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ABOUT COVER
Peear-Reviewer of World Journal of Clinical Cases, Dr. Adonis Protopapas is a gastroenterology Resident at the first Propaedeutic Department of Internal Medicine of the Aristotle University of Thessaloniki (Greece), located at the A.I.E.P.A Hospital. He earned his Bachelor's degree in 2015 from the Democritus University of Thrace, followed by three Master’s of Science degrees, with specializations in clinic pharmacology, medical research methodology, and healthcare management. His research interests are mainly focused on the area of hepatology, although he also participates in various projects related to endoscopy and inflammatory bowel disease. He is particularly fascinated by research on cirrhosis and its complications. (L-Editor: Filipodia)

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Retrospective Study

Virus load and virus shedding of SARS-CoV-2 and their impact on patient outcomes

Pei-Fen Chen, Xia-Xia Yu, Yi-Peng Liu, Di Ren, Min Shen, Bing-Sheng Huang, Jun-Ling Gao, Zheng-Yang Huang, Ming Wu, Wei-Yan Wang, Li Chen, Xia Shi, Zhao-Qing Wang, Ying-Xia Liu, Lei Liu, Yong Liu

ORCID number: Pei-Fen Chen 0000-0001-9904-6997; Xia-Xia Yu 0000-0003-4811-5125; Yi-Peng Liu 0000-0003-4983-8173; Di Ren 0000-0002-4552-249X; Min Shen 0000-0003-1319-2534; Bing-Sheng Huang 0000-0002-1183-7506; Jun-Ling Gao 0000-0001-9174-1709; Zheng-Yang Huang 0000-0001-6387-570X; Ming Wu 0000-0002-0800-5506; Wei-Yan Wang 0000-0001-7591-6943; Li Chen 0000-0002-3472-1522; Xia Shi 0000-0002-3245-0693; Zhao-Qing Wang 0000-0002-1461-1625; Ying-Xia Liu 0000-0002-5059-6290; Lei Liu 0000-0003-2027-4271; Yong Liu 0000-0002-5208-888X.

Author contributions: Liu Y and Liu L designed and coordinated this study; Chen PF, Liu Y, and Ren D contributed to data collection and management; Liu Y, Yu XX, Gao JL, Huang ZY and Huang BS contributed to data analysis and draft writing; Liu L and Huang BS contributed to the final approval of the version to be submitted; and all authors read and approved the final manuscript.

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Abstract

BACKGROUND
Understanding a virus shedding patterns in body fluids/secretions is important to determine the samples to be used for diagnosis and to formulate infection control measures.

AIM
To investigate the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) shedding patterns and its risk factors.

METHODS
All laboratory-confirmed coronavirus disease 2019 patients with complete medical records admitted to the Shenzhen Third People’s Hospital from January 28, 2020 to March 8, 2020 were included. Among 145 patients (54.5% males; median age, 46.1 years), three (2.1%) died. The broncho-alveolar lavage fluid (BALF) had the highest virus load compared with the other samples. The viral load peaked at admission ($3.3 \times 10^8$ copies) and sharply decreased 10 d after admission.

RESULTS
The viral load was associated with prolonged intensive care unit (ICU) duration. Patients in the ICU had significantly longer shedding time compared to those in the wards ($p < 0.0001$). Age > 60 years [hazard ratio (HR) = 0.6; 95% confidence interval (CI): 0.4-0.9] was an independent risk factor for SARS-CoV-2 shedding, while chloroquine (HR = 22.8; 95%CI: 2.3-224.6) was a protective factor.

CONCLUSION
BALF had the highest SARS-CoV-2 load. Elderly patients had higher virus loads, which was associated with a prolonged ICU stay. Chloroquine was associated with shorter shedding duration and increased the chance of viral negativity.

Key Words: COVID-19; Virus shedding; Viral load; Patient outcome; China; Infectious disease

INTRODUCTION
The coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus that is closely related to SARS-CoV, which caused an outbreak in 2003[1]. The outbreak of COVID-19 was first reported in December 2019 in Wuhan, China[2,3]. Such outbreak can cause emotional distress and anxiety[4], which can occur even in people not at high risk of getting sick, in the face of a virus that the common people are unfamiliar with[5]. The common signs
of COVID-19 include fever, cough, and shortness of breath\[^9\]. While a significant proportion of patients develop neurological manifestations, especially olfactory and gustatory dysfunction\[^8\]\[^10\]. There is no specific treatment, but supportive care is necessary in severe and critical cases\[^8\]\[^9\]. Acute respiratory distress syndrome and sepsis were reported in 100% of the patients with confirmed COVID-19 who died\[^11\].

