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## Fungal infections in mechanically ventilated patients with COVID-19 during the first wave: the French multicentre MYCOVID study

Jean-Pierre Gangneux, Eric Dannaoui, Arnaud Fekkar, Charles-Edouard Luyt, Françoise Botterel, Nicolas de Prost, Jean-Marc Tadié, Florian Reizine, Sandrine Houzé, Jean-François Timsit, et al.

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1 **High prevalence of fungal infections in mechanically ventilated COVID-19 patients in**  
2 **the ICU: The French multicenter MYCOVID study**

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5 Jean-Pierre Gangneux, Eric Dannaoui, Arnaud Fekkar, Charles-Edouard Luyt, Françoise  
6 Botterel, Nicolas De Prost, Jean-Marc Tadié, Florian Reizine, Sandrine Houze, Jean-François  
7 Timsit, Xavier Iriart, Béatrice Riu-Poulenc, Boualem Sendid, Saad Nseir, Florence Perlat,  
8 Florent Wallet, Patrice Le Pape, Emmanuel Canet, Ana Novara, Melek Manai, Estelle  
9 Perraud, Arnaud Thille, Sophie Brun, Yves Cohen, Alexandre Alanio, Bruno Mégarbane,  
10 Muriel Cornet, Nicolas Terzi, Lionel Lamhaut, Estelle Sabourin, Guillaume Desoubeaux,  
11 Stephan Ehrmann, Christophe Hennequin, Muriel Fartoukh, Gilles Nevez, Cécile Aubron,  
12 Valérie Letcher-Bru, Ferhat Meziani, Julien Mayaux, Antoine Monsel, Marion Blaize,  
13 Frédérique Boquel, Florence Robert-Gangneux, Yves Le Tulzo, Philippe Seguin, Hélène  
14 Guegan, Brice Autier, Matthieu Lesouhaitier, Romain Pelletier, Sorya Belaz, Christine  
15 Bonnal, Antoine Berry, Jordan Leroy, Nadine François, Chantal Fradin, Jean-Christophe  
16 Richard, Sylvie Paulus, Laurent Argaud, Damien Dupont, Jean Menotti, Florent Morio,  
17 Stéphane Gaudry, Carole Schwebel, Cécile Garnaud, Juliette Guitard, Solène Le Gal,  
18 Dorothee Quinio, Jeff Morcet, Bruno Laviolle, Jean-Raph Zahar, Marie-Elisabeth Bougnoux

19  
20

21 CHU de Rennes, Rennes (JP Gangneux, JM Tadié, F Reizine, F Robert-Gangneux, Y Le  
22 Tulzo, P Seguin, H Guegan, B Autier, M Lesouhaitier, R Pelletier, S Belaz, J Morcet, B  
23 Laviolle) ; CHU Hôpital Européen Georges Pompidou – APHP, Paris (E Dannaoui, A  
24 Novara, M Manai) ; CHU La Pitié-Salpêtrière – APHP, Paris (A Fekkar, CE Luyt, J Mayaux,  
25 A Monsel, M Blaize) ; CHU Henri Mondor – APHP, Créteil (F Botterel, N De Prost, F  
26 Boquel) ; CHU Bichat – APHP, Paris (S Houze, JF Timsit, C Bonnal) ; CHU de Toulouse,  
27 Toulouse (X Iriart, B Riu-Poulenc, A Berry, J Leroy, N François) ; CHU de Lille, Lille (B  
28 Sendid, S Nseir, C Fradin) ; Hospices civils de Lyon, Lyon (F Persat, F Wallet, JC Richard, S  
29 Paulus, L Argaud, D Dupont, J Menotti) ; CHU de Nantes, Nantes (P Le Pape, E Cannet, F  
30 Morio) ; CHU de Poitiers, Poitiers (E Perraud, A Thille, S Gaudry) ; CHU Avicenne – APHP,  
31 Bobigny (S Brun, Y Cohen, C Garnaud, JR Zahar) ; CHU Lariboisière/Saint-Louis – APHP,  
32 Paris (B Megarbane, A Alanio) ; CHU de Grenoble, Grenoble ( M Cornet, N Terzi, L  
33 Lamhaut, C Schwebel, C Garnaud) ; CHU Necker Enfants malades – APHP, Paris (L  
34 Lamhaut, E Sabourin, ME Bougnoux) ; CHU de Tours, Tours (G Desoubeaux, S Ehrmann, J  
35 Guitard) ; CHU Saint-Antoine/Tenon – APHP, Paris (C Hennequin, M Fartoukh) ; CHU de  
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38

39 *1 Covering letter*

40 *2 Manuscript including tables and panels*

41 *3 Figures*

42 *4 Authors statement form (see next section)*

43 *5 Declaration of interests and source of funding statements (see next section)*

44 *6 In-press papers—one copy of each with acceptance letters*

45 *7 Protocols and CONSORT details for randomised controlled trials (see Articles)*

46 *Texte: 3500 mots*

47 *30 réf*

48 **Summary** (300 mots)

49

## 50 **Background**

51 Patients with severe COVID-19 have clearly emerged as a population with a high risk of  
52 invasive fungal infections (IFI). However, the prevalence of IFI has not yet been  
53 prospectively assessed in large populations of mechanically ventilated COVID-19 patients.  
54 We aimed to determine the prevalence of IFI, the risk factors, and the mortality associated  
55 with COVID-19-associated pulmonary aspergillosis (CAPA) in mechanically ventilated  
56 patients in the ICU.

## 57 **Methods**

58 We performed a national multicenter observational cohort study among 18 French ICUs and  
59 enrolled 576 mechanically ventilated COVID-19 patients. Patients were systematically  
60 screened for fungal microorganisms once/twice a week during the period of mechanical  
61 ventilation up to ICU discharge, and CAPA was defined according to recent definitions. The  
62 trial is registered at ClinicalTrials.gov, NCT04368221.

63

## 64 **Findings**

65 In total, 509 patients with  $\geq 3$  screening samples were analyzed (mean age of 59.4 years and  
66 78.6% were male). The prevalence of proven/probable CAPA was 17.1% (87/509) and 4.7%  
67 (24/509) for possible CAPA. Thirty-eight (7.5%) patients experienced other IFIs: 32  
68 candidemia (6.3%), six mucormycosis (1.2%), and one fusariosis. Four patients had a positive  
69 PCR for *Pneumocystis jirovecii*. Multivariate analysis of factors associated with death in the  
70 entire study population showed three significant factors: age  $\geq 62$  (HR: 1.83 [95% CI 1.35-  
71 2.48]), solid organ transplantation (2.33 [1.43-3.79]), and proven/probable CAPA (1.77  
72 [1.25-2.48]). Severity scores were significantly higher for CAPA and mortality was  
73 significantly higher in cases of proven/probable and possible CAPA than no CAPA: 59.8%,  
74 45.8%, and 30.9%, respectively ( $p < 0.0001$ ). Anti-IL6-treated patients were characterized by  
75 a significantly younger age, dexamethasone treatment (6.64 [2.89-15.23]), positive  
76 galactomannan antigenemia (5.01 [1.47-17.09]), and proven/probable/possible CAPA (2.60  
77 [1.11-6.06]).

