Received: 23 June 2020 | Accepted: 10 July 2020

DOI: 10.1002/jmv.26307

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Association between detectable SARS-COV-2 RNA in anal swabs and disease severity in patients with coronavirus disease 2019

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Funding information
Infectious Disease Specialty of Guangzhou High-level Clinical Key Specialty, Grant/Award Number: 2019-2021; Chinese 13th Five-Year National Science and technology major project, Grant/Award Number: 2018ZX10302103-002

Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA was found in the intestines and feces, but its clinical significance is not completely clear. We aim to characterize the longitudinal test results of SARS-CoV-2 RNA in anal swabs and to explore the association with disease severity.

Methods: We included laboratory-confirmed coronavirus disease 2019 (COVID-19) patients, who were hospitalized in Guangzhou Eighth People's Hospital and excluded those who had not received anal swabs for SARS-COV-2 RNA testing. Epidemiological, clinical, and laboratory data were obtained. Throat swabs and anal swabs were collected periodically for SARS-COV-2 RNA detection.

Results: Two hundred and seventeen eligible patients (median aged 50 years, 50.2% were females) were analyzed. 21.2% (46/217) of the patients were detected with SARS-CoV-2 RNA in anal swabs. The duration of viral RNA was longer, but the viral load was lower in anal swabs than throat swabs in the early stage of the disease. During a median follow-up of 20 days, 30 (13.8%) patients were admitted to the intensive care unit (ICU) for high-flow nasal cannula or higher-level oxygen support measures to correct hypoxemia. Detectable viral RNA in anal swabs (adjusted hazard ratio [aHR], 2.50; 95% confidence interval [CI], 1.20-5.24), increased C-reactive protein (aHR, 3.14; 95% CI, 1.35-7.32) and lymphocytopenia (aHR, 3.12; 95% CI, 1.46-6.67) were independently associated with ICU admission. The cumulative incidence of ICU admission was higher among patients with detectable viral RNA in anal swabs (26.3% vs 10.7%, $P = .006$).

Abbreviations: ACE2, angiotensin-converting enzyme 2; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AS, anal swab; AST, aspartate aminotransferase; bpm, beats per minute; COVID-19, coronavirus disease 2019; Ct, cycle threshold; ECMO, extracorporeal membrane oxygenation; FiO2, oxygen concentration; GI, gastrointestinal; HR, hazard ratio; ICU, intensive care unit; IQR, interquartile range; LPV/r, lopinavir/ritonavir; N, nucleocapsid protein; ORF1ab, open reading frame 1ab; PaO2, oxygen partial pressure; RNA, ribonucleic acid; RT-PCR, reverse-transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, sequential organ failure assessment; SaO2, oxygen saturation; TS, throat swab.

Conclusion: Detectable SARS-CoV-2 RNA in the digestive tract was a potential warning indicator of severe disease.

KEYWORDS
anal swab, COVID-19, disease severity, SARS-CoV-2 RNA

1 | BACKGROUND

During the last few months, coronavirus disease 2019 (COVID-19) has become a global public health threat and caused millions of infections and deaths.[1] Research has revealed that the pathogen is a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belonging to the same family of viruses responsible for the SARS and the Middle East respiratory syndrome (MERS).[2-4] Although most cases are mild with a good prognosis, the mortality rate of severe patients is considerable.[5] At present, no vaccines or specific antiviral drugs are available for prevention or treatment of COVID-19. Early differentiation of severe cases from mild cases is very helpful to reduce the mortality rate. However, effective early warning indicators of severe disease are still lacking so far.

SARS-CoV-2 RNA can be detected not only in the respiratory tract, but also in the blood, digestive tract, and feces.[6-8] Several studies have found that the positive rate of SARS-CoV-2 RNA in anal swabs (AS) is higher than that in nasopharyngeal swabs and sputum samples during convalescence,[9-11] suggesting that SARS-CoV-2 might actively infect and replicate in the gastrointestinal (GI) tract.[12] Wong et al[13] found patients with more severe disease tended to have a higher detection rate of fecal SARS-CoV-2 RNA. Our previous study suggests that detectable SARS-CoV-2 RNA in blood is an indicator of further clinical severity.[14] So, the relationship of viral load between the respiratory tract and digestive tract, and its association with the severity of the disease is still unclear.

In this study, we aimed to characterize the longitudinal test results for SARS-CoV-2 RNA in the digestive tract and to explore the association between detectable viral RNA and disease severity in patients with COVID-19.

2 | METHODS

2.1 | Study population

Guangzhou Eighth People's Hospital is one of the designated hospitals for patients with COVID-19 and hospitalized around 85% of the confirmed cases in Guangzhou. All patients were diagnosed with COVID-19 by means of reverse-transcriptase polymerase chain reaction (RT-PCR) assayed in throat swabs (TSs) before hospitalization, according to World Health Organization interim guidance and the new coronavirus pneumonia prevention and control program (in Chinese).[15,16] We retrospectively included laboratory-confirmed cases with COVID-19 from 20 January to 20 February 2020, and excluded patients who did not have AS tests during hospitalization. Patients were followed up until 1 June 2020, or the day when patients recovered and discharged from hospital, or were transferred to the designated hospital for critically ill patients, or died. This study was approved by the Ethics Committee of Guangzhou Eighth People's Hospital. Written informed consent was obtained from all screened patients.

