Infectivity of Adult and Pediatric COVID-19 Patients

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Abstract

We report infectivity of adult and pediatric COVID-19 patients in presence of viral shedding and anti-SARS-CoV-2 antibody response

A total of 408 consequent samples from eleven adult and five pediatric patients with SARS-CoV-2 infection were included. The samples every second day from saliva, nasopharynx, feces, serum, urine, tear were studied by RT-PCR and viral culture. Anti-SARS-CoV-2 antibodies were measured.

The median duration of RNA shedding in all specimens was 7(2-15) days in adults and 5(3-19) days in children. The median duration from onset of symptoms to admission was three days.The viral RNA was positive in 44.7 % of the nasopharynx and 37.6% of saliva samples up 16 days in adults and 19 days in chldren. The latest viral culture positivity was detected on day 8 of symptoms in nasopharynx. The viral RNA was found in 6.1% of feces, 4.4% ofserum, 4.3 % of tear, 2.9% of urine. The earliest seroconversion was the 7^{th} day for adults and 8^{th} day for children. Atthe 14^{th} day, total antibody positivity was 78% in adults, and 80% in children. After seroconversion, the viral RNA was still detected in the nasopharynx and saliva of three patients, however, the infectious virus was not present.

Earlier hospital admission could be associated with shorter SARS-CoV-2 RNA shedding. The infectivity of patient is very low after 8 days of symptoms. The risk of fecal-oral transmission is very low, and strict hand hygiene measures could be preventive. The positive antibody test result could be used as a discharge criterion.

Introduction

The epidemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in Wuhan and has spread worldwide(1). In SARS-CoV-2 infection, detailing the dynamics of the viral shedding is critical for describing the risk of transmission (2-4). Understanding the risk of transmission is necessary for the management of the cases regarding the timing of discharge or de-escalation of infection control measures (3, 5). The antibody response with viral shedding will provide a broader perspective on the course of the infection process.

Detection of viral RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) is recommended for COVID-19 diagnosis and viral RNA test results are used for decision of patient discharge and infection prevention and control precautions (6). However, viral RNA positivity does not indicate the presence of infectious viral particles, and there are limited studies showing live viruses in clinical samples (2, 7, 8). The live virus was detected usually in the nasopharynx, tear, and saliva (7). Besides, few studies reported the live virus in feces, urine of COVİD patients (2, 9, 10).

The dynamics of the SARS CoV-2 infection differ in adults and children. Milder symptoms with prolonged viral RNA shedding may lead children to be considered as a silent source of transmission (11). To clarify the risk of transmission, testing of live viruses in pediatric patients needs to be investigated.

By this study we described the features of shedding of the viral RNA and live virus in nasopharyngeal, saliva, tear, urine, blood, and feces in both adult and children groups, including antibody response together with their clinical and laboratory findings. We aimed to present data in a prospective cohort for the clinical practice of diagnosis and infection control measures.

Methods

Study population

The patients diagnosed with laboratory-confirmed SARS-CoV-2 infection in Koc University Hospital between March 2020 and June 2020 were included in the study. The cases were classified as mild, moderate, severe, or critical (if exist) according to the WHO Interim Guidance of Clinical Management of COVID-19 (27 May 2020). (12). Written informed consent was obtained from each enrolled patient or parents. Patients who were followed as an outpatient and those discharged were followed up by phone calls until the 14^{th} day of admission and invited to the hospital to give clinical samples on previously planned days. Exclusion criteria were patients with the indefinite time of illness onset.

Clinical classification, RNA testing results, demographic, clinical, laboratory, radiologic, and therapeutic information of the patients were obtained from electronic medical records. Laboratory results included routine blood biochemistry, complete blood count, lymphocyte count, interleukin-6, C-reactive protein. Chest computed tomography was obtained on admission. The dates of illness onset were also recorded at admission. While mild and some moderate cases were given hydroxychloroquine (HQ), more severe patients had been given favipiravir, and tocilizumab when needed, in addition to supportive therapy according to the national guide. Institutional Review Board of Koç University Committee on Human Research approved the study.

Serial samples (nasopharyngeal swabs, saliva, urine, tears, stool, and serum) from all patients were collected every other day within the first week of their diagnosis, and also at the 10, 14, 21 days. The samples were transferred within one hour and stored in -80 °C.

