Living SARS-CoV-2 in feces suggesting possible fecal-oral contamination

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Abstract

After we observed a COVID-19 epidemic cluster associated with ruptured sewage pipe, we inoculated 106 stool samples from 46 patients to search for living SARS-CoV-2 and could isolate 2 strains. These 2 isolates were obtained from a unique patient with kidney transplantation under immunosuppressive therapy who was admitted for severe diarrhea. This report emphasizes that immune-compromised patients have probably massive multiplication of virus in the gastrointestinal tract and confirms possible fecal-oral transmission of SARS-CoV-2.

Introduction

In Wuhan, China, a group of patients was diagnosed with a severe respiratory disease of unknown origin, in December 2019. The pathogen responsible for this disease was identified as a new strain of coronavirus, called Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2), and the associated disease was named "COronaVIrus 2019 Disease (COVID-19) [1]. SARS-CoV-2 is considered as acquired through the respiratory tract after inhalation of particles or contact of face mucosa with contaminated hands. This is the reason the major recommendations to avoid infection are of wearing masks and washing hands frequently. However, viral RNA has been detected in stool samples of people infected with the virus. These patients, who had confirmed infection with SARS-CoV-2, presented gastrointestinal symptoms such as diarrhea and vomiting. These observations revealed the involvement of the gastrointestinal tract in the infection [2]. Some reports have shown that gastrointestinal infections can occur before respiratory symptoms. In about 10% of people infected with the virus, diarrhea and nausea occurred 1 to 2 days before the occurrence of fever and respiratory symptoms [3,4]. Finally, we could observe that SARS-CoV-2 can grow in Coco-2 cells, polarized cells derivate from colorectal cancer [5]. In a recent work performed in our institute we identified a cluster of cases associated to a specific clone called genotype Marseille 1 [6]. The index case was imported from Tunisia and the first cases thereafter diagnosed associated to ships connecting North Africa to Marseille, in travelers, but also several in crew members exposed to a ruptured sewage pipe. We thus raised the possibility of fecal-oral transmission. Indeed, the reports of viable SARS-CoV-2 in stool are scarce as three studies only reported the presence of viable virus in stool samples [7–9]. These studies showed positive relationship between high viral load in stools with isolation of the virus from the stools. In the present work, all SARS-CoV-2 PCR-positive samples from stools obtained in our laboratory were inoculated to evaluate the presence of viable virus.

Materials and methods

From March 4, 2020 to April 29, 2020, 128 stool samples (0.2 g in 1ml of buffer, Sigma Virocult®, Elitech, Puteaux, France) from 54 patients were tested positive for SARS-CoV-2 by PCR targeting E gene [10]. Of these, 106 frozen samples from 46 patients and conserved at -80◦C were available for viral isolation. After thawing, 500 µL diluted sample was mixed with 150µl of HBSS buffer and then filtered using a 0.22-µm pore-sized centrifugal filter (Merck Millipore, Darmstadt, Germany). Four wells of Vero E6 cells were each inoculated with 50µl of the filtrate as previously described [11]. The unique modification to the original protocol was that after the first week of subculture, instead of two blind sub-cultures each week we performed 5. Once a cytopathic effect was detected in the well, the content of the well was collected. 600 µL was frozen to conserve the virus and 200µl was used to perform the SARS-CoV-2 qPCR for confirmation of presumptive identification then genome sequencing [12].

Results

Four weeks after the inoculation, thus at the third subculture, two samples showed cytopathic effects that appeared as a group of rounded cells. All other inoculations remained negative after the 5th sub-culture. RT-PCR performed on the two supernatants confirmed that cytopathic effect was due to growing SARS-CoV-2. Genomic analysis showed that the two strains were from the different genotypes 20A/25563T-1b and 20B-1A respectively [13]. These 2 stool samples at a PCR cycle threshold (Ct) of 33.2 and 33.4 respectively with live SARS-CoV-2, were collected on April 14th and 15th from the same patient. This patient was a 62-year-old man who was kidney transplanted 21 years ago. He had also diabetes, hypertension, and overweight. He consulted in the emergency department on April 13th because since ten days, he presented asthenia, anorexia, diarrhea and weight loss, without dyspnea (Figure 1). COVID-19 pneumonia was diagnosed on chest CT that showed formal