2.2 | Virological detection

TSs and ASs were collected periodically for SARS-COV-2 RNA detection. Virological detection was carried out on the platform of Da'an Gene Corporation, Sun Yat-sen University, Guangzhou, China. Viral RNA extraction and RT-PCR were performed following the standard protocol.[14,17] Viral RNA was extracted with a Nucleic Acid Isolation Kit on an automatic workstation Smart 32, both being provided by Da'an Gene Corporation, Sun Yat-sen University. A 200 μL sample was used for extraction following the standard protocol, and viral RNA was finally eluted with 60 μL elution buffer. RT-PCR reagent was used following the RNA extraction. Two PCR primer and probe sets, targeting open reading frame 1ab (ORF1ab, forward primer: CCCTGTGGGTTTTACACTTAA; reverse primer: ACGATTGTGCATCAGCTGA; fluorescent probe: 5′-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3′) and nucleocapsid protein (N, forward primer: GGGGAACTTCTCCTGCTAGAAT; reverse primer: CAGACATTTTGCTCTCAAGCTG; fluorescent probe: 5′-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3′) separately, were added into the same reaction.[18,19] Positive and negative controls were included for each batch of detection. A cycle threshold value (C_t value) of 40 or more for both genes was defined as negative, and a C_t value of less than 40 for both genes was defined as positive. Samples with a single C_t value less than 40 required confirmation by retesting.

2.3 | Data collection

The medical records, nursing records, and laboratory reports of patients were analyzed to obtain data of demographic status (eg, age and gender), underlying comorbidities (eg, diabetes, hypertension, and cardiovascular disease), epidemiological characteristics (eg, recent exposure history), clinical symptoms and signs (eg, fever, cough, and dyspnea), laboratory findings (eg, complete blood count, coagulation test, and blood chemistry), chest computed tomographic scans and treatment measures (eg, antiviral therapy, corticosteroid therapy, and respiratory support). Data were entered into a computerized database and reviewed by a trained team of physicians.

2.4 | Definition

On the basis of the new coronavirus pneumonia prevention and control program promulgated by the National Health Commission of China, patients were divided into four clinical classifications.[16] Mild status was defined as having mild clinical symptoms but no signs of pneumonia on imaging. Moderate status was defined as having fever and respiratory symptoms, and/or signs of pneumonia on imaging. Severe status must meet any of the following conditions (a) respiratory rate (RR)≥ 30 breaths/min; (b) finger oxygen saturation (SaO_2) at rest ≤93%; (c) arterial blood oxygen partial pressure (PaO_2)/oxygen concentration (FiO_2) ≤300 mm Hg (1mm Hg = 0.133kPa). Critical status must meet any of the following conditions: (a) respiratory failure requiring mechanical ventilation; (b) shock; (c) patients with another organ functional failure need to be admitted to intensive care unit (ICU) for treatment.

2.5 | Statistical analysis

Data were expressed as counts and percentages for categorical variables and as mean and standard deviation or median and interquartile range (IQR) for continuous variables. Qualitative and quantitative differences between subgroups were analyzed using the χ^2 test or Fisher's exact tests for categorical parameters and Student *t* test or Mann-Whitney test for continuous parameters, as appropriate. Cox regression models were performed to evaluate the association between baseline parameters and ICU admission. The log-rank test was performed to examine differences in the risk of ICU admission. All statistical tests were two-sided. Statistical significance was taken as $P < .05$. All analyses were performed with SPSS software, version 26.0 (IBM, Armonk, NY).

