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.

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Letter to the Editor

Temporal viral loads in respiratory and gastrointestinal tract and serum antibody responses during SARS-CoV-2 infection in an Italian pediatric cohort

To the Editor

SARS-CoV-2 is currently the world's most pressing public health threat and has a significant impact on the lives of people around the world. The viral infection causes a series of respiratory manifestations, including severe respiratory distress, indicating that the virus most likely infects respiratory epithelial cells and spreads mainly via respiratory tract from human to human. Although respiratory symptoms are the hallmark of SARS-CoV-2 infection, gastrointestinal manifestations have also been reported in affected patients, with diarrhea presenting both in adults and children (2%–49.5%), while vomit appears more common in children (6.5%–66.7%) suggesting that the virus may also spread through the gastrointestinal tract [1–4].

To further investigate the dynamic profiles of viral shedding in pediatric SARS-CoV-2 infection, we examined SARS-CoV-2 RNA in rectal swabs and urine by real-time PCR and compared them with nasopharyngeal swabs in a group of 23 Italian children with COVID-19, in parallel with sierology.

According to our hospital guidelines for patients with SARS-CoV-2 infection, the decision to discharge positive patients is based on the resolution of clinical symptoms. The decision to discontinue transmission-based precautions is based on negative results of two respiratory swabs collected at day 14 and 14 + 2 from hospital discharge.

Of the 23 patients, 16 were males and 7 females with ages ranging from 1 month to 15 years. Of note, 5 patients were neonates. Ten had a confirmed contact with a positive familiar case, eight had a contact with a familiar suspected case and in 5 the contact was unknown. Upon admission 20/23 patients had fever, which resulted higher than ≥38 °C in 17/23. Other symptoms included cough, rinorrhea and gastrointestinal manifestations. Fever was absent in 2 patients and one patient was asymptomatic. This latter patient came to our attention because her parents had suspected SARS-CoV-2 infection. Patients 22 and 23 had clinical features of hyperinflammation compatible with a Kawasaki-like disease. Comorbidities were present in 7 patients. Chest X ray performed in most patients showed interstitial pneumonia. Patients 9 and 10 were transferred to PICU for non-invasive ventilation and assisted invasive ventilation respectively due to severe respiratory distress (Supplementary Table 1). Patient 9 had a congenital cardiopathy. All patients had a favourable outcome.

All patients tested positive for SARS-CoV-2 in the respiratory specimens at hospital admission and resulted negative at the outpatient control on day 14, confirmed at ≥24 h apart (data not shown). The study was approved by the local ethics committee in March 2020. Informed written consent was signed by at least one parent and patient's data were de-identified.

In thirteen patients, rectal swabs collected in 2 to 3 occasions during follow up, in an interval period of 14–38 days after hospital discharge, tested negative at all times. Three out of these 13 children had a rectal swab available also at hospital admission which tested negative as well.

In the remaining 10 children, rectal swabs tested positive at least once. For 4 out of these 10 children, rectal swabs were available at the hospital admission and tested positive concomitantly with the positivity of nasopharyngeal swabs. Among them, pt.3 still tested positive at the 12th days from hospital discharge and tested negative on the 30th day; pt. 9 rectal swab still tested positive at 14th, 18th, and at 33th day from hospital discharge; pt.10 rectal swab still tested positive at the 14th day from hospital discharge and tested negative on the 18th and 33th day; pt.21 rectal swab tested negative at the 16th and 18th day from hospital discharge (Fig. 1). The mean time of hospitalization was 7.89 ± 5.65 days.

For the remaining 6 children rectal swabs were not available at hospital admission but at different times during follow up. In 4 out of these 6 patients, rectal swabs tested negative between the 19th-52th day from hospital discharge, whereas in pt. 2 and pt. 19 they still tested positive at the last evaluation after hospital discharge, which occurred at the 36th and 55th day, respectively, (Fig. 1A).

At hospital admission, gastrointestinal symptoms, such as diarrhea and/or vomiting were present in 8 out of the 23 patients; 3 (pts. 5, 10, 21) out of these 8 patients tested positive for rectal swabs at admission.

SARS-CoV- 2 was not detected in any urine specimen collected simultaneously with rectal swabs for all patients (data not shown).

The antibody response of IgG class to SARS-CoV-2, performed on a blood sample from 19 patients, collected at a mean time of 17.39 ± 6.34 days from hospital discharge, was detected in all tested patients. On Western blot (WB), all patients tested positive for nucleocapsid protein (NP), and some for envelop protein (E) as well (Fig. 1B); on Enzyme Linked Immunosorbent Assay) (ELISA) all children tested positive for Spike protein (S) (data not shown).

