Test-based De-isolation in COVID-19 Immunocompromised patients: Ct value versus SARS-CoV-2 viral cultures


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Highlights:
De-isolation of immunocompromised COVID-19 patients is challenging

Test-based rather than symptom-based approach is suggested

The mean Ct values for negative viral cultures was 20.5 in our case series

A test-based approach could lead to prolonged quarantine of non-infectious patients

The de-isolation of immunocompromised patients needs disease-specific studies

Abstract:

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

Methods: We evaluated this strategy by comparing SARS-CoV-2 viral load (Ct values) and viral cultures at the time of hospital discharge in a series of immunocompetent (6 patients) and immunocompromised (5 solid organ transplants, 1 patient with lymphoma and one patient with hepatocellular carcinoma) COVID-19 patients.

Results: 3/13 (23%) patients had positive viral cultures: one patient with lymphoma at day 16 and two immunocompetent patients at day 7 and 11. Of the patients, 80% had negative viral cultures and had mean Ct value 20.5. None of the solid organ transplants recipients had positive viral cultures.

Conclusion: The mean Ct values for negative viral cultures was 20.5 in our case series of immunocompromised patients. Unlike hematological malignancies, none of the solid organ transplants had positive viral cultures. Adopting the test-based approach for all immunocompromised patients may lead to prolonged quarantine. Large scale studies in
Introduction:
With the ongoing coronavirus disease 19 (COVID-19) pandemic, there is an increasing number of immunocompromised patients being infected with the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) worldwide including solid organ transplant recipients (Elias M et al, 2020). Immunocompromised patients may have prolonged viral shedding and thus may be unrecognized sources of SARS-CoV-2 transmission (Baang JH et al, 2021). Critically ill patients had positive infectious SARS-CoV-2 cultures for 20 days while those with mild disease had positive viral cultures for 8-10 days post infection (van Kampen JJA et al, 2021) (Wölfel R et al, 2020). A symptom-based strategy for removing isolation of immunocompromised patients was published and calls for isolation for 20 days post symptom onset, compared to 10 days of isolation in 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 B 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.). In this study, we evaluated this approach in a case series of immunocompromised patients.
patients and we correlated the results of SARS-CoV-2 RT-PCR 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. We included hospitalized COVID-19 patients who agreed to participate in the study. 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 at 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, used for the diagnosis of COVID-19, was performed on the Abbott m2000sp and Abbott m2000rt platforms for nucleic acid extraction and amplification respectively. The assay targets the RdPp and N genes with a detection limit of 100 RNA molecules c/ml (Bultery LS 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 previously described (Azhar EI 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 our previously published protocol (Azhar EI et al., 2020) and used as a positive control. This sample had a titer of $3.16 \times 10^5$ TCID$_{50}$/ml.

**Detection of replicating SARS-CoV-2**

Samples were diluted at 1:10 dilution in DMEM with 2% FBS, inoculated onto Vero E6 cells in 6 well plates in duplicates, and incubated for 1hr at 37°C. Inocula were then removed and replaced with 2 mL DMEM with 2% FBS. Plates were incubated at 37°C and a 5% CO$_2$ atmosphere for 3 days or until cytopathic effect (CPE) was observed in 85-90% of cells of the positive control samples with daily examination for CPE. This viral isolation system has a sensitivity of 3.16 TCID$_{50}$/ml as tested by serial dilution of the control sample.

**Statistical analysis:**

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

**Results:**

In this study, we included 13 patients (7 male and 6 female patients). The mean age±SD was 53±17.4 years. There were 7 immunocompromised and 6 immunocompetent patients who underwent viral culture in addition to SARS-CoV-2 PCR testing at the time of hospital discharge.

The immunocompromised group constituted 5 solid organ transplants and 2 patients with malignancy (lymphoma and hepatocellular carcinoma). The average duration after transplantation was 5.2±1.8 years. Out of 7 immunocompromised patients, 2 (28.5%) had
severe pneumonia on high flow oxygen, 3 (43%) had pneumonia and 2 (28.5%) had upper respiratory tract infection.

