COVID-19 Associated Pulmonary Aspergillosis: A Case Series

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ABSTRACT

Coronavirus disease 2019 (COVID-19) has been a worldwide pandemic with several problems, one of which is the lack of definitive treatment. COVID-19-associated pulmonary aspergillosis (CAPA), the presence of invasive pulmonary aspergillosis (IPA) in COVID-19 patients, is one of the concerning secondary infections associated with higher mortality and worse clinical outcomes. Diagnosing CAPA may be challenging due to the possible absence of classic host factors and clinical symptoms or obscured radiological findings. We described two CAPA cases, which were suspected due to persistent respiratory failure despite standard treatment of COVID-19 with additional therapies and antimicrobial agents for secondary infections, eventually diagnosed with serum galactomannan testing. Clinical conditions of both patients improved significantly after the administration of voriconazole. This case series emphasizes the importance of being aware of clinical suspicions indicating CAPA followed by galactomannan testing as a relatively fast, noninvasive test for its diagnosis, which leads to appropriate antifungal treatment.

Keywords: *Aspergillosis, Aspergillus, COVID-19, COVID-19-associated pulmonary aspergillosis (CAPA), invasive pulmonary aspergillosis (IPA).*

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has been a pandemic with several issues, including the lack of definitive treatment. Secondary infection in patients with COVID-19 has been a serious concern. One concerning pathogen among these is *Aspergillus,* which can cause invasive pulmonary aspergillosis (IPA). IPA in patients with COVID-19 is referred to as COVID-19-associated pulmonary aspergillosis (CAPA). A systematic review found that the overall CAPA incidence was 13.5%, with the majority requiring invasive mechanical ventilation. CAPA was also found to have a mortality rate as high as 48.4% with prolonged mechanical ventilation use and hospitalization.[1] Patients with CAPA may not present with the classic clinical criteria or radiological findings of IPA. Difficulties in differentiating IPA from other secondary infections in COVID-19 have caused delayed or missed diagnosis of CAPA.[1,2] We describe two cases of CAPA diagnosed with clinical suspicion in COVID-19 patients with respiratory failure and persistently high oxygen supplementation needs despite standard medical therapy, including anti-inflammatory, immunomodulating, and antimicrobial agents for secondary infection.

CASE ILLUSTRATION

Case 1

A 70-year-old woman was admitted with a fever three days before hospital admission. The fever was unmeasured at home but was felt to be not high. The patient also complained of productive cough with difficult expectoration. The patient denied nasal discharge or blockage, shortness of breath, loss of smell or taste, nausea, vomiting, or changes in the stool. The patient had a history of diabetes mellitus and was routinely consuming pioglitazone. There was no past medical history of hypertension, cardiovascular diseases, tuberculosis, stroke, kidney or liver diseases, asthma, and allergies. The patient was in close contact with her son, who was confirmed to have COVID-19. The patient had already received two doses of Coronavac vaccination.

On initial physical examination, the patient was alert, blood pressure 138/78 mmHg, heart rate 87 bpm, axillary temperature 36.8°C with tachypnea of 22 breaths per minute and SpO_2 91% on room air which improves to the respiratory rate of 18 breaths per minute and SpO_2 98% with 4 LPM of oxygen via nasal cannula. Minimal crackles were heard on both lungs, while other general exams revealed normal results.

Two days before admission, the patient had already undergone a chest x-ray which showed bilateral interstitial pneumonia and a positive SARS-CoV-2 naso-oropharyngeal RT-PCR swab test. On the day of admission (D1), additional lab exams were done (**Table 1**). The patient was assessed with severe COVID-19 and type 2 diabetes mellitus. Initial treatment consisted of IV remdesivir 200 mg (loading dose) followed by 100 mg qd, dexamethasone 6 mg IV qd, ranitidine 50 mg IV bid, paracetamol 500 mg PO tid, N-acetylcysteine 200 mg tid, zinc 20 mg bid, vitamin D3 1000 U bid, subcutaneous heparin 5000 IU qd, and basal-bolus insulin regiment to regulate blood glucose.

On the night of D5 of hospitalization, the patient had a rapid desaturation until she only maintained SpO_2 91% with 15 LPM of oxygen via a non-rebreathing mask (NRM). On the following day (D6), the high-flow nasal cannula (HFNC) was used, and SpO_2 of 96% could be maintained with FiO_2 80% and 40 LPM flow. A repeat chest x-ray showed heterogeneous opacities on both lungs' middle to lower section, indicating bilateral pneumonia (**Figure 1**). The antimicrobial levofloxacin 750 mg IV qd was started, and dexamethasone was also increased to 5 mg bid. On D7, the blood interleukin-6 (IL-6) level was measured at 5.79 pg/mL. The patient's required amount of oxygen supplementation gradually increased, and dyspnea remained unresolved. On the D12, the patient was administered two doses of convalescent plasma therapy of 200 mL.

