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Short Communication

Test-based de-isolation in COVID-19 immunocompromised patients: Cycle threshold value versus SARS-CoV-2 viral culture



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ABSTRACT

Background: Immunocompromised patients with coronavirus disease 2019 (COVID-19) have prolonged infectious viral shedding for more than 20 days. A test-based approach is suggested for de-isolation of these patients.

Methods: The strategy was evaluated by comparing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load (cycle threshold (Ct) values) and viral culture at the time of hospital discharge in a series of 13 COVID-19 patients: six immunocompetent and seven immunocompromised (five solid organ transplant patients, one lymphoma patient, and one hepatocellular carcinoma patient).

Results: Three of the 13 (23%) patients had positive viral cultures: one patient with lymphoma (on day 16) and two immunocompetent patients (on day 7 and day 11). Eighty percent of the patients had negative viral cultures and had a mean Ct value of 20.5. None of the solid organ transplant recipients had positive viral cultures.

Conclusions: The mean Ct value for negative viral cultures was 20.5 in this case series of immunocompromised patients. Unlike those with hematological malignancies, none of the solid organ transplant patients had positive viral cultures. Adopting the test-based approach for all immunocompromised patients may lead to prolonged quarantine. Large-scale studies in disease-specific populations are needed to determine whether a test-based approach versus a symptom-based approach or a combination is applicable for the de-isolation of various immunocompromised patients.

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Introduction

With the ongoing coronavirus disease 19 (COVID-19) pandemic, an increasing number of immunocompromised patients are becoming infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) worldwide, including solid organ transplant recipients (Elias et al., 2020). Immunocompromised patients may have prolonged viral shedding and thus may be unrecognized sources of SARS-CoV-2 transmission (Baang et al., 2021). It has been reported that critically ill patients have positive infectious SARS-CoV-2 cultures for 20 days, while those with mild disease have positive viral cultures for 8–10 days post infection (van Kampen et al., 2021; Wölfel et al., 2020). A symptom-based strategy for ending the isolation of immunocompromised patients has been published that calls for isolation for 20 days post symptom onset, compared to 10 days of isolation for immunocompetent patients (Discontinuation of Transmission-Based Precautions and Disposition of Patients with SARS-CoV-2 Infection in Healthcare Settings, CDC, n.d. 2021). A recent case report showed that an immunocompromised patient had prolonged infectious SARS-CoV-2 shedding for 143 days post symptom onset (Choi et al., 2020). Thus, a test-based approach for de-isolation of immunocompromised patients was suggested (Discontinuation of Transmission-Based Precautions and Disposition of Patients with SARS-CoV-2 Infection in Healthcare Settings, CDC, n.d. 2021).

This study was performed to evaluate this approach in a case series of immunocompromised patients. The results of SARS-CoV-2 RT-PCR were correlated with viral cultures to evaluate the association of infectiousness and persistent PCR positivity.

Materials and methods

The study was approved by King Faisal Specialist Hospital and Research Center in Jeddah, Saudi Arabia (IRB 2020-19). Hospitalized

COVID-19 patients who agreed to participate in the study were included. The recruitment was based on patient approval and consent, a positive nasopharyngeal swab for SARS-CoV-2 by RT-PCR, and the willingness of the participant to provide a follow-up nasopharyngeal swab for SARS-CoV-2 testing at the time of discharge from the hospital. Charts were reviewed for demographics, comorbidities, clinical course, outcome, and immunosuppressive medications. SARS-CoV-2 viral cultures were performed on follow-up nasopharyngeal swabs of patients on the day of hospital discharge. Baseline nasopharyngeal swabs at the time of SARS-CoV-2 diagnosis were not available for viral culture testing.

SARS-CoV-2 RT-PCR

The Abbott RealTime SARS-CoV-2 EUA test was used for the diagnosis of COVID-19. The test was performed on the Abbott m2000sp and Abbott m2000rt platforms for nucleic acid extraction and amplification, respectively. The assay targets the RNA-dependent RNA polymerase and N genes, with a detection limit of 100 RNA copies/ml (Bulterys et al., 2020).

