Clinical predictive risk factors prolonged the duration of SARS-CoV-2 clearance in 279 moderate COVID-19 patients

A multicenter retrospective cohort study

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Abstract

The results of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid as one of the criteria has been widely applied to assess whether the coronavirus disease 2019 (COVID-19) patients could discharge, however, the risk factors that affect the duration of the SARS-CoV-2 clearance remained to be an enigma. Our research was to identify risk factors correlated with prolonged duration of the SARS-CoV-2 clearance in moderate COVID-19 patients.

We retrospectively analyzed 279 consecutive ordinary COVID-19 patients in 3 hospitals in Hubei province including Huangshi Hospital of Infectious Disease, Wuhan Thunder God Mountain Hospital, and Tongji Hospital. Eight clinical characters were contained as risk factors. We used a logistic regression model and nomogram to assess the possibility that the SARS-CoV-2 nucleic acid may turn negative in 14 days.

Time from symptoms onset to diagnosis (odds ratio [OR] = 3.18; 95% confidence interval [CI] 1.56–6.46; P = .001), time from onset use of antiviral drugs to onset of symptoms (OR = 0.41; 95% CI 0.23–0.72; P = .02), and bacterial coinfection (OR = 0.07; 95% CI 0.01–0.86; P = 0.038) were independent risk factors for the duration of SARS-CoV-2 nucleic acid clearance. The regression model showed good accuracy and sensitivity (area under the curve = 0.96). Nomogram was also provided to predict the negative conversion rate of SARS-CoV-2 nucleic acids within 14 days.

Time from symptoms onset to diagnosis, time from onset use of antiviral drugs to onset of symptoms, and bacterial coinfection were independent risk factors for the time of SARS-CoV-2 nucleic acid turning negative in ordinary COVID-19 patients. However, the age, gender, underlying disease, fungal coinfection, and duration use of antiviral drugs were irrelevant factors.

Abbreviations: ALT = alanine aminotransferase, BCI = bacterial coinfection, COVID-19 = coronavirus disease 2019, DOADU = duration of antiviral drug use, DOSC = duration of SARS-CoV-2 clearance, FCI = fungal coinfection, HLOS = hospital length of stay, NOUD = number of underlying diseases, ROC = receiver operating characteristic, RT-PCR = reverse transcription polymerase chain reaction, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, TFOUADTOO = time from onset use of antiviral drugs to onset of symptoms, TFSOTD = time from symptom onset to diagnosis, UD = underlying disease.

Keywords: COVID-19, retrospective cohort study, risk factor, SARS-CoV-2 virus clearance

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XW, SZ, and CL contributed equally to this work.

Our research was in accordance with the ethical standards of the responsible committee on human experimentation. The study is conducted on already available data. Ethical approval was approved by the Institutional Research Ethics Committee of the Huangshi Hospital of Infectious Disease (Number: HSZY-FJ-2020-002-01, Supplement 1, Supplemental Digital Content, http://links.lww.com/MD2/A539).

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Coronavirus disease 2019 (COVID-19) is an emergent, bursting infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has accounted for the global pandemic since it was first found in Wuhan, Hubei Province, driving to millions of death, extensive healthcare support, and substantial economic losses. SARS-CoV-2 nucleic acids detection is one of the gold standards for COVID-19 diagnosis. Also, negative nucleic acid detection with respiratory tract samples for 2 consecutive times (the sampling interval is at least 24 hours) is one of the judging criteria for recovery or relaxing isolation. Therefore, addressing the risk factors associated with the time of viral excretion is always significant.

