Title: Dynamic viral SARS-CoV-2 RNA shedding in in children: preliminary data and clinical consideration of Italian regional center

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ABSTRACT

We evaluated SARS-CoV-2-RNA clearance in 22 children . The estimation of positivity at day 14 from symptom onset is 52% for nasopharyngeal swab and 31% for stool swab. These data underline the significance of nasopharyngeal and stool swab for detecting infected children; further studies are needed for transmissibility.

BACKGROUND

The outbreak of severe acute respiratory syndrome caused by a novel coronavirus infection (SARS-CoV-2) has rapidly spread worldwide since its onset in Wuhan throughout December 2019 with the declaration of Public Health Emergency of International Concern by World Health Organization (WHO) on March 11th 2020.

Globally, the proportion of SARS-CoV-2 among children is small compared to the other age groups. A recent review of 72.314 cases by the Chinese Center for Disease Control and Prevention showed only 1% aged 9 years or younger and 1% aged 10 to 19 years [1].

In contrast with adults, children infected by SARS-CoV-2 seem to have milder clinical course and good prognosis [2]. The most common signs and symptoms are cough, pharyngeal erythema, fever, upper respiratory symptoms, fatigue and gastrointestinal symptoms including diarrhea and vomiting [3-4].

At the pandemic onset, children could have constitute a possible source of spreading, even with paucity of symptoms. Few data on viral RNA clearance and route of transmission of SARS-CoV-2, are available [5]. Still a large debate is on oral-fecal transmission and the length of contagiousness. Considering the possible role of "silent" source of infection [6], children primarily need to be investigated on this issue, in order to direct the possible counter actions to face this pandemic.

We present preliminary data on viral RNA clearance of SARS-CoV-2 in a series of 22 children admitted at Bambino Gesù Pediatric Hospital (BGPH) COVID Center.

METHODS

Twenty-two pediatric patients, with infection confirmed by nasopharyngeal swab SARS-COV-2 nucleic acid test, are included. All patients were followed in inpatient setting from March 16th to April 8th 2020 at a COVID center created for the SARS-CoV-2 pandemic in the first week of March 2020. The clinical records were reviewed for collecting demographic information, contact history,

previous history, clinical symptoms and laboratory findings. Microbiological data consisted of RT-PCR for SARS-CoV-2 RNA on nasopharyngeal, conjunctival swab and on stool and urine samples. These tests were repeated every 2-3 days until two consecutive negative results in the absence of new symptoms (See Supplement 1 for SARS-CoV-2 nucleic acid detection). The follow-up was updated to April 12th 2020;13 patients were discharged at this date. The duration of symptoms and of RNA shedding was considered from the illness onset the date of last follow-up for symptoms and to the date of last swab for viral shedding. The Kaplan-Meier method was used to estimate the duration of symptoms and viral RNA shedding for symptomatic patients; swab positive patients were censored at the date of last swab. The study was approved by the local Institutional Review Board.

RESULTS

Twenty-two patients were followed in inpatient setting at the BGPH COVID Center; the sex ratio (F/M) was 7/15 and median age was 84 months (range 8 days- 210 months). The patients' characteristics and symptoms were summarized in **Table 1**. Four patients were asymptomatic. None suffered for immunodeficiency or received any immunosuppressive medication; two patients had an underlining condition (Angelman syndrome, suspected genetic syndrome and Autism). Regarding the four newborns, the tests were performed after the discovery of the positive results of the mother (one case) and a midwife (the other 3 cases): in all cases the mother was positive. Two of the newborns were completely asymptomatic, the other two presented low-grade fever and hyporexia, not initially related with SARS-CoV2 infection. At the last follow-up on April 12th 2020, symptoms regressed in all 18 symptomatic patients, the median symptoms length was 8 days (range 1-21). A family cluster was identified in 19 patients. At diagnosis, stool was positive for SARS-CoV-2 in 15/22 (68%) patients, urine in 1/22 (4.5%) and conjunctival swab in 2/22 patients (9.1%). At last follow-up on April 12th 2020, 13 patients were discharged (median length of stay: 7 days, range 3-15 days): the nasopharyngeal swab persisted positive in 7/13 patients, 54%, (95% CI 25 -

81) and the stool swab persisted positive in 6/9, 67% (95% CI 30-93). The nasopharyngeal swab was negative at a median of 8 days (range: 2-17 days) from the date of symptoms onset and the stool swab was negative at a median of 14 days from the date of symptoms onset (range 10-15 days). The viral RNA shedding on stool and nasopharyngeal swab at diagnosis and discharge are summarized in **Table 1** where the differences in symptoms occurrence according to positive stool swab are summarized. Overall, the estimation of persistence of viral RNA shedding at day 14 from symptom onset is 52% (95% CI 21-76) for nasopharyngeal swab, and 31% (95% CI 5-63) for stool swab (see **Figure 1** and **2**). **Figure 1** and **Figure 2** represented the estimation of symptoms improvement, nasopharyngeal and stool RNA clearance over time in our population. At day 11, estimated proportion of patients with symptoms is 6% (95% CI 4-22%): indeed, 94% of population had no symptoms at day 11.

