Risk factors associated with prolonged viral clearance in patients with a refractory course of COVID-19□a retrospective study

Weitao Zhuang [Equal first author, 1, 2], **Shujie Huang** [Equal first author, 1, 2], **Dongya Wang** [3, 4], **Lulu Zha** [3, 4], **Wei Xu** [5], **Guibin Qiao** [Corresp. 1]

[1] Department of Thoracic Surgery, Guangdong Provincial People's Hospital, Guangzhou, Guangdong, China

[2] Shantou University Medical College, Shantou, Guangdong, China

[3] General Hospital of Southern Theater Command, Chinese People's Liberation Army, Guangzhou, Guangdong, China

[4] Huoshenshan Hospital, Wuhan, China

[5] School of Public Health and Management, Chongqing Medical University, Chongqing, China

Corresponding Author: Guibin Qiao
Email address: guibinqiao@126.com

Background: This study aimed to characterize a cohort of patients with a refractory course of COVID-19, and to investigate factors associated with the duration of viral clearance (DoVC).

Materials & Methods: A total of 68 patients who had ≥2 COVID-19-related admissions were retrospectively enrolled from Huoshenshan Hospital. Univariate analysis and multivariate analysis were performed to examine the potential association between clinicopathologic characteristics and the DoVC.

Results: The median DoVC in the overall study cohort was 48 days (ranged from 11 to 104 days). Multivariate analysis indicated that fever at illness onset [Hazard ratio (HR) = 3.967, 95%CI, 1.583−9.942, p = 0.003], serum level of aspartate aminotransferase > 21.7 IU/L (HR = 3.188, 95% CI, 1.299−7.827, p =0.011), and titer of SARS-CoV-2 IgG > 141.69 (HR = 3.718, 95% CI, 1.553−8.904, p = 0.003) were the three independent risk factors associated with delayed viral clearance.

Conclusion: Identification of risk factors associated with delayed viral clearance helped to provide customized information for clinical practice in patients with a refractory course of COVID-19. The current study suggested a longer observation period and serial follow-up testing after the first discharge are necessary for COVID-19 patients who had fever at illness onset, impaired hepatic function or increased titer of SARS-CoV-2 IgG.

Risk factors associated with prolonged viral clearance in patients with a refractory course of COVID-19: a retrospective study

Weitao Zhuang[1,2], M.D., Shujie Huang[1,2], M.D., Dongya Wang[3,4], B.S.N., Lulu Zha[3,4], B.S.N., Wei Xu, Ph.D.[5], Guibin Qiao[1], M.D., Ph.D.

Affiliations:

1. Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, China;

2. Shantou University Medical College, Shantou 515041, China;

3. Huoshenshan Hospital, Wuhan 430113, China;

4. General Hospital of Southern Theater Command, Chinese People's Liberation Army, Guangzhou, 510010, China

5. School of Public Health and Management, Chongqing Medical University, Chongqing 400016, China

* Weitao Zhuang and Shujie Huang contributed equally to this work

Corresponding to:

Guibin Qiao, M.D., Ph.D.

Department of Thoracic Surgery

Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences

106 Zhongshan Second Road, Guangzhou 510080, China.

Email: guibinqiao@126.com

Abstract

Background: This study aimed to characterize a cohort of patients with a refractory course of COVID-19, and to investigate factors associated with the duration of viral clearance (DoVC).

Materials & Methods: A total of 68 patients who had ≥2 COVID-19-related admissions were retrospectively enrolled from Huoshenshan Hospital. Univariate analysis and multivariate analysis were performed to examine the potential association between clinicopathologic characteristics and the DoVC.

Results: The median DoVC in the overall study cohort was 48 days (ranged from 11 to 104 days). Multivariate analysis indicated that fever at illness onset [Hazard ratio (HR) = 3.967, 95%CI, 1.583−9.942, p = 0.003], serum level of aspartate aminotransferase > 21.7 IU/L (HR = 3.188, 95% CI, 1.299−7.827, p =0.011), and titer of SARS-CoV-2 IgG > 141.69 (HR = 3.718, 95% CI, 1.553−8.904, p = 0.003) were the three independent risk factors associated with delayed viral clearance.

Conclusion: Identification of risk factors associated with delayed viral clearance helped to provide customized information for clinical practice in patients with a refractory course of COVID-19. The current study suggested a longer observation period and serial follow-up testing after the first discharge are necessary for COVID-19 patients who had fever at illness onset, impaired hepatic function or increased titer of SARS-CoV-2 IgG.

Keywords: COVID-19; Readmission; Reinfection; Refractory; Viral Clearance

Introduction

As the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sweeps across the globe, there is rising concern that the patients who recovered from COVID-19 may be at risk of reinfection. It was reported that a small percentage of patients were tested positive again after recovery from a previous episode of COVID-19 infection.[1,2] However, the mechanism of reinfection/reactivation has not been clarified, which created an

uncertainty of the criteria to define clinical and virological recovery.[3] Miscellaneous hypotheses are coming out to address this scientific question, such as false positive results from the leftover genetic material,[4,5] persistence of virus within body, cross-contamination from another virus, incorrect sampling methods,[6] thermal inactivation of samples,[7] impaired immune response,[5] even storage of virus in exosomes and extracellular vesicles.[8] Given the false negative rates were as high as 30%−60% for SARS-CoV-2 PCR testing in upper respiratory tract or sputum specimens,[9,10] it is not easy to distinguish between reinfection and prolonged viral clearance.[11,12] Underlying mechanisms and corresponding patient characteristics of reinfection or prolonged viral clearance may be quite different, which requires personalized treatment strategy. As medical institutions are overwhelmed by the cumulating patients, what kind of care should be provided to reinfected patients, and how to deal with the patients with a refractory course of disease, remain to be answered by both physicians and researchers.

The median duration of viral shedding was reported to be about 20 days in many studies.[13-17] However, prolonged viral clearance is not a rare phenomenon, with the longest duration being 83 days.[18-20] In this regard, a longer period of post-discharged quarantine and medical observation has been suggested.[1,18,20] Identifying the patients who are at risk of reinfection or delayed viral clearance might be the first important step to tackle the abovementioned challenge. This may help with optimization of treatment and prevention of transmission of the disease. Recently, studies are emerging to identify the risk factors associated with longer viral clearance.[14,15,17,21] However, risk factors of prolonged duration of viral clearance (DoVC) in patients with a refractory course of COVID-19 are less clear and required further investigation.

In this study, we characterized a cohort of patients who had ≥2 COVID-19-related hospitalizations, with the purpose to investigate the clinicopathologic risk factors associated with the refractory course of COVID-19.

65 # Patients and Methods

66 **Participants**

67 A total of 68 inpatients from Huoshenshan Hospital between February 8, 2020 to March 31, 2020 were
68 enrolled in this retrospective study. All patients had a previous history of COVID-19-related hospitalization
69 before admitted to Huoshenshan Hospital. The severity of disease was evaluated based on the sixth version of
70 Chinese clinical guideline for COVID-19.[22] Mild cases referred to mild clinical symptom with no sign of
71 pneumonia on imaging. General cases were defined as positive clinical symptoms along with pneumonia on
72 thoracic imaging. Patients who met any of the following criteria were classified as severe cases: respiratory rate
73 $\geq$ 30 per minute; pulse oxygen saturation $\leq$ 93% at resting status; arterial partial pressure of oxygen
74 (PaO2)/oxygen concentration (FiO2) $\leq$ 300 mmHg; patients with > 50% progression of lesion volume within 24
75 to 48 hours on chest imaging. Patients who presented with any of the following conditions were defined as
76 critical cases: Respiratory failure and requiring mechanical ventilation; shock; organ failure that requires ICU
77 care. Patients could be discharged after they met the following criteria[13]: absence of fever for at least 3 days,
78 remission of clinical symptoms or chest imaging, and two consecutive negative results for SARS-CoV-2 RNA
79 in respiratory tract samples obtained at least 24 hours apart. This study was approved by the institutional review
80 boards of Huoshenshan Hospital (No. IEC-AF-SL-02), Guangdong Provincial People's Hospital
81 (No.GDRE20200141H) and General Hospital of Southern Theater Command (No.202039), and was carried out
82 in accordance with the Declaration of Helsinki. Electronic form of informed consents to use their demographic
83 and clinical information were collected from all participants in advance.

84

85 **Data collection**

A standardized sheet for data collection was used to retrieve the demographic, clinical, laboratory and treatment information from electronic medical records. Given the relatively high rate of false negative nucleic acid testing by nasal/pharyngeal swab in the previous studies,[9,10] we raised the standard to define viral clearance, which was in accordance with the abovementioned standard criteria of discharge from hospital.[13] Therefore, the DoVC was calculated from the date of illness onset (as stated in the history of present illness) to the date of discharge from Huoshenshan hospital. All data were double-checked by two researchers (W. Z. and S. H.) independently for accuracy.

Laboratory tests

Respiratory samples were tested for SARS-CoV-2 RNA by the real-time reverse transcriptase polymerase chain-reaction (RT-PCR) test (BioGerm, Shanghai, China), following WHO guideline for qRT-PCR.[23,24] SARS-CoV-2 IgM and IgG antibodies in blood samples were measured using chemiluminescent immunoassay (Shenzhen Yahuilong Biotechnology Co., Ltd) as per manufacturer's the protocol. The titer larger than 10 of either IgM or IgG were considered positive infection for SARS-CoV-2.

Treatment

All patients were provided with necessary supportive care during the first and second hospitalizations, including but not limited to oxygen, antipyretics, antitussive, expectorant, antidiabetic, and antihypertensive agents. For etiological treatment, antivirals, antibiotics, traditional Chinese herbal tea or Chinese patent medicine were used either separately or in combination.

Statistical analysis

Data of baseline characteristics such as demographic information, reasons of readmission, symptoms and signs, comorbidities and treatment were presented as frequency (percentage), while data of laboratory results were presented as median (interquartile range, IQR). The medians of continuous variables were used as cut-off values to separate the patients into two groups for the sake of comparison, except for C-reactive protein and procalcitonin, where the upper limits of normal range were used. Univariate analysis was performed by using log-rank test to evaluate the association of patient characteristics with DoVC. All variables with p values < 0.1 were included in multivariate analysis. Subsequently, log minus log function was used to identify the covariates which did not satisfy the proportional hazard assumption, and these covariates were excluded for the subsequent multivariate analysis. Multivariate analysis was conducted using Cox regression model to identify independent risk factors of prolonged viral clearance. Cases with missing data were excluded from the Cox regression model. A sensitivity analysis was performed to assess the robustness of the results (see Supplementary table S1, S2). Finally, Kaplan-Meier method was used to visualize the impact of these risk factors on the DoVC.

Results

Baseline characteristics

The mean age of patients with a refractory course of COVID-19 was 52.4 years old, and 42 out of 68 patients were male. According to the sixth version of Chinese clinical guideline for COVID-19,[22] 54 out of 68 (79.4%) patients were classified as general cases, while the others were severe cases. The number of patients who were readmitted to hospitals because of recurrence of symptoms, possible reinfection with positive nucleic acid testing again or referral with persistent symptoms was 3 (4.4%), 12 (17.6%) and 53 (78.0%), respectively. Fever (76.5%), cough (69.1%), shortness of breath (41.2%) and fatigue (32.4%) were the four most prevalent

128 sign and symptoms at illness onset among these patients. Hypertension (27.9%) was the most common
129 comorbidity, followed by type II diabetes mellitus (17.6%) (Table 1).
130
131 **Clinicopathologic information**
132 Complete blood count and blood biochemistries such as inflammatory seromarkers, renal, cardiac and
133 hepatic function tests were measured upon admission to Huoshenshan Hospital (Table 2). Of the 68 individuals,
134 sixty had white blood cell counts in the normal range; 9 patients had increased platelet counts; 30 patients had
135 increased C-reactive protein (CRP). Twelve out of 39 patients had increased procalcitonin (PCT). Almost all
136 patients were normal regrading cardiac, renal and hepatic function tests. Thirty-six patients were tested for the
137 serum antibodies to SARS-CoV-2, of which all patients were positive for SARS-CoV-2 IgG, while only
138 86.1% of patients were positive for SARS-CoV-2 IgM. In this study cohort, 28 out of 68 patients (41.2%)
139 were treated with antiviral agents, including ribavirin, arbidol, oseltamivir and entecavir. Forty out of 68 patients
140 (58.8%) were treated with antibiotics, which were mainly cephalosporin and floxacin. Notably, 95.6% of patients
141 received traditional Chinese herbal tea or Chinese patent medicine, such as Lianhua Qingwen Capsule.
142 Corticosteroid was mainly provided to the severe or critical cases. Twenty-seven out of 68 patients had low
143 sleep quality and required prescription of hypnotics. The median time from illness onset to the second time
144 of admission was 24 days (ranged from 3 to 65 days). The median DoVC in the overall study cohort was
145 48 days (ranged from 11 to 104 days). A half of the patients had a DoVC in the range of 31 to 60 days, and
146 the DoVC was longer than 2 months in 30.9% of patients.
147
148 **Risk factors associated with longer viral clearance**

