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Journal of the Formosan Medical Association 120 (2021) 2186–2190

Available online at www.sciencedirect.com

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Case Report

Evolution of SARS-CoV-2 neutralizing antibody in an HIV-positive patient with COVID-19

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Received 9 February 2021; received in revised form 24 March 2021; accepted 11 April 2021

KEYWORDS
Antiretroviral therapy;
Coronavirus;

We presented the clinical course and immune responses of a well-controlled HIV-positive patient with COVID-19. The clinical presentation and antibody production to SARS-CoV-2 were similar to other COVID-19 patients without HIV infection. Neutralizing antibody reached a plateau from 26th to 47th day onset but decreased on 157th day after symptoms.

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https://doi.org/10.1016/j.jfma.2021.04.010
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Enzyme-linked immunosorbent assay (ELISA);
Plaque reduction assay;
Severe acute respiratory syndrome

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Introduction

Coronavirus disease 2019 (COVID-19) has become pandemic and continues to cause a high number of casualties. Individuals affected by COVID-19 who are aging with comorbidities and immunocompromised status appear to have poor clinical outcome. A cohort study by Williamson et al., which included 10,926 COVID-19-related mortality cases in the United Kingdom, indicated that mortalities was associated with immunocompromised status, including malignancy (non-hematological and hematological), solid-organ transplantation and rheumatic disorders.[1] Nevertheless, the impact of HIV infection on the survival of people living with HIV (PLWH) who concurrently have COVID-19 is less clear.[2] Several reports suggested that PLWH might be at an increased risk of severe COVID-19 disease, especially among those with lower CD4 cell counts or unsuppressed HIV viral replication.[3] However, for those who are receiving antiretroviral therapy (ART) with sustained viral suppression and recovery of CD4 count, the clinical course and immune response to COVID-19 might be modified.[4] Here we present the clinical course and antibody response of an individual with HIV infection who was affected by COVID-19.

Case report

The 38-year-old man had developed watery diarrhea four days prior to this admission, when he was traveling in the Philippines. Two days later, a fever of 37.4 °C and dry cough ensued. He was admitted to a negative-pressure isolation room at the National Taiwan University Hospital upon his return to Taiwan with deteriorating symptoms. SARS-CoV-2 was later identified by real-time reverse transcription-polymerase chain reaction (RT-PCR) assay of a throat swab specimen. He had had HIV infection four years earlier before this admission and had achieved successful, sustained viral suppression with coformulated abacavir, lamivudine, and dolutegravir. His latest CD4 count was 807 cells/μL four months earlier. Blood examinations showed mild leukopenia (3390 cells/μL) and lymphopenia (1389 cells/μL) upon admission. No elevation of C-reactive protein or aminotransferases was noted. The chest radiograph on admission was normal. His symptoms soon resolved within three days after admission. However, SARS-CoV-2 virus was persistently detectable by RT-PCR assays until the 45th day after the onset of the symptoms.

Laboratory investigations

SARS-CoV-2 viral isolation

Sputum or throat swab specimens obtained from the patient with COVID-19 were maintained in the viral—transport medium. The specimens were propagated in Vero E6 cells in DMEM supplemented with 2 μg/mL of tosylsulfonyl phenylalantyl chloromethyl ketone (TPCK) -trypsin (Sigma—Aldrich). Culture supernatants were harvested when more than 70% of cells showing cytopathic effects. The full-length genomic sequences of the derived clinical isolates [SARS-CoV-2/NTU04/TWN/human/2020 (Accession ID EPI_ISL_422407)] were determined and submitted, along with the patients' travel history and basic information, to the GISAID database.

SARS-CoV-2 antibody detection

Serum specimens of the patient were collected once or twice per week during the hospital stay. Follow-up serum specimens obtained at out-patient clinic three months after discharge was also submitted for antibody detection. Enzyme-linked immunosorbent assay (ELISA) (*recom*Well SARS-CoV-2 IgG: (REF 7304), *recom*Well SARS-CoV-2 IgA: (REF 7305), Mikrogen Diagnostik, Germany) and Western blotting (*recom*Line SARS-CoV-2 IgG [Aviditat] (REF 7374), Mikrogen Diagnostik, Germany) were performed to detect antibodies against SARS-CoV-2 antigens, including seasonal coronaviruses HCoV (229E, NL63, OC43, HKU1) nucleocapsid protein, SARS-CoV-2 nucleocapsid protein (NP), SARS-CoV-2 receptor binding domain (RBD) protein and SARS-CoV-2 spike surface (S1) protein.

