Dynamic surveillance of SARS-CoV-2 shedding and neutralizing antibody in children with COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China and quickly spread globally. In this study, we investigated the characteristics of viral shedding from different sites and the neutralizing antibody (NAb) response during the acute and convalescent phases of nine children with COVID-19. SARS-CoV-2 was detected in their nasopharyngeal swabs (9/9, 100%), stool samples (8/9, 89%), and oropharyngeal swabs (3/9, 33%) but was not detected in their serum and urine samples. The median duration of viral shedding detected in nasopharyngeal swabs, oropharyngeal swabs, and stools was 13, 4, and 43 days respectively, and the maximum duration of viral shedding detected from stools was 46 days after discharge. In children, nasopharyngeal swabs appear to be a more sensitive specimen type for the diagnosis of COVID-19 compared with oropharyngeal swabs. Three of eight patients produced NAb in the acute phase, and NAb were detected in all eight patients with convalescent sera. The results of this study provide valuable information for the diagnosis and surveillance of COVID-19 and development of SARS-CoV-2 vaccines for use in children.

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by the Mann–Whitney U test. Correlations were calculated using the Spearman correlation.

Of the nine patients, five (cases 1–5) patients had only mild upper respiratory tract infection (URTI) manifestations on admission, and the other four (cases 6–9) had chest radiographs showing evidence of pneumonia. The median age of the patients was 85 months (range, 7–139 months), and four patients (44%) were male. Cough (78%), fever (67%), and sore throat (56%) were the major symptoms. Levels of C-reactive protein, procalcitonin, and IL-6 were elevated in 4 (44%), 6 (67%), and 2 (22%) patients respectively, and lymphocyte counts were normal in all patients. No significant differences in the common laboratory results were observed between the URTI and pneumonia patients (Table S1).

The SARS-CoV-2 full genome sequences from four patients (two patients in each of the two groups) were found to be identical or nearly identical to the reference sequence (NC_045512.2). There was one variable nucleotide and one variable amino acid in case 3, seven variable nucleotides and three variable amino acids in case 5, three variable nucleotides and three variable amino acids in case 9, and no variable nucleotides in case 6.

All patients had SARS-CoV-2 shedding in their nasopharyngeal swabs (NSs). The median duration of viral shedding from the day of illness onset to the last positive nasopharyngeal swab collected as part of clinical care was 13 days (range, 6–24 days), and there was no significant difference between URTI and pneumonia patients (p = 0.539). Only three of nine patients had oropharyngeal swabs (OS) that were SARS-CoV-2 RNA-positive for a median duration of four days (range, 3–10 days), and all three had pneumonia (Figure 1). SARS-CoV-2 RNA was not detected in the serum or urine samples.

Except for one patient who was negative for SARS-CoV-2 RNA upon hospital admission, all patients had SARS-CoV-2 shedding in their stools for a median duration of 43 days (range, 28–66 days). All eight of these patients had persistent viral shedding in their stools after discharge, and the median duration from the day of discharge to the day of last positive collected stool at follow-up was 22.5 days (range, 4–46) (Figure 1).

Among the eight patients in acute phase (1–4 days after illness onset), NAb were produced in the sera of three patients (IC50 > 80) (Figure 1). However, viral shedding in the respiratory tract was still persistent in these three patients, and the viral shedding duration after NAb development was as long as 19 days in case 5. NAb were detected in all eight patients with convalescent sera (Figure 1), and the median IC50 was 1,483.9 (range, 307.2–5,925.4) (Table S2). A positive correlation was observed between the duration of viral shedding in stool and the NAb titres of convalescent sera (r = 0.810, p = 0.015), whereas no correlation was observed between the duration of viral shedding in NSs and the NAb titres (r = 0.275, p = 0.509).

Oropharyngeal swabs were used much more frequently than NSs in China during the COVID-19 outbreak because they are more convenient to collect. Here, we conducted consecutive surveillance of SARS-CoV-2 RNA in nine children with COVID-19 after admission and discharge. SARS-CoV-2 was detected in all the NSs and 33% of oropharyngeal swabs, indicating that, in children, NSs appear to be a more sensitive specimen type for COVID-19 diagnosis compared with oropharyngeal swabs. A report from 353 adult patients also demonstrated a higher detection rate in NSs than in oropharyngeal swabs (19.0% vs. 7.6%, respectively) [4]. Thus, choosing NSs as the proper type of upper respiratory tract specimen for molecular diagnosis of COVID-19 is recommended [5,6]. One reason for the higher positive rate of SARS-CoV-2 in NSs is that the respiratory tract is the major route of SARS-CoV-2 transmission. Nasal epithelial cells, including goblet cells and ciliated cells, show the highest expression of SARS-CoV-2 entry receptor ACE2 among cells in the airway, suggesting that the nasal epithelial cells might be the first loci of original infection [7]. Furthermore, it is easier to obtain high-quality specimens from children’s nasopharynx than from their oropharynx, because oropharyngeal sampling often elicits the gag reflex and can make patients, especially children, less compliant.

