

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, Francoise 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, Stephan Ehrmann, Christophe Hennequin, Muriel Fartoukh, Gilles Nevez, Cécile Aubron, 11 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, Sorva 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 Dorothée 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,
- Bobigny (S Brun, Y Cohen, C Garnaud, JR Zahar); CHU Lariboisière/Saint-Louis APHP,
 Paris (B Megarbane, A Alanio); CHU de Grenoble, Grenoble (M Cornet, N Terzi, L
- Paris (B Megarbane, A Alanio); CHU de Grenoble, Grenoble (M Cornet, N Terzi, L
 Lamhaut, C Schwebel, C Garnaud); CHU Necker Enfants malades APHP, Paris (L
- 33 Lamhaut, C Schwebel, C Garnaud); CHU Necker Enfants malades APHP, Paris (L
- 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
- 36 Brest, Brest (G Nevez, C Aubron, S Le Gal, D Quinio) ; CHU de Strasbourg, Strasbourg (V
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- 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

Patients with severe COVID-19 have clearly emerged as a population with a high risk of invasive fungal infections (IFI). However, the prevalence of IFI has not yet been prospectively assessed in large populations of mechanically ventilated COVID-19 patients. 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

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

- 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 >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 fungel pathogenes in severe COVID 10 patients

need of active surveillance for fungal pathogens in severe COVID-19 patients.

83 Funding

- 84 Pfizer Inc., NY, USA
- 85
- 86

Key words: Covid-19 associated pulmonary aspergillosis (CAPA), invasive pulmonary
aspergillosis (IPA) Acute respiratory distress syndrome (ARDS), mechanical ventilation,
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

97 Flowchart en .ppt

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.^{1, 2} Patients with severe COVID-19 have also emerged as a population with a high risk of fungal infections.^{3, 4} COVID-19 is associated with epithelial lung damage, 104 lymphopenia, dysfunction of the cell response, and the use of broad spectrum antibiotics, 105 dexamethasone, and immunosuppressive therapies.⁵ Thus, adherence of Aspergillus conidia 106 107 may be promoted by prior damage of the lungs due to SARS- CoV-2 infection and multiple risk factors may lead to tissue invasion after conidia germination.^{6,7} 108

During the COVID-19 pandemic, several case series have been published that suggest an increased risk of IPA in patients with severe COVID-19 (COVID-19-associated pulmonary aspergillosis - CAPA), with an estimated prevalence of <5% to >30%, whereas the prevalence of candidemia and other fungal infections is still poorly known.⁵ However, the heterogeneity of monitoring protocols, diagnostic tools, and severity of COVID-19 patients included in various studies (all hospitalized COVID-19 patients, only patients in the ICU, or only patients 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 published in The Lancet Infectious Diseases journal.⁸ Consensual recommendations are 117 essential because patients who have been infected during the pandemic do not met the usual 118 119 EