The clinical manifestations and interval changes of reverse-transcriptase quantitative polymerase chain reactions among different specimens of coronavirus disease 2019 patients

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Abstract

Background: Since December 2019, a number of cases and deaths due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic have been reported worldwide. In spite of clinical manifestations similar to the SARS-CoV epidemic in 2003, affected organs and severity are yet to be defined. Moreover, viral load alterations and viral shielding among different specimens remained scarce. Therefore, clarifying clinical presentations and correlations among viral loads, disease severity, and viral shielding of SARS-CoV-2 infection is crucial in the disease prevention.

Methods: The clinical courses of SARS-CoV-2 cases were presented through Gantt charts. Laboratory examinations and reverse-transcriptase quantitative polymerase chain reactions (RT-qPCR) among different specimens were tested periodically. Cycle thresholds (C_T) were recorded and presented as viral loads.

Results: From March 2020 to April 2020, 4 SARS-CoV-2 cases were presented, of which, cases 1 and 2 manifested the symptoms severer than cases 3 and 4, along with higher serum lactate dehydrogenase levels and graded for lymphocytopenia. Case 4 initially exhibited anosmia but recovered within a short period. Curves of the C_T of all the cases, except case 2, concaved upward after prescribing hydroxychloroquine (HCQ) and azithromycin. Except for case 4, the C_T in most stool specimens remained undetectable; however, none of the cases presented gastrointestinal symptoms. Surprisingly, the C_T values of the saliva specimens were inconsistent with those of the nasopharyngeal swabs and sputum.

Conclusion: SARS-CoV-2 manifests various symptoms. Sudden onset of central nervous system symptoms should be considered. The timing of HCQ and azithromycin administration might be a key factor in the viral load reduction. Positive prediction values of RT-qPCR of different specimens should be tested carefully to prevent false-negative results.

Keywords: Central nervous system; Cycle threshold; Hydroxychloroquine; Reverse-transcriptase quantitative polymerase chain reaction; Severe acute respiratory syndrome coronavirus 2

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The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 151-157.

Received July 24, 2020; accepted August 4, 2020.

doi: 10.1097/JCMA.0000000000000451.

1. INTRODUCTION

Since December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak started from Wuhan City, Hubei province, China, has resulted in millions of confirmed cases and hundreds of thousands of deaths within a short period. World Health Organization (WHO) has named the novel coronavirus disease as coronavirus disease 2019 (COVID-19). Among the confirmed cases in Taiwan, coronavirus, in addition to the host's respiratory system, could also affect other system such as the central nervous system. However, the data of viral shielding and the positivity of different specimen types remained scarce. We demonstrate the clinical manifestations, laboratory data, and viral load alterations during the hospitalization of

the confirmed COVID-19 cases at the Taipei Veterans General Hospital (TPEVGH).

2. METHODS

2.1. Patient Source

All 4 confirmed cases (1 male and 3 females) were from Taipei Veterans General Hospital (TPEVGH) from March 2020 to April 2020.

2.2. Data Collection

For all the confirmed COVID-19 cases at TPEVGH, the nasopharyngeal, sputum, saliva, and stool samples were tested periodically by reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) before the patients were discharged from isolation by Taiwan Centers for Disease Control (TCDC).[1] Laboratory data, including hemograms and biochemistry, daily body temperature, oxygen demands, and oxygenations, were collected.

2.3. Data Analysis

The clinical course of each patient was briefly summarized. Daily body temperatures and oxygen demands were demonstrated as Gantt charts. Body temperature of <37.5 °C was defined as "normal," while that of >38.3°C was defined as "high-grade fever," and the middle values were defined as "low-grade fever." The fraction of inspired oxygen (FiO_2) of >60% was defined as "high oxygen demand," while that of <40% was defined as "low oxygen demand," and the middle values were interpreted as "medium oxygen demand." RNA was extracted from the specimens collected and stored in the virus transport medium (VTM) and subsequently analyzed for the envelope protein (E) gene, the RNA-dependent RNA polymerase (RdRP) gene, and the ribonuclease P (RNase P) gene using RT-qPCRs to detect and estimate the viral shedding and viral loads at different time points postsymptoms development. The RT-qPCR testing was reported nondetectable, and the cycle threshold (C_T) was defined as 50 when no signals were detected, and all the tests with the $C_T > 40$ were defined as "no detection." C_T between 38 and 40 was defined as "weakly positive," 30–37 as "positive," and <30 as "strongly positive." C_T of the RNase P gene indicates the presence of the host gene or the housekeeping gene, and the C_T of gene E or RdRP gene subtracted from the RNase P gene was assumed to represent the viral load of each specimen. This study was approved by the intuitional review board of TPEVGH.