In univariate analyses, several clinicopathologic characteristics were found to be associated with the DoVC, including fever (p = 0.016), shortness of breath (p = 0.024), and fatigue (p = 0.04) at onset, as well as serum levels of CRP (p = 0.0026) and SARS-CoV-2 IgG (p = 0.0096) (Table 1, Table 2). Thirty-six patients with complete data were included in the Cox model. Sensitivity analyses suggested no statistically significant difference in baseline and clinical characteristics between overall study cohort and patients included in Cox model (see Supplementary table S1, S2).Multivariate analysis suggested that fever at illness onset [Hazard ratio (HR) = 3.967, 95%CI, 1.583−9.942, p = 0.003], serum level of aspartate aminotransferase > 21.7 IU/L (HR = 3.188, 95% CI, 1.299−7.827, p =0.011), and titer of SARS-CoV-2 IgG > 141.69 (HR = 3.718, 95% CI, 1.553−8.904, p = 0.003) were the three independent risk factors associated with longer viral clearance in the refractory COVID-19 patients (Table 3). The proportional curves of positive viral test specified by three independent factors were depicted using Kaplan-Meier method (Figure 1). The estimated median DoVC was significantly longer in patients with fever at admission (53 days vs. 41 days, p = 0.016), with a titer of SARS-CoV-2 IgG higher than 141 AU/ml (68 days vs. 56 days, p = 0.0096). Patients with mildly impaired liver function (with AST > 21.7IU/L) were deemed to have a statistically boundary significant longer DoVC (56 days vs. 42 days, p = 0.051).

Discussion

The DoVC is central for decision-making of nosocomial isolation precaution and post-discharge quarantine. Due to the high infectivity of virus and the overloaded medical system, a refractory clinical course of COVID-19 could be rather annoying for both patients and medical workers. However, there is still a lack of clinical

guideline to deal with this particular group of patients. The current study documented the demographical, epidemiological, laboratory, and many other clinical data in a cohort of patients with ≥2 COVID-19-related hospitalizations, with the purpose to identify risk factors of prolonged hospitalization or viral clearance in these patients. In this retrospective study, 12 out of 68 (17.6%) were considered as possible reinfection (Table 1), given that they were negative for viral RNA in the former two consecutive nucleic acid tests. However, there was no concrete evidence to support this assumption as the false negative rate of nucleic acid testing was too high. Therefore, they were collectively recognized as patients with a refractory clinical course of COVID-19, along with those with recrudescent or persistent symptoms. It should be noted that this definition did not necessarily signify a severe or critical disease.

Before the cross-study comparison, it is worth noting that the definition of duration of viral shedding or viral clearance has not been unified in the previous studies.[14,17,21] In consideration of the high false negative rate of nucleic acid testing, we adopted a more conservative criteria to define the date of discharge as the endpoint of viral clearance. As two patients were still in hospitalization due to the complications from other organs despite their negative viral RNA results, DoVC may be slightly overestimated in this study cohort. The median DoVC was 48 days in our study, which was similar to the finding of one previous study (median = 53.5 days, IQR 47.75-60.5 days).[18] The duration of viral shedding was about 20 days in many other studies, which was much shorter because the patients with multiple COVID-19-related admissions were usually not enrolled.[13-17] It should be noted that nasal or pharyngeal swabs were mostly used for nucleic testing in these studies, but persistent SARS-CoV-2 RNA has been found elsewhere (e.g. anal swab or stool sample) after negative conversion of nasopharyngeal RT-PCR test.[9,25]

In the current study, the DoVC had a very wide range (Table 2), which indicated that the further

191 stratification among this population might be helpful for personalized management. We have identified the
192 presence of fever at illness onset, AST > 21.7 IU/L and titer of SARS-CoV-2 IgG > 141.69 to be the three
193 independent risk factors for longer viral clearance in the refractory COVID-19 patients (Table 3, Figure 1).
194 Several other studies have also revealed the presence of fever was significantly associated with prolonged
195 DoVC.[26,27] These patients were hypothesized to be more severely affected by the SARS-CoV-2 infection in the
196 lungs and thus the prolonged DoVC.[26] Fever at illness onset may indicate a cytokine storm or a higher viral load
197 at the time of symptom onset,[28] as the viral load was found to associated with more severe COVID-19 and longer
198 virus-shedding period.[29] Mildly abnormal liver function test was frequently reported in COVID-19 patients,
199 especially the elevated plasma AST.[26,30,31] Although a serum level of AST > 21.7 IU/L in our study not
200 necessarily signified clinical relevance, it was significantly associated with a delayed viral clearance (HR =
201 3.188, 95% CI, 1.299–7.827, p=0.011). Drug-induced liver injury should be taken into consideration,[30] because
202 a longer hospital stay usually required more medication, which were potentially hepatotoxic. Other possible
203 underlying mechanisms included direct viral hepatitis or immune-mediated inflammatory response.[26] The
204 presence of angiotensin-converting enzyme 2 (ACE2) receptor on cholangiocytes and hepatocytes suggested a
205 plausible mechanism of SARS-CoV-2 related hepatotoxicity.[32] Interestingly, a high level of SARS-CoV-2 IgM
206 was also found to be related to the prolonged viral shedding in a previous study,[18] which was instead echoed by
207 the level of IgG in our study. The larger magnitude of SARS-CoV-2 IgG, along with presence of fever at illness
208 onset, might imply a higher viral load or stronger immune response to the virus. Nevertheless, the underlying
209 mechanism requires further investigation.

