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BRIEF COMMUNICATION

Bronchoalveolar lavage-based COVID-19 testing in patients with cancer

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Abstract
Objective/Background: A few case reports in the setting of coronavirus disease 2019 (COVID-19) and multiplex polymerase chain reaction (PCR)-based assays for common respiratory pathogens have shown a higher yield of bronchoalveolar lavage (BAL) samples than upper airway specimens in immunocompromised patients.
Methods: A retrospective study was conducted reviewing patients diagnosed with COVID-19 at the Medical College of Wisconsin (Milwaukee, WI, USA) between March 13, 2020 and June 11, 2020. All patients tested positive for SARS-CoV-2 via real-time reverse transcriptase PCR (RT-PCR), through a nasopharyngeal or a bronchoscopy specimen.
Results: During the study period, 53 bronchoscopy procedures were performed at the institution, of which five patients tested positive for COVID-19. Of the five patients, three underwent BAL testing based on high clinical suspicion for COVID-19 after the nasopharyngeal (NP) swab(s) was negative. All three patients had underlying cancers and had lymphopenia for a considerable duration prior to being diagnosed with COVID-19. Two patients had better outcomes that could

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be attributed to earlier BAL specimen testing resulting in timely medical intervention.
Conclusion: This study underscores the need for early lower respiratory tract sampling, whenever possible, in patients with cancer and prolonged lymphopenia. High clinical suspicion ought to supersede false-negative NP reverse transcriptase—PCR as early bronchoscopic evaluation in cancer patients, who are either receiving active treatment or are immunosuppressed, can allow timely institution of efficacious treatment, enrollment into clinical trials, as well as effective infection control. In the apt clinical setting in patients with cancer, presumptive treatment may also be considered to minimize exposure to healthcare providers and proceduralists.

Introduction

Coronavirus disease 2019 (COVID-19) is diagnosed by a qualitative reverse transcriptase—polymerase chain reaction (RT-PCR) assay that detects severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a nasopharyngeal (NP) or an oropharyngeal (OP) swab specimen [1,2]. Infection due to SARS-CoV-2 leads to poor outcomes in patients with underlying malignancy or those receiving active cancer treatment [3—5]. Timely diagnosis is critical to curbing mortality in this cohort of patients [6]. Herein, we report three cases with prolonged lymphopenia due to underlying cancers that were diagnosed with bronchoalveolar lavage (BAL) specimen, highlighting false negativity with NP specimen testing in this cohort of patients [2].

Methods

A retrospective study was performed with informed consent of the patients/guardians. Between March 13, 2020 and June 11, 2020, 1,516 patients tested positive for COVID-19 at the Medical College of Wisconsin (Milwaukee, WI, USA). All patients tested positive for SARS-CoV-2 via real-time reverse transcriptase PCR (RT-PCR), through a nasopharyngeal or a bronchoscopy specimen. The study was approved by the Medical College of Wisconsin's Institutional Review Board.

Results

During the study period, 53 bronchoscopy procedures were performed at the institution, of which five patients tested positive for COVID-19. Of the five patients, three underwent BAL testing based on high clinical suspicion for COVID-19 after NP swab(s) was negative. The patients are presented.

Case 1

A 76-year-old male patient, with hypertension, hyperlipidemia, pulmonary embolism, and myelodysplastic syndrome who had undergone allogeneic stem cell transplantation with a matched unrelated donor (alloMUD), was admitted with dyspnea, fever, malaise, and intermittent confusion. The patient did not report any cough, sore throat, rhinorrhea, nausea, vomiting, or diarrhea. Patient underwent alloMUD from a peripheral blood source with fludarabine and busulfan conditioning 2.5 months prior to this admission (Day + 71) and was receiving sirolimus for graft-versus-host disease (GvHD) prophylaxis. Chimerism studies showed 94% donor cells, 2 months post-transplantation. His post-transplant course was complicated by skin GvHD for which he was receiving high-dose corticosteroid. Of note, he resided at a rehabilitation facility and was admitted 4 weeks prior with similar symptoms and tested negative for COVID-19 twice during that admission. Additionally, the patient also tested negative via NP swab a day prior to the current admission.

Upon presentation, he had a fever of 103°F (39.5 °C), tachycardia (heart rate: 153 beats/minute), and tachypnea (respiratory rate: 30 breaths/minute) but sustained oxygen saturation above 96% on ambient air. The following day, he developed hypotension and hypoxia requiring supplemental oxygen up to 6 L/minute via nasal cannula (NC). Initial blood workup was significant for leukocytosis, hyperlactatemia, and an elevated lactate dehydrogenase (LDH: 375 U/L. A COVID-19 PCR from NP swab was negative. Chest X-ray (CXR) showed central opacities and small bilateral effusion. After initial stabilization, his clinical condition deteriorated with fever, tachypnea, tachycardia, and worsening hypoxia on the 4th day of admission. A computed tomography (CT) scan of the chest showed bilateral ground-glass opacities (GGO) and cavitation in the right upper lobe. The clinical characteristics are listed in Table 1. Broad antimicrobial coverage was continued. The patient underwent bronchoscopy on the 8th day of hospitalization, and BAL testing was positive for COVID-19. With worsening hypoxia, the patient was intubated on the 10th day and received remdesivir (compassionate usage) and convalescent plasma via a clinical trial. His clinical status worsened further with multiorgan failure, acute respiratory distress syndrome, and rising inflammatory markers (ferritin: 50,068 ng/mL; LDH: 7,213 U/L; C-reactive protein [CRP]: 31.9 mg/dL) (Table 2). The patient was transitioned to comfort care in consultation with family and passed away on the 12th day.

Case 2

An 88-year-old male patient, with a history of hypertension, hyperlipidemia, atrial fibrillation, and IgG kappa multiple myeloma, was admitted with fever, hypoxia, and generalized weakness. He had been receiving corticosteroids at a dose equivalent to prednisone 20 mg daily, in combination

Table 1 Clinical, Disease, and Laboratory Characteristics of Cancer Patients with COVID-19 Tested Through BAL.

	Patient #1	Patient #2	Patient #3
Demographics			
Age, years	76	88	77
Sex	M	M	M
Race	Caucasian	Caucasian	African American
Comorbidities	HTN, HLD, PE	HTN, HLD, BPH, AF	HTN, DM, HCV cirrhosis, ESLD
Smoking status	Former	Former	Former
Cancer related			
Underlying cancer	Myelodysplastic syndrome	Multiple myeloma	Hepatocellular carcinoma
Cancer status at the time of COVID-19 diagnosis	Engrafted; complete remission	Responding to DRD	Remission (await OLTx)
Duration between cancer and COVID-19 diagnoses	9 months	4.5 years	6 years
Active cancer treatment	Yes	Yes	Yes
Cancer treatment	Allogeneic MUD PBSCT (2.5 months prior to COVID-19)	DLD	TACE
Anti-infective prophylaxis before COVID-19	Acyclovir, dapsone, voriconazole	Acyclovir	N/A
COVID-19 related			
COVID-19 symptoms	Dyspnea, fever, malaise	Dyspnea, fever, weakness	Fever, dysgeusia, fatigue
Potential exposure to COVID-19	Subacute rehabilitation	None	None
Day of symptoms when tested positive for COVID-19	Day 8 (Positive from BAL; NP swab negative 4 times while inpatient)	Day 10	Day 8
Source of testing	BAL	BAL	BAL
Days between first negative NP swab and positive BAL	6 weeks	3 days	2 days
Number of negative NP swabs before positive BAL	4	1	1
Admission CXR/Chest CT	Bilateral GGO with cavitation	Bilateral GGO with consolidative opacities	Bilateral GGO with consolidative opacities
LOHS	11	12	5
ICU admission	Yes	No	No
Mechanical ventilation	Yes	No	No
Duration of oral corticosteroid prior to COVID-19	2 months	6 months	No
Duration of lymphopenia prior to COVID-19	2 months	4 months	6 months
Median absolute lymphocyte count before COVID-19 (range)[a]	0.49 (0.39—0.92) $\times$ $10^3/\mu L$	0.38 (0.08—0.68) $\times$ $10^3/\mu L$	0.50 (0.31—0.97) $\times$ $10^3/\mu L$
Day of clinical deterioration after hospital admission	Day 9	N/A	N/A
Survival status	Dead	Alive	Alive
Duration from symptom onset and death	5 weeks	N/A	N/A

Note. AF = atrial fibrillation; BAL = bronchoalveolar lavage; BPH = benign prostatic hyperplasia; DLD = daratumumab, lenalidomide, dexamethasone; ESLD = end-stage liver disease; GGO = ground-glass opacities; HCV = hepatitis C virus; HLD = hyperlipidemia; HTN = hypertension; ICU = intensive care unit; LOHS = length of hospital stay; MUD = matched unrelated; N/A = not applicable; NP = nasopharyngeal; OLTx = orthotopic liver transplantation; PBSCT = peripheral blood stem cell transplantation; PE = pulmonary embolism; TACE = trans-arterial chemoembolization.

