



## CAPA Case Report

Recovery of a triazole-resistant *Aspergillus fumigatus* in respiratory specimen of COVID-19 patient in ICU – A case reportThéo Ghelfenstein-Ferreira<sup>a,\*</sup>, Anastasia Saade<sup>b</sup>, Alexandre Alanio<sup>a,c</sup>, Stéphane Bretagne<sup>a,c</sup>, Raffael Araujo de Castro<sup>c</sup>, Samia Hamane<sup>a</sup>, Elie Azoulay<sup>b</sup>, Swann Bredin<sup>b</sup>, Sarah Dellièvre<sup>a</sup><sup>a</sup> Laboratoire de Parasitologie – Mycologie, Hôpital Saint-Louis (AP-HP), Université de Paris, France<sup>b</sup> Médecine Intensive et Réanimation, Hôpital Saint-Louis (AP-HP), Université de Paris, France<sup>c</sup> Unité de Mycologie Moléculaire, Institut Pasteur, CNRS URA 3012, Centre National de Référence des Mycoses Invasives et des Antifongiques, URA, 3012, Paris, France

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## ABSTRACT

Although invasive pulmonary aspergillosis (IPA) is typically described in immunocompromised host, patient with severe influenzae can develop IPA. Similarly, patients with severe COVID-19 complicated with IPA are increasingly reported. Here, we describe a case of invasive aspergillosis with triazole-resistant *A. fumigatus* (TR34/L98H mutation) in a 56-year-old patient with COVID-19 in intensive care unit. This report highlights the need to define the available tools for diagnosis of invasive aspergillosis in severe COVID-19 patients.

## 1. Introduction

*Aspergillus fumigatus* is an opportunistic fungal pathogen that causes invasive pulmonary aspergillosis (IPA) in severely immunocompromised host. However, in the past two decades, IPA was reported in critically ill patients without any classical immunosuppression factors [1]. These patients did not meet the host criteria of the European Organization for the Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) [2]. Blot *et al.* defined new criteria to classify intensive care unit (ICU) patients suspect of IPA as “putative” aspergillosis. Early diagnosis of IPA in immunocompromised patients remains a real challenge. In severe patients [3], the diagnostic process is even more difficult, as patients present with atypical and unspecific clinical pictures. Malnutrition, diabetes mellitus, pulmonary disorders and corticosteroid use were found as underlying predisposing conditions for the development of IPA in ICU patients [4]. Among pulmonary disorders, severe influenza pneumonia was clearly identified as an independent risk factor for IPA. Indeed, the incidence of IPA in ICU patients admitted for severe influenza reaches up to 19% [5]. Consequently, occurrence of IPA in acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 is highly expected. In ICU-hospitalized patients with severe COVID-19, preliminary reports showed 19–33% of COVID-19-associated pulmonary aspergillosis (CAPA) [6,7]. Azole antifungal therapy is the first line of

treatment for IPA, but due to emerging azole-resistance amongst *A. fumigatus*, local prevalence of azole-resistance needs to be taken into account when initiating antifungal therapy. Susceptibility testing is of huge importance to detect azole-resistance early in the course of disease to enable effective treatment. We report a case of CAPA due to a triazole-resistant *A. fumigatus*.

## 2. Case presentation

A 56-year-old man, janitor with a history of diabetes mellitus, hypertension, hyperlipidemia, and obesity (body mass index: 31 kg/m<sup>2</sup>) was admitted (day 0) to the intensive care unit (ICU) of Saint-Louis Hospital (Paris, France) for ARDS. His medication consisted of an anti-hypertensive association with angiotensin II receptor blockers and calcium channel blockers, metformin, glimepiride, atorvastatin. He has been under inhaled fluticasone propionate/salmeterol for the suspicion of early stage chronic obstructive pulmonary disease (COPD). He reported intermittent fever (>38.3 °C), muscle ache for 2 weeks. Nonproductive cough and progressively increasing dyspnea started 7 days ago. He received 8 days of amoxicillin and 5 days of pristinamycin for the suspicion of bacterial pneumonia. Time course of the patient is detailed in Fig. 1.

