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Epidemiology of Invasive Pulmonary Aspergillosis Among Intubated Patients With COVID-19: A Prospective Study

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(See the Editorial Commentary by Brüggemann et al on pages e3615–6.)

Background. We evaluated the incidence of invasive pulmonary aspergillosis among intubated patients with critical COVID-19 and evaluated different case definitions of invasive aspergillosis.

Methods. Prospective, multicenter study in adult patients with microbiologically confirmed COVID-19 receiving mechanical ventilation. All included participants underwent a screening protocol for invasive pulmonary aspergillosis with bronchoalveolar lavage galactomannan and cultures performed on admission at 7 days and in case of clinical deterioration. Cases were classified as coronavirus-associated pulmonary aspergillosis (CAPA) according to previous consensus definitions. The new definition was compared with putative invasive pulmonary aspergillosis (PIPA).

Results. 108 patients were enrolled. Probable CAPA was diagnosed in 30 (27.7%) patients after a median of 4 (2–8) days from intensive care unit (ICU) admission. Kaplan-Meier curves showed a significantly higher 30-day mortality rate from ICU admission among patients with either CAPA (44% vs 19%, $P = .002$) or PIPA (74% vs 26%, $P < .001$) when compared with patients not fulfilling criteria for aspergillosis. The association between CAPA (OR, 3.53; 95% CI, 1.29–9.67; $P = .014$) or PIPA (OR, 11.60; 95% CI, 3.24–41.29; $P < .001$) with 30-day mortality from ICU admission was confirmed, even after adjustment for confounders with a logistic regression model. Among patients with CAPA receiving voriconazole treatment (13 patients; 43%) a trend toward lower mortality (46% vs 59%; $P = .30$) and reduction in galactomannan index in consecutive samples were observed.

Conclusions. We found a high incidence of CAPA among critically ill COVID-19 patients and its occurrence seems to change the natural course of disease.

Keywords. SARS-CoV-2; COVID-19; severe respiratory failure; aspergillosis; voriconazole.

The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–associated coronavirus disease 2019 (COVID-19) is a major threat for global health. Approximately 14–30% of hospitalized patients diagnosed with COVID-19 develop a severe respiratory failure requiring intensive care [1–3]. Among ventilated patients with COVID-19, preliminary studies have reported a high incidence of invasive aspergillosis that may affect up to 30% of intubated patients [4–6]. These observations mirror previous reports of invasive pulmonary aspergillosis complicating severe influenza in patients admitted for intensive care unit (ICU) care [7, 8].

Despite the high number of case reports of COVID-19–associated aspergillosis, a standardized case definition for this infection is lacking. Recently revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions of possible, probable, and proven invasive aspergillosis [9] in immunocompromised patients, which rely on characteristic radiological features of invasive mold disease (ie, nodular lesions with or without halo signs, cavitation), are difficult to apply in critically ill patients with COVID-19 who often have less-specific radiological signs of infection in the presence of acute respiratory distress syndrome (ARDS) [10]. Previous investigations have proposed a unique definition of putative invasive pulmonary aspergillosis (PIPA) for patients with *Aspergillus*-positive lower respiratory tract cultures (entry criterion) with compatible signs and symptoms of pneumonia, abnormal chest X-ray or computed tomography imaging, and either host immunosuppressive risk factors or lack of bacterial growth in lower respiratory cultures or the presence of a positive cytological smear demonstrating branching hyphae [11]. Recently, an expert consensus proposed a case definition for influenza-associated pulmonary aspergillosis (IAPA) based on galactomannan (GM) testing on serum or respiratory specimens. A similar definition was also proposed

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for coronavirus-associated pulmonary aspergillosis (CAPA) complicating severe COVID-19 cases [12].

In this study we aimed to describe the incidence and outcome of CAPA in a larger cohort of ventilated patients with COVID-19. Additionally, we aimed to evaluate the prognostic impact of different aspergillosis case definitions in this setting.

METHODS

Design and Setting

We performed a prospective multicenter cohort study in patients with laboratory-confirmed SARS-CoV-2 virus infection, hospitalized from 22 February through 20 April 2020 in 4 ICUs from 3 hospitals in Bologna, Italy: 1 tertiary 1420-bed teaching hospital and 2 tertiary hospitals with 870 and 320 beds, respectively. The numbers of maximum ICU beds per hospital available during the epidemic peak were 77, 22, and 13 respectively. Diagnostic testing for COVID-19 and hospitalization was performed according to local policy and clinical judgment and was not dictated by study protocol. Data were collected anonymously and managed using REDCap electronic data capture tools [13, 14]. The study was approved by the ethics committee Comitato Etico Indipendente di Area Vasta Emilia Centro (no. 283/2020/Oss/AOUBo).

Participants

Participants were consecutive adult (≥18 years) patients diagnosed with SARS-CoV-2 infection and requiring ICU admission for mechanical ventilation. Exclusion criteria were as follows: (1) early (<48 hours) ICU discharge and (2) ICU admission for reasons other than ARDS.

Study Procedures

For all participants, a screening protocol for invasive aspergillosis was proposed and consisted of bronchoalveolar lavage (BAL) performed on ICU admission (0–2 days), at day 7 (±2 days) from the first day of mechanical ventilation, and if the patient showed evidence of clinical disease progression, which was defined by either (1) worsening of fever or (2) increases in respiratory secretions or deterioration in respiratory status after a period of clinical stability. Samples were processed for GM detection and cultures. Additionally, BAL samples that tested positive for GM were stored at −80°C and later analyzed using a commercial quantitative real-time *Aspergillus* polymerase chain reaction (PCR) assay (described below). Therefore, the results of PCR assay were not reported to clinicians. Direct cytological examination of BAL samples was deferred due to COVID-19 safety concerns.

Severe COVID-19 cases were treated with hydroxychloroquine, lopinavir–ritonavir or darunavir–cobicistat, intravenous tocilizumab (6 mg/kg in 1–2 doses within 12–24 hours) or subcutaneous tocilizumab administered in 2 simultaneous doses of 162 mg, methylprednisolone 1 mg/kg for 5–7 days, and low-molecular-weight heparin (LMWH) at a daily dosage of 60–100 mg according to body weight.

Microbiological Analysis

The presence of SARS-CoV-2 was detected by reverse transcriptase (RT)–PCR assay. Briefly, universal transport medium (UTM)-RT swab specimens (Copan, Italy) were immediately tested or stored at 4°C until processed, no more than 48 hours. Total genomic DNA/RNA was extracted from 280 µL of the clinical swab sample by Nuclisens EasyMag (BioMerieux, Marcy l'Etoile, France) following the manufacturer's instructions. Detection of SARS-CoV-2 virus was performed by real-time RT-PCR following the World Health Organization and/or Centers for Disease Control and Prevention protocol in a QuantStudio S5 Real-time PCR system (ThermoFisher).

The GM antigen index was measured with a sandwich enzyme-linked immunosorbent assay (ELISA; Platelia Aspergillus; Bio-Rad Laboratories) in BAL and serum specimens. BAL samples were further analyzed by culture for filamentous fungi and quantitative real-time PCR for *Aspergillus* genus as follows: A 10-µL volume of BAL fluid was cultured on Sabouraud Chloramphenicol agar tubes (Vakutainer Kima, Padova, Italy) at 30°C for up to 5 days. As soon as molds were visible they were subcultured on Sabouraud Dextrose Agar plates (Vakutainer Kima) for 2 to 3 days at 30°C. Fungus identification was performed by microscopic examination of lactophenol cotton blue-stained slides and by a matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry instrument (Bruker, Italy), following the manufacturer's instructions.

The residual volume was frozen at −20°C until used for PCR analysis. DNA extraction for PCR analysis was performed on an ELITe InGenius automated platform as well as RT-PCR using the *Aspergillus* spp. ELITe MGB kit (Elitgroup, Puteaux, France). The DNA was extracted from a 1-mL volume of BAL fluid and was eluted in a 200-µL saline solution prior to DNA amplification in the same platform. RT-PCR for *Aspergillus* genus was performed by an *Aspergillus* spp. ELITe MGB kit, which was CE-In-vitro diagnostic (CE-IVD) validated on a diverse range of sample types. The target region was the ribosomal DNA18S (rDNA18S), and the human B-globin gene was used as an internal standard. The fungal DNA copy number was expressed as copies/mL in relation to an rDNA18s standard curve.

Variables and Definitions

Microbiological diagnosis of SARS-CoV-2 infection was defined as a positive RT-PCR test on respiratory specimens. These consisted of nasopharyngeal swabs or BAL in all cases.

Invasive pulmonary aspergillosis was defined according to the recently proposed CAPA definition for COVID-19–positive patients admitted to the ICU with pulmonary infiltrates (entry criterion) who had at least 1 of the following: serum GM index more than 0.5 or BAL GM index more than 1.0 or positive *Aspergillus* BAL culture or cavitating infiltrate (not attributed to another cause) in the area of the pulmonary infiltrate [12].

To assess the prognostic performance of different cases definitions, we compared cases of CAPA and cases of PIPA, which were defined according to AspICU study group criteria [11].

