COVID-19

Detected SARS-CoV-2 in Ascitic Fluid Followed by Cryptococcemia: a Case Report

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Abstract

SARS coronavirus-2 (SARS-CoV-2) detection in different clinical specimens has raised important insights about its pathogenesis, but some details remain to be understood. In that respect, disrupt viral control seen in solid organ transplant patients on chronic immunosuppression can help unveil pathogenic mechanisms and characterize new coronavirus disease-19 (COVID-19) immunological and clinical aspects, as well as secondary complications. We herein report a case of SARS-CoV-2 detection in ascitic fluid from a kidney transplant patient with decompensated cirrhosis and COVID-19 and then discuss about immune, cellular, and virological aspects of such clinical presentation of the disease, which also included a disseminated infection, demonstrated by viral detection in his blood sample. We subsequently discuss about the fatal outcome caused by a secondary bloodstream infection by Cryptococcus neoformans. This unprecedented case report presents ascitic fluid as a novel specimen in which SARS-CoV-2 can be detected. Immune dysregulation and cumulative risk factors may lead to secondary infections by opportunistic agents, including Cryptococcus neoformans.

Keywords Cryptococcus · Cirrhosis · COVID · Coinfection · Viremia · Kidney transplant

Introduction

SARS coronavirus-2 (SARS-CoV-2) affects primarily upper and lower respiratory tract, but detection in different clinical specimens, including serum and stool, has raised important insights about its kinetics and pathogenesis [1].

In that respect, disruption in viral control seen in solid organ transplant patients on chronic immunosuppression [2] can help pathogenic mechanisms and characterize new coronavirus disease-19 (COVID-19) immunological and clinical aspects, as well as secondary complications.

We herein report an unprecedented case of SARS-CoV-2 detection in ascitic fluid from a kidney transplant patient with decompensated cirrhosis and COVID-19 and then discuss about immune, cellular, and virologic aspects of such clinical presentation of the disease, which also included a disseminated infection, demonstrated by viral detection in his blood sample. We then discuss about the fatal outcome caused by a secondary bloodstream infection by *Cryptococcus neoformans*.

Case Report

A 75-year-old white male with a history of hypertension, and a kidney transplant from a deceased donor 3 years prior, presented in the emergency department on May 18, 2020, 5 days following the onset of dry cough and progressive dyspnea, without fever or other respiratory or gastrointestinal tract symptoms. He was on immunosuppressive therapy with 4 mg tacrolimus and 5 mg prednisone and denied previous opportunistic infections. Physical examination at admission revealed an axillary temperature of 37.1 °C, blood pressure of 160/90 mmHg, heart rate of 104 per minute, respiratory rate of 25 breaths per minute, and oxygen saturation of 92% while

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breathing ambient air. Abdominal examination demonstrated mild ascites with collateral circulation.

He was diagnosed with liver cirrhosis 2 years ago and denied any prior decompensation, including no history of ascites. During investigation at the time, concomitant portal hypertension and thrombosis were also found but were stable since then without anticoagulation. Previous serologies were negative for HIV, hepatitis C, syphilis, and schistosomiasis. His hepatitis B serostatus was positive anti-HBs (255 UI/L), positive anti HBc, and negative HBsAg. Also, he had undetectable hepatitis B viral loads on two consecutive PCR exams.

Initial laboratory findings showed normal white cell count but severe lymphopenia (162 per mm^3) as well as other cirrhosis dysfunctions including thrombocytopenia, increased international normalized ratio (INR), and hypoalbuminemia. Creatinine was 1.5 times its baseline value of 2.4 mg/dl, procalcitonin was elevated at 3.92 ng/dl, and d-dimer was above 20 μg/mL (maximum test detection). Total bilirubin was normal at 0.67 mg/dL, as were transaminases (AST: 33 U/L; ALT: 20 U/L). Evolutive laboratory exams may be seen in Table 1.

Chest computed tomography scan showed bilateral ground-glass opacities with some areas of consolidation (Fig. 1), and specimens from his nasopharyngeal swab were positive for COVID-19 on real-time reverse-transcriptase polymerase chain reaction (RT-PCR).

As supplemental oxygen (O2) with a nasal cannula was administered at 2 L/min, his pulse oximetry went up to 97%, and he was started on ceftriaxone and clarithromycin. Tacrolimus was suspended, and prednisone dose was increased to 30 mg daily.

On day 2 of hospitalization (day 8 of illness), his breathing pattern worsened, as he needed an increase in nasal oxygen flow to 6 L/min, and so did his ascites. A diagnostic and therapeutic paracentesis was performed, and 2 l of light yellow ascitic fluid were subsequently removed. Laboratory analysis showed a total cell count of 133 per mm^3 (macrophages: 60%, mesotelyocytes: 24%, and lymphocytes: 16%), and no red blood cells were detected. Total protein and albumin levels were 0.8 g/dL and 0.6 g/dL, respectively. Direct smears and cultures were negative for bacteria, fungi, and mycobacteria, but RT-PCR for SARS-CoV-2 in ascitic fluid was positive. Serum and fecal samples were also positive (Table 2). A rapid serological test performed on day 10 of symptoms was positive for IgM and IgG antibodies.

