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Associations of clinical characteristics and treatment regimens with the duration of viral RNA shedding in patients with COVID-19

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Abstract

Objective: The novel coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a global pandemic, but the factors influencing viral RNA shedding, which would help inform optimal control strategies, remain unclear.

Methods: The clinical course and viral RNA shedding pattern of 267 consecutive symptomatic COVID-19 patients admitted to the hospital from January 20, 2020 to March 15, 2020 were evaluated retrospectively.

Results: The median duration of viral RNA shedding was 12 days (interquartile range 8–16 days) after the onset of illness. Of the 267 patients included in this study, 65.2% had viral RNA clearance within 14 days, 88.8% within 21 days, and 94.4% within 28 days. Older age (hazard ratio (HR) 0.99, 95% confidence interval (CI) 0.98–1.00; p = 0.04), time lag from illness onset to hospital admission (HR 0.91, 95% CI 0.88–0.94; p < 0.001), diarrhea (HR 0.59, 95% CI 0.36–0.96; p = 0.036), corticosteroid treatment (HR 0.60, 95% CI 0.39–0.94; p = 0.024), and lopinavir/ritonavir use (HR 0.70, 95% CI 0.52–0.94; p = 0.014) were significantly and independently associated with prolonged viral RNA shedding.

Conclusions: Early detection and timely hospital admission may be warranted for symptomatic COVID-19 patients, especially for older patients and patients with diarrhea. Corticosteroid treatment is associated with prolonged viral RNA shedding and should be used with caution. Lopinavir/ritonavir use may be associated with prolonged viral RNA shedding in non-severe patients; further randomized controlled trials are needed to confirm this finding.

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Introduction

At the end of 2019, a new type of pneumonia caused by a novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2), known officially as coronavirus disease 2019 (COVID-19), first emerged in Wuhan, China. It spread rapidly throughout China and became a global pandemic (Huang et al., 2020; Li et al., 2020; Guan et al., 2020; Arshad Ali et al., 2020), posing enormous challenges to global public health.

The duration of viral shedding is critical for determining control strategies to manage the disease and to reduce transmission. Several small-scale studies have reported the viral RNA shedding pattern of SARS-CoV-2 (Hu et al., 2020; Xu et al., 2020). Hu et al. found that viral RNA-negative conversion in patients with COVID-19 occurred within 7 days after the first positive reverse transcription PCR test in 10.2% of patients, within 14 days in 62.7%, and within 21 days in 91.2% (Hu et al., 2020). Xu et al. reported a median duration of SARS-CoV-2 RNA shedding of 15 days after the onset of illness and that prolonged viral RNA shedding was independently associated with male sex, delayed hospital admission, and invasive mechanical ventilation (Xu et al., 2020). Prolonged viral RNA shedding was also observed after steroid treatment, although the effect was rendered insignificant after controlling for confounders (Xu et al., 2020). However, these
studies were limited by small sample sizes and might have had insufficient power to identify other significant risk factors associated with the duration of viral RNA shedding. Therefore, further in-depth studies are warranted to characterize the viral RNA shedding pattern and identify associated risk factors for optimizing disease control strategies.

Accumulating epidemiological studies have reported the clinical characteristics of COVID-19 in China (Huang et al., 2020; Li et al., 2020; Guan et al., 2020; Yang et al., 2020; Mo et al., 2020; Wang et al., 2020; Chen et al., 2020). Most of these studies with large sample sizes have reported data from Wuhan, the center of the outbreak, where a sudden surge in demand for medical care influenced timely treatment, resulting in serious illness. Studies on COVID-19 in lower incidence areas may provide additional insights into the clinical course of affected patients. Guangzhou is a densely populated metropolitan city in Southern China. In 2003, Guangzhou was heavily hit by severe acute respiratory syndrome (SARS). The lessons learned from SARS have provided some experiences for the management of COVID-19. Between January 20, 2020 and March 15, 2020, 347 patients were confirmed to have COVID-19 in Guangzhou. The majority of these patients (n = 284) were admitted to Guangzhou Eighth People’s Hospital, which was a designated hospital for infectious disease. This study was performed to summarize the pattern of SARS-CoV-2 RNA shedding and evaluate the associations between clinical characteristics and the duration of viral RNA shedding in patients with COVID-19.