criteria. SARS-CoV-2 PCR that was performed on nasopharyngeal swab 4 times on April 13th and 14th was negative. It was positive once on nasopharyngeal swab on April 15th at a Ct of 33.5. Culture was negative for this swab but direct amplification and sequencing on sample allowed to determine it was a 20A/25563T-1b genotype. Laboratory findings revealed acute kidney injury and mild inflammation. Maintenance immunosuppressive treatment consisted in tacrolimus 6.5 mg/day and prednisone 5 mg/day. Treatment with azithromycin was given for 5 days, hydroxychloroquine for 10 days and ceftriaxone for 7 days from April 14th. The dose of tacrolimus was temporarily divided by 2. Diarrhea ceased on April 15th. Acute functional renal failure secondary to diarrhea corrected after refilling and discontinuing diuretics and ACE inhibitors. C reactive protein normalized on April 18th. Stool and nasopharyngeal SARS-CoV-2 PCR were negative on April 28th.

Discussion

Until now, rare reports have shown the presence of viable SARS-CoV-2 in stool in spite common detection of viral RNA [7–9]. The isolation or detection of viruses with two different genotypes in a same patient is curious but not unique [14]. Our observation confirms that viral excretion from the digestive tract can last longer than that from the respiratory tract, since fecal samples remained positive for SARS-CoV-2 RNA for approximately 5 weeks after respiratory tract samples became negative for SARS-CoV-2 RNA [15]. Interestingly, our patient had low viral excretion in upper respiratory sample, probably because these samples were sampled late in the evolution. The fact that 2 stool samples with a viral load comparable to that of culture negative naso-pharyngeal sample suggest that there was continued viral multiplication in the digestive tract. But we cannot exclude that viral load in stool could be higher but seem lower due to PCR inhibitors present is stool. Whatever, in a large nationwide French cohort consisting of 279 kidney transplant recipients with Covid-19 [16], a 22.8% 30-day mortality rate was reported and diarrhea was the third most frequent symptom on

admission (43.5%) after fever (80%) and cough (63.6%). Gastrointestinal symptoms were significantly more frequent than that previously reported in general population studies conducted both in China (3−5%) [17,18], and in the USA (24%) [19]. These data taken together highlight the importance of testing for the presence of SARS-CoV-2 in kidney transplant recipients, in case of gastroenteritis, especially in the stools. SARS-CoV-2 is mainly transmitted through the respiratory tract, but gastrointestinal symptoms such as diarrhea and vomiting developed by some patients followed by RT-PCR detection then culture as in the present work have shown that the virus can survive in the digestive tract [20]. As previous studies have shown that human coronaviruses, such as SARS-CoV and Middle Eastern Respiratory Syndrome (MERS-CoV), may be transmitted through fecal-oral way [20], the possibility of such route on infection is questionable for SARS-CoV-2. The presence of viral RNA in wastewater samples collected from hospital, in raw wastewater and in wastewater sample after secondary treatment as the presence of viable virus in stool confirms the possibility of fecal-oral transmission.

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Ethical approval : The protocol was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (N°: 2020-01)