The factors dictating the severity of illness and outcome among patients with COVID-19 are still not well defined. Observational studies reported that the median duration of viral shedding was 20.0 d (IQR: 17.0-24.0) among survivors\[^10\]\[^11\]. In some other coronavirus respiratory illnesses, the higher virus load and longer shedding duration were related to a worse outcome\[^10\]\[^11\]. The longest observed duration of viral shedding in survivors was 37 d\[^11\]. The viral shedding duration among patients with COVID-19 has been reported to be associated with age and comorbidities\[^6\]\[^8\]\[^9\]. Prolonged Middle East respiratory syndrome (MERS) viral shedding in the respiratory tract was associated with severe outcomes in patients with MERS\[^10\]\[^11\]. In addition, studies suggested that patients with MERS-CoV requiring intensive care unit (ICU) admission had a higher viral load than the patients not requiring ICU admission\[^11\]. Nevertheless, whether the virus load and shedding duration of SARS-CoV-2 are associated with the severity of illness is still unknown.

Data on viral load and shedding in the respiratory tract are limited, and risk factors for viral shedding have yet to be fully clarified. COVID-19 has proven to be highly infectious and is transmitted person-to-person\[^11\]. Respiratory droplets were suspected to be the main route of transmission for SARS-CoV\[^12\]\[^13\] and SARS-CoV-2\[^14\]. Aerosols and fomite are possible transmission routes since SARS-CoV-2 survives for 0.8-6.8 h on different surfaces\[^14\]. It is of great epidemiologic significance to understand the virus shedding patterns in different body fluids and secretions to determine which samples are the most suitable for diagnosis, and how to formulate appropriate infection control measures and isolation duration. Most importantly, virus shedding could be used as a reliable marker to scrutinize the effectiveness of antiviral drugs.

This study aimed to examine the distribution of COVID-19 virus in the tissues of the patients and the shedding pattern of COVID-19 virus in the respiratory secretions and to investigate potential factors associated with viral load and shedding and patient outcomes.

**MATERIALS AND METHODS**

**Viral loads and cycle threshold value for SARS-CoV-2 virus**

A cycle threshold value (Ct value) < 37 was defined as a positive test, and a Ct value of ≥ 40 was defined as a negative test. A medium load, defined as a Ct value of ≥ 37 but < 40, required retesting. By regression analysis of the standard curves provided by the manufacture (Shanghai Jienuo Co., Ltd., Shanghai, China) (Supplementary Figure 1), the following equations of the standard curve (plot of Ct values against the log of the standard sample amount) were determined for SARS-CoV-2 (R\(^2\) = 0.995): Ct = -3.39 × log(copies) + 40.52.

**Data collection**

The following data were extracted from the medical records: Demographics (age, sex, body mass index, history of Hubei contact, and smoking), symptoms (at admission and in the ICU), comorbidities (hypertension, diabetes, heart diseases, and chronic obstructive pulmonary disease), laboratory findings at admission (temperature, white blood cells, platelets, lymphocytes, interleukin (IL)-6, serum creatinine, respiratory tract viral load, and PaO\(_2\)/FiO\(_2\)), severity scores [Acute Physiology And Chronic Health Evaluation II (APACHEII), Sequential Organ Failure Assessment (SOFA), and Glasgow Coma Scale (GCS)], therapy, outcomes (viral shedding, ICU stay, hospital stay, death, and discharge).

**Statistical analysis**

Continuous variables are presented as mean values with 95% confidence intervals (CI). The means for continuous variables were compared using the independent t test when the data were normally distributed (Kolmogorov-Smirnov test); otherwise, the Mann-Whitney U-test was used. Data with non-Gaussian distribution from repeated measures were compared using the generalized linear mixed model. The proportions for categorical variables were compared using the χ\(^2\)-square test, while the Fisher’s exact test was used when the data were limited. Multivariable regression analysis or time-dependent Cox regression was conducted to assess the relative influence of virus
shedding on ICU admission and hospital duration. P values ≤ 0.05 were considered statistically significant. All analyses were performed using R (http://www.R-project.org) and EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc. Boston, MA, United States).