210 Although less importantly, attention should also be paid to the symptoms such as shortness of breath, fatigue
211 and the serum level of CRP (Table 1, Table 2). However, we did not observe the impact of age, comorbidities,

212 severity of disease and treatment on the DoVC. Previous studies revealed multiple risk factors associated with
213 prolonged viral shedding, including age,[14,21] sex,[17] fever[15,33] or chest tightness[21] at admission, comorbidities such
214 as hypertension and diabetes mellitus,[26] time from symptom onset to admission,[15,17] albumin level,[16] antiviral
215 treatment,[14,16] invasive mechanical ventilation,[17] and length of hospital stay.[15] What is noteworthy is that many
216 studies directly divided the patients into two groups using a cutoff value in the DoVC and perform multivariate
217 analysis by logistic regression, instead of using Kaplan-Meier method and Cox regression model.[14,15,17] This
218 might have led to a loss of detailed temporal information, and thus the inaccuracy of results.

219 Although this study is bolstered by its design and methodological strength, it is also limited by its
220 retrospective nature and small sample size. Not all tests were performed and monitored during hospitalization in
221 all patients, especially the serology testing for SARS-CoV-2. Therefore, patients with incomplete data were
222 excluded from multivariable regression analysis, which limited its statistical power despite a sensitivity analysis
223 has been done. The patients with multiple hospitalizations might potentially subject to recall bias in retrieving
224 the treatment history. Additionally, we only enrolled patients with multiple COVID-19-related hospitalizations,
225 therefore, the results and conclusion should be customized to this specific group of patients, which limits the
226 generalizability of this study. Finally, the ability to associate clinicopathologic characteristics with DoVC might
227 be limited by using a convenient instead of statistically determined sample size.

228 In conclusion, this study investigated the risk factors of delayed viral clearance in patients with more than
229 one COVID-19-related hospitalizations. Patient characteristics such as fever at illness onset, mildly impaired
230 liver function, and a high serum level of SARS-CoV-2 IgG were associated with delayed viral clearance.
231 Therefore, a longer observation period and serial follow-up tests after the first discharge are necessary for
232 COVID-19 patients who are at high risk of reinfection or prolonged viral clearance.

Acknowledgements

We thank all the patients who provided consents to participate in this retrospective study.

Funding

This study was funded by the 2020 Guangdong Provincial Special Project for Popularization of Science and Technology Innovation (grant number: 2020A1414070007).

References

1. Mei Q, Li J, Du R, Yuan X, Li M, Li J. Assessment of patients who tested positive for COVID-19 after recovery. Lancet Infect Dis. 2020. doi: 10.1016/S1473-3099(20)30433-3

2. Smith J. South Korea reports more recovered coronavirus patients testing positive again. Available from: https://wwwreuterscom/article/us-health-coronavirus-southkorea/south-korea-reports-more-recovered-coronavirus-patients-testing-positive-again-idUSKCN21V0JQ.

3. Alvarez-Moreno CA, Rodriguez-Morales AJ. Testing Dilemmas: Post negative, positive SARS-CoV-2 RT-PCR - is it a reinfection? Travel Med Infect Dis 2020; 35: 101743. doi: 10.1016/j.tmaid.2020.101743

4. Chaturvedi R, Naidu R, Sheth S, Chakravarthy K. Efficacy of Serology Testing in Predicting Reinfection in Patients with SARS-CoV-2. Disaster Med Public Health Prep 2020: 1-7. doi: 10.1017/dmp.2020.216

5. Batisse D, Benech N, Botelho-Nevers E, Bouiller K, Collarino R, Conrad A. Clinical recurrences of COVID-19 symptoms after recovery: viral relapse, reinfection or inflammatory rebound? J Infect 2020. doi: 10.1016/j.jinf.2020.06.073.

