SARS-CoV-2 Viral Load in Clinical Samples of Critically Ill Patients

Yongbo Huang[#1], Sibei Chen[#1], Zifeng Yang[1], Wenda Guan[1], Dongdong Liu[1], Zhimin Lin[1], Yu Zhang[1], Zhiheng Xu[1], Xiaoqing Liu[*1], Yimin Li[*1]

Institution

1 State Key Laboratory of Respiratory Diseases, Guangzhou Institute of Respiratory Health, First Affiliated Hospital of Guangzhou Medical University,

[#]These authors contributed equally to this work.

* Corresponding authors:

Dr. Yimin Li (dryiminli@vip.163.com) and Dr. Xiaoqing Liu (lxq1118@126.com), The First Affiliated Hospital Guangzhou Medical University, 151 Yanjiang Street West, Guangzhou, Guangdong, 510120, China

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Introduction

An outbreak caused by a newly identified coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, China in December 2019(1), and has since spread across mainland China and to other countries. The clinical spectrum of the coronavirus disease 2019 (COVID-19) ranges from asymptomatic to severe condition with 5.0% of patients admitted to the Intensive Care Unit (ICU) (2, 3). Reverse-transcriptase–polymerase-chain-reaction (real-time RT-PCR) assays are recommended for the diagnosis of SARS-CoV-2 infection (4). A previous study reported the SARS-CoV-2 viral load in upper respiratory specimens of COVID-19 patients (5). Here, we investigate the viral load in specimens from multiple sites and the duration of viral shedding in respiratory tract samples from laboratory-confirmed critically ill patients with COVID-19 requiring ICU admission.

Methods

We conducted a retrospective, descriptive study which included 16 consecutive critically ill patients with COVID-19 admitted to the ICU of The First Affiliated Hospital of Guangzhou Medical University. The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University. The requirement for informed consent was waived for the retrospective collection of data. A protocol was developed for sample collection when the first patient was admitted to our ICU, as follows: serial samples from the upper respiratory tract (throat and nasal swabs), lower respiratory tract (sputum or endotracheal aspirate (ETA)) were collected daily

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during the first week after admission and, every 2 to 3 days after the first week, until two sequential negative results were obtained or the patient was discharged from the ICU. Plasma, serum, conjunctival swabs and urine sample were also collected in the first week after ICU admission. 15 patients tested negative in these samples and in remaining patient, sample collection was discontinued when two sequential negative results were obtained. Fecal samples were collected when available, and if unavailable, anal swabs were collected instead. Gastric fluid samples were collected only in patients with an in-dwelling gastric tube. Most sampling was done according to the designed protocol (Supplementary datasheets). Swab samples were immediately placed into sterile tubes containing 3 mL of viral transport medium (VTM). The specimens were sent to the virology laboratory of our hospital for sample processing and viral RNA extraction. 0.25 ml of liquid samples (VTM or directly from biological specimens) were used for RNA extraction. Viral RNA of SARS-Cov-2 was detected according to the recommendation by the Chinese Center for Disease Control and Prevention (CDC). (http://www.chinacdc.cn/jkzt/crb/zl/szkb_11803/jszl_11815/202001/t20200123_211378.html). Two target genes, open reading frame1ab (ORF1ab) and nucleocapsid protein (N), were simultaneously amplified and tested using real-time RT–PCR assay. The viral load was indicated as cycle threshold (Ct) value of N gene of SARS-CoV-2. A positive and a negative control were included in the assay, according to the manufacturer's protocol. A Ct value of <40 was defined as positive for SARS-CoV-2 RNA and >40 was defined as negative. Samples with a Ct value between 37 to 40,

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were retested, at least twice. Ct values of all samples collected and tested are shown in supplementary datasheets.

Results

A total of 16 patients (13 men and 3 women; median age, 59.5 years; range, 26 to 79 years) who were admitted to our ICU from January 26 through February 25, 2020 were included in this study. Twelve patients were imported cases who had recently returned from Hubei Province, and four had exposure to patients with confirmed SARS-CoV-2 infection. Most patients (75 %) had at least one preexisting chronic condition. All patients showed evidence of pneumonia in chest radiographs and 15 patients were diagnosed with Acute Respiratory Distress Syndrome (ARDS, 8 with moderate ARDS and 7 with severe ARDS) upon admission. A total of 4 (25 %) patients were supported with noninvasive positive pressure ventilation and 12 (75%) with invasive mechanical ventilation, Extracorporeal membrane oxygenation (ECMO) was applied in 5 (31%) patients (Table 1). As of March 20, nine patients were discharged from the ICU and all 16 patients were alive.

