J Infect Chemother 27 (2021) 1122–1125

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Note

Investigation of nasopharyngeal viral load at discharge in patients with COVID-19

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ARTICLE INFO

Article history:
Received 18 November 2020
Received in revised form
26 February 2021
Accepted 29 March 2021
Available online 8 April 2021

Keywords:
COVID-19
SARS-CoV-2
Infectivity
Viral load

ABSTRACT

This study aimed to assess the nasopharyngeal viral load at discharge or time of discontinued isolation in coronavirus 2019 (COVID-19) patients admitted to our hospital and discharged under the current symptom-based criteria in Japan.

Patients diagnosed with COVID-19 by reverse transcription polymerase chain reaction and hospitalized at Toyama University Hospital were included in the analysis. Nasopharyngeal viral load was measured when symptom-based criteria for discharge or end of isolation in the accommodations were met, and examined the relationship between viral load and days after onset or age. From the perspective of virus isolation limit, the amount of infectious viral load was defined at 50 copies/μL by nasopharyngeal sample.

Thirty-three patients with laboratory-confirmed COVID-19 were included in the analysis, after excluding critical and fatal cases. Mean nasopharyngeal viral load at discharge or end of isolation was 1.90 log-copies/μL, and 64% of patients were discharged with over 50 copies/μL. No correlation was apparent between age and viral load at discharge, and viral load remained relatively high at discharge or end of isolation in all age groups.

Although attempts at infectious virus isolation are necessary, infection control precautions even after discharge or discontinued isolation in accommodations may be needed, as the date of onset mostly depended on self-reporting by patients.

Currently, novel coronavirus disease 2019 (COVID-19) is primarily diagnosed by the detection of severe acute respiratory syndrome coronavirus 2019 (SARS-CoV-2) RNA via reverse transcription polymerase chain reaction (RT-PCR) or by viral culture and demonstration of cytopathic effect. Although RT-PCR only identifies viral RNA and cannot determine whether infectious virus remains present, infectiousness can be inferred from the viral load. The RT-PCR threshold cycle (Ct) represents the number of PCR cycles required to detect SARS-CoV-2 RNA, with lower values indicating higher viral load and, by implication, higher infectiousness [1]. The exact RT-PCR Ct values and viral loads from nasopharyngeal swabs or saliva associated with the presence of infectious SARS-CoV-2 remains unclear, but infectious virus has been isolated from specimens with an RT-PCR Ct as low as 34 [2]. On the other hand, virus isolation has been reported as almost impossible for Ct values of 33–35 [3].

Ct values are generally used for estimating the viral load because of the correlation between the Ct value and the viral load [4]. They can fluctuate depending on the pre-analytical process such as the sample collections and the protocols, however, the viral load provide the beneficial information to understand the pathogenesis. For example, the minimum viral loads of culture positive nasopharyngeal specimens are reportedly 12–252 copies/μL [5–7]. In

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https://doi.org/10.1016/j.jiac.2021.03.023
1341-321X/

addition, we previously reported that viral loads was higher among symptomatic cases who transmitted to others compared to those who did not transmit to others [8]. In this study, the differences in the viral load gradually disappeared, and viral loads reached to approximately at 50 copies/μL about 10 days after the onset. Thus, we defined the minimum viral load associated with infectivity as 50 copies/μL in this study.

Virus shedding is highest on the day of onset, then gradually declines. However, shedding may persist, with positive PCR test results seen even after symptoms have subsided. For this reason, the current criteria for discharge or end of isolation in Japan have been changed from the conventional method based on the results of PCR testing (test-based strategy) to a method using subjective symptoms as an index (symptom-based strategy). However, uncertainty remains regarding the date of symptom onset, as this has relied on self-reported information from the patient. Moreover, one report described virus isolation from patient specimens on day 13 of illness [2]. Concerns therefore remain regarding the presence of infectious patients excreting virus even after meeting the current discharge criteria.

This study therefore assessed the nasopharyngeal viral load at discharge or end of isolation in COVID-19 patients admitted to our hospital and discharged after meeting the current symptom-based criteria in Japan.

Patients diagnosed with COVID-19 by RT-PCR of nasopharyngeal swabs and hospitalized at Toyama University Hospital between August 8 and October 5, 2020 were included in this study. Patients were divided into 4 groups according to the severity of symptoms: mild, moderate, severe, or critical. Patients who showed sufficient improvement of symptoms and were judged as able to be followed-up at the accommodation facility for the rest of the medical treatment period were moved from the hospital to the accommodation facility. The date of discharge from hospital or end of isolation in the accommodation facility was determined based on the symptom-based criteria in Japan, defined as: “At least 10 days have passed since symptoms first appeared, at least 72 hours have passed since last fever without the use of fever-reducing medications, and symptoms (e.g., cough, shortness of breath) have improved.” [9].