[a] Normal range of absolute lymphocyte count: 0.90—3.20 $\times$ $10^3/\mu L$.

with chemotherapy, for approximately 6 months. Upon presentation, the patient was febrile up to 100.5°F (38 °C) and needed 4 L/minute of supplemental oxygen via NC. He was receiving chemotherapy with daratumumab, lenalidomide, and dexamethasone for multiple myeloma and had pancytopenia (white blood cell count [WBC]: 2.8 $\times$ $10^3/\mu L$; absolute neutrophil count: 2.6 $\times$ $10^3/\mu L$; absolute lymphocyte count: 0.08 $\times$ $10^3/\mu L$; hemoglobin: 7.8 g/dL; platelets at baseline: 58 $\times$ $10^3/\mu L$). The patient did not have renal or hepatic dysfunction, and a coagulation panel was unremarkable. A CT scan suggested a multifocal pneumonia and showed bilateral GGO, particularly in the right upper lobe

Table 2 Patients' Status.

	CRP[c]	Ferritin[d]	LDH[e]	D-Dimer[f]	COVID-19 treatment
Patient 1	D9: 24.2 **D11: 31.9**	Pre-COVID: 518 D9: 10,973 **D11: 50,068**	Pre-COVID: 330 D6: 592 D9: 898 **D11: 7,213**	D9: 1.73 **D11: 2.88**	Remdesivir[a], convalescent plasma (10th day)[b]
Patient 2	D1: 7.8 **D5: 15.6** D6: 12.8 D8: 8.8 D10: 4.1 D12: 2	Pre-COVID: 142 Pre-symptomatic: 542 **D5: 2,587** D6: 1,982 D8: 1,692 D10: 1,068 D12: 780	Pre-COVID: 260 **D5: 370** D8: 342 D10: 284 D12: 264	D4: 11.26 D5: 5.82 D6: 8.43 **D8: 19.78** D10: 4.95 D12: 4.31	Convalescent plasma (5th day of admission)[b]
Patient 3	**D4: 11.1** D6: 6.6	Pre-COVID: 115 **D4: 958** D6: 888	**D4: 362**	**D4: 1.25** D6: 1.15	None

Note. CRP = C-reactive protein; LDH = lactate dehydrogenase. Data in **BOLD** represents day of peak levels.
[a] Compassionate usage.
[b] Clinical trial.
[c] Normal range of CRP = 0.00—0.50 mg/dL.
[d] Normal range of ferritin = 30.0—400.0 ng/mL.
[e] Normal range of LDH = 135—225 U/L.
[f] Normal range of D-Dimer = $\leq$ 0.69 mg/L FEU

and consolidative opacities. Infectous work up including COVID-19 PCR from NP swab, respiratory culture, community-acquired bacteria and atypical organisms, community respiratory viruses, and cytomegalovirus PCR in the blood was negative. Because of no improvement in hypoxia with antimicrobials and high clinical suspicion of COVID-19, he underwent a BAL on the 4th day of admission. PCR for COVID-19 returned positive on the BAL specimen. The patient was treated with convalescent plasma on the 5th day of admission. Thereafter, the patient's clinical status continued to improve, he was progressively weaned off supplemental oxygen, inflammatory markers trended down (Table 2), and he was discharged well on the 12th day.

Case 3

A 77-year-old male patient, with hepatitis C-related hepatocellular carcinoma who recently underwent a bridging transarterial chemoembolization while awaiting orthotopic liver transplantation and a medical history significant for hypertension, hyperlipidemia, and diabetes mellitus, reported malaise, subjective fever, change in taste sensation, and worsening dyspnea for almost a week. The patient was noted to be febrile (102.8°F [39.2 °C]) and needed supplemental oxygen of 2 L/minute via NC. Initial blood workup demonstrated a normal WBC count, preserved liver and kidney function, and baseline thrombocytopenia (122 $\times$ 10^3/μL) and hyponatremia (132 mEq/L). Furthermore, the patient had lymphopenia for at least 6 months. COVID-19 PCR from a NP swab was negative. CXR did not demonstrate focal opacities, consolidation, or effusion. Infectious work up for bacterial, viral, and endemic fungi was negative. The patient was presumptively treated with broad-spectrum antimicrobials. A CT scan of the chest obtained due to worsening hypoxia showed unilateral upper lobe GGO and scattered nodules. He underwent a BAL on the 3rd day of admission, and PCR performed on the BAL specimen returned positive for COVID-19. Inflammatory markers are listed in Table 1. Broad-spectrum antimicrobials were discontinued. His clinical condition improved, inflammatory markers trended down, and he was discharged on the 5th day of admission with self-isolation advice. Interestingly, patient had a second negative COVID-19 NP swab, 1 day after the positive test from the BAL specimen.