On day 4, he required intubation as his respiratory status deteriorated (PaO2/FiO2 ratio: 110). Immunosuppression was then switched to 50 mg intravenous hydrocortisone every 6 h. His ventilation parameters improved in the following days, but his graft dysfunction did not—Doppler ultrasonography of graft was normal—and he was started on hemodialysis. On day 12, despite treatment with meropenem, vancomycin, and fluconazole, he had a refractory septic shock. Blood cultures

from day 12 and 16 were negative for bacteria, but both samples were positive for yeasts, which were *post-mortem* identified as *Cryptococcus neoformans*. Tracheal aspirate cultures from the same days were negative for bacteria, fungi, and mycobacteria. The patient died on day 18.

Methods

Nasal and oropharyngeal swabs were collected and stored in 2 mL of sterile Ringer's lactate solution. Ascitic fluid was collected with a sterile syringe following abdominal punction with aseptic technique. Blood was collected in a serum separator tube and then centrifuged according to the Centers for Disease Control and Prevention (CDC) guidelines. Stool was collected with a sterile container.

Ribonucleic acid (RNA) extraction was performed in all four specimens with QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), following manufacturer's instructions. Viral detection was performed with AgPath-ID One-Step RT-PCR Reagents (ThermoFisher Scientific, Austin, USA), according to manufacturer's instructions, in a total of 20 μL reaction volume, containing 5.0 μL of purified RNA, primers, and probes (400 nM and 200 nM, respectively), aiming at the N1 and N2 targets of the SARS-CoV-2 nucleoprotein gene in accordance with CDC guidelines. Samples with cycle threshold (Ct) values below 40 were considered positive. Rapid serological test was performed with serum sample following manufacturer's instructions (Zhuhai Livzon Diagnostics).

Results may be seen in Table 2.

Discussion

A great variety of clinical features have been attributed to COVID-19 in both immunocompetent and immunocompromised populations, including cough and dyspnea [2, 3] similar to the ones initially reported by this patient. As disease severity progresses, pulmonary injury is found to be a common feature [3], and previous lung autopsy reports have attributed to endothelialitis with intracellular viruses [4]. Also, as it appears to incite a high rate of thrombotic events [5], it might also explain further aggravation of his previous portal vein thrombosis observed with this patient, which could possibly justify the acute worsening of his ascites on the second day of hospitalization.

Nevertheless, SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) as a cell receptor to invade human cells [6], and since it is expressed in a wide variety of human tissues, including enterocytes from ileum and colon [7], it would explain detection of viral RNA in his fecal sample, and it might have contributed to local viral spread,

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Table 1 Evolutive blood test results on initial days of hospitalization compared with previous results

Laboratory findings	Normal range	22/04/2020 No symptoms	18/05/2020 Hospital day 1 Illness day 5	19/05/2020 Hospital day 2 Illness day 6	20/05/2020 Hospital day 3 Illness day 7	22/05/2020 Hospital day 5 Illness day 9	23/05/2020 Hospital day 6 Illness day 10
Absolute white-cell count (per mm^3)	3500–10,500	2560	5700	4580	7880	5430	6730
Absolute neutrophil count (per mm^3)	1700–8000	1802	5547	4278	7636	5197	6192
Absolute lymphocyte count (per mm^3)	900–2900	586	162	229	150	114	269
Platelet count (per mm^3)	150,000–450,000	45,000	48,000	44,000	56,000	64,000	63,000
Hemoglobin (g/dL)	13.5–17.5	11.7	11.3	10.3	10.9	9.9	9.9
Hematocrit (%)	39–50	34	32	30	32.1	29	30.2
Other findings							
Procalcitonin (ng/mL)	< 0.06	–	–	3.92	–	–	–
Lactate dehydrogenase (U/L)	< 250	–	366	–	–	–	–
D-dimer (ug/FEU)	< 0.5	–	>20	>20	–	>20	–
Troponin (pg/mL)	< 14	–	65	60	108	–	–
Creatine kinase (U/L)	39–308	–	–	–	135	–	–
Creatinine (mg/dL)	0.7–1.2	2.5	3.68	4.15	4.55	4.89	4.68
Urea (mg/dL)	10–50	80	126	142	184	218	234
Total bilirubin (mg/dL)	0.3–1.2	1.19	–	0.67	–	0.49	0.47
Aspartate aminotransferase (U/L)	< 40	34	39	33	–	35	17
Alanine aminotransferase (U/L)	< 41	36	22	20	–	21	18
Gamma glutamyl transferase (U/L)	< 60	75	–	114	–	–	–
Albumin (g/dL)	3.5–5.2	–	–	2.6	–	–	–
International normalized ratio (INR)	0.8–1.2	1.35	1.25	1.34	–	–	1.34
Alpha fetoprotein (ng/dL)	< 7	3.8	–	1.95	–	–	–
Sodium (mmol/L)	137–148	145	142	148	143	147	147
Potassium (mmol/L)	3.5–5	4.7	4.6	5.2	4.4	4.4	3.2
Calcium (mmol/L)	1.15–1.32	–	–	–	–	1.13	–

