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

About 5% of patients with coronavirus disease 2019 (COVID-19) 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 patients with influenza, 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 European Organization for Research and Treatment of Cancer Mycoses Study Group (EORTC-MSG) consensus criteria used to define aspergillosis in immunocompromised patients.[5] Therefore, an algorithm to discriminate *Aspergillus* spp colonisation from putative IPA was developed for patients in ICU on the basis of mycological criteria combining culture from respiratory specimens and galactomannan detection in the bronchoalveolar lavage (BAL) and serum.[4,6]

Parallelling what has been reported in influenza patients, we designed this prospective observational study to investigate IPA risk in critically ill patients with COVID-19. The patients were classified by means of the EORTC-MSG criteria[5] (if immunocompromised) or the influenza-associated IPA criteria[4] combined with serum β-D-glucan and quantitative real-time PCR (qPCR)[7] done in the serum or pulmonary specimens (if non-immunocompromised). Putative IPA was considered if *Aspergillus* spp were identified in BAL culture; or if two of the following conditions were met (ie, presence of *Aspergillus* spp in bronchial aspiration [BA] culture; positive *Aspergillus fumigatus* qPCR in BAL, BA, or serum;[8] galactomannan

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	Putative invasive pulmonary aspergillosis patients (sex, age)								Probable IPA patient (sex, age)
	Patient 1 (male, 53 years)	Patient 2 (female, 59 years)	Patient3 (female, 69 years)	Patient 4 (female, 63 years)	Patient 5 (male, 43 years)	Patient 6 (male, 79 years)	Patient 7 (male, 77 years)	Patient 8 (female, 75 years)	Patient 9 (male, 47 years)
Risk factors of severe COVID-19	Hypertension, obesity, ischaemic heart disease	Hypertension, diabetes, obesity	Hypertension, obesity	Hypertension, diabetes, ischaemic heart disease	Asthma	Hypertension	Hypertension, asthma	Hypertension, diabetes	None
EORTC risk factors	None	None	None	None	Steroids	None	None	None	Myeloma, steroids
APACHE II score	26	16	11	20	8	16	25	21	10
Thoracic CT-scan/x-ray*	Typical COVID-19	Typical COVID-19	Typical COVID-19	Typical COVID-19	Typical COVID-19	Typical COVID-19, segmental lung atelectasis	Typical COVID-19, emphysema	Typical COVID-19	Typical COVID-19 + one peripheral nodule
Anti-COVID-19 therapies	LPV–RTV	LPV–RTV, AZI	LPV–RTV	LPV–RTV	AZI	LPV–RTV, HCQ, AZI	LPV–RTV, HCQ, AZI	LPV–RTV, AZI	No
Steroids to treat pneumonia†	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No
Renal replacement therapy	Yes	No	No	Yes	No	No	Yes	No	No
Vasopressor	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Pulmonary specimen‡	BAL	BAL	BA	BAL	BAL	BAL	BAL	BAL	BA
Invasive pulmonary aspergillosis diagnosis									
BAL culture§	–	+	+	–	+	+	+	+	+
BAL/BA qPCR¶	–	–	23·9	–	–	34·5	29·0	31·7	–
BAL galactomannan index	0·89	0·03	ND	0·15	0·12	0·05	3·91	0·36	ND
Serum qPCR¶	–	–	–	ND	–	–	–	–	–
β-D-glucan, pg/mL	523	ND	7·8	105	7	23	135	450	14
Serum galactomannan index	0·13	0·04	0·03	0·51	0·04	0·02	0·37	0·37	0·09
Number of mycological criteria	2	1	2	2	1	2	3	3	1
Antifungal therapy	None	None	None	None	None	None	VRC	CSP	None
Outcome	Alive	Alive	Alive	Death (day 0)	Alive	Alive	Death (day 18)	Death (day 11)	Death day 3)

EORTC=European Organization for Research and Treatment of Cancer. APACHE= Acute Physiology and Chronic Health Enquiry. LPV–RTV=lopnavir–ritonavir combination. AZI=azithromycin. HCQ=hydroxychloroquine. BAL=Bronchoalveolar lavage. BA=bronchial aspiration. ND=not done.VRC=voriconazole. CSP=caspofungin. *Thoracic CT scan was done in Pt3, Pt4, Pt5, 5 days (median) before respiratory specimens. †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; ‡No endotracheal or endobronchial lesion was observed. §–=negative; +=positive with *Aspergillus fumigatus* identification. ¶qPCR=quantitative real-time PCR (–, negative; if positive, number of quantification cycles).