Cell line and SARS-CoV-2 culture

Assays to detect infectious SARS-CoV-2 were performed in the biosafety level 3 laboratory of the Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University. Vero E6 cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS), as described previously (Azhar et al., 2020). A human SARS-CoV-2 patient isolate (SARS-CoV-2/human/SAU/85791C/2020, GenBank accession number [MT630432](#)) was inoculated onto the Vero E6 cells according to a previously published protocol (Azhar et al., 2020) and used as a positive control. This sample had a titer of 3.16×10^5 TCID₅₀/ml.

Table 1

Summary of the clinical and microbiological characteristics of the 13 study patients (seven immunocompromised and six immunocompetent).

	Age (years) Sex	Diagnosis and medications	Clinical course	Days post symptom onset	PCR result	Ct value	Viral culture
Immunocompromised patients							
1	34 Female	Cardiac transplant in 2014 on FK, MMF and prednisolone, epilepsy	Severe pneumonia on high-flow nasal cannula	D3 D26	Positive Positive	NA 22.87	NA Negative
2	71 Male	Renal transplant in 2014 on FK, MMF and prednisolone, DM, HTN, and CAD	Severe pneumonia on high-flow nasal cannula	D3 D17	Positive Positive	11.58 23.12	NA Negative
3	75 Male	Renal transplant in 2014 on FK, MMF and prednisolone, HTN, BPH	Pneumonia on low-flow nasal cannula	D6 D19	Positive Positive	8.82 13.88	NA Negative
4	46 Female	Lymphoma on rituximab	Pneumonia on low-flow nasal cannula	D1 D16	Positive Positive	15.83 21.07	NA Positive
5	26 Male	Renal transplant in 2018 on FK, MMF and prednisolone, DM	Pneumonia, not requiring oxygen	D4 D12	Positive Positive	10.38 27.57	NA Negative
6	38 Female	Renal transplant in 2014 on FK, AZA and prednisolone, APS, and hypothyroidism	Upper respiratory tract infection	D1 D9	Positive Positive	2.8 14.84	NA Negative
7	69 Male	DM, HTN, IHD, CLD, hepatocellular cancer on sorafenib	Upper respiratory tract infection	D1 D12	Positive Positive	12.86 21.01	NA Negative
Non-immunocompromised patients							
8	60 Female	DM, HTN, hypopituitarism	Severe pneumonia admitted to ICU, intubated and ventilated	D5 D23	Positive Positive	5 17.5	NA Negative
9	30 Male	Von Willebrand disease	Severe pneumonia on high-flow nasal cannula	D8 D11	Positive Positive	9.54 17.53	NA Negative
10	74 Male	DM, HTN, CAD	Severe pneumonia on high-flow nasal cannula	D1 D24	Positive Positive	20.01 27.50	NA Negative
11	54 Female	DM, HTN	Severe pneumonia on high-flow nasal cannula	D3 D13	Positive Positive	6.77 19.38	NA Negative
12	66 Female	Asthma, HTN	Pneumonia, not requiring oxygen	D4 D7	Positive Positive	6 15.34	NA Positive
13	48 Male	Hypothyroidism	Pneumonia, not requiring oxygen	D3 D11	Positive Positive	3.33 12.90	NA Positive

APS, anti-phospholipid syndrome; AZA, azathioprine; BPH, benign prostatic hyperplasia; CAD, coronary artery disease; CLD, chronic liver disease; DM, diabetes mellitus; FK, tacrolimus; HTN, hypertension; ICU, intensive care unit; IHD, ischemic heart disease; MMF, mycophenolate mofetil; NA, sample not available for testing. All recruited cases survived until the end of the study.

Detection of replicating SARS-CoV-2

Samples were diluted at 1:10 dilution in DMEM with 2% FBS, inoculated onto Vero E6 cells in six-well plates in duplicate, and incubated for 1 h at 37 °C. Inocula were then removed and replaced with 2 ml DMEM with 2% FBS. The plates were incubated at 37 °C and a 5% CO₂ atmosphere for 3 days or until a cytopathic effect (CPE) was observed in 85–90% of cells of the positive control samples, with daily examination for the CPE. This viral isolation system has a sensitivity of 3.16 TCID₅₀/ml, as tested by serial dilution of the control sample.

Statistical analysis

Categorical variables were presented as frequencies and percentages, while continuous variables were presented as the mean ± standard deviation (SD). Differences in categorical variables were examined using Fisher's exact test, while differences in continuous variables were examined using the Mann-Whitney test. All P-values were two-tailed. A P-value <0.05 was considered as significant. IBM SPSS Statistics software release 25.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses.