## 78 **Interpretation**

79 This study shows the dramatic prevalence of CAPA and candidiasis associated with a high  
80 mortality rate in mechanically ventilated COVID-19 patients. These findings highlight the  
81 need of active surveillance for fungal pathogens in severe COVID-19 patients.

82

## 83 **Funding**

84 Pfizer Inc., NY, USA

85

86

87 **Key words:** Covid-19 associated pulmonary aspergillosis (CAPA), invasive pulmonary  
88 aspergillosis (IPA) Acute respiratory distress syndrome (ARDS), mechanical ventilation,  
89 ICU, Candidemia, anti-IL6

90

91

## 92 **Research in context a faire**

93 *Autres Recos:*

94 *We use a comma before the final “and” or “or” in a list of items • Type decimal points midline (ie, 23.4, not*  
95 *23.4). To create a midline decimal on a PC: hold down ALT key a*

96 *Figures : no less than 300 dpi when set at its final printed size. Ideal file formats are TIF or JPG*



98 **Introduction**

99 Fungal infections are known to be among the infectious complications related to the damage  
100 caused by viral pulmonary infections, particularly in intensive care unit (ICU) patients with  
101 severe acute respiratory distress syndrome (ARDS). Indeed, invasive pulmonary aspergillosis  
102 (IPA) is now a well-known complication of the clinical course of critically ill patients with  
103 influenza.<sup>1, 2</sup> Patients with severe COVID-19 have also emerged as a population with a high  
104 risk of fungal infections.<sup>3, 4</sup> COVID-19 is associated with epithelial lung damage,  
105 lymphopenia, dysfunction of the cell response, and the use of broad spectrum antibiotics,  
106 dexamethasone, and immunosuppressive therapies.<sup>5</sup> Thus, adherence of *Aspergillus* conidia  
107 may be promoted by prior damage of the lungs due to SARS- CoV-2 infection and multiple  
108 risk factors may lead to tissue invasion after conidia germination.<sup>6, 7</sup>

109 During the COVID-19 pandemic, several case series have been published that suggest an  
110 increased risk of IPA in patients with severe COVID-19 (COVID-19-associated pulmonary  
111 aspergillosis - CAPA), with an estimated prevalence of <5% to >30%, whereas the prevalence  
112 of candidemia and other fungal infections is still poorly known.<sup>5</sup> However, the heterogeneity  
113 of monitoring protocols, diagnostic tools, and severity of COVID-19 patients included in  
114 various studies (all hospitalized COVID-19 patients, only patients in the ICU, or only patients  
115 in the ICU with ARDS) make it difficult to interpret the data published in the literature.

116 It is only recently that specific criteria for the case definition and diagnosis of CAPA were  
117 published in The Lancet Infectious Diseases journal.<sup>8</sup> Consensual recommendations are  
118 essential because patients who have been infected during the pandemic do not meet the usual  
119 EORTC/MSG criteria for invasive aspergillosis, as there are no host-related risk factors nor  
120 specific clinical features.<sup>9</sup> In addition, the more focused AspICU algorithm to diagnose  
121 invasive pulmonary aspergillosis (IPA) in critically ill patients does not include new  
122 diagnostic developments and appears to be ill adapted to COVID-19 patients.<sup>10, 11</sup>  
123 Furthermore, the typical radiological features of aspergillosis may be difficult to assess in  
124 ARDS-associated COVID-19.<sup>12</sup> Finally, the sensitivity and specificity of current mycological  
125 tests have still not been completely evaluated in this specific population.

126 Here, we sought to characterize the prevalence, risk factors, and outcome of invasive fungal  
127 infections (IFI), including CAPA and candidemia, in COVID-19 infected and mechanically  
128 ventilated patients in the ICU using the new definitions of CAPA and a standardized and  
129 rigorous protocol registered at ClinicalTrials.gov ID: NCT04368221.

130

## 131 **Methods**

### 132 **Study design and participants**

133 We performed a national multicenter observational cohort study among ICU COVID-19  
134 patients to determine the burden of IFI. Adult patients ( $\geq 18$  years) were eligible for enrolment  
135 if they had an RT-PCR-confirmed diagnosis of SARS-CoV-2 infection and required  
136 mechanical ventilation for ARDS. Patients were consecutively included between February  
137 and July 2020 in 18 participating university hospital ICUs across France (**figure S1**). The  
138 patients' clinical and biological data were collected as part of their follow-up from admission  
139 to discharge from the ICU. Data were collected anonymously and managed using eCRF  
140 available at ClinicalTrials.gov ID: NCT04368221.

141 The MYCOVID study was conducted in full concordance with the principles of the  
142 Declaration of Helsinki and the laws, and regulations of France. This study was approved by  
143 the French authorities (Comité Consultatif sur le Traitement de l'information en matière de  
144 Recherche dans le domaine de la Santé, and Commission Nationale de L'Informatique et des  
145 Libertés) and registered as No. 910346. According to French policy, a non-opposition  
146 statement was obtained for all included patients or, where applicable, a relative or their legal  
147 representative, signifying that all received written detailed information about the objectives of  
148 the study and were free to request withdrawal of their data at any time. The study protocol  
149 was reviewed and approved by the ethics committee of Rennes University Hospital, Rennes,  
150 France (Approval no. 20.56). The study was registered at clinicaltrial.gov under the number  
151 NCT04368221.

152

### 153 **Procedures**

154 In each participating ICU, patients with ARDS-associated COVID-19 who were mechanically  
155 ventilated were systematically screened for fungal respiratory microorganisms once or twice a  
156 week during the period of mechanical ventilation up to ICU discharge, resulting in a  
157 minimum of three samples if discharge occurred after 15 days (**figure 1A**). Respiratory  
158 samples included tracheal aspiration (TA), and/or bronchial aspiration (BA), and/or  
159 bronchioloalveolar lavage (BAL). The local microbiological laboratory in each participating  
160 center processed the fungal cultures of the samples. Real-time quantitative PCR (RT-qPCR)  
161 for *Aspergillus*, *Pneumocystis jirovecii*, and Mucorales were also performed on the same  
162 samples either locally or following referral to a specialized laboratory. The other

163 microbiology samples and tests were performed according to the local ICU hospital standard  
164 of care. Specific blood biomarkers (qPCR for either *Aspergillus* or Mucorales and  
165 galactomannan antigenemia) were sought if respiratory samples were positive either for  
166 filamentous fungi or *P. jirovecii*. Demographic and clinical data (underlying diseases, gravity  
167 scores (Simplified Acute Physiology Score II [SAPS II], Sequential Organ Failure  
168 Assessment [SOFA], anti-COVID-19 treatments), laboratory data (lymphopenia, viral  
169 detection of HSV and CMV, bacterial and fungal ventilated-associated pneumonia [VAP],  
170 and candidemia), and antifungal treatments received were recorded in the eCRF.