DISCUSSION

Infants and young children are generally considered at high risk for viral respiratory tract infection as respiratory syncytial virus and influenza virus; the respiratory tract immaturity and immune system contribute to severe viral respiratory disease in this age group. The SARS-CoV-2 epidemiology and clinical course in childhood perplex clinicians, epidemiologists, and scientists.

In our series, the SARS-CoV-2 is confirmed to have lower incidence and milder symptoms [2-4] compared to adults, as was reported also in SARS-CoV and MERS-CoV epidemics [7, 8].

The major concern of our data is fecal RNA shedding reported in 68% of patients independently from gastrointestinal symptoms and with relatively slow RNA clearance. Indeed, about 50% of patients were discharged with positive stool swab; in children who initially tested positive for SARS-COV-2 on feces, the median time to regression of fecal RNA shedding from symptoms onset was 14 days, with an estimated still positive stool swab at day 14 from symptom onset in 31% of patients. For nasopharyngeal swab, 52% of patients were still positive at day 14 from symptom onset; considering the small patient population and incomplete observation, the Kaplan–Meier

method is used for this estimation. Our data are consistent with the pediatric experience reported by Lo at al. [9] who underlined the common gastrointestinal involvement confirmed by fecal RNA shedding in absence of specific symptoms. Moreover, the stool swabs remained positive even when nasopharyngeal swab was negative and a longtime RNA shedding was suggested by our estimation curve in a third of the population as observed by Xu et al. [10] Although both cited reports are on limited pediatric population, the viral RNA shedding from digestive system raise the possibility of fecal–oral transmission. The fecal–oral transmission exists with other respiratory viruses but an evidence of replication-competent virus in fecal swabs is necessary to support the extra pulmonary transmission. In adults, the data about the fecal viral RNA shedding is more consistent [11, 12]; the SARS-CoV-2 RNA and viral nucleocapsid were observed in gut biopsy in intra cellular staining and vital viral load was confirmed in stool samples [13]. The clinical relevance of fecal viral RNA shedding both in terms of infectiousness and transmissibility need to be confirmed. Indeed, as suggested by Yeo et al. [14], further studies should investigate the oral-fecal transmission of SARS-CoV-2 including environmental analysis on sewage water to determine whether the virus remains viable in conditions favorable for transmission. Obviously, the oral-fecal transmission of SARS-CoV-2 is a main concern in pediatric population.

We suggest a close monitoring of SARS-CoV-2 patients after discharge with stool swab added to the nasopharyngeal swab-testing in order to identify a cured child and to clarify the children's role in transmission chain. Obviously, an additional cost for the National Health System has to be considered. The implication of potentially oral-fecal transmission is more notable considering children's specific characteristics especially for no continent children; the viral spread with oral-fecal transmission is well known in school communities, above all in younger children, with consequent implications on social and public health policies. Indeed, our data could suggest a substantial proportion of viral RNA shedding likely occurred after last symptoms. More inclusive criteria for contact tracing should be considered for effective control of the outbreak to capture potential transmission events the days before and following the symptoms.

According to Kelvin [6], we supported the idea that children are susceptible to SARS-CoV-2 infection but are less likely to be symptomatic or to present severe symptoms, raising the option that children could be facilitators of viral transmission. However, the importance of children in transmitting the virus remains uncertain. Probably the pediatric infections partially contributed to "undocumented infections" [15] that explain the rapid geographic spread of SARS-CoV2 with its challenging containment.

Our data suggest the importance of fecal swab along with nasopharyngeal swab for monitoring viral RNA shedding in children, and to clearly identify cured patient. Further studies are needed to clarify the potential infectiousness and the role of viral RNA shedding in pediatric population in sustaining SARS-CoV-2 transmission and its relationship with clinical course.