There was no significant improvement in the patient's clinical condition after convalescent plasma therapy, ten days of dexamethasone, and levofloxacin (**Table 2**). In addition, procalcitonin level was not high (0.14 nG/mL). Therefore, a secondary infection caused by aspergillosis or cytomegalovirus was suspected. The patient was tested for galactomannan, anti-cytomegalovirus (anti-CMV) antibodies, and CD4 count on D17. Anti-CMV IgM was nonreactive while IgG was

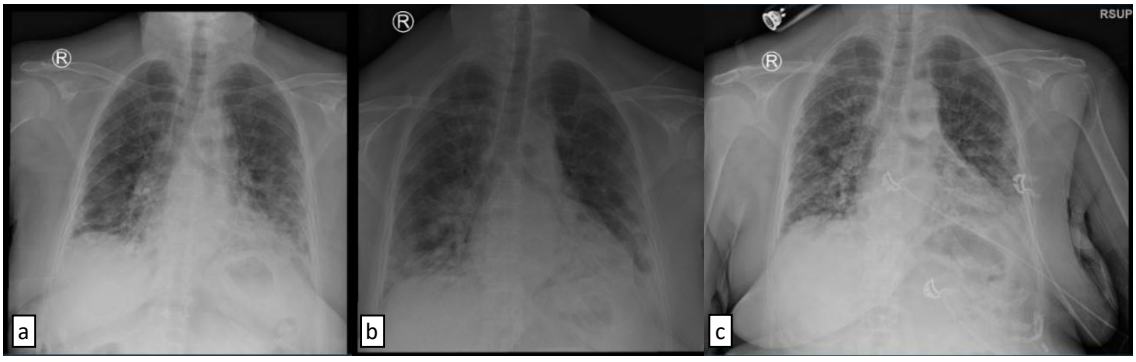


Figure 1. Serial Chest X-ray on: (a) Day 6; (b) Day 16; and (c) Day 26 of Hospitalization of Patient 1.

Table 1. Clinical condition and laboratory results of patient 1.

Days of hospitalization	D1	D5	D6	D12	D17	D22	D27
Temp. (^{0}C)	36.2	37	36.2	36.9	36.8	36.7	36.6
RR (times/min)	18	24	26	23	17	22	27
SpO_2 (%)	98	91	96	97	97	96	97
O_2 therapy device	NC	NRM	HFNC	HFNC	HFNC	HFNC	NRM
Flow (LPM)	4	15	40	60	60	45	15
FiO_2			80	85	90	50	
ROX index			4.62	4.96	6.34	8.73	
Leukocyte (/uL)	5920	11060	10260	12580		12610	12610
Neutrophil (/uL)	4458	9890	9410	11290		10520	9880
Lymphocyte (/uL)	959	680	650	660		1070	1380
Neu/Lym ratio (NLR)	4.65	14.54	14.48	17.11		9.83	7.16
ALT (U/L)	68			80		35	27
AST (U/L)	39			71		27	34
Procalcitonin (nG/mL)	0.07	0.09	0.15	0.14	0.16	0.05	
CRP (mg/L)		114.6	191.7	33.7	45.7	20.5	
aPTT (s)				28.4 (31.5)	52.6 (32.3)	48.6 (32.0)	26.3 (33.3)
Fibrinogen	725.4						
D-dimer (ug/mL)	990		1890	1130	1060	800	

Table 2. Notable therapies of Patient 1.

Days of hospitalization	D1	D5	D6	D12	D17	D22	D27
Antiviral	Remdesivir IV						
Corticosteroid	Dexamethasone IV					Methylprednisolone PO	
Antimicrobial	Levofloxacin IV (14 days)						
Colchicine	Colchicine PO						
Convalescent Plasma (CP)	CP						
Antifungal	Voriconazole IV						

reactive 341.7 U/mL, CD4 was 38% with an absolute count of 489 /uL. On D20, the patient underwent a second SARS-CoV-2 RT-PCR, which had a negative result. Dexamethasone was reduced to 5 mg qd. The Galactomannan test revealed a positive result (2.04) on D21, and the patient was assessed with invasive pulmonary aspergillosis (IPA). Voriconazole was started on D22 with the dose of 6 mg/kgBW IV q12h (loading dose) followed by 4 mg/kgBW IV q12h from the second to the fourteenth day. She was transferred to the medical non-COVID-19 high-care unit (HCU) after the third negative SARS-CoV-2 RT-PCR result. On D27, oxygen supplementation was stepped down from HFNC to 15 LPM via NRM. After that, the patient's clinical condition improved, and three days later, the patient could maintain SpO2 of 95% with 3 LPM of oxygen via nasal cannula.

