Medical Mycology, 2020, 0, 1–5
doi:10.1093/mmy/myaa078
Advance Access Publication Date: 0 2020
Brief Report

Brief Report

Pulmonary aspergillosis in critically ill patients with Coronavirus Disease 2019 (COVID-19)

Damien Dupont[1,2,†], Jean Menotti[1,3,†], Jean Turc[4], Charline Miossec[1], Florent Wallet[5,6], Jean-Christophe Richard[7], Laurent Argaud[8], Sylvie Paulus[9], Martine Wallon[1,2], Florence Ader[10] and Florence Persat[1,3,*]

[1]Institut des Agents Infectieux, Parasitologie et Mycologie Médicale, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, F-69004, France, [2]Physiologie intégrée du système d'éveil, Centre de Recherche en Neurosciences de Lyon, INSERM U1028-CNRS UMR 5292, Faculté de Médecine, Université Claude Bernard Lyon 1, Bron, F-69500, France, [3]EA7426 PI3 - Inflammation and Immunity of the Respiratory Epithelium, Université Claude Bernard Lyon 1, Pierre-Bénite, F-69495, France, [4]Service de Réanimation Chirurgicale et Anesthésiologie, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, F-69437, France, [5]Service de Réanimation Médicale, Hôpital Lyon Sud, Hospices Civils de Lyon, Pierre-Bénite, F-69495, France, [6]Laboratoire des Pathogènes Emergents, Fondation Mérieux, Centre International de Recherche en Infectiologie, Institut National de la Santé et de la Recherche Médicale U1111, Lyon, F-69007, France, [7]Service de Médecine Intensive Réanimation et Surveillance Continue Médicale, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, F-69004, France, [8]Service de Médecine Intensive-Réanimation, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, F-69437, France, [9]Service d'Anesthésie-Réanimation du Pôle Est, Hôpital Louis Pradel, Hospices Civils de Lyon, Bron, F-69500, France and [10]Service de Maladies Infectieuses et Tropicales, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, F-69004, France

*To whom correspondence should be addressed. Florence Persat, PhD, Institut des Agents Infectieux, Parasitologie et Mycologie Médicale, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, F-69004, France. Tel: +33472001516; E-mail: florence.persat@chu-lyon.fr

†These authors contributed equally to this work.

Received 28 May 2020; Revised 4 August 2020; Accepted 11 August 2020; Editorial Decision 10 August 2020

Abstract

Occurrence of putative invasive pulmonary aspergillosis was screened in 153 consecutive adult intensive care unit (ICU) patients with respiratory samples addressed for mycological diagnosis during a 6-week period at the emergence of coronavirus disease 2019 (COVID-19) pandemic. Positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR) was observed for 106 patients (69.3%). Nineteen of them (17.9%) with positive *Aspergillus* results were considered as having putative invasive pulmonary aspergillosis. These observations underline the risk of pulmonary aspergillosis in COVID-19 patients, even in patients not previously known to be immunosuppressed, advocating active search for *Aspergillus* infection and prompt antifungal treatment. Standardized surveillance protocols and updated definitions for ICU putative invasive pulmonary aspergillosis are needed.

Lay Abstract

Adult ICU patients with respiratory samples addressed for mycological diagnosis were screened during the emergence of COVID-19 pandemic. Positive SARS-CoV-2 PCR was observed for 106 patients, nineteen of them (17.9%) having aspergillosis. This underlines the risk of aspergillosis in COVID-19 patients.

Key words: *Aspergillus*, pulmonary aspergillosis, COVID-19, intensive care unit, acute respiratory distress syndrome, COVID-19 associated pulmonary aspergillosis (CAPA).

During the coronavirus disease 2019 (COVID-19) pandemic, a risk of secondary pulmonary infections, including aspergillosis, was mentioned in patients suffering from acute respiratory distress syndrome (ARDS).[1] This was congruent with the well-established risk of invasive pulmonary aspergillosis (IPA) in patients with severe influenza.[2,3] A prospective study was conducted in Lyon teaching Hospitals, in order to estimate the occurrence of IPA and describe patient characteristics. Patients were included from March 1 to April 11, during the period of active circulation of the virus in this area in France, in adult patients admitted to five intensive care units (ICU) for whom at least one sample was sent to the mycology laboratory. Patients with only sputum samples were excluded.