RESULTS

Data collection and patients
All consecutive patients with confirmed COVID-19 admitted to the Shenzhen Third People’s Hospital from January 28, 2020 to March 8, 2020, were screened for eligibility. The Shenzhen Third People’s Hospital is the only designated infectious disease hospital responsible for the treatments of COVID-19 in Shenzhen, China. This retrospective study was approved by the Institutional Review Board of Shenzhen Third People’s Hospital. Informed written consent was obtained from all subjects prior to the study.

All patients with COVID-19 included in this study were diagnosed according to World Health Organization interim guidance[19]. The hospitalized patients with more than one positive nucleic acid test for SARS-CoV-2 virus at least one day apart and with complete medical records were included in the analysis. To investigate the possible transmission capacity, the specimens were obtained from a variety of sources, including sputum, nasopharyngeal swabs, blood, endotracheal aspirate, saliva, and eye discharges.

Characteristics of the specimens
The study initially screened 1461 patients, and 111 were excluded for missing virological records (Figure 1). Among those 1350 patients with COVID-19, 7404 virological tests were performed. Of 6959 (94.0%) nasopharyngeal swab specimens, 41.6% were positive for COVID-19. Among 144 (1.9%) blood samples, 14.6% showed positive results. Among 213 (2.9%) bronchoalveolar lavage fluid (BALF) specimens, 56.3% were positive. Among 47 (0.6%) saliva samples, 29.8% were positive. Among 38 (0.5%) eye discharge samples, 15.8% were positive. In addition, two cerebrospinal fluid (CSF, 0.03%) samples were available, and both were negative. One (0.01%) anus swab sample was negative for COVID-19 (Supplementary Table 1).

Characteristics of the patients
The patients with incomplete medical records were excluded, and 145 patients with full inpatient records were included (Figure 1). Among them, 79 (54.28%) were males, and the mean age was 46.1 (95%CI: 42.6-49.9) years. Of these patients, 107 (73.8%) were admitted to the isolation wards, and 38 (26.2%) were admitted and transferred to the ICU. The median duration from the first symptoms to hospital admission was 4.6 d (95%CI: 4.1-5.1 d) (Table 1). Hypertension [26 (17.9%)], cardiovascular disease [18 (12.4%)], and diabetes [11 (7.6%)] were the most common comorbidities. During the first 3 d after admission, 142 were treated with interferon, 16 (11.0%) received no antiretrovirals (ARV), while 84 (57.9%) received one ARV, 38 (26.2%) received two ARVs, and seven (4.8%) received three ARVs.

In 92 (63.5%) patients, viral negativization took longer than 2 wk. These patients were older (50.3 vs 39.6 years) and were more likely to have hypertension and heart diseases compared with patients who achieved viral negativization in less than 2 wk (Table 1). In addition, they had lower platelet and higher IL-6 and serum creatinine levels. Their conditions were more severe, with higher SOFA and APACHEII scores.

Virus load in different sample types
The mean viral load at admission for the 145 patients was 1.16 × 10^4 copies/mL (26.7 ± 4.4 in Ct values), but it could reach 11.7 in Ct value (3.3 × 10^6 copies/mL). The mean viral load in the respiratory tract was 4.9 × 10^5 copies/mL. The median viral load in nasopharyngeal swab samples was 1.32 × 10^6 copies/mL (26.6 ± 4.4 in Ct value); from sputum samples, it was 2.9 × 10^6 copies/mL (32.2 in Ct value); from BALF it was 5.7 × 10^6 copies/mL (24.5 ± 3.6 in Ct value); and from blood samples, it was 4.2 × 10^6 copies/mL (28.2 ± 7.4 in Ct value) (Supplementary Figure 1). The BALF had the highest virus load compared with the other samples.