**Patients and methods**

**Study design and participants**

This was a retrospective study of consecutive patients with COVID-19 who were admitted to Guangzhou Eighth People’s Hospital between January 20, 2020 and March 15, 2020. The hospital was responsible for admitting the majority of COVID-19 confirmed cases in the Guangzhou area. Patients were admitted from outpatient clinics or transferred from other hospitals. All confirmed COVID-19 cases were isolated until viral RNA tests were negative at least twice sequentially. The study was approved by the Ethics Committee of Guangzhou Eighth People’s Hospital, and all patients provided written informed consent (No. 202001134).

**Definitions**

COVID-19 infection was confirmed by positive SARS-CoV-2 RNA test from throat swab samples, using a reverse transcription quantitative PCR method according to the manufacturer’s protocol (Daan Gene Co. Ltd, Guangzhou, China). A cycle threshold (CT) value of 40 or more was defined as negative.

According to the Chinese COVID-19 guidelines (National Health Commission & State Administration of Traditional Chinese Medicine of China, 2020), the severity of COVID-19 infection was grouped into four categories: mild, moderate, serious, and critical illness. Patients with mild illness did not demonstrate findings on chest computed tomography (CT). Those with moderate illness showed features of pneumonia on chest CT, but the symptoms were mild. Serious illness was defined by the presence of at least one of the following criteria: (1) breathing rate higher than 30/ min; (2) pulse oximeter oxygen saturation (SaO2) less than 93% at rest; and (3) ratio of partial pressure of arterial oxygen (PaO2) to fraction of inspired oxygen (FiO2) less than 300 mmHg (1 mmHg = 0.133 kPa). Critical illness was defined by presence of at least one of the following criteria: (1) occurrence of respiratory failure requiring mechanical ventilation; (2) shock; and (3) failure of other organs requiring treatment in the intensive care unit (ICU). Patients fulfilling all of the following criteria were discharged: (1) afebrile for more than 3 days, (2) improved respiratory symptoms, (3) resolution of infiltration shadows on pulmonary imaging, and (4) clearance of viral RNA. The latter was defined if a nucleic acid test was negative in at least two sequential respiratory tract samples collected at more than a 24-h interval (National Health Commission & State Administration of Traditional Chinese Medicine of China, 2020). In this study, viral RNA tests on respiratory tract samples were repeated every 2 days starting on the day of admission, and the date of the first negative test of the sequential negative tests was defined as the day of viral RNA clearance.

**Data collection**

The records of patients with COVID-19 infection were extracted from the hospital electronic medical system. Demographic, clinical, laboratory, imaging, and treatment data were collected and reviewed by trained physicians.

**Statistical analysis**

The statistical analysis was performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as the median with interquartile range (IQR), and categorical variables were expressed as the number and percentage (%). The Breslow test was used to evaluate the impact of demographic and clinical factors on the duration of viral RNA shedding. Variables with a p-value of less than 0.05 in the univariate test were included in the multivariable Cox regression model. A p-value of less than 0.05 (two-tailed) was considered statistically significant.

**Results**

**Baseline characteristics**

Overall, 284 patients were admitted to the hospital during the study period, accounting for 82% of the confirmed cases in Guangzhou (n = 347) during this period. After excluding two patients who recovered without a valid viral RNA clearance date, two patients who were transferred to the hospital with viral RNA shedding, and 13 asymptomatic individuals whose date of illness onset was not clear (median duration of viral shedding was 5 days (IQR 3–9 days) since the first positive test), a total of 267 patients were included in the current study (Figure 1). The median age was 49 years (IQR 34–62 years), and three patients were adolescents (age <16 years). A total of 121 (45.3%) patients were male, and 24 (9.0%) patients were smokers. The estimated median incubation period was 6 days (IQR 4–9 days), and the median time lag from illness onset to hospital admission was 4 days (IQR 2–7 days).

Among these patients, 166 (62.2%) had a history of exposure to the area of the epicenter (Hubei Province) or close contact with people from Hubei. Of the included patients, 105 (39.3%) had at least one comorbidity, including hypertension (21.3%), diabetes (8.6%), cardiovascular disease (7.5%), and malignancy (1.9%). Fever (67.0%) and cough (58.4%) were the most common symptoms. The majority of patients (66.3%) had a normal body temperature at the time of hospital admission (Table 1). More than half of the patients (60.3%) had elevated D-dimer levels, and 37.6% had elevated procalcitonin levels. A decreased lymphocyte count was observed in 33.7% of patients (Table 2). According to the severity of clinical manifestations and chest imaging, 26 (9.7%) patients were considered to be mildly ill, 197 (73.8%) moderately ill, 40 (15.0%) seriously ill, and four (1.5%) critically ill. No significant differences were observed in disease severity based on sex or smoking status.
Older age, comorbid disease, and delayed hospital admission were more likely to result in serious and critical illness (Supplementary Material, Table S1).