Results

A total of 13 patients were included in this study (seven male and six female). The mean age was 53 ± 17.4 years. There were seven immunocompromised patients and six immunocompetent patients who underwent viral culture in addition to SARS-CoV-2 PCR testing at the time of hospital discharge.

The immunocompromised group comprised five solid organ transplant recipients and two patients with malignancy (lymphoma and hepatocellular carcinoma). The average duration after transplantation was 5.2 ± 1.8 years. Of the seven immunocompromised patients, two (28.5%) had severe pneumonia and were on high-flow oxygen, three (43%) had pneumonia, and two (28.5%) had an upper respiratory tract infection.

Among the six patients in the immunocompetent group, hypertension (n = 4, 67%) and diabetes (n = 3, 50%) were the most common comorbidities. One patient (17%) was intubated and ventilated, three patients (50%) had severe pneumonia and were on high-flow oxygen, and two patients (33%) had pneumonia, not requiring oxygen (Table 1).

Viral cultures and viral load (cycle threshold (Ct) values)

Among the 13 patients, three (23%) had positive viral cultures (Table 2): one patient with lymphoma (on day 16) and two non-

Table 2
Association between viral culture results, immune status of the patients, Ct values, and days from symptom onset to time of viral culture.

	Negative viral culture	Positive viral culture	Total	P-value
Number (%) of patients				
Immunocompromised	6 (85.7%)	1 (14.3%)	7 (100.0%)	0.559
Immunocompetent	4 (66.7%)	2 (33.3%)	6 (100.0%)	
Total	10 (76.9%)	3 (23.1%)	13 (100.0%)	
Days from symptom onset to time of viral culture (mean ± SD)				
Immunocompromised	15.8 ± 6.2	16.0 ± 0	15.9 ± 5.6	>0.99
Immunocompetent	17.8 ± 6.7	9.0 ± 2.8	14.8 ± 7.0	0.133
Total	16.6 ± 6.1	11.3 ± 4.5	15.4 ± 6.0	0.161
Ct values compared to viral culture results (mean ± SD)				
Immunocompromised	20.5 ± 5.3	21.1 ± 0	20.6 ± 4.8	>0.99
Immunocompetent	20.5 ± 4.8	14.1 ± 1.7	18.4 ± 5.0	0.133
Total	20.5 ± 4.8	16.4 ± 4.2	19.6 ± 4.8	0.287

SD, standard deviation.

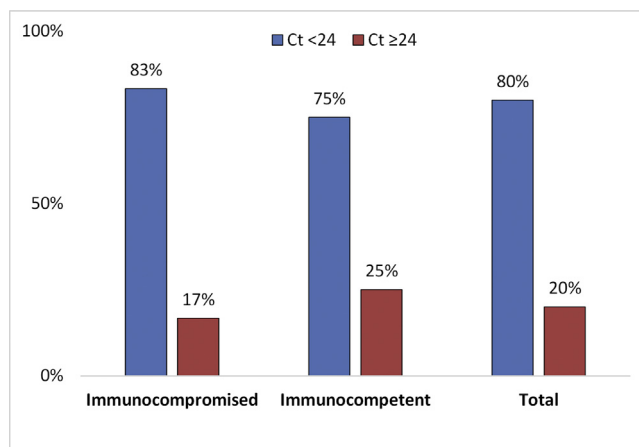


Figure 1. Graph showing patients with negative cultures (n = 10) and the percentage of those with a Ct value >24 and a Ct value <24 among immunocompetent and immunocompromised patients.

immunocompromised patients (on day 7 and day 11). Ten patients (77%) had negative viral cultures on day 9–26. The average time from symptom onset to follow-up viral culture was 15.9 ± 5.6 days, with no difference by viral culture results (P = 0.161) or by immune status (P = 0.628). The average PCR Ct value for the immunocompromised patients was 20.6 ± 4.8, with almost identical results in those with negative and positive culture (P > 0.99). The average PCR Ct value for immunocompetent patients was 18.4 ± 5.0, with higher (but non-significant) results in those with negative culture compared to those with positive culture (20.5 ± 4.8 versus 14.1 ± 1.7, P = 0.133). As shown in Figure 1, eight (80%) out of the 10 patients with negative viral cultures had Ct values less than 24. This percentage was 83% in immunocompromised patients compared with 75% in immunocompetent patients (P > 0.99).

