

Electronic Supplementary Material

Epidemiological, Clinical and Serological Characteristics of Children with Coronavirus Disease 2019 in Wuhan: A Single-centered, Retrospective Study

Dan Luo^{1,2} • Zhi Xia³ • Heng Li³ • Danna Tu³ • Ting Wang¹ • Wei Zhang¹ • Lu Peng¹ • Wenfu Yi¹ • Sai Zhang¹ • Junhua Shu³ • Hui Xu³ • Yong Li³ • Buyun Shi³ • Chengjiao Huang³ • Wen Tang³ • Shuna Xiao³ • Xiaolan Shu³ • Yan Liu¹ • Yuan Zhang¹ • Shan Guo² • Zhi Yu² • Baoxiang Wang² • Yuan Gao² • Qinxue Hu⁴ • Hanzhong Wang¹ • Xiaohui Song⁵ • Hong Mei² • Xiaoqin Zhou³✉ • Zhenhua Zheng¹✉

1. CAS Key Laboratory of Special Pathogens and Biosafety, Center for Emerging Infectious Diseases, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
2. Department of Gastroenterology, Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430015, China
3. Department of Pediatrics, Women and Children's Hospital of Hubei Province, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430070, China
4. State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
5. Department of Obstetrics, Wuhan Maternal and Child Healthcare Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430015, China

Supporting information to DOI: 10.1007/s12250-020-00333-z

Supplementary Materials

Methods

Definitions of Suspected and Confirmed Cases with COVID-19 and Clinical Presentations

1. According to the recommended definitions by the WHO interim guidance and the new coronavirus pneumonia prevention and control program (7th edition) of National Health Commission of the People's Republic of China, a suspected COVID-19 case was defined by the following criteria: (A) patients with acute respiratory illness, but with no other etiology that fully explains the clinical presentation, and a history of travel to or residence in epidemic areas within 14 days prior to symptom onset; (B) patients with any acute respiratory illness, who has been in contact with a confirmed or probable case of COVID-19 disease within 14 days prior to the onset of symptoms; (C) patients with severe acute respiratory infection, who requires hospitalization, and has no other etiology that fully explains the clinical presentation. A confirmed case was defined as a person with a laboratory confirmed infection of the COVID-19 virus by SARS-CoV-2 nucleic acid test, irrespective of the clinical signs and symptoms (WHO 2020; National Health Commission of the People's Republic of China 2020).
2. The incubation period was defined as the time from exposure to the onset of illness, which was estimated among patients who could provide the exact date of close contact with individuals with confirmed or suspected SARS-CoV-2 infection.
3. Fever was defined as having an axillary temperature of 37.5 °C or higher.
4. Lymphocytopenia was defined as having a lymphocyte count of $<1.2 \times 10^9$ cells per liter.

Laboratory testing

SARS-CoV-2 Nucleic Acid Detection

All swab samples were sent to the Hubei Provincial Center for Disease Control and Prevention for detection of SARS-CoV-2 nucleic acid using real-time quantitative polymerase chain reaction (RT-PCR) assay. Extraction of nucleic acids from the swab samples were performed with the commercialized nucleic acid extraction kits.

The following two sequences of SARS-CoV-2 were adopted for the RT-PCR assays. For open reading frame 1 ab fragment, the forward primer was 5'-CCCTGTGGGTTTTACTTAA-3', the reverse primer was 5'-ACGATTGTGCATCAGCTGA-3', and probe was 5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'.

For the N region of SARS-Cov-2 sequence, the forward primer was 5'-GGGGAACTTCTCCTGCTAGAAT-3', the reverse primer was 5'-CAGACATTTTGTCTCAAGCTG-3', and the probe was 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'.

Amplifications were firstly done at 50 °C for 10 min and then at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 55 °C for 40 s.

The cycle threshold (Ct) of less than 37 indicated the SARS-CoV-2 being detected, whereas the Ct of 40 or greater indicated negative findings. A Ct of higher than 37 but lower than 40 was considered susceptible value, and should be subject to re-detecting.