3 | RESULTS

3.1 | Patient characteristics

From 20 January to 20 February 2020, 297 laboratory-confirmed patients with COVID-19 were hospitalized in Guangzhou Eighth People's Hospital. After excluding 80 patients who did not receive AS tests during hospitalization, 217 patients were included in this study. The median age was 50 years (IQR, 36-63), 109 (50.2%) were female and 148 (68.2%) were imported cases. The median duration from disease onset to hospital admission was 4 days (IQR, 2-7). Ninty (41.5%) patients had one or more comorbidities including hypertension (49 [22.6%]), diabetes (17 [7.8%]), cardiovascular disease (9 [4.1%]), chronic liver disease (15 [6.9%]), chronic kidney disease (5 [2.3%]), pulmonary tuberculosis (3 [1.4%]), and other comorbidities (28 [12.8%]). AS tests for SARS-CoV-2 RNA were performed at a median of 8 days (QIR, 4-19) after admission, and the results indicated 21.2% (46/217) of patients were detectable. A total of 52 (24.0%) patients presented with at least one GI symptoms on admission, including anorexia (38 [17.5%]), diarrhea (17 [7.8%]), nausea (9 [4.1%]), vomiting (4 [1.8%]), and abdominal pain (3 [1.4%]). During hospitalization, 66 (30.4%) patients had occurrence of GI symptoms, including anorexia (33 [15.2%]), diarrhea (30 [13.8%]), abdominal pain (12 [5.5%]), nausea (3 [1.4%]), and vomiting (2 [0.9%]). The prevalence of GI symptoms that occurred during hospitalization was higher in patients with detectable than those with undetectable SARS-CoV-2 RNA in ASs (47.8% vs 25.7%, $P = .004$). The difference was mainly contributed by anorexia (26.1% vs 12.3%, $P = .021$) and diarrhea (21.7% vs 11.7%, $P = .080$). Patients with detectable SARS-CoV-2 RNA in ASs had higher levels of systolic and diastolic blood pressure and higher proportions of hypertension (34.8% vs 19.3%, $P = .026$) compared with those with undetectable tests. Other comorbidities like diabetes (6.5% vs 8.2%, $P = .709$), cardiovascular disease (6.5% vs 3.5%, $P = .363$), chronic liver disease (6.5% vs 7.0%, $P = .906$), chronic kidney disease (2.2% vs 2.3%, $P = .947$), and pulmonary tuberculosis (2.2% vs 1.2%, $P = .512$) were comparable between these two groups. Other characteristics between them are shown in Table 1. In brief, the demographic status, epidemiological characteristics, clinical symptoms and signs, laboratory and imaging findings, and treatments were comparable between the two groups.

3.2 | Longitudinal change of AS tests

The longitudinal changes of SARS-CoV-2 RNA in ASs and TSs among the 46 patients who had detectable viral RNA in ASs are shown in Figure 1. Most patients were tested for viral RNA in ASs at intervals of 3 to 6 days. Fifteen patients had AS tests within 3 days after admission, among which 11 presented positive for viral RNA. The median duration from admission to the negative conversion of viral RNA was longer in ASs than in TSs (19 days vs 11 days, $P = .007$). Seventeen (37.0%) patients presented detectable viral RNA in ASs after negative conversion in TSs. Thirteen (28.3%) and 9 (19.6%) out of the 46 patients remained viral RNA detectable in ASs for up to 3 and 4 weeks after admission, respectively. The median cycle threshold (C_t) values (C_t = ORF1ab + N) of the AS tests on admission, week 1, week 2, and week 3 were ($C_t = 39 + 37$), ($C_t = 39 + 37$), ($C_t = 39 + 38$), and ($C_t = 39.5 + 38$), respectively. The median C_t values of the throat swab tests on admission, week 1, week 2, and week 3 were ($C_t = 34 + 31.5$), ($C_t = 35.5 + 34$), ($C_t = 39 + 36.5$), and ($C_t = 38.5 + 35.5$), respectively. The C_t values of the ORF1ab genes in AS tests were higher than those in throat swab tests on admission (39 vs 34, $P = .010$) and week 1 (39 vs 35.5, $P = .069$) (Figure 2A), although not all the differences are statistically significant. Similarly, the C_t values of the N genes in AS tests tended to be higher than those in throat swab tests on admission (37 vs 31.5, $P = .078$) and week 1 (37 vs 34, $P = .056$) (Figure 2B). One week after admission, there was no difference in the C_t values between ASs and TSs (Figure 3).

3.3 | Disease severity and clinical outcomes

Of the 217 hospitalized patients with COVID-19, 201 (92.6%) patients were diagnosed with mild/moderate status, and 16 (7.4%)

TABLE 1 Characteristics of patients with COVID-19, according to SARS-CoV-2 RNA detection in anal swabs[a]