Treatment

Most patients received antibiotic treatment (75.3%) and oxygen therapy (66.7%). Two (1.4%) patients received mechanical ventilation, and 22 (8.2%) patients were admitted to the ICU. Twenty-nine (10.9%) patients were treated with corticosteroid. Lopinavir/ritonavir was the most frequently used antiviral drug and was used in 28.1% of patients, followed by arbidol (24.7%), oseltamivir (13.1%), and chloroquine (9.0%) (Table 3).

Outcomes and viral RNA shedding patterns

At the time of reporting (March 20, 2020), 264 symptomatic patients (99.6%) had recovered and had been discharged from the hospital, with a median hospital stay of 18 days (IQR 13–25 days, range 6–46 days). One patient died without viral RNA shedding, and the remaining two patients without viral RNA shedding were transferred to another hospital. The median duration of viral RNA shedding after illness onset was 12 days (IQR 8–16 days, maximum 45 days). Accumulatively, 20.2% of patients achieved viral RNA clearance within 7 days, 65.2% within 14 days, 88.8% within 21 days, and 94.4% within 28 days after the onset of illness (Figure 2).

Factors and therapeutic regimens associated with the duration of viral shedding

We compared the duration of viral shedding by demographic and clinical characteristics. As shown in Tables 1–3, the time lag from illness onset to hospital admission, older age, disease severity, diarrhea as a symptom, high lactic acid level, reduced serum albumin level, ICU care, oxygen therapy, use of antibiotics, corticosteroid treatment, and use of lopinavir/ritonavir were associated with prolonged viral shedding in the univariate analysis. However, in the multivariable Cox regression analysis, only age, time lag between illness onset and hospital admission, diarrhea as a symptom, corticosteroid treatment, and lopinavir/ritonavir use remained significantly associated with delayed viral RNA clearance (Table 4).

Discussion

This study is novel in investigating the whole course of viral shedding in a relatively large sample size COVID-19 patients. In this retrospective cohort, 88.8% of patients with COVID-19 achieved viral RNA clearance within 21 days after the onset of illness, and 94.4% within 28 days after the onset of illness. Older age, longer time from the onset of illness to hospital admission, diarrhea as a symptom, corticosteroid use, and lopinavir/ritonavir use were associated with delayed clearance of viral RNA. Findings from this study have important implications for optimizing treatment options and public control strategies.

Older age and the time lag from illness onset to hospital admission were two major determinants of the persistence of viral RNA. Although the immune system weakens with aging (Sadighi Akha, 2018), delayed hospital admission might result in disease progression, especially in older patients. Indeed, more than 34% of patients in this cohort who were 65 years or older experienced serious or critical illness. Therefore, special attention should be given to older patients who tend to have more severe illness.
Table 1
Baseline clinical characteristics and duration of viral RNA shedding in patients with COVID-19.