FOOTNOTE PAGE

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TABLE 1: patient characteristics

SEX RATIO (F/M)		7/15
Age (median mo , range)		84 (8 days- 210 mo)
Symptoms	None	4/22 (18%)
	Fever	15/18 (83%)
	Respiratory symptoms	10/18 (55%)
	Diarrhea and vomiting	7/18 (39%)
	Seizure	3/18 (17%)
Symptoms in positive Stool Swab	Respiratory symptoms	7/15 (47%)
	Diarrhea and vomiting	3/15 (20%)
Symptom Length (median days, range)		8 (2-21)
Nasopharyngeal Swab	Positive at admission	22/22 (100%)
	Positive at discharge	7/13 (54%)
	Positive NS length (median days, range)	8 (2-17)
Stool Swab	Positive at admission	15/22 (68%)
	Positive at discharge	6/13 (46%)
	Persistent Positive at discharge	6/9 (67%)
	Positive SS length (median days, range)	14 (10-14)

Table 1 Legend: mo, months; Symptoms in Stool Swab positive, only patients with stool swab positive were considered and symptoms calculated in this population; NS, nasopharyngeal swab; SS, stool swab; Positive NS or SS length was calculated from symptom onset to the date of negative swab only for patients with negative swab; Positive at discharge was calculated considering only patients discharged; Persistent Positive at discharge were considered patients with SS positive at discharge out of patients with positive SS at admission in hospital.

Figure 1 Legend: Representation of viral RNA shedding from rhino-pharyngeal tract according to Kaplan-Meyer method. RF: rhinopharyngeal. Day 0 represents the onset of symptoms. The asymptomatic patients were excluded from the Kaplan Meier analysis.

Figure 2 Legend: Representation of viral RNA shedding in stools according to Kaplan-Meyer method. Day 0 represents the onset of symptoms. The asymptomatic patients were excluded from the Kaplan Meier analysis.

Figure 1

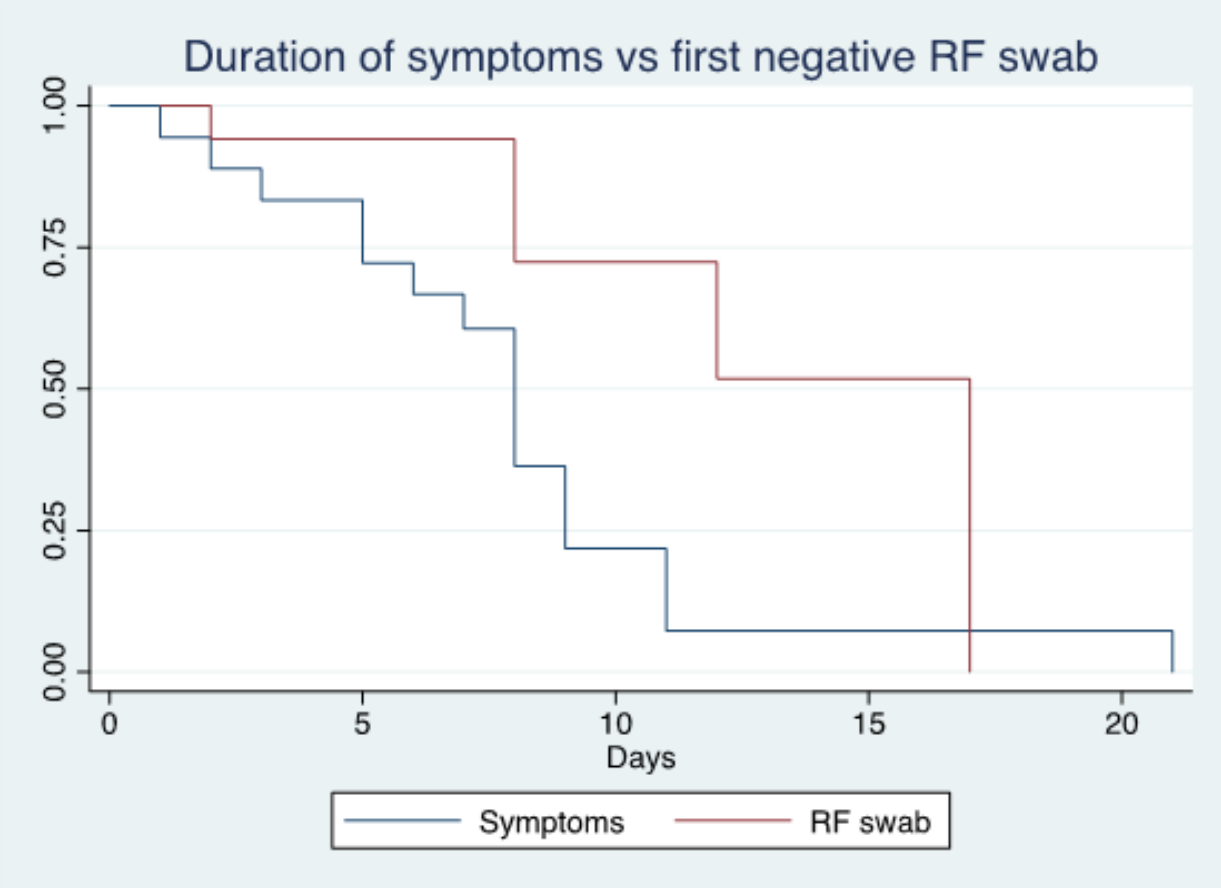

Figure 2