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

Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study

Michele Bartoletti[1], Renato Pascale[1], Monica Cricca[2], Matteo Rinaldi[1], Angelo Maccaro[1], Linda Bussini[1], Giacomo Fornaro[1], Tommaso Tonetti[3], Giacinto Pizzilli[3], Eugenia Francalanci[1], Lorenzo Giuntoli[4], Arianna Rubin[1], Alessandra Moroni[2], Simone Ambretti[2], Filippo Trapani[1], Oana Vatamanu[1], Vito Marco Ranieri[3], Andrea Castelli[5], Massimo Baiocchi[5], Russell Lewis[1], Maddalena Giannella[1] and Pierluigi Viale[1], for the PREDICO study group[6].

[1]Infectious Diseases Unit, Department of Medical and Surgical Sciences, Policlinico Sant'Orsola, Bologna, Italy.

[2]Operative unit of Microbiology, University of Bologna, Policlinico Sant'Orsola, Bologna, Italy.

[3]Intensive Care Unit, Department of Medical and Surgical Sciences, Policlinico Sant'Orsola, Bologna, Italy

[4]Intensive Care Unit, Maggiore Hospital, Bologna, Italy

[5]Cardio-Thoracic Anesthesiology Unit, S.Orsola Malpighi University Hospital, Bologna, Italy.

[6]PREDICO study group listed in the Acknowledgement section.

Corresponding author:

Michele Bartoletti, MD, PhD

Infectious Diseases Unit, Sant'Orsola Malpighi Hospital

Via Massarenti 11, 40137, Bologna, Italy

Tel +390512143199, fax +39051343500

email: m.bartoletti@unibo.it

SUMMARY

Using a prospective surveillance based on galactomannan and cultures on bronchoalveolar lavage in mechanical ventilated patients with COVID-19, we found a high incidence of invasive pulmonary aspergillosis. The occurrence of aspergillosis changes significantly the natural history of the disease.

ABSTRACT

Background

In this study we evaluated the incidence of invasive pulmonary aspergillosis among intubated patients with critical coronavirus disease 2019 (COVID-19) and evaluated different case definitions of invasive aspergillosis.

Methods:

Prospective, multicentre study on adult patients with microbiologically confirmed COVID-19 receiving mechanical ventilation. All included participants underwent 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

A total of 108 patients were enrolled. Probable CAPA was diagnosed in 30 (27.7%) of patients after a median of 4 (2-8) days from intensive care unit (ICU) admission. Kaplan-Meier curves showed a significant higher 30-day mortality rate from ICU admission among patients with either CAPA (44% vs 19%, p= 0.002) or PIPA (74% vs 26%, p<0.001) when compared with patients not fulfilling criteria for aspergillosis. The association between CAPA [OR 3.53 (95%CI 1.29-9.67), P=0.014] or PIPA [OR 11.60 (95%CI 3.24-41.29) p<0.001] 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=0.30) and reduction of galactomannan index in consecutive samples was observed.

Conclusion

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

Keywords: SARS-CoV-2, COVID-19, severe respiratory failure, aspergillosis, voriconazole

INTRODUCTION

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 Organisation 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 mould disease (i.e. nodular lesions with or without halo signs, cavitation) are difficult to apply in critically-ill COVID-19 patients 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 risk 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 testing on serum or respiratory specimens. A similar definition was also proposed for coronavirus associated pulmonary aspergillosis (CAPA) complicating severe COVID-19 cases[12].

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

METHODS

Design and setting

We performed a prospective multicentre cohort study of patients with laboratory-confirmed SARS-CoV2 virus infection, hospitalized from February 22 through April 20, 2020 in four intensive care units (ICUs) from 3 Hospitals in Bologna, Italy: 1 tertiary 1420-bed teaching and 2 tertiary hospitals with 870 and 320 beds, respectively. The number of maximal ICU beds per hospital available during the epidemic peak were 77, 22 and 13 respectively. Diagnostic testing for COVID-19 and hospitalization were performed according to local policy and clinical judgment and were 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 Ethic Committee (Comitato Etico Indipendente di Area Vasta Emilia Centro, n. 283/2020/Oss/AOUBo).

Participants

All consecutive adult (≥18 years) patients diagnosed with SARS-CoV-2 infection and requiring ICU admission for mechanical ventilation. Exclusion criteria were: i) early (<48h) ICU discharge ii) ICU admission for reason other than acute respiratory distress syndrome (ARDS).

Study Procedures

For all participants a screening protocol for invasive aspergillosis was proposed and consisted in 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 a) worsening of fever or b) increases in respiratory secretions or deterioration in respiratory status after a period of clinical stability. Samples were processed for galactomannan (GM) detection and cultures. Additionally, BAL samples that tested positive for galactomannan were store at -80°C and later analysed using a commercial quantitative real-time Aspergillus PCR assay (described below). Therefore, the result of PCR assay was 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 12-24h) or subcutaneous tocilizumab administered in two simultaneous doses of 162 mg, methylprednisolone 1mg/kg for 5-7 days and low molecular weight heparin (LMWH) at daily dosage of 60-100 mg according to body weight.

Microbiology analysis

The presence of SARS-Cov2 was detected by RT-PCR assay. Briefly, UTM-RT swab specimens (Copan, Italy) were immediately tested or stored at 4ºC 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 manufacturer's instructions. Detection of SARS-CoV-2 virus was performed by real time RT-PCR following the WHO and/or CDC protocol in a QuantStudio S5 Real-time PCR system (ThermoFisher, USA).

The galactomannan antigen index was measured with a sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia™ Aspergillus; Bio-Rad Laboratories) in BALs and serum

specimens. BALs were further analysed by culture for filamentous fungi and quantitative real time-PCR for *Aspergillus* genus as follows A ten-microliter volume of BAL were cultured on Sabouraud Chloramphenicol agar tubes (Vakutainer Kima, Padova, Italy) at 30°C for up to five days. As soon as molds were visible they were subcultured on Sabouraud Dextrose Agar plates (Vakutainer Kima, Padova, Italy) for 2 to 3 days at 30 °C. Fungi identification was performed by microscopic examination of lactophenol cotton-blue stained slides and by MALDI-TOF Mass Spectrometry Instrument (Bruker, Italy), following manufacturer's instructions.

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

Variables and definitions

Microbiological diagnosis of SARS-CoV2 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 consisting in COVID-19 positive patients admitted to the ICU with pulmonary infiltrates (entry criterion) who had at least one of the following: serum GM index >0.5 or BAL GM index >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 was defined according with AspICU criteria[11].

Exposure variables were assessed at hospital admission and included: age, sex, body mass index. Underlying conditions were recorded according to 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 ≥ 15 days, uncontrolled HIV infection (<200CD^4/mm^3). Regarding the SARS-CoV2 infection, we collected symptoms vital signs, 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). 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 mean and standard deviation if normally distributed or as median and interquartile range (IQR) if non-normally distributed.

For group comparison, Student t test, Mann-Whitney test, and ANOVA or Kruskall-Wallis were used for quantitative variables normally distributed, skewed distributed and for >2 groups, respectively. Pearson's $\chi 2$ test (Fisher exact test where appropriate) for categorical variables. Shapiro Wilk's and Kolmogorov-Smirnov test, 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 analysed 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 form ICU admission at univariate analysis. To assess the impact on mortality of CAPA and PIPA definitions we, first compared survivors and non-survivors after 30 days form ICU admission at 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 galactomannan index were alternatively included to assess their effect on the mortality model. All statistical analysis was performed with Stata-IC 16 (College Station, Texas) and R version 3.5 (R Core team, Vienna, Austria).

