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Isolation of *Aspergillus* spp. in respiratory samples of patients with COVID-19 in a Spanish Tertiary Care Hospital

Iker Falces-Romero[1] | Mario Ruiz-Bastián[1] | Beatriz Díaz-Pollán[2] | Emilio Maseda[3] | Julio García-Rodríguez[1] | SARS-CoV-2 Working Group

[1]Clinical Microbiology and Parasitology Department, University Hospital La Paz, Madrid, Spain

[2]Infectious Diseases Unit, University Hospital La Paz, Madrid, Spain

[3]Department of Anesthesia and Surgical Intensive Care, University Hospital La Paz, Madrid, Spain

Correspondence
Iker Falces-Romero, Clinical Microbiology and Parasitology Department, University Hospital La Paz, Paseo de la Castellana 261, Madrid 28046, Spain.
Email: falces88@gmail.com

Summary

Background: Invasive pulmonary aspergillosis (IPA) is a complication of respiratory bacterial and viral infections such as coronavirus disease 2019 (COVID-19).

Patients/Methods: In University Hospital La Paz (Madrid, Spain), we reviewed the clinical and demographic characteristics of 10 patients with positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) PCR and *Aspergillus* spp. isolate in respiratory samples. We also recovered results of galactomannan tests in serum and/or bronchoalveolar lavage (BAL) samples.

Results: Eight male and two female from 51 to 76 years were recovered. They had reported risk factors to develop IPA (haematological malignancies, immunosuppression, diabetes, obesity, intensive care unit stay, among others). Azole susceptible *Aspergillus fumigatus* was isolated in nine patients and *Aspergillus nidulans* was isolated in one patient. Only one case was classified as probable aspergillosis, seven cases as putative aspergillosis, and two cases were not classifiable. Eight patients received antifungal treatment. Seven patients died (70%), two are still inpatient due to nosocomial infections and one was discharged referred to another institution.

Conclusions: This clinical entity has high mortality, and therefore, it should be performed surveillance with early galactomannan tests and cultures in respiratory samples in order to improve the outcome of the patients with this condition.

KEYWORDS

Aspergillus, COVID-19, immunosuppression, respiratory samples

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, has emerged from Wuhan City (Hubei, China) and has become pandemic across the world.[1] This infection can cause severe respiratory disease with important rate of intensive care unit (ICU) admissions.[2] Bacterial and fungal infections are complications of this viral pneumonia[3,4] due to the severe damage of lung tissue, cytokine storm and immune-paralysis caused by viral infection-induced acute respiratory distress syndrome (ARDS).[1,5] Invasive pulmonary aspergillosis (IPA) has been reported in other respiratory virus such as *Influenza* viruses.[6] In this case, risk factors are haematological malignancies, solid transplant recipients, ICU stay, diabetes, chronic obstructive pulmonary disease (COPD), systemic corticosteroid administration or chronic kidney disease, among others.[5,7] It is essential to study if these factors or similar are related to the risk of developing COVID-19 associated pulmonary aspergillosis (CAPA), as well as to clarify clinical significance of isolation of *Aspergillus* spp. in respiratory samples in these patients.

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The aim of this study was to review the isolates of *Aspergillus* spp. from respiratory samples of patients with COVID-19 during the three months of maximum incidence of COVID-19 in the University Hospital La Paz in Madrid, Spain.

2 | PATIENTS AND METHODS

We performed a retrospective observational study of patients with positive PCR for SARS-CoV-2 and isolation of *Aspergillus* spp. in respiratory samples in the same episode during three months (March-May 2020) in University Hospital La Paz (Madrid, Spain), our tertiary care centre with 1286 beds. In these three months, we had a total of around 5,700 patients with positive SARS-CoV-2 PCR in the hospital (including our healthcare workers). All demographic, clinical and microbiological data were recovered from the clinical records of the patients.