| Characteristic                                      | Median (IQR) value or number (%) | Median (IQR) duration of viral shedding since illness onset (days) | p-Value |
|-----------------------------------------------------|----------------------------------|---------------------------------------------------------------|---------|
| Age (years)                                         |                                   |                                                               |         |
| <16                                                 | 49 (34–62)                        | 8.0 (6.0–15.0)                                               | 0.018*  |
| 16–49                                               | 131 (49.1)                        | 11.0 (7.0–15.0)                                             |         |
| ≥65                                                 | 91 (33.3)                         | 13.0 (10.0–17.0)                                            |         |
| Sex                                                 |                                   |                                                               |         |
| Female                                              | 146 (54.7)                        | 12.0 (9.0–17.0)                                             | 0.985   |
| Male                                                | 121 (45.3)                        | 12.0 (8.0–16.0)                                             |         |
| Smoking                                             |                                   |                                                               |         |
| No                                                  | 243 (91.0)                        | 12.0 (9.0–17.0)                                             | 0.447   |
| Yes                                                 | 24 (9.0)                          | 12.0 (6.0–15.8)                                             |         |
| History of exposure to Hubei or close contact with people from Hubei | |                                                               | 0.196   |
| No                                                  | 101 (37.8)                        | 12.0 (7.0–16.5)                                             |         |
| Yes                                                 | 166 (62.2)                        | 12.0 (10.0–16.3)                                            |         |
| Estimated incubation period (days)                  | 6.0 (4.0–9.0)                     | 12.5 (10.0–16.0)                                            | 0.383   |
| <7                                                  | 206 (77.2)                        | 12.0 (7.0–16.5)                                             |         |
| ≥7                                                  | 47 (17.6)                         | 12.0 (7.8–17.0)                                             |         |
| NA                                                  | 14 (5.2)                          | 12.0 (7.8–17.0)                                             |         |
| Time lag from illness onset to hospital admission (days) | 4.0 (2.0–7.0)                     | 10.0 (6.0–15.0)                                             | <0.001* |
| <4                                                  | 113 (49.8)                        | 14.0 (11.0–17.0)                                            |         |
| ≥4                                                  | 134 (50.2)                        | 14.0 (11.0–17.0)                                            |         |
| Severity of disease                                 |                                   |                                                               | <0.001* |
| Mild                                                | 26 (9.7)                          | 10.0 (6.0–15.0)                                             |         |
| Moderate                                            | 197 (73.8)                        | 12.0 (8.0–16.0)                                             |         |
| Serious                                              | 40 (15.0)                         | 14.0 (12.0–23.5)                                            |         |
| Critical                                             | 4 (1.5)                           | 32.0 (16.0–39.0)                                             |         |
| Comorbidities                                       |                                   |                                                               | 0.178   |
| No                                                  | 162 (60.7)                        | 12.0 (7.0–16.3)                                             |         |
| Yes                                                 | 105 (39.3)                        | 13.0 (10.0–16.5)                                            |         |
| Hypertension                                        |                                   |                                                               | 0.415   |
| No                                                  | 210 (78.7)                        | 12.0 (8.0–17.0)                                             |         |
| Yes                                                 | 57 (21.3)                         | 12.0 (10.0–16.0)                                            |         |
| Diabetes                                             |                                   |                                                               | 0.808   |
| No                                                  | 244 (91.4)                        | 12.0 (8.3–16.0)                                             |         |
| Yes                                                 | 23 (8.6)                          | 11.0 (8.0–19.0)                                             |         |
| Cardiovascular disease                              |                                   |                                                               | 0.795   |
| No                                                  | 247 (92.5)                        | 12.0 (8.0–17.0)                                             |         |
| Yes                                                 | 20 (7.5)                          | 12.5 (10.0–15.5)                                            |         |
| Chronic liver disease                               |                                   |                                                               | 0.152   |
| No                                                  | 257 (96.3)                        | 12.0 (8.0–16.0)                                             |         |
| Yes                                                 | 10 (3.7)                          | 16.0 (10.8–27.5)                                            |         |
| Respiratory disease                                 |                                   |                                                               | 0.375   |
| No                                                  | 258 (96.6)                        | 12.0 (8.8–17.0)                                             |         |
| Yes                                                 | 9 (3.4)                           | 12.0 (6.0–13.0)                                             |         |
| Malignancy                                          |                                   |                                                               | 0.551   |
| No                                                  | 262 (98.1)                        | 12.0 (8.0–16.0)                                             |         |
| Yes                                                 | 5 (1.9)                           | 14.0 (10.0–18.5)                                            |         |
| Symptoms and signs                                  |                                   |                                                               |         |
| Maximum temperature on admission (°C)               | 37.0 (36.7–37.8)                  | 12.0 (8.0–15.5)                                             | 0.065   |
| <37.5                                               | 173 (66.3)                        | 12.0 (8.0–15.5)                                             |         |
| 37.5–37.9                                          | 31 (11.9)                         | 13.0 (10.0–16.0)                                            |         |
| 38.0–38.9                                          | 50 (19.2)                         | 15.0 (10.0–20.0)                                            |         |
| ≥39.0                                               | 7 (2.7)                           | 10.0 (10.0–27.0)                                            |         |
| Fever before admission                              |                                   |                                                               | 0.162   |
| No                                                  | 88 (33.0)                         | 12.0 (7.0–16.0)                                             |         |
| Yes                                                 | 179 (67.0)                        | 12.0 (10.0–17.0)                                            |         |
| Cough                                               |                                   |                                                               | 0.313   |
| No                                                  | 111 (41.5)                        | 12.0 (8.0–17.0)                                             |         |
| Yes                                                 | 156 (58.4)                        | 12.5 (9.3–16.8)                                             |         |
|Expectoration                                        |                                   |                                                               | 0.297   |
| No                                                  | 193 (72.2)                        | 12.0 (8.0–17.0)                                             |         |
| Yes                                                 | 74 (27.7)                         | 12.5 (10.0–16.3)                                            |         |
| Fatigue                                             |                                   |                                                               | 0.690   |
| No                                                  | 207 (77.5)                        | 12.0 (8.0–17.0)                                             |         |
| Yes                                                 | 60 (22.5)                         | 12.0 (10.0–16.0)                                            |         |
| Anorexia                                            |                                   |                                                               | 0.846   |
| No                                                  | 220 (82.4)                        | 13.0 (8.8–17.0)                                             |         |
| Yes                                                 | 47 (17.6)                         | 12.0 (10.0–16.0)                                            |         |
| Muscle pain                                         |                                   |                                                               | 0.085   |
| No                                                  | 221 (82.8)                        | 12.0 (8.0–16.0)                                             |         |
| Yes                                                 | 46 (17.2)                         | 14.0 (10.8–19.0)                                            |         |
| Sore throat                                         |                                   |                                                               | 0.433   |
| No                                                  | 233 (87.3)                        | 12.0 (8.0–17.0)                                             |         |
| Yes                                                 | 34 (12.7)                         | 12.5 (10.0–15.5)                                            |         |
Table 1 (Continued)