Among the 6 immunocompetent group, hypertension (N=4, 67%) and diabetes (N=3, 50%) were the most common comorbidities. One patient (17%) was intubated and ventilated, 3 patients (50%) had severe pneumonia on high flow oxygen and 2 patients (33%) had pneumonia (table 1).

**Viral cultures and viral load (Ct values):**

Among the 13 patients, 3 (23%) patients had positive viral cultures (Table 2); one patient with lymphoma at day 16 and two non-immunocompromised patients at day 7 and 11. Ten patients (77%) had negative viral cultures at days 9-26. The average time from symptom onset to follow up viral culture was 15.9±5.6 with no difference by viral culture results (p=0.161), and by immune status (p=0.628). The average PCR Ct values in 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 values in immunocompetent patients was 18.4±5.0, with higher (but non-significant) results in those with negative culture compared with positive culture (20.5±4.8 versus 14.1±1.7, p=0.133). As shown in Figure 1, 8 (80%) out of 10 patients with negative viral cultures had Ct values less than 24. This percentage was 83% in immunocompromised compared with 75% in immunocompetent patients (p>0.99).

**Discussion:**

In this study, 3/13 (23%) of samples had positive viral cultures, the mean Ct value for negative cultures was <20.5 and mean symptom to time test was 16.6 days. The positive rate of infectious SARS-CoV-2 culture was variable in previous studies depending on disease severity and symptom to time of test. Bullard et al reported viral positivity rate of 26/90 (28.9%) up to 8 days post symptom onset and the median Ct value for negative viral culture
was <23 (Bullard J et al, 2020). Details of disease severity and extent of immunosuppression were not described in this study. Basile et al described 56/243 (24%) viral positivity rate in 195 patients with various disease severity with a mean symptom to test time of 4.5 days. The positivity rate was 15%, 45% and 82% in outpatients, inpatients and ICU patients respectively. The positivity rate was also different according to the duration of symptom to test time. It was 80%, 45% and 4% in the first week, second week and third week respectively (Basile K et al, 2020).

In our study, most of the samples with negative viral cultures had Ct values 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 will lead to prolonged quarantine. Previous studies showed that samples with Ct values less than 24 were more likely to have positive viral cultures compared to samples with Ct values more than 24 (Jefferson T et al., 2020). A recent study showed that patients may still have infectious viral shedding with high Ct value more than 25 (Folgueira MD et al., 2021). Folgueira showed that 5% and 10% of patients with mild and severe disease respectively and 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 our study, 80% of patients had negative viral cultures with Ct values <25. Our study population had 50% immunocompromised patients unlike the other 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 be still infectious while patients with low Ct values may be not 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 CG et al 2020). In a recent unpublished study, the Ct values of superspreaders and non-superspreaders were not
significantly different with overlapping values indicating that the Ct values are not reliable indicators for SARS-CoV-2 transmission (Tian D, under review).

In our cohort, one patient with lymphoma who received rituximab had positive viral cultures more than 20 days from onset of symptoms similar to the other reports of prolonged infectious viral shedding in patients with hematological diseases and B cell dysfunction (Hensley MK 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 and testing should be used to remove 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 SF et al. 2021). A risk-based approach for infectious viral shedding in immunocompromised patients would be useful to identify patients that require quarantine more than 20 days, taking into consideration the variable course and outcome in various immunocompromised patients (Fung M et al., 2020).

Our results are limited by the small sample size and lack of serial cultures in each patient. Future large studies of SARS-CoV-2 viral cultures in specific populations such as solid organ transplants, HIV infected individuals and patients on biological agents are needed to validate our 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.

**Contributions:**

Study design (ANA, EIA), Data collection (MFM, MAAH, MAAM, DTB, GEA, LKH), Data analysis (AE, ANA, SP), writing the manuscript (ANA, SAE), reviewing the manuscript
(JAA, HAB, IK, EIA, SP), perform the test (AD, EIA, AMT, SIA, SAE, AMH, TAA, LHB), providing patients (ANA, NAZ, RSA)

Funding:

None

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.