Two commercial real-time PCR were used indistinctly for the diagnosis of COVID-19 in nasopharyngeal swabs and bronchial aspirates (BAS): SARS-CoV-2 real-time PCR Kit (Vircell, Granada, Spain) and TaqMan 2019 nCoV Assay Kit v1 (Thermo Fisher, Waltham, MA, USA). Sputa, BAS and bronchoalveolar lavages (BAL) of the patients were cultured in standard fungal culture media. *Aspergillus* spp. strains were identified by culture characteristics, microscopic morphology and matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS – Bruker Daltonics, Billerica, MA, USA) and susceptibility test to antifungal agents was performed by gradient diffusion strips according to the methodology of Clinical Laboratory Standards Institute (CLSI).[8] Galactomannan assays were performed in serum and/or BAL with Platelia Aspergillus antigen ELISA kit (Bio-Rad, Hercules, CA, USA) with a cut-off index of 0.5 for both samples. COVID-19 associated IPA cases were classified according to EORTC/MSG criteria[9] and *Asp*ICU algorithm.[10] According to Koehler et al[3] a confirmed COVID-19 case with ICU stay was considered to meet the host criterion 'Congenital or acquired immunodeficiency' in *Asp*ICU algorithm.[10] This retrospective study had the approval of the Clinic Research Ethics Committee of University Hospital La Paz with the code PI-4244.

3 | RESULTS

Ten patients, eight male and two female with positive SARS-CoV-2 PCR and *Aspergillus* spp. isolates in respiratory samples were recovered (age range 51-76 years, median age 69.5 years old). The patients had classic risk factors to develop pulmonary aspergillosis, such as diabetes, obesity, COPD, male sex and old age (≥65 years old). In four patients, the immunosuppressed underlying condition was due to haematological diseases, HIV and ankylosing spondylitis HLA B27+ under treatment with golimumab. Seven patients had an ICU stay with mechanical ventilation and all patients received corticosteroids with different administration routes as part of the anti-COVID-19 treatment (Table 1).

PCR for SARS-CoV-2 diagnosis was performed in nine patients in samples from nasopharyngeal swab and in the other patient, PCR was performed in BAS. In four patients PCR was performed before hospital stay (days −3 to −1, in Emergency Department), in two patients at the moment of ICU admission (day 0) and in four remaining patients several days after hospital stay (day + 2 to + 35) (Table 1). After the COVID-19 diagnosis, *Aspergillus* spp. isolates grew on culture media from deep respiratory samples: eight BAS (time range after positive PCR: 2-46 days, median days: 13 days), one sputum (day + 11 after positive PCR) and one BAL (day + 9 after positive PCR). In two patients the isolate grew on two and five consecutive BAS cultures respectively (Table 1). *Aspergillus fumigatus* was isolated in nine patients and *Aspergillus nidulans* in one patient. Susceptibility to antifungal agents was performed in seven strains (Table 1). All tested antifungals showed good in vitro activity in *A fumigatus* strains and in the case of *A nidulans*, only amphotericin B did not show good activity (MIC = 2 μg/mL). Galactomannan assays were performed in only three patients, one with positive values in serum (1.97) and BAL (3.87) and another with positive values in two consecutives BAL samples (2.16, 1.11). The third patient had a negative galactomannan test in serum (0.22). Nine patients underwent imaging procedures by chest X-ray or radiographs and only in one patient chest computerised tomography (CT Scan) was employed. In seven cases, it was observed bilateral basal or interstitial infiltrates and in five patients there were present ground-glass opacities. Clinically, cough, fever, dyspnoea and/or respiratory insufficiency was observed in nine patients. Only one case was classified as probable aspergillosis, seven cases as putative aspergillosis, and two cases were not classifiable.

To treat COVID-19 infection, all patients received hydroxychloroquine, four patients received tocilizumab and four patients received lopinavir/ritonavir. Eight patients received different intravenous antifungal therapies (four sequential and one combined treatments) due to the isolation of *Aspergillus* spp. (Table 1). Final outcome was seven deaths, two prolonged ICU hospitalisations and only one hospital discharge (the patient was referred to other hospital and survived).

4 | DISCUSSION

Fungal infections associated to COVID-19 have been reported since the pandemic originated, especially in ICU stay patients[1-7] and similarly to secondary infections associated to *Influenza* viruses. Most of the risk factors previously mentioned for IPA are common in both virus.[6] In our series, four out of ten patients had an immunosuppression due to haematological malignancies and/or after receiving immunosuppressive therapy. According to EORTC/MSG criteria,[9] there was only one case classified as probable IPA (imaging procedure was done by CT) and according to *Asp*ICU algorithm[10] there were seven cases classified as putative IPA and the two remaining cases were not able to be classified by neither algorithm because of lack of ICU stay (criterion of *Asp*ICU algorithm) CT