Viral nucleic acid detection

RNA from nasopharyngeal, urine, saliva, serum, tear, and semen samples were extracted manually using QIAamp Viral RNA Kit (Qiagen) with Qiacube HT (Qiagen) as instructed by the manufacturer. RNA isolation from fecal samples was done with the Quick-RNA™ Fecal/Soil Microbe Microprep Kit (ZYMO Research) following the manufacturer's instructions. Quantitative reverse transcription PCR (qRT-PCR) was conducted with primers and Taqman probes targeting nucleocapsid N1 and RNA dependent RNA polymerase (RdRp) genes using Light Cycler 480 Probe Master (Roche) in a LightCycler 480 Real-Time PCR System (Roche) (13). Ct values of >40.0 were considered negative.

Viral Culture

SARS-CoV-2 RT-PCR positive specimens were cultured in the biosafety level-3 (BSL-3) laboratory to detect the live virus. Briefly, 2.5 x 10^5 Vero-CCL81 cells/ml were seeded in 96 well tissue culture plate in Dulbecco's Modified Eagle Medium (DMEM) supplemented with antibiotic/antimycotic (GIBCO) and heat-inactivated fetal bovine serum (5% FBS). When the cells reach 90% confluency, 100 ml of 2-fold serially diluted stool, urine, saliva, serum, nasopharyngeal swab, and tear samples were inoculated. At 7 days post-infection, 500 µl of culture supernatants were transferred on the confluent monolayers of Vero-CCL81 cells in 6 well plates. The cytopathic effect was examined daily up to 5 days; RT-PCR was performed from culture supernatants that induced visible CPE under the light microscope(14).

Antibody Detection

In serum samples of the patients, anti-SARS-Cov-2 total immunoglobulins directed toward nucleocapsid protein of virus were measured with the Electro-chemiluminescence immunoassay (ECLIA) method using Roche Elecsys Anti-SARS-CoV-2 assay via Roche Cobas-6000 analyzer (Roche, Mannheim, Germany). The CV% value for the intermediate precision in positive samples was; 2.3-6.5%. The positivity was defined as a cut-off index (ratio) of ≥1.0.

We summarized and presented data by using STATA 16v (USA).

Results

In total, 11 adult and five children patients with mild, moderate, or severe COVID-19 were included. In adults, the median age was 65 years (24-91) and 64% was female. In children, the median age was 10.2 (3-17) and 60% was female. The median duration from onset of symptoms to admission was three days.

In the adult group, three patients (27.2%) had severe, five (45.4%) had moderate, and three (27.2%) had mild disease. Eight out of 11 (73%) adults had pneumonia, and two (20%) were transferred to ICU. Two children (3 months old and 8 years old) had a mild infection without any specific treatment. Two children (14 and 17 years old), who had a moderate infection with pneumonia were hospitalized. A 9 years old child who had been hospitalized for suspected juvenile idiopathic arthritis (JIA), and diagnosed with mild COVID-19, based on lack of lung involvoment. Macrophage activation syndrome (MAS) was diagnosed on the day of SARS-CoV-2 positivity based on the presence of hemophagocytosis in the bone marrow. This patient was followed up in ICU.

Three children and seven (44%) adults received hydroxychloroquine. Among adults, six (55%) received favipiravir with a median duration of 5 days, and two received tocilizumab. On admission, 6/11 (55%) adults had lymphopenia (<1000 × 109/L). The fatality of one adult patient (91 years old) out of three (27.2%) was attributed to COVID-19. The clinical and demographic features were presented in table 1.

In total, 408 samples from 11 adults and 5 children were studied. In adults SARS-CoV-2 RNA was detected in 41.8% (23/55) of nasopharyngeal, 37.5% (21/56) of saliva, 5.6% (3/53) of feces, 5.3% (3/56) of tear, 5.5% (3/54) of serum, 3.5% (2/56) of urine samples, none of the semen samples were positive. In

children, 58.3% (7/12) of nasopharyngeal, 38.46% (5/13) of saliva, 8.33% (1/12) of feces samples were positive. None of the urine, tear, and serum was positive (Table 2).

In all samples, the median duration of SARS-CoV-2 RNA shedding was 7 (2-15) days in adults and 5 (3-19) days in children. Nasopharyngeal and saliva samples, or both of them were found to be positive within the five days of symptom onset in all screened patients. In nasopharyngeal samples from adults, the latest viral RNA positivity was seen on day 15 and in children on day five. In saliva, the latest viral RNA detection time was the day on 10 in adults and day 19 in children. In serum samples from adults, viral RNA was detected within five days of symptoms and in urine within seven days. No viral RNA was seen in the serum and urine of the children. In feces from adults, viral RNA was first detected on day 3 and the latest RNA positivity was detected on day 14 of the symptoms. In the children group, one feces sample out of 12 (day 10 of the symptoms) was found to be positive for viral RNA. (Figure 1a and 1b).

In viral culture studies, SARS-CoV-2 was grown in 7 of the 23 nasopharyngeal samples (30.4%) of adult patients, who had PCR positivity. No viral growth was detected from the sites other than nasopharyngeal ones, despite their low Ct values in RT-PCR. The median duration of infectious virus shedding in nasopharynx was 4 days, and the latest infectious virus isolation time was day 8 after the onset of the symptoms. After 8 days of onset of symptoms, there was no culture positivity. The culture was positive in one clinically severe patient despite three days of favipiravir use.

The earliest seroconversion was detected on day 7 in adults and day 8 in children. The total antibody was detected in 8 out of 11 (78%) of adults and 4 out of 5 (80%) of children within the 14thday symptom onset. In 9 (89%) of adults, antibody positivity was seen at the 29thday of symptom onset. Two adult patients died before seroconversion in day 8 and day 11 of onset of symptoms. After antibody production, the viral RNA was still detected in the nasopharynx of one severe adult patient and saliva of one mild pediatric patient however live virus did not exist in these samples (supplement). In one 74-year-old female patient, who had a moderate condition with intracranial hemorrhage and diabetes, no SARS-CoV-2 antibody was detected on her last sample on the 16th day of onset of symptoms. One pediatric patient in the moderate condition who was diagnosed as MAS did not develop an antibody response at the 29^{th} day onset of symptoms.

Discussion

We report the dynamics of infectious SARS-CoV-2 viral shedding in adult and pediatric patients in mild, moderate, and severe clinical conditions. We assessed viral shedding and infectivity in the presence of anti-SARS-CoV-2 antibody response. This information is critical to creating algorithms for diagnosis, prevention, and control precautions during the hospital stay and after discharge.

In our study, the median duration of SARS-CoV-2 RNA shedding was 7 days in adults, and 5 days in children. The longest duration of viral RNA shedding was 19 days in a saliva sample of a child. In our study, the median duration from onset of symptoms to admission was three days. In other studies, the

median viral RNA shedding time was reported as 17 days (15, 16). The prolonged shedding time was reported to be associated with high body temperature on admission, male sex, mechanical ventilation, delayed hospital admission, and hospital length of stay (15, 16). Being in parallel with these findings, we can explain our short viral RNA shedding time with the earlier hospital admission which provided effective and early case management with favipiravir and hydroxychloroquine, supportive treatment. The culture was positive in one clinically severe patient despite three days of favipiravir use.

In viral culture studies**,** the duration of infectious virus shedding was between 2-8 days after onset of the symptoms. Our study included mild, moderate, and severe cases. Our results were concordant with the findings that were limited with adult mild cases reported by Wölfel et al. that could not detect live virus after 8 days of the symptoms (3). Another study reported similar results without disease severity assessment (7).

In our study, RT-PCR positivity was obtained in all nasopharynx and/or saliva samples within five days of infection, and Ct values of nasopharyngeal and saliva RT-PCR tests increased after the first week of the disease. In recent reports, SARS-CoV-2 viral RNA was detected in saliva samples with higher viral loads than nasopharyngeal samples, and saliva was questioned for its role in the fast-spreading nature of the epidemic (8, 17, 18). We found viral shedding among 37.5% of the adult, and 38.4% pediatric saliva samples. In one patient (41 years old male), the virus was detected only in the saliva sample on admission, all other samples were negative. In our study, the latest saliva PCR positivity after the onset of symptoms was on day 10 in adults and day 19 in a pediatric patient with MAS. In another study, the viral RNA shedding in saliva was reported to be positive within 11 days (8). We did not find live virus in viral cultures of saliva samples. Saliva viral culture positivity was reported in previous studies (19). Based on these findings, we suggest that saliva could be a good diagnostic specimen, and the risk of virus transmission by saliva is lower than nasopharyngeal secretions.

In our patients, two adults with moderate and one child with mild clinical manifestations experienced diarrhea. None of these patient's fecal samples were found to be positive for SARS-CoV-2 with RT-PCR. The prolonged shedding of viral RNA in feces raises concerns about the timing of discontinuing precautions during the hospital stay or after the discharge of the patients. These concerns are more important for children especially those who do not have toilet training or have poorer hand hygiene. In our study we detected viral RNA in feces of 3 adults at the 3, 7, and 14^{th} days after onset of symptoms, and 10^{th} day of the pediatric patient. In a study, the viral RNA was tested positive in feces of an adult patient 47 days after onset of the symptoms (20). Children show longer fecal shedding compared to adults, in some cases shedding over 70 days was reported (21-23). However, the presence of viral RNA in fecal specimens could not be considered as evidence of replicating live virus and do not confirm the potential for fecal-oral transmission. In our study, the viral culture of RT-PCR positive fecal specimens yielded no viral growth. Two studies reported the presence of the live virus in the stool samples (2, 24). To date, however, there have been no published reports of transmission of SARS-CoV-2 through feces. These results suggested that the risk of fecal-oral transmission is very low, and proper hand hygiene with no

extra precautions for prevention of fecal transmission could be appropriate during the hospital stay or after discharge.

Although there is a low prevalence of SARS-CoV-2 in tears, exposure to tear may increase the risk for transmission to health care workers especially ophthalmologists. We found viral shedding in 3 out of 56 (5.3 %) tear samples in adults and one sample in children. We did not find an infectious virus in tear samples. Infectious virus was reported in an ocular swab specimen of a patient with conjunctivitis (10). The prevalence of shedding of the viral RNA in urine samples is very low (2, 6) and live virus in urine samples was detected in only one case report (9). We detected viral RNA in two urine samples of a patient with mild symptoms, however, live viruses did not exist in these samples. Based on our results, the risk of transmission through urine is very low.

The presence of viral RNA detection in serum of COVID-19 patients was reported; however, SARS-CoV-2 could not be cultured from serum (25). In accordance with the previous reports, we detected SARS-CoV-2 RNA 3 out of 54 (5.5 %) serum samples in 2 adults without the viral growth in cell culture and none of the serum samples were positive in children.

Serological diagnosis is very important especially for patients with low viral load and could not be diagnosed by RT-PCR tests. But, the timing of antibody tests is critical for an accurate diagnosis. In our study, the rates of total SARS-CoV-2 antibody were reached 88.8 % (8/9) in adults and 80% (4/5) in pediatric patients until the 14^{th} day of symptom onset. Similar to our findings, Zhoa et al observed that total antibody positivity began to increase from the first week to 15 days of symptom onset (26). In another study conducted with 18 patients, positive antibody results were obtained at the end of the first week of disease (27). In our study, the delayed antibody response was observed in two patients with comorbidities (diabetes and juvenile idiopathic arthritis). Studies pointed out that delayed antibody response was associated with low viral load or impairment of adaptive immune response (28, 29). In our two patients (an adult and a pediatric case) viral RNA shedding lasted after seroconversion. The lack of infectious viruses in all samples after seroconversion suggested to us that a positive antibody test result could be used as a discharge criterion.

The strength of our study is having clinical cases, molecular, and viral culture studies in the same institute. Examining the patients in our institute shortened transportation time to the laboratory, all collected samples were processed and stored within one hour without a loss in infectivity. Therefore, we were able to isolate the live virus in samples with high Ct values. The number of samples per patient was 25 on average. Asymptomatic and critical patients were not studied. Fatal cases were not excluded. Viral dynamics at different sites should be further explored in larger populations of COVID-19 patients.

In conclusion, earlier hospital admission could be associated with shorter SARS-CoV-2 RNA shedding. Salivacoıuld be a good diagnostic specimen. The risk of fecal-oral transmission is very low, and strict hand hygiene measures could be preventive with no extra precautions. Positive antibody test result could be used as a discharge criterion, based on the lack of infectious virus in all samples after seroconversion.

Declarations

Competing interests: The authors declare no competing interests.

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Figures

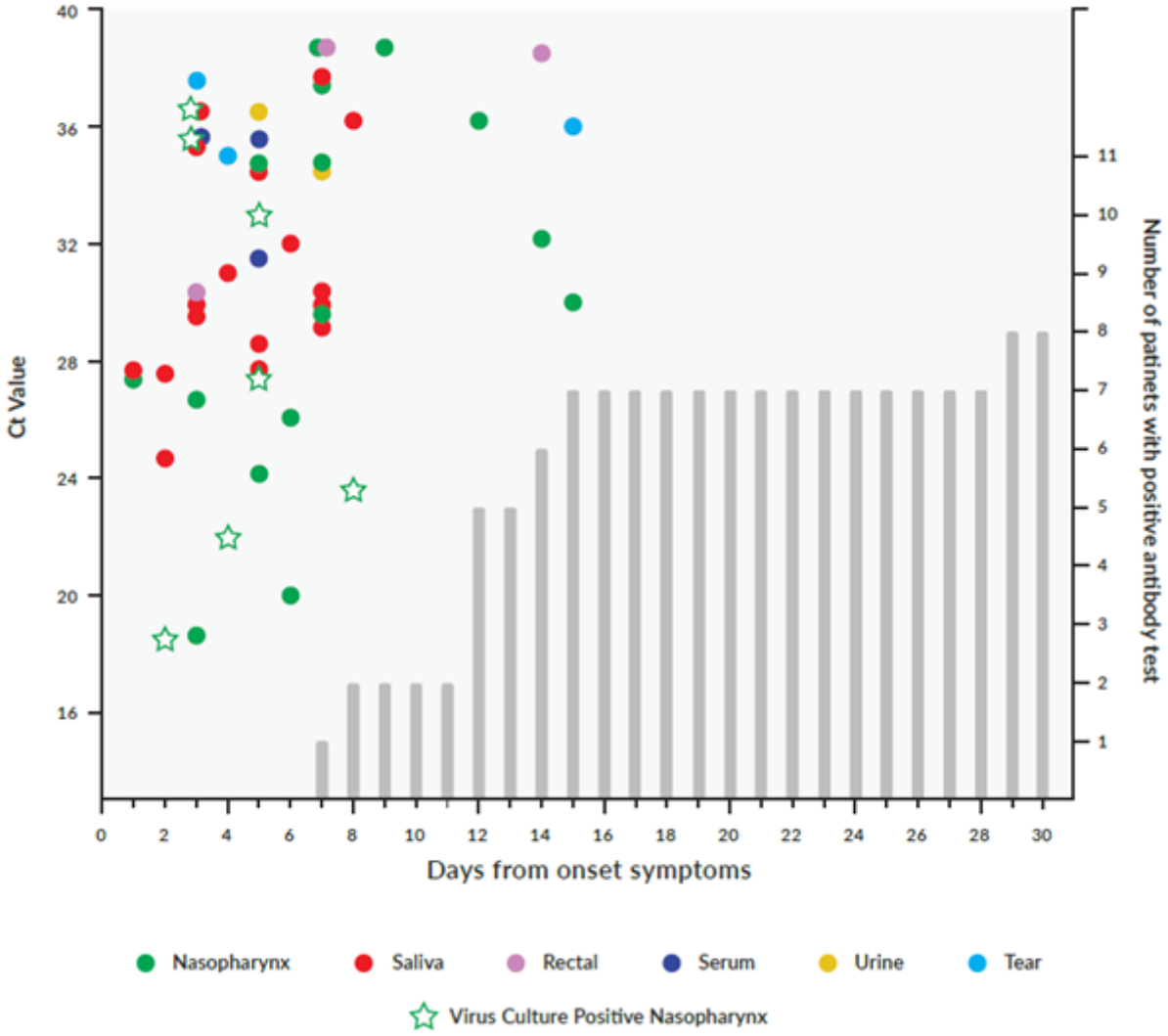

Figure 1

Viral shedding dynamics of adult and children patients.

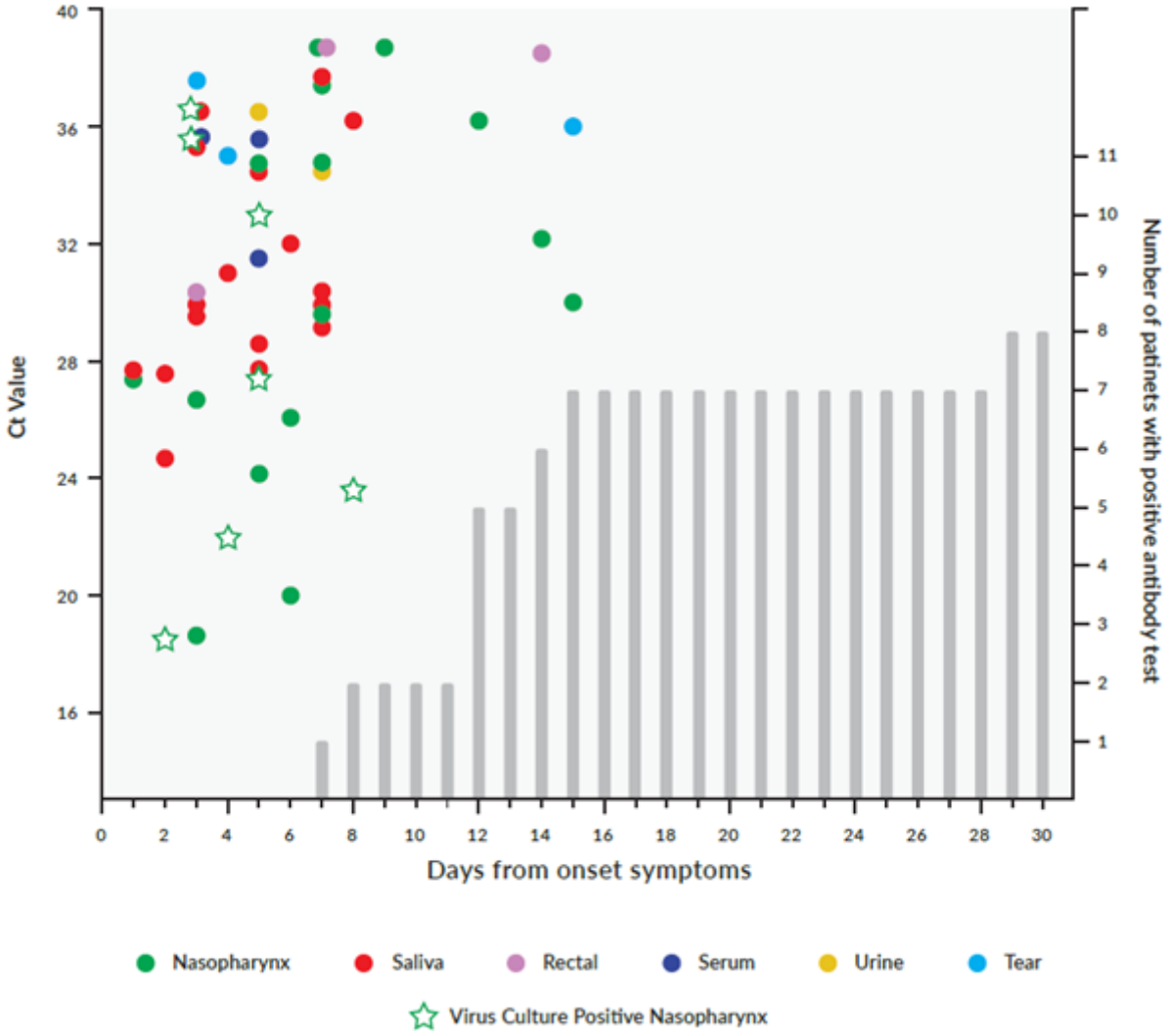

Figure 1

Viral shedding dynamics of adult and children patients.

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