The median viral shedding duration in paediatric oropharyngeal swabs was only four days, much shorter than that in adults (median, 20 days) [8], probably because paediatric patients had milder disease severity than did adult patients [9]. Here, all patients with positive oropharyngeal swabs showed evidence of pneumonia by chest radiography; however, more cases and evidence are needed to elucidate the relationship between oropharynx virus excretion and disease severity. The observation that virus was still detected in NSs after oropharyngeal swab results turned negative suggests that NSs might be more reliable for surveilling SARS-CoV-2 shedding in the respiratory tract.

Our findings demonstrate a much higher positive rate of virus detection in the stool samples of children (8/9, 89%) than in those of adults (27%–50%) [10,11]. A study conducted in Guangzhou showed eight of ten children with COVID-19 had SARS-CoV-2 rRT-PCR-positive rectal swabs, which is similar to our finding [12]. It is worth noting that SARS-CoV-2 shedding in the stool after discharge lasted for as long as 46 days (Case 8), which is the longest duration reported to date [13]. Live SARS-CoV-2 is present in stool samples [14], but it remains unclear whether transmission can occur via virus-contaminated faeces. The infectivity of SARS-CoV-2-positive faeces needs to be tested by virus culture and animal models. Because the current discharge criteria for COVID-19 are based on
Figure 1. SARS-CoV-2 shedding and NAb response in children with COVID-19 according to the day of illness onset. (NS, nasopharyngeal swab; OS, oropharyngeal swab; N, IC<sub>50</sub> < 80; +, IC<sub>50</sub> = 80–499; 2+, IC<sub>50</sub> = 500–999; 3+, IC<sub>50</sub> = 1000–2000; 4+, IC<sub>50</sub> > 2000).
obtaining a negative viral RNA test on respiratory specimens, we should be cautious when caring for children after discharge who may have persistent viral shedding in their stools. When such children are discharged, physicians should recommend home quarantine and even virus monitoring.

We observed that three of eight patients induced NAbs in the acute phase of SARS-CoV-2 infection. However, viral shedding was persistent after NAb production, indicating that the NAbs generated in the acute phase were insufficient to clear the SARS-CoV-2 quickly and clearance of SARS-CoV-2 may require time and the development of high NAb titres.

A study conducted in 175 adult patients showed that about 30% of recovered patients generated very low NAb titres (IC₅₀ < 500), with 10 patients whose NAb titres were below the limit of detection [15]. In comparison, only one case in our study generated a low NAb titre (IC₅₀: 307.2) in the convalescent phase, and all other patients with convalescent sera produced medium to high NAb titres (IC₅₀ > 500). Together, these findings indicate that children develop a robust NAb response after SARS-CoV-2 infection, and humoral immunity may play a more critical role in the recovery of paediatric patients than in that of adult patients.

The NAb titres in convalescent sera were also observed to have a moderate positive correlation with the duration of viral shedding in stool. The patients with longer durations of viral shedding in stool probably had higher viral loads in the intestine, which might stimulate stronger humoral immune responses against SARS-CoV-2. From another perspective, the high NAb titres during the convalescent period may not effectively shorten the duration of viral shedding in the faeces after recovery. However, given the small sample size of this study, more research is needed to clarify the correlation between NAb production and viral shedding in children with COVID-19.

In conclusion, our study demonstrates the features of SARS-CoV-2 shedding and NAb response in paediatric patients with COVID-19. The results provide valuable information for COVID-19 diagnosis and surveillance in children and for SARS-CoV-2 vaccine development.

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References
[1] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727–733.
[2] World Health Organization. Novel coronavirus (2019-nCoV): situation report-109. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200508-covid-19-sitrep-109.pdf
[3] Xia S, Liu M, Wang C, et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res. 2020;30(4):343–355.
[4] Wang X, Tan L, Wang X, et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 333 patients received tests with both specimens simultaneously. Int J Infect Dis. 2020;94:107–109.
[5] Loeffelholz MJ, Tang Y. Laboratory diagnosis of emerging human coronavirus infections – the state of the art. Emerg Microbes Infec. 2020;9(1):747–756.
[6] Tang Y, Schmitz JE, Persing DH, et al. The laboratory diagnosis of COVID-19 infection: current issues and challenges. J Clin Microbiol. 2020. doi:10.1128/JCM.00512-20 [Epub ahead of print].
[7] Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med. 2020. doi:10.1038/s41591-020-0868-6 [Epub ahead of print].
[8] 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. The Lancet. 2020;395(10229):1054–1062.
[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. doi:10.1093/cid/ciaa198. [Epub ahead of print].
[10] Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA. 2020. doi:10.1001/jama.2020.3204 [Epub ahead of print].
[11] Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect, 2020;9(1):386–389.
[12] 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;26(4):502–505.
[13] Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol Hepatol. 2020;5(5):434–435.
[14] Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020. doi:10.1001/jama.2020.3786 [Epub ahead of print].

[15] Wu F, Wang A, Liu M, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. BioRxiv. 2020 April 20. https://doi.org/10.1101/2020.03.30.20047365.