|                              | Median [IQR] value or number (%) | Median [IQR] duration of viral shedding since illness onset (days) | p-Value |
|------------------------------|----------------------------------|---------------------------------------------------------------|---------|
| **Catarrh symptoms**         |                                  |                                                               |         |
| No                           | 234 (87.6)                       | 12.0 (9.0–17.0)                                               | 0.317   |
| Yes                          | 33 (12.4)                        | 12.0 (8.0–15.0)                                               |         |
| **Headache**                 |                                  |                                                               |         |
| No                           | 236 (88.4)                       | 12.0 (9.0–17.0)                                               | 0.288   |
| Yes                          | 31 (11.6)                        | 11.0 (9.0–15.0)                                               |         |
| **Shortness of breath**      |                                  |                                                               |         |
| No                           | 238 (89.1)                       | 12.0 (8.0–16.0)                                               | 0.122   |
| Yes                          | 29 (10.9)                        | 13.0 (10.0–24.5)                                              |         |
| **Chest distress**           |                                  |                                                               |         |
| No                           | 246 (92.1)                       | 12.0 (9.0–16.0)                                               | 0.594   |
| Yes                          | 21 (7.9)                         | 11.0 (9.0–28.0)                                               |         |
| **Diarrhea**                 |                                  |                                                               |         |
| No                           | 248 (92.9)                       | 12.0 (8.0–16.0)                                               | 0.042*  |
| Yes                          | 19 (7.1)                         | 15.0 (12.0–20.0)                                              |         |
| **Nausea**                   |                                  |                                                               |         |
| No                           | 253 (94.8)                       | 12.0 (8.0–17.0)                                               | 0.671   |
| Yes                          | 14 (5.2)                         | 12.5 (10.0–17.0)                                              |         |
| **Vomiting**                 |                                  |                                                               |         |
| No                           | 260 (97.4)                       | 12.0 (8.8–17.0)                                               | 0.844   |
| Yes                          | 7 (2.6)                          | 13.0 (10.0–16.0)                                              |         |

IQR, interquartile range; NA: not available.
Data are presented as the number (%) for categorical variables and as the median [IQR] for continuous variables. The Breslow test was used to test the difference in duration of viral RNA shedding by demographic and clinical characteristics.

* p < 0.05.

Table 2
Baseline laboratory findings and viral RNA clearance in patients with COVID-19.