6. Law SK, Leung AWN, Xu C. Is reinfection possible after recovery from COVID-19? Hong Kong Med J 2020; 26(3): 264-5. doi: 10.12809/hkmj208601

254 7. Pan Y, Long L, Zhang D, Yuan T, Cui S, Yang P, Wang Q, Ren S. Potential False-Negative Nucleic Acid Testing
255 Results for Severe Acute Respiratory Syndrome Coronavirus 2 from Thermal Inactivation of Samples with Low Viral
256 Loads. Clin Chem. 2020;66(6):794-801. doi: 10.1093/clinchem/hvaa091
257 8. Elrashdy F, Aljaddawi AA, Redwan EM, Uversky VN. On the potential role of exosomes in the COVID-
258 19 reinfection/reactivation opportunity. J Biomol Struct Dyn 2020: 1-12. doi: 10.1080/07391102.2020.1790426
259 9. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in Different Types of Clinical
260 Specimens. JAMA 2020, 12;323(18):1843-1844. doi: 10.1001/jama.2020.3786.
261 10. Li Y, Yao L, Li J, Chen L, Song Y, Cai Z, Yang C. Stability issues of RT-PCR testing of SARS-CoV-2 for
262 hospitalized patients clinically diagnosed with COVID-19. J Med Virol. 2020;92(7):903-8. doi: 10.1002/jmv.25786
263 11. Man Z, Jing Z, Huibo S, Bin L, Fanjun Z. Viral shedding prolongation in a kidney transplant patient with
264 COVID-19 pneumonia. Am J Transplant 2020. doi: 10.1111/ajt.15996
265 12. Xiao AT, Tong YX, Zhang S. False negative of RT-PCR and prolonged nucleic acid conversion in COVID-
266 19: Rather than recurrence. J Med Virol 2020. doi: 10.1002/jmv.25855
267 13. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J,
268 Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in
269 Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62. doi: 10.1016/S0140-6736(20)30566-
270 3
271 14. Yan D, Liu XY, Zhu YN, Huang L, Dan BT, Zhang GJ, Gao YH. Factors associated with prolonged viral
272 shedding and impact of lopinavir/ritonavir treatment in hospitalised non-critically ill patients with SARS-CoV-
273 2 infection. Eur Respir J. 2020;56(1). doi: 10.1183/13993003.00799-2020
274 15. Qi L, Yang Y, Jiang D, Tu C, Wan L, Chen X, Li Z. Factors associated with the duration of viral shedding

in adults with COVID-19 outside of Wuhan, China: a retrospective cohort study. Int J Infect Dis. 2020;96:531-7. doi: 10.1016/j.ijid.2020.05.045

16. Fu Y, Han P, Zhu R, Bai T, Yi J, Zhao X, Tao M, Quan R, Chen C, Zhang Y, He Q, Jing M, Xiong X, Tian D, Yan W. Risk factors for viral RNA shedding in COVID-19 patients. Eur Respir J. 2020;56(1). doi: 10.1183/13993003.01190-2020

17. Xu K, Chen Y, Yuan J, Yi P, Ding C, Wu W, Li Y, Ni Q, Zou R, Li X, Xu M, Zhang Y, Zhao H, Zhang X, Yu L, Su J, Lang G, Liu J, Wu X, Guo Y, Tao J, Shi D, Yu L, Cao Q, Ruan B, Liu L, Wang Z, Xu Y, Liu Y, Sheng J, Li L. Factors associated with prolonged viral RNA shedding in patients with COVID-19. Clin Infect Dis. 2020. doi: 10.1093/cid/ciaa351

18. Li N, Wang X, Lv T. Prolonged SARS-CoV-2 RNA shedding: Not a rare phenomenon. J Med Virol 2020. doi: 10.1002/jmv.25952

19. Yang JR, Deng DT, Wu N, Yang B, Li HJ, Pan XB. Persistent viral RNA positivity during the recovery period of a patient with SARS-CoV-2 infection. J Med Virol 2020. doi: 10.1002/jmv.25940

20. Liu WD, Chang SY, Wang JT, Tsai MJ, Hung CC, Hsu CL, Chang SC. Prolonged virus shedding even after seroconversion in a patient with COVID-19. J Infect. 2020. doi: 10.1016/j.jinf.2020.03.063

21. Hu X, Xing Y, Jia J, Ni W, Liang J, Zhao D, Song X, Gao R, Jiang F. Factors associated with negative conversion of viral RNA in patients hospitalized with COVID-19. Sci Total Environ. 2020;728:138812. doi: 10.1016/j.scitotenv.2020.138812

22. National Health Commission of China. Chinese management guideline for COVID-19 (version 6.0). Published February 19, 2020. https://www.chinadaily.com.cn/pdf/2020/1.Clinical.Protocols.for.the.Diagnosis.and.Treatment.of.COVID-

19.V7.pdf (accessed Feb 20, 2020; in Chinese).

23. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brunink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP, Drosten C. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3). doi: 10.2807/1560-7917.ES.2020.25.3.2000045

24. Organization WH. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. Interim guidance. Jan 17, 2020. . https://wwwwhoint/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 (accessed Feb 20, 2020)

25. Kipkorir V, Cheruiyot I, Ngure B, Misiani M, Munguti J. Prolonged SARS-Cov-2 RNA Detection in Anal/Rectal Swabs and Stool Specimens in COVID-19 Patients After Negative Conversion in Nasopharyngeal RT-PCR Test. J Med Virol 2020. doi: 10.1002/jmv.26007

26. Zhou Y, Ding F, Bao W, Xue Y, Han L, Zhang X, Zhang P, Ji Y, Yin D, Bao A, Luo S, Xu Z, Liu J, Zhang M. Clinical features in coronavirus disease 2019 (COVID-19) patients with early clearance and prolonged shedding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. Ann Transl Med. 2021;9(8):665. doi: 10.21037/atm-21-445.

27. Bennasrallah C, Zemni I, Dhouib W, Sriha H, Mezhoud N, Bouslama S, Taboubi W, Beji MO, Kacem M, Abroug H, Fredj MB, Loussaief C, Belguith AS. Factors associated with a prolonged negative conversion of viral RNA in patients with COVID-19. Int J Infect Dis. 2021;105:463-9. doi: 10.1016/j.ijid.2021.02.089.

28. Chau AS, Weber AG, Maria NI, Narain S, Liu A, Hajizadeh N, Malhotra P, Bloom O, Marder G, Kaplan B. The Longitudinal Immune Response to Coronavirus Disease 2019: Chasing the Cytokine Storm. Arthritis Rheumatol.

2021;73(1):23-35. doi: 10.1002/art.41526.

29. Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LLM, Zhang W. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis. 2020;20(6):656-7. doi: 10.1016/S1473-3099(20)30232-2.

30. Bertolini A, van de Peppel IP, Bodewes F, Moshage H, Fantin A, Farinati F, Fiorotto R, Jonker JW, Strazzabosco M, Verkade HJ, Peserico G. Abnormal liver function tests in COVID-19 patients: relevance and potential pathogenesis. Hepatology. 2020. doi: 10.1002/hep.31480

31. Zarifian A, Bidary MZ, Arekhi S, Rafiee M, Gholamalizadeh H, Amiriani A, Ghaderi MS, Khadem-Rezaiyan M, Amini M, Ganji A. Gastrointestinal and hepatic abnormalities in patients with confirmed COVID-19: a systematic review and meta-analysis. J Med Virol. 2020. doi: 10.1002/jmv.26314

32. Chai X, Hu L, Zhang Y, Han W, Lu Z, Ke A, Zhou J, Shi G, Fang N, Fan J, Cai J, Fan J, Lan F. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. bioRxiv 2020.02.03.931766. doi: 10.1101/2020.02.03.931766.

33. Lu Y, Li Y, Deng W, Liu M, He Y, Huang L, Lv M, Li J, Du H. Symptomatic Infection is Associated with Prolonged Duration of Viral Shedding in Mild Coronavirus Disease 2019: A Retrospective Study of 110 Children in Wuhan. Pediatr Infect Dis J. 2020;39(7):e95-e9. doi: 10.1097/INF.0000000000002729

Figure Legend

Figure 1. Proportional curves of positive viral testing. Comparison of duration of viral clearance between patients (A) with or without fever at onset; (B) with lower (≤141.69) or higher (> 141.69) levels of SARS-

337 CoV-2 IgG; (C) with lower (≤ 21.7 IU/L) or higher (> 21.7 IU/L) levels of aspartate aminotransferase (AST).

338 Log-rank test was used for comparison.

Table 1(on next page)

Table 1 Baseline characteristics of patients with a refractory clinical course of COVID-19

Table 1 Baseline characteristics of patients with a refractory clinical course of COVID-19

Baseline Characteristics	Study cohort = 68 Frequency (%) / Mean ± SD	p value*
Age (years)	52.4 ± 14.1	0.99
≤60	45 (66.2)	
>60	23 (33.8)	
Male	42 (61.8)	0.64
Reasons of readmission		0.69
Recurrence of symptoms	3 (4.4)	
Possible reinfection with positive nucleic acid testing again	12 (17.6)	
Referral with persistent symptoms	53 (78.0)	
Symptoms and signs at illness onset		
Fever	52 (76.5)	**0.016**
Shortness of breath	28 (41.2)	**0.024**
Dyspnea	5 (7.4)	0.18
Chest tightness	11 (16.2)	0.58
Myalgia	13 (19.1)	0.90
Dry cough	16 (23.5)	0.41
Productive cough	31 (45.6)	0.56
Fatigue	22 (32.4)	**0.04**
Diarrhea	5 (7.4)	0.59
Headache	3 (4.4)	0.98
Comorbidities		
Hypertension	19 (27.9)	0.53
Type II diabetes mellitus	12 (17.6)	0.74
Coronary artery disease	4 (5.9)	0.31
COPD	3 (4.4)	0.91
Hepatitis B	3 (4.4)	0.52
Malignancy	3 (4.4)	0.65
Smoker	6 (8.8)	0.99
Alcohol user	3 (4.4)	0.46
Severity of COVID-19 pneumonia		0.42