Case 2

A 51-year-old man was admitted with shortness of breath four days prior to hospitalization. The patient initially had a fever, headache, muscle pain, and occasional dry cough in the past week before hospitalization. He initially did not have breathing problems and denied complaints of nasal discharge or blockage, loss of smell or taste, or any other complaints. Two days later, he underwent a SARS-CoV-2 antigen test, and the results came out positive. He then started self-isolation and developed shortness of breath four days before hospitalization. The patient had a history of diabetes mellitus and routinely consumed glimepiride 2 mg qd and metformin 500 mg tid.

He also had a 4-year history of hypertension treated with irbesartan. There was no past medical history of cardiovascular diseases, kidney or liver diseases, asthma, and allergies. The patient was not vaccinated for COVID-19.

On initial physical examination, the patient was alert, blood pressure 133/81 mmHg, heart rate 87 bpm, axillary temperature 36.5°C with tachypnea of 30 breaths per minute, and SpO_2 93% with 3 LPM of oxygen via nasal cannula. Slight rales were heard on both lungs, while other general exams were normal. Initial laboratory and chest x-ray were done (**Figure 2**). The patient's D-dimer was elevated, and the circulating IL-6 level was high (**Table 3**). Chest x-ray showed bilateral alveolar opacities in the perihilar and paracardial area consistent with bilateral pneumonia. The patient was assessed with severe confirmed COVID-19, type 2 diabetes mellitus, and hypertension. Initial treatment consisted of IV remdesivir 200 mg (Loading dose) followed by 100 mg qd (D2-D14), dexamethasone 6 mg IV qd, paracetamol 1000 mg IV qd, vitamin C 1000 mg IV bid, vitamin D3 5000 mg PO qd, Zinc 20 mg PO bid, heparin 10.000 U/24h (2 mL/h), and basal-bolus insulin to regulate blood sugar.

Nasopharyngeal swab for SARS-CoV-2 RT-PCR was obtained and the result was positive (ORF1b Cq = 27.20, RdRP Cq = 30.24). On D4, 5 LPM of oxygen via nasal cannula was needed to maintain SpO2 of 96%, and levofloxacin 750 mg IV qd was started for secondary infection. The patient's oxygen desaturation worsened that he needed 15 LPM of oxygen via NRM on D5. The deterioration continued as the patient fell into acute respiratory distress syndrome (ARDS) on D6. He was transferred to the HCU, and HFNC was initiated in addition to increasing the dose of dexamethasone to 5 mg bid, the addition of colchicine 0.5 mg tid for five days, and treatment with tocilizumab was planned. On the night of D6, the D-dimer result was extremely high at 30.990 ug/mL, warranting escalation of the anticoagulant. Heparin was switched to enoxaparin 0.6 cc bid, and antioxidant therapy with N-acetylcysteine 5000 mg IV qd was started. Tocilizumab 400 mg IV was administered on D9, followed by 200 mg IV 12 hours after. On D13, the meropenem 3 gr IV qd was added.

On D14, due to the need for supplementary oxygen still being high and ROX index had not reached safe levels after standard therapy including antiviral remdesivir, corticosteroid, tocilizumab, and adequate antibiotics (**Table 4**), fungal infection was suspected, and serum sample for Galactomannan test was taken. SARS-CoV-2 RT-PCR was reassessed the next day (D15), and it was still positive but with a higher CT value (ORF1b Cq = 38.04 dan RdRP Cq = 38.77).

On D16, the result of the serum galactomannan test came out positive (2.03), hence invasive pulmonary aspergillosis (IPA) was assessed, and voriconazole 6 mg/kgBW q12h IV loading dose followed by 4 mg/kgBW IV q12h for fourteen days was administered. Anti-CMV was also checked, but only IgG was reactive of 107.4 U/mL. Three days later (D20), oxygen supplementation could be deescalated to 15 LPM via NRM, and RT-PCR was shown to be negative, so the patient was considered to be moved to a non-isolation HCU. Repeat chest x-ray on D21 showed reduced lung opacities with signs of pulmonary fibrosis. The patient's clinical condition improved gradually after that, and he was discharged from the hospital a few days later.