Conflict of Interest:

The authors declare no conflict of interest

Acknowledgement:

We would like to thank our patients who contributed to this work.
References


Tian D, Lin Z, Kriner EM, Esneault DJ, Tran J, DeVoto JC et al. SARS-CoV-2 load does not predict transmissibility in college students. medRxiv preprint doi: https://doi.org/10.1101/2021.03.02.21252105


Figure Legend:

The graph shows patients with negative cultures (n=10) and the percentage of those with Ct value $>$ 24 and Ct value $<$ 24 in immunocompetent and immunocompromised patients.
Table 1: Summary of the clinical and microbiological characteristics of 13 patients (7 immunocompromised and 6 immunocompetent)

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis and Medications</th>
<th>Clinical course</th>
<th>Days post symptom onset</th>
<th>PCR Result</th>
<th>Ct Value</th>
<th>Viral culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>Female</td>
<td>Cardiac transplant in 2014 on FK, MMF and Prednisolone, Epilepsy</td>
<td>Severe pneumonia on high flow nasal cannula</td>
<td>D3 D26</td>
<td>Positive</td>
<td>22.8 7</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>Male</td>
<td>Renal transplant in 2014 on FK, MMF and prednisolone, DM, HTN and CAD</td>
<td>Severe pneumonia on high flow nasal cannula</td>
<td>D3 D17</td>
<td>Positive</td>
<td>11.5 8 23.1 2</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>Male</td>
<td>Renal transplant in 2014 on FK, MMF and Prednisolone, HTN, BPH</td>
<td>Pneumonia on low flow nasal cannula</td>
<td>D6 D19</td>
<td>Positive</td>
<td>8.82 13.8 8</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>Female</td>
<td>Lymphoma on Rituximab</td>
<td>Pneumonia on low flow nasal cannula</td>
<td>D1 D16</td>
<td>Positive</td>
<td>15.8 3 21.0 7</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>Male</td>
<td>Renal transplant in 2018 on FK, MMF and prednisolone, DM</td>
<td>Pneumonia Not requiring oxygen</td>
<td>D4 D12</td>
<td>Positive</td>
<td>10.3 8 27.5 7</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>Female</td>
<td>Renal transplant in 2014 on FK, AZA and Prednisolone, APS and hypothyroidism</td>
<td>Upper respiratory tract infection</td>
<td>D1 D9</td>
<td>Positive</td>
<td>2.8 14.8 4</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>69</td>
<td>Male</td>
<td>DM, HTN, IHD, CLD, hepatocellular cancer on sorafenib</td>
<td>Upper respiratory tract infection</td>
<td>D1 D12</td>
<td>Positive</td>
<td>12.8 6 21.0 1</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>Female</td>
<td>DM, HTN, Hypopituitarism</td>
<td>Severe pneumonia admitted in ICU intubated and ventilated</td>
<td>D5 D23</td>
<td>Positive</td>
<td>5 17.5</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>Male</td>
<td>Von Willebrand Disease</td>
<td>Severe pneumonia on high flow nasal cannula</td>
<td>D8 D11</td>
<td>Positive</td>
<td>9.54 17.5 3</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>74</td>
<td>Male</td>
<td>DM, HTN, CAD</td>
<td>Severe pneumonia on high flow nasal cannula</td>
<td>D1 D24</td>
<td>Positive</td>
<td>20.0 1 27.5 0</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>54</td>
<td>Female</td>
<td>DM, HTN</td>
<td>Severe pneumonia on high flow nasal cannula</td>
<td>D3 D13</td>
<td>Positive</td>
<td>6.77 19.3 8</td>
<td>Negative</td>
</tr>
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</tr>
<tr>
<td>12</td>
<td>66</td>
<td>Female</td>
<td>Asthma, HTN</td>
<td>Pneumonia Not requiring oxygen</td>
<td>D4 D7</td>
<td>Positive Positive</td>
<td>6 15.3 4</td>
<td>NA Positive</td>
</tr>
<tr>
<td>13</td>
<td>48</td>
<td>Male</td>
<td>Hypothyroidism</td>
<td>Pneumonia Not requiring oxygen</td>
<td>D3 D11</td>
<td>Positive Positive</td>
<td>3.33 12.9 0</td>
<td>NA Positive</td>
</tr>
</tbody>
</table>

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

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