SARS-CoV-2 Antibody Detection

All serum samples were separated by centrifugation at 3,000 ×g for 15 minutes, followed by 56 °C for 30 minutes of inactivation, and then stored at -20 °C until use. In-house anti-SARSr-CoV (SARS-related coronaviruses) IgM and IgG ELISA kits were developed using bat SARSr-CoV Rp3 nucleocapsid protein (NP) as antigen, which shared above 90% amino acid identity to all SARSr-CoV (including SARS-CoV-2), as reported previously (Zhou *et al.* 2020; Zhang *et al.* 2020; Ge *et al.* 2013). For IgM detection, anti-human IgM (μ chain) were coated on ELISA plates and incubated with the diluted serum for 40 minutes at 37 °C, followed by RP3 NP-HRP conjugated (Kyab Biotech Co., Ltd, Wuhan, China). For IgG detection, these ELISA plates were coated with recombinant N protein and incubated with the diluted serum for 60 minutes at 37 °C, followed by the anti-Human IgG-HRP conjugated monoclonal antibody (Kyab Biotech Co., Ltd, Wuhan, China). The optical density (OD) values were read at 450-630 nM.

Antiviral Treatment

1. Current recommended dose and duration of oseltamivir for treatment of influenza (Ward *et al.* 2005; Morioka *et al.* 2010):

	Dose	Duration
Infant (≤1 year)	3-4mg/kg twice daily	5 days
Children (> 1year)		
≤15kg	30mg twice daily	5 days
15-23kg	45mg twice daily	5 days
23-40kg	60mg twice daily	5 days
≥40kg	75mg twice daily	5 days

2. The recommended dose and duration of interferon alpha inhalation is 2-4ug/kg, twice daily for 5-7 days (National Health Commission of the People's Republic of China 2020).

Results

Identification of Patients and Epidemiological Characteristics

Among 56 suspected patients, 14 patients were positive for SARS-CoV-2 nucleic acid (named group NP) and diagnosed as laboratory confirmed cases with COVID-19 in hospital. Among the 42 patients with SARS-CoV-2 nucleic acid negative, 20 cases were SARS-CoV-2 antibody positive (named group AP), with 2 cases of merely IgM positive, 13 merely IgG positive and 5 both IgM and IgG positive. Furthermore, 11 of 13 patients had an unambiguous exposure history to SARS-CoV-2 in 14 days and were scanned with abnormalities in chest CT. The

remaining two patients also presented with typical CT images showing ground-glass opacity and wedge-shaped consolidation near the pleura. Additionally, in group NP, 6 cases were conducted to serological detection, and were SARS-CoV-2 antibody positive. Collectively, a total of 26 patients in both groups were antibody positive. By combining nucleic acid and antibody detection, 14 patients in group NP and 20 patients in group AP were finally identified as the research objects in the present study ([Supplementary Fig. S1](#)).

Radiologic Characteristic

The CT images of a patient presented with the radiological characteristic of COVID-19, which consisted of ground-glass opacity and wedge-shaped patchy shadowing near the pleura in the right middle and lower lung field ([Supplementary Fig. S2](#)).

Reference:

- World Health Organization (WHO) (2020). Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. Available online: [https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-\(ncov\)-infection-is-suspected](https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected). Accessed on 26 August 2020.
- National Health Commission of the People's Republic of China (2020) New coronavirus pneumonia prevention and control program (7th ed) (In Chinese). Available online: <http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml>. Accessed on 26 August 2020.
- Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579: 270-273.
- Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, Wang YY, Xiao GF, Yan B, Shi ZL, Zhou P (2020) Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect* 9: 386-389.
- Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Cramer G, Zhang SY, Wang LF, Daszak P, Shi ZL (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538.
- Ward P, Small I, Smith J, Suter P, Dutkowski R (2005) Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. *J Antimicrob Chemother* 55 Suppl 1, i5-i21.
- Morioka I, Arakawa S, Matsuo M (2010) Clinical features of, treatments and preventions for newborns with pandemic influenza A (H1N1) 2009. *Nihon Rinsho* 68: 1656-1658. (In Japanese)