Exposure variables were assessed at hospital admission and included age, sex, and body mass index. Underlying conditions were recorded according to the Charlson comorbidity index [15]. Immunosuppression included neutropenia (neutrophil count <500/mm^3), solid-organ transplantation, hematopoietic stem cell transplantation, corticosteroid therapy at a dosage higher then or equivalent to prednisone 16 mg/day for 15 or more days, uncontrolled human immunodeficiency virus (HIV) infection (<200 CD^4/mm^3). With regard to SARS-CoV-2 infection, we collected symptoms, vital signs, and laboratory and radiological tests, at hospitalization and during follow-up, and treatments received. Clinical severity at hospitalization and ICU admission was recorded according to sequential organ failure assessment (SOFA) score. Endpoint variables were assessed from hospital admission to discharge. We collected duration of mechanical ventilation, in-hospital all-cause mortality, and date of hospital discharge.

Statistical Analysis

For descriptive analysis, categorical variables are presented as counts and percentages, continuous variables as means and standard deviations if normally distributed, or as medians and interquartile ranges (IQRs) if non–normally distributed.

For group comparisons, Student's *t* test, Mann-Whitney test, and analysis of variance or Kruskal-Wallis test were used for normally distributed quantitative variables, skewed distributed, and for more than 2 groups, respectively. Pearson's chi-square test (or Fisher's exact test, where appropriate) for categorical variables. Shapiro-Wilk's and Kolmogorov-Smirnov tests, as well as visual methods, were applied to test for normality.

Incidence rates of CAPA were calculated per 10 000 ICU patient-days, and 95% confidence intervals (CIs) for the incidence rates were estimated under the assumption of a Poisson distribution. Survival was analyzed by Kaplan-Meier curves. The impact of PIPA and CAPA definitions on survival status of COVID-19 was assessed by the log-rank test after 30 days from ICU admission by univariate analysis. To assess the impact on mortality using CAPA and PIPA definitions we first compared survivors and nonsurvivors after 30 days from ICU admission by univariate analysis. Age, sex, SOFA score at ICU admission, and need for renal replacement therapy were included in a logistic regression model for 30-day mortality. Thereafter, the variables PIPA, CAPA, and first GM index were alternatively included to assess their effect on the mortality model. All statistical analyses were performed with STATA-IC 16 (StataCorp, College Station, TX) and R version 3.5 (R Core Team, Vienna, Austria).

RESULTS

During the study period 822 patients with a diagnosis of COVID-19 were admitted to the 3 centers. Of these, 185 (22%) were admitted to the ICU and 163 (20%) were intubated. Screening for aspergillosis was performed in 108 patients; this group was selected as the study cohort. Main reasons for protocol exclusion were early (<48 hours) extubation (12 cases), ICU admission and intubation for reasons other than ARDS (13 cases), and noncompliance to the protocol (30 cases). In these latter cases, bronchoscopies were not performed for safety concerns (16 cases) or GM was not tested because of insufficient BAL quantity (8) or by mistake (5) (Figure 1).

Overall, the median (IQR) age was 64 (57–70) years and 83 (78%) patients were male. The median age-adjusted Charlson comorbidity index was 2.5 (1–4). At ICU admission the median

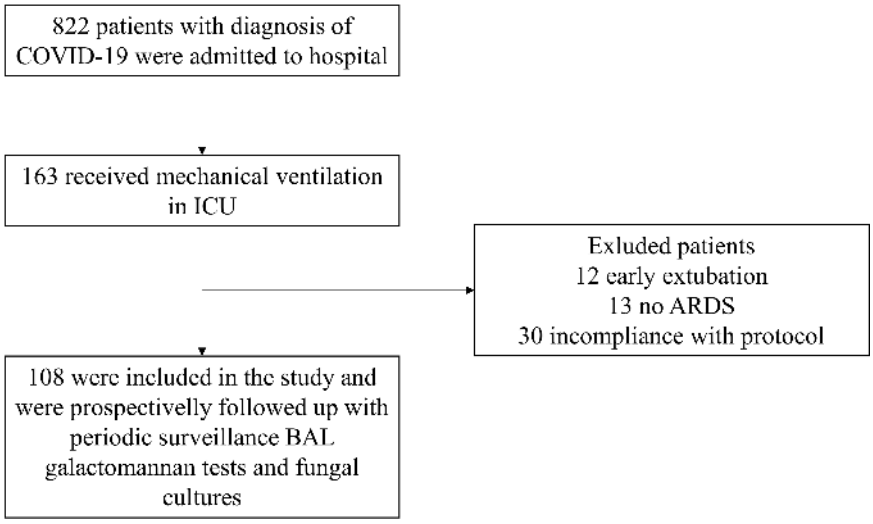

Figure 1. Study flowchart. Abbreviations: ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; COVID-19, coronavirus disease 2019; ICU, intensive care unit.

(IQR) SOFA score was 4 (3–5). A total of 189 samples from BAL were obtained and analyzed (Table 1), with a median (IQR) of 2 (1–3) samples per patient. Positive GM (index >1.00) was found on admission in 14 of 108 (13%) cases, and on second or subsequent determinations in 9 (8%) or 5 (5%) cases, respectively. The median (IQR) GM index in positive samples was 3.73 (1.76–5.07). On bronchoscopic examination, pseudomembranous plaques or ulcers were visible in 6 patients. In all of these cases BAL GM was positive. No tracheal or lung biopsies were performed as most patients (82/108, 76%) were receiving therapeutic dosages of LMWH.

According to CAPA criteria, probable aspergillosis was diagnosed in 30 (27.7%) cases after a median of 4 (2–8) days from intubation and a median of 14 (11–22) days from COVID-19 symptom onset. The incidence of probable CAPA was 38.83 per 10 000 ICU patient-days. A comparison of patients with and without probable CAPA is depicted in Table 2. Briefly, the only factor associated with CAPA was chronic steroid therapy ($P = .02$) at dosages higher than or equivalent to prednisone 16 mg/day for at least 15 days.

Simultaneous serum and BAL GM samples were only available in 59 patients. Among these, CAPA was diagnosed in 16 patients. Only 1 patient fulfilling the CAPA definition had a positive serum GM (GM index >0.5). Using BAL GM as a diagnostic benchmark, specificity, sensitivity, and positive- and negative-predictive values of serum GM for CAPA diagnosis were 6%, 34%, 19%, and 49%, respectively.

Cultures of BAL revealed growth of *Aspergillus* spp. in 20 of 108 (18%) cases and *Aspergillus fumigatus* was isolated in 16 (15%) patients. When applying the AspICU algorithm, PIPA was diagnosed in 19 (17.6%) patients. Detailed microbiological findings in cases classified as CAPA or PIPA are summarized in Table 1.

Impact of Aspergillosis Case Definition on Mortality

The last evaluable follow-up date was 19 May 2020. The median patient follow-up time was 31 (20–43) days. At that time, 54 (50%) patients were discharged, 44 (41%) had died, and in 9 patients the follow-up was ongoing. Differences between survivors and nonsurvivors are reported in Table 3. Kaplan-Meier curves showed a significantly higher 30-day mortality rate from ICU admission among patients with either probable CAPA when compared with patients without CAPA (44% vs 19%; $P = .002$) (Figure 2A) or PIPA when compared with patients without PIPA (74% vs 26%; $P < .001$) (Figure 2B). Diagnosis of CAPA was associated with 30-day mortality from ICU admission (odds ratio [OR], 3.53; 95% CI, 1.29–9.67; $P = .014$), even after adjustment for age (OR, .99; 95% CI, .94–1.06; $P = .99$), need for renal replacement therapy (OR, 3.02; 95% CI, 1.11–8.19; $P = .015$), and SOFA score at ICU admission (OR, 1.38; 95% CI, 1.07–1.73; $P = .004$) with a logistic regression model. After repeating a similar logistic regression model using a probable PIPA variable instead of CAPA, PIPA was independently associated with mortality (OR, 11.60; 95% CI, 3.24–41.29; $P < .001$).

Prognostic Implication of Initial Bronchoalveolar Lavage Galactomannan Index

The relationship between initial BAL GM index and 30-day survival is shown in Figure 3. The odds of death within 30 days of ICU admission increased 1.41-fold (1.10–1.81; $P = .007$) for each point increase in the initial BAL GM index. When adjusted for age, need for renal replacement therapy, and SOFA score at ICU admission, the initial BAL GM index was still independently associated with increased odds of death within 30 days of ICU admission (OR, 1.44; 95% CI, 1.08–1.94; $P = .014$).

Table 1. Comparison of Microbiological Tests Among Patients With COVID-19 With Pulmonary Aspergillosis Classified With Either Coronavirus-associated Pulmonary Aspergillosis or Putative Invasive Pulmonary Aspergillosis

Test	Total (N = 108)	CAPA (n = 30)[a]	PIPA (n = 19)[a]	Colonization or No Aspergillosis (n = 77)
Cultures	20 (18)	19 (63)	19 (100)	1 (1)
Aspergillus fumigatus	16 (15)	15 (50)	15 (79)	1 (1)
Aspergillus niger	3 (3)	3 (100)	3 (16)	0 (0)
Aspergillus flavus	1 (1)	1 (3)	1 (5)	0 (0)
BAL-positive GM (index >1)	30 (28)	30 (100)	18 (95)	0 (0)
Positive BAL GM on first determination (day 0–2)	14 (13)	14 (47)	11 (58)	0 (0)
Positive BAL GM on second determination (day 5–9)	9 (8)	9 (30)	4 (21)	0 (0)
Other BAL GM determination	5 (5)	7 (23)	4 (21)	0 (0)
BAL GM value, index, median (IQR)	0.14 (0.09–1.27)	3.5 (1.72–4.7)	3.73 (1.76–5.07)	0.09 (0.07–0.18)
Positive serum GM (index >0.5)	1 (1)	1 (3)	1 (5)	0 (0)
Serum GM value, index, median (IQR)[b]	0.06 (0.03–0.09)	0.06 (0.03–0.11)	0.06 (0.04–0.18)	0.06 (0.03–0.08)
Positive *Aspergillus* PCR[c]	26/67 (38)	20/30 (67)	19/19 (100)	5/36 (14)