Lower respiratory tract samples (LRT) including Broncho-Alveolar Lavage (BAL), Endo-Tracheal Aspiration (ETA), and Bronchial Aspiration (BA) received at the Mycology laboratory from Hospices Civils de Lyon (HCL) intensive care unit (ICU) adult patients during this 6-week period were processed according to standard mycological procedures. Calcofluor direct examination (Becton-Dickinson, Franklin Lakes, NJ, USA) and cultures on Can2 and Sabouraud mycological media (bioMérieux, Marcy l'Etoile, France) were performed. Identification was obtained by MALDI-TOF (VITEK® MS, bioMérieux). Additionally, serum and/or BAL galactomannan (GM) *Aspergillus* antigen was performed by ELISA (Platelia™ *Aspergillus* antigen, BioRad, Marnes, France), with a cut-off index of 1 as recently recommended.[2,4]

We collected results of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) PCR in respiratory samples (nasopharyngeal, tracheal aspirate and/or BAL samples) from all included patients, independently. Clinical characteristics (Table 1) were retrieved from the medical record database. Ethical clearance was granted as part of the HCL Global COVID Research Initiative: patients were informed that their clinical and biological data could be used for research purposes; no patient opposed. Putative IPA definition cases followed the *Asp*ICU criteria (*Aspergillus* positive culture on respiratory samples from at risk patient with abnormal pulmonary imagery),[5] with inclusion of BAL GM results.[2,4] COVID-19 diagnosis was considered as a risk factor, as previously reported by Koehler et al.[6]

Among the 153 patients screened for fungal infection, i) 106 had a positive SARS-CoV-2 PCR result during the study period (69.3%), ii) Twenty-three patients had at least one microbiological finding evocative of putative IPA: a positive *Aspergillus* culture ($n = 19$), a positive GM assay in BAL ($n = 6$), or both ($n = 2$). Blood GM test was performed for 12 patients among these 23 patients. Only one was positive (index: 2.41), associated with a positive LRT culture. Sex ratio was 3.6 (18 males/5 females). Median age was 69 [62, 73] years. Positive samples for *Aspergillus* detection were sent 6 days [1, 9] after the start of ventilation. Mycological positive results were given to clinicians from 12 [7.25, 15] days after ICU admission.

Among the 23 patients with microbiological findings consistent with putative IPA, 19 patients had a positive SARS-CoV-2 PCR, in the context of classical clinical symptoms (fever, cough, dyspnea, myalgia or headaches). Four patients had putative IPA with repeatedly negative SARS-CoV-2 PCR. These four patients died during their ICU stay. One had risk factor for aspergillosis (COPD). All had concurrent fungal (*Pneumocystis jirovecii* pneumoniae ($n = 2$), mucormycosis ($n = 1$), and candidemia ($n = 1$)), bacterial or viral infections.

Characteristics of the 19 patients are summarized in Table 1. Fifteen presented lymphocytopenia at admission. The most frequent underlying diseases was arterial hypertension ($n = 7$; 36.8%) and type-2 diabetes mellitus ($n = 7$; 36.8%). Three had recent history of malignancy (follicular lymphoma, $n = 1$; colon cancer, $n = 1$; urothelial carcinoma, $n = 1$), not considered as risk factors for IPA by EORTC/MSG.[4] Seven patients received steroids, six for hemodynamic or renal failures and one for COVID-19 treatment (methylprednisolone 40 mg bid). No patient received steroid at dose and length of treatment considered as as risk factor for IPA.[4] Three patients (no 6, 15, 16) received hydroxychloroquine for 10, 5, and 2 days, respectively. Respiratory risk factors were reported for seven patients, three of them having two risk factors: COPD ($n = 4$; 21.1%), asthma ($n = 4$; 21.1%), or a history of tuberculosis ($n = 2$; 10.5%). The remaining 12 patients had no identified risk factors for *Aspergillus* infection. All patients suffered from either mild ($n = 2$), moderate ($n = 13$), or severe ($n = 4$) ARDS at the time of sampling (Berlin definition),[7] all requiring invasive mechanical ventilation and prone positioning. Radiological features revealed ground glass opacities typical of COVID-19 lesions, with condensation ($n = 13$; 68.4%) and pulmonary embolism ($n = 5$; 26.3%). Nine patients presented other computed tomography (CT) scan features: emphysema ($n = 5$; 26.3%), cavitation ($n = 2$; 10.5%), nodule ($n = 2$; 10.5%), bronchiectasis ($n = 2$; 10.5%) and secondary infection signs ($n = 5$; 26.3%).