3. RESULTS

3.1. Case 1

A 59-years-old man with a history of hypertension and coronary artery disease under the prescription of amlodipine, atorvastatin, and aspirin and travel history of Kuala Lumpur, Dubai, Munich, and Frankfurt from March 1, 2020, to March 16, 2020, showed sore throat accompanied by intermittent low-grade fever as initial symptoms three days before diagnosed with confirmed COVID-19. He was admitted to the negative pressure isolation ward on March 17, 2020. Grade 2 lymphocytopenia and deterioration of serum lactate dehydrogenase (LDH) level were presented upon admission. However, neither patchy pneumonia nor infiltration observed in his chest radiogram and the oxygen demand remained normal. Empirical antibiotics with levofloxacin (03/17–03/20) and antiviral agent oseltamivir (3/17–3/21) were administered. However, the serial of chest radiograms from March 21, 2020, showed progress in pneumonia along with increasing oxygen demand, deterioration of absolute lymphocyte count, and serum LDH level. Therefore, hydroxychloroquine (HCQ) (3/21–3/31), azithromycin (3/22–3/28), and moxifloxacin (3/28–3/30) were administrated. Oxygen demand, lymphocytopenia, and serum LDH level improved significantly 2 weeks after the onset of the symptoms. After notifying the TCDC about his 3 negative RT-qPCR results for SARS-CoV-2, he was shifted from isolation after 33 days and discharged from hospital 36 days after the onset of the symptoms.

3.2. Case 2

A 75-years-old woman with a history of hypertension and type-2 diabetes mellitus with the subscription of bisoprolol and metformin, and under adjuvant hormone therapy with Tamoxifen for breast cancer postmodified radical mastectomy 5 years ago showed dry cough and fever 2 days after returning from her journey to Peru, Brazil, and France between 18 February 2020 and 18 March 2020. Three days later, she was confirmed of having COVID-19 and admitted to the negative pressure isolation ward. Her hemogram was normal, and an empirical antibiotic with levofloxacin was administrated (03/21-03/31). However, acute respiratory failure with hypoxemia was noted on March 31, 2020, 12 days after the onset of the symptoms, an endotracheal tube was inserted, and she was transferred to TPEVGH on the same day. Grade 1 lymphocytopenia was also observed on day 12. Venous-venous extracorporeal membrane oxygenation (V-V ECMO) was applied on day 13 due to deterioration of oxygen demand and acute respiratory distress syndrome. HCQ was administered from day 12 to 21. Significant improvement in the chest radiograms was observed since day 19, along with improved absolute lymphocyte counts and serum LDH levels. Interleukin-6 (IL-6) level was about 25 pg/mL. The anti-IL-6 receptor antibody, tocilizumab, was prescribed twice (320 and 240 mg) on day 21, and improvement in the chest radiogram was observed. However, oxygen demand increased and the RT-qPCR signal turned positive from weak positive again on day 23, along with the deterioration of lymphocytopenia. Regimens of antibiotics were adjusted accordingly, resulting into gradual improvement in the condition. V-V ECMO and the mechanical ventilator were weaned on day 36 and 45, respectively. The patient was moved from isolation ward after 3 detrimental results of RT-qPCR on May 7, 2020, 49 days after the onset of the symptoms, and was transferred to general ward for further care.

3.3. Case 3

A 39-years-old female without any history of any systemic disease worked as a designer in London from January 19, 2020, to March 23, 2020. There were >100 confirmed COVID-19 cases in the community she lived in London. Upon experiencing fever on March 30, 2020, she visited the emergency room, and next day, was found positive for SARS-CoV-2 infection. She was admitted to the negative pressure isolation ward once TCDC notified her positive result 3 days after the onset of the symptoms. Serial hemograms remained normal during the entire course, and neither lymphocytopenia nor deterioration of serum LDH level was observed. HCQ was prescribed from day 5 to 11 and no antibiotic was administered during hospitalization. Increasing infiltration over the right lower lung field was observed initially, which was resolved on day 10. She was moved from isolation after 3 detrimental results of RT-qPCR on April 21, 2020, 23 days after the onset of the symptoms.