Discussion

In this study, three of the 13 (23%) samples had positive viral cultures; the mean Ct value for negative cultures was 20.5 and the mean time from symptom onset to testing was 16.6 days. The positive rate of infectious SARS-CoV-2 culture has been variable in previous studies, depending on the disease severity and time from symptom onset to testing. Bullard et al. reported a viral positivity rate of 28.9% (26/90) up to 8 days post symptom onset, and the median Ct value for negative viral culture was 23 (Bullard et al., 2020). Details of disease severity and the extent of immunosuppression were not described in that study. Basile et al. described a 24% (56/243) viral positivity rate in 195 patients with disease of varying severity, with a mean time between symptom onset and testing of 4.5 days. The positivity rate was 15% in outpatients, 45% in inpatients, and 82% in ICU patients. The positivity rate also differed according to the duration between symptom onset and testing. It was 80% in the first week, 45% in the second week, and 4% in the third week (Basile et al., 2020).

In the present study, most of the samples with negative viral cultures had Ct values of less than 24. These patients recovered and were at least 10 days post symptom onset. Extending the isolation of these patients based on the results of Ct values would lead to prolonged quarantine. Previous studies have shown that samples with Ct values less than 24 are more likely to have positive viral cultures compared to samples with Ct values greater than 24 (Jefferson et al., 2020). A recent study showed that patients may still have infectious viral shedding with a high Ct value greater than 25 (Folgueira et al., 2021). Folgueira et al. showed that 5% of patients with mild disease and 10% of patients with severe disease

who had Ct values >35 had positive viral cultures. In addition, 10% of patients with Ct values <25 had negative viral culture and they were 10 days post symptom onset. In the present study, 80% of patients had negative viral cultures, with Ct values <25. The study population included 50% immunocompromised patients; this differs from the previous study, which included only 21% immunocompromised patients.

Together, all of these results demonstrate the challenges of adopting a test-based approach for de-isolation of immunocompromised patients, as patients with high Ct values may still be infectious, while patients with low Ct values may not be infectious. Until better diagnostic modalities other than viral cultures are developed, this technique remains the gold standard method for identifying the infectivity of COVID-19 patients (Huang et al., 2020). In a recent unpublished study, the Ct values of superspreaders and non-superspreaders did not differ significantly; values were overlapping, indicating that the Ct value is not a reliable indicator for SARS-CoV-2 transmission (Tian, under review).

In the study cohort, one patient with lymphoma who received rituximab had positive viral cultures more than 20 days after the onset of symptoms, which is similar to other reports of prolonged infectious viral shedding in patients with hematological diseases and B cell dysfunction (Hensley et al., 2021). None of the patients with solid organ transplants had positive viral cultures for more than 20 days post symptom onset and they did not receive B cell depleting agents such as rituximab. It can be postulated that immunocompromised patients with B cell depletion are those who would benefit the most from the test-based protocol for de-isolation, while for others, a combination of clinical response and testing should be used to release patients from isolation. This recommendation is consistent with our understanding of the major role that antibodies have in clearing virus and protecting against re-infection (Lumley et al., 2021). A risk-based approach for infectious viral shedding in immunocompromised patients would be useful to identify patients who should quarantine for more than 20 days, taking into consideration the variable course and outcomes in various immunocompromised patients (Fung and Babik, 2021).

The results of this study are limited by the small sample size and lack of serial cultures for each patient. Future large studies of SARS-CoV-2 viral cultures in specific populations such as solid organ transplant recipients, HIV-infected individuals, and patients on biological agents are needed to validate the study findings and determine whether a test-based approach versus a symptom-based approach or a combination is applicable for the de-isolation of various immunocompromised patients.

Author contributions

Study design: ANA, EIA. Data collection: MFS, MAAH, MAAM, DTB, GEA, LKH. Data analysis: AE, ANA, SP. Writing the manuscript: ANA, SAE. Reviewing the manuscript: JAA, HAB, IK, EIA, SP. Performing the test: AD, EIA, AMT, SIA, SAE, AMH, TAA, LHB. Providing patients: ANA, NAZ, RSA.

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This research project was approved by the Institutional Research Board of King Faisal Specialist Hospital and Research Center in Jeddah (IRB 2020-19).

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