Association between virus load and disease severity
The patients in the ICU had higher virus loads than those in wards. The average Ct
### Table 1 Characteristics and outcomes of the patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All</th>
<th>Viral shedding (d)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 14</td>
<td>≥ 14</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>53 (37%)</td>
<td>92 (63%)</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>46.1 (42.6-49.9)</td>
<td>39.6 (34.2-45.9)</td>
<td>50.3 (46.1-54.9)</td>
</tr>
<tr>
<td>Sex, male, n (%)</td>
<td>79 (54.5%)</td>
<td>25 (47.2%)</td>
<td>54 (58.7%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6 (22.9-24.2)</td>
<td>22.9 (21.9-24.1)</td>
<td>23.9 (23.2-24.7)</td>
</tr>
<tr>
<td>History of Hubei contact</td>
<td>129 (89.0%)</td>
<td>50 (94.3%)</td>
<td>79 (85.9%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>3 (2.1%)</td>
<td>0</td>
<td>3 (3.3%)</td>
</tr>
<tr>
<td>Symptom to admission (d)</td>
<td>4.6 (4.1-5.1)</td>
<td>4.4 (3.6-5.3)</td>
<td>4.7 (4.1-5.3)</td>
</tr>
<tr>
<td>Symptom to ICU (d)</td>
<td>12.0 (10.8-13.4)</td>
<td>10.5 (8.7-12.7)</td>
<td>12.4 (10.9-14.2)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>26 (17.9%)</td>
<td>5 (9.4%)</td>
<td>21 (22.8%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11 (7.6%)</td>
<td>4 (7.6%)</td>
<td>7 (7.6%)</td>
</tr>
<tr>
<td>Heart disease</td>
<td>18 (12.4%)</td>
<td>3 (5.7%)</td>
<td>15 (16.3%)</td>
</tr>
<tr>
<td>COPD</td>
<td>4 (2.8%)</td>
<td>1 (1.9%)</td>
<td>3 (3.3%)</td>
</tr>
<tr>
<td>Laboratory findings on admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.4 (37.3-37.5)</td>
<td>37.3 (37.0-37.5)</td>
<td>37.5 (37.3-37.6)</td>
</tr>
<tr>
<td>WBC (× 10⁹/L)</td>
<td>4.84 (4.58-5.12)</td>
<td>4.89 (4.46-5.36)</td>
<td>4.82 (4.49-5.16)</td>
</tr>
<tr>
<td>PLT (× 10¹²/L)</td>
<td>175.51 (166.47-185.05)</td>
<td>201.84 (185.82-219.25)</td>
<td>161.75 (151.91-172.23)</td>
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<tr>
<td>LYMPH (× 10⁹/L)</td>
<td>1.20 (1.10-1.29)</td>
<td>1.21 (1.06-1.37)</td>
<td>1.19 (1.07-1.31)</td>
</tr>
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<td>IL-6 (ug/L)</td>
<td>18.24 (14.93-22.29)</td>
<td>12.55 (8.58-18.36)</td>
<td>21.72 (17.34-27.20)</td>
</tr>
<tr>
<td>CR (mg/L)</td>
<td>67.47 (64.12-71.00)</td>
<td>62.03 (57.87-66.50)</td>
<td>70.87 (66.21-75.86)</td>
</tr>
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<td>Viral load in respiratory tract [log (copies)]</td>
<td>3.69 (3.06-5.07)</td>
<td>3.36 (2.49-4.83)</td>
<td>4.22 (3.10-5.12)</td>
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<tr>
<td>PAO₂/FIÓ₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 100</td>
<td>1 (0.7%)</td>
<td>0</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>≥ 100, &lt; 200</td>
<td>8 (5.9%)</td>
<td>3 (6.4%)</td>
<td>5 (5.7%)</td>
</tr>
<tr>
<td>≥ 200, &lt; 300</td>
<td>19 (14.1%)</td>
<td>7 (14.9%)</td>
<td>12 (13.6%)</td>
</tr>
<tr>
<td>≥ 300</td>
<td>107 (79.3%)</td>
<td>37 (78.7%)</td>
<td>70 (79.6%)</td>
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<tr>
<td>Severity score</td>
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<tr>
<td>APACHEII</td>
<td>4.2 (3.7-4.7)</td>
<td>3.4 (2.8-4.1)</td>
<td>4.8 (4.2-5.5)</td>
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<td>SOFA</td>
<td>1.7 (1.5-1.9)</td>
<td>1.4 (1.2-1.7)</td>
<td>1.9 (1.6-2.2)</td>
</tr>
<tr>
<td>GCS</td>
<td>14.8 (14.5-15.2)</td>
<td>15.0 (15.0-15.0)</td>
<td>14.7 (14.2-15.5)</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fever</td>
<td>123 (84.8%)</td>
<td>41 (77.4%)</td>
<td>82 (89.1%)</td>
</tr>
<tr>
<td>Sore muscles</td>
<td>32 (22.1%)</td>
<td>13 (24.5%)</td>
<td>19 (20.7%)</td>
</tr>
<tr>
<td>Cough</td>
<td>84 (57.9%)</td>
<td>31 (58.5%)</td>
<td>53 (57.6%)</td>
</tr>
<tr>
<td>ARV in the first 3 d</td>
<td>0.008</td>
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<tr>
<td>None</td>
<td></td>
<td></td>
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<tr>
<td>Kaletra</td>
<td>40 (27.6%)</td>
<td>8 (15.1%)</td>
<td>32 (34.8%)</td>
</tr>
<tr>
<td>Kaletra &amp; Other ARV</td>
<td>40 (27.6%)</td>
<td>19 (35.9%)</td>
<td>21 (22.8%)</td>
</tr>
<tr>
<td>Other ARV but Kaletra</td>
<td>49 (33.8%)</td>
<td>16 (30.2%)</td>
<td>33 (35.9%)</td>
</tr>
<tr>
<td>ARV in the first 3 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16 (11.0%)</td>
<td>10 (18.9%)</td>
<td>6 (6.5%)</td>
</tr>
<tr>
<td>1</td>
<td>84 (57.9%)</td>
<td>22 (41.5%)</td>
<td>62 (67.4%)</td>
</tr>
<tr>
<td>2</td>
<td>38 (26.2%)</td>
<td>18 (34.0%)</td>
<td>20 (21.7%)</td>
</tr>
</tbody>
</table>
Continuous variables were described by mean values with 95% confidence intervals, while categorical variables were presented using percentages. BMI: Body mass index; ICU: Intensive care unit; WBC: White blood count; PLT: Platelet; LYMPH: Lymphocyte; IL-6: Interleukin 6; CR: Serum creatinine; APACHEII: Acute Physiology And Chronic Health Evaluation II; SOFA: The Sequential Organ Failure Assessment; GCS: Glasgow Coma Scale; COPD: Chronic obstructive pulmonary disease; ARV: Antiretroviral therapy; HFO: High flow oxygen; MV: Mechanical ventilation; NMV: Non-invasive ventilation; CRRT: Continuous Renal Replacement Therapy.