Table: **Clinical characteristics of nine critically ill patients with COVID-19 probable (n=1) and putative invasive pulmonary aspergillosis (n=8)**

index >0·8 in BAL;[5] galactomannan index >0·5 in serum; and β-D-glucan >80 pg/mL in serum).

27 successive mechanically ventilated patients with COVID-19 (18 male and nine female, median age 63 years [IQR 56-71]) were included. Specimens (20 BALs and seven BAs) were obtained on day 3 [IQR 1–6] post-intubation. Probable IPAs were diagnosed in one patient (4%) and putative IPAs were diagnosed in eight patients (30%; table). Putative IPA diagnosis relied on *Aspergillus* spp identification in BAL culture (n=2) and validation of 2 or more mycological criteria (n=6).

History of hypertension was reported more frequently in the patients with IPA (seven of nine *vs* six of 18, p=0·046). No other significant differences were observed in terms of age, EORTC-MSG risk factors for IPA, time between onset of symptoms and intubation and time between onset of symptoms or intubation and *Aspergillus* spp respiratory specimen collection, severity, laboratory data, non-COVID CT-scan images, and steroid administration. Antifungal therapy was initiated in two of nine (22%) patients with IPA. Mortality rate did not differ between IPA and non-IPA patients (four of nine [44%] *vs* seven of 18 [39%], p=0·99.

We found putative IPA in almost one-third of our mechanically ventilated patients with COVID-19—a similar prevalence to that observed in patients with influenza.[3,4] One patient with myeloma presented with probable IPA on the basis of EORTC criteria[5] with one nodule on chest x-rays in addition to the typical COVID-19-attributed lesions.

Since CT and BAL are extremely difficult to do in patients with life-threatening COVID-19, mycological data collection is essential to allow IPA diagnosis. We strongly support adding β-D-glucan in serum and qPCR in serum and respiratory specimens to the accepted mycological work-up (ie, BAL culture and galactomannan testing)[4,6] until the most sensitive and specific biomarkers are identified in this setting. Serum galactomannan was negative in eight of nine (89%) patients, suggesting a lesser degree of *Aspergillus* invasiveness or early IPA diagnosis, since respiratory specimens were obtained shortly after intubation. Galactomannan was negative in our two patients receiving hydroxychloroquine, which is thought to have a negative effect on this measurement.[9]

We believe that IPA is more probable if at least two mycological criteria are met. However, three patients had *Aspergillus fumigatus* 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 accepted,[4,6] would have resulted in underestimating the frequency of putative IPA (22% rather than 30% in our study).

Despite similar IPA prevalence in critically ill patients with COVID-19 and influenza, the contribution of *Aspergillus* to the patient presentation in each illness might be different. In our patients with IPA, 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,[10,11] our findings support systematic screening for *Aspergillus* infection markers in critically ill patients with COVID-19. Although oseltamivir-induced inhibition of the host neuraminidase activity has been suggested as a possible molecular mechanism leading to decreased anti-*Aspergillus* protective immunity in patients with influenza, the exact reasons for increased vulnerability of the patients with COVID-19 to *Aspergillus* remain to be identified as well as the contribution of *Aspergillus* to COVID-19-related lung inflammation.

The authors declare no competing interests. This study was part of the COVID-ICU registry and the French COVID-19 cohort registry done by the REACTing consortium. Our institutional ethics committee approved the study (IDRCB, 2020-A00256-33; CPP, 11-20 20.02.04.68737).

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