3.4. Case 4

A 22-years-old student in New York City with a history of migraine and allergic rhinitis presented nasal congestion on April 6, 2020, and anosmia on the next day. She visited the emergency room and was found positive for SARS-CoV-2 infection. She was admitted to the negative pressure isolation ward 3 days after onset of the symptoms. Fever was recorded on the arrival day, but no fever episode

was recorded after admission. Neither lymphocytopenia nor deterioration of serum LDH level was observed. Pneumonia was also not observed during hospitalization. An empirical antibiotic ceftriaxone and antiviral agent oseltamivir were prescribed upon admission, but both were discontinued on day 2. HCQ was prescribed from day 5 to 12, and azithromycin was prescribed from day 9 to 13. Anosmia resolved gradually. She was then moved from isolation and discharged after 3 detrimental negative results of RT-qPCR on May 10, 2020, 23 days after the onset of the symptoms.

3.5. Clinical Manifestations

Among all, cases 1 and 2 presented severer symptoms and disease status than cases 3 and 4. In case 1, the patient presented mild respiratory symptoms and high-grade fever in the first 9 days. Since pneumonia developed on day 8 after onset of the symptoms, HCQ, and azithromycin were prescribed, and fever patterns and pneumonia were improved gradually. Fever subsided and symptoms relieved since day 16. In case 2, the patient initially exhibited mild respiratory symptoms and high-grade fever. However, ARDS occurred on day 12 after onset of the symptoms. Mechanical ventilator was applied on day 12, and ECMO was applied on day 13, and HCQ was prescribed on day 12. Fever subsided after ECMO application but deteriorated since day 26, 5 days after anti-IL-6 receptor antibody infusion, and body temperature fluctuated in the next 18 days. In case 3, fever was recorded only on the first day when symptoms developed. We did not file any postadmission episode of fever. HCQ alone was prescribed from day 5 to 11. Infiltration over the right lower lung field increased mildly on day 10 without developing any severe respiratory symptom. In case 4, the patient presented mild respiratory symptoms initially accompanied with anosmia. Fever was recorded only once on day 3. HCQ and azithromycin were prescribed on days 5 and 9; anosmia was improved gradually (Fig. 1A).

Oxygen demand was also recorded (Fig. 1B). In case 1, although high-grade fever and pneumonia were observed in the first 2 weeks, the oxygen demands remained nearly normal. The patient only needed nasal cannula support in the second week when pneumonia was developed. In case 2, the oxygen demands deteriorated after symptoms developed and worsened on days 12 and 13 and required nearly 100% pure oxygen support. Oxygen demands decreased to 40%–60% FiO_2 on day 14 but deteriorated again on day 19 and 20 and stabilized after anti-IL-6 receptor infusion on day 21. The oxygen demand became nearly normal, and ECMO and mechanical ventilator weaned on day 36 and 45, respectively. In cases 3 and 4, oxygen demands remained normal, and patients did not require additional oxygen support during the entire courses.

3.6. Laboratory Examinations

Serial laboratory examinations were monitored periodically during hospitalizations of all the COVID-19 patients (Fig. 2). Compared with cases 1 and 2, cases 3 and 4 presented milder disease severity and laboratory examinations. Left shifting in the hemograms was observed commonly in the early stage of disease in cases 1 and 2, which remained relatively normal in cases 3 and 4 and were only observed when pulmonary infiltration increased in case 3 (Fig. 2A). Cases 3 and 4 showed normal absolute lymphocyte counts, while different grades of lymphocytopenia were observed along with varying severities of disease in cases 1 and 2. Lymphocytopenia was improved while disease severity progressed markedly (Fig. 2B). The disease severity significantly correlated to the platelet count (Fig. 2C) and serum LDH level (Fig. 2D). When the disease became severer, the lower platelet count and the higher serum LDH level were observed.