Data are presented as n (%) unless otherwise indicated.

Abbreviations: BAL, bronchoalveolar lavage; CAPA, coronavirus-associated pulmonary aspergillosis; COVID-19, coronavirus disease 2019; GM, galactomannan; IQR, interquartile range; PCR, polymerase chain reaction; PIPA, putative invasive pulmonary aspergillosis.

[a]18 patients with PIPA were also classified in the CAPA group. One patient had positive cultures for *Aspergillus fumigatus* but GM on BAL was 0.663.

[b]Performed in only 59 patients.

[c]Performed in only 67 patients.

Table 2. Comparison of Patients with COVID-19 Fulfilling Criteria of Probable Coronavirus-associated Pulmonary Aspergillosis (CAPA) With Patients Without CAPA

	CAPA (n = 30)	No CAPA (n = 73)	*P*
Demographics			
Age, median (IQR), years	63 (57–70)	63 (57–70)	.86
Male	24 (80)	83 (77)	.80
Underlying diseases			
Obesity	10 (37)	34 (49)	.36
BMI, median (IQR), kg/m^2	28 (26–31)	29 (26–31)	.92
Hypertension	16 (59)	49 (65)	.64
Diabetes mellitus	5 (17)	13 (17)	.99
Coronary disease	3 (10)	9 (11)	.99
Cerebrovascular disease	3 (10)	1 (1.4)	.06
Chronic kidney disease	6 (20)	6 (8)	.08
COPD	4 (13)	13 (17.8)	.10
Malignancies	2 (7)	5 (6)	.99
Solid-organ transplant	1 (3)	4 (5)	.99
Chronic steroid treatment	5 (17)	2 (3)	.02
Hemodialysis	3 (12)	3 (5)	.36
Charlson index, median (IQR)	3 (1–4)	2 (1–4)	.51
Symptoms at hospital admission			
Fever			
>38°C	16 (55)	61 (83)	.009
<38°C	8 (28)	6 (8)	
Cough	16 (57)	44 (62)	.51
Dyspnea	22 (79)	62 (84)	.57
Laboratory tests at admission			
White blood cells, median (IQR), 10^9/L	9.7 (4.9–14.0)	7.1 (5.2–10.1)	.13
Neutrophils, median (IQR), 10^9/L	8.0 (3.9–13.4)	5.9 (4.0–8.8)	.24
Lymphocytes, median (IQR), 10^9/L	0.76 (0.55–1.10	0.84 (0.50–1.01)	.67
Creatinine, median (IQR), mg/dL	1.0 (0.77–2.05)	1.00 (0.77–1.38)	.38
CRP, median (IQR), mg/dL	11 (5–18)	11.8 (6.5–19.9)	.37
LDH, median (IQR), IU/L	375 (311–500)	389 (286–524)	.67
SOFA score	3 (2–4)	3 (1–4)	.81
COVID-19 treatment			
Hydroxychloroquine	28 (93)	73 (94)	.99
Azithromycin	9 (30)	31 (40)	.38
Lopinavir	12 (40)	27 (35)	.61
Darunavir	2 (7)	6 (8)	.99
Remdesivir	3 (10)	5 (6)	.68
Tocilizumab	22 (73)	57 (78)	.80
Corticosteroids	18 (60)	34 (46.6)	.29
Prednisone equivalents, median (IQR), mg	100 (89–129)	107 (70–133)	.89
ICU admission			
Time from symptom onset to ICU admission, median (IQR), days	8 (4–13)	9 (7–11)	.73
Time from hospital admission to ICU, median (IQR), days	3 (0–6)	3 (1–4)	.68
First partial arterial oxygen pressure to fraction of inspired oxygen after intubation, mm/Hg	153 (102–232)	153 (98.7–200)	.50
Prone positioning	22 (76)	56 (83)	.22
RRT	11 (37)	20 (26)	.34
Inotropic support	19 (63)	50 (72)	.47
Days of mechanical ventilation	13 (7–23)	16 (10–16)	.09
ICU length of stay, days	16 (9–27)	21 (13–31)	.08

Data are presented as n (%) unless otherwise indicated.