There were significant differences among patients with COVID-19 for the viral clearance duration. Part of them kept SARS-CoV-2 nucleic acid long-term positive, others did not. Prolonged viral shedding in the respiratory tract is associated with severe patient outcomes with seasonal influenza and the Middle East Respiratory Syndrome Coronavirus. Kinds of researches are now focusing on the duration of virus clearance, showing that the median duration of SARS-CoV-2 excretion was 14 days. However, the clinical predictive risk factors associated with it are still a controversial issue. Several studies have documented sex, disease severity, APACHE II score, immunoglobulin use, and lymphocyte function were factors associated with a prolonged duration of SARS-CoV-2, while corticosteroid treatment and antiviral therapy did not affect it. Alanine aminotransferase, glucose, and high-density lipoprotein cholesterol were proved to be independent factors that affected the time of nucleic acid turning negative. Identifying the risk factors associated with SARS-CoV-2 clearance duration from clinical characteristics could provide references to assess the recovery or relaxing isolation time.

2. Methods

2.1. Study design and participants

Our retrospective cohort study included 3 cohorts of inpatients from Huangshi Hospital of Infectious Disease, Wuhan Thunder God Mountain Hospital, and Tongji Hospital. All inpatients who have diagnosed with mild COVID-19 according to Guidelines for the Diagnosis and Treatment of Novel Coronavirus (COVID-19) Infection by the National Health Commission (Trial Version 8) (10), and discharged from February 1, 2020 to April, 30 2020 were included in our research (Fig. 1). These 3 hospitals were the COVID-19 designated hospitals in Wuhan and Huangshi, which was the second-worst of the COVID-19 pandemic in Hubei Province, and was also the neighboring city of Wuhan. We excluded those patients with untrustworthy medical history for a long course (≥14 days) before hospital admission, who died in 14 days or had poor medical records during the hospital.

Our research had got ethical approval by the Institutional Research Ethics Committee of the Huangshi Hospital of Infectious Disease (Number: HSZY-PJ-2020-002-01, Supplement 1, Supplemental Digital Content, http://links.lww.com/MD2/A535).

2.2. Data collection

We extracted basic information (gender, age, telephone number, job, and address), medical characteristics, which included number and type of underlying disease, type of COVID-19, date of admission to hospital, date of discharge from hospital, date of symptoms onset, date of diagnosis with COVID-19, date of first time SARS-CoV-2 turning negative, date of the patients first using the antiviral medications, time from onset use of antiviral drugs to onset of symptoms, duration of antiviral use, and other important medical records.
bacterial coinfection, fungal coinfection, and clinical outcomes. All collected information was computerized in Epidata, one of the convenient tools for managing clinical information. All data were first checked by 2 physicians (CL and YQ), then the ombudsman (TH and YZ) would inspect randomly to ensure that data were accurately recorded and correct any difference in interpretation between the 2 primary reviewers.

2.3. Laboratory procedures

Methods for laboratory confirmation of SARS-CoV-2 infection were described as follows: (1) the count of peripheral leucocyte and lymphocyte were declined or normal; the C response protein and erythrocyte sedimentation rate were elevated and calcitininog retained normal. A small part of patients may present with d-dimer elevating; (2) SARS-CoV-2 acid can be detected in nasopharyngeal swabs, sputum, and other lower respiratory secretions, blood, feces, and urine; (3) SARS-CoV-2 specific IgM and IgG antibody were positive, but the positive rate may be low within 1 week of the onset. After alleviation of symptoms of fever, cough, and dyspnoea, we collected nasal swabs specimens every other day for SARS-CoV-2 reverse transcription-polymerase chain (RT-PCR) re-examination, but this was just a qualitative test. If the patients were absent of fever for at least 3 days, the lung computed tomography had improved drastically, the respiratory symptoms relieved, and 2 consecutive reverse transcription-polymerase chain (RT-PCR) re-examination, if the patients were absent of fever for at least 3 days, the lung computed tomography had improved drastically, the respiratory symptoms relieved, and 2 consecutive throat-swab samples negative for SARS-CoV-2 RNA, they were able to be discharged from hospitals.

