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ACCEPTED MANUSCRIPT

SARS-CoV-2 in pleural fluid in a kidney transplant patient

SARS-CoV-2 in pleural fluid

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Abstract

Coronavirus disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has quickly spread all over the globe from China. Pleural involvement is not common; around 5-10% of patients can develop pleural effusion and little is known about the involvement of pleural structures in this new infection.

A 61-year-old male kidney transplant patient with a history of multiple biopsy-confirmed acute rejections and chronic allograft rejection was admitted to our COVID-19 Unit with dry cough, exertional dyspnea, oliguria and abdominal distension. Lung ultrasound imaging, chest X-ray and CT scan showed left pleural effusion and atelectasis of the neighboring lung parenchyma. RT-PCR was positive for SARS-CoV-2 in the pleural fluid and cytology showed mesothelial cells with large and multiple nuclei, consistent with a cytopathic effect of the virus.

This is one of few reports describing detection of SARS-CoV-2 in the pleural fluid and to the best of our knowledge, is the first to document the simultaneous presence of a direct cytopathic effect of the virus on mesothelial cells in a kidney transplant patient with COVID-19 pneumonia. The pleura proved to be a site of viral replication where signs of a direct pathological effect of the virus on cells can be observed, as we report here. RT-PCR for SARS-CoV-2 should be part of routine examination of pleural effusion even in patients with mild respiratory symptoms or with comorbidities that seem to explain the cause of effusion.

Keywords: COVID-19, SARS-CoV-2, pleural fluid, transplant

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection spread rapidly around the world from China. Common symptoms of Coronavirus disease 2019 (COVID-19) at presentation include fever, dyspnoea, dry cough, fatigue and diarrhoea (1). Nasal congestion and anosmia have also been reported (1, 2). Interstitial pneumonia is the major clinical manifestation and around 10% of patients develop severe acute respiratory distress syndrome (ARDS) (3, 4). Pleural involvement is not common; around 5-10% of patients can develop pleural effusion but little is known about involvement of pleural structures in this new infection (5).

This is one of few reports describing detection of SARS-CoV-2 in pleural fluid, and to the best of our knowledge, it is the first to document the simultaneous presence of a direct cytopathic effect of the virus on mesothelial cells in a kidney transplant patient with COVID-19 pneumonia.

Case report

A 61-year-old male kidney transplant patient with a history of multiple biopsy-confirmed acute rejections and chronic allograft rejection was admitted to our COVID-19 Unit on 9th April 2020. Renal function was severely reduced and serum creatinine measured 3 weeks before admission was 7.59 mg/dl (glomerular filtration rate 16 ml/min), although he was not yet on dialysis. His maintenance immunosuppressant therapy consisted of prednisone, tacrolimus and mycophenolate mofetil.

On admission, the patient presented with mild respiratory symptoms, oliguria and abdominal distension. He reported a dry cough and exertional dyspnea in the previous week and a nasopharyngeal swab was positive for SARS-CoV-2. Since serum creatinine was 11.1 mg/dl, hemodialysis was immediately begun. He also showed severe anemia and leucopenia while C-reactive protein was 4.00 mg/dl (see Table 1 for complete lab data). Chest auscultation revealed wheezing, basal right crackles, basal left reduction of physiological vesicular murmur and dullness to percussion. Lung ultrasound imaging indicated an interstitial syndrome with bilateral diffuse multiple B lines and left basal pleural effusion. Chest X-ray showed left pleural effusion and a CT scan confirmed the left pleural effusion and atelectasis of the neighboring lung parenchyma with no signs of viral parenchymal involvement (Figure 1a).

The laboratory characteristics of the fluid were compatible with transudative effusion (Table 2). Reverse transcriptase-polymerase chain reaction (RT-PCR) was positive for SARS-CoV-2 and cytology showed mesothelial cells with large multiple nuclei, consistent with a cytopathic effect of the virus (Figure 1b); microbiology was negative.

The patient was treated with methylprednisolone 1.5 mg/kg/day for 5 days while the other immunosuppressants were suspended. Respiratory condition improved rapidly and the nasopharyngeal swab for SARS-CoV-2 became negative 8 days after admission. Unfortunately, renal function did not improve and long-term hemodialysis was begun. During hospitalization, the patient also developed a perforated diverticulum and left hemicolectomy was necessary. Despite the difficulties encountered during hospital stay, the patient recovered completely and was discharged 28 days after admission.