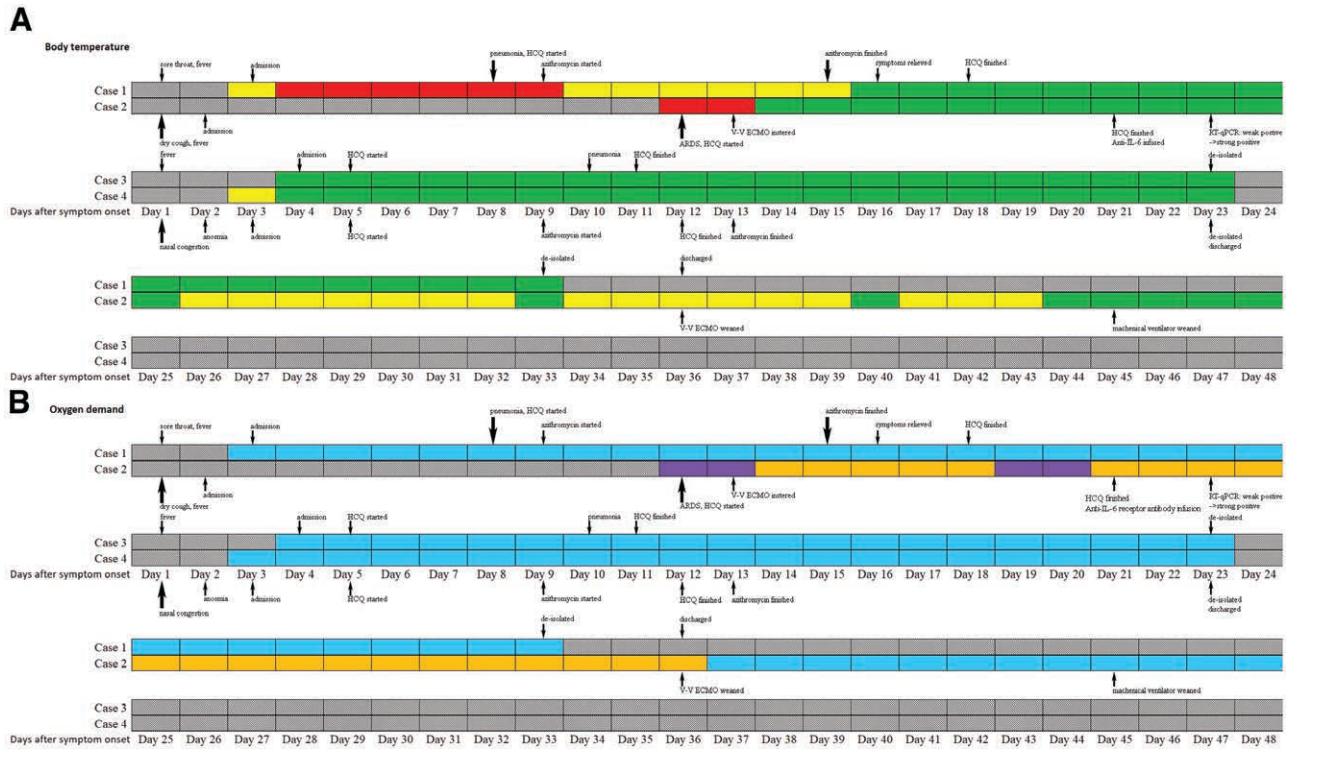

Fig. 1 Gantt chart of cases in Taipei Veterans General Hospital. (A) Body temperatures. (B) Oxygen demands. HCQ, hydroxychloroquine; V-V ECMO, venous-venous extracorporeal membrane oxygenation; ARDS, acute respiratory distress syndrome.