Recent reports have underlined that stool specimens from patients with COVID-19 may be positive for SARS-CoV-2 [3,5,6]. However, with the exception of two anecdotical Italian infants with SARS-CoV-2 infection who tested positive on rectal swabs [7], no systematic evaluation of fecal and urine transmission has been performed yet in SARS-CoV-2 Italian infected children. Among the 23 children studied, rectal swabs tested positive in 10 (43.5%), even after negativization of the nasopharyngeal swabs. Of note, 4 out of these 10 children were tested concomitantly with nasopharyngeal and rectal swabs during hospital admission and gave positive results in both specimens. Our data confirm previous findings [3,5,6] suggesting that SARS-CoV-2 may have specific tropism for mucosal epithelial cells and that, compared with respiratory specimens, rectal swabs may remain positive longer. Notably none of the urine specimens collected at the same time of rectal swabs tested positive for SARS-CoV-2, suggesting its preferential localization at, and

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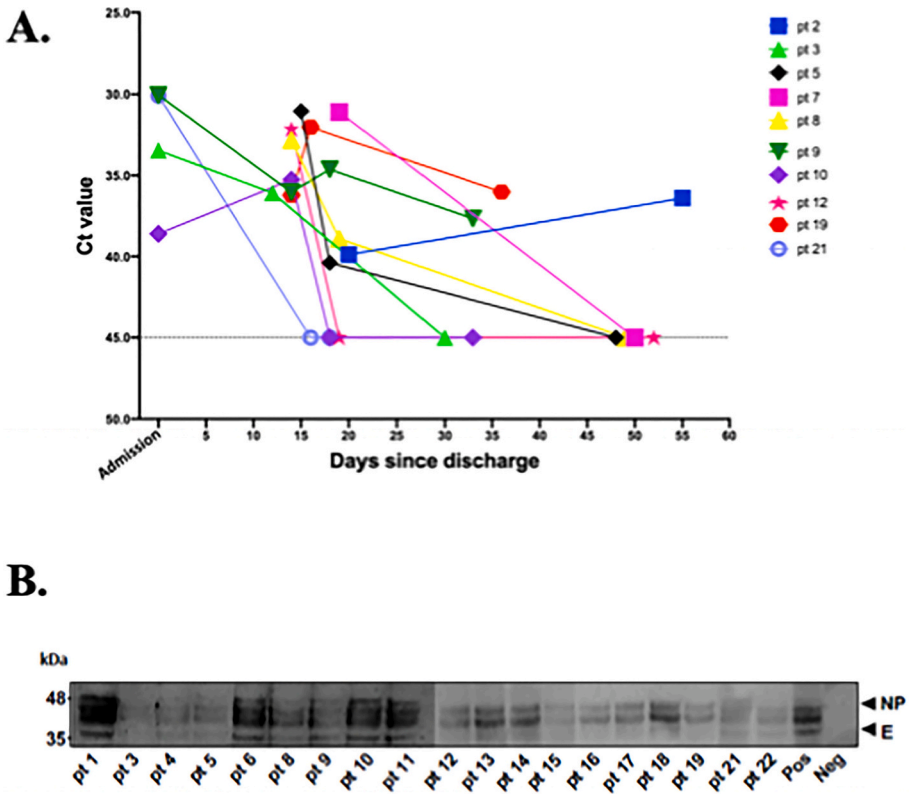

Fig. 1. A. Cronological changes in Ct values of NP gene by real-time RT-PCR. Ct value of NP gene on real-time RT-PCR detected in rectal swabs from 10 infected children. The Ct value is inversely related to viral RNA copy numbers and a value of 45 means the virus is molecularly undetectable. B. Western blot of SARS-CoV-2 infected Vero E6 cells using childrens' plasma, and plasma from positive (Pos) and negative (Neg) donors.

spreading through, mucosal surfaces rather than by systemic route. The positivity of rectal swabs for SARS-CoV-2 raises the issue of the clinical significance of this finding. Our data suggest that the detection of SARS-CoV-2 in rectal swabs doesn't associate necessarily with gastrointestinal symptoms, and that the gastrointestinal symptoms in SARS-CoV-2 infected children are not necessarily related to positive rectal swabs. It is thus becoming evident that a possible viral transmission by non-respiratory tract (i.e. gastrointestinal) may exist and therefore additional caution should be given in the case of management of young children by their caregivers, in order to limit this possibility.

All patients had a favourable outcome. With the exception of 4 children (pts. 1,9,10,22) who had a severe clinical course, including the 2 who required admission to PICU, the remaining had a mild disease. These 4 children had higher mean values of anti-S IgG levels (45.40 ± 12.62) as compared to patients with milder disease (28.52 ± 16.11). Of note, in a recent SARS-CoV-2 macaque model, anti-spike IgG stimulated pulmonary proinflammatory responses and caused acute lung injury [8,9]. In this animal model, the detrimental effect of anti-spike IgG was attributable to the effector wound-healing macrophages, which was mediated via the Fcgamma receptor. It is tempting to speculate that this phenomenon may apply also in humans, but the lack of sequential evaluation of antibody titers over time, doesn't allow to draw any conclusive remarks. Serial antibody responses in adults with different severity of clinical course might help in defining the protective or detrimental role of the antibody response even on the light of possible therapeutic use of convalescent plasma.

In conclusion, our study shows that SARS-CoV-2 may be present in the gastrointestinal tract in pediatric patients even after nasopharyngeal swabs test negative, with evident implications for caregiving measures in order to restrict viral diffusion. On the other hand, it emerges also that sierology may result more reliable than swabs in terms of identifying SARS-CoV-2 infection in children, a finding that has to be confirmed in more numerous cohorts of infected pediatric patients.

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