RESULTS

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

Overall, median (IQR) age was 64 (57-70) years and 83 (78%) were male. The median age-adjusted Charlson comorbidity index was 2.5 (1-4). At ICU admission the median (IQR) SOFA score was 4 (3-5). A total of 189 samples from BAL were obtained and analysed (Table 1), with a median (IQR) of 2 (1-3) samples for patients. Positive GM (index >1.00) was found on admission in 14/108 (13%) cases, 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, plaques pseudomembranous or ulcers were visible in 6 patients. In all 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 form intubation and a median of 14 (11-22) days from COVID-19 symptoms onset. The incidence density of probable CAPA was 38.83 per 10,000 ICU patient-days. A comparison of patients with and without probable CAPA in depicted in Table 2. Briefly, the only factor associated to CAPA was chronic steroid therapy (p=0.02) at dosage higher then or equivalent to prednisone 16 mg/day for at least 15 days.

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

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

Impact of aspergillosis case definition on mortality

The last evaluable follow-up date was May 19,2020. The median patient follow-up time was 31 (20-43) days. At this time, 54 (50%) patients were discharged, 44 (41%) died and, in 9 patients the follow-up was ongoing. Differences between survivors and non-survivors 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= 0.002; Figure 2, panel A) or PIPA when compared with patients without PIPA (74% vs 26%, p<0.001 Figure 2, panel B) . CAPA diagnosis was associated with 30-day mortality from ICU admission [OR

3.53(95%CI 1.29-9.67), P=0.014] even after adjustment for age [OR 0.99(95% CI 0.94-1.06), p=0.99], need for renal replacement therapy [OR 3.02(95%CI 1.11-8.19), p=0.015] and SOFA score at ICU admission [OR 1.38(95%CI 1.07-1.73), p=0.004] with a logistic regression model. After repeating a similar logistic regression model using probable PIPA variable instead of CAPA, PIPA was independently associated to mortality [OR 11.60(95%CI 3.24-41.29) p<0.001].

Prognostic implication of initial BAL galactomannan index

The relationship between initial BAL galactomannan index and 30-day survival is show in Figure 3. The odds of death within 30 days of ICU admission increased 1.41-fold (1.10-1.81; p=0.007) for each point increase in the initial BAL galactomannan index. When adjusted for age, need for renal replacement therapy and SOFA score at ICU admission the initial BAL galactomannan 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=0.014).

Effect of antifungal treatment

Among patients fulfilling probable CAPA definition a total of 16 (53%) of patients received antifungal of which 13 (43%) were treated with voriconazole. Reason for non-treatment was post-mortem diagnosis or clinical decision not to start antifungal therapy in 7 cases, each.

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

DISCUSSION

In this study we evaluated the incidence of pulmonary aspergillosis among mechanical ventilated critically ill COVID-19 patients. Definition of invasive pulmonary aspergillosis in patients with severe COVID-19 infection in the ICU remains difficult. AspICU algorithm, based on *Aspergillus* spp cultures compatible signs and symptoms, host factors and abnormal imaging was previously validated in the context of critically ill ICU patients and it allow to identify PIPA cases[11]. In our study, all patients satisfied the criteria of symptomatic disease and abnormal chest imaging. However, both these criteria are also compatible with COVID-19 itself. Host factors included in the AspICU algorithm and the EORTC/MSG definitions may be useful in evaluating risk for invasive aspergillosis in general population but could not be adaptable in the context of ARDS caused by respiratory viruses. Moreover, in most laboratory like ours, direct smear evaluation was not currently performed in COVID-19 patients due to safety concerns. Similarly, lung biopsies or autopsied may be limited for personnel safety reason. In our study, of 16 deceased patients with CAPA diagnosis autopsy was performed in only 4. In two cases evidence of fungal tissue invasion was found.