Virus neutralization assay

Neutralizing antibody titer to SARS-CoV-2 was tested by plaque reduction neutralization assay from the serum specimens sequentially collected. The serum specimens used in these assays were heat-inactivated at 56 °C for 30 min, and then diluted in serum-free DMEM media.

Plaque reduction neutralization assay was performed in triplicate in 24-well tissue culture plates. The clinical isolates of SARS-CoV-2 used in the assay were SARS-CoV-2/NTU04/TWN/human/2020 (Accession ID EPI_ISL_422407) from the patient, and SARS-CoV-2/NTU03/TWN/human/2020 (Accession ID EPI_ISL_413592), which exhibits the D614G mutation. SARS-CoV-2 (50—100 plaque forming unit, pfu) was incubated with diluted test sera at different time points for 1 h at 37 °C before

adding to the Vero E6 cell monolayer for another 1 h. Subsequently, virus-serum mixtures were removed and the cell monolayer was washed once with phosphate buffered saline before covering with DMEM media containing 2% fetal bovine serum (FBS) and 1% methylcellulose for 5—7 days. The cells were fixed with 10% formaldehyde overnight. After removal of overlay media, the cells were stained with 0.7% crystal violet and the plaques were counted.

Results

Anti-SARS-CoV-2 IgG and IgA responses of the patient were first detected by ELISA on the 18th day after initial symptoms, which lasted until the day of his discharge from the hospital (day 48 after symptom onset). The antibody responses against SARS-CoV-2 via Western Blotting are shown in Fig. 1. Antibodies against seasonal coronaviruses HCoV (229E/NL63/OC43/HKU1) nucleocapsid protein were detected from the very early sample (the fourth day after symptoms onset) until the last serum sampled on the 157th day. Antibodies against SARS-CoV-2 specific protein (NP/RBD/S1) were firstly detected on the 13th day, and remained detectable on the 157th day.

The results of plaque reduction neutralizing assay are shown in Fig. 2 and Table 1. Neutralizing effect was not significantly observed from the serum sampled on the ninth day after the symptom onset, while the serum specimens collected on the 26th and 47th day continued to have a considerable neutralizing effect. However, such neutralizing effect gradually diminished over time, from 61% to 36% of the serum specimens collected from 47th to 158th day, with an 80-fold dilution, respectively. Compared with the neutralizing effect of the serum collected from this patient against SARS-CoV-2 with (NTU03) or without (NTU04) D614G mutation, we found that the neutralizing effect was detected earlier against the virus without D614G mutation (NTU04). However, a similar kinetics of neutralizing effect against SARS-CoV-2 with or without D614G mutation was still observed.

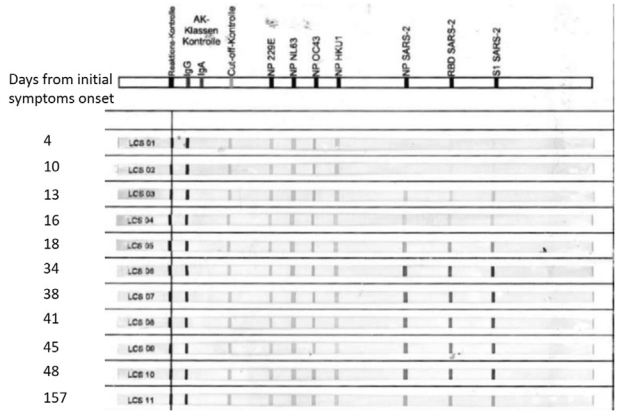

Figure 1 Antibody detection against SARS-CoV-2 by Western blot of the patient coinfected with HIV and SARS-CoV-2 on different dates.

Discussion

We present the clinical course of COVID-19 and detection of neutralizing antibody against SARS-CoV-2 of an individual coinfected with HIV and SARS-CoV-2. The findings of COVID-19 of mild severity in this individual with HIV infection was similar to those without HIV infection as recent studies described, in whom prolonged virus shedding was often noted.[5,6] In our case, SARS-CoV-2 could only be readily isolated from the respiratory specimens collected on the first day of admission. While PLWH with uncontrolled HIV or lower CD4 counts tend to have poor outcome when infected with SARS-CoV-2, the recent study by Dandachi et al. did not demonstrate excess morbidity and mortality among PLWH affected by COVID-19 who had achieved HIV viral suppression with ART.[7] However, for PLWH who had multiple comorbidities and presented with severe COVID-19, the mortality rate remained high despite higher CD4 counts.[2,8]

