2020.20025619.
[20] T. Novel Coronavirus Pneumonia Emergency Response Epidemiology, The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China, Zhonghua Liu Xing Bing Xue Za Zhi 41 (2020), pp. 145-151.
[21] Chen X, Ling J, Mo P et al., Restoration of leukomonocyte counts is associated with viral clearance in COVID-19 hospitalized patients, medRxiv (2020), p. 2020.2003.2003.20030437.
[22] Jin Y-H, Cai L, Cheng Z-S, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res 2020;7. 4-4.
[23] Varga SM, Sant AJ. Editorial: orchestration of an immune response to respiratory pathogens. Front Immunol 2019;10:690.
[24] Wan S, Yi Q, Fan S et al., Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP), medRxiv (2020), p. 2020.2002.2010.20021832.
[25] Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020. pp. S2213–2600(2220)30076-X.
[26] Liao M, Liu Y, Yuan J et al., The landscape of lung bronchoalveolar immune cells in COVID-19 revealed by single-cell RNA sequencing, medRxiv (2020), p. 2020.2002.2023.20026690.
[27] Procedures and instructions of national immunization programm for children, 2016 edition (in Chinese, 2016 December 29th). http://www.nhc.gov.cn/jkj/s3581/201701/a91fa2f3f9264cc186e1dee4b1f24084.shtml.
[28] Bangham CR, Openshaw PJ, Ball LA, King AM, Wertz GW, Askonas BA. Human and murine cytotoxic T cells specific to respiratory syncytial virus recognize the viral nucleoprotein (N), but not the major glycoprotein (G), expressed by vaccinia virus recombinants. J Immunol (Baltimore Md. : 1950) 1986;137:3973–7.
[29] Raiden S, Sananez I, Remes-Lenicov F, et al. Respiratory syncytial virus (RSV) infects CD4+ T cells: frequency of circulating CD4+ RSV+ T cells as a marker of disease severity in young children. J Infect Dis 2017;215:1049–58.
[30] Schmidt ME, Varga SM. Cytokines and CD8 T cell immunity during respiratory syncytial virus infection, Cytokine (2018), pp. S1043-4666(1018)30297-30297.
[31] Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020.
[32] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020.
[33] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507–13.
[34] Li Y, Wang K, Zhang B, et al. Salivary mycobiome dysbiosis and its potential impact on bacteriome shifts and host immunity in oral lichen planus. Int J Oral Sci 2019;11.
[35] Yu J, Liu W, Chen W, et al. Experts opinion on the laboratory diagnosis and treatment of invasive fungal infection secondary to severe novel coronavirus pneumonia (in Chinese). Chin J Mycol 2020;15:1–5.
[36] Li AM, Ng PC. Severe acute respiratory syndrome (SARS) in neonates and children. Arch Dis Child Fetal Neonatal Ed 2005;90:F461–5.
[37] Gu H, Xie Z, Li T, et al. Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. Sci Rep 2016;6:19840.
[38] Zou Z, Yan Y, Shu Y, et al. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. Nat Commun 2014;5. 3594-3594.
[39] Yang P, Gu H, Zhao Z, et al. Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. Sci Rep 2014;4. 7027-7027.
[40] Li M, Wang J. Prospect of ambroxol in the treatment of COVID-2019 The Chinese Journal of Clinical Pharmacology (in Chinese, Epub ahead of print, 2020 February 2nd) http://kns.cnki.net/kcms/detail/11.2220.R.20200223.2334.002.html, .
[41] Yang X, Zhao X. Associativity from Children with 2019 Novel Coronavirus Disease Journal of Pediatric Pharmacy (in Chinese, Epub ahead of print, 2020 March 10th) http://kns.cnki.net/kcms/detail/50.1156.R.20200309.0900.002.html, (2020).
[42] Netea MG, Quintin J, van der Meer JWM. Trained immunity: a memory for innate host defense. Cell Host Microbe 2011;9:355–61.
[43] Netea MG, Joosten LAB, Latz E, et al. Trained immunity: a program of innate immune memory in health and disease. Science 2016;352.