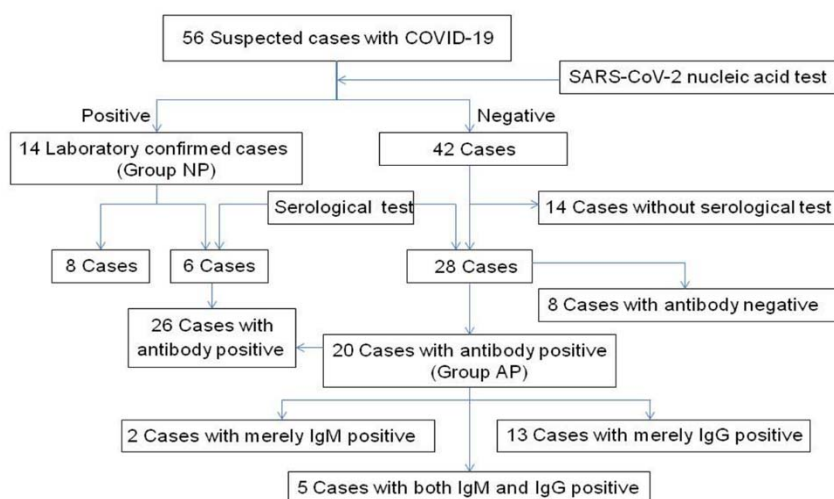


Fig. S1 Retrospective analysis flow diagram.

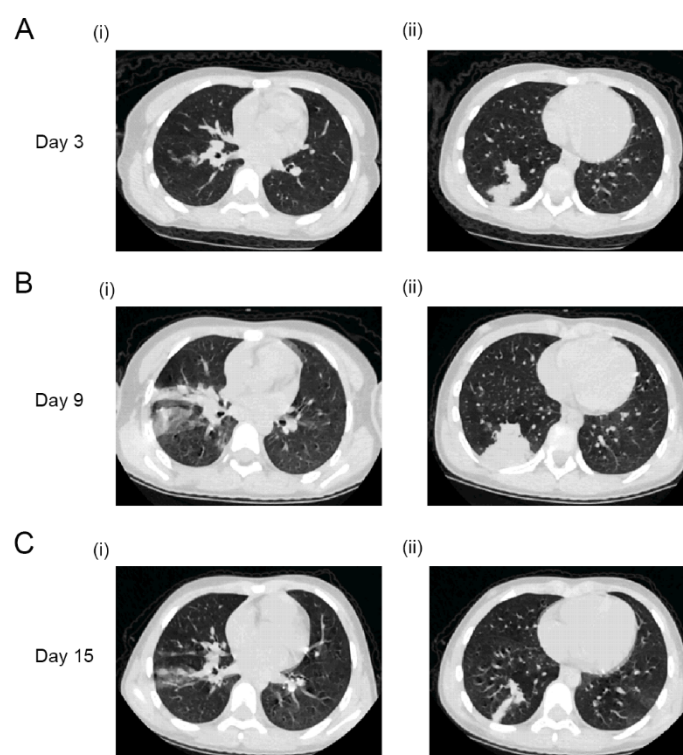


Fig. S2 Chest CT images of a nine-year-old girl with COVID-19, whose SARS-CoV-2 RNA and IgM was both positive and SARS-CoV-2 IgG was negative. (A) Chest CT images obtained on day 3 after onset show a small amount of ground glass opacity in right lung (i) and right lower pneumonia with atelectasis (ii). (B) Chest CT images taken on day 9 after onset show ground glass opacity and local wedge-shaped patchy shadowing in the right middle (i) and right lower lung field, and the atelectasis absorption in right lung is not obvious (ii). (C) Chest CT images taken on day 15 after onset show the partially absorption of ground glass opacity and local patchy shadowing in the right middle (i), and partially re-expansion of the segmental atelectasis in lower lung field after the treatment (ii).