171 The local investigators were requested to classify patients as being colonized or infected with  
172 proven or putative pulmonary aspergillosis according to the AspICU classification currently  
173 being used at the time of the study. During the analysis of data, the CAPA consensual  
174 definitions were published by Koehler et al. and were thus also used for the statistical  
175 analysis.

176

## 177 **Outcomes**

178 The outcomes of interest were the prevalence of IFIs and fungal respiratory colonization and  
179 their impact on ICU mortality. CAPA was defined and classified according to the most recent  
180 definitions.<sup>8</sup> The characteristics of CAPA patients were compared to those of non-CAPA  
181 patients and to proven/putative IPA according to the AspICU algorithm. Candidemia was  
182 defined as one or more positive blood cultures for *Candida* spp. For patients with a positive  
183 qPCR for *P. jirovecii*, the Ct was recorded but it was not possible to discriminate between  
184 infection and colonization. Invasive mucormycosis were defined by the local investigators  
185 according to the clinical signs associated to a positive specific qPCR or culture from  
186 respiratory samples and/or a positive blood qPCR for the same genus. The diagnosis of other  
187 invasive fungal infections was performed on a case-by-case basis for respiratory samples with  
188 positive cultures for other filamentous fungi.

189

## 190 **Statistical analysis**

191 An independent data monitoring committee provided an external medical and statistical  
192 review of the efficacy and safety data. Continuous variables are presented as means, with  
193 standard deviations, and medians (interquartile range) and categorical variables as numbers  
194 (percentage), with the corresponding 95% confidence interval. Subgroups (for example,  
195 positive and negative patients) were compared using standard statistical approaches: chi<sup>2</sup> tests  
196 for categorical variables and Student t or Wilcoxon rank sum tests for continuous variables.

197 Multivariate analysis using a Cox model or logistic regression was performed to determine the  
198 parameters most highly associated with invasive fungal infections and mortality. Survival  
199 curves were constructed using the Kaplan-Meier method and were compared using the log  
200 rank test. Two-sided tests were performed and considered statistically significant for p-values  
201 <0.05. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary,  
202 NC, USA). The full population for prevalence calculations was defined as patients who did  
203 not oppose participation in the study, met the eligibility criteria, and were systematically  
204 screened for fungal respiratory microorganisms once or twice a week from entry to discharge  
205 from the ICU.

206

### 207 **Role of the funding source**

208 The study sponsor (Pfizer Inc., NY, USA) was not involved in the study design, collection,  
209 analysis, or interpretation of the data, writing of the report, or the decision to submit the paper  
210 for publication. The corresponding author had full access to all the data and the final  
211 responsibility to submit the manuscript for publication. All authors vouch for the accuracy  
212 and completeness of the data, data analyses, and interpretation and fidelity to the protocol and  
213 approved the final manuscript for submission.

214

### 215 **Results**

216 Between February 29 and July 9, 2020, 576 mechanically ventilated COVID-19 patients, from  
217 18 French ICUs, were enrolled in the MYCOVID study. The final analysis was carried out on  
218 509 patients, as 11 were withdrawn/excluded and 56 did not undergo complete mycological  
219 screening or follow-up (<3 screening samples) (**figure 1B**). The mean number of samples  
220 (respiratory and serum) was  $6.6 \pm 3.8$  (median 6.0 [95% CI 6.3-7.0]) for the whole population.  
221 The number of included patients and the period of inclusion according to the center are  
222 presented in **figure S1**.

223

224



225 *Baseline characteristics*

226 The mean duration of ICU stay was 32.7±24.1 days (median 26.0, minimum 1, maximum  
 227 204). The baseline characteristics of the 509 patients included in the statistical analysis are  
 228 shown in **table 1**. The mean age was 59.4±12.5 years (median 60.7) and 79% were male. The  
 229 mean body mass index was 29.3±5.6 (median 28.4 [95% CI 28.7-29.8]). The baseline  
 230 characteristics and comorbidities were typical of COVID-19, with 32.9% (28.8-37.1) of  
 231 patients presenting with diabetes mellitus, 50.1% (45.7-54.5) with high blood pressure, and  
 232 64.2% (59.9-68.4) with initial lymphopenia. Anti-COVID-19 treatments are listed in **table 1**.  
 233 The mean duration of mechanical ventilation was 27.1±19.8 days (25.5-28.9). Severity  
 234 scores (SAPS II and SOFA) are presented in **table 1** and 186/509 patients died in the ICU  
 235 (36.5% [32.3-40.9]).

236

237

238 **Table 1. Demographic and baseline characteristics and clinical course data for 509**  
 239 **mechanically ventilated COVID-19 patients in the intensive care unit (ICU).**

240

	<b>Demographic characteristics</b> Mean ± SD, and 95% CI
Sex at birth (509)	
- Female	109 (21.4% [17.9 - 25.2])
- Male	400 (78.6% [74.8 - 82.1])
Age, years (509)	59.4 ± 12.5 (58.1 - 60.4)
Weight, kg (490)	86.9 ± 17.6 (85.3 - 88.5)
Height, cm (490)	172.4 ± 9.1 (171.6 - 173.2)
BMI, kg/m <sup>2</sup> (490)	29.3 ± 5.6 (28.8 - 29.8)
	<b>Baseline characteristics</b> n (% , [95% CI])
Obesity	62 (12.2% [9.5 - 15.3])
Dyslipidemia	21 (4.1% [2.6 - 6.2])
Diabetes mellitus	167 (32.9% [28.8 - 37.1])
High blood pressure	254 (50.1% [45.7 - 54.5])
COPD	34 (6.7% [4.7 - 9.2])
Asthma	14 (2.8% [1.5 - 4.6])
Initial lymphopenia	323 (64.2% [59.9 - 68.4])
Transplantation	35 (6.9% [4.8 - 9.4])
Hematological malignancy	6 (1.2% [0.4 - 2.6])
All immunosuppression	55 (10.8% [8.2 - 13.8])
Lopinavir+Ritonavir (Kaletra)	111 (21.8% [20.7 - 33.9])
Remdisivir	20 (3.9% [2.4 - 6.0])
Oseltamivir	40 (7.9% [5.7 - 10.6])