Until now, there are limited therapeutic agents approved for treating COVID- 19, such as remdesvir, under certain clinical conditions. Aside from searching for potential therapeutics, decoding SARS-CoV-2 immunity to facilitate research and development of vaccine is urgent to end the pandemic. The kinetics of neutralizing effect in our patient echo the findings of the study by Wang et al., in which virus-specific antibodies of immunocompetent patients reached the peak about one month after the onset of initial symptoms and might gradually decline over a 3-month period.[9]

Our study suggests that PLWH with high CD4 counts are capable of producing considerable neutralizing antibodies against SARS-CoV-2, which was comparable with the findings by Brochot et al., that neutralizing antibodies reached a plateau 2 weeks after symptom onset and then declined in the majority of inpatients without HIV infection.[10]

The difference of the neutralizing effects against SARS-CoV-2 with or without D614G mutation is still debating. D614G spike mutation might increase SARS-CoV-2 susceptibility to neutralization; however, the observations by Weissman et al. and Lee et al. suggested that D614G mutation did not impact the neutralization capacity of the elicited antibodies.[11,12]

Previous studies also highlighted that the disease severity could be associated with neutralizing antibody responses.[10,13,14] While the impact of HIV infection on kinetics of neutralizing antibody detection remains unclear, our observation suggests that PLWH with higher CD4 counts should not be excluded from being considered eligible for vaccination if the vaccination for SARS-CoV-2 is to be scaled up. However, since SARS-CoV-2 does not induce a prolonged neutralizing antibody response, investigations to identify the most effective vaccination strategies among PLWH are warranted.

In summary, the clinical presentations of COVID-19 in this PLWH who had achieved good viral suppression of HIV-1 and had high CD4 counts were similar to those reported among affected individuals without HIV infection. PLWH with higher CD4 counts could still generate considerable neutralizing antibodies against SARS-CoV-2.

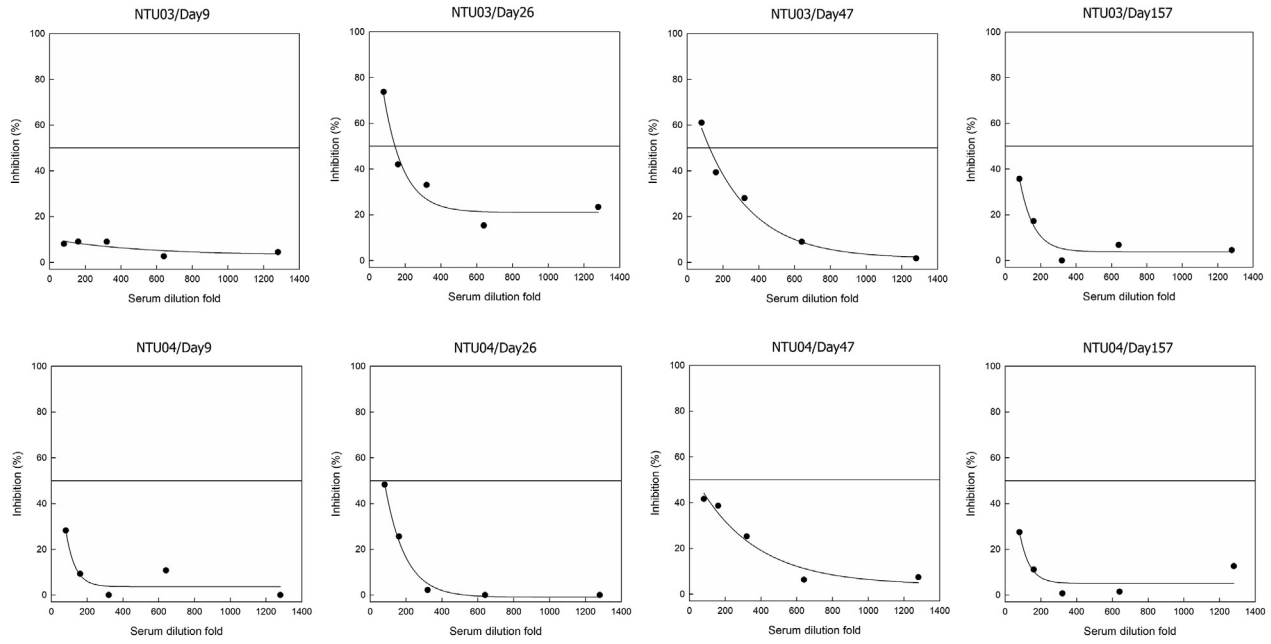

Figure 2 SARS-CoV-2 inhibition by the patient's serum samples collected on 9th, 26th, 47th and 157th day after the onset of symptoms.