Discussion

SARS-CoV-2 infection may present with different symptoms that express direct or indirect involvement of various organs and systems (5). In most patients, imaging of the lung shows ground glass opacities and crazy paving pattern in the early phases, and later larger consolidations in the basal or dependent lung regions, readily visible by CT (6). Due to its safety, repeatability, absence of radiation, low cost and point of care use, ultrasound imaging of the lungs has shown good clinical value in COVID-19 patients (7). Despite the high sensitivity of these techniques, pleural effusion has only occasionally been reported in COVID-19 (5). In our experience, the incidence of pleural effusion in hospitalized COVID-19 patients is 7.5%; in most cases it was mild, not requiring drainage.

Mei et al. recently published a case report of a COVID-19 patient whose pleural fluid RT-PCR was positive for SARS-CoV-2 (8). The present report is the first concerning pleural effusion in a kidney transplant patient with COVID-19. In our case RT-PCR of pleural fluid was positive for SARS-CoV-2, and we also documented mesothelial cells with large multiple nuclei, consistent with a cytopathic effect of the virus. Unfortunately, we were unable to perform electron microscopy.

Dysregulated and/or exaggerated cytokine and chemokine responses in SARS-CoV-2 infection have been reported in many studies. Cytokine release syndrome is a systemic inflammatory response, that can be triggered by infection, certain drugs and other factors. It has been demonstrated in COVID-19 patients (9). *In vitro* experiments show that delayed release of cytokines and chemokines occurs in respiratory epithelial cells, dendritic cells and macrophages in the early stage of SARS-CoV-2 infection, and that the cells secrete low levels of interferon antiviral factors and high levels of proinflammatory cytokines (interleukins IL-1 β , IL-6, and tumor necrosis factor) and chemokines (10). No specific antiviral therapy for SARS-CoV-2 infection has yet been found, although most reports suggest that immunomodulation therapy can play a positive role. Blockade of IL-6 and IL-1 has shown promising results and high doses of steroids prove to reduce mortality, moderating cytokine release (9, 11).

The angiotensin converting enzyme 2 (ACE2) receptor proves to play a crucial role in viral entry into cells and its reduced transmembrane expression is associated with increased risk of ARDS in infected subjects (12). Drugs interfering with ACE2 receptor show promising positive effects, making the receptor a major focus in the search for new therapies (13).

In solid organ transplant recipients, such as our patient, the risk of pneumonia and development of ARDS is expected to be higher, although a number of reports have indicated a similar incidence to that of the general population (14, 15). The hypothesis that post-transplant immunosuppression can somehow protect patients against the hyperinflammatory syndrome resulting from the cytokine storm induced by SARS-CoV-2 is intriguing and needs further confirmation (9, 16). In the present case, despite the setbacks our patient had to face (severe kidney failure and intestinal perforation requiring dialysis and surgery, respectively), the outcome was positive and the patient was eventually discharged.

Diagnostic and therapeutic procedures, such as thoracentesis, must only be performed in COVID-19 patients if there are strict clinical indications. All safety criteria for operators performing collection and

analysis of samples must comply with international standards. In our case, pleural fluid was drawn at the patient's bedside in the COVID-19 isolation ward. The pleural fluid samples were treated according to current national and international regulations. All diagnostic laboratories in our hospital operate at biosecurity levels 2, 3 and 4, and are able to handle biological samples potentially infected with SARS-CoV 2 (17).

Conclusions

Although pleural involvement is not common in COVID-19, patients should be checked for the presence of effusion. Signs of a direct pathological effect on pleural cells can even be observed in cases with mild/moderate pneumonia, as we report in this case, suggesting that the pulmonary and pleural compartments may behave distinctly and that participation of the pleura is not always a result of pulmonary spread. However, we do not have reliable data to support this hypothesis, verification of which will require further studies. RT-PCR for SARS-CoV-2 should be part of routine examination of pleural effusion, even in patients with mild respiratory symptoms or with comorbidities that seem to explain the cause of effusion. How to treat SARS-CoV-2 infection is still debated. Immune modulation has shown promising results and several trials are underway (11). Solid organ transplant patients offer a unique *in vivo* model of biological responses to this new virus and can be useful to help understand response to therapy. Pleural drainage should nevertheless be an aspect of non-pharmacological therapy in selected COVID-19 patients to improve respiratory dynamics and prognosis.

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Declaration of financial/other relationships

The contents of the paper and the opinions expressed within are those of the authors, and it was the decision of the authors to submit the manuscript for publication.