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; IQR, interquartile range; ICU intensive care unit; LDH, lactate dehydrogenase; RRT, renal replacement therapy; SOFA, sequential organ failure assessment.

Effect of Antifungal Treatment

Among patients fulfilling the probable CAPA definition, a total of 16 (53%) patients received antifungal treatment, of whom 13 (43%) were treated with voriconazole. Reasons for nontreatment were postmortem diagnosis or clinical decision not to start antifungal therapy in 7 cases each.

Table 3. Differences Between Survivors and Nonsurvivors After 30 Days From Intensive Care Unit Admission

	Survivors (n = 72)	Nonsurvivors (n = 36)	*P*
Demographics			
Age, median (IQR), years	64 (58–70)	63 (58–72)	.98
Male	53 (73)	30 (83)	.36
Underlying diseases			
Obesity	26 (42)	18 (51)	.52
BMI, median (IQR), kg/m^2	28 (26–31)	30 (26–31)	.28
Hypertension	44 (64)	21 (64)	.99
Diabetes mellitus	12 (16)	6 (16)	.99
Coronary disease	8 (11.1)	4 (11)	.99
Cerebrovascular disease	0 (0)	4 (11)	.01
Chronic kidney disease	5 (7)	7 (19)	.10
COPD	12 (17)	5 (14)	.78
Malignancies	4 (6)	3 (8)	.64
Solid-organ transplant	3 (4)	2 (6)	.99
Chronic steroid treatment	2 (3)	5 (15)	.04
Hemodialysis	1 (2)	5 (18)	.02
Charlson index, median (IQR)	2 (1–3)	3 (2–5)	.16
Laboratory tests at admission			
White blood cells, median (IQR), 10^9/L	8.2 (4.5–13.1)	10.3 (7.3–13.9)	.87
Neutrophils, median (IQR), 10^9/L	6.1 (4.0–9.0)	8.3 (5.3–14.9)	.99
Lymphocytes, median (IQR), 10^9/L	0.84 (0.59–1.0)	0.74 (0.59–1.00)	.92
Creatinine, median, mg/dL	0.90 (0.72–1.1)	1.04 (0.8–2.1)	.03
CRP, median (IQR), mg/dL	12.7 (6.8–19.8)	11.3 (7.7–21.2)	.98
LDH, median (IQR), IU/L	382 (301–511)	418 (275–544)	.71
SOFA score	3 (3–4)	4 (3–7)	.03
COVID-19 treatment			
Hydroxychloroquine	67 (93)	34 (94)	.99
Azithromycin	24 (33)	16 (44)	.29
Lopinavir	22 (28)	7 (19)	.23
Darunavir	1 (5)	7 (8)	.99
Remdesivir	3 (4)	5 (14)	.11
Tocilizumab	55 (76)	27 (75)	.99
Corticosteroids	32 (44)	24 (67)	.04
ICU admission			
Time from symptom onset to ICU admission, median (IQR), days	10 (7–13)	7 (5–10)	.02
Time from hospital admission to ICU, median (IQR), days	3 (1–5)	4 (0–7)	.26
First partial arterial oxygen pressure to fraction of inspired oxygen after intubation, mm/hG	173 (107–216)	123 (86–172)	.04
Prone positioning	53 (81)	28 (78)	.79
RRT	13 (18)	18 (50)	.001
Inotropic support	15 (79)	54 (67)	.41
Tracheostomy	42 (62)	23 (66)	.83

Data are presented as n (%) unless otherwise indicated.
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; IQR, interquartile range; ICU intensive care unit; LDH, lactate dehydrogenase; RRT, renal replacement therapy; SOFA, sequential organ failure assessment.

Among patients with probable CAPA, voriconazole treatment was associated with a trend toward lower mortality (Figure 3A). Additionally, among 13 patients with at least 2 consecutive GM tests performed within 7 days, a trend toward GM index reduction over time was observed (Figure 3B and 3C).

DISCUSSION

In this study we evaluated the incidence of pulmonary aspergillosis among mechanical ventilated, critically ill patients with COVID-19. The definition of invasive pulmonary aspergillosis in patients with severe COVID-19 infection in the ICU remains difficult. The AspICU algorithm, based on *Aspergillus* spp. culture compatible signs and symptoms, host factors, and abnormal imaging, was previously validated in the context of critically ill ICU patients and allows the identification of PIPA cases [11]. In our study, all patients satisfied the criteria of symptomatic disease and abnormal chest imaging. However, both of these criteria are also compatible with COVID-19 itself. Host factors included in the AspICU algorithm and the EORTC/MSG