Characteristics	All patients (n = 217)	AS detectable (n = 46)	AS undetectable (n = 171)	*P* value[b]
Age, y	50 (36-63)	53 (41-62)	48 (34-63)	.711
Female sex, n (%)	109 (50.2)	20 (43.5)	89 (52.0)	.302
Cases imported from Hubei, n (%)	148 (68.2)	33 (71.7)	115 (67.3)	.562
Any comorbidity, n (%)	90 (41.5)	23 (50.0)	67 (39.2)	.186
Days from illness onset to admission (day)	4 (2-7)	4 (2-6)	5 (2-7)	.237
Symptoms on admission				
Fever, n (%)	155 (71.4)	33 (71.7)	122 (71.3)	.958
Highest temperature (°C)	38.1 (37.6-38.7)	38.0 (37.5-38.8)	38.1 (37.7-38.6)	.907
Cough, n (%)	131 (60.4)	31 (67.4)	100 (58.5)	.273
Sputum production, n (%)	67 (30.9)	17 (37.0)	50 (29.2)	.315
Dyspnea, n (%)	29 (13.4)	9 (19.6)	20 (11.7)	.164
GI symptoms, n (%)	52 (24.0)	10 (21.7)	42 (24.6)	.691
Diarrhea, n (%)	17 (7.8)	3 (6.5)	14 (8.2)	.709
Abdominal pain, n (%)	3 (1.4)	1 (2.2)	2 (1.2)	.512
Anorexia, n (%)	38 (17.5)	7 (15.2)	31 (18.1)	.645
Nausea, n (%)	9 (4.1)	2 (4.3)	7 (4.1)	.939
Vomiting, n (%)	4 (1.8)	0 (0.0)	4 (2.3)	.581
Other symptoms[c], n (%)	93 (42.9)	18 (39.1)	75 (43.9)	.565
GI symptoms occurred during hospitalization, n (%)	66 (30.4)	22 (47.8)	44 (25.7)	.004
Diarrhea, n (%)	30 (13.8)	10 (21.7)	20 (11.7)	.080
Abdominal pain, n (%)	12 (5.5)	2 (4.3)	10 (5.8)	.693
Anorexia, n (%)	33 (15.2)	12 (26.1)	21 (12.3)	.021
Nausea, n (%)	3 (1.4)	1 (2.2)	2 (1.2)	.512
Vomiting, n (%)	2 (0.9)	1 (2.2)	1 (0.6)	.380
Vital signs on admission				
Respiratory rate (bpm)	20 (18-20)	20 (18-20)	20 (18-20)	.464
Heart rate (bpm)	84 (78-95)	85 (79-94)	84 (78-97)	.910
Systolic pressure (mm Hg)	126 (118-138)	130 (122-143)	125 (117-137)	.035
Diastolic pressure (mm Hg)	82 (75-90)	85 (76-94)	80 (74-89)	.037
Laboratory findings				
C-reactive protein (mg/L)	10 (10-30)	10 (10-24)	10 (10-31)	.439
C-reactive protein ≥10 mg/L, n (%)	90 (41.5)	18 (39.1)	72 (42.1)	.716
Procalcitonin (ng/mL)	0.04 (0.03-0.09)	0.05 (0.04-0.11)	0.04 (0.03-0.09)	.440
<0.05 ng/mL, n (%)	90/153 (58.8)	15/31 (48.4)	75/122 (61.5)	.419
≥0.05 to <0.10 ng/mL, n (%)	28/153 (18.3)	8/31 (25.8)	20/122 (16.4)	...
≥0.10 to <0.50 ng/mL, n (%)	33/153 (21.6)	8/31 (25.8)	25/122 (20.5)	...
≥0.50 ng/mL, n (%)	2/153 (0.1)	0/31 (0.0)	2/122 (1.6)	...
Leukopenia, n (%)	46/192 (24.0)	8/41 (19.5)	38/151 (25.2)	.452
Neutropenia, n (%)	32/192 (16.7)	7/41 (17.1)	25/151 (16.6)	.937
Lymphocytes (10^9/L)	1.3 (1.0-1.9)	1.3 (0.9-1.6)	1.4 (1.0-1.9)	.832
<0.5 10^9/L, n (%)	8/192 (4.2)	4/41 (9.8)	4/151 (2.6)	.116
≥0.5 to <1.0 10^9/L, n (%)	44/192 (22.9)	10/41 (24.4)	34/151 (22.5)	...
≥1.0 10^9/L, n (%)	140/192 (72.9)	27/41 (65.9)	113/151 (74.8)	...
Thrombocytopenia, n (%)	27/192 (14.1)	7/41 (17.1)	20/151 (13.2)	.532
Prothrombin time, s	13.5 (13.1-14.0)	13.5 (13.1-13.9)	13.5 (13.1-14.1)	.646
APTT, s	39.8 (36.5-42.6)	41.2 (37.4-43.6)	40.0 (36.7-44.2)	.296
Total bilirubin, μmol/L	9 (7-14)	10 (7-13)	9 (7-14)	.532
Albumin, g/L	40 (36-43)	40 (36-44)	40 (37-42)	.739
ALT elevation, n (%)	26/197 (13.2)	7/39 (17.9)	19/148 (12.8)	.412

(Continues)

TABLE 1 (Continued)

Characteristics	All patients (n = 217)	AS detectable (n = 46)	AS undetectable (n = 171)	*P* value[b]
AST elevation, n (%)	31/190 (16.3)	8/40 (20.0)	23/150 (15.3)	.478
Increased creatinine, n (%)	34/183 (18.6)	9/37 (24.3)	25/146 (17.1)	.314
Increased creatine kinase, n (%)	20/179 (11.1)	6/38 (15.8)	14/141 (9.9)	.309
Lactate dehydrogenase, U/L	193 (153-246)	187 (150-290)	194 (153-244)	.787
Imaging findings				
Pneumonia, n (%)	179 (82.5)	35 (76.1)	144 (84.2)	.198
Hydrothorax, n (%)	13/208 (6.3)	1/44 (2.3)	12/164 (7.3)	.220
Pulmonary consolidation, n (%)	18/208 (8.7)	1/44 (2.3)	17/164 (10.4)	.090
Treatment				
Oxygen inhalation, n (%)	145 (66.8)	29 (63.0)	116 (67.8)	.540
Antibacterial agents, n (%)	142 (65.4)	29 (63.0)	113 (66.1)	.700
Anticoronavirus treatment				
LPV/r, n (%)	77 (35.5)	21 (45.7)	56 (32.7)	.104
Arbidol, n (%)	93 (42.9)	18 (39.1)	75 (43.9)	.565
Chloroquine phosphate, n (%)	29 (13.4)	5 (10.9)	24 (14.0)	.575
Oseltamivir, n (%)	57 (26.3)	17 (37.0)	40 (23.4)	.063
Glucocorticoid, n (%)	42 (19.4)	7 (15.2)	35 (20.5)	.424
Immunoglobulin, n (%)	28 (12.9)	6 (13.0)	22 (12.9)	.975