To overcome all 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. PIPA criteria do not consider non-culture-based methods such as BAL GM or BAL/serum PCR. These latter 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 30/108 (28%) patients fulfilling probable CAPA criteria. The incidence rate ratio of positive BAL galactomannan with CT findings during the COVID period (38.83 per 10,000 unit days) vs. 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); $p<0.0001$. However, these estimates are likely biased towards higher number of cases during the COVID period

because serial BAL testing was performed in the COVID group but not the non-COVID historical controls. These results are slightly higher to prevalence of aspergillosis complicating severe influenza cases found in previous studies (7-19%) [7, 8]. Similarity between severe IAPA and CAPA could be expected given the extensive damage 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 symptoms onset and CAPA diagnosis was 14 days. Other studies performed on IAPA found comparable timing between influenza onset and aspergillosis[16, 17]. Likewise influenza, severe COVID-19 is characterized by lymphopenia. This factor was previously associated to development of invasive aspergillosis[18].

It was recently hypothesized that the uncontrolled cytokine storm may play role in determining disease progression for COVID-19 patients[19]. Consistently, most patients received corticosteroids and tocilizumab in our cohort. This heavy use of immunomodulant drugs may had 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 cases[8, 20]. Conversely, cytokine storm may add challenges to 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 best antifungal treatment for CAPA are lacking. Most common recommendation of use voriconazole or isavuconazole in the setting of aspergillosis complicating severe influenza cases are based on studies performed in immunocompromised[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 galactomannan index was observed. Unfortunately, the relatively low sample size prevents any firm conclusion about antifungal treatment.

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 hematological patients 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 COVID-19 patients and that its occurrence seems to change the natural history of disease. The use of CAPA criteria for diagnosis of invasive aspergillosis may provide earlier diagnosis than AspICU criteria and might prioritize prompt antifungal treatment.

ACKNOWLEDGEMENT

We thank the PREDI-CO study group.

[6]PREDICO study group

Luigi Raumer, Luca Guerra, Fabio Tumietto, Alessandra Cascavilla, Eleonora Zamparini, Gabriella Verucchi, Simona Coladonato, Stefano Ianniruberto, Luciano Attard, Marina Tadolini Francesca Volpato, Giulio Virgili, Nicolò Rossi, Elena Rosselli Del Turco, Viola Guardigni, Giovanni Fasulo, Nicola Dentale, Ciro Fulgaro, Giorgio Legnani, Emanuele Campaci, Cristina Basso, Alberto Zuppiroli, Amalia Sanna Passino, Giulia Tesini, Lucia Angelelli, Adriana Badeanu, Agostino Rossi, Giulia Santangelo, Flovia Dauti, Vidak Koprivika, Nicholas Roncagli, Ioannis Tzimas, Guido Maria Liuzzi, Irid Baxhaku, Letizia Pasinelli, Mattia Neri, Tommaso Zanaboni, Francesco Dell'Omo, Alice Gori, Idina Zavatta, Stefano Antonini, Chiara Pironi, Elena Piccini, Luca Esposito, Alessandro Zuccotti, Giacomo Urbinati, Agnese Pratelli, Alberto Sarti, Michela Semprini, Enrico Evangelisti, Mara D'Onofrio, Giuseppe Sasdelli, University of Bologna, Bologna, Italy. Elisabetta Pierucci; Intensive Care Unit, Department of Medical and Surgical Sciences, Policlinico Sant'Orsola, Bologna, Italy. Paolo Gaibani, Giada Rossini, Caterina Vocale; Centro di riferimento regionale per le emergenze microbiologiche (CRREM), Clinical Microbiology Unit, Department of Experimental, Diagnostic and Specialty Medicine, Policlinico Sant'Orsola, Bologna, Italy.

FUNDING

No external funding was received for the present study.

CONFLICT OF INTERESTS

Authors state no conflict of interest related to the content of the present study.