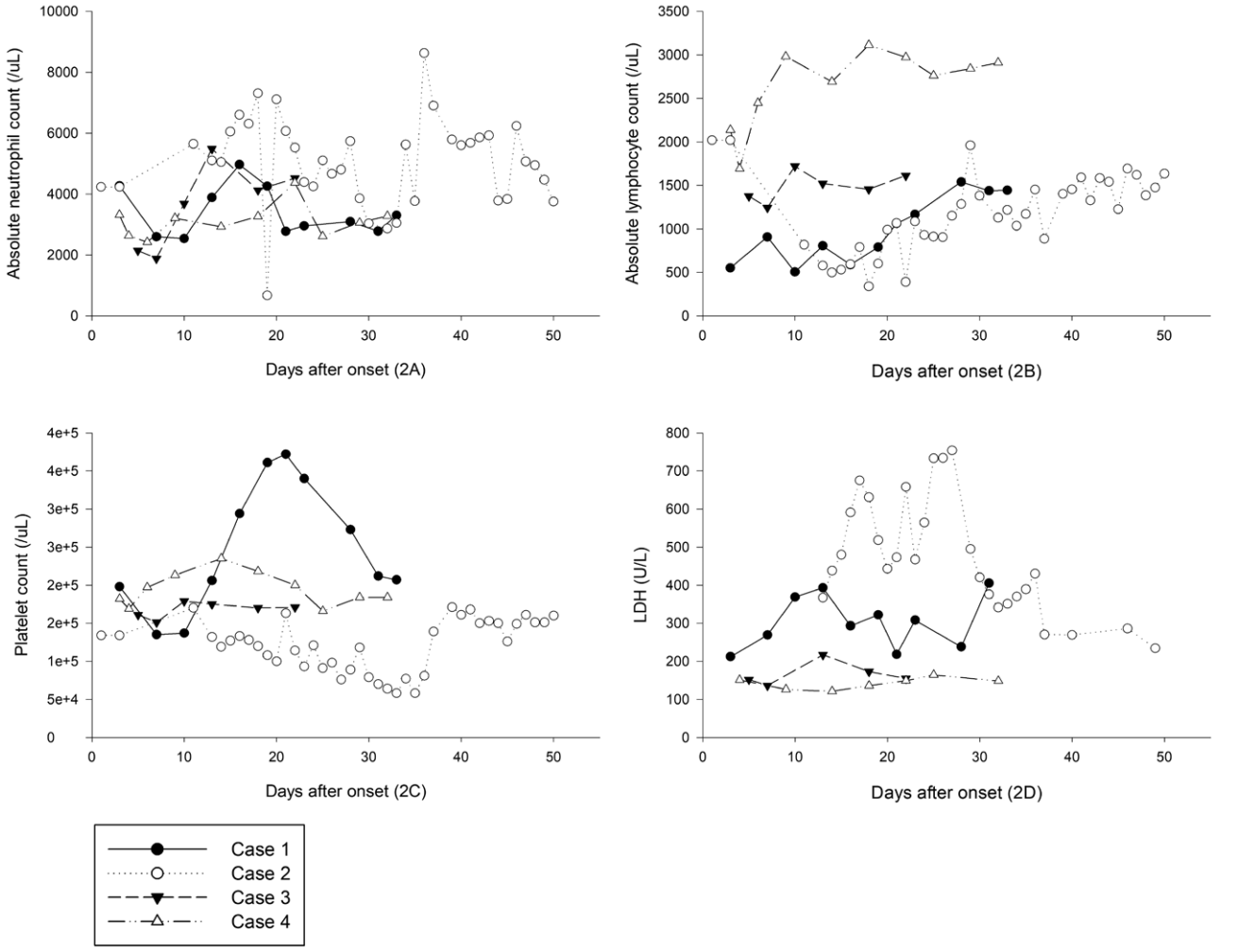

Fig. 2 Laboratory examinations of cases in Taipei Veterans General Hospital: (A) absolute neutrophil counts; (B) absolute lymphocyte counts; (C) platelet counts; and (D) serum lactate dehydrogenase levels.

3.7. Cycle Threshold Alterations

Among the nasopharyngeal specimens, signals of both E and RdRP genes were strongly positive at the beginning but turned weak and became undetectable at about 30 days after onset of the symptoms in case 1 (Fig. 3A,B). In case 2, positive signals were recorded and fluctuated in the first two-third of the hospitalization period, which then turned negative. Interestingly, the signals turned positive from weakly positive upon anti-IL-6 receptor antibody infusion (Fig. 3C,D).

On the contrary, signals in case 3 turned weak from strong positive and became undetectable within 10 days, which was quicker than in cases 1 and 2 (Fig. 4A,B). In case 4, the first signals of both E and RdRP genes were undetectable, which was reported as negative result. All the others remained nearly strong positive in the first half of the clinical course but fluctuated in the E gene in the second half course. Signals became undetectable in the RdRP gene in about 20 days after onset of the symptoms (Fig. 4C,D). Signals of most of the saliva specimens were undetectable. Only strong positive signals of the E gene were recorded after anti-IL-6 receptor antibody infusion in case 2, whereas the signals of the RdRP gene still remained undetectable. In most of the sputum specimens collected in cases 2 and 3, signals altered along with the nasopharyngeal specimens. Stool specimens were also collected and tested, for which, only 1 strong positive signal was recorded in case 4 when symptoms developed at the beginning and all the others remained undetectable.