LRT cultures yielded *Aspergillus fumigatus* in 14 of the SARS-CoV-2 patients and other *Aspergillus* species for two patients. According to the ICU-IPA definition, our patients may be considered with putative IPA, if the viral infection is considered as a risk factor. Nine patients were given voriconazole for at least 48 hours. Although not significant, there was a trend towards a lower mortality rate at 42 days after mycological

Table 1. Characteristics of Coronavirus disease 2019 (COVID-19) ICU adult patients with positive microbiological criteria for putative invasive pulmonary aspergillosis.

Case nr.	Age	Sex	Underlying diseases	O_2-therapy	ARDS[a] at the time of the respiratory sampling	Lymphocyte count at ICU admission (G/L) (N1-4 G/L)	CT findings		*Aspergillus* spp. positive LRT culture			GM in BAL	Delay in days			Antifungal treatment (days)	42-day outcome/ ICU entry
							COVID-19 lesions[b]	Other lesions	LRT type	Branching hyphae on DE	*Aspergillus* species		ICU admission/PA Dg	COVID-19/ PA Dg	MV start/PA Dg		
1	86	M	Cardiopathy	SV	Mild	**0.72**[d]	Moderate	NA	BA	No	*A. fumigatus*	ND	4	10	NA	No	Alive
2	79	F	Colon cancer, AHT, COPD	MV	Moderate	2.17	Severe	NA	BAL	No	*A. fumigatus*	ND	7[e]	7[e]	7[e]	No	Death at day 3
3	78	M	COPD, AHT, type 2 diabetes mellitus, urothelial carcinoma	MV	Moderate	**0.65**	Moderate	Emphysema	BA	No	*A. fumigatus*	ND	9	8	7	No	Death at day 13
4	77	M	Asthma, COPD, ABPA	MV	Severe	**0.45**	Severe	Emphysema	BA	No	*A. fumigatus*	ND	10	7	7	No	Death at day 10
5	76	M	No	MV	Moderate	**0.22**	Severe	Emphysema, secondary infection	BA	No	*A. fumigatus*	BAL at day-10 /BA Index = 0.076	14	3	10	Vorico 42 days (-14 days with caspo)	Alive
6[c]	73	F	Hypothyroidia	MV	Moderate	2.67	Presence	Pulmonary embolism	BAL	No	*A. fumigatus*	Index = 0.805	23	23	21	Vorico 42 days	Alive
7	72	M	Type 2 diabetes mellitus, AHT, carcinoma, renal insufficiency	MV	Moderate	**0.21**	Severe	Nodule, Secondary infection, bronchiectasis	No	NA	NA	Index > 3.483	15	14	11	Vorico 14 days	Alive
8	72	M	Schizophreny, glaucoma	MV	Moderate	**0.49**	Severe	Pulmonary embolism	ETA	No	No	BAL at day-12/ETA Index = 1.913	15	15	11	No	Alive
9	72	M	Type 2 diabetes mellitus, AHT	MV	Mild	**0.66**	Presence	NA	BAL	Yes	*A. fumigatus*	ND	19	22	15	Vorico 12 days	Alive
10	70	M	Asthma, type 2 diabetes mellitus, tuberculosis in 2012	MV	Moderate	**0.70**	Presence	Emphysema, nodule, cavitation, secondary infection	BAL	Yes	*A. fumigatus*	Index > 3.045	12	12	1	Vorico 12 days (overdosing)	Death at day 25
11	69	M	AHT	MV	Moderate	**0.51**	Critical	Pulmonary embolism	BAL	No	*A. fumigatus*	ND	4	3	3	No	Alive
12	68	F	COPD, asthmatic bronchitis	MV	Moderate	**0.68**	Critical	Pulmonary embolism, cavitation	ETA	Yes	*A. fumigatus,*	ND	10	14	7	Vorico At least 45 days (underdosing)	Alive
13	67	M	Type 2 diabetes mellitus, AHT, cardiopathy	MV	Moderate	**0.87**	Severe	Pulmonary embolism	BAL	ND	ND	Index = 1.232	10	11	10	No	Alive
14	63	M	Follicular lymphoma in remission	MV	Severe	**0.60**	Critical	Secondary infection	BAL	No	*A. fumigatus*	Index = 0.923	19	19	13	No	Death at day 20
15	62	M	Tuberculosis in the infancy	MV	Severe	**0.31**	Severe	Emphysema	ETA	No	*A. calidoustus*	neg	13	12	13	No	Death at day 36
16	62	M	Renal insufficiency	MV	Moderate	**0.46**	Severe	Secondary infection	BA	NR	*A. fumigatus*	ND	9	9	7	Vorico 42 days	Alive
17	58	F	Type 2 diabetes mellitus, AHT, HIV	MV	Moderate	1.97	Severe	Secondary infection	BA	No	*A. niger*	ND	2	2	2	No	Alive