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Table 1. Comparison of microbiological tests among COVID-19 patients with pulmonary aspergillosis classified with either coronavirus-associated pulmonary aspergillosis (CAPA) or putative invasive pulmonary aspergillosis (PIPA)

Test	Total N= 108 (%)	CAPA n=30 (%)*	PIPA n=19 (%)*	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	1 (1)	1 (3)	1 (5)	0 (0)

(index > 0.5)				
Serum GM value, index, median (IQR)[#]	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[§]	26/67 (38)	20/30 (67)	19/19 (100)	5/36 (14)

Abbreviations: BAL bronchoalveolar lavage, CAPA coronavirus associated pulmonary aspergillosis, PIPA putative invasive pulmonary aspergillosis

*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

[#] performed in only 59 patients

§ performed in only 67 patients

Table 2. Comparison of patients with COVID-19 fulfilling criteria probable coronavirus-associated pulmonary aspergillosis (CAPA) with patients without CAPA

	CAPA **N=30 (%)**	**Non-CAPA** **N=73 (%)**	**p**
Demographics			
Age, years, mean (±SD)	63 (57-70)	63 (57-70)	0.86
Male	24 (80)	83 (77)	0.80
Underlying diseases			
Obesity	10 (37)	34 (49)	0.36
BMI, median (IQR)	28 (26-31)	29 (26-31)	0.92
Hypertension	16 (59)	49 (65)	0.64
Diabetes mellitus	5 (17)	13 (17)	0.99
Coronary disease	3 (10)	9 (11)	0.99
Cerebrovascular disease	3 (10)	1 (1.4)	0.06
Chronic kidney disease	6 (20)	6 (8)	0.08
COPD	4 (13)	13 (17.8)	0.10
Malignancies	2 (7)	5 (6)	0.99
Solid organ transplant	1 (3)	4 (5)	0.99
Chronic steroid treatment	5 (17)	2 (3)	0.02
Haemodialysis	3 (12)	3 (5)	0.36
Charlson index, median (IQR)	3 (1-4)	2 (1-4)	0.51
Symptoms at hospital admission			
Fever			0.009
> 38°C	16 (55)	61 (83)	
< 38°C	8 (28)	6 (8)	

Cough	16 (57)	44 (62)	0.51
Dyspnoea	22 (79)	62 (84)	0.57
Laboratory tests at admission			
White Blood Cells (10^9/L) median (IQR)	9.7 (4.9-14.0)	7.1 (5.2-10.1)	0.13
Neutrophils (10^9/L) median (IQR)	8.0 (3.9-13.4)	5.9 (4.0-8.8)	0.24
Lymphocytes (10^9/L) median (IQR)	0.76 (0.55-1.10	0.84 (0.50-1.01)	0.67
Creatinine (mg/dL), median	1.0 (0.77-2.05)	1.00 (0.77-1.38)	0.38
CRP (mg/dl), median (IQR)	11 (5-18)	11.8 (6.5-19.9)	0.37
LDH (IU/L), median (IQR)	375 (311-500)	389 (286-524)	0.67
SOFA score	3 (2-4)	3 (1-4)	0.81
COVID19 treatment			
Hydroxychloroquine	28 (93)	73 (94)	0.99
Azithromycin	9 (30)	31 (40)	0.38
Lopinavir	12 (40)	27 (35)	0.61
Darunavir	2 (7)	6 (8)	0.99
Remdesivir	3 (10)	5 (6)	0.68
Tocilizumab	22 (73)	57 (78)	0.80
Corticosteroids	18 (60)	34 (46.6)	0.29
Prednisone equivalents (mg) median (IQR)	100 (89-129)	107(70-133)	0.89
ICU admission			
Time from symptoms onset to ICU admission, days median (IQR)	8 (4-13)	9 (7-11)	0.73
Time from hospital admission to ICU days median (IQR)	3 (0-6)	3 (1-4)	0.68
First partial arterial O2 pressure to	153 (102-232)	153 (98.7-200)	0.50

fraction of inspired O2 after intubation			
Prone positioning	22 (76)	56 (83)	0.22
RRT	11 (37)	20 (26)	0.34
Inotropic support	19 (63)	50 (72)	0.47
Days of mechanical ventilation	13 (7-23)	16 (10-16)	0.09
ICU length of stay	16 (9-27)	21 (13-31)	0.08