All detectable C_T values of E and RdRP genes were subtracted with corresponding values of the RNase P gene to normalize the levels of 2 genes among different specimens. Among different specimens and patients, nasopharynx and sputum specimens were tested with the highest levels of both E and RdRP genes in the early disease phase, and these levels decreased correspondingly in cases 1, 3, and 4. In case 2, similar trends of the E gene levels in nasopharynx and sputum specimens were observed but fluctuated in the RdRP gene. Among the patients, the levels of E and RdRP genes in saliva specimens fluctuated, and no significant correlation to the clinical courses was observed. No target genes were detected in most of the stool specimens except in case 4. ΔC_T was –1.91 in the E gene and 0.27 in the RdRP gene.

4. DISCUSSION

Initially, case 4 in TPEVGH presented both respiratory symptoms and anosmia. Viruses might affect multiple systems and show varying symptoms. Viruses such as human immunodeficiency virus, human polyomavirus, and herpes simplex virus are well known neurotropic viruses, which infect the nervous system (NS) through either bloodstream or directly.[2,3] Such viruses might infect immune-function cells thereby resulting in the disruption of

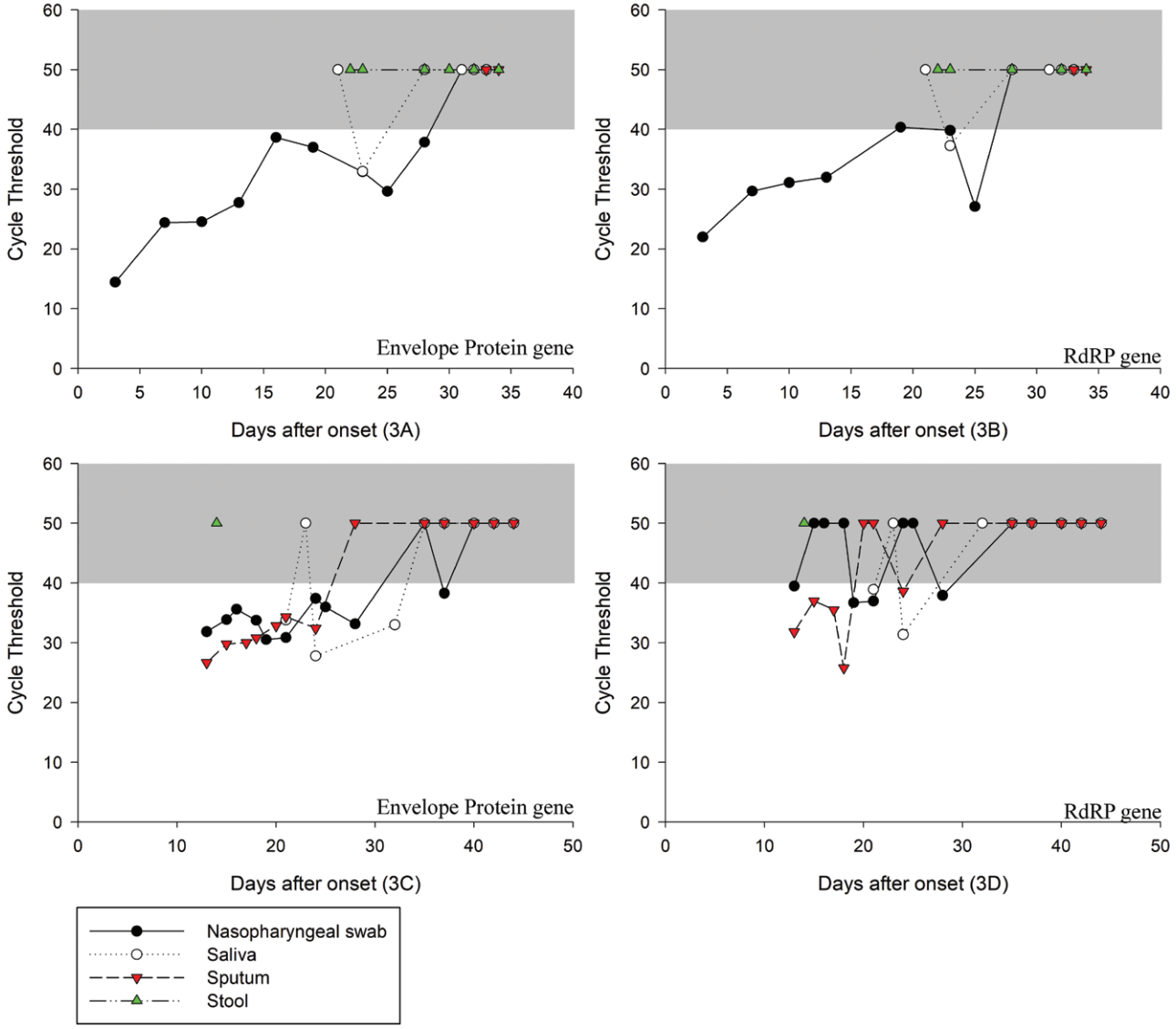

Fig. 3 Cycle thresholds of envelop protein and RNA-dependent RNA polymerase genes of cases in Taipei Veterans General Hospital: (A,B) case 1; (C,D) case 2.

blood-brain barrier.[4] However, coronavirus was known to manifest respiratory symptoms commonly. Recently, coronavirus has been observed for having the ability to invade the central nervous system (CNS),[5,6] and a significant portion of coronavirus-infected patients exhibiting CNS symptoms has been reported.[6–8] In such cases, betacoronavirus was reported frequently; however, the mechanism remained scarce.[9] Some patients infected by SARS-CoV in 2003 showed effect on CNS, which led to permanent CNS damage. Anosmia and ageusia were both reported previously.