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Co-infection of human immunodeficiency virus, herpes simplex virus-2 and SARS-CoV-2 with false-negative real-time polymerase chain reaction

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Dear Sir,

The role of real-time polymerase chain reaction (RT-PCR) is pivotal in the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The coronavirus disease 2019 (COVID-19) pandemic started from Wuhan, China in December 2019, and the disease is still evolving.⁽¹⁻³⁾ False-negative RT-PCR has been reported in human immunodeficiency virus (HIV) patients with COVID-19.⁽⁴⁾ However, there is scarce data on the features of COVID-19 in these patients.⁽⁵⁾ We present a case of co-infection of HIV-1, herpes simplex virus-2 (HSV-2), and SARS-CoV-2 with false-negative RT-PCR in a critically ill patient.

The first case of COVID-19 in Saudi Arabia was confirmed on 2 March 2020, and currently more than 316,000 people have been infected and more than 3,900 patients have died.^(6,7) In the month of May 2020, at the peak of the COVID-19 outbreak in our country, a previously healthy 40-year-old woman was admitted to the emergency department with four days of fever (38.5°C), persistent cough, myalgias and dyspnoea. Her saturation of peripheral oxygen was 70% on room air, and she had haemodynamic instability. Her score on the Glasgow Coma Scale was 9 out of 15, with no other neurological pathology. She was intubated, mechanically ventilated and transferred to the intensive care unit (ICU) designated for COVID-19. Laboratory findings were normal, apart from lymphocytopenia ($0.69 \times 10^9/L$; normal $1.1\text{--}3.2 \times 10^9/L$), and increased levels of C-reactive protein (706 mg/L; normal 0–5 mg/L) and lactate dehydrogenase (990 units/L; normal 100–190 units/L). The rest of the biochemical report was within normal limits.

The patient underwent a full diagnostic work-up for viral, bacterial and systemic disorders. Cardiac enzymes, electrocardiogram and echocardiography were normal. Chest computed tomography (CT) depicted bilateral ground-glass opacities and consolidations (Fig. 1a). She was

tested for SARS-CoV-2 infection by RT-PCR assays (targeting for RdRp gene, E gene, and N gene of SARS-CoV-2), which were performed with nasopharyngeal swabs using QuantiNova Probe RT-PCR kit (Qiagen) in a Light-Cycler 480 real-time PCR system (Roche, Basel, Switzerland).^(8,9) Specifically, each 20- μ l reaction mixture contained 10 μ l of 2 \times QuantiNova probe RT-PCR master mix, 0.2 μ l of QN Probe RT-Mix, 1.6 μ l of each 10 μ M forward and reverse primer, 0.4 μ l of 10 μ M probe, 1.2 μ l of RNase-free water, and 5 μ l of TNA as the template. The thermal cycling condition was 10 minutes at 45°C for reverse transcription, 5 minutes at 95°C for PCR initial activation, and 45 cycles of 5 seconds at 95°C and 30 seconds at 55°C. The assays were performed as previously described.⁽¹⁰⁾ However, RT-PCR results for SARS-CoV-2 were negative.

Emergency central nervous system (CNS) CT and angiography revealed diffuse white matter hypodensities and brain oedema (Fig. 1b & C). At that time, the differential diagnosis included stroke, encephalitis and posterior reversible encephalopathy syndrome, among others. Upon ICU admission (Day 1), lopinavir/ritonavir (400 mg/100 mg, two tablets of 200 mg/50 mg twice daily), acyclovir (10–15 mg/kg intravenously every 8 hours), ceftriaxone (1g intravenously twice daily), dexamethasone (10 mg intravenously once daily), therapeutic low-molecular-weight heparin (adjusted for her body weight and renal function) and ICU supportive care were administered by the treating ICU team.⁽¹¹⁾ The patient also received acute respiratory distress syndrome net and prone positioning ventilation, maintaining a partial arterial pressure of oxygen to fractional inspired concentration of oxygen ratio of 160. Cerebrospinal fluid (CSF) analysis revealed slightly elevated opening pressure of 22 cm CSF (normal 10–20 cm), 205 white blood cells/ μ L (85% lymphocytes) with normal protein and glucose levels. PCR in the serum detected HIV-1 (plasma viral copies 3.000/ μ L; CD4 and CD8 -T cells 350/ μ L and 684/ μ L, respectively). In the CSF, HSV-2-DNA was found by means of HSV-1/2 PCR kit (Qiagen, Valencia, CA, USA).