Figure 1: Patient flowchart. SARS-Cov-2: Severe acute respiratory syndrome coronavirus 2.
Figure 2 Box plot showing the cycle threshold value differences at the day of hospital admission. A: Duration of intensive care unit stay (< 7 vs ≥ 7 d) (one-sided t test, P = 0.02); B: Vital status (death or not) (one-sided t test, P < 0.0001); C: Procedures (mechanical ventilation needed or not) (one-sided t test, P = 0.009); D: Results at admission (interleukin 6) (one-sided t test, P = 0.05). Ct: Cycle threshold; MV: Mechanic ventilation; IL-6; Interleukin 6; ICU: Intensive care unit.

Moreover, patients with IL-6 > 30 µg/L had higher Ct values compared to those with IL-6 < 30 µg/L (P = 0.05) (Figure 2D). The average Ct values did not significantly differ in terms of comorbidities, sex, and Hubei contact (all P > 0.05).

Duration of SARS-CoV-2 viral shedding
The average duration of SARS-CoV-2 viral shedding was 17.3 d (95%CI: 15.8-18.9 d). For the ward patients, the average duration of SARS-CoV-2 viral shedding was 12.1 d (95%CI: 11.0-13.3 d), while for the ICU patients, it was 24.4 d (95%CI: 22.6-26.3 d) (P < 0.0001). Patients with lower first positive Ct values tended to have longer viral shedding time (P = 0.0176).