2.4. Definitions

Fever was defined as the axillary temperature of at least 37.3°C. Ordinary COVID-19 were defined according to Guidelines for the Diagnosis and Treatment of COVID-19: (1) meeting one of the evidence: (i) real-time RT-PCR detection of SARS-CoV acid shows positive, (ii) the tested virus gene is highly homologous to SARS-CoV-2, (iii) serum SARS-CoV-2 IgM and IgG antibody are positive, and (iv) serum SARS-CoV-2 specific IgG antibody in the recovery phase is 4 times or higher than that in the acute phase, (2) patient has the symptoms of cough, expectoration, chest pain, dyspnea, and fever, and (3) chest computed tomography imaging findings of pneumonia.

2.5. Statistical analysis

Statistical analysis was performed using SPSS IBM version 24 and R version 3.6.3. We used binary logistic regression to establish a regression equation between relevant risk factors and the duration of SARS-CoV-2 elimination. All relevant risk factors including gender, age, number of underlying diseases, time from symptom onset to diagnosis, time from the onset use of antiviral drugs to the onset of symptoms, duration of antiviral use, bacterial coinfection, and fungal coinfection were put into the logistic regression equation as a predictor variable in model 1 using Enter method. P value <.1 for each risk factor was considered to be significant. Model 2 was established after removing those insignificant variables that screened in model 1, P value <.05 was defined as statistically significant. A receiver operating characteristic curve with the corresponding area under the curve was performed to assess the fitting degree between 2 models by R packages “AER,” “robust,” “qcc,” “foreign,” and “rms.”

3. Results

3.1. Patients’ characteristics of study

The study finally included 279 patients with a common type of COVID-19, 156 (55.9%) male and 123 (44.1%) female. The mean age (95% CI) was 50.55 (36.07–65.03), most of them were between 18 and 65 (Table 1). 180 (64.5%) patients had underlying

| Characteristics | Estimates |
|-----------------|-----------|
| Gender          |           |
| Male            | 156 (55.0%) |
| Female          | 123 (44.1%) |
| Mean (95% confidence interval) of age (year) | 50.55 (36.07–65.03) |
| <18             | 3 (1.1%)   |
| 18–48           | 123 (44.1%) |
| 49–65           | 99 (33.5%) |
| >65             | 54 (19.3%) |
| UD              |           |
| no              | 180 (64.5%) |
| yes             | 99 (35.5%) |
| NOUDD           |           |
| 1               | 71 (25.4%) |
| 2               | 18 (6.4%)  |
| ≥3              | 0 (0%)     |
| Name of antiviral drugs |       |
| Arbidol         | 216 (77.4%) |
| Recombinant human interferon-α-2b (FN-α-2b) | 216 (77.4%) |
| Ribavirin       | 48 (17.2%)  |
| Hydroxychloroquine Sulfate | 3 (1.1%) |
| Lopinavir/Ritonavir Oral solution | 48 (17.2%) |
| Oxemertvir      | 12 (4.3%)  |
| BCI             |           |
| yes             | 201 (72%)  |
| no              | 78 (28%)   |
| FCI             |           |
| yes             | 6 (2.2%)   |
| no              | 273 (97.8%)|
| Clinical outcomes* |       |
| Cured           | 261 (93.5%) |
| Improved        | 12 (4.3%)  |
| Aggravated      | 6 (2.2%)   |
| Died            | 0 (0%)     |
| Mean (95% confidence interval) of TFSOTD (day) | 4.77 (1.39–10.93) |
| Mean (95% confidence interval) of TFOUADTOOS (day) | 6.22 (1.8–10.64) |
| Mean (95% confidence interval) of DOSC (day)† | 14.03 (6.71–20.74) |
| ≤14 days        | 117 (41.9%) |
| >14 days        | 162 (58.1%) |
| Mean (95% confidence interval) of HLOS (day) | 19.32 (13.11–25.53) |
| Mean (95% confidence interval) of DOADU (day) | 18.03 (10.71–25.35) |

BCI = bacterial coinfection. COVID-19 = coronavirus disease 2019. DOADU = duration of antiviral drug use. DOSC = duration of SARS-CoV-2 clearance, FCI = fungal coinfection. HLOS = hospital length of stay. NOUDD = number of underlying diseases. SARS-CoV-2 = severe acute respiratory syndrome coronavirus. TFOUADTOOS = time from onset use of antiviral drugs to onset of symptoms. TFSOTD = time from symptom onset to diagnosis. UD = underlying disease.

