Prevalence of putative invasive pulmonary aspergillosis in critically ill patients with COVID-19

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High prevalence of putative invasive pulmonary aspergillosis in critically ill COVID-19 patients

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About 5% of coronavirus disease 2019 (COVID-19) patients require intensive care unit (ICU) management.[1] These patients are at high risk of developing secondary infections including invasive pulmonary aspergillosis (IPA).[2] First reported with H1N1 influenza, IPA represents a frequent (20-30%) and early-onset complication (median, 3 days post-ICU admission) in critically ill influenza patients leading to enhanced illness severity and mortality (40-60%).[3,4] Most cases have been observed in non-immunocompromised patients, questioning the applicability of the EORTC-MSG consensus criteria used to define aspergillosis in immunocompromised patients.[5] Therefore, an algorithm to discriminate *Aspergillus* colonization from putative IPA was developed in ICU patients based on mycological criteria combining culture from respiratory specimens and galactomannan detection in the bronchoalveolar lavage (BAL) and serum.[4,6]

Paralleling what has been reported in influenza patients, we designed this prospective observational study to investigate IPA risk in critically ill COVID-19 patients. The patients were classified using the EORTC-MSG criteria[5] (if immunocompromised) and the influenza-associated IPA criteria[4] combined with serum beta-D-glucan and quantitative real-time PCR (qPCR)[7] performed in the serum and/or pulmonary specimens (if non-immunocompromised). Putative IPA was considered if (i) *Aspergillus spp.* was identified in BAL culture; or (ii) two of the following conditions were met, i.e. presence of *Aspergillus spp.* in bronchial aspiration (BA) culture; positive *Aspergillus fumigatus* qPCR in BAL, BA or serum;[8] galactomannan index >0.8 in BAL;[5] galactomannan index >0.5 in serum; and beta-D-glucan >80 pg/mL in serum. Noteworthy, direct examination of respiratory specimens was not performed to avoid operator contamination.

Twenty-seven successive mechanically ventilated COVID-19 patients (18M/9F, median age, 63 years [range, 43-79]) were included. Specimens (20 BALs/7 BAs) were obtained on day3 [1-6] post-intubation. Probable and putative IPAs were diagnosed in one (4%) and eight patients (30%), respectively (Table 1). Putative IPA diagnosis relied on *Aspergillus* identification in BAL culture (N=2) and validation of ≥2 mycological criteria (N=6).

History of hypertension was significantly more frequently reported in the IPA patients (7/9 *versus* 6/18, p=0.04). No other significant differences were observed in terms of age, EORTC-MSG risk factors for IPA, time between onset of symptoms and intubation and times between onset of symptoms or intubation and *Aspergillus* respiratory specimen collection, severity, laboratory data, non-COVID CT-scan images, and steroid administration. Antifungal therapy was initiated in 2/9 IPA patients. Mortality rate did not differ between IPA and non-IPA patients (4/9 *versus* 7/18, p=0.9).

Here we found putative IPA in almost one third of our mechanically ventilated COVID-19 patients, at a similar rate to that which has been observed in influenza patients.[3,4] One patient with myeloma presented a probable IPA based on the EORTC criteria[5] with one nodule on chest X-Rays in addition to the typical COVID-19-attributed lesions.

Since CT-scan and BAL are extremely difficult to perform in life-threatening COVID-19 patients, mycological data collection is essential to allow IPA diagnosis. We strongly support adding beta-D-glucan and qPCR in serum and respiratory specimens to the currently accepted mycological work-up (i.e. BAL culture and galactomannan testing)[4,6] until the most sensitive and specific biomarkers are determined in this setting. Serum galactomannan was negative in 8/9 patients, suggesting lesser degree of aspergillus invasiveness or early IPA diagnosis since respiratory specimens were obtained shortly after intubation. Interestingly, galactomannan was negative in our two patients receiving hydroxychloroquine thought to have a negative effect on this measurement.[9]

We are convinced that IPA is more likely if at least two mycological criteria are met, such as in six of our patients. One patient presented positive serum beta-D-glucan (>80pg/mL) and galactomannan index (>0.5) without *Aspergillus* detection in the BAL. Three others had *Aspergillus fumigatu*s culture without positive qPCR detection or galactomannan antigen in the BAL or BA. Not considering positive culture alone as a diagnostic criterion in accordance with what is currently accepted,[4,6] would have resulted in underestimating the frequency of putative IPA (22% rather than 30% in our study).