On examination, the heart rate was 99 beats/min, respiratory rate 34 breaths/min, blood pressure 132/92 mmHg, temperature 38 °C and

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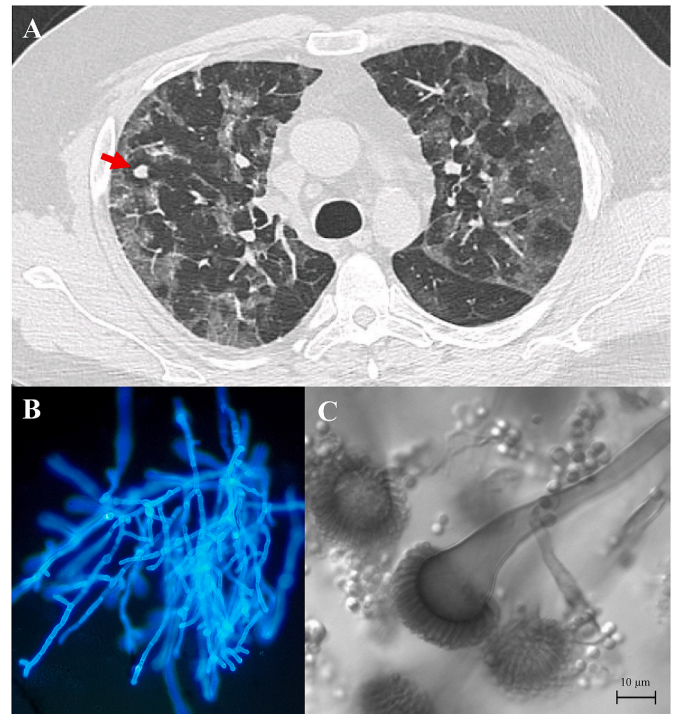
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oxygen saturation 95% on high flow nasal oxygenation. Arterial blood gas on 100% oxygen showed a pH of 7.41, pCO<sub>2</sub> 27.9 mmHg, pO<sub>2</sub> of 87.9 mmHg (PaO<sub>2</sub>/FiO<sub>2</sub>: 87.9). White blood-cell count was 10,720 cells per mm<sup>3</sup> (normal range, 4,000 to 10,000) with 82.6% neutrophils (8,900 cells per mm<sup>3</sup>) and 12.7% lymphocytes (1,370 cells per mm<sup>3</sup>), lactate dehydrogenase blood level was 882 U per liter (normal range, 240 to 480), CRP 206 mg per deciliter (normal value < 5), ferritin 556 ng per milliliter (normal range, 30 to 400) and D-Dimer 2390 ng per milliliter (normal value < 500). In addition, interleukin-6 level was 93.6 pg per milliliter (normal value < 7). His Sepsis-related Organ Failure Assessment (SOFA) score at ICU admission was 8. He was diagnosed with hypoxemic respiratory failure (PaO<sub>2</sub>/FiO<sub>2</sub> <100) and rapidly required mechanical ventilation and norepinephrine. He also presented with diabetic ketoacidosis (pH 7.3, glucose level 20.5 mmol per liter, ketonemia and bicarbonate ions level 17.2 mmol per liter) requiring continuous insulin infusion, and acute kidney failure without criteria for renal replacement therapy (creatinine level 2.76 mg per deciliter, 221 μmol per liter).

Chest computed tomography (CT) showed multiple bilateral ground-glass opacities with a crazy paving pattern characteristic of COVID-19 (Fig. 2A). In addition, 3 pulmonary nodules (7mm, 1mm, 1mm) were visualized in the right upper lobe. Nasopharyngeal swab real-time reverse-transcriptase PCR (RealStar® SARS-CoV-2 Kit, Altona Diagnostics) performed at the admission was positive for SARS-CoV-2 on the 2 targets (E and S genes) with quantification cycle (Cq) values of 23.3 and 22.8 respectively. He received a 7-day course of dexamethasone (20 mg) and 1 infusion of eculizumab (1200 mg) on day 2 (clinicaltrials.gov, NCT04346797). The patient was also treated with broad-spectrum antibiotic regimen, including piperacillin-tazobactam, cefotaxime and spiramycin which were stopped upon negative tracheal aspirate BioFire® FilmArray® pneumonia plus panel (BioFire Diagnostics, Biomerieux) on day 4.

At admission, screening for fungal infections with serum galactomannan index (Platelia® Aspergillus enzyme immunoassay, Biorad) and serum β-D-Glucan (Fungitell®, Cape Code diagnostics) was negative (0.07 and 10.4 pg per milliliter, respectively).

