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CHEST

First Detection of SARS-CoV-2 by Real-Time Reverse Transcriptase-Polymerase Chain Reaction Assay in Pleural Fluid

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Coronavirus disease 2019 (COVID-19) is a pandemic infection due to the spread of a novel coronavirus (severe acute respiratory syndrome coronavirus 2), resulting in a wide range of clinical features, from asymptomatic carriers to ARDS. The gold standard for diagnosis is nucleic acid detection by real-time reverse transcriptase-polymerase chain reaction in nasopharyngeal swabs. However, due to limitations in this technique's sensitivity, thoracic imaging plays a crucial, complementary role in diagnostic evaluation and also allows for detection of atypical findings and potential alternative targets for sampling (eg, pleural effusion). Although less common, pleural involvement has been described in a minority of patients. This report describes the first case of reverse transcriptase-polymerase chain reaction detection of severe acute respiratory syndrome coronavirus 2 in pleural fluid obtained by means of ultrasound-guided thoracentesis, and its main characteristics are detailed. Pleural effusion is not a common finding in COVID-19 infection, but a prompt recognition of this potential localization may be useful to optimize diagnostic evaluation as well as the management of these patients.

CHEST 2020; 158(4):e143-e146

KEY WORDS: CT-guided biopsy; diagnostic yield; meta-analysis; pleural lesions; safety; ultrasound-guided biopsy

In December 2019, an outbreak of novel coronavirus disease 19 (COVID-19, 2019-nCoV, or severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) occurred in Wuhan, China. It has since dramatically spread worldwide.

The cornerstone of diagnosis in this context is nucleic acid detection by real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) in nasopharyngeal swabs. However, a not negligible false-negative rate has been reported in the literature for this technique.[1,2] Sensitivity seems to be influenced by several factors, including selected "intrinsic" patient characteristics (ie, stage of disease, viral load), as well as technical aspects in collecting and managing specimens.[3,4]

Thoracic imaging, in particular CT scanning and thoracic ultrasound (TUS) technique, plays a complementary, key

ABBREVIATIONS: COVID-19 = coronavirus disease 19; RT-PCR = reverse transcriptase-polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TUS = thoracic ultrasound

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DOI: https://doi.org/10.1016/j.chest.2020.05.583

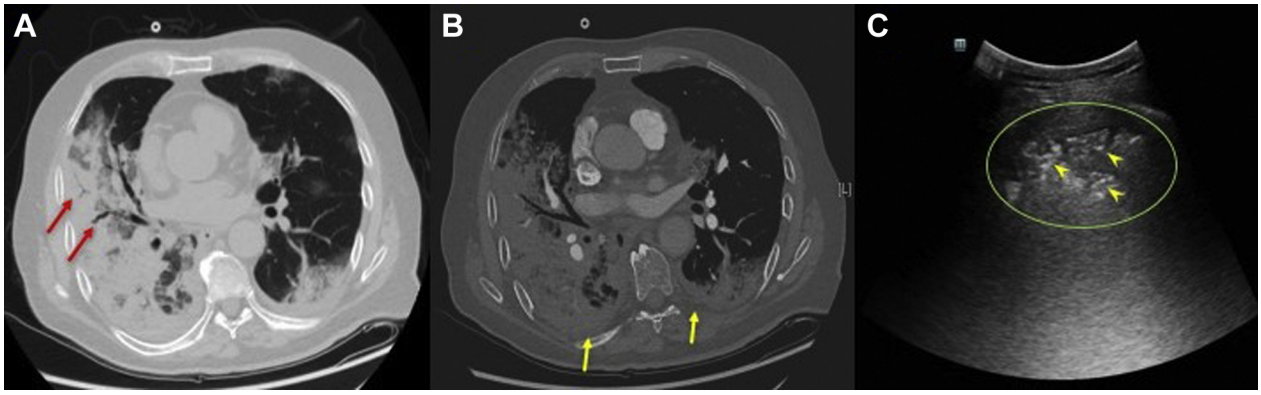

Figure 1 – *A-C, Thoracic imaging at baseline. A, High-resolution CT imaging showed multiple bilateral consolidations with inner air bronchogram sign (red arrows) with predominant right-side distribution. B, Contrast-enhanced CT scan ruled out pulmonary embolism, revealing small bilateral pleural effusion (yellow arrows). C, Thoracic ultrasound by convex probe array in right mid-axillary line confirmed demarcated consolidation (green circle) with inner air bronchogram sign (arrowhead).*

role in the diagnostic evaluation of COVID-19. Both these procedures allow for increased detection of disease in the proper clinical setting and description of further atypical features, as well as potential targets for sampling (eg, pleural effusions).[1,2,5,6] Although less common, pleural involvement has been described in a substantial minority of cases (pleural thickening, 32%; pleural effusion, 5%),[7] and it has been significantly associated with a worse prognosis.[8]