* We defined clinical outcomes as follows: (1) recovery, the following conditions shall be met: (i) the body temperature is normal for 3 consecutive days; (ii) respiratory symptoms disappeared completely; and (iii) the nucleic acid test of respiratory tract samples is negative for 2 consecutive times (the sampling interval is at least 1 day); (2) condition improved, meet any of the following 2 items: (i) the clinical symptoms are significantly relieved and (ii) the nucleic acid of sputum, nasopharynx test paper, and other respiratory tract samples are negative for 2 consecutive times (the sampling interval should be at least 24 hours); and (3) condition worsen, 1 of the following situations occurs: (i) shortness of breath and respiratory rate (RR) ≥30 times/min; (ii) oxygen saturation ≤93% in resting state, (iii) arterial oxygen partial pressure (PaO2)/fraction of inspired oxygen (FiO2) ≤300 mmHg, (iv) shock, (v) respiratory failure and requiring ventilator support, and (vi) organ failure needing intensive care.

† We defined SARS-CoV-2 negative nucleic acid as follows: the nucleic acid test of respiratory tract samples was negative for 2 consecutive times (the sampling time was at least 24 hours).
diseases, among them 71 (25.4%) participants had only 1 kind of basic disease, 18 (6.4%) had 2. All patients had accepted antiviral therapy, Arbidol, and Recombinant human interferon-α-2b were the most common antiviral drug in the 3 hospitals (216 patients vs 216 patients). The second were Ribavirin and Lopinavir/Ritonavir Oral solution (48 patients vs 48 patients). Twelve patients used Osimertiniv, only 3 had received Hydroxychloroquine Sulfate. A 201 (72%) patients got bacterial coinfection, this was much more than patients diagnosed with fungous coinfection (6 for 2.2%); of the 279 participants, 261 (93.5%) were cured, 12 (4.3%) improved, 6 (2.2%) aggravated, and no one died. These participants had a mean (95% CI) time from onset use of antiviral drugs to onset of symptoms of 6.22 (1.8–10.64) day. The average time from symptom onset to diagnosis was 4.77 (95% CI: 3.19–6.34) days, a mean time from diagnosis to SARS-CoV-2 nucleic acid converting negative of 14.03 (6.71–20.74) days, a mean length of stay of 19.32 (13.11–25.53) days, a mean antiviral use duration of 19.32 (13.11–25.53) days.

### 3.2. Association between risk factors and SARS-CoV-2 nucleic acids negative conversion within 14 days before variate adjusted

We got 8 risk factors in the logistic regression model 1 ($R^2=0.87$, $P<.001$), the details were provided in Table 1. It indicated that gender (OR, 0.48; 95% CI, 0.09–2.45, $P=.38$), age (OR, 0.99; 95% CI, 0.93–1.05, $P=.67$), number of underlying diseases (OR, 0.73; 95% CI, 0.17–3.11; $P=.67$), duration of antiviral drug use (OR, 1.13; 95% CI, 0.92–1.38; $P=.237$), and fungal coinfection (OR, 0.00; 95% CI, 0.00–0.00; $P=1.000$) had nothing to do with the duration of SARS-CoV-2 nucleic acids clearance; patients who underwent longer time from symptom onset to diagnosis had a greater risk of prolonged SARS-CoV-2 clearance (OR, 3.18; 95% CI, 1.56–6.46; $P=.001$), however, using antiviral drugs as early as possible after emergence of clinical symptoms (OR, 0.41; 95% CI, 0.23–0.72; $P=.002$) and avoiding bacterial infection (OR, 0.07; 95% CI, 0.01–0.86; $P=.038$) were helpful for SARS-CoV-2 clearance.