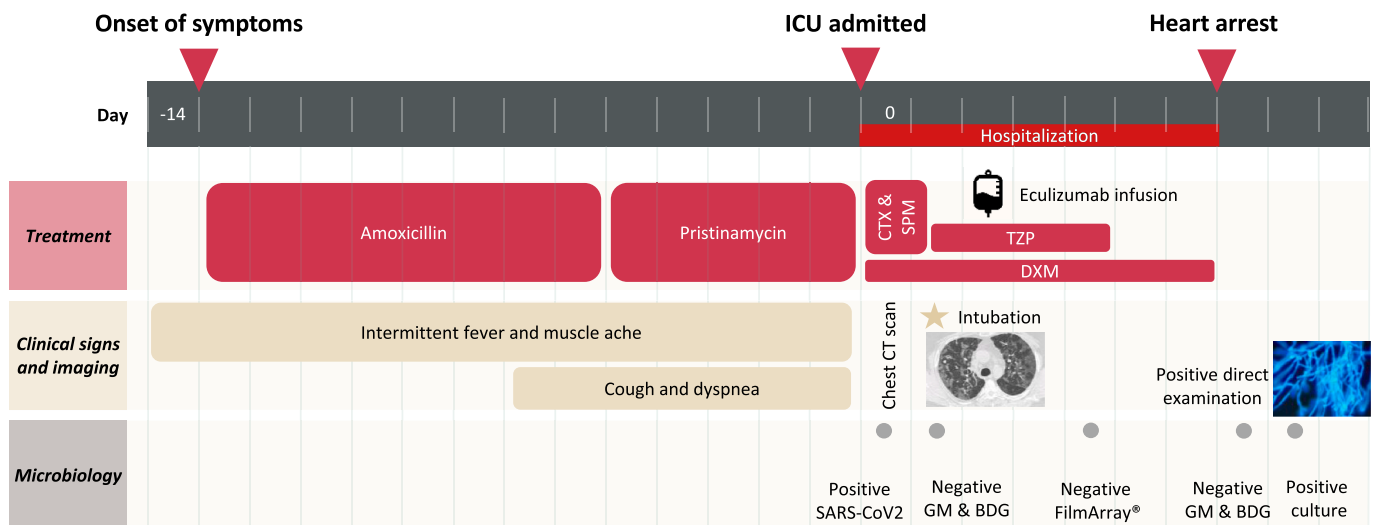
On day 6, his condition worsened with increasing vasopressor and oxygen need. Tracheal aspirate showed branching hyphae on direct examination with calcofluor white (Fig. 2B). Culture on Malt Extract Agar (VWR, Avantor) and Sabouraud Dextrose Agar with Chloramphenicol media (Bio-Rad) isolated *A. fumigatus* (20 CFU per 50 μL) after 1 day at 30 °C (Fig. 2C). Specific *A. fumigatus* quantitative PCR [8] (Af qPCR) performed on tracheal aspirate was positive with Cq value of



**Fig. 2.** (A) Chest CT on day 14 of symptom onset, which demonstrates bilateral ground glass opacities with a 7mm nodule in the right upper lobe. (B) Direct examination of the sample with calcofluor white, original magnification x200. (C) Strain culture of *Aspergillus fumigatus* isolates from tracheal aspirate.

26.3. Bacterial culture on chocolate (PolyViteX®, BioMérieux) and blood (Columbia agar + 5% sheep blood, BioMérieux) agar media remained negative after 5 days of incubation. Concomitantly, serum galactomannan index (0.05), serum β-D-Glucan (<7.8 pg per milliliter) and serum qPCR-Af were negative. On the same day, transthoracic echocardiogram showed hypertrophy and dilation of the right ventricle. D-dimer level increased >20000 ng per milliliter with fibrinogen 3.85 g per liter. The patient evolved unfavorably and died of a cardiac arrest suspect of massive pulmonary embolism. In this case, we were not able to efficiently treat the patient as the evolution was unfortunately too rapid. Autopsy was prohibited due to SARS-CoV-2 infection.

Antifungal susceptibility testing using EUCAST method and



**Fig. 1.** Time course of the patient with COVID-19-associated pulmonary aspergillosis. First day of hospitalization is considered day 0. CTX, cefotaxime; SPM, spiramycin; DXM, dexamethasone; TZP, piperacillin-tazobactam; GM, serum galactomannan; BDG, serum β-D-Glucan.

according to EUCAST clinical breakpoints [9] showed the following minimal inhibitory concentrations (MIC) of posaconazole (0.5 µg per milliliter, resistant MIC >0.25), itraconazole (>8 µg per milliliter, resistant MIC >1), voriconazole (2 µg per milliliter, resistant MIC >1) and isavuconazole (4 µg per milliliter, resistant MIC >1). DNA was extracted and *cyp51A* gene was sequenced as previously described [10]. TR34/L98H mutation was identified.