|                              | Median [IQR] value or number (%) | Median [IQR] duration of viral shedding since illness onset (days) | p-Value |
|------------------------------|----------------------------------|---------------------------------------------------------------|---------|
| **WBC count (×10⁹/L)**       |                                  |                                                               |         |
| <3.5                        | 5.19 (4.16–6.39)                 | 17.0 (9.3–32.5)                                              | 0.182   |
| 3.5–9.5                     | 55 (20.6)                       | 12.0 (8.3–16.0)                                              |         |
| >9.5                        | 200 (74.9)                      | 12.0 (8.0–16.0)                                              |         |
| Neutrophil count (×10⁹/L)    |                                  |                                                               |         |
| <1.8                        | 3.02 (2.18–3.93)                 | 12.0 (8.5–19.5)                                              | 0.430   |
| 1.8–6.1                     | 33 (12.5)                       | 12.0 (8.0–16.0)                                              |         |
| >6.1                        | 214 (81.1)                      | 14.0 (10.0–27.5)                                             |         |
| Lymphocyte count (×10⁷/L)    |                                  |                                                               |         |
| <111                        | 1.37 (1.00–1.94)                 | 10.0 (8.0–15.0)                                              | 0.426   |
| 1.1–3.2                     | 90 (33.7)                       | 12.5 (9.0–16.0)                                              |         |
| >3.2                        | 170 (63.7)                      | 12.0 (8.0–17.0)                                              |         |
| Platelet count (×10⁹/L)      |                                  |                                                               |         |
| ≥125                        | 195 (159–246)                   | 12.0 (8.0–16.0)                                              | 0.928   |
| <125                        | 251 (94.0)                      | 12.0 (8.0–17.0)                                              |         |
| Hemoglobin (g/dl)            |                                  |                                                               |         |
| ≥12.5                       | 13.8 (12.4–15.5)                | 12.0 (9.0–16.0)                                              | 0.575   |
| <12.5                       | 223 (83.8)                      | 11.0 (10.3–18.8)                                             |         |
| ALT (U/L)                    |                                  |                                                               |         |
| ≤40                         | 206 (82.7)                      | 12.0 (8.0–17.0)                                              | 0.483   |
| >40                         | 43 (17.3)                       | 13.0 (10.0–17.0)                                             |         |
| AST (U/L)                    |                                  |                                                               |         |
| ≤35                         | 18.6 (15.1–26.3)                | 12.0 (9.0–16.0)                                              | 0.316   |
| ≥35                         | 215 (86.3)                      | 13.0 (8.8–20.0)                                              |         |
| Creatine kinase (U/L)        |                                  |                                                               |         |
| ≤310                        | 52 (36–84)                      | 12.0 (9.0–17.0)                                              | 0.794   |
| >310                        | 242 (97.6)                      | 12.5 (7.5–24.0)                                              |         |
| Procalcitonin (ng/ml)        |                                  |                                                               |         |
| ≤0.05                       | 0.0 (0.0–1.0)                   | 12.0 (9.0–16.0)                                              | 0.496   |
| ≥0.05                       | 161 (62.4)                      | 12.0 (8.0–17.5)                                              |         |
| Albumin (g/l)                |                                  |                                                               |         |
| ≥40                         | 38.9 (33.3–42.1)                | 11.0 (6.5–15.0)                                              | 0.003*  |
| <40                         | 109 (40.8)                      | 13.0 (10.0–17.0)                                             |         |
| Total bilirubin (µmol/l)     |                                  |                                                               |         |
| ≤26                         | 9.9 (6.9–14.7)                  | 12.0 (8.0–16.0)                                              | 0.129   |
| >26                         | 238 (89.1)                      | 14.0 (10.0–20.5)                                             |         |
| Creatine (µmol/l)            |                                  |                                                               |         |
| ≤117 (male), ≤81 (female)    | 63.1 (52.0–76.9)                | 12.0 (8.0–16.0)                                              | 0.242   |
| >117 (male), >81 (female)    | 248 (92.9)                      | 13.0 (10.0–17.0)                                             |         |
| LDH (U/L)                    |                                  |                                                               |         |
| ≤250                        | 190 (155–255)                   | 12.0 (8.0–16.0)                                              | 0.394   |
| >250                        | 199 (74.5)                      | 12.0 (10.0–19.0)                                             |         |
| Lactic acid (mmol/l)         |                                  |                                                               |         |
| ≤1.4                        | 1.8 (1.4–2.2)                   |                                                               | 0.025*  |
Table 2 (Continued)

| Parameter | Median (IQR) value or number (%) | Median (IQR) duration of viral shedding since illness onset (days) | p-Value |
|-----------|----------------------------------|---------------------------------------------------------------|---------|
| ≤22       | 212 (79.4)                       | 12.0 (8.0–16.0)                                              |         |
| >22       | 55 (20.6)                        | 13.0 (10.0–20.0)                                             | 0.730   |
| D-dimer (µg/l) ≤1000 | 1150 (790–1755)         | 12.0 (8.0–16.0)                                              |         |
| >1000     | 96 (39.7)                        | 12.0 (9.0–17.0)                                              |         |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; LDH, lactate dehydrogenase; WBC, white blood cell.

Data are presented as the number (%) for categorical variables and as the median (IQR) for continuous variables. The Breslow test was used to compare the difference in duration of viral RNA shedding by groups.

* p < 0.05.