The viral load was measured using a nasopharyngeal swab at discharge or end of isolation after meeting the requisite criteria, and we examined the relationship between viral load and days after onset or age.

In quantitative measurement by RT-PCR, nasal swab specimens were pretreated with 500 μL of Sputazyme (Kyokuto Pharmaceutical, Tokyo, Japan). After centrifugation at 20,000×*g* for 30 min at 4 °C, the supernatant was used for RNA extraction. A total of 60 μL of RNA solution was obtained from 140 μL of supernatant using a QIAamp ViralRNA Mini Kit (QIAGEN, Hilden, Germany) or Nippongene Isospin RNA Virus (Nippon Gene Co., Tokyo, Japan) according to the instructions from the manufacturers. Viral loads of SARS-CoV-2 were quantified based on an N2-gene-specific primer/probe set by quantitative RT-PCR according to the protocol of the Japan National Institute of Infectious Diseases [10]. The quality of quantification was controlled by AcroMetrix COVID-19 RNA Control (Thermo Fisher Scientific, Fremont, CA). The detection limit was approximately 0.4 copies/μL (2 copies/5 μL).

After excluding 6 critical and 2 fatal cases, 33 patients were included in the present analysis. Baseline characteristics of the patients are summarized in Table 1. Median age was 52 years, comprising 2 patients (6.1%) ≤20 years old, 21 patients (63.6%) at 18–64 years old, and 10 patients (30.3%) ≥65 years old. Patients comprised 17 males (51.5%) and 16 females (48.5%). Thirty-two patients (97.0%) were symptomatic, and 1 patient (3.0%) was asymptomatic. Nine patients (27.3%) were classified as showing mild disease, 9 patients (57.6%) were moderate, and 4 patients (12.1%) were severe. Most of the patients had a significantly reduced viral load at discharge or end of isolation compared to at admission (Fig. 1). One of them had a higher viral load at discharge than at admission. Eight patients were discharged or ended isolation in the accommodation at 10 days after symptoms onset, among whom only 2 patients (25%) were discharged or ended isolation in the accommodation with a nasopharyngeal viral load of ≤50 copies/μL (Fig. 2). In total, 64% of patients were discharged or ended isolation with over 50 copies/μL. No correlation was identified between these viral loads and days after onset or age, and viral load remained high at discharge or end of isolation in the accommodation in some patients, regardless of age.

Currently, a symptom-based strategy is being used in Japan to determine discharge and end of accommodation treatment for

Table 1
Basic characteristics of the patients.

Characteristics	Study population
Age, median, y	52.0
≦20, n (%)	2 (6.1)
21–64, n (%)	21 (63.6)
≧65, n (%)	10 (30.3)
Sex	
Male, n (%)	17 (51.5)
Presence of symptoms, n (%)	
Asymptomatic	1 (3.0)
Symptomatic	32 (97.0)
Mild	9 (27.3)
Moderate	19 (57.6)
Severe	4 (12.1)
Coexisting conditions, n (%)	
Any coexisting condition	15 (45.5)
Chronic lung disease	1 (3.0)
Diabetes mellitus	4 (12.1)
Hypertension	8 (24.2)
Immune deficiency	1 (3.0)
Cardiovascular disease	2 (6.1)
Cerebrovascular accident	0 (0)
Renal disease	3 (9.1)
Received hemodialysis	0 (0)
Cognitive impairment	0 (0)
Obesity	10 (30.3)
Medication, n (%)	
no medication	26 (78.8)
Steroid	3 (9.1)
Heparin	2 (6.1)
Favipiravir	6 (18.2)
Remdesivir	3 (9.1)

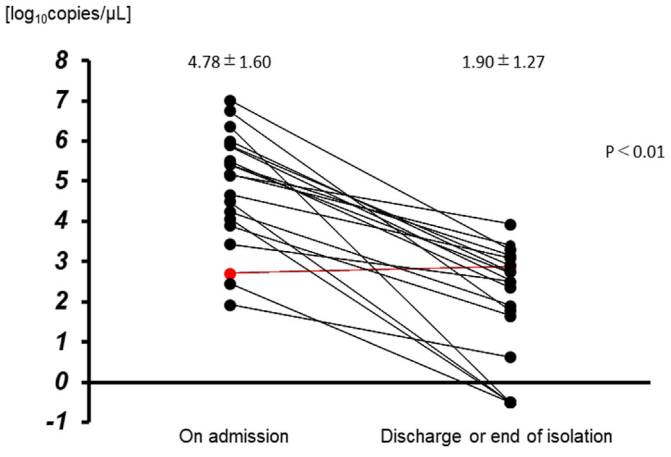

Fig. 1. Relationship between the nasopharyngeal viral load at the time of discharge or discontinuing isolation and days after symptom onset.