Abbreviations: BMI body mass index; COPD chronic obstructive pulmonary disease; CRP C-reactive protein; GCS Glasgow coma scale; LDH lactate dehydrogenase; MAP mead arterial pressure; PR pulse rate; IQR interquartile range; IU international unit; ICU intensive care unit; RRT renal replacement therapy; SOFA sequential organ failure assessment

Table 3. Differences between survivors and non-survivors after 30 days from intensive care unit admission

	Survivors N=72 (%)	**Non-Survivors N= 36 (%)**	**P**
Demographics			
Age, years, mean (±SD)	64 (58-70)	63 (58-72)	0.98
Male	53 (73)	30 (83)	0.36
Underlying diseases			
Obesity	26 (42)	18 (51)	0.52
BMI, median (IQR)	28 (26-31)	30 (26-31)	0.28
Hypertension	44 (64)	21 (64)	0.99
Diabetes mellitus	12 (16)	6 (16)	0.99
Coronary disease	8 (11.1)	4 (11)	0.99
Cerebrovascular disease	0 (0)	4 (11)	0.01
Chronic kidney disease	5 (7)	7 (19)	0.10
COPD	12 (17)	5 (14)	0.78
Malignancies	4 (6)	3 (8)	0.64
Solid organ transplant	3 (4)	2 (6)	0.99
Chronic steroid treatment	2 (3)	5 (15)	0.04

Haemodialysis	1 (2)	5 (18)	0.02
Charlson index, median (IQR)	2 (1-3)	3 (2-5)	0.16
Laboratory tests at admission			
White Blood Cells (10^9/L) median (IQR)	8.2 (4.5-13.1)	10.3 (7.3-13.9)	0.87
Neutrophils (10^9/L) median (IQR)	6.1 (4.0-9.0)	8.3 (5.3-14.9)	0.99
Lymphocytes (10^9/L) median (IQR)	0.84 (0.59-1.0)	0.74 (0.59-1.00)	0.92
Creatinine (mg/dL), median	0.90 (0.72-1.1)	1.04 (0.8-2.1)	0.03
CRP (mg/dl), median (IQR)	12.7 (6.8-19.8)	11.3 (7.7-21.2)	0.98
LDH (IU/L), median (IQR)	382 (301-511)	418 (275-544)	0.71
SOFA score	3 (3-4)	4 (3-7)	0.03
COVID19 treatment			
Hydroxychloroquine	67 (93)	34 (94)	0.99
Azithromycin	24 (33)	16 (44)	0.29
Lopinavir	22 (28)	7 (19)	0.23
Darunavir	1 (5)	7 (8)	0.99
Remdesivir	3 (4)	5 (14)	0.11
Tocilizumab	55 (76)	27 (75)	0.99
Corticosteroids	32 (44)	24 (67)	0.04
ICU admission			
Time from symptoms onset to ICU admission, days median (IQR)	10 (7-13)	7 (5-10)	0.02
Time from hospital admission to ICU days median (IQR)	3 (1-5)	4 (0-7)	0.26
First partial arterial O2 pressure to	173 (107-216)	123 (86-172)	0.04

fraction of inspired O2 after intubation			
Prone positioning	53 (81)	28 (78)	0.79
RRT	13 (18)	18 (50)	0.001
Inotropic support	15 (79)	54 (67)	0.41
Tracheostomy	42 (62)	23 (66)	0.83

Abbreviations: BMI body mass index; COPD chronic obstructive pulmonary disease; CRP C-reactive protein; GCS Glasgow coma scale; LDH lactate dehydrogenase; MAP mead arterial pressure; PR pulse rate; IQR interquartile range; IU international unit; ICU intensive care unit; RRT renal replacement therapy; SOFA sequential organ failure assessment

Figure 1. Study flowchart

Figure 2. Kaplan Maier survival curves for 30-day mortality from ICU admission. Patients were stratified as having probable coronavirus-associated pulmonary aspergillosis (CAPA) (Panel A) or putative pulmonary aspergillosis (PIPA) (panel B)

Figure 3. Relationship between initial bronchial alveolar lavage galactomannan index and 30-day mortality.