Abbreviations: APTT, activated partial thromboplastin time; ALT, alanine aminotransferase; AS, anal swabs; AST aspartate aminotransferase; bpm, beats per minute; COVID, novel coronavirus (SARS-CoV-2)-infected disease; GI, gastrointestinal; IQR, interquartile range; LPV/r, lopinavir/ritonavir.
[a]Data are presented as medians (IQR) or n (%). The increase and decrease of laboratory indicators are compared with the normal range of local laboratory testing.
[b]Qualitative and quantitative differences were analyzed using the χ^2 test or Fisher's exact tests for categorical parameters and the Student *t* test or Mann-Whitney test for continuous parameters, as appropriate. All statistical tests were two-sided.
[c]Other symptoms included myalgia, fatigue, sore throat, and headache.

severe/critical status on admission. During a median follow-up of 20 days (IQR, 14-26), 174 (80.2%) patients were diagnosed with mild/moderate status, and 43 (19.8%) severe/critical status. A total of 30 (13.8%) patients were admitted to ICU for high-flow nasal cannula or higher-level oxygen support measures to correct hypoxemia, among whom 12 (5.5%) patients needed mechanical ventilation and 4 (1.8%) patients used extracorporeal membrane oxygenation (ECMO). 3.7% (8/217) patients were transferred to the designated hospital for critically ill patients in Guangzhou due to the deterioration of their illness. Only one (0.5%) patient (82-year-old male) died of multiple organ failure even though receiving ECMO treatment. As of 1 June 2020, all the remaining 208 (95.9%) patients had recovered and were discharged from Guangzhou Eighth People's hospital.

The disease severity and clinical outcomes between patients with detectable and undetectable SARS-CoV-2 RNA in ASs are shown in Table 2. The proportion of ICU admission was higher in the detectable group than the undetectable group (26.1% vs 10.5%, *P* = .007). In addition, patients with detectable viral RNA in ASs had a higher trend of severe/critical status (on admission, 13.0% vs 5.8%, *P* = .097) and mechanical ventilation (10.9% vs 4.1%, *P* = .074). Patients with detectable viral RNA in ASs had significantly longer duration from admission to positive-to-negative conversion of TSs viral RNA (11 days vs 8 days, *P* = .027) and hospitalization stay (22 days vs 20 days, *P* = .031).

3.4 | Factors associated with ICU admission

Table 3 shows the factors associated with ICU admission during hospitalization. In the univariate Cox regression analysis, age (>60 vs ≤60 years), sex (male vs female), comorbidity (yes vs no), GI symptoms throughout the hospitalization (yes vs no), detectable viral RNA in ASs (yes vs no), C-reactive protein (>10 vs ≤10 mg/L) and lymphocyte count (≤1.0 vs >1.0 × 10^9/L) were associated with ICU admission. In multivariate analysis, detectable viral RNA in ASs (adjusted hazard ratio [aHR], 2.50; 95% confidence interval [CI], 1.20-5.24, *P* = .015), C-reactive protein (aHR, 3.14; 95% CI, 1.35-7.32, *P* = .008), and lymphocyte count (aHR, 3.12; 95% CI, 1.46-6.67, *P* = .003) were independently associated with ICU admission. The 7-day and 14-day cumulative incidence of ICU admission was 23.9% and 26.3% among patients with detectable RNA in ASs, and 8.8% and 10.7% among patients with undetectable RNA in ASs, respectively. The 21-day cumulative incidence of ICU admission was higher among patients with detectable RNA in ASs than patients with undetectable RNA (26.3% vs 10.7%, *P* = .006) (Figure 3).

4 | DISCUSSION

This study found that 21.2% of COVID-19 patients were detectable for SARS-CoV-2 RNA in ASs, and longer duration with lower levels of