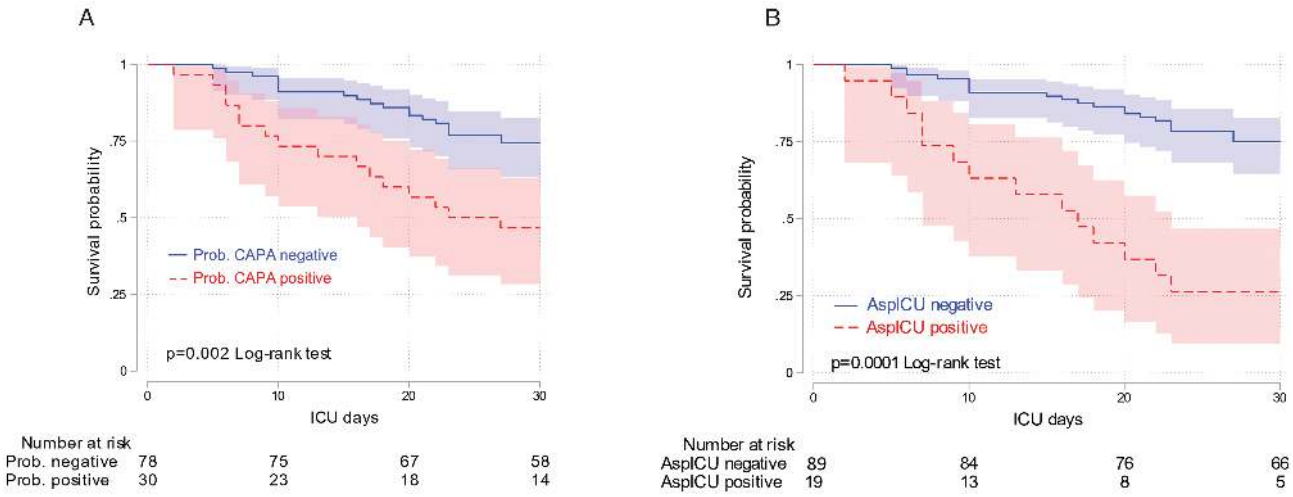

Figure 2. Kaplan-Meier survival curves for 30-day mortality from ICU admission. Patients were stratified as having probable CAPA (*A*) or PIPA AspICU criteria fullfilled (*B*). Abbreviations: CAPA, coronavirus-associated pulmonary aspergillosis; ICU, intensive care unit; PIPA, putative invasive pulmonary aspergillosis; prob., probable.

definitions may be useful in evaluating the risk of invasive aspergillosis in the general population but are not adaptable to the context of ARDS caused by respiratory viruses. Moreover, in most laboratories such as ours, direct smear evaluation is not currently performed in patients with COVID-19 due to safety concerns. Similarly, lung biopsies or autopsy samples may be limited for personnel safety reasons. In our study, of 16 deceased patients with a CAPA diagnosis, autopsy was performed in only 4. In 2 cases, evidence of fungal tissue invasion was found.

To overcome these limitations, we tried to validate a novel proposed definition of CAPA based on recently published expert consensus definitions [12]. When we evaluated the survival impact of PIPA and probable CAPA criteria, both were independent predictors of mortality. We believe that the use of CAPA criteria could be more useful in clinical practice for guiding clinical decisions. The PIPA criteria do not consider non–culture-based methods such as BAL GM or BAL/serum PCR. These latter methods may allow prompt earlier diagnosis and treatment of aspergillosis, potentially leading to improved patient survival.

According to this new definition, we were able to identify 30 of 108 (28%) patients fulfilling probable CAPA criteria. The incidence rate ratio of positive BAL GM with computed tomography findings during the COVID-19 period (38.83 per 10 000 unit days) versus the same months (February to April) of the previous year in the same ICUs (9.69 per 10 000 unit days) was 4.04 (95% CI, 1.77–9.91; $P < .0001$). However, these estimates are likely biased towards higher numbers of cases during the COVID-19 period because serial BAL testing was performed in the COVID-19 group but not in the non–COVID-19 historical controls. These results are slightly higher than the prevalence of aspergillosis complicating severe influenza cases found in previous studies (7–19%) [7, 8]. The similarity between severe IAPA and CAPA could be expected given the extensive damage