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Authors contribution

DB performed the literature search, data collection, data analysis and interpretation and wrote the manuscript. FF, EDV, MAM, LV, MGD, GG, AG, MGC, EB, SS, SV, RG and BF performed data analysis and interpretation. All authors contributed equally to clinical management of the patient during his hospital stay. All authors are guarantors of the paper, taking responsibility for the integrity of the work as a whole.

All authors read and approved the final version of the manuscript.

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CAPTIONS

Figure 1: A: Chest CT after intravenous administration of only 60 ml of contrast medium. Virtual monoenergetic reconstruction (55 KeV) of dual energy CT data shows pleural effusion and atelectasis of the neighbouring lung parenchyma and excludes active foci of bleeding. B: Microvacuolated macrophages and scattered mesothelial cells with enlarged multiple nuclei suggesting viral infection.

Table 1: Lab findings on admission to hospital

Table 2: Features of pleural fluid

Table 1

C-reactive protein	4.00 mg/dL
Lactate dehydrogenase	262 IU/L
D-dimer	478 ug/L
Ferritin	925 ng/mL
White blood cells	$2.63 \times 10^3/\text{mmc}$
• Neutrophils	67.8%
• Lymphocytes	20.5%
• Monocytes	9.1%
• Eosinophils	1.5%
• Basophils	1.15
Red blood cells	$2.19 \times 10^6/\text{mmc}$
Hemoglobin	6.2 g/dL
Hematocrit	19.4%
Mean Corpuscular Volume	88.6 fL
Mean Corpuscular Hemoglobin	28.3 pg
Mean Corpuscular Hemoglobin Concentration	32.0 g/dL
Red blood cell Distribution Width	16.8%
Platelets	$148 \times 10^3/\text{mmc}$
Glucose	71 mg/dL
Creatinine	11.1 mg/dL
Blood Urea Nitrogen	212 mg/dL
Cholesterol	161 mg/dL
Total proteins	4.2 g/dL
Albumin	2.4 g/dL
Bilirubin	0.3 mg/dL
Glutamic oxaloacetic transaminase	10 IU/L
Glutamate-pyruvate transaminase	8 IU/L

Table 2

Appearance	clear
Colour	yellow
Total protein	2 g/dL
Lactate dehydrogenase	79 U/L
White cell count	25/mcl (80% mononuclear cells)

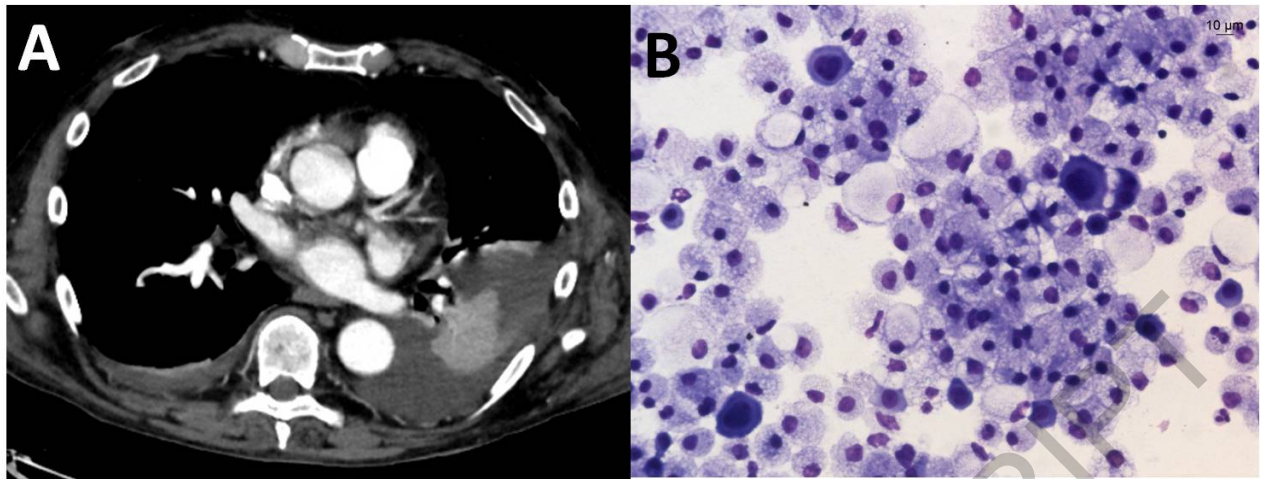


Fig 1