Despite similar IPA rates in critically ill COVID-19 and influenza patients, the contribution of *Aspergillus* to the patient presentation in each illnesses may be different. In our IPA patients, death including in the two patients who received anti-*Aspergillus* treatment was not related to aspergillosis but to bacterial septic shock complicated by multiorgan failure.

Consistent with others,[10] our findings support systematic screening for Aspergillus infection markers in critically ill COVID-19 patients. Although oseltamivir-induced inhibition of the host neuraminidase activity has been suggested as possible molecular mechanism leading to decreased anti-*Aspergillus* protective immunity in influenza patients, the exact reasons for increased vulnerability of the COVID-19 patient to *Aspergillus* remain to be determined as well as *Aspergillus* contribution to COVID-19-reated lung inflammation.

Conflict of interest

The authors declare no conflict of interest

Ethics

This study was part of the COVID-ICU registry and the French COVID-19 cohort registry conducted by the REACTing consortium and directed by INSERM and ISARIC. Our institutional ethics committee approved the study (N°, IDRCB, 2020-A00256-33; CPP, 11-20 20.02.04.68737). When possible, signed informed consent was obtained from the patients or the next of kin.

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Patients (gender, age)	Risk factors of severe COVID-19	EORTC risk factors	APACHE II score	Thoracic CT-scan/X-Ray*	Anti-COVID-19 therapies	Steroids to treat pneumonia†	Renal replacement therapy	Vasopressor	Pulmonary specimen§	IPA diagnosis						Number of mycological criteria	Antifungal therapy	Outcome
										BAL Culture‡	BAL/BA qPCR¥	BAL galactomannan index	Serum qPCR¥	beta-D-glucan (pg/mL)	Serum galactomannan index			
Putative IPA patients																		
Pt1 (M, 53)	HT, obesity, IHD	None	26	Typical COVID-19	LPV/RTV	Yes	Yes	Yes	BAL	-	-	0.89	-	523	0.13	2	None	Alive
Pt2 (F, 59)	HT, diabetes, obesity	None	16	Typical COVID-19	LPV/RTV, AZI	No	No	Yes	BAL	+	-	0.03	-	ND	0.04	1	None	Alive
Pt3 (F, 69)	HT, obesity	None	11	Typical COVID-19	LPV/RTV	Yes	No	Yes	TA	+	23.9	ND	-	7,8	0.03	2	None	Alive
Pt4 (F, 63)	HT, diabetes, IHD	None	20	Typical COVID-19	LPV/RTV	Yes	Yes	Yes	BAL	-	-	0.15	ND	105	0.51	2	None	Death (day0)
Pt5 (M, 43)	Asthma	Steroids	8	Typical COVID-19	AZI	No	No	No	BAL	+	-	0.12	-	7	0.04	1	None	Alive
Pt6 (M, 79)	HT	None	16	Typical COVID-19, segmental	LPV/RTV, HCQ, AZI	Yes	No	Yes	BAL	+	34.5	0.05	-	23	0.02	2	None	Alive

				lung atelectasis														
Pt7 (M, 77)	HT, asthma	None	25	Typical COVID-19, emphysema	LPV/RTV, HCQ, AZI	Yes	Yes	Yes	BAL	+	29.0	3.91	-	135	0.37	3	VRC	Death (day18)
Pt8 (F, 75 yr)	HT, diabetes	None	21	Typical COVID-19	LPV/RTV, AZI	Yes	No	Yes	BAL	+	31.7	0.36	-	450	0.37	3	CSP	Death (day11)
Probable IPA patient																		
Pt9 (M, 47)	None	Myeloma, steroids	10	Typical COVID-19 + one peripheral nodule	No	No	No	Yes	TA	+	-	ND	-	14	0.09	1	None	Death (day3)

HT, hypertension; IHD, ischemic heart disease; LPV/RTV, lopnavir/ritonavir combination; AZI, azithromycin; HCQ, hydroxychloroquine; IPA, invasive pulmonary aspergillosis; VRC, voriconazole; CSP, caspofungin; EORTC, European Organization for Research and Treatment of Cancer; [†]Steroid regimen, dexamethasone intravenous dose of 20 mg once daily from day 1 to day 5, followed by 10 mg once daily from day 6 to day 10; [£]Culture (-, negative; +, positive with *Aspergillus fumigatus* identification); [¥]qPCR, quantitative real-time PCR (-, negative; if positive, number of quantification cycles) Thoracic CT-scan/X-Ray[*]: Thoracic Ct scan was performed in Pt3, Pt4, Pt5, five days (median) before respiratory specimens. Pulmonary specimen[$]: BAL, Bronchoalveolar lavage; BA, Bronchial Aspiration; No endotracheal/endobronchial lesion was observed.