Mild	0
General	54 (79.4)
Severe	14 (20.6)
Critical	0

*Univariate analysis to determine the association of baseline characteristics with duration of viral clearance by using log-rank test. COPD, chronic obstructive pulmonary diseases.

Table 2(on next page)

Table 2 Clinicopathologic characteristics of patients with a refractory clinical course of COVID-19

1 **Table 2** Clinicopathologic characteristics of patients with a refractory clinical course of COVID-19

Clinicopathologic characteristics	Study cohort = 68 Frequency (%) / Median (Q1, Q3)	Normal range	p value*
Laboratory results			
WBC # (×10^9/L)	5.95 (5.03, 7.10)	3.5−9.5	0.81
RBC # (×10^12/L)	4.10 (3.80, 4.45)	4.3−5.8	0.49
Platelet # (×10^9/L)	234.00 (192.25, 291.75)	125−350	**0.053**
NLR	2.30 (1.76, 3.36)		0.12
C-reactive protein (mg/L)	2.81 (1.49, 8.62)	0−4	**0.026**
Normal	38 (55.9)		
Increased	30 (44.1)		
Procalcitonin (not tested = 29) (ng/ml)	0.05 (0.03, 0.06)	0−0.05	0.17
Normal	27 (69.2)		
Increased	12 (30.8)		
Albumin (g/L)	38.15 (35.08, 40.55)	40−55	0.46
ALT (IU/L)	30.15 (16.40, 47.27)	9−50	0.25
AST (IU/L)	21.70 (16.97, 27.73)	9−60	**0.051**
HBDH (IU/L)	152.90 (128.60, 191.68)	24−190	0.54
LDH (IU/L)	181.15 (126.48, 239.65)	120−250	0.66
CK (IU/L)	45.55 (34.28, 69.68)	24−190	0.94
CK-MB (IU/L)	7.75 (6.40, 11.50)	0−24	0.93
SARS-CoV-2 IgM (not tested = 32) (AU/ml)	36.19 (16.75, 89.03)	< 10	0.27
SARS-CoV-2 IgG (not tested = 32) (AU/ml)	141.69 (114.55, 178.33)	< 10	**0.0096**
Treatment details			
Antivirals	28 (41.2)	—	**0.052**
Antibiotics	40 (58.8)	—	0.16
Traditional Chinese medicine	65 (95.6)	—	0.13
Corticosteroid	12 (17.6)	—	0.45
Hypnotics	27 (39.7)	—	0.57
Duration of viral clearance	48 (33, 65)	—	—
≤30 days	13 (19.1)		
31-60 days	34 (50.0)		

> 60 days	21 (30.9)

* Univariate analysis to determine the association of clinicopathologic characteristics with duration of viral clearance by using log-rank test. Patients were categorized into two groups based on the median value of laboratory results except for C-reactive protein and procalcitonin. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; HBDH,α-Hydroxybutyrate dehydrogenase; LDH, lactase dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase isoenzyme.

Table 3(on next page)

Table 3 Multivariate analysis using Cox regression model (n=36)

Table 3 Multivariate analysis using Cox regression model (n=36)

Variables (Reference)	Hazard Ratio	95% Confidence Interval		p value
		Lower	Upper	
Fever (No)				
Yes	3.967	1.583	9.942	**0.003**
Shortness of breath (No)				
Yes	1.832	0.737	4.555	0.192
Fatigue (No)				
Yes	0.855	0.337	2.174	0.743
Use of antiviral medication (No)				
Yes	0.732	0.324	1.594	0.728
Platelet # (≤234 × 10^9/L)				
> 234 × 10^9/L	0.493	0.214	1.136	0.097
AST (≤21.7 IU/L)				
> 21.7 IU/L	3.188	1.299	7.827	**0.011**
SARS-CoV-2 IgG (≤141.69)				
> 141.69	3.718	1.553	8.904	**0.003**

CRP, C-reactive protein; AST, aspartate aminotransferase; SARS-CoV-2 IgG, Severe acute respiratory syndrome coronavirus 2 immunoglobulin G.

Figure 1

Figure 1 Proportional curves of positive viral testing.