Smooth curves were fitted for Ct value and the illness onset to hospital admission (Figure 3A). Virus load peaked at admission and sharply decreased within 10 d after admission, after which it gradually dropped until reaching a low level or negativization. Moreover, the results also indicated the longer the time from symptom to hospital admission, the longer was the duration of viral shedding (Figure 3C). We further compared the proportion of patients who tested positive for SARS-CoV-2 RNA over time after admission, and significant differences were seen in the duration of viral shedding for the elderly population (Figure 3B), patients with PaO2/FiO2 < 100 (Figure 3D), and chloroquine prescribed within 3 d after admission (Figure 3E).

Risk factors for prolonged viral shedding
All available data from 145 patients were incorporated in a time-dependent Cox proportional hazards model. Chloroquine was positively correlated with virus shedding, increasing the possibility of viral shedding. Age > 60 years was negatively correlated with viral shedding and could potentially decrease the chance of viral shedding and was thus associated with longer shedding duration. Age > 60 years [hazard ratio (HR) = 0.6; 95%CI: 0.4-0.9] was an independent risk factor for SARS-CoV-2 shedding, while chloroquine (HR = 22.8; 95%CI: 2.3-224.6) was a protective factor (Table 2); immunoglobulin, antibiotics, steroid, PaO2/FiO2 ratio, and the neutrophil-lymphocyte ratio were not independently associated with virus shedding (Table 2). Viral shedding was associated with longer ICU duration, but not with hospital stay and in-hospital mortality (Table 3).

DISCUSSION
In this study, we analyzed the virus nucleic acid content of SARS-CoV-2 from different samples, described the features of virus load and virus shedding, identified the risk factors for prolonged SARS-CoV-2 RNA shedding and evaluated the impact of prolonged SARS-CoV-2 RNA shedding on patients’ outcomes, including ICU duration and in-hospital mortality.

SARS-CoV-2 was detected in samples from eye discharge, blood, saliva, nose swab, and BALF. Although the SARS-CoV-2 virus is mainly distributed in the respiratory system, it can also be found in the digestive tract, blood, and eye discharge, which may contribute to the high contagious capacity of the virus[20]. In the present study, SARS-CoV-2 was detected in the nasopharyngeal swab, blood, BALF, saliva, and eye discharge. It was not found in the CSF and anus swab, but these two sample types
The shedding pattern of SARS-CoV-2 RNA demonstrated that the virus load increased sharply before admission and dropped dramatically thereafter. Based on the samples collected from 145 patients, the mean virus load at admission was $1.16 \times 10^4$ copies/mL, which is basically similar to SARS\cite{12}, instead of 1000 times higher, as reported\cite{21}, and the highest viral load was $3.3 \times 10^8$ copies/mL according to the previous research\cite{21}. Moreover, the present study suggested that the viral load peaked at admission, decreased right after the start of treatment, decreased sharply within 10 d after admission, and dropped gradually until reaching a quite low level or negativization. In addition, the mean duration of SARS-CoV-2 viral shedding was much longer in ICU patients compared to those in the wards (21.8 vs. 14.8 d). The shedding duration of SARS-CoV-2 RNA was reported to be considerably longer than that of MERS and SARS\cite{15, 22}. Moreover, the duration of virus shedding was influenced by the time between the onset of illness and admission, where a later admission was associated with longer virus shedding, higher ICU admission, and lower survival probability. Those data suggest that the early detection and clinical intervention would help shorten the viral shedding duration, lower the risk of ICU admission, and increase the survival probability.

This study suggested that senior age was an independent risk factor for virus shedding. Previous research suggested that advanced age is associated with prolonged illness and poor outcomes in patients hospitalized with COVID-19\cite{23-25}. The present study further demonstrated that the elderly had higher initial viral loads in contrast to