Discussion

Emerging data related to the kinetics of SARS-CoV-2 transmission indicates active viral replication in the upper respiratory tract early and in the lung parenchyma later during illness in immunocompetent patients [7]. The considerably higher mortality in the immunocompromised hosts could be due, in part, to delayed diagnosis owing to preferentially abundant angiotensin converting enzyme 2 (ACE2) expression in the lower tract [8,9]. Several reports have shown more abundant ACE2 expression in the lower airway epithelial cells than in the NP cells among patients with comorbidities who develop severe COVID-19 [10]. Solitary cases in the setting of COVID-19 and multiplex PCR-based assays for common respiratory pathogens have also shown an incremental yield in BAL samples in the immunocompromised patients [11,12]. Additionally, SARS-CoV-2 could have higher receptor tropism in the lower respiratory tract. This is corroborated by relative oligosymptomatic transmission as well as a lack of reports of olfactory or gustatory dysfunction in the immunocompromised patients. Patient #1 did not report upper respiratory tract symptoms and tested negative for SARS-CoV-2 four times via NP swabs, in the preceding 5 weeks, prior to testing positive in a BAL specimen. In contrast to patient #1, patients #2 and #3 had better out-

comes likely due to earlier BAL specimen testing resulting in timely medical intervention. Serum interleukin or cytokine levels were not checked in the current study. However, elevated CRP levels are shown to correlate with poor outcomes in COVID-19 (Table 2) [13].

Protracted lymphopenia may result in active viral replication predominantly in the lower tract early during the course of illness and prolonged shedding of replication-incompetent virus from the upper airway later. A recent study examining in situ pulmonary expression of SARS-CoV-2 in autopsy cases preferentially detected the virus in pneumocytes during the early acute phase of illness [14]. Another study evaluating 678 patients with COVID-19 showed that recent chemotherapy was associated with high viral load at the time of admission and this, in turn, was independently associated with risk of intubation and mortality [15]. Protracted lymphopenia leads to high viral load in cancer patients. This often coincides with chronic use of steroids that results in immunomodulation, effector T-cell suppression, and repression of proinflammatory cytokines hampering the innate immune response to SARS-CoV-2 [16].

The three cases further highlight the importance of clinical decision making and high pre-test probability of COVID-19 in select patients, when the actual predictive value of NP swab-based diagnosis is plagued by sampling issues and lack of sensitivity, and when bronchoscopic diagnosis may not always be feasible (the tenuous clinical status of cancer patients) or practical (exposure to healthcare providers from aerosol-generating procedures). Thus, it remains unclear yet if alternate, noninvasive sampling methods (such as deep cough sputum samples) have any higher yield than NP or OP specimens.

Conclusion

The study underscores a potentially reversed viral shedding pattern in immunocompromised patients due both to an inability to contain the virus early–numerical and functional compromise in effector T-cells–and an abundance in the lower tract early–ACE2 overexpression as well as preferential viral tropism. Hence, an understanding of analytic and clinical sensitivities of a diagnostic test is imperative. The underlying immune status should determine decision making related to the site of specimen, method of collection, the anticipated burden of organism, severity, and timing of symptoms and illness. Clinical decision making–driven by presentation, radiology, exposure, and inflammatory markers–ought to supplant diagnostic testing results, particularly in patients with cancer. High clinical suspicion ought to supersede false-negative NP RT-PCR as early bronchoscopic evaluation in cancer patients, who are either receiving active treatment or are immunosuppressed, can allow timely institution of efficacious treatment, enrollment into clinical trials, as well as effective infection control. At the same time, the risk of exposure to the healthcare providers and proceduralists should be balanced against the benefit of performing early diagnostic bronchoscopy. Prudence is equally vital and, in apt clinical settings in patients with cancer, presumptive treatment may also be considered to minimize exposure to healthcare providers.

Authors' contributions

MBA and MH designed the study. MBA drafted the manuscript. MBA, SC, SA, AD, BG, and MH contributed to patient care. MBA, BB, MBG, SC, AD, BT, BG, and MH critically revised the manuscript. All authors approved the final version.

Ethics approval and consent to participate

The study was approved by the Medical College of Wisconsin's institutional review board.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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