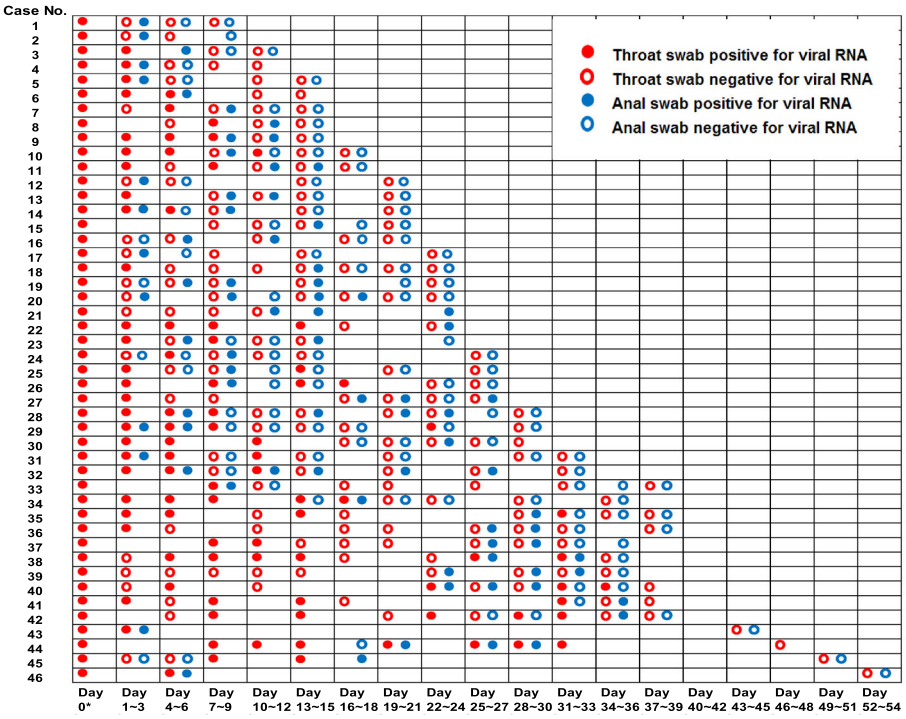

FIGURE 1 Longitudinal results of anal swabs and throat swabs among the 46 patients with detectable SARS-CoV-2 RNA in the anal swab

the virus was found in ASs than in TSs. Patients with detectable viral RNA in ASs had a significantly higher risk of ICU admission. These findings may provide critical information for quickly establishing a COVID-19 hierarchical management system that can greatly reduce the development of severe disease and mortality rates.

The demographic characteristics, clinical symptoms, laboratory index, and imaging findings were not different between patients with ASs positive and those with negative results. However, we found that patients with detectable viral RNA in ASs were more likely to develop GI symptoms such as anorexia and diarrhea. A recent study involving 84 hospitalized health care workers with COVID-19 found the positive rate of viral RNA in stool samples was higher in patients with diarrhea than those without diarrhea (69% vs 17%, $P < .001$).[20] These pieces of evidence together indicate that intestinal infection of novel coronavirus is related to the GI symptoms in COVID-19 patients.

A recent study has found that angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV-2 attachment and entry.[3] Besides the lung, ACE2 is also present in the epithelia of the small

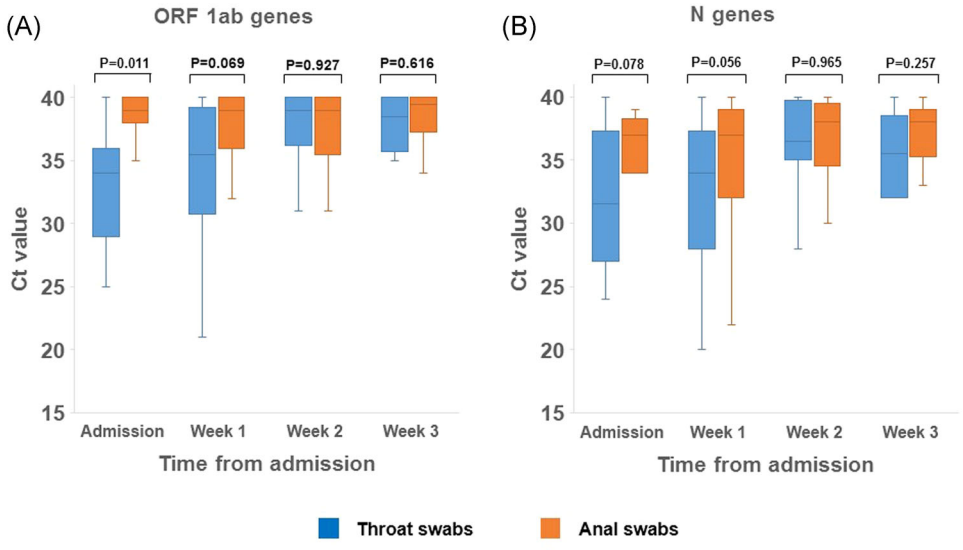

FIGURE 2 Cumulative incidence of intensive care unit (ICU) admission in patients with detectable and undetectable SARS-CoV-2 RNA in the anal swab

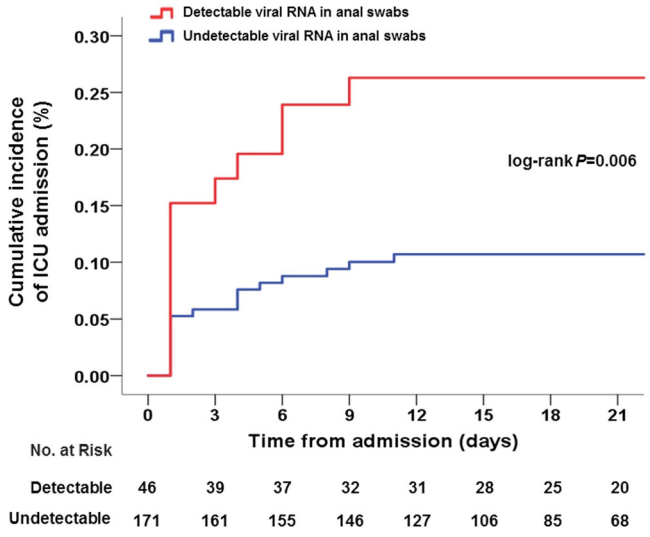

FIGURE 3 Comparision of longitudinal cycle threshold (C_t) values between anal swabs (AS) and throat swabs (TS). A, C_t values of the open reading frame 1ab (ORF1ab) genes. B, C_t values of the nucleocapsid protein (N) genes