[44] Mackaness GB. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J Exp Med 1969;129:973–92.
[45] Berg RE, Crossley E, Murray S, Forman J. Memory CD8+ T cells provide innate immune protection against Listeria monocytogenes in the absence of cognate antigen. J Exp Med 2003;198:1583–93.
[46] Welsh RM, Selin LK. No one is naive: the significance of heterologous T-cell immunity. Nat Rev Immunol 2002;2:417–26.
[47] Miller A, Reandelar MJ, Fasciglione K, Roumenova V, Li Y, Otazu GH. Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study, medRxiv (2020), p. 2020.2003.2024.20042937.
[48] 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.
[49] Xu X-W, Wu X-X, Jiang X-G, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ 2020;368. m606-m606.

[50] Rato S, Rausell A, Munoz M, Telenti A, Ciuffi A. Single-cell analysis identifies cellular markers of the HIV permissive cell. PLoS Pathog 2017;13.
[51] Patil VS, Madrigal A, Schmiedel BJ, et al. Precursors of human CD4(+) cytotoxic T lymphocytes identified by single-cell transcriptome analysis. Sci Immunol 2018;3.
[52] Xin G, Zander R, Schauder DM, et al. Single-cell RNA sequencing unveils an IL-10-producing helper subset that sustains humoral immunity during persistent infection. Nat Commun 2018;9.
[53] Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020. S0092-8674(0020)30262-30262.
[54] Si Jh, Tan Jj, Yang G, Tang Lx. Some medical staff positive for serum SARS coronavirus antibody IgG have only mild symptoms, Nan fang yi ke da xue xue bao = Journal of Southern Medical University 26 (2006), pp. 220-221.
[55] Xu H-F, Xu R-H, Xu J-G, et al. Study on the dynamic prevalence of serum antibody against severe acute respiratory syndrome coronavirus in employees from wild animal market in Guangzhou. Zhonghua Liu Xing Bing Xue Za Zhi 2006;27:950–2.
[56] Liu J-H, Ma S-X, Ouyang X-L, et al. Determination and comparison of anti-SARS antibody in children and adults. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2004;12:217–9.
[57] Cao L, Wang T-Y, Chen H-Z, et al. A preliminary investigation on the serological and epidemiological characteristics of severe acute respiratory syndrome in children. Zhonghua Er Ke Za Zhi 2004;42:840–4.
[58] Li L-H, Shi Y-L, Li P, et al. Detection and analysis of SARS coronavirus-specific antibodies in sera from non-SARS children. Di Yi Jun Yi Da Xue Xue Bao 2003;23:1085–7.
[59] Zhao L-Q, Qian Y, Zhu R-N, et al. Serological analysis of SARS Coronavirus in children diagnosed clinically as severe acute respiratory syndrome cases during SARS epidemic in Beijing. Zhonghua Er Ke Za Zhi 2006;44:262–6.
[60] C. National Health Committee of the People's Republic of. New research from scientific research unit shows that SARS antibodies are found in 40% of children (in Chinese). http://www.nhc.gov.cn/wjw/zcjd/201304/706f5fa3371d44228b757aeee1904d14.shtml files/18/706f5fa3371d44228b757aeee1904d14.html.
[61] R.L. Kruse, Therapeutic strategies in an outbreak scenario to treat the novel coronavirus originating in Wuhan, China, F1000Res 9 (2020), pp. 72-72.
[62] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020.
[63] Sun C, Qiu X, Gao Y, Zhang L, Li N, Liu K. Exploring preventive measures for COVID-19 based on the existing virus vaccines Shandong Science (in Chinese, Epub ahead of print, 2020 March 3rd) http://kns.cnki.net/kcms/detail/37.1188.N.20200303.1027.002.html.
[64] Can a century-old TB vaccine steel the immune system against the new coronavirus? | Science | AAAS. https://www.sciencemag.org/news/2020/03/can-century-old-tb-vaccine-steel-immune-system-against-new-coronavirus files/2/can-century-old-tb-vaccine-steel-immune-system-against-new-coronavirus.html.