The median days from the onset of symptoms to ICU admission was 12.0 days (Q1, Q3: 9.0, 16.5). During the ICU stay, nasal swab samples from 13 patients (81%) and throat swab samples from 10 patients (63%) tested positive for SARS-CoV-2, but lower respiratory specimens (sputum or ETA) were positive in all 16 patients (100%). Viral RNA was also detected in urine (1 patient), conjunctival swab (1 of 15 patients; one patient refused to provide a conjunctival swab) and gastric fluid (6 of 13

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patients). SARS-CoV-2 viral RNA was also detected in fecal samples from 11 patients (69%) and anal swabs (4 patients). In one patient, viral RNA was present in all types of specimens taken, suggesting that infection in this patient may be systemic (Table 1).

We analyzed the viral load and duration of virus shedding in nasal, and throat swabs and lower respiratory specimens, in relation to the day of symptom onset (Fig. 1 A). Surprisingly, 11 patients (69%) showed prolonged viral shedding in lower respiratory specimens, beyond 28 days after the onset of symptoms. As of March 20, the longest observed period of viral shedding in lower respiratory tract specimens was 55 days (Patient # 4, supplementary datasheets). In addition, lower respiratory tract specimens (sputum or ETA) had significantly higher SARS-CoV-2 viral RNA levels (inversely related to the Ct value) than nasal and throat swab specimens (Figure 1B). Our results indicated that samples from the lower respiratory tract had the highest viral load but slowest resolution of viral shedding in comparison with throat and nasal swab samples.

Discussion

It is generally believed that the lung is the major target organ of SARS-CoV-2; however, we detected viral RNA in numerous different clinical samples including conjunctival swab, blood samples, gastric juice, feces, anal swab and urine in critically ill patients. Wang et al. tested 1070 specimens collected from 205 patients with COVID-19 and found that virus could be detected in different types of clinical

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specimens including respiratory tract samples, feces and blood (6). Although the detection of viral RNA does not always equate the presence of infectious virus and viral RNA shedding of SARS-CoV-2 does not equate with infectivity. Our colleague (Sun et al., manuscript under review) had previously succeeded in isolating infectious virus from urine sample from one of our patients. This suggests that the SARS-CoV-2 virus can replicate in extrapulmonary sites, as which has been previously observed in some severe viral pneumonia patients, such as those caused with by the highly pathogenic avian influenza (7). However, transmission of the SARS-CoV-2 via extra-respiratory routes such as fecal–oral transmission in SARS-CoV-2 spread must be further investigated. Our findings are in concordance with reports showing that ACE2, the putative cell entry receptor of SARS-CoV-2, is widely expressed in a variety of epithelial cells in multiple organs (8). It is still unclear whether replication of SARS-CoV-2 in extrapulmonary organs contributes to organ injury and dysfunction, considering that secondary organ injury owing to hypoxia, tissue hypoperfusion and inflammation is common in critically ill patients.

Zou. et al. found that SARS-CoV-2 viral RNA could be weakly detected in nasal and throat swabs after 14 days from symptoms onset (5). Pan. et al. reported viral loads from different types of clinical specimens collected from 82 infected individuals within a maximum 15 days after symptoms onset (9). Zhou. et al. found that median duration of viral shedding in throat swabs was 20.0 days in COVID-19 survivors (10). Here, we found that SARS-CoV-2 viral RNA could be detected in sputum or ETA beyond 28 days from symptoms onset in 11 patients (69%), as well as in

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extrapulmonary samples from these critically ill patients. These findings have important implications for assessing of transmission risk and the protection of ICU staff and strengthen the importance of effective antiviral treatment for critically ill patients with COVID-19.

This study is limited by a small number of critically ill patients and no non-survivor data as there were no death case in our ICU during the study period. In addition, we were not able to sample consistently according to the designed protocol and sampling was discontinued after patients were discharged to the hospital's isolation ward. Longitudinal studies in a larger cohort would help to further understand the viral load and shedding of COVID-19 patients.

In conclusion, critically ill patients infected with SARS- Cov-2 demonstrated higher viral load and prolonged shedding in lower respiratory tract specimens, as compared with upper respiratory tract specimens. Sampling from lower respiratory tract may be required to assess the true viral clearance in such patients.