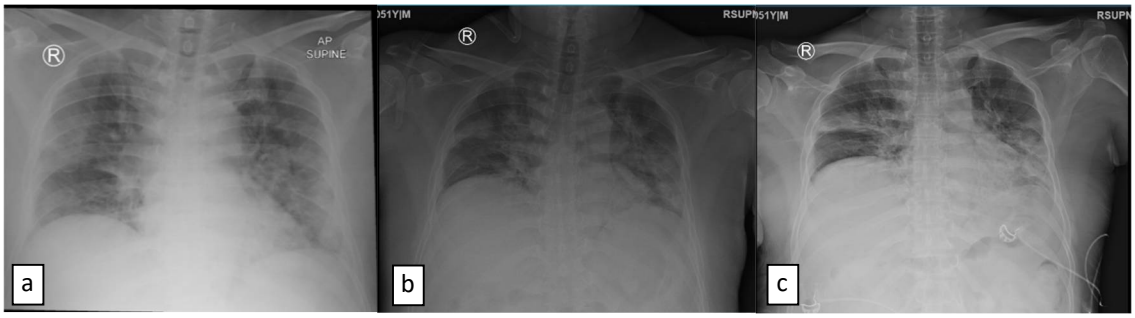
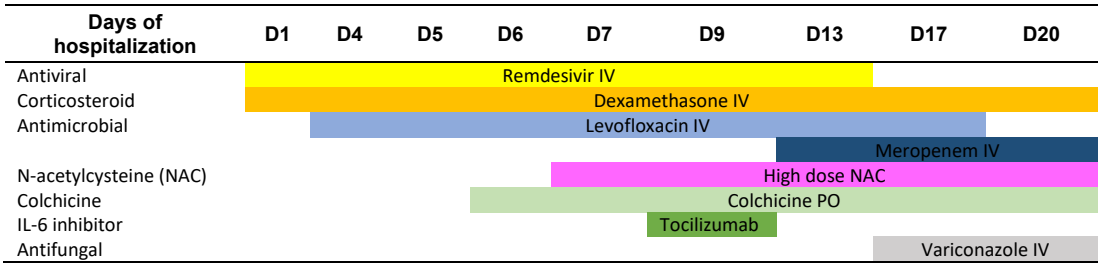


Figure 2. Serial Chest X-ray on: (a) Day 1; (b) Day 6; and (c) Day 21 of Hospitalization of Patient 2.

Table 3. Clinical condition and laboratory results of Patient 2.

Days of hospitalization	D1	D4	D5	D6	D7	D9	D13	D17	D20
Temp. (^{0}C)	36.5	36.4	36.9	35.6	36.5	36	36.8	36.1	36.
RR (times/min)	30	24	32	28	38	26	22	22	24
SpO_2 (%)	93	96	97	97	95	95	97	98	95
O2 therapy device	NC	NC	NRM	HFNC	HNC	HFNC	HFNC	HFNC	NRM
Flow (LPM)	3	5	15	60	65	70	60	80	15
FiO_2			100	80	97	95	70	95	
ROX index				4.33	2.58	3.85	6.30	4.69	
Leukocyte (/uL)	8460	12660		14940					
Neutrophil (/uL)	6240	10840		13460					
Lymphocyte (/uL)	1610	1030		990					
Neu/Lym ratio (NLR)	3.88	10.52		13.60					
ALT (U/L)	45			43					
AST (U/L)	52			26					
Procalcitonin (nG/mL)	0.68			0.11					
CRP (mg/L)	55			72.9					
aPTT (s)					31.2 (31.7)	32.6 (30.9)			
D-dimer (ug/mL)	960			30990			4110		
IL-6	75.18								

Table 4. Notable therapies of Patient 2.



DISCUSSION

COVID-19-associated pulmonary aspergillosis (CAPA) is defined as invasive pulmonary aspergillosis (IPA) occurring in coronavirus disease 19 (COVID-19) patients.[1] Patients with CAPA often do not have classic host factors for invasive fungal infections. Extensive pulmonary epithelial damage, dysregulation of the immune response, lymphopenia, or treatment factors such as widespread broad-spectrum antibiotic use in intensive care units, corticosteroid therapy, and anti-interleukin 6 (IL-6) treatment are some of the several mechanisms are thought to predispose COVID-19 patients to IPA.[3,4] The varied time to CAPA diagnosis from illness onset of about 8 to 16 days might be caused by difficulty differentiating IPA from COVID-19 pneumonia with other causes of secondary infections[1]. According to various studies, the diagnostic criteria for CAPA were based on the same criteria for IPA mentioned before, mostly using the AspICU criteria.[1,3] The diffuse bilateral lung infiltrates in COVID-19 patients might obscure important radiologic clues for IPA.[5] An awareness of clues suggesting CAPA followed by prompt diagnostic testing for *Aspergillus* is crucial in preventing the delay of diagnosing and treating CAPA.