Cefotaxime	25 (4.9% [3.2 - 7.2])
Rovamycin	15 (2.9% [1.7 - 4.8])
Azithromycin	10 (2.0% [0.9 - 3.6])
Hydroxychloroquine	167 (32.9% [28.8 - 37.1])
Dexamethasone	202 (40.1% [35.8 - 44.5])
Anti-IL6	38 (7.5% [5.4 - 10.2])
Anti-IL1	16 (3.1% [1.8 - 5.1])
Prone position	394 (77.6% [73.7 - 81.1])
<b>Clinical course data</b>	
Mean $\pm$ SD, and 95% CI	
Duration of mechanical ventilation, days (508)	27.1 $\pm$ 19.8 (25.4 - 28.9)
SAPS II – admission (485)	44.1 $\pm$ 16.2 (42.6 - 45.5)
SOFA – admission (398)	7.4 $\pm$ 3.9 (7.0 - 7.8)
SOFA – day 7 (367)	8.7 $\pm$ 4.2 (8.3 - 9.1)
SOFA – day 15 (261)	8.5 $\pm$ 4.6 (7.9 - 9.0)
SOFA - discharge (285)	6.1 $\pm$ 6.0 (5.4 - 6.8)
Death in ICU (509)	186 (36.5% [32.3 - 40.9])

241 BMI: body-mass index, COPD: chronic obstructive pulmonary disease, SAPS II: Simplified Acute Physiology  
242 Score II; SOFA: Sequential Organ Failure Assessment.

243

#### 244 *Prevalence of secondary infections*

245 The prevalence of secondary infections is presented in [table 2](#). We compared the observed  
246 prevalence of invasive aspergillosis using either the definition according to Koehler et al.<sup>8</sup>  
247 (i.e. proven/probable/possible CAPA) or that according to Blot et al.<sup>11</sup> (i.e. proven/putative  
248 IPA). In total, 111/509 (21.8%) patients were classified as having CAPA according to  
249 Koehler et al.;<sup>8</sup> 87/509 (17.1%) fulfilled the criteria for proven/probable CAPA and 24/509  
250 (4.7%) for possible CAPA. Use the AspICU classification published by Blot et al.<sup>11</sup> resulted  
251 in 57/509 (11.2%) patients being classified by the local investigators as proven/putative IPA  
252 and 50/509 (9.8%) as colonized. Concerning other IFIs, 38 patients (7.5%) experienced  
253 another invasive fungal infection, sometimes with co-infections with one or two different  
254 fungi, consisting of 32 cases of candidemia (6.3%), six of invasive mucormycosis (1.2%),  
255 and one of invasive fusariosis. Of note, four patients had a positive PCR for *Pneumocystis*  
256 *jirovecii* that could be classified either as infection or colonization. Aside from fungal  
257 infections, 374 (73.5%) patients were diagnosed with bacterial ventilator-associated  
258 pneumonia (VAP), 76 (15.5%) with a HSV1 viral infection, and 49 (10.0%) with a CMV  
259 infection ([table 2](#)). Overall, 162 (31.8%) patients received antifungal treatment during the

260 mechanical ventilation period, among them 58/162 received more than one antifungal).  
 261 Voriconazole and caspofungin were the two most frequently administered antifungal drugs  
 262 (table 2).

263  
 264  
 265  
 266

**Table 2. Prevalence of infections in 509 mechanically ventilated COVID-19 patients in the intensive care unit (ICU) and antifungal treatments.**

	Prevalence of infections n (%), [95% CI]	267 268 269	*CA PA acco rdin g to Koe hler et al. <sup>8</sup> , pr/p b: prov en/p roba ble, pos: poss ible
CAPA status*			
- pr/pb invasive aspergillosis	87 (17.1% [13.9 - 20.6])		
- pos invasive aspergillosis	24 (4.7% [3.0 - 6.9])		
AspICU status#		273	
- pr/pu invasive aspergillosis	57 (11.2% [8.6 - 14.3])	274	
- Aspergillus colonization	50 (9.8% [7.4 - 12.7])	275	
Other invasive fungal infections (one or more)	38 (7.5% [5.3 - 10.1])	276	
- Candidemia	32 (6.3% [4.3 - 8.8])		
- Invasive mucormycosis	6 (1.2% [0.4 - 2.5])		
- Invasive fusariosis	1 (0.2% [0.0 - 1.1])		
Bacterial VAP	374 (73.5% [69.4 - 77.3])		
CMV infection	49 (10.0% [7.5 - 13.0])	283	
HSV1 infection	76 (15.5% [12.4 - 19.0])	284	
	<b>Duration of antifungal treatment (days)</b>		#Ac cord ing to Blot et al. <sup>11</sup> , pr/p u:
	Mean ± SD (min, median, max)		
Fluconazole (n=30)	19.2 ± 20.4 (2, 14, 84)		
Caspofungin (n=70)	7.9 ± 9.1 (1, 5, 60)		
Liposomal amphotericin B (n=34)	11.8 ± 27.5 (1, 4, 156)		
Voriconazole (n=73)	15.2 ± 16.1 (1, 10, 88)		
Isavuconazole (n=15)	12.3 ± 6.1 (3, 12, 26)		
		294	

295 proven/putative

296  
 297

298 *Invasive aspergillosis*

299 We used the case definition of CAPA according to Koehler et al.<sup>8</sup> in all subsequent analyses.  
 300 Invasive aspergillosis was the most prevalent IFI in this cohort. We analyzed the risk factors  
 301 and impact of CAPA on mortality. The main significant differences between the  
 302 proven/probable/possible CAPA group and the non-CAPA group were the mean age of the  
 303 patients (62.2±13.3 versus 58.6±12.2, respectively,  $p=0.007$ ) and an immunosuppressed  
 304 status (16.2% versus 9.3%, respectively,  $p=0.038$ ), particularly transplantation (11.7% versus  
 305 5.5%, respectively,  $p=0.023$ ) (table 3). Comparison of the proven/probable CAPA group with  
 306 others (including possible CAPA and non-CAPA) showed significant differences for exactly  
 307 the same variables: higher mean age ( $p=0.025$ ), total immunosuppression ( $p=0.034$ ), and  
 308 transplantation ( $p=0.05$ ). Thirty-eight patients received anti-IL6 treatment for COVID-19,

309 showing a statistical difference between the CAPA group (15.3%) and non-CAPA group  
 310 (5.3%,  $p=0.0016$ ).

311 The mean duration of mechanical ventilation was  $31.3 \pm 24.5$  days for patients with CAPA  
 312 ( $32.1 \pm 25.3$  days for proven/probable CAPA and  $28.1 \pm 21.7$  for possible CAPA) versus  
 313  $26.0 \pm 18.1$  for patients without CAPA ( $p=0.013$ ). The severity scores were significantly  
 314 higher for the CAPA patients (table 3). Survival curves show that mortality was significantly  
 315 different between the three groups: 30.9%, 45.8%, and 59.8% for the non-CAPA, possible  
 316 CAPA, and proven/probable CAPA groups, respectively ( $p<0.0001$ ) (figure 2). Seventy-  
 317 seven percent (67/87) of proven/probable CAPA patients received at least one antifungal  
 318 agent (33/67 received more than one antifungal). Among them, 67.2% (45/67) received  
 319 voriconazole, 31.3% (21/67) liposomal amphotericin B, 29.9% (20/67) caspofungin, 16.4%  
 320 (11/67) isavuconazole, and 16.4% (11/67) other antifungal drugs, alone or in combination.