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Figure legend

Figure 1 Viral load detected in respiratory specimens obtained from critically ill patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A). Aggregated cycle threshold (Ct) values of Nucleocapsid protein gene of SARS-COV2 in serial throat swabs, nasal swabs and sputum/endotracheal aspirate (ETA) samples in 16 patients, according to days after symptom onset. B). Box plot of lowest Ct values in throat swabs, nasal swabs and sputum/ETA samples during the entire ICU stay among patients with COVID-19. Box and whiskers plot features are as follows: central line in the box is the median, bottom line of the box is first quartile (25%) and top line of box is third quartile (75%). Bottom of whiskers is maximum Ct value; top of whiskers is minimum Ct value. Groups were compared using Kruskal Willis test with Dunn's multiple comparison test. * $p<0.01$.

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Table 1. Baseline and clinical characteristics, main interventions, and detection of SARS-CoV-2 in specimens from patients with the SARS-CoV-2 infection admitted to the ICU

Variables	All patients (n=16)
Age (year), median (Range)	59.5 (26 – 79)
Male sex, n (%)	13 (81%)
Body mass index, kg/m^2, median (Q1, Q3)	24.1 (22.0 – 27.5)
Chronic conditions, n (%)	12 (75%)
Diabetes	6 (37%)
Chronic cardiac disease	10 (63%)
Chronic pulmonary disease	5 (31%)
Chronic neurologic disease	2 (13%)
Any malignancy	1 (6%)
Liver disease	2 (13%)
Smoker (including ex-smoker), n (%)	9 (56%)
Exposure, n (%)	
Exposure to Hubei	12 (75%)
Exposure to confirmed patients	4 (25%)
Days from onset of symptoms to ICU admission, median (Q1, Q3)	12.0 (9.0 – 16.5)
Ratio of PaO_2 to FiO_2 (mmHg) on day 1, mean ± SD	120.7 ± 60.8
APACHE II score on day 1, mean ± SD	16.4 ± 7.8

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SOFA score on day 1, mean ± SD	6.9 ± 3.8
Acute respiratory distress syndrome, n (%)	15 (94%)
Mild ARDS	0
Moderate ARDS	8 (50%)
Severe ARDS	7 (44%)
Mechanical ventilation during ICU stay, n (%)	
Non-invasive	4 (25%)
Invasive	12 (75%)
Extracorporeal membrane oxygenation during ICU stay y, n (%)	5 (31%)
Positive for SARS-CoV-2 during ICU stay, n / patients tested	
Nasal swab	13/16
Throat swab	10/16
Sputum/ETA	16/16
Conjunctival swab	1/15
Blood	1/16
Urine	1 /16
Gastric fluid	6 /13
Feces	11 /16
Anal swab	4/15

Abbreviations: APACHE II=Acute Physiology and Chronic Health Evaluation II.

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ARDS=Acute respiratory distress syndrome. ETA=endotracheal aspirate. FiO_2=fraction of inspired oxygen. PaO_2=partial pressure of oxygen. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. SD = standard deviation. SOFA=Sequential Organ Failure Assessment. ICU=intensive care unit.

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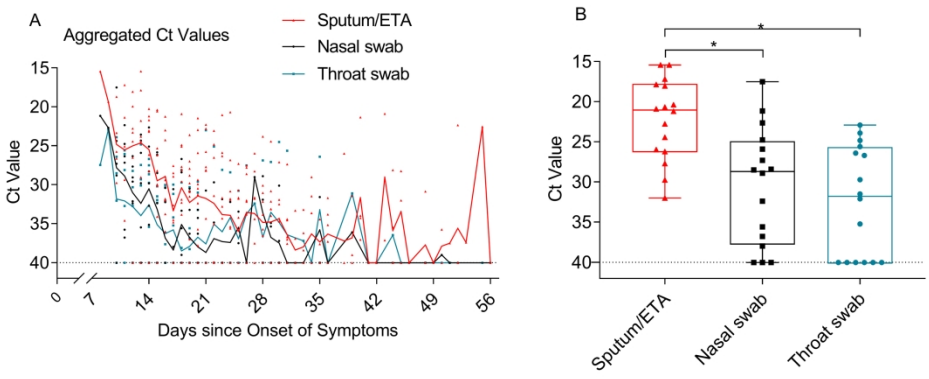

Viral load detected in respiratory specimens obtained from critically ill patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

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