TABLE 1 Clinical and demographic characteristics of ten patients with COVID-19 and isolation of *Aspergillus* spp. in respiratory samples

Case	Sex/ Age (years)	Underlying disease/ Risk factors	Hospital stay	SARS-COV-2 PCR (+)[a]	Isolate/ Susceptibility test. MIC (μg/mL)[a]	GM[b]
1	M/72	Myelodysplastic syndrome. HIV. COPD	Non-ICU stay (43 days)	Nasopharyngeal swab (day + 35)	*A fumigatus* in BAS (day + 18). VOR: 0.06, POS: 0.12, ISA: 0.03, CAS: 0.03, AMB: 0.5.	Serum (1.97) BAL (3.87) Serum (0.12) Serum (0.08)
2	M/67	DM 2. COPD. Obesity	ICU stay (10 days). MV	BAS (day + 5)	*A fumigatus* in BAS (day + 7)	No
3	M/70	DM2. Obesity	ICU stay (15 days). MV	Nasopharyngeal swab (day 0)	*A fumigatus* in BAS (day + 10). VOR: 0.03, POS: 0.12, ISA: 0.06, CAS: 0.25, AMB: 0.12.	No
4	M/51	Ankylosing spondylitis HLA B27+ (golimumab). DM2	ICU stay (42 days). MV	Nasopharyngeal swab (day 0)	*A fumigatus* in 2 BAS (days + 7, +18). VOR: 0.12, POS: 0.06, ISA: 0.06, CAS: 0.12, AMB: 0.12.	No
5	M/56	Acquired haemophilia A (prednisone)	ICU stay (>100 days). MV	Nasopharyngeal swab (day −1)	*A fumigatus* in 5 BAS (days + 15, +23, +30, +40, +51). VOR: 0.12, POS: 0.25, ISA: 0.12, CAS: 0.25, AMB: 0.12.	No
6	F/69	DM2	ICU stay (20 days). MV	Nasopharyngeal swab (day −2)	*A nidulans* in BAS (day + 7). VOR: 0.25, POS: 0.12, ISA: 0.03, CAS: 0.12, AMB: 2.	No
7	F/76	Hypothyroidism	ICU stay (34 days). MV	Nasopharyngeal swab (day −3)	*A fumigatus* in BAS (day + 25). VOR: 0.094, POS: 0.25, ISA: 0.012, CAS: 0.12, AMB: 0.19.	No
8	M/73	DM2	ICU stay (>100 days). MV	Nasopharyngeal swab (day + 2)	*A fumigatus* in BAS (day + 48). VOR: 0.12, POS: 0.12, ISA: 0.12, CAS: 0.25, AMB: 0.25.	Serum (0.22)
9	M/71	Chronic lymphocytic leukaemia. Chronic ischaemic heart disease. COPD	Non-ICU stay (17 days)	Nasopharyngeal swab (day −1)	*A fumigatus* in sputum (day + 11)	No
10	M/66	COPD	Non-ICU stay (18 days)	Nasopharyngeal swab (day + 12)	*A fumigatus* in BAL (day + 9)	BAL (2.16) BAL (1.11)

Abbreviations: AMB, Amphotericin B; ANI, Anidulafungin; BAL, Bronchoalveolar lavage; BAS, Bronchial aspirate; CAS, Caspofungin; COPD, Chronic Obstructive Pulmonary Disease; CT, Chest computerised tomography; DM2, Type 2 Diabetes Mellitus; GM, Galactomannan; HCQ, Hydroxychloroquine; ICU, Intensive care unit; INH, Inhaled; ISA, Isavuconazole; IV, Intravenous; L/R, Lopinavir/Ritonavir; MIC, Minimum inhibitory concentration; MICA, Micafungin; MV, Mechanical ventilation; N/C, Not classifiable; PO, Oral; POS, Posaconazole; RX, X-Ray or radiograph; TCZ, Tocilizumab; VOR, Voriconazole.

[a]Positive SARS-CoV-2 PCR and isolates of *Aspergillus* spp. in respiratory samples regarding hospital stay day (admission).

[b]Galactomannan values ≥ 0.5 were considered positive in serum and BAL.

[c]According to: Donnelly JP, Chen SC, Kauffman CA et al Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis*. 2019 Dec 5:ciz1008.

[d]According to: Blot SI, Taccone FS, Van den Abeele et al A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med* 2012;186:56-64.