### 3.3. Association between risk factors and SARS-CoV-2 nucleic acids negative conversion within 14 days after variate adjusted

After adjusted those 5 factors in logistic regression model 1, we had established logistic regression model 2 ($R^2=0.85$, $P<.001$), the results were shown in Table 2. In this model, time from symptom onset to diagnosis (OR, 3.18; 95% CI, 1.56–6.46; $P=.001$), time from onset use of antiviral drugs to onset of symptoms (OR, 0.41; 95% CI, 0.23–0.72; $P=.002$), and bacterial coinfection (OR, 0.07; 95% CI, 0.01–0.86; $P=.038$) were risk factors associated with prolonged SARS-CoV-2 elimination. It demonstrated that diagnosing and treating COVID-19 as early as possible, preventing bacterial infection were of great importance for shorting the duration of SARS-CoV-2 clearance. In addition, as shown in Figure 2, regression model 2 showed robust accuracy and good sensitivity (area under the curve=0.96), and the predicted probability was consistent with the actual probability (mean absolute error=0.015) (Fig. 3). A chi-square test was run to assess the fitting degree between the 2 models, the result showed that model 2 was as good as model 1 ($P=.97$).

We had established a nomogram based on model 2. According to this nomogram, we could estimate the negative conversion rate of SARS-CoV-2 nucleic acids within 14 days (Fig. 4).
4. Discussion

COVID-19 is still gaining ground around the world, perceptions of factors affecting the duration of viral clearance are still controversial. Thus, we conducted a retrospective cohort study of 279 hospitalized confirmed SARS-CoV-2 infection patients from 3 hospitals in Hubei Province, they were Huangshi Hospital of Infectious Disease, Wuhan Thunder God Mountain Hospital, and Tongji Hospital. All these patients belonged to the common type of COVID-19. We identified that time from symptoms onset to diagnosis, time from onset use of antiviral drugs to onset of symptoms, and bacterial coinfection were independent risk factors associated with the duration of SARS-CoV-2 clearance, while age, gender, number of underlying diseases, time of antiviral use, and fungal coinfection were not.

Recent studies have shown that 74% to 86% of patients hospitalized with COVID-19 were at least 50 years old,[11] the median age was 54 years.[12] Those individuals aged >65 years were more likely associated with severe disease.[13] But in our study, we did not identify age to be an independent risk factor for virus excretion. For 77.6% patients were young adults and midlife (18 ≤ age < 65), they had a good immune response and physiological functions. Previous studies evaluated 25% of COVID-19 patients had comorbidities, while it raised to 60% to 90% of hospitalized patients.[11,14,15] The most common underlying diseases in COVID-19 patients included hypertension (48%–57%), diabetes (17%–34%), and cardiovascular disease (21%–28%). A small proportion had chronic kidney disease (3%–13%), chronic pulmonary disease (4%–10%), malignancy (6%–8%), and chronic liver diseases.[16] COVID-19 patients with more underlying comorbidities above related to worse prognosis[17] and prolonged virus shedding.[18] However, in our retrospective cohort study, this relationship was undiscovered. This indicated the number of underlying diseases might affect patients’ prognosis, but had nothing to do with the virus clearance duration.

As for the association between gender and the duration of SARS-CoV-2 viral shedding, it was a controversial issue. Several recent studies found that male sex was an independent risk factor for prolonged SARS-CoV-2 viral clearance.[7] Men experienced a higher incidence of mortality than females.[19] Imbalanced levels of angiotensin-converting enzyme 2 (ACE2) play an essential role in the sex-based differences in responding to the disease.[20] SARS-CoV-2 has a high-affinity ACE2 receptor in type 2 alveolar epithelial cells. Our study and other similar researches suggested
that gender was not an independent risk factor relevant to the duration of SARS-CoV-2 viral excretion.