321

322

323

324 **Table 3. Demographic and baseline characteristics, immunosuppressive and**  
 325 **antimicrobial agents, and severity scores between CAPA and non-CAPA patients under**  
 326 **mechanical ventilation.**  
 327

	pr/pb/pos CAPA* (n=111)	Non-CAPA* (n=398)	
Onset factors of CAPA* (n, %)	n, value or % (95% CI)	n, value or % (95% CI)	p
Sex at birth			0.84
Female (109, 21%)	23, 20.7% (13.6-29.5)	86, 21.6% (17.7-26.0)	
Male (400, 79%)	88, 79.3% (70.5-86.4)	312, 78.4% (74.0-82.3)	
Age, years	111	398	0.005
Mean $\pm$ SD, (95% CI)	62.2 $\pm$ 13.3 (59.7-64.7)	58.6 $\pm$ 12.2 (57.4-59.8)	
Weight, kg	104	387	0.07
Mean $\pm$ SD, (95% CI)	84.1 $\pm$ 18.5 (80.5-87.7)	87.3 $\pm$ 17.3 (85.9-89.4)	
BMI, kg/m <sup>2</sup>	102	384	0.11
Mean $\pm$ SD, (95% CI)	28.5 $\pm$ 5.4 (27.4-29.6)	29.5 $\pm$ 5.7 (28.9-30.1)	
Obesity (62, 12.2%)	9 8.1% (3.8-14.8)	53 13.3% (10.1-17.1)	0.14
Diabetes mellitus (167, 32.9%)	41 36.9% (28.0-46.6)	126 31.7% (27.2-36.6)	0.31
High blood pressure (254, 50.1%)	57 51.8% (42.1-61.4)	197 49.6% (44.6-54.7)	0.68
COPD (34, 6.7%)	11 9.9% (5.1-17.0)	23 5.8% (3.7-8.5)	0.12
Asthma (14, 2.8%)	5 4.5% (1.5-10.2)	9 2.3% (1.0-4.2)	0.20
Transplantation (35, 6.9%)	13 11.7% (6.4-19.2)	22 5.5% (3.5-8.2)	0.023
Hematological malignancy	0	6	0.35

(6, 1.2%)	0.0% (0.0-3.3)	1.5% (0.6-3.3)	
All immunosuppression	18	37	0.038
(55, 10.8%)	16.2% (9.9-24.4)	9.3% (6.6-12.6)	
Lopinavir+Ritonavir (Kaletra)	21	90	0.40
(111, 21.8%)	18.9% (12.1-27.5)	22.6% (18.6-27.0)	
Remdisivir	4	16	1.0
(20, 3.9%)	3.6% (1.0-9.0)	4.0% (2.3-6.4)	
Oseltamivir	11	29	0.42
(40, 7.9%)	9.9% (5.1-17.0)	7.3% (4.9-10.3)	
Cefotaxime	5	20	0.82
(25, 4.9%)	4.5% (1.5-10.2)	5.0% (3.1-7.7)	
Rovamycin	6	9	0.11
(15, 2.9%)	5.4% (2.0-11.4)	2.3% (1.0-4.2)	
Azithromycin	4	6	0.24
(10, 2.0%)	3.6% (1.0-9.0)	1.5% (0.6-3.3)	
Hydroxychloroquine	30	137	0.17
(167, 32.9%)	27.0% (19.0-36.3)	34.5% (29.8-39.4)	
Dexamethasone	48	154	0.34
(202, 40.1%)	44.0% (34.5-53.9)	39.0% (34.1-44.0)	
Anti-IL6	17	21	0.0016
(38, 7.5%)	15.3% (9.2-23.4)	5.3% (3.3-8.0)	
Anti-IL1	3	13	1.0
(16, 3.1%)	2.7% (0.6-7.7)	3.3% (1.8-5.5)	
Duration of mechanical ventilation (508)	111	397	0.013
	31.3 ± 24.5 (26.6-35.9)	26.0 ± 18.1 (24.2-27.8)	
Prone position	89	305	0.52
(394, 77.6%)	80.2% (71.5-87.1)	76.8 (72.4%-80.9)	
SAPS II – admission	107	378	0.021
(485, 44.1 ± 16.2)	47.3 ± 17.5 (43.9-50.6)	43.2 ± 15.7 (41.6-44.8)	
SOFA – admission	100	298	0.22
(398, 7.4 ± 3.9)	7.8 ± 3.7 (7.1-8.6)	7.3 ± 4.0 (6.8-7.7)	
SOFA – day 7	93	273	0.010
(367, 8.7 ± 4.2)	9.6 ± 3.7 (8.8-10.4)	8.4 ± 4.4 (7.9-8.9)	
SOFA – day 15	78	183	0.075
(261, 8.5 ± 4.6)	9.2 ± 4.2 (8.3-10.2)	8.1 ± 4.8 (7.4-8.8)	
SOFA - discharge	81	204	<0.0001
(285, 6.1 ± 6.0)	8.3 ± 6.1 (7.0-9.7)	5.3 ± 5.8 (4.5-6.1)	

328 \*According to Koehler et al.<sup>8</sup>. SAPS II: Simplified Acute Physiology II, SOFA: Sequential Organ Failure  
329 Assessment, BMI: body-mass index, COPD: chronic obstructive pulmonary disease.  
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### Mortality

The severity of the SAPS II and SOFA scores was significantly associated with death in the ICU: 186/509 patients (36.5% [95% CI 32.3-40.9], [table 4](#)).

Three host factors were significantly associated with overall mortality in the ICU: older age ( $p=0.0001$ ), immunosuppression ( $p=0.019$ ), and, in particular, transplantation ( $p=0.0008$ , [table 4](#)). Among the treatments and management measures administered in the ICU to the patient population, the combination of lopinavir+ritonavir (Kaletra) ( $p=0.035$ ) and anti-IL1 ( $p=0.036$ ) were associated with death in the ICU, whereas cefotaxime was associated with survival ( $p=0.0005$ , [table 4](#)). The duration of mechanical ventilation was longer for survivors ( $28.9 \pm 21.2$  [95% CI 26.5-31.2]) than for the patients who died ( $24.1 \pm 16.6$  [21.7-26.5], [table 4](#)). Other treatment or management measures showed no significant statistical differences in terms of mortality in the ICU ([table 4](#)).