Table 3

Treatments and duration of viral RNA shedding in patients with COVID-19.

| Treatment           | Number (% of patients) | Median (IQR) duration of viral shedding since illness onset (days) | p-Value |
|---------------------|------------------------|---------------------------------------------------------------|---------|
| ICU care            |                        |                                                              |         |
| No                  | 245 (91.8)             | 12.0 (8.0–16.0)                                              | 0.04*   |
| Yes                 | 22 (8.2)               | 14.0 (12.0–20.5)                                             |         |
| Oxygen therapy      |                        |                                                              |         |
| No                  | 89 (33.3)              | 11.0 (7.0–15.0)                                              | 0.012*  |
| Yes                 | 178 (66.7)             | 13.0 (9.8–17.0)                                              |         |
| Mechanical ventilation |                      |                                                              |         |
| No                  | 265 (99.3)             | 12.0 (8.0–16.0)                                              | 0.068   |
| Yes                 | 2 (0.7)                | 24/37                                                        |         |
| Antibiotics         |                        |                                                              |         |
| No                  | 66 (24.7)              | 11.0 (7.0–15.0)                                              | 0.017*  |
| Yes                 | 201 (75.3)             | 13.0 (9.0–17.0)                                              |         |
| Ribavirin           |                        |                                                              |         |
| No                  | 265 (99.3)             | 12.0 (8.0–16.5)                                              | 0.783   |
| Yes                 | 2 (0.7)                | 10/13                                                        |         |
| Chloroquine         |                        |                                                              |         |
| No                  | 243 (91.0)             | 12 (9.0–16.0)                                                | 0.933   |
| Yes                 | 24 (9.0)               | 13.0 (8.0–17.0)                                              |         |
| Corticosteroid      |                        |                                                              | <0.001* |
| No                  | 238 (89.1)             | 12.0 (8.0–16.0)                                              |         |
| Yes                 | 29 (10.9)              | 18.0 (12.5–27.0)                                             |         |
| Oseltamivir         |                        |                                                              |         |
| No                  | 232 (86.9)             | 12.0 (8.0–16.0)                                              | 0.147   |
| Yes                 | 35 (13.1)              | 15.0 (10.0–17.0)                                             |         |
| Lopinavir/ritonavir |                        |                                                              |         |
| No                  | 192 (71.9)             | 12.0 (8.0–16.0)                                              | 0.034*  |
| Yes                 | 75 (28.1)              | 14.0 (10.0–19.0)                                             |         |
| Arbidol             |                        |                                                              | 0.348   |
| No                  | 201 (75.3)             | 12.0 (8.0–16.0)                                              |         |
| Yes                 | 66 (24.7)              | 13.0 (9.5–17.3)                                              |         |

ICU, intensive care unit; IQR, interquartile range. The Breslow test was used to compare the difference in time to viral RNA clearance by groups.

* p < 0.05.

Corticosteroid treatment has been reported to be associated with prolonged viral RNA shedding in patients with Middle East respiratory syndrome (MERS) (Arabi et al., 2018) and SARS (Lee et al., 2004) in observational studies. Xu et al. reported that corticosteroid use was also associated with prolonged viral RNA shedding in patients with COVID-19, although the effect was rendered insignificant in the multivariable models (Xu et al., 2020), probably due in part to an insufficient sample size to detect the difference. In line with previous reports (Russell et al., 2020; Shang et al., 2020), the present analysis indicated that corticosteroid use was independently associated with delayed viral RNA clearance even after controlling for major confounders, including age and disease severity, calling for their cautious use in patients with COVID-19.

Lopinavir/ritonavir is a fixed-dose combination of anti-HIV protease inhibitors acting against the viral 3CL protease (Nukoolkarn et al., 2008). Although it has been used to treat COVID-19 (National Health Commission & State Administration of Traditional Chinese Medicine of China, 2020), no benefits were observed with lopinavir/ritonavir compared with standard care in a controlled clinical trial involving patients with severe COVID-19 in Wuhan (Cao et al., 2020). Our findings are in line with those of the controlled trial. Moreover, apart from a lack of benefit, the use of lopinavir/ritonavir tended to delay viral RNA clearance, even after controlling for confounding factors, including disease severity. The slightly different findings between these two studies might be explained by the fact that most patients in our cohort had moderate illness. In support of our perspective, the median duration of viral RNA shedding was significantly longer in cases of mild-to-moderate illness treated with lopinavir/ritonavir (14.0 days [IQR 8.3–18.8] vs 11.0 days [IQR 8–15]; p = 0.043), but not in cases of serious-to-critical illness (14.0 days [IQR 12.0–32.0] vs 15.0 days [IQR 12.0–22.0]; p = 0.604). Additionally, because of a lack of study controls, we could not rule out the possibility that some unadjusted potential confounding factors biased the findings. Most recently, a randomized controlled trial indicated the superior benefits of triple combination of interferon beta-1b, lopinavir/
ritonavir, and ribavirin compared with lopinavir/ritonavir alone for alleviating symptoms, shortening the hospital stay, and reducing the duration of viral shedding in patients with mild-to-moderate COVID-19 infection (Hung et al., 2020). Therefore, the findings regarding the negative effect of lopinavir/ritonavir on viral RNA clearance should be interpreted with caution. Further randomized controlled trials are needed to confirm this finding.