Pleural fluid characteristics in these patients have never been described, and there are no reports on RT-PCR detection in pleural samples. Here, we describe the first case of RT-PCR detection of SARS-CoV-2 in pleural fluid obtained by means of TUS-guided thoracentesis and report its main characteristics.

Case Report

On March 25, 2020, a 72-year-old man was admitted to our Pulmonology Unit with a 5-day history of dry cough, fever up to 39°C, fatigue, and positive RT-PCR assay for SARS-CoV-2 in nasopharyngeal swabs, demonstrating a high viral load (174,000,000 copies/mL of swab solution). He was a nonsmoker, and his medical history was unremarkable apart from mild hypertension.

The physical examination revealed a body temperature of 38.7°C, BP of 124/76 mm Hg, pulse of 115 beats/min,

TABLE 1] Laboratory and Clinical Features at Baseline and at Day 6

Parameter	Reference Range	Day 1	Day 6
WBC count (per mmc)	4-10,000	6,200	2,870
Platelet count (per mmc)	150-4,000,000	153,000	217,000
Hemoglobin, g/dL	12.5-17	14.2	14.1
Absolute lymphocyte count (per mmc)	1,000-4,000	415	761
Lactate dehydrogenase, U/L	≤ 240	270	257
C-reactive protein, mg/dL	≤ 0.6	30.4	1.9
Procalcitonin, ng/mL	≤ 0.05	0.44	0.03
IL-6, pg/mL	<5	84	186
Total protein, g/dL	6-8	4.9	5.2
Albumin, g/dL	4-4.76	1.86	2.49
Alanine aminotransferase, U/L	≤ 40	46	29
Aspartate aminotransferase, U/L	≤ 40	27	26
Creatinine, mg/dL	0.6-1.40	0.85	0.71
D-dimer, ng/mL	0-355	706	1684
Brain natriuretic peptide, ng/mL	≤ 150	…	28
Ratio partial pressure of oxygen/F_{IO_2}	na	175	142

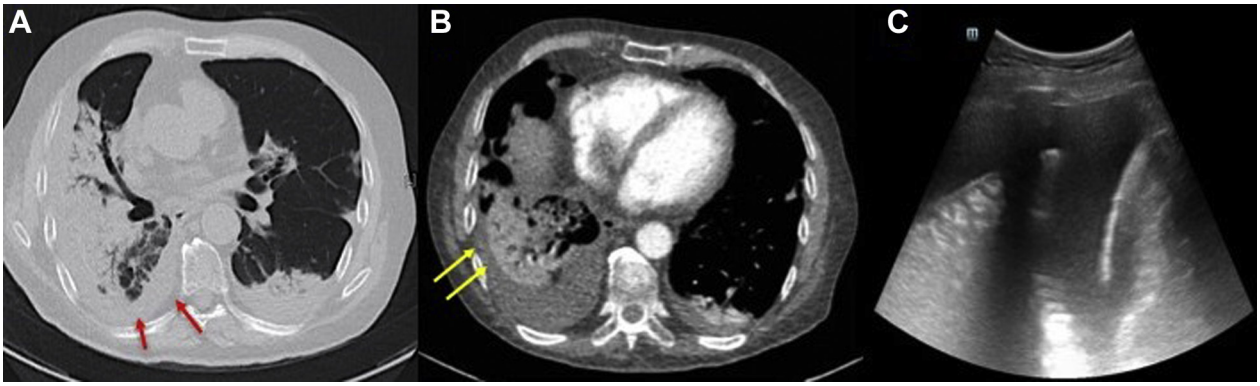

Figure 2 – *A-C, Thoracic imaging at day 6. A, Six days, later high-resolution CT imaging showed persistent multiple bilateral consolidations with predominant right-side distribution and increasing of bilateral pleural effusion (red arrows). B, Contrast-enhanced CT scan confirmed a large amount of right-sided pleural effusion with a slight enhancement on parietal pleura (yellow arrows). C, Thoracic ultrasound in the right mid-axillary line revealed moderate pleural effusion with atelectasis of the lower lobe.*

respiratory rate of 23 breaths/min, and oxygen saturation of 93% on oxygen mask at 50% of fraction inhaled oxygen.