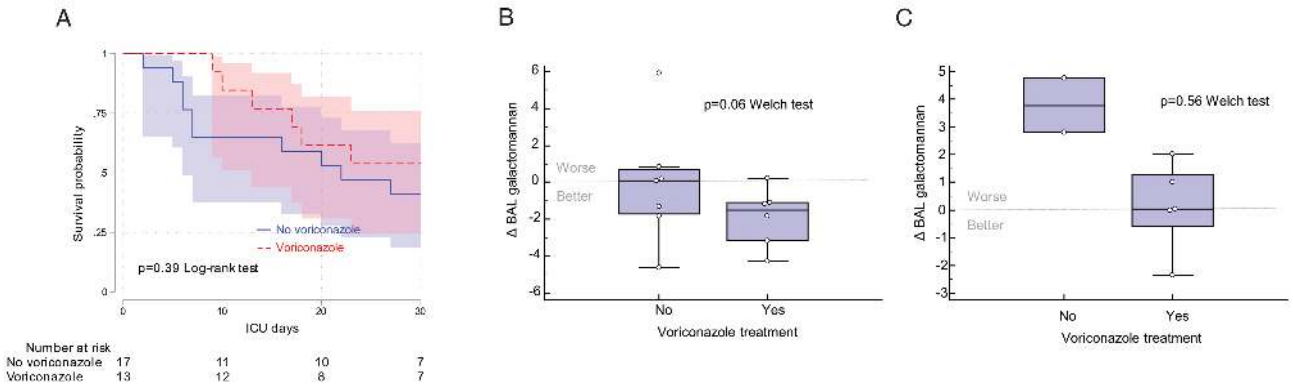

Figure 3. Effect of voriconazole treatment among patients with probable CAPA. Comparison of patients receiving or not receiving voriconazole treatment (*A*). Reduction in BAL galactomannan index over time among patients with CAPA treated with voriconazole observed within 7 days (13 patients) (*B*) or and within 14 days (7 patients) (*C*). Abbreviations: BAL, bronchoalveolar lavage; CAPA, coronavirus-associated pulmonary aspergillosis; ICU, intensive care unit.

to the respiratory epithelium associated with both infections, which is considered a key predisposing event for semi-invasive and invasive pulmonary aspergillosis in these populations [12]. In our study, the median number of days between COVID-19 symptom onset and CAPA diagnosis was 14 days. Other studies performed on IAPA found comparable timing between influenza onset and aspergillosis [16, 17]. Like influenza, severe COVID-19 is characterized by lymphopenia. This factor was previously associated with the development of invasive aspergillosis [18].

It was recently hypothesized that an uncontrolled cytokine storm may play a role in determining disease progression for patients with COVID-19 [19]. Consistently, most patients received corticosteroids and tocilizumab in our cohort. This heavy use of immunomodulating drugs may have contributed to the high prevalence of CAPA in our study. Similar findings were observed in a large number of studies, including those focused on patients with severe influenza [8, 20]. Conversely, a cytokine storm may add challenges to the diagnosis of bacterial and fungal infection as symptoms and radiological findings may overlap. Therefore, a screening algorithm for CAPA, as performed in our study, may provide prompt diagnosis and treatment.

Studies on the best antifungal treatment for CAPA are lacking. The most common recommendations are the use of voriconazole or isavuconazole in the setting of aspergillosis complicating severe influenza cases and are based on studies performed in immunocompromised individuals [10]. In this report most patients received voriconazole. Although this study was not designed to address this point in explorative analysis, an interesting trend toward higher survival (Figure 4) or reduced BAL GM index was observed. Unfortunately, the relatively low sample size prevents any firm conclusions on antifungal treatment.

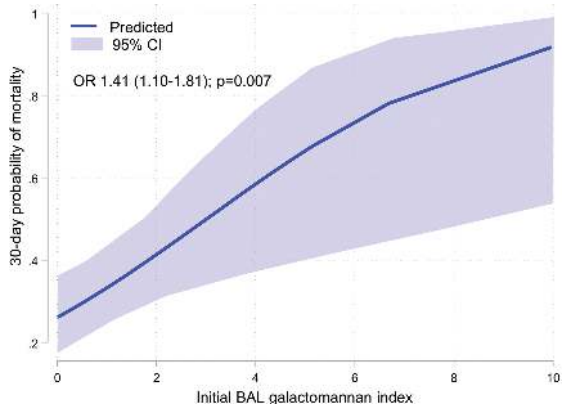

Figure 4. Relationship between initial BAL galactomannan index and 30-day mortality. Abbreviations: BAL, bronchoalveolar lavage; CI, confidence interval; OR, odds ratio.

Another interesting finding in our study is the correlation between the magnitude of the BAL GM index and 30-day patient mortality. Similar results were also found in studies on serum GM in patients with hematologic disease and may be the expression of higher fungal burden [21]. If confirmed by further studies, BAL GM may be useful to prioritize antifungal treatment to patients considered at higher risk of mortality.

In conclusion, we found a high incidence of invasive aspergillosis among critically ill patients with COVID-19 and that its occurrence seems to change the natural course of disease. The use of CAPA criteria for the diagnosis of invasive aspergillosis may allow earlier diagnosis than AspICU criteria and might prioritize prompt antifungal treatment.

Notes

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