[10–13] A significant proportion of COVID-19 patients was also reported with CNS involvement but mainly in patients with high disease severity;[14–16] however, this patient in TPEVGH did not develop any severe disease and recovered from anosmia within a short period, which was contrary to the previously reported cases. The correlations among disease severity, viral load, and CNS damage were defined. However, further study should be considered. Furthermore, the influence of COVID-19 in the CNS manifestations should be observed in a more extended period.

HCQ and azithromycin were reported as optimal treatments previously, where significant reductions in viral load were observed in the patients treated with both HCQ alone and HCQ plus azithromycin.[17,18] Accordingly, HCQ was prescribed to all the confirmed cases in TPEVGH. Although, the effects of HCQ and azithromycin were controversial in the recent study.[19] Surprisingly, the overall clinical conditions and the C_T curves in cases 1, 3, and 4 concaved upward upon HCQ administration, indicating reduced viral load. However, we did not observe a significant reduction in viral load in case 2. The C_T remained comparable after HCQ administration, for which, the timing for HCQ administration might be a key factor. HCQ and azithromycin combination was not prescribed in case 3 due to high risk of inducing cardiac arrhythmia.[20]

In the SARS-CoV infection, viral loads in the respiratory specimens peaked in the first 10 days, followed by immediate decrease; however, viral loads in the stool specimens correlated to the gastrointestinal symptoms.[21] None of the confirmed cases at TPEVGH was observed with gastrointestinal symptoms; however, a vast amount of virus was detected in the stool specimen of case 4. Stool and urine from the SARS-CoV patients were tested by PCR, which remained positive even after disinfection. Hence, although some evidence indicated that sewage from the patients was not contagious, the transmissibility was