Among secondary infections, only fungal co-infections were significantly associated with death. In addition to the survival curves showing mortality to be significantly higher for CAPA patients ([figure 2](#)), invasive aspergillosis was associated with death in the univariate analysis ([table 4](#)), regardless of the case definition used: proven/probable/possible CAPA ( $p<0.0001$ ), proven/probable CAPA ( $p<0.0001$ ), possible CAPA ( $p=0.01$ ), and proven/putative IPA ( $p=0.011$ ). The detection of *Aspergillus* in respiratory samples by culture or PCR was also associated with death ( $p=0.005$  and  $p=0.05$ , respectively, data not shown), regardless of the aspergillosis status. The introduction of anti-*Aspergillus* triazole (voriconazole and/or isavuconazole) did not modify mortality ([figure 3](#),  $p=0.86$ ), nor did other antifungal drugs increase survival.

Aside from invasive *Aspergillus* infections, among the other infections, only candidemia was associated with mortality ( $p=0.017$ , [table 4](#)). There was no significance for bacterial VAP, nor HSV1 or CMV infections. We then performed a multivariate analysis of factors associated with death, which identified three significant factors: age  $\geq 62$  (HR 1.83 [95% CI 1.35-2.48]), solid organ transplantation (HR 2.33 [1.43-3.79], and proven/probable CAPA (HR 1.77 [1.25-2.48], [figure 4](#)). Of note, possible CAPA and candidemia, although not significantly associated with death, showed, however, a trend toward higher mortality (HR 1.37 [0.74-2.55] and 1.19 [0.71-2.01], respectively).

364 **Table 4. Baseline characteristics, host factors, treatments, and secondary infections**  
 365 **associated with death in the intensive care unit (ICU) of 509 mechanically ventilated**  
 366 **COVID-19 patients.**  
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Factors (n, %)	Death in the ICU (n=186)	Survival (n=323)	p
	n, %, (95% CI)	n, %, (95% CI)	
Sex at birth			0.19
Female (109, 21%)	34, 18.3% (13.0-24.6)	75, 23.2% (18.7-28.2)	
Male (400, 79%)	152, 81.7% (75.4-87.0)	248, 76.8% (71.8-81.3)	
Age, years	62.3 ± 10.9	57.6 ± 13.1	0.0001
Mean ± SD, (95% CI)	(60.7-63.9)	(56.2-59.1)	
Weight, kg	86.8 ± 17.8	86.8 ± 17.5	0.93
Mean ± SD, (95% CI)	(84.2-89.5)	(85.0-88.9)	
BMI, kg/m <sup>2</sup>	29.2 ± 5.6	29.3 ± 5.7	0.77
Mean ± SD, (95% CI)	(28.3-30.0)	(28.7-30.0)	
<b>Baseline characteristics</b>			
	n (%), [95% CI]		
Obesity (62, 12.2%)	17 9.1% (5.4-14.2)	45 13.9% (10.3-18.2)	0.11
Dyslipidemia (21, 4.1%)	8 4.3% (1.9-8.3)	13 4.0% (2.2-6.8)	0.88
Diabetes mellitus (167, 32.9%)	61 33.0% (26.3-40.3)	106 32.8% (27.7-38.2)	1.0
High blood pressure (254, 50.1%)	101 54.6% (47.1-61.9)	153 47.5% (42.0-53.1)	0.12
COPD (34, 6.7%)	17 9.1% (5.4-14.2)	17 5.3% (3.1-8.3)	0.09
Asthma (14, 2.8%)	8 4.3% (1.9-8.3)	6 1.9% (0.7-4.0)	0.10
Initial lymphopenia (323, 64.2%)	127 68.6% (61.4-75.3)	196 61.6% (56.0-67.0)	0.11
Transplantation (35, 6.9%)	22 11.8% (7.6-17.4)	13 4.0% (2.2-6.8)	0.0008
Hematological malignancy (6, 1.2%)	3 1.6% (0.3-4.7)	3 0.9% (0.2-2.7)	0.67
All immunosuppression (55, 10.8%)	28 15.1% (10.2-21.0)	27 8.4% (5.6-11.9)	0.019
Lopinavir+Ritonavir (Kaletra) (111, 21.8%)	50 26.9% (20.7-33.9)	61 18.9% (14.8-23.6)	0.035
Remdisivir (20, 3.9%)	7 3.8% (1.5-7.6)	13 4.0% (2.2-6.8)	0.88
Oseltamivir (40, 7.9%)	20 10.8% (6.7-16.1)	20 6.2% (3.8-9.4)	0.09
Cefotaxime (25, 4.9%)	1 0.5% (0.0-3.0)	24 7.4% (4.8-10.9)	0.0005
Rovamycin (15, 2.9%)	5 2.7% (0.9-6.2)	10 3.1% (1.5-5.6)	0.79
Azithromycin (10, 2.0%)	3 1.6% (0.3-4.6)	7 2.2% (0.9-4.4)	0.75
Hydroxychloroquine (167, 32.9%)	51 27.4% (21.1-34.4)	116 36.0% (30.8-41.5)	0.0502
Dexamethasone (202, 40.1%)	84 45.7% (38.3-53.1)	118 36.9% (31.6-42.4)	0.052
Anti-IL6 (38, 7.5%)	17 9.2% (5.4-14.3)	21 6.5% (4.1-9.8)	0.28
Anti-IL1	10	6	0.036

(16, 3.1%)	5.4% (2.6-9.7)	1.9% (0.7-4.0)	
Time in the ICU before mechanical ventilation (508)	186 0.1 ± 2.3 (-0.2-0.5)	322 -0.6 ± 5.6 (-1.2-0.00)	0.11
Duration of mechanical ventilation (508)	186 24.1 ± 16.6 (21.7-26.5)	322 28.9 ± 21.2 (26.5-31.2)	0.01
Prone position (394, 77.6%)	147 79.0% (72.5-84.6)	247 76.7% (71.7-81.2)	0.58

**Clinical course data**

Mean ± SD, median, and (95% CI)