Interestingly, another RT-PCR test for SARS-CoV-2 performed on nasopharyngeal specimens (24 hours apart) was persistently negative.

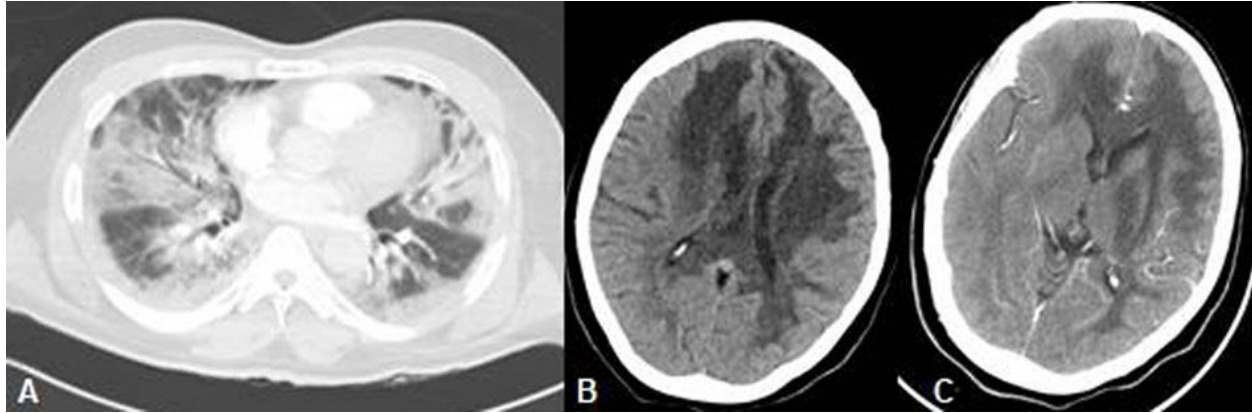


Fig. 1 (a) CT image of the chest shows bilateral diffuse peripheral ground-glass opacities and consolidations with mainly lower lobe distribution. (b) Brain CT and (c) CT angiography images show diffuse white matter hypodensities in the frontal-parietal region, extending to the corpus callosum, basal ganglia and brain stem (mainly on the left side).

On Day 3, the patient developed septic shock and multisystem organ failure. Antibiotics were upgraded to meropenem (2 g intravenously every 8 hours), and vancomycin (1g intravenously every 12 hours). Vasopressors and fluid resuscitation were initiated. The patient developed acute kidney injury with associated acidosis and hyperkalaemia, and underwent continuous renal replacement therapy as per Kidney Disease Improving Global Outcomes 2019 guidelines.⁽¹²⁾ On Day 4 post-ICU admission, the third RT-PCR test performed for COVID-19 was positive (31 copies for the E gene and 29 copies for the RdRp gene assays per reaction). Rescue therapies were considered; however, the patient developed ventricular tachycardia and cardiac arrest. No electrolyte disturbances were observed. Despite strenuous resuscitation efforts, the patient succumbed to the disease. Blood cultures for common bacterial and fungal infections were

negative. CSF *Cryptococcal* and blood *Toxoplasma gondii* antigen serology tests were also negative. Cultures derived from endotracheal tube aspirates were negative for common bacteria, while microscopy examination, which was used for detection of *Pneumocystis species*, was also negative. In addition, tests that were performed for systemic disorders (i.e. autoimmune disorders, including antiphospholipid antibodies) were negative.

