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Plasma as an alternative COVID-19 diagnostic specimen in a hospitalized patient negative for SARS-CoV-2 by nasopharyngeal swab

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ABSTRACT

We present the case of an inpatient with pneumonia and repeatedly negative nasopharyngeal SARS-CoV-2 testing. In such challenging cases, alternative diagnostic options include lower respiratory tract and plasma SARS-CoV-2 RNA testing, of which the latter may be particularly useful where bronchoscopy is deferred due to clinical factors or transmission risk.

A 40-year-old male known for gout presented to the emergency department with a 3-day history of fever, shortness of breath, non-productive cough, nausea, and vomiting. On presentation, the patient was febrile to 38.4°C, with a pulse of 109, and oxygen saturation of 97% on room air. His physical exam revealed diffuse bilateral crackles. Complete blood count was unremarkable, and lactate dehydrogenase (LDH) was mildly increased to 264 U/L (reference range 135-225). Other laboratory values were within normal limits including basic metabolic panel, lactate, liver enzymes and lipase. The chest x-ray demonstrated multifocal pneumonia, and chest CT showed bibasilar and right upper lobe ground glass opacities (Fig. 1).

Real-time reverse transcription polymerase chain reaction (rRT-PCR) testing of a nasopharyngeal swab specimen by the Xpert Xpress SARS-CoV-2 Assay (Cepheid; Sunnyvale, CA) was negative. Testing for routine respiratory viruses was also performed by the Xpert Flu/RSV Assay (Cepheid) and the ePlex Respiratory Pathogen Panel (GenMark Diagnostics; Carlsbad, CA), and were negative. Blood cultures showed no growth at five days.

The patient received treatment with ceftriaxone, azithromycin, and oxygen support (1–2 L/min). Given the radiologic findings and persistent clinical suspicion for coronavirus disease 2019 (COVID-19), repeat nasopharyngeal swab testing for SARS-CoV-2 using the Stanford Emergency Use Authorization (EUA) laboratory-developed test was performed and was negative (U.S. Food and Drug Administration, 2020). Bronchoscopy was considered but deferred due to the infection control risk. A laboratory-developed ELISA targeting the SARS-CoV-2 spike protein receptor binding domain (RBD) at day four of hospitalization showed a borderline IgM positive result at an optical density of 0.45 (negative; reference range <0.4), and IgG negative at 0.23 (negative; reference range <0.3) (Roltgen et al., 2020).

The patient was discharged home eight days following admission. The working diagnosis was that of SARS-CoV-2 pneumonia; no antiviral therapy was administered as he was considered ineligible due to a lack of positive nucleic acid amplification testing. Four months after initial presentation, he has not had medical follow-up at our center.

Retrospective rRT-PCR and serologic testing of residual plasma specimens was performed to confirm the diagnosis of COVID-19. SARS-CoV-2 RNA was detected from plasma samples drawn on days 4 and 5 of admission. The day 4 specimen was drawn the same day as the IgM-positive specimen, and was positive by rRT-PCR targeting the *E* gene at a cycle threshold (Ct) of 37.3, and by rRT-PCR targeting the N2 region of the nucleoprotein (*N*) gene at a Ct of 34.0 (Bulterys et al., 2020). The plasma specimen from the following day was positive only by the N2 rRT-PCR at a Ct of 36.3. Plasma specimens drawn 6 and 7 days following initial presentation were *E* and N2 rRT-PCR negative; however serologic testing of these specimens