SAPS II – admission (485, 44.1 ± 16.2)	180 46.8 ± 16.1 (44.4-49.2)	305 42.5 ± 16.1 (40.6-44.3)	0.004
SOFA – admission (398, 7.4 ± 3.9)	142 7.9 ± 3.9 (7.2-8.5)	256 7.2 ± 3.9 (6.7-7.6)	0.08
SOFA – day 7 (366 8.7 ± 4.2)	128 10.2 ± 4.1 (9.4-10.9)	239 7.9 ± 4.1 (7.4-8.4)	0.0001
SOFA – day 15 (261, 8.5 ± 4.6)	96 10.7 ± 4.1 (9.9-11.6)	165 7.1 ± 4.4 (6.5-7.8)	0.0001
SOFA - discharge (285, 6.1 ± 6.0)	109 12.6 ± 4.5 (11.7-13.4)	176 2.1 ± 2.1 (1.8-2.4)	0.0001
CAPA pr/pb/pos* (111, 21.8%)	63 33.9% (24.6-45.3)	48 14.8% (9.9-21.5)	<0.0001
CAPA pr/pb* (87, 17.1%)	52 28.0% (21.6-35.0)	35 10.8% (7.7-14.7)	<0.0001
CAPA pos* (24, 4.7%)	11 5.9% (3.0-10.3)	13 4.0% (2.2-6.8)	0.33
AI AspICU pr/pu# (57, 11.2%)	31 16.7% (11.6-22.8)	26 8.0% (5.3-11.6)	0.003
Other IFI (38, 7.5%)	21 11.3% (7.1-16.7)	17 5.3% (3.1-8.3)	0.013
Candidemia (32, 6.3%)	18 9.7% (5.8-14.9)	14 4.3% (2.4-7.2)	0.017
Bacterial VAP (374, 73.5%)	137 73.7% (66.7-79.8)	237 73.4% (68.2-78.1)	0.94
CMV infection (49, 10.0%)	22 12.2% (7.8-17.8)	27 8.7% (5.8-12.4)	0.22
HSV1 infection (76, 15.5%)	30 16.6% (11.5-22.8)	46 14.8% (11.1-19.3)	0.61

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371 *Anti-IL6 treatment*

372 Among the 38 patients who received anti-IL6 for COVID-19, 15.3% were in the  
 373 proven/probable/possible CAPA group versus 5.3% in the non-CAPA group ( $p=0.0016$ , **table**  
 374 **3**). Comparison between only the proven/probable CAPA patients and the others showed a  
 375 similar statistically significant difference ( $p=0.006$ ). Although there was no significant link  
 376 between anti-IL6 treatment and overall mortality (**table 4**), we investigated whether anti-IL6  
 377 could be a significant onset factor for CAPA. The significant factors associated with anti-IL-6  
 378 treatment by univariate analysis are summarized in **table 5**. Among them, four factors were  
 379 identified by multivariate analysis. Dexamethasone treatment (6.64 [95% CI 2.89-15.23]),  
 380 positive galactomannan antigenemia (5.01 [1.47-17.09]), and proven/probable/possible  
 381 CAPA (2.60 [1.11-6.06]) were highly significantly associated with anti-IL6 treatment and an  
 382 age  $\geq 62$  was significantly associated with the absence of anti-IL6 treatment (0.25 [0.11-  
 383 0.58]).

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386 **Table 5. Uni- and multivariate analysis of factors associated with anti-IL6 treatment in**  
 387 **509 intubated and mechanically ventilated COVID-19 patients.**  
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Factors	Anti-IL6 treatment	No anti-IL6	Univariate	Multivariate
	n (% [95% CI])	n (% [95% CI])	P	OR (95% CL)
Age, years	38	468	0.001	0.25
Mean $\pm$ SD (95% CI)	53.1 $\pm$ 12.8 (48.9 - 57.3)	59.9 $\pm$ 12.3 (58.8 - 61.0)		(0.11-0.58) for age $\geq$ 62
Solid organ transplantation	38	468	0.0383	-
Yes	6 (15.8% [6.0 - 31.3])	29 (6.2 [4.2% - 8.8])		
No	32 (84.2% [68.7 - 94.0])	439 (93.8% [91.2 - 95.8])		
Total immunodepression	38	468	0.0145	-
Yes	29 (76.3% [59.8 - 88.6])	46 (9.8% [7.3 - 12.9])		
No	9 (23.7% [11.4 - 40.2])	422 (90.2% [87.1 - 92.7])		
Dexamethasone	38	464	< 0.0001	6.64 (2.89-15.23)
Yes	29 (76.3% [59.8 - 88.6])	173 (37.3% [32.9 - 41.9])		
No	9 (23.7% [11.4 - 40.2])	291 (62.7% [58.1 - 67.1])		
<i>Aspergillus</i> culture in respiratory samples	38	468	0.040	-
Negative	29 (76.3% [59.8 - 88.6])	414 (88.5% [85.2 - 91.2])		
Positive	9 (23.7% [11.4 - 40.2])	54 (11.5% [8.8 - 14.8])		
Galactomannan antigenemia	38 (0)	468 (0)	0.002	5.01 (1.47-17.09)
Negative	31 (81.6% [65.7 - 92.3])	448 (95.7% [93.5 - 97.4])		
Positive	7 (18.4% [7.7 - 34.3])	20 (4.3% [2.6 - 6.5])		

Proven/Probable CAPA	38 (0)	468 (0)	0.004	-
No	25 (65.8% [48.6 - 80.4])	394 (84.2% [80.6 - 87.4])		
Yes	13 (34.2% [19.6 - 51.4])	74 (15.8% [12.6 - 19.4])		
Proven/probable/possible			0.002	2.60
CAPA	38 (0)	468 (0)		(1.11-6.06)
No	21 (55.3% [38.3 - 71.4])	374 (79.9% [76.0 - 83.5])		
Yes	13 (34.2% [19.6 - 51.4])	74 (15.8% [12.6 - 19.4])		
Among yes, possible	4 (10.5% [2.9 - 24.8])	20 (4.3 [2.6 - 6.5])		

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## DISCUSSION

395 To our knowledge, this is the largest cohort study to date to investigate the prevalence of IFI  
396 among mechanically ventilated ICU patients infected with SARS-CoV-2, along with the  
397 associated mortality and the risk factors for such secondary infections. Among this  
398 homogeneous population, we were surprised to record 106/509 (20.8%) patients (representing  
399 150 episodes) with proven or probable IFI, which increased to 130/509 (25.5%) when  
400 including possible IFI. The high prevalence observed in the MYCOVID study may be related  
401 to several factors, such as the homogeneous severe COVID-19 population with ARDS under  
402 study, the rigorous monitoring dedicated to fungal diagnosis, the role of previous  
403 comorbidities, and the role of the treatment received. Covid-19 associated aspergillosis are the  
404 most prevalent fungal infections in this cohort. The vast majority of the already published data  
405 included heterogeneous COVID-19 populations and mainly used the AspICU case definitions.  
406 By contrast, we also used the recent CAPA definitions and included only the most severe  
407 mechanically ventilated patients, which represents a higher-risk subgroup of patients than all  
408 those infected with SARS-CoV-2. Using the most recent and consensual definitions in the  
409 literature,<sup>8</sup> we identified a high prevalence of CAPA. Indeed, we observed 17.1%  
410 proven/probable CAPA and 4.7% possible CAPA cases, allowing us to refine the prevalence  
411 of CAPA among mechanically ventilated COVID-19 patients. Highly variable IPA prevalence  
412 estimates, ranging from 2% to 27.7%, have been reported in 10 studies that included more  
413 than 80 patients.<sup>13-22</sup> Such varying prevalence may be related to variable definitions of IPA,  
414 the monocentric nature of most of the studies, and the relatively low number (<150) of  
415 patients included in 8 of the 10 studies. Furthermore, the higher prevalence of IPA observed in  
416 the MYCOVID study may be the result of under-diagnosis in previous studies, as several  
417 authors did not provide their diagnostic criteria or rhythm of monitoring and used the AspICU  
418 case definition.