### Table 2 Univariate and multivariate regression analyses of the risk factors for viral shedding

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th></th>
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<th></th>
<th>Multivariate</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td>$P$ value</td>
<td>HR (95%CI)</td>
<td>$P$ value</td>
<td>HR (95%CI)</td>
<td>$P$ value</td>
<td>HR (95%CI)</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Sex, male</td>
<td>1.1 (0.7, 1.6)</td>
<td>0.757</td>
<td>0.8 (0.5, 1.3)</td>
<td>0.422</td>
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<tr>
<td>Age (yr)</td>
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<td>&lt; 60</td>
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<tr>
<td>$\geq$ 60</td>
<td>0.5 (0.4, 0.8)</td>
<td>0.002</td>
<td>0.6 (0.4, 1.0)</td>
<td>0.030</td>
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<tr>
<td>Diabetes</td>
<td>0.9 (0.5, 1.8)</td>
<td>0.870</td>
<td>1.4 (0.7, 2.7)</td>
<td>0.370</td>
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<tr>
<td>IVIG</td>
<td>0.5 (0.3, 0.7)</td>
<td>&lt;0.001</td>
<td>0.6 (0.3, 1.2)</td>
<td>0.182</td>
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<td></td>
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</tr>
<tr>
<td>Antibiotics</td>
<td>0.5 (0.4, 0.8)</td>
<td>0.002</td>
<td>0.7 (0.4, 1.3)</td>
<td>0.273</td>
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<tr>
<td>Steroids</td>
<td>0.5 (0.3, 0.7)</td>
<td>&lt;0.001</td>
<td>0.8 (0.4, 1.7)</td>
<td>0.588</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PAO$_2$/FIO$_2$</td>
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<td></td>
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<td></td>
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<tr>
<td>&lt; 300</td>
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<tr>
<td>$\geq$ 300</td>
<td>1.3 (0.8, 2.2)</td>
<td>0.247</td>
<td>1.0 (0.5, 1.7)</td>
<td>0.898</td>
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<tr>
<td>Chloroquine</td>
<td>26.8 (3.0, 239.3)</td>
<td>0.003</td>
<td>19.0 (1.9, 186.2)</td>
<td>0.012</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NLR</td>
<td>1.0 (0.9, 1.0)</td>
<td>0.386</td>
<td>1.0 (1.0, 1.1)</td>
<td>0.632</td>
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</tbody>
</table>

IVIG: Intravenous immunoglobulin; NLR: Neutrophil-lymphocyte ratio.

### Table 3 Impact of severe acute respiratory syndrome coronavirus 2 viral shedding on outcomes

<table>
<thead>
<tr>
<th></th>
<th>Non-adjusted</th>
<th>Model I</th>
<th>Model II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td>$P$ value</td>
<td>HR (95%CI)</td>
</tr>
<tr>
<td>ICU duration</td>
<td>0.52 (0.90-0.96)</td>
<td>0.02</td>
<td>0.49 (0.42-0.94)</td>
</tr>
<tr>
<td>Hospital stay</td>
<td>0.15 (-0.01,0.32)</td>
<td>0.07</td>
<td>0.11 (-0.05-0.26)</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>1.07 (0.98, 1.17)</td>
<td>0.15</td>
<td>0.06 (-0.04-0.16)</td>
</tr>
</tbody>
</table>

Data was presented as HR (95%CI) with $P$ value for death and coefficient (95%CI), $P$ value for intensive care unit length and hospital length. Model I, adjusted for age and sex; Model II, adjusted for age, sex, and body mass index. ICU: Intensive care unit.
Figure 3 Factors associated with the duration of viral shedding. A: Changes in cycle threshold values during hospitalization using generalized additive models (F: 46.54, P < 0.0001, adjusted for sex and age); B: From symptom onset to hospital admission ($\chi^2$: 85.31, P < 0.0001); C: Cumulative proportions of patients with symptom onset to admission < 7 d, 7-14 d, and > 14 d ($\chi^2$ = 76.58, P < 0.001); D: Cumulative proportions of patients with $\text{PaO}_2/\text{FiO}_2$ < 100, 100-200, > 200 ($\chi^2$ = 53.74, P < 0.0001) at admission; E: Cumulative proportion of patients with chloroquine or without (P < 0.0001). Ct: Cycle threshold; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

younger patients. A higher viral load may reflect a disability of the immune system to contain viral proliferation, thus contributing to the prolonged viral shedding period. Another independent risk factor was the use of chloroquine within 3 d after admission. The interim conclusions from the published literature is that there is no current evidence of use of chloroquine for treatment of COVID-19. Chloroquine should be restricted to clinical trials with strict vigilance and follow-up\cite{26}. The results in the present study indicated that chloroquine could greatly increase the probability of viral negativity by 19.0 times while shortening the virus shedding duration. This finding is consistent with previous publications\cite{5,27}. Nevertheless, due to the limitation of observational studies, randomized clinical trials are necessary before any definitive conclusions on the effectiveness of chloroquine can be reached.