### 3. Discussion

Here, we report the case of a patient with severe SARS-CoV-2 infection complicated with a putative IPA. The isolated strain of *A. fumigatus* was triazole-resistant and TR34/L98H mutation was recovered from sequencing *cyp51A* gene. Triazole-resistance frequency varies considerably among geographic regions. The prevalence is less than 1% in France [10] and reaches 10% in the Netherlands [11], complicating the management and worsening the prognosis in patients treated for aspergillosis [12]. In this case, the patient was azole-naive which precludes the resistance development through azole therapy. In Europe, wood from the sawmill, wood chippings, flower bulb waste, green waste materials and soil from market garden were described as environmental hotspots for triazole-resistance selection of *A. fumigatus* [13–15]. Our patient worked as a janitor, performing various tasks such as gardening and may have manipulated organic matter containing triazole-resistant isolates of *A. fumigatus*. Hospital-acquired infection is unlikely since nosocomial infection with *A. fumigatus* are defined as infection occurring after 7 days of admission [16] while the strain was recovered at day 6 in our patient. This report emphasizes the need to screen isolates for pan-azole resistance on a routine basis that can be performed by broth microdilution reference method [9] or less time-consuming method such as gradient concentration strips [17], agar supplemented with various concentrations of azoles [18] or PCR screening for specific mutations [19].

CAPA was only recently described [20,21]. Misclassification between *Aspergillus* colonization and putative IPA may impact the management of the patients and their outcome. Depending on the chosen classification, our case could be characterized differently as infection or colonization. For this purpose, guidelines were compared to solve this issue (Table 1). (i) EORTC/MSGERC classification from 2019 is not suitable due to the lack of host factors in our patient. (ii) According to Blot *et al.* criteria, this case is classified as a putative aspergillosis. (iii) Using Schauvlieghe *et al.* criteria for IAPA, this case would be considered as a colonization rather than an infection as only positive culture from BAL is an accepted criterion. However, fungal load in the patient tracheal aspirate was significant (20 CFU per µl) at day 1 of culture and the direct examination was positive. (iv) Verweij *et al.* [22] recently proposed a consensus case definition of IAPA and CAPA which classifies our case as colonization. A positive tracheal aspirate is an accepted criterion if cavitary infiltrates are observed on CT imaging while our patient's chest CT showed ground glass opacities with nodules.

Patient classification as *Aspergillus* colonization or infection remains variable depending on the classification used, which is well illustrated herein, hence the difficulty to compare different studies and the need to find consensus on these definitions. This difficulty is further increased by the complexity of the aspergillosis diagnosis in ICU. In patients with COVID-19 hospitalized in ICU, medical imaging of the lungs is less specific than in neutropenic patients due to underlying COVID-19 damage. Furthermore, serum galactomannan lacks sensitivity in non-neutropenic patients [23] which could also be expected with serum quantitative PCR [8]. Both were negative in this patient. In previous studies only 3/20 patients (0 to 40%) with CAPA had positive serum galactomannan [3,6,7]. The lack of accurate marker may be an argument for colonization, showing the absence of angioinvasion. More studies compiling available cases are needed to select appropriate markers to define viral ARDS-associated pulmonary aspergillosis.

**Table 1**

Categorization of reported case of pulmonary aspergillosis according to various guidelines.

	Donnelly <i>et al.</i> , 2019	Blot <i>et al.</i> , 2012	Schauvlieghe <i>et al.</i> , 2018	Verweij <i>et al.</i> , 2020
<i>Host factor</i>	no	Glucocorticoid treatment	NA	COVID-19
<i>Clinical criteria</i>	NA	Dyspnea Worsening respiratory insufficiency	Dyspnea Worsening respiratory insufficiency	NA
<i>Imaging criteria</i>	Dense well-circumscribed lesion	Abnormal chest CT scan	Any infiltrate on chest CT scan	Infiltrate on chest CT scan
<i>Mycology criteria</i>	<i>Aspergillus</i> -positive culture on tracheal aspirate	<i>Aspergillus</i> -positive culture in lower respiratory tract	NFR (only BAL required)	<i>Aspergillus</i> -positive culture on tracheal aspirate <sup>a</sup>
<i>Diagnosis</i>	Colonization	Putative invasive aspergillosis	Colonization	Colonization

NA, Not applicable; CT scan, computed tomography scan.

BAL, bronchoalveolar lavage.

<sup>a</sup> Tracheal aspirate accepted if cavitary infiltrates are observed on CT scan.

Post-mortem autopsy may assist in identifying colonization or proven infection, unfortunately in this case it was not performed because of the risk of contamination. Here, the patient death was likely not related to aspergillosis, but clinically attributed to presumed pulmonary embolism.

More studies are needed to evaluate the impact of *Aspergillus* colonization or putative IPA on patients' outcome. It remains unclear whether the presence of *Aspergillus* is a prognosis marker of severity or is responsible for the clinical worsening of the patient potentially leading to death.

### Declaration of competing interest

There was no funding for this manuscript. All authors declare that they have no conflicts of interest regarding this manuscript.

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