intestine and endothelial cells.[21,22] In addition, SARS-CoV-2 RNA has been found in patient feces, and Lu et al further found viral RNA exists in the esophagus, stomach, duodenum, and rectum specimens.[8,23] In this study, we found over one in five patients were detected with viral RNA in ASs. Liu et al observed four patients had detectable SARS-CoV-2 RNA by RT-PCR in ASs from 69 patients who had recovered from COVID-19. Among these four patients, three had positive results in nasopharyngeal swabs, and the positive results on the respiratory tract were observed before the digestive tract.[24] Kipkorir et al summed up the recent evidence of prolonged SARS-CoV-2 detection in anal/rectal swabs and stool specimens in COVID-19 patients after negative conversion in nasopharyngeal RT-PCR test and found that the pooled prevalence estimate for prolonged rectal/anal/stool SARS-CoV-2 RNA was 32%, highlighting the potential of GI shedding of the virus even in asymptomatic patients.[25] Peng et al detected SARS-CoV-2 RNA in urine and blood specimens and anal and oropharyngeal swabs. Although they found symptoms related to infection of these systems may not be present, they still believed testing different specimen types may be useful for monitoring disease changes and progression, and for establishing a prognosis.[26] Our longitudinal study found a positive coloration between ASs and disease severity, further supporting their conclusion. Consistent with previous reports,[9,10,27] we found the duration of viral was longer in ASs than in TSs, suggesting persistent fecal viral shedding and potential fecal-oral transmission.

The transmission ability of the virus is greatly correlated with the viral load. However, little is known about the viral load of SARS-CoV-2 in the digestive tract. In this study, we found the C_t values were higher in ASs than those in TSs in the early stages of COVID-19. The high Ct values in ASs approximately indicated low levels of virus in these specimens.[11] In the middle and later stages of the disease, there was no significant difference in viral load between them, and both tended to be negative. It is suggested that during the recovery period of the disease, the virus in the respiratory tract and digestive tract are gradually eliminated. However, it is still uncertain when the patient will not be contagious. As we only detected the viral RNA but could not isolate the

TABLE 2 Disease severity and clinical outcomes of the studied patients

Variables[a]	All patients (n = 217)	AS detectable (n = 46)	AS undetectable (n = 171)	*P* value
Clinical classifications on admission				
Mild/moderate status, n (%)	201 (92.6)	40 (87.0)	161 (94.2)	.097
Severe/critical status, n (%)	16 (7.4)	6 (13.0)	10 (5.8)	...
Clinical classifications during hospitalization				
Mild/moderate status, n (%)	174 (80.2)	34 (73.9)	140 (81.9)	.229
Severe/critical status, n (%)	43 (19.8)	12 (26.1)	31 (18.1)	...
Admitted to ICU, n (%)	30 (13.8)	12 (26.1)	18 (10.5)	.007
Mechanical ventilation, n (%)	12 (5.5)	5 (10.9)	7 (4.1)	.074
Use of ECMO, n (%)	4 (1.8)	1 (2.2)	3 (1.8)	1.000
Duration from admission to positive-to-negative conversion of TS viral RNA (days)	8 (5-13)	11 (7-16)	8 (5-12)	.027
Duration from admission to improvement of pneumonia (days)	8 (7-14)	11 (7-18)	8 (6-13)	.067
Outcomes				
Transferred for advanced treatment, n (%)	8 (3.7)	4 (8.7)	4 (2.3)	.021
Death, n (%)	1 (3.7)	1 (2.2)	0 (0.0)	...
Recovered and discharge from hospital, n (%)	208 (95.9)	41 (89.1)	167 (97.7)	...
Duration of hospitalization (days)[b]	20 (14-26)	22 (18-30)	20 (13-26)	.031

Abbreviations: AS, anal swabs; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; IQR, interquartile range; TS, throat swabs.
[a]Data are presented as medians (IQR) or n (%).
[b]The calculation of the duration of hospitalization excluded the eight patients who were transferred to the designated hospital for advanced treatment.