The effectiveness of several clinical treatments was also examined. Contrary to the
intuitive experience, the present study suggests that immunomodulation, such as thymosin and intravenous immunoglobulin (IVIG), had no impact on the pattern of virus shedding. This effect has been explored in other researches, and the studies for IVIG were inconclusive due to potential confounding effects of patient comorbidities, stage of illness, or effect of other treatments\[28,29\]. Our findings suggest that immunomodulation treatment might not reduce the shedding duration after adjusting for the confounding factors. Similar findings were observed about the effect of antiviruses treatment. Kaletra, a combination of lopinavir and ritonavir for human immunodeficiency virus treatment, has shown efficacy in a case report in China\[25\], but after adjusting for the confounding factors, it was not superior to ribavirin plus interferon. It is also true for other antiviral drugs. Thus, any wide administration of this antiviral treatment (AVT) should be carefully evaluated by randomized clinical trials.

This study has some limitations. First, due to the retrospective study design, not all comorbidities were well documented in all patients. Therefore, their role might be underestimated in identifying the risk factors for virus shedding. Second, the patients received AVT empirically, which greatly varied in the selection of antiviral drugs and duration, thus making it extremely challenging to evaluate the role of antiviral drugs. Third, the estimated duration of viral shedding was limited by the frequency of respiratory specimen collection, lack of quantitative viral RNA detection, and a relatively low positive rate of SARS-CoV-2 RNA detection in throat swabs. Fourth, by excluding patients still in hospital as of March 8, 2020, and the low mortality in our group, the impact of virus shedding on mortality might be underestimated. Finally, the interpretation of our findings is limited by the sample size. Nevertheless, by including all adult patients in the designated hospitals in Shenzhen admitted for COVID-19, we believe that our study population is representative of the cases diagnosed and treated in a large city outside Wuhan.

CONCLUSION

The present study is the first to examine the patterns of SARS-CoV-2 virus shedding and its risk factors. The results strongly suggest that SARS-CoV-2 was identified in a wide range of samples, including the respiratory tract, blood, and eye discharge. In addition, the data suggested that earlier hospitalization might help reduce the virus-shedding period and ICU stay. Regarding clinical therapy, the analysis did not confirm the effectiveness of immunomodulation or AVT, but chloroquine could have the potential to shorten the duration and increase the possibility of shedding, although with limited power due to the small sample size. The findings of this retrospective study have important implications for the clinical treatment and policy making with regard to reducing the damage of the current pandemic caused by SARS-CoV-2.

ARTICLE HIGHLIGHTS

Research background
The outbreak of coronavirus disease 2019 (COVID-19) cause emotional distress and anxiety worldwide. However, the factors for the severity of illness and its outcomes still remain unclear.

Research motivation
The goal of this study was to characterize the viral shedding patterns and risk factors in hospitalized patients with COVID-19.

Research objectives
The study aimed to identify the characteristics of viral load and shedding, the risk factors affecting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus clearance and to evaluate the effect of prolonged viral shedding on the outcome of the patients.

Research methods
This was a retrospective study on all laboratory-confirmed COVID-19 patients with complete medical records admitted to the Shenzhen Third People’s Hospital from
January 1, 2020 to March 8, 2020. A total of 7404 virological tests in 1350 patients were analyzed to identify the pattern of virus load in different samples. Furthermore, 145 patients with full inpatient records were statistically analyzed to reveal the risk factors associated with the viral shedding and ICU admission by multivariate Cox regression.

**Research results**
SARS-CoV-2 virus was identified in a wide range of samples, including eye discharge. Earlier hospitalization might help reduce the virus-shedding period and intensive care unit (ICU) stay. Chloroquine is associated with the shortened shedding duration.

**Research conclusions**
Among various samples the SARS-CoV-2 virus, bronchoalveolar lavage fluid had the highest SARS-CoV-2 load. Elderly patients had higher virus loads, which was associated with a prolonged ICU stay. Chloroquine was associated with a shorter shedding duration and increased the chance of viral negativity.

**Research perspectives**
The findings about the virus shedding patterns and its risk factors suggested that early hospitalization has the potential to reduce the virus shedding time and the ICU stay. Also, the confirmation of effectiveness of immunomodulation and chloroquine might help in clinical treatment and policy making.

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**REFERENCES**


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