[e]According to: Koehler P, Cornely OA, Böttiger BW et al COVID-19 associated pulmonary aspergillosis. *Mycoses* 2020;63:528-534.

scans (criterion of EORTC/MSG). The classification of IPA is very complex and non-specific for SARS-CoV-2 infection. Furthermore, ground-glass opacities and other patterns are unspecific and common to COVID-19 pneumonia and IPA. Galactomannan test in serum is of little value in non-neutropenic patients.[10] In our series, only one case (neutropenic patient) had positive values in serum and BAL. In the other case, the patient (non-neutropenic) had positive values in two BAL samples after positive BAL culture. Due to

Imaging procedures	Corticoids treatment	Anti-COVID-19 treatment	Antifungal treatment (iv)	EORTC[c]	*Asp*ICU[d,e]	Outcome
CT: Interstitial infiltrates. Ground-glass opacities	iv, po, inh	HCQ	VOR 300 mg/12 h (32 days) CAS 50 mg/24 h (10 days) (Combined treatment)	Probable	N/C	Died (day + 43)
RX: Interstitial infiltrates. Basal consolidation. Pleural effusion	iv	HCQ	No	N/C	Putative	Died (day + 10)
RX: Bibasal infiltrates	iv	HCQ L/R	AMB 240 mg/24 h (2 days)	N/C	Putative	Died (day + 15)
RX: Bilateral interstitial opacities	iv	HCQ	AMB 300 mg/24 h (2 days) ISA 200 mg/24 h (18 days) AMB 250 mg/24 h (3 days) (Sequential treatment)	N/C	Putative	Died (day + 42)
RX: Ground-glass opacities.	iv, po	HCQ TCZ L/R	MICA 100 mg/24 h (15 days) VOR 300 mg/12 h (3 days) ISA 200 mg/24 h (15 days) AMB 250 mg/24 h (3 days) (Sequential treatment)	N/C	Putative	Still inpatient
RX: Ground-glass opacities	iv, inh	HCQ TCZ	AMB 200 mg/24 h (7 days) VOR 250 mg/12 h (1 day) (Sequential treatment)	N/C	Putative	Died (day + 20)
RX: Ground-glass opacities	iv, po	HCQ	No	N/C	Putative	Died (day + 34)
RX: Interstitial infiltrates	iv	HCQ TCZ	ANI 100 mg/24 h (2 days) AMB 250 mg/24 h (26 days) AMB 300 mg/24 h (7 days) (Sequential treatment)	N/C	Putative	Still inpatient
RX: Bilateral diffuse opacities	po, inh	HCQ L/R	VOR 200 mg/12 h (4 days)	N/C	N/C	Died (day + 17)
RX: Interstitial infiltrates. Ground-glass opacities	iv, inh	HCQ TCZ L/R	VOR (posology not available, 6 weeks)	N/C	N/C	Alive (Referred to other hospital) (day + 18)

the risk of false negative results in immunocompetent patients, in patients without risk factors or culture-positive BAL, additional galactomannan test on BAL can be recommended to further strengthen the diagnosis.[10]

Due to bad clinical state, eight patients received antifungals due to *Aspergillus* spp. isolation. Two remaining patients did not receive any therapy because they died few days after known the culture result. However, five patients finally died despite the treatment and

two patients still inpatient in ICU for more than 100 days due to COVID-19 and several nosocomial complications. More studies are needed in order to elucidate the real clinical significance of isolating *Aspergillus* spp. or other fungi in COVID-19 cases. Nevertheless, it is a clinical entity which has high mortality and it should be recommended to perform fungal cultures with successive galactomannan tests in deep respiratory samples and/or serum in order to discard the presence of *Aspergillus* spp. in COVID-19 patients with risk factors to develop CAPA.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTION

Iker Falces-Romero: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Methodology (lead); Writing-original draft (lead); Writing-review & editing (lead). **Mario Ruiz-Bastián:** Conceptualization (equal); Data curation (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Beatriz Díaz-Pollán:** Data curation (equal); Writing-review & editing (equal). **Emilio Maseda:** Data curation (equal); Writing-review & editing (equal). **Julio García-Rodriguez:** Conceptualization (equal); Data curation (equal); Supervision (equal); Writing-review & editing (equal).

ORCID

Iker Falces-Romero https://orcid.org/0000-0001-5888-7706

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