Table 1. *Continued*

Case nr.	Age	Sex	Underlying diseases	O_2-therapy	ARDS[a] at the time of the respiratory sampling	Lymphocyte count at ICU admission (G/L) (N1-4 G/L)	CT findings		*Aspergillus* spp. positive LRT culture			GM in BAL	Delay in days			Antifungal treatment (days)	42-day outcome/ ICU entry
							COVID-19 lesions[b]	Other lesions	LRT type	Branching hyphae on DE	*Aspergillus* species		ICU admission/PA Dg	COVID-19/ PA Dg	MV start/PA Dg		
18	51	M	Type 2 diabetes mellitus, obesity, asthma	MV, vvECMO	Severe	2.34	Severe	NA	BAL	ND	*A. fumigatus*	ND	10	11	10	Vorico 14 days (overdosing)	Death at day 29
19	44	M	Chronic B hepatitis	MV	Moderate	0.71	Severe	NA	ETA	No	*A. fumigatus*	BAL at day-7/ ETA index = 3.227	4	5	3	Vorico 49 days	Alive

ABPA, allergic bronchopulmonary aspergillosis; AHT, arterial hypertension; ARDS, acute respiratory distress syndrome; BA, bronchial aspiration; BAL, bronchoalveolar lavage; caspo, caspofungin; COPD, chronic obstructive pulmonary disease; CT, computed tomography; DE, direct examination; Dg, diagnosis; ETA, endotracheal aspiration; GM, galactomannan antigen; HIV, human immunodeficiency virus; LRT, low respiratory tract sample; MV, mechanical ventilation; NA, non applicable; ND, not done; PA, pulmonary aspergillosis; SV, spontaneous ventilation; Vorico, voriconazole; vvECMO, veno-venous extracorporeal membrane oxygenation.

[a]Cf Berlin definition.

[b]COVID-19 lesions: ground glass opacities, crazy paving, condensations (subpleural localization). Lesion extensions: moderate (<30%), severe (30–75%), critical (>75%).

[c]In bold, patients with hydroxychloroquine treatment (see the text).

[d]In bold, patients with lymphocytopenia.

[e]Post-mortem diagnosis.

diagnosis in antifungal-treated patients (3 deaths/9; 33.3%), compared to untreated patients (5 deaths/10; 50%).

This study reports a series of 19 putative IPA among 106 ICU patients with COVID-19 (17.9%) and provides three important findings. First, it highlights that severe SARS-CoV-2 infection should be considered as a risk factor for IPA, as recently reported.[6,8,9] Second, this higher risk for IPA occurs even in patients not previously known to be immunosuppressed, as reported with flu patients. Indeed, in our series, only three patients out of 19 presented with a previous history of cancer. Interestingly, respiratory risk factors classically associated with the presence of *Aspergillus* in the airway, such as COPD, asthma or previous history of tuberculosis, were reported for seven patients. Third, these observations highlight the need to monitor specifically COVID-19 ICU patients for IPA, since the association of these two pathogens is emerging. Further data are required to assess to what extend IPA worsens patients prognosis.[10]