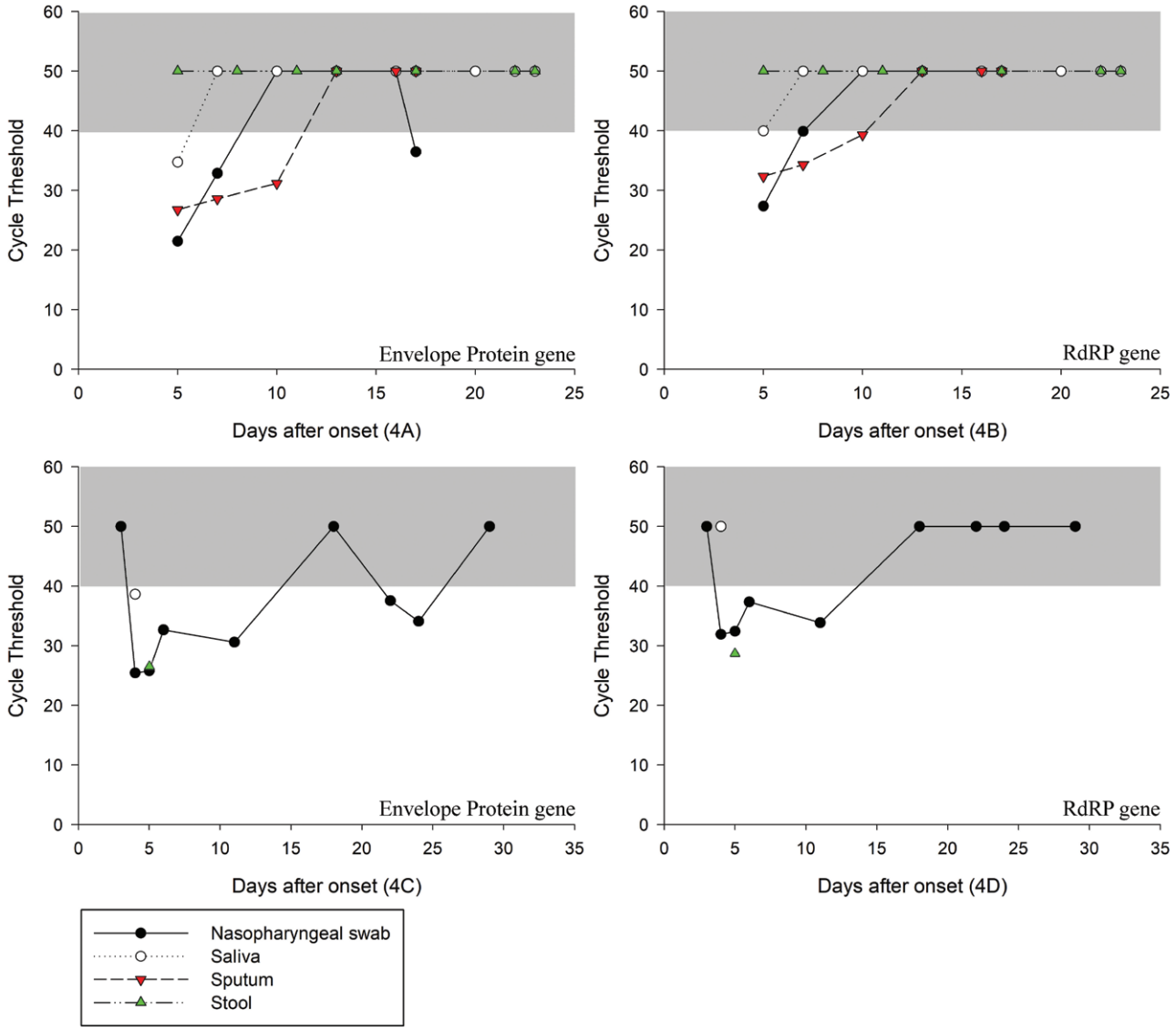

Fig. 4 Cycle thresholds of envelop protein and RNA-dependent RNA polymerase genes of cases in Taipei Veterans General Hospital. (A,B) case 3; (C,D) case 4.

still defined,[22] indicating that the sewage from the SARS-CoV-2 patients should be dealt carefully. Clinically, acquiring saliva specimens from the patients would be much safer than performing the nasopharyngeal or throat swab tests. Saliva specimen was reported as a tool to screen the SARS-CoV and SARS-CoV-2 infections[23] and showed higher consistency than the nasopharyngeal specimen.[24,25] However, the RT-qPCR results of the saliva specimens were inconsistent with nasopharyngeal specimens or sputum of the confirmed cases from TPEVGH. The method for acquiring a saliva specimen and its positive predictive value should be further modified and examined.

Due to limited COVID-19 confirmed cases at TPEVGH, the results should not be generalized to all the conditions. More cases and information on COVID-19 should be included to support the results we have observed in this study.

In conclusion, SARS-CoV-2 manifests various symptoms and affects multiple systems. Any symptom similar to viral infection should be examined carefully, especially sudden onset of CNS symptoms. Viral shielding varies in different stages of the disease. The positive prediction values for different specimens by RT-qPCR should be tested carefully to prevent false-negative results.

ACKNOWLEDGMENTS

This study was supported by the grant from the TPEVGH (grant numbers V109E-004-2(110)). We thank everyone at TPEVGH who made great efforts in COVID-19 prevention and treatment during the pandemic, especially to all who worked in the isolation wards and infection control staff.

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