Table 1 Inhibition of two virus strain by tested serum of different concentration derived from different time via plaque reduction assay.

	1:80		1:160		1:320		1:640		1:1280	
	NTU03	NTU04	NTU03	NTU04	NTU03	NTU04	NTU03	NTU04	NTU03	NTU04
Day 9	8%	28%	9%	9%	9%	0	3%	11%	5%	0
Day 26	74%	48%	42%	26%	33%	2%	15%	0	23%	0
Day 47	61%	42%	39%	39%	28%	25%	9%	6%	2%	7%
Day 157	36%	28%	17%	11%	0	1%	7%	1%	5%	13%

*Data shown in percentage of plaque reduction.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This study is supported by the National Taiwan University Hospital TOP DOWN Project (NO. 109-P13 and NO. 109-P14).

References

1. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020;**584**:430—6.
2. Blanco JL, Ambrosioni J, Garcia F, Martínez E, Soriano A, Mallolas J, et al. COVID-19 in patients with HIV: clinical case series. *Lancet HIV* 2020;**7**:e314—6.
3. Vizcarra P, Pérez-Elías MJ, Quereda C, Moreno A, Vivancos MJ, Dronda F, et al. Description of COVID-19 in HIV-infected individuals: a single-centre, prospective cohort. *Lancet HIV* 2020;**7**:e554—64.
4. Ho HE, Peluso MJ, Margus C, Matias Lopes JP, He C, Gaisa MM, et al. Clinical outcomes and immunologic characteristics of Covid-19 in people with HIV. *J Infect Dis* 2020 Jun 30:jiaa380 [Epub ahead of print].
5. Liu WD, Chang SY, Wang JT, Tsai MJ, Hung CC, Hsu CL, et al. Prolonged virus shedding even after seroconversion in a patient with COVID-19. *J Infect* 2020;**81**:318—56.
6. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;**581**:465—9.
7. Dandachi D, Geiger G, Montgomery MW, Karmen-Tuohy S, Golzy M, Antar AAR, et al. Characteristics, comorbidities, and outcomes in a multicenter registry of patients with HIV and coronavirus disease-19. *Clin Infect Dis* 2020 Sep 9:ciaa1339 [Epub ahead of print].
8. Collins LF, Moran CA, Oliver NT, Moanna A, Lahiri CD, Colasanti JA, et al. Clinical characteristics, comorbidities and outcomes among persons with HIV hospitalized with coronavirus disease 2019 in Atlanta, Georgia. *AIDS* 2020;**34**:1789—94.
9. Wang K, Long QX, Deng HJ, Hu J, Gao QZ, Zhang GJ, et al. Longitudinal dynamics of the neutralizing antibody response to SARS-CoV-2 infection. *Clin Infect Dis* 2020 Aug 3:ciaa1143 [Epub ahead of print].
10. Brochot E, Demey B, Touzé A, Belouzard S, Dubuisson J, Schmit JL, et al. Anti-spike, anti-nucleocapsid and neutralizing

antibodies in SARS-CoV-2 inpatients and asymptomatic individuals. *Front Microbiol* 2020;**11**:584251.

11. Weissman D, Alameh MG, de Silva T, Collini P, Hornsby H, Brown R, et al. D614G spike mutation increases SARS CoV-2 susceptibility to neutralization. *Cell Host Microbe* 2021 Jan 13;**29**(1):23—31. e4. [Epub ahead of print].
12. Lee CY, Amrun SN, Chee RS, Goh YS, Mak TM, Octavia S, et al. Human neutralising antibodies elicited by SARS-CoV-2 non-D614G variants offer cross-protection against the SARS-CoV-2 D614G variant. *Clin Transl Immunol* 2021 Feb 22;**10**(2):e1241 [Epub ahead of print].
13. Ko JH, Joo EJ, Park SJ, Baek JY, Kim WD, Jee J, et al. Neutralizing antibody production in asymptomatic and mild COVID-19 patients, in comparison with pneumonic COVID-19 patients. *J Clin Med* 2020;**9**:2268.
14. Liu L, To KK, Chan KH, Wong YC, Zhou R, Kwan KY, et al. High neutralizing antibody titer in intensive care unit patients with COVID-19. *Emerg Microb Infect* 2020;**9**:1664—70.