Figure 4. Effect of voriconazole treatment among patients with probable coronavirus-associated pulmonary aspergillosis (CAPA). Comparison of patients receiving or not voriconazole treatment (A). Reduction of bronchoalveolar lavage 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).

Figure 1

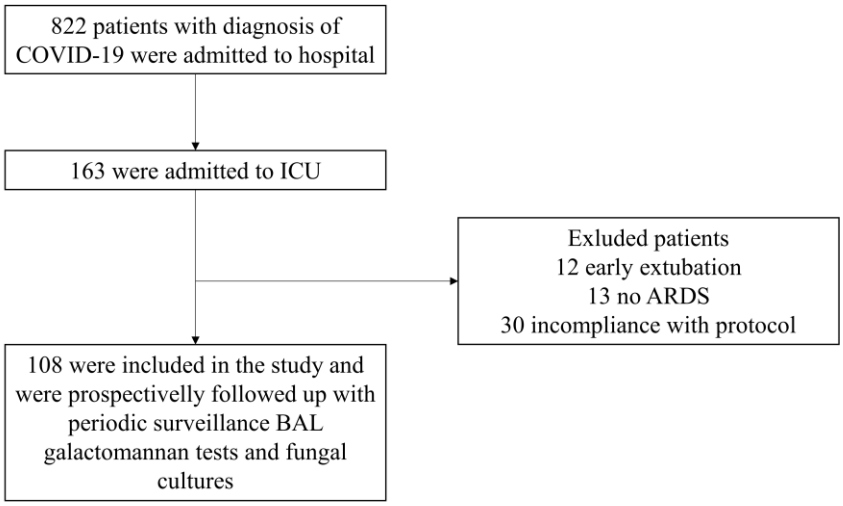

Figure 2

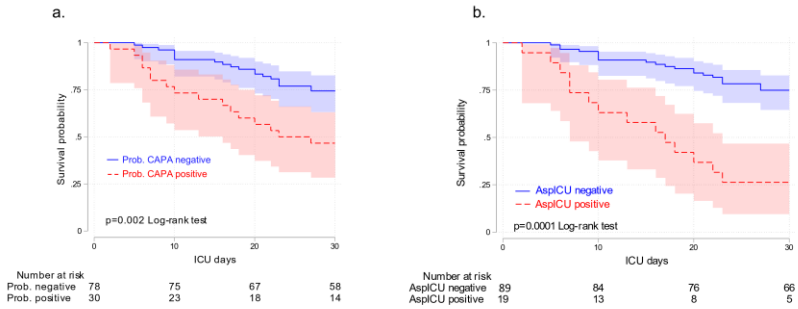

Figure 3

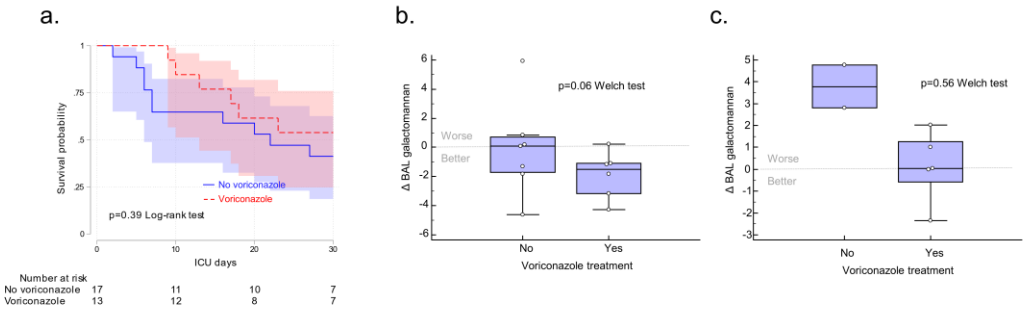

Figure 4