Emerging data have reported diarrhea as the most common gastrointestinal manifestation of COVID-19 and have found an association with prolonged viral shedding (Cheung et al., 2020; Pan et al., 2020). Additionally, a recent study involving 232 confirmed COVID-19 patients in China revealed that patients with diarrhea as a symptom had a higher risk of progression to severe disease compared with their counterparts (Wan et al., 2020). Likewise, the present study identified 7.1% of patients with diarrhea, which was independently associated with delayed viral RNA clearance, even after controlling for major confounders. The detailed mechanisms of prolonged viral RNA shedding in patients with diarrhea remain unclear. The SARS-CoV-2 receptor, angiotensin-converting enzyme 2 (ACE2), is abundantly expressed in the epithelial cells of the lung and intestinal enterocytes (Lamers et al., 2020; Hamming et al., 2004). Therefore, the prolonged viral RNA shedding associated with diarrhea as a symptom might reflect a higher viral burden in these patients. Given the growing number of patients with COVID-19 infection, our data suggest that particular attention should be given to patients with diarrhea as a symptom.

In this study, the majority of patients had viral RNA clearance within 3 weeks, which is consistent with other reports (Hu et al., 2020; Xu et al., 2020). However, a small proportion of patients had viral shedding for more than 1 month, including patients with mild illness. Although detectable viral RNA does not mean transmissibility, prolonged viral shedding reinforces the importance of continued isolation for recovered patients. In support of this perspective, the Chinese guidelines suggest at least 14 days of continued isolation for COVID-19 patients after discharge, which has been found to have a satisfactory effect on reducing further transmission (National Health

Figure 2. Cumulative viral RNA clearance rate by severity of COVID-19. The diagram shows the cumulative clearance rate within 7 days, 14 days, 21 days, 28 days, 35 days, and 42 days. The number (%) of patients is presented.
Commission & State Administration of Traditional Chinese Medicine of China, 2020).

The median duration of viral shedding after the onset of illness was 12 days in this study. In comparison, Xu et al. (2020) reported a duration of 15 days, which might reflect the less severe nature of the disease in our patients. Additionally, the difference might be attributed to the shorter time from onset of illness to hospital admission in our study, at 4 days (IQR 2–7 days), compared with the 5 days (IQR 3–8 days) reported by Xu et al. The shorter duration of viral shedding in our study emphasizes the importance of early detection and timely supportive care for symptomatic patients with COVID-19.

This study has some limitations. First, the viral RNA test was repeated every 2 days after hospitalization. Therefore, there might be some bias in the duration of viral RNA shedding. Second, owing to the small sample size and the observational nature of the study, the data regarding the efficacy of treatment with antiviral drugs should be interpreted with caution. Third, the majority of patients had moderate illness and the results might not apply to patients with serious or critical illness. Lastly, this study was conducted as a retrospective single-center study. However, the hospital managed more than 80% of the confirmed COVID-19 cases in the Guangzhou area and the findings from this cohort should represent the epidemic features of COVID-19 in that area.

In conclusion, older age, delayed hospital admission, diarrhea as a symptom, corticosteroid use, and lopinavir/ritonavir use were independently associated with prolonged SARS-CoV-2 RNA shedding in the respiratory tract. The study findings reinforce the importance of early detection and timely supportive treatment for patients with COVID-19, especially for older patients and patients with diarrhea as a symptom. Corticosteroid use was associated with delayed clearance of viral RNA and should be used with caution. Lopinavir/ritonavir use might be associated with delayed SARS-CoV-2 RNA clearance in patients with non-severe illness. However, this finding should be interpreted with caution and further randomized controlled studies are needed to confirm this finding.

Author contributions

XC, BZ, WH, JW, and YZ designed the study. XC, JZ, WH, XH, JC, HZ, SQ, and JW contributed to disease diagnosis and treatment and data collection. XC, BZ, and YZ analyzed the data. XC, BZ, WH, JC, JW, and YZ wrote the manuscript. All authors contributed to the discussion, and reviewed and approved the final version of the manuscript. JW and YZ finalized the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.06.091.

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