This brief case report, albeit with limitations, carries important messages. Life-threatening COVID-19 can present with acute respiratory distress syndrome, sepsis, multisystem organ failure, neurological manifestations and thromboembolic phenomena.⁽¹⁻³⁾ Notably, devastating CNS pathology, ranging from meningoencephalitis to stroke and acute disseminated encephalomyelitis, has been reported in patients with severe COVID-19.⁽¹³⁻¹⁵⁾ In our patient, contrast brain CT did not depict any ring enhancement; however, the possibility of other diagnoses, such as toxoplasmosis or CNS lymphoma related to HIV, cannot be excluded. Unfortunately, magnetic resonance imaging was not performed as the patient deteriorated rapidly and could not be transported safely outside of the ICU. Also, the possibility of stroke remains a differential diagnosis and the detection of HSV-2 in the CSF cannot be ignored. This is a rare (< 2%) cause of HSV encephalitis and has a bad prognosis, although it is rare for HSV-2 encephalitis to present with brain oedema and mass effect.⁽¹⁶⁾ We are uncertain how our patient was infected with SARS-CoV-2 and HIV, as her past medical history and list of conducts were not available. Hence, the exposure risk could not be properly evaluated, which is a major limitation of this case report, preventing its generalisability. Nevertheless, our patient had a relatively high HIV viral load with preserved CD4 count and CD4/CD8 ratio. Therefore, we speculate that our patient could have acute HIV infection or HIV seroconversion illness, which is associated with CNS pathology,⁽¹⁷⁾ although its link to COVID-19 is not yet elucidated.

In our case, cultures for fungal infections, microscopy for detection of *pneumocystis species*, as well as serology antigen tests for *Cryptococcus* and *Toxoplasma species* were all negative; however, opportunistic infections cannot be definitively excluded. Initial empiric treatment could have included trimethoprim/sulfamethoxazole, but the decision was not made by the treating team. Despite all applied empiric and supportive therapies, the patient had a fulminant clinical course and succumbed due to the development of cardiac arrhythmias, which led to cardiac arrest, although no electrolyte disturbances were observed. Hence, the possibility of COVID-19-related myocarditis or stress cardiomyopathy should be considered, as reported in other studies.⁽¹⁸⁻²⁴⁾ Post-mortem autopsy reports were not available in our case, as it is not allowed by the current legislation in Saudi Arabia. In our untreated HIV patient, the false-negative result of RT-PCR for COVID-19 cannot be clearly attributed to the suppressive effect of anti-HIV-1 agents.^(4,5) Stability issues and test fluctuations due to insufficient viral material of the obtained nasopharyngeal specimen might have been responsible for the persistently negative RT-PCR.⁽²⁵⁾ Notably, testing for SARS-CoV-2 could also be directed toward lower respiratory tract specimens (i.e. endotracheal aspirate and bronchoalveolar lavage specimens), although this remains controversial, or alternate specimens (i.e. stool), which along with serology examinations, may be helpful diagnostic adjuncts.⁽²⁶⁻²⁸⁾

In our patient, the full picture of epidemiologic and exposure risk background was not available, which further complicated the evaluation of her immune system response to the viral infections. Scarce data exists in the literature on the laboratory and clinical features of COVID-19 in patients with active HIV infection.^(4,5) The role of RT-PCR is pivotal in the diagnosis of SARS-CoV-2 infection, but in the face of inconclusive results, the integration of chest CT in our diagnostic arsenal remains mandatory.^(29,30) Moreover, the natural course of SARS-CoV-2

viraemia remains obscure, as re-infections and/or recurrently positive RT-PCR results were reported.⁽³⁰⁻³²⁾

In conclusion, co-infection of SARS-CoV-2 with other viruses may result in unpredictable dysregulations of the immune system response that are not fully understood. The diagnostic and therapeutic challenges are obvious. Further research is required to investigate co-infections of SARS-CoV-2 with other viruses, as well as the diagnostic performance of RT-PCR in such cases.

Yours sincerely,

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This study was approved by the Institutional Review Board of King Saud Medical City, Riyadh, Kingdom of Saudi Arabia (H-01-R-053, IORG0010374#, H1RI-07 May-20-01). Written informed consent was obtained from the patient's legal representative post-mortem for publication of this study for scientific purposes.

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