419 We identified several factors associated with CAPA. Patients with CAPA were significantly  
420 older, their disease was more severe based on the SOFA score on day 7, and they were more  
421 frequently under immunosuppressive therapy, particularly for organ transplantation. They also  
422 more frequently received anti-IL6 treatment, which was, furthermore, independently  
423 associated with CAPA by multivariate analysis. The benefit/risk ratio of anti-IL6 treatment  
424 needs to be carefully evaluated due to the conflicting results concerning its use and clinical  
425 interest for COVID-19 patients.<sup>23, 24</sup> Other risk factors, not investigated in this study, could be

426 linked to environmental exposure. Indeed, several teams have modified their existing air  
427 treatment to protect healthcare workers from aerosolization by creating negative pressure  
428 zones, which could potentially increase the risk of *Aspergillus* exposure.

429 Certain other respiratory viruses, such as influenza, also predispose patients to IPA.<sup>1</sup> In such  
430 patients, infection by respiratory viruses results in epithelial damage and disruption of normal  
431 ciliary clearance, aiding the invasion of pulmonary tissues by conidia. The prevalence of IPA  
432 in patients infected with SARS-CoV-2 appears to be similar than the known prevalence  
433 during severe seasonal influenza,<sup>1</sup> despite the accumulation of numerous facilitating factors,  
434 including leucopenia/lymphopenia, T-cell perturbations, and treatment with corticosteroids,  
435 anti-IL1, and anti-IL6.

436 One main result of this study is the significantly higher mortality of CAPA patients, both by  
437 uni- and multivariate analysis, reaching 59·8% for proven/probable and 45·8% for possible  
438 CAPA versus 30·9 in the absence of CAPA. One limit to this work is that it was not designed  
439 to evaluate the efficacy of antifungal treatment. Such elevated mortality warrants  
440 consideration of early antifungal strategies, such as antifungal prophylaxis for high-risk  
441 patients, as well as the implementation of air-treatment measures. Of note, we observed a  
442 pejorative outcome using the AspICU classification for proven/putative AI (31/57, 54·4%)  
443 similar to that for proven/probable CAPA (52/87, 59·8%), even for patients categorized as  
444 colonized (18/50, 36%), compared to non-CAPA patients. A search for discrepancies in the  
445 classification between CAPA and AspICU case definitions showed that 36 patients classified  
446 as CAPA were classified as “only” colonized using the AspICU case definition. Mortality  
447 was, however, as high as 61·1% (22/36). Although many authors have suggested that the  
448 presence of *Aspergillus* in respiratory specimens from COVID-19-infected patients is a  
449 manifestation of colonization, we advocate using the more robust CAPA definitions and  
450 considering any marker indicating the presence of *Aspergillus* as an indication to perform a  
451 BAL, as also suggested by van Grootveld.<sup>25</sup> In the MYCOVID study, all positive diagnostic  
452 procedures on respiratory and/or blood samples were statistically linked to increased mortality  
453 and should be considered.

454 Aside from aspergillosis, the MYCOVID study highlights the occurrence of other fungal  
455 infections, with a high prevalence of 7·5%, of which the most frequent was candidemia in  
456 6·3% of patients. Several authors have noted a global increase in the incidence of candidemia  
457 in COVID-19 patients, even candidemia due to *Candida auris*.<sup>26, 27</sup> Among them, a recent

458 review reported an overall incidence varying from 0·7% to 23·5%, of which most occurred in  
459 the ICU.<sup>26</sup> The reasons for such an increased incidence in this specific population is as yet  
460 unknown. COVID-19 patients are exposed to multiple risk factors for candidemia, such as  
461 antibiotic therapy, corticosteroids, immunosuppressive therapy, and long ICU stays. Several  
462 studies have suggested that direct disruption of the intestinal barrier caused by COVID-19  
463 may be an additional risk factor for candidemia.<sup>21, 28</sup> Indeed, it is known that enterocytes are  
464 infected by SARS-CoV-2, suggesting a possible link with *Candida* gut translocation.<sup>29</sup>  
465 Recently, a pilot study found enrichment of the fecal mycobiome with *Candida* and  
466 *Aspergillus* during the hospitalization of 30 patients with COVID-19 relative to controls.<sup>30</sup>  
467 However, more studies are needed to determine whether such alterations can explain the  
468 observed increased incidence of candidemia. The high mortality of patients with candidemia  
469 in the MYCOVID study (56·3%) warrants the analysis of risk factors and the establishment of  
470 optimal management and prevention of candidemia in this context.

471 This study highlights the high prevalence and mortality of invasive aspergillosis and  
472 candidiasis in ARDS-associated COVID-19 patients relative to that due to bacterial and viral  
473 secondary infections.

474 Antifungal drugs with high efficacy and low toxicity are available, along with non-invasive  
475 diagnostic tools, to allow the efficient management of IFI. The high mortality currently  
476 associated with CAPA demonstrates the importance of improving the management of this  
477 infectious complication. Overall, these findings highlight the need for a high index of  
478 suspicion and the implementation of active surveillance.

479 Further studies should determine if an early antifungal treatment or prophylaxis is needed if  
480 risk factors, such as those determined in this study, are present.

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### 483 **Contributors**

484 JPG, ED, JRZ, and MEB conceived and designed the trial and wrote the manuscript. BL and  
485 JM contributed to the protocol and design of the study. All other authors contributed to the  
486 implementation of the study, inclusion of patients, and data collection. All authors critically  
487 reviewed and approved the final version.

488

### 489 **Declaration of interests**

490

491 **Acknowledgments**

492 We thank

493 Relecture, stats, We also thank the volunteers who participated in this study.

494

495 **Figure legends**

496 **Figure 1.** Study design (A) and study flowchart (B)

497 **Figure 2.** Survival probability according to CAPA status

498 **Figure 3.** Probability of survival of proven/probable/possible CAPA patients receiving, or  
499 not, anti-*Aspergillus* treatment with voriconazole and/or isavuconazole

500 **Figure 4.** Multivariate analysis of factors associated with death

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600 Koehler Philip

601 MARR Kierien, University of Seattle

602 Benoit Misset



603 Frédéric Lamothe  
604 Stijn Blot, University of Gant  
605 Vanderdonk  
606 D Denning, University of Manchester  
607 TORRES Antonio, University of Barcelona  
608 Rodriguez-Bano Jesus, University of Sevilla  
609 MUNOZ P, University of Madrid  
610 WELTE Tobias, University School of Hanver  
611 SHORR Andrew, Washington Dc,  
612 KANJ Souha, American University of Beirut  
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