BAL, if possible, should be used for standard mycological culture and GM detection on the rationale that the deeper the sample, the higher the probability of IPA. Koehler et al.[6] recommended GM detection in ETA as well, however ETA is not validated by the manufacturer. Also, GM detection is more sensitive in BAL than in blood in non-neutropenic patients who are more likely to have a non angioinvasive IPA, as opposed to neutropenic patients.[11,12] Monitoring blood antibody levels might still be of interest in patients who are mildly immunocompromised and/or have underlying respiratory diseases. *Aspergillus* PCR assay on LRT samples may also be proposed to increase diagnostic sensitivity.[10]

Since the EORTC-MSG consensus criteria for IPA in immunocompromised patients are inappropriate for ICU patients,[4,13] a specific definition is needed for these patients.[14] COVID-19 and other viral infections associated with ARDS might be considered as a host risk factor in ICU by analogy with flu infection.[2] Noteworthy, more precise other criteria, particularly mycological criteria (number or nature of the respiratory samples) are needed since the putative IPA classification used in this study might have led to an excess of IPA, compared to chronic pulmonary aspergillosis and *Aspergillus* colonization of the respiratory tract.[15] An updated definition and standardized diagnostic procedures would then benefit patients and serve as a basis for optimizing clinical management and assessing treatment efficacy.

Acknowledgment

The authors thank Michelle Grange for English editing of the manuscript.

Declaration of interest

J.M. has received a research grant outside the submitted work from MSD and aid for attending symposia from Gilead, MSD, Pfizer and Roche Diagnostics; F.P. has received aid for attending symposia from Gilead and Pfizer. All other authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Yang X, Yu Y, Xu J et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020; 8: 475–481.
2. Schauwvlieghe AFAD, Rijnders BJA, Philips N et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med*. 2018; 6: 782–792.
3. Vanderbeke L, Spriet I, Breynaert C et al. Invasive pulmonary aspergillosis complicating severe influenza: epidemiology, diagnosis and treatment. *Curr Opin Infect Dis*. 2018; 31: 471–480.
4. 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; doi: 10.1093/cid/ciz1008.
5. Blot SI, Taccone FS, Van Den Abeele AM et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med*. 2012; 186: 56–64.
6. Koehler P, Cornely OA, Bottiger BW et al. COVID-19 associated pulmonary aspergillosis. *Mycoses*. 2020, doi: 10.1111/myc.13096.
7. Ranieri VM, Rubenfeld GD, Thompson BT et al. Acute respiratory distress syndrome: The Berlin definition. *JAMA*. 2012; 307: 2526–2533.
8. Alanio A, Dellière S, Fodil S et al. High prevalence of putative invasive pulmonary aspergillosis in critically ill COVID-19 patients. doi: https://doi.org/10.1101/2020.04.21.20064915.
9. van Arkel ALE, Rijpstra TA, Belderbos HNA, van Wijngaarden P, Verweij PE BR. COVID-19 associated pulmonary aspergillosis. *Am J Respir Crit Care Med*. 2020; 202: 132–135.
10. Gangneux JP, Bougnoux ME, Dannaoui E et al. Invasive fungal diseases during COVID-19: we should be prepared. *J Mycol Med*. 2020; 30: 100971.
11. Zhou W, Li H, Zhang Y et al. Diagnostic value of galactomannan antigen test in serum and bronchoalveolar lavage fluid samples from patients with nonneutropenic invasive pulmonary aspergillosis. *J Clin Microbiol*. 2017; 55: 2153–2161.
12. Bergeron A, Porcher R, Sulahian A et al. The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. *Blood*. 2012; 119: 1831–1837.
13. Vandewoude KH, Vogelaers D, Blot SI. Aspergillosis in the ICU: the new 21st century problem? *Med Mycol*. 2006; 44: 71–76.
14. Bassetti M, Scudeller L, Giacobbe DR et al. Developing definitions for invasive fungal diseases in critically ill adult patients in intensive care units. Protocol of the FUNgal infections Definitions in ICU patients (FUNDICU) project. *Mycoses*. 2019; 62: 310–319.
15. Verweij PE, Gangneux JP, Bassetti M et al. Diagnosing COVID-19-associated pulmonary aspergillosis. *Lancet Microbe*. 2020; 1: e53–e55.