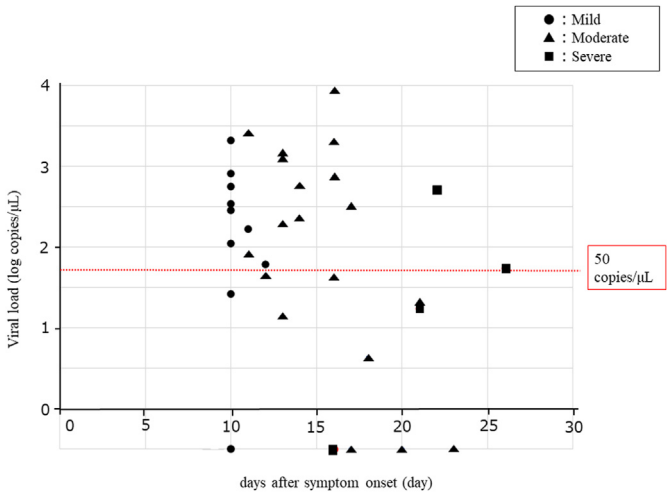

Fig. 2. Nasopharyngeal viral load at admission and discharge or end of isolation. Red circles represent one case who had a higher viral load at discharge than at admission.

COVID-19 patients. Symptom relief is defined as "no fever despite no use of fever-reducing medications and improvement of symptoms (e.g., cough, shortness of breath)", but infectiousness may not have completely disappeared under those conditions in some patients. Virus has reportedly been isolated from a sample with a Ct value of 34 and from a sample 13 days after onset [2]. Some patients showed viral loads ≤50 copies/μL at discharge or end of accommodation treatment, but many cases were discharged with a high viral load. Based on such results, although we did not try to the isolation of infectious virus from the samples, it seems likely that some patients are excreting virus even after meet the current discharge criteria. The current symptom-based strategy may have resulted in discharge or end of isolation with patients in an infectious state.

None of the COVID-19 patients who have been admitted to our hospital and discharged after meeting the criteria have required readmission. In addition, no cases of secondary infection originating from the patient have been confirmed after discharge. Although previous studies demonstrated some cases in which the PCR test became positive again after discharge, and the subjective symptoms recurred, suggesting a relapse of COVID-19 [11,12]. However, there have been no reports that described the secondary infection spread from the case of relapse. The effects of continued infection prevention measures even after discharge and the existence of asymptomatic secondary infections cannot be ruled out, and there are still many unclear points regarding the possibility of infection in patients who have relapsed or after discharge. Further evidence is expected to accumulate.

The present study contained some cases in which the viral load was not sufficiently reduced at the time of discharge or the end of isolation. It remains unclear how viral load changes over time in relapsed cases. However, because the increase of the viral load after the convalescent of the disease can imply the relapse, it may be necessary to measure the viral load and confirm its improvement.

There are several limitations in our research. First, we evaluated only the viral load but not attempted to isolate the virus. In addition, we have not evaluated factors that affect infectivity other than viral load, such as the status of infection protection at the time of exposure. Therefore, it may be difficult to evaluate the infectivity of the patients after discharge from the hospital. Second, compared to the liquid specimens, it is difficult to know the exact sample volume of the nasopharyngeal swab specimens. Therefore, the viral loads in the present study roughly indicate estimated values.

Although isolation of infectious virus from samples at discharge or end of isolation is necessary, infection control precautions are needed even after discharge or end of isolation at the accommodation, because of the uncertain nature of the date of onset based on self-reports from patients.

Funding

This study was supported by the Research Program on Emerging and Re-emerging Infectious Diseases from AMED Grant No. JP20he0622035.

Ethical approval

This study was performed in accordance with the Helsinki Declaration and approved by the Ethical Review Board of the University of Toyama (approval No.: R2019167).

ICMJE statement

All authors meet the ICME authorship criteria.

Authors contribution

YF, HK, YT, MK, Y.Murai, KK, AU, Y.Miyajima, and KK contributed to the acquisition of data, participated in study design, analyzed and interpreted the data, and drafted the manuscript. Y.Morinaga contributed to the viral load measurement. IS and YY were clinical investigators of the trials and responsible for the medical care of trial participants, communication with the research ethics committee, protocol, informed consent, data integrity and reporting. YY was responsible for the overall organization and coordination of the trial. All authors contributed to the writing of the final manuscript. All members of the present study team contributed to the management or administration of the trial.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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