Fig. 1 Chest computed tomography from day 1 of hospitalization (day 7 of illness) showing ground-glass opacities with some areas of consolidation
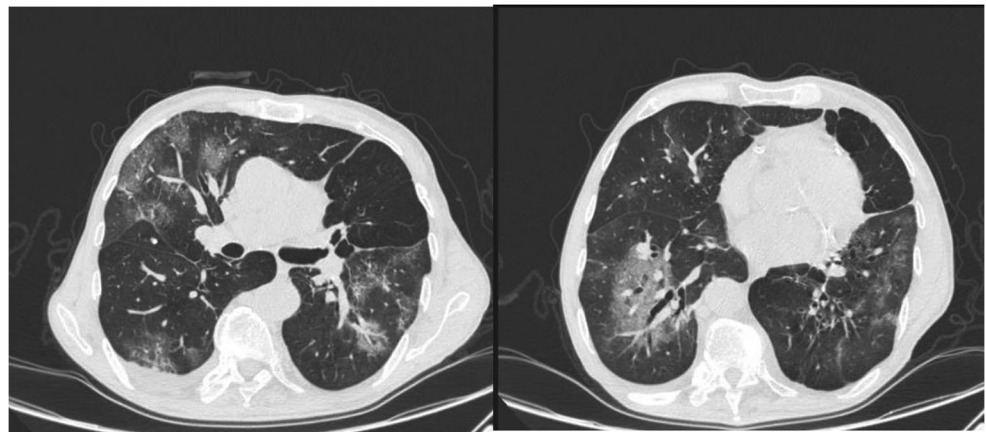
including to peritoneum. But more importantly, macrophages also express the ACE2 receptor [8], and, despite their importance in antiviral defense mechanisms in general, more attention has been given to the hypothesis that, in the case of SARS-CoV-2, they might also enable viral anchoring, replication, and possibly spread to other organs [8]. Since macrophages were predominant in this patient's ascitic fluid, direct viral injury might also have played a role on his initial clinical presentation. Interestingly, infectious peritonitis is a well-described condition in felines that is caused by a coronavirus through a similar immune pathway [9]. On the other hand, while reports on patients with cirrhosis and SARS-CoV-2 show a high rate of decompensation with ascites [10], viral etiology in this clinical scenario has not been very well described thus far.

At the same time, viral RNA was not detected in samples of ascitic fluid from surgical patients according to previous studies [11, 12], so it remains a matter demanding further investigation, especially because these reports present a small number of patients requiring emergency operations and no information regarding co-morbidities or immune status.

In the case of solid organ transplant patients, chronic immunosuppressive therapy impairs T cell immunity [2], and disruption in viral control may lead to higher rates of viral replication [3, 13]. While this could have contributed to a disseminated, more severe infection in this patient, viral RNA has been detected in blood samples from critically ill, immunocompetent patients as well [14].

Also, transient lymphopenia is a prominent feature in SARS-CoV-2, and it can also be a marker of severe disease by inducing immune dysregulation with T cell exhaustion and depletion of the TCD4 + lymphocyte subset [15, 16]. At the same time, TCD4+ cells' decline has a clear association with higher risk of cryptococcosis in different conditions [17, 18]. Moreover, such risk is also seen with solid organ transplant patients [19], as well as in cirrhotic patients in general, who additionally present higher incidence of disseminated infections [19]. Therefore, it can be highly debatable whether the SARS-CoV-2 infection itself and its immune dysregulation could have triggered disseminated cryptococcosis in this patient, or if he accumulated too many prior risk factors, or perhaps a combination of both. In any case, other fungal secondary infections have been reported with COVID-19 [20], and clinicians should be aware of the possibility of such scenario with *Cryptococcus* species as well, especially in populations with cumulative risk factors [17–19].

Conclusion

In summary, this unprecedented case report presents ascitic fluid as a novel specimen in which SARS-CoV-2 can be detected. Immune dysregulation and cumulative risk factors may

Table 2 Specimen tested for COVID-19 using RT-PCR technique according to date of exam

Specimen	18/05/2020 Illness day 5	20/05/2020 Illness day 7	22/05/2020 Illness day 9	23/05/2020 Illness day 10
Nasopharyngeal swab	Positive (CT 14–15)	–	–	
Ascitic fluid	–	Positive (CT 34–38)	–	
Serum	–	–	Positive (CT 31–33)	
Stool	–	–	–	Positive (CT 25–26)

Sample was considered positive if cycle threshold (CT) value is < 40

lead to secondary infections by opportunistic agents, including *Cryptococcus neoformans*. More studies are required to better elucidate viral dynamics, immune response, and different clinical presentations in the immunocompromised, especially because of such diverse disease spectrum as seen with COVID-19.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval This case report was approved by the Local Ethics Committee (CAAE: 33911720.3.0000.5505).

Consent for Publication Consent for publication from this patient was not possible because he died. No close relatives were able to be reached despite several attempts. No detailed information regarding his identification was published on this manuscript.

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