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] In the ICU, 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 rate (40-60%).[3,4] Interestingly, most influenza-associated IPA cases were observed in non-immunocompromised patients, thus questioning the applicability of the EORTC-MSG consensus criteria used largely to define aspergillosis in immunocompromised patients.[5] Therefore, in the ICU patients without the usual risk factors, a clinical algorithm to discriminate *Aspergillus* colonization from putative IPA was recently developed based on mycological criteria combining visualization and culture of *Aspergillus* 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 bi-center study to investigate the risk of IPA in critically ill COVID-19 patients, especially since these patients were likely to receive immunomodulatory therapies. The patients were classified using the influenza-associated IPA criteria in the ICU[4] completed with beta-D-glucan measurement and quantitative real-time PCR (qPCR) in BAL. Putative IPA was considered if one of the following conditions was met, i.e. 1- presence of *Aspergillus fumigatus* in culture; 2- BAL galactomannan index >0.8 AND beta-D-glucan >80 pg/mL; 3- *Aspergillus fumigatus* qPCR with quantification cycle <35 in pulmonary specimens;[7] and/or 4- serum beta-D-glucan >80pg/mL AND serum galactomannan index >0.5. Of note, direct examination of respiratory specimens was not performed to avoid operator contamination.

Twenty-seven successive mechanically ventilated COVID-19 patients with pneumonia admitted between March 16th and 28th to our two ICUs were included (Table 1). Respiratory specimens (20 BALs and 7 bronchial aspirations) were obtained on day 3 [1-6] post-intubation. Putative IPA was diagnosed in 9/27 patients (33%) including six patients validating ≥2 mycological criteria and three patients with only *Aspergillus fumigatus* identification in the respiratory specimen culture. History of hypertension was significantly more frequently reported in the patients with putative IPA than patients without aspergillosis (p=0.04). No other significant differences were observed in terms of age, invasive aspergillosis EORTC-MSG risk factors, time between onset of symptoms and intubation and times between onset of symptoms or intubation and *Aspergillus* respiratory

specimen collection, ARDS severity, clinical and laboratory data, non-COVID CT-scan images and steroid administration. No other immunosuppressant drug was administered in the ICU. Specific anti-*Aspergillus* therapy was initiated in only one of the nine patients with putative IPA. In this patient initially receiving caspofungin to treat concomitant invasive blood *Candida glabrata* infection, antifungal treatment was switched to voriconazole. No significant increase in fatality rate was observed in the patients with putative IPA (3/9 *versus* 3/18, p=0.4), although so far, 2/9 and 8/18 patients are still intubated in each group, respectively.

Here we found putative IPA in almost one third of our successive critically ill COVID-19 patients, at a similar rate to what has been observed in influenza patients.[3,4] Interestingly, when respiratory specimens were positive for *Aspergillus*, serum galactomannan was negative. One case had positive serum beta-D-glucan and galactomannan without *Aspergillus* detection in the BAL. Our findings strongly suggest that mechanically ventilated COVID-19 patients should be systematically screened for *Aspergillus* infection markers. Three of our cases had *Aspergillus fumigatu*s culture with no positive qPCR detection or galactomannan antigen in the BAL. Interestingly, not considering positive culture alone as a diagnostic criterion, by contrast to what is currently accepted,[4,6] would have resulted in underestimating the frequency of putative IPA (22% rather than 33% 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. Here, none of our nine putative IPA patients except one received anti-*Aspergillus* treatment. In the treated patient, antifungal treatment was adapted to cover both candida and *Aspergillus* infections. In the IPA group, the three fatalities were not related to aspergillosis but to bacterial septic shock complicated by multiorgan failure.

Finally, although oseltamivir-induced inhibition of the host neuraminidase activity has been suggested as a possible molecular mechanism leading to anti-aspergillus protective immunity decrease in influenza patients, the exact reasons for increased vulnerability of the COVID-19 patient to *Aspergillus* remain to be determined as well as the *Aspergillus* contribution to COVID-19-reated lung inflammation.

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Table 1. Characteristics of 27 critically ill COVID-19 patients according to the clinical classification of aspergillosis. Comparisons were performed using Chi-2 or Mann-Whitney tests, as appropriate.

Parameters	Putative aspergillosis (n=9)	No aspergillosis (n=18)	p
Age (years)	63	63	1.0
Sex ratio (M/F)	1.3	2.6	0.4
Diabetes, %	33	33	1.0
Hypertension, %	78	33	**0.04**
Obesity, %	33	17	0.4
Ischemic heart disease, %	22	33	0.7
Aspergillosis host factors, %†	22	17	1.0
Time from symptoms start to tracheal intubation (days)	7 [3-10]	8 [5-13]	0.2
Time from tracheal intubation to *Aspergillus* pulmonary specimen (days)	6 [1-11]	2 [0-10]	0.1
Time from symptoms start to *Aspergillus* pulmonary specimen (days)	12 [7-15]	11 [5-21]	0.7
Viral load, (cycles)	24 [17-37]	27 [15-34]	1.0
PaO_2/FiO_2 (mmHg)	167 [88-290]	163 [42-480]	0.8
Non-COVID CT scan signs, %	67	43	1.0
BAL macrophages, % (N=20)	26 [8-55]	34 [4-49]	0.8
BAL neutrophils, % (N=20)	47 [10-82]	30 [13-87]	1.0
BAL lymphocytes, % (N=20)	12 [6-48]	24 [1-53]	0.6
Vasopressors, %	89	67	0.4
Renal replacement, %	30	22	0.7
Steroid administration in the ICU, %††	67	72	1.0
Deaths, %	33	17	0.4

†based on EORTC MSG criteria for invasive aspergillosis, including steroids (n=3) and haematological malignancies (n=2); ††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; BAL, bronchoalveolar lavage; ICU, intensive care unit; CT scan, computerized tomography scanner.