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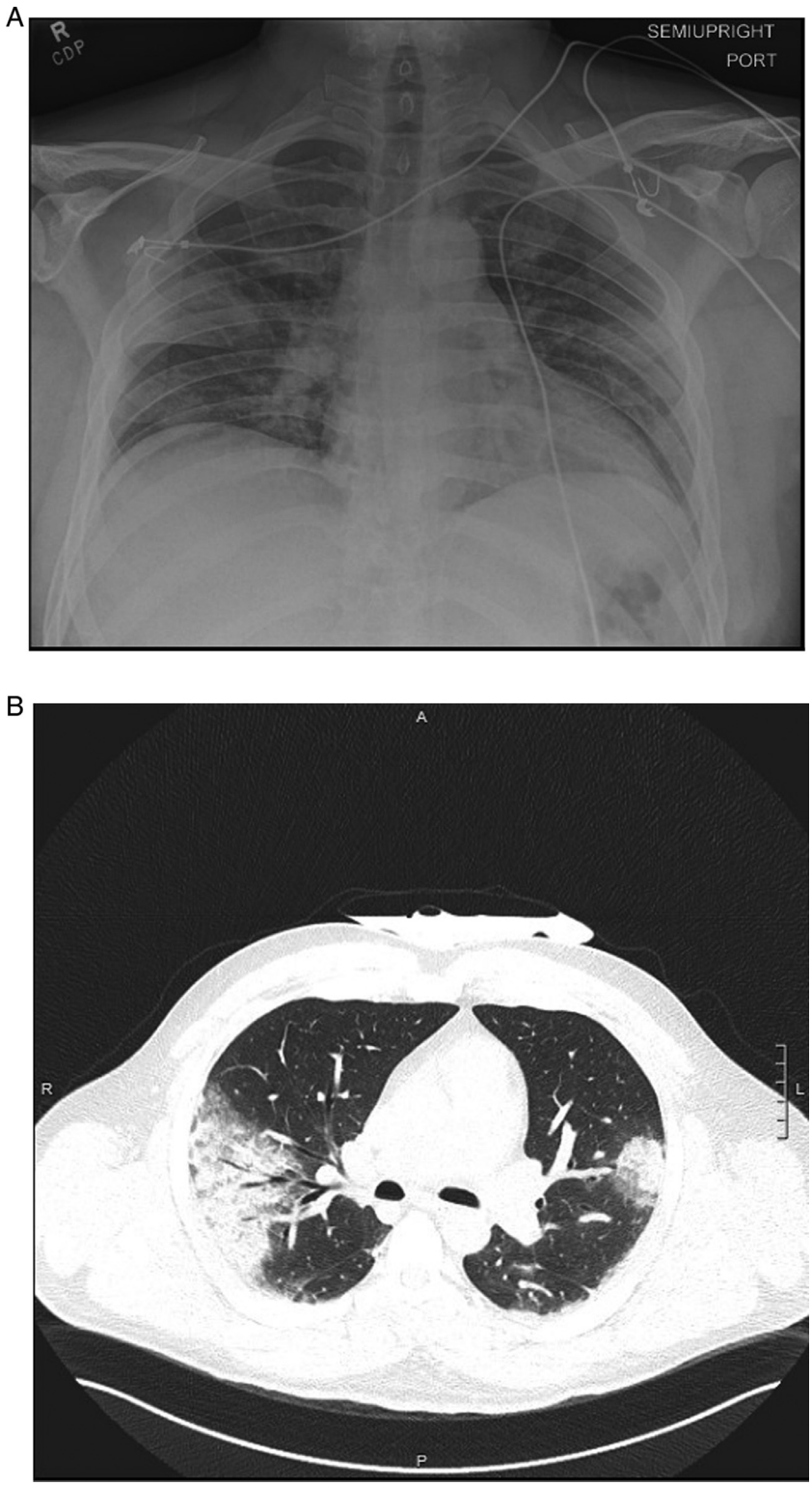

Fig. 1. (A) Chest x-ray on admission revealed multifocal pneumonia. (B) Chest CT scan on day 1 of admission revealed bibasilar and right upper lobe ground glass opacities.

confirmed SARS-CoV-2 IgA and IgG seroconversion against RBD, the S1 region of the spike protein, and the nucleocapsid protein (Hanson et al., 2020, Roltgen et al., 2020).

Upper respiratory sampling is the most common means to confirm the diagnosis of acute COVID-19 infection, and performs adequately in most cases. However, this clinical case illustrates the important challenge of confirming an acute diagnosis of COVID-19 infection in the presence of repeatedly negative rRT-PCR testing of upper respiratory tract specimens and an isolated, weak-positive IgM. Despite the high analytical sensitivity of nucleic acid amplification testing, decreased clinical sensitivity of upper tract specimens may be observed in COVID-19 patients presenting with pneumonia (Parikh et al., 2020, Winichakoon et al., 2020, Woloshin et al., 2020). When clinical suspicion persists despite negative nucleic acid results from upper respiratory tract samples, lower tract sampling by bronchoscopy may be considered. However, this aerosol-generating

procedure is often deferred, due to its attendant transmission risks to healthcare workers.

Importantly, this case provides evidence that plasma may serve as a lower-risk, diagnostic alternative to bronchoalveolar lavage fluid, specifically when upper respiratory tract testing is negative, and imaging is consistent with viral pneumonia. Recent work from several groups has demonstrated that plasma SARS-CoV-2 RNA is a marker for COVID-19 severity (Hogan et al., 2020, Pinsky and Hogan, 2020, Prebensen et al., 2020), indicating that this plasma approach may be particularly effective in identifying patients that have developed lower tract disease.

Plasma SARS-CoV-2 RNA testing requires that the laboratory perform a separate validation for this specimen type, which requires additional time and resources. However, given that plasma is a commonly used matrix for virology molecular testing, current routine processes are well suited to address this specimen type. For institutions where plasma is not validated as a specimen type, testing may be considered at the discretion of the medical director, and results may be issued with a disclaimer that the specimen type is nonvalidated. Furthermore, laboratory staff must be aware of validated specimen types to avoid unnecessary test request rejection. Clinical laboratories must have an on-call physician to address these issues on an ad hoc basis, if no policy is explicitly in place for routine receipt of these specimen types.

In summary, the diagnosis of acute COVID-19 relies primarily on nucleic acid amplification testing of upper respiratory specimens. However, lower respiratory tract and plasma SARS-CoV-2 nucleic acid amplification testing may also be considered; and the latter may be of particular utility in cases where bronchoscopy is deferred due to clinical factors or transmission risk. Confirmation of SARS-CoV-2 infection is paramount to prevent lapses in infection control, and to enable timely initiation of therapy when indicated.

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Declaration of competing interest

The authors report no conflicts of interest relevant to this article.

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