TABLE 3 Factors associated with ICU admitted among patients with COVID-19

	Admitted to ICU			
	Univariate		Multivariate[a]	
Variables	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (>60 vs ≤60 y)	2.12 (1.04-4.34)	.040		
Sex (male vs female)	2.16 (1.01-4.61)	.047		
Comorbidity (yes vs no)	3.02 (1.41-6.45)	.004		
GI symptoms[b] (yes vs no)	2.55 (1.17-5.56)	.019		
Detectable viral RNA in AS (yes vs no)	2.64 (1.27-5.49)	.009	2.50 (1.20-5.24)	.015
C-reactive protein (>10 vs ≤10 mg/L)	4.15 (1.85-9.31)	.001	3.14 (1.35-7.32)	.008
Lymphocyte count (≤1.0 vs >1.0 × 10^9/L)	4.57 (2.22-9.42)	<.001	3.12 (1.46-6.67)	.003

Abbreviations: AS, anal swabs; GI, gastrointestinal; HR, hazard ratio; ICU, intensive care unit.
[a]Factors associated with ICU admission were analyzed by the Cox regression model (forward stepwise).
[b]GI symptoms including presence on admission and new occurrences during hospitalization.

live virus, the transmission ability among these patients is still unclear. One of the limitations of PCR testing is the inability to differentiate between actual viral replication and the detection of nonviable, and therefore noninfectious, viral material.[28]

The novel finding of this study was the association between AS test results for SARS-CoV-2 RNA and disease severity. Patients with detectable viral RNA in ASs had a higher cumulative incidence of ICU admission, a sign of disease deterioration. After adjusting for known risk factors including age, sex, comorbidities, GI symptoms, C-reactive protein, and lymphocyte count, this study showed that detectable viral RNA in ASs was independently associated with ICU admission. Patients with detectable viral RNA in ASs had a 2.5 times higher risk of ICU admission than those with detectable viral RNA in ASs. Our previous small sample size, cross-sectional study found that the presence of viral RNA in the blood was positively correlated with disease severity.[14] The present large sample size and follow-up study clarified the relationship between viral RNA in the digestive tract and disease severity. Recent studies have suggested that the disease severity may be related to age, concomitant disease, lymphocytes, interleukin-6, sequential organ failure assessment (SOFA) score, and D-dimer, but the association with virus distribution is unclear so far.[29-31] Although patients with detectable viral RNA in ASs had a higher proportion of hypertension, further analysis shows that hypertension was not independently associated with ICU admission. This study revealed the relationship between the virus in the digestive tract and the severity of COVID-19, highlighting the need to screen the virus in the digestive tract.

The reason why patients with viruses in the digestive tract may have more serious diseases remains largely unknown, one of the possible reasons is the rampant coronavirus replication in the pulmonary alveolus. The actively replicating virus may break through the alveolar vessel leakage into the blood flow, and infect the intestinal epithelium. Compared with patients with undetectable viral RNA, patients with detectable viral RNA in ASs had lower C_t values in the TSs (median: $C_t = 34.5 + 36$ vs $C_t = 39 + 40$), indicating higher viral load. Another possible reason is delayed virus clearance. As mentioned above, SARS-CoV-2 RNA was found in the esophagus, stomach, duodenum, and rectum specimens,[8] due to the large number and wide distribution of ACE2,[21] the digestive tract may serve as an extrapulmonary site for viral replication and storage.[32] These factors may cause delayed elimination of SARS-CoV-2 from the respiratory system, leading to disease progression. We found patients who were positive for viral RNA both in TSs and ASs had delayed clearance of virus, delayed improvement of pneumonia, and longer duration of hospitalization than patients who were positive for viral RNA only in TSs, despite having the same treatment strategy. Further verification is needed.

Our study has some limitations. First, this study mainly summarized from clinical phenomena and laboratory test results, the data regarding cytokine storm and viral genome are lacking. Second, in the early stages of the epidemic, paired and serial specimens of TSs and ASs were not obtained at every time point, and the prevalence of detectable viral RNA in ASs could be underestimated. Third, the prevalence of COVID-19 in Guangzhou is relatively low, and most patients were of non-severe type. Therefore, the number of patients with a serious disease is limited, and the predictors of this study need to be further verified. Fourth, due to the rapid outbreak of the epidemic, we do not have more details about the patient's treatment history. Whether these treatment histories affect the present results needs further investigation.

In summary, we found a longer duration of the virus but lower viral load in the digestive tract than in the respiratory tract, and detectable SARS-CoV-2 RNA in the digestive tract was a potential warning indicator of severe disease. Screening the virus in the digestive tract, close monitoring, and early intervention in patients with the detectable virus are needed.

ACKNOWLEDGMENTS

This study was funded by the Infectious Disease Specialty of Guangzhou High-level Clinical Key Specialty (2019-2021) and the Chinese 13th Five-Year National Science and technology major project (2018ZX10302103-002). The grant support is independent of the investigation process and write-up.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

LL, WC, and WL conceived the study and designed the protocol. XT and YL gave instructions. ZX contributed to statistical analysis and interpretation of data. LL and WL drafted the manuscript. FL, FH, and LL were in charge of nuclear acid RT-PCR. YL and CW reviewed the data independently. YC, XC, and XO contributed to conducting the study and collecting data. All authors had full access to the final version of the manuscript and agreed to its submission.

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How to cite this article: Lin W, Xie Z, Li Y, et al. Association between detectable SARS-COV-2 RNA in anal swabs and disease severity in patients with coronavirus disease 2019. *J Med Virol*. 2021;93:794–802. https://doi.org/10.1002/jmv.26307