The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) stated that the diagnosis of invasive fungal diseases needs to fulfill certain host factors, clinical features (certain radiologic findings for IPA), and mycological evidence. A high index of suspicion may arise

from host factors such as immunocompromise, neutropenia, hematologic malignancies, prolonged corticosteroids use, treatment with T-cell or B-cell immunosuppressants, and other factors.[2] Severe COVID-19 with ARDS might also be considered as a host criterion (acquired immunodeficiency).[8] The clinical criteria for IPA from the AspICU are also often used. Fulfilling the criteria requires one of the following signs and symptoms: refractory fever despite three or more days of antibiotic therapy; recrudescent fever lasting 48 hours or more despite antibiotic therapy; pleuritic chest pain/rub, dyspnea; hemoptysis; or worsening respiratory failure despite antibiotic therapy and ventilatory support.[9] Diagnostic investigation for CAPA is triggered in COVID-19 patients with refractory respiratory failure for more than five to fourteen days despite having already received all support recommended for patients with COVID-19 who are critically ill.[3] Both patients have in common a persistent respiratory failure lasting more than fourteen days after COVID-19 symptom onset, showed by the intractably high oxygen supplementation need to maintain a stable SpO_2 despite receiving the antiviral remdesivir, dexamethasone, adequate antimicrobial agents, and even additional therapies such as convalescent plasma (case 1) and the IL-6 inhibitor tocilizumab (case 2). This raised the clinical suspicion of IPA, which is called CAPA, in COVID-19 patients.

Thoracic high-resolution computed tomography scan (HRCT scan) is the preferred radiologic modality for IPA. HRCT scan might show either: dense, well-circumscribed lesions with or without a halo sign; air crescent sign; pleural-based cavitation; or wedge-shaped and segmental or lobar consolidation. The halo sign is a localized ground-glass appearance representing hemorrhagic infarction surrounding a nodule or consolidation.[6,7]

The definitive diagnosis of invasive aspergillosis requires either a positive culture of samples from an ordinarily sterile site (e.g., a brain abscess) or positive results of both histologic testing and culture of a sample from the affected organ. The fast progression of the disease and other factors that might make invasive procedures not feasible makes it challenging to diagnose the patient before further clinical deterioration or even death. These reasons cause up to 40% of invasive aspergillosis to be missed clinically and are only diagnosed postmortem.[6] Therefore, using faster, noninvasive modalities, such as serum biomarkers (galactomannan and beta-D-glucan assays) and/or with microscopic examination and culture of sputum samples, is preferred as a first step to diagnose IPA.

The antigen test for *Aspergillus* typically relies on the detection of galactomannan, which is a polysaccharide constituent of *Aspergillus* cell walls released during growth. Galactomannan can be detected in serum, plasma, BAL fluid, sputum, or other body fluids.[6,10] Galactomannan test of serum was done on the two patients. Both patients had a positive result of 2.04 (patient 1) and 2.03 (patient 2), exceeding the high cut-off of 1.0-1.5, with a positive predictive value (PPV) for IPA as high as 100%.[11,12] The presence of clinical suspicion supported by a positive galactomannan test is usually enough evidence of diagnosis aspergillosis and to start preemptive antifungal therapy.

The recommended treatment of invasive aspergillosis is initial intravenous (IV) antifungal therapy. Voriconazole and isavuconazole are the preferred first-line agents, while caspofungin, posaconazole, micafungin, and lipid Amphotericin B are second-line agents.[6] Both patients received voriconazole IV and showed clinical improvements after administration of the drug.

CONCLUSION

COVID-19-associated pulmonary aspergillosis (CAPA) is a concerning secondary infection in patients with COVID-19. Aside from being associated with higher mortality and poorer clinical outcomes, CAPA might not present with the classic host factor, clinical criteria, or radiological findings of invasive pulmonary aspergillosis (IPA). Awareness of patients with suspicion of CAPA is crucial, particularly concerning the worsening of persistence of respiratory failure in COVID-19 patients in whom clinical conditions do not improve despite anti-inflammatory, immunomodulating agents

and appropriate antimicrobial therapy in addition to standard therapy, especially after two weeks of symptom onset. The Galactomannan test is a useful, relatively quick, noninvasive test for diagnosing IPA, including CAPA. The use of antifungals in these patients is essential and may significantly improve their outcomes.

DECLARATION OF CONFLICTING INTERESTS

The Authors declare that there is no conflict of interest.

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