To the best of our knowledge, there was no specific antiviral medication for SARS-CoV-2 until now, and most of the drugs undergoing clinical tests with COVID-19 patients were designed for influenza, HIV, Ebola, etc. Guidelines recommended antiviral drugs, including Interferon-alpha, Ribavirin, Hydroxychloroquine Sulfate, and Arbidol. Moreover, the guideline defined that the treatment course was no more than 10 days. Actually, this was not the case in practice. On the one hand, we lacked awareness for this emergent infectious disease at the beginning of the pandemic, as well as antiviral drug use. On the other hand, for most doctors, antiviral drugs may be just placebo treatments. In our study, their was no association between the treatment course of antiviral medications and the duration of virus clearance, which was consistent with previous research. Overall, we needed more studies to reveal the mechanism and metabolic process of antiviral drugs on SARS-CoV-2 in vivo to better understand the effect on virus clearance.

By reviewing former researches, bacterial pathogens were usually found in respiratory viral infectious diseases such as influenza, which was an important reason affecting the prednisone and needs timely diagnosis and therapy. Severe influenza patients with bacterial coinfection rates were about 20% to 30%. In our research, bacterial coinfection was more common (72%) among COVID-19 individuals. In contrast, they had a low rate (2.2%) of fungal infection. And, COVID-19 patients combined with bacterial infection needed a longer duration of SARS-CoV-2 nucleic acid clearance. In patients with influenza, it has been hypothesized that the virus may lead to the damage of the lower airway epithelial cells, coupled with the dysfunction of mucociliary, which can promote the bacteria sucked from the nasopharynx or combine with the cell surface, and cause further damage by inhibiting the repair and regeneration of the epithelial cell layer. Given the knowledge of the pathophysiology of SARS-CoV-2 is still developing, our understanding of the pathogenesis of bacterial and fungal coinfection is incomplete. Whether the above conclusion applies to COVID-19 needs further researches.

Currently, there were few studies about the relationship between time from symptom onset to diagnosis, time from onset use of antiviral drugs to onset of symptoms, and SARS-CoV-2 cleaning time. Our results showed both of them were independent risk factors. We speculated that the longer the time from symptom to diagnosis, the less likely they could receive systematic medical services in the early stage of the disease, such as antiviral and anti-inflammatory treatment. Besides, maybe these people ignored subtle clinical symptoms so that he missed the best therapy opportunity. A cohort study suggested that early and timely antiviral therapy with mild COVID-19 may significantly slow down disease progression and improve prognosis. However, more relevant studies need to be carried out.

A systematic analysis for COVID-19 in different countries and regions showed that the average hospital length of stay for ordinary COVID-19 patients in China was 14 days, longer than 5 days in other countries. This may be due to the differences in diagnosis, discharge standards, and medical resources. SARS-CoV-2 mainly infected respiratory and digestive tract mucosa. Viremia was less likely to occur, and the characteristics of constant virus mutation led to a shorter duration of patients' antiviral immunity, they may be at risk of reinfection. There was a few SARS-CoV-2 reinfection cases that had been reported. Additionally, there was no correlation between the detection results of viral nucleic acid and the symptoms of the disease. For the common type of COVID-19 patients, once ensuring resolution of symptoms and evidence of 2 negative PCR samples at least 24 hours apart, they were recommended to discharge from hospital, and isolated at home if necessary.

Several shortages of this study need to be acknowledged. First, we only observed 279 hospitalizations of ordinary COVID-19 patients and excluded asymptomatic SARS-CoV-2 infected individuals, and the conclusion cannot fully explain the duration of SARS-CoV-2 clearance in all COVID-19 patients. Second, the risk factors included in our study are limited. There may be other related factors we do not detect. Third, during the initial of the pandemic, lack of awareness of COVID-19, and the available reagent for SARS-CoV-2 RNA detection, the real-time from symptoms onset to diagnosis might be shorter.

5. Conclusion
In summary, in this study, we found that diagnosing and treating COVID-19 as early as possible, preventing bacterial infection were of great importance for shorting the duration of SARS-CoV-2 clearance. The age, gender, number of underlying diseases, duration of antiviral use, and fungal coinfection had no impact on SARS-CoV-2 clearance. However, further rigorously designed researches should be conducted to reveal and assess the clinical predictive risk factors relevance of our proposed indicators.

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