Chest radiography showed bilateral infiltrates, with prevalent distribution on the right side, and a CT scan confirmed bilateral, multilobar ground-glass opacities with multifocal consolidations, predominantly in the lower lobes and small bilateral pleural effusion; contrast-enhanced CT imaging was negative for pulmonary embolism (Figs 1A, 1B). TUS examination by convex probe in the right mid-axillary line revealed demarcated consolidation with an inner air bronchogram sign (Fig 1C). Laboratory results documented lymphopenia (415/mmc), elevated levels of lactate dehydrogenase (270 U/L), D-dimer (706 ng/mL), IL-6 (84 pg/mL), and C-reactive protein (30.4 mg/dL) (Table 1). Results of urinary antigen tests for *Legionella pneumophila* and *Streptococcus pneumoniae* were negative.

The patient was started on oral antiviral therapy with lopinavir/ritonavir 400/100 mg bid and hydroxychloroquine 200 mg bid, prophylactic antibiotic therapy (ceftriaxone), intermittent noninvasive ventilation (pressure support ventilation with 10 cm H_2O inspiratory positive airway pressure level and 6 cm H_2O expiratory positive airway pressure), and supportive care.

Due to worsening of respiratory symptoms and gas exchanges, the patient's CT scan and TUS evaluations

TABLE 2] Pleural Fluid Characteristics

Parameter	Results
Appearance	Clear
Color	Yellow
Total protein, g/dL	2.3 g/dL
Cholesterol, mg/dL	50 mg/dL
Lactate dehydrogenase, U/L	168 U/L
Glucose, mg/dL	115 mg/dL
WBC count	120/mcl (92% of mononucleated cells)
Cytology	Reactive mesothelial cells and lymphocytes
Microbiology	Negative
SARS-CoV-2 (RT-PCR)	
Qualitatative (positive/negative)	Positive
Quantitative (copies/mL)	6,776

RT-PCR = reverse transcription real-time polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

were repeated after 6 days, and both showed persistence of lung consolidations, mainly in the right lower lobes, and significant right pleural effusion (Fig 2). TUS-guided thoracentesis was therefore performed, removing 600 mL of clear yellow pleural fluid; this sample was sent for differential cell counts, chemical analysis, cultures, cytologic examination, and SARS-CoV-2 RT-PCR. Cell count examination revealed predominant mononucleated cells (92%); chemical parameters showed an exudate according to the criteria of Light et al,[9] pH was 7.35, and results of microbiologic tests for detection of both anaerobic and aerobic bacteria, mycobacteria, and fungi were negative. Cytologic analysis documented reactive mesothelial cells and lymphocytes. The SARS-CoV-2 RT-PCR assay revealed the presence of virus at a moderate viral load (6,776/mL) (Table 2).

Following removal of the pleural fluid, the patient's dyspnea and respiratory failure progressively improved. No recurrence of pleural effusion was observed at daily TUS assessment over the following days.

Discussion

The current diagnostic approach to COVID-19 disease mainly relies on positive RT-PCR assay for SARS-CoV-2 in nasopharyngeal swabs; the sensitivity of this technique is limited, however, especially in later stages with predominant involvement of the lower respiratory tract. For this reason, RT-PCR assay is currently performed on other biological materials, such as BAL fluid and stool.[10] To the best of our knowledge, this case is the first of SARS-CoV-2 detection in pleural fluid.

In the current case, the recognition of a significant pleural effusion was also essential for optimizing patient prognosis, as fluid removal substantially contributed to improvement in respiratory dynamics, leading to better lung expansion (especially during ventilatory positive pressure support).

A further relevant message of this case is the key role of longitudinal TUS evaluation. This evaluation offers the advantage of being low cost, nonionizing, and available at the bedside, leading to reduced risk of transmission for health-care workers during patient transportation and avoidance of having to sanitize larger areas of equipment (just the probe is sanitized instead of the whole radiologic suite).

Pleural effusion is not a common finding in COVID-19 infection. However, clinicians should be aware of this potential disease localization, as its prompt recognition may be useful to optimize diagnostic evaluation in patients with negative upper respiratory tract RT-PCR, as well as management of these patients.

Acknowledgments

Financial/nonfinancial disclosures: None declared.

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