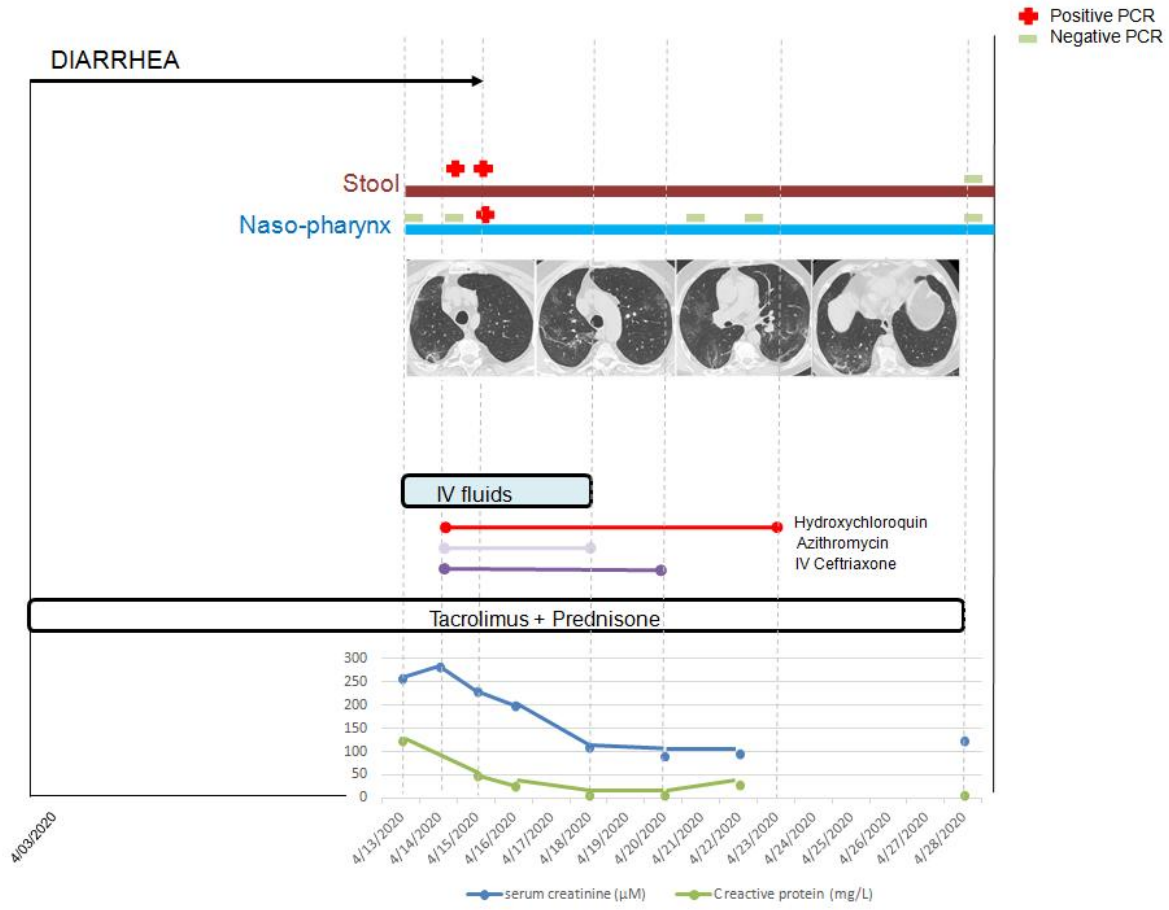
Informed consent: All subjects provided informed consent in accordance with the Declaration of Helsinki.

Figures titles and notes

Figure 1. Clinical, biological, virological and treatment timeline during the course of COVID-19

IV: intravenous

Gastroenteritis began 10 days before hospitalization. Diarrhea ceased on April 15. SARS-CoV-2 PCR was positive first in the stool and then in the pharynx. Typical COVID-19 pneumonia existed on the CT-scan while the patient presented no dyspnea. Acute functional renal failure corrected after refilling and discontinuing diuretics and ACE inhibitors. The dose of tacrolimus was temporarily divided by 2. Treatment with azithromycin was given for 5 days, hydroxychloroquine for 10 days and ceftriaxone for 7 days. C reactive protein normalized on April 18.


Positive PCR
Negative PCR
DIARRHEA
Stool
Naso-pharynx
IV fluids
Hydroxychloroquin
Azithromycin
IV Ceftriaxone
Tacrolimus + Prednisone
300
250
200
150
100
50
0
4/03/2020
4/13/2020
4/14/2020
4/15/2020
4/16/2020
4/17/2020
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4/19/2020
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4/25/2020
4/26/2020
4/27/2020
4/28/2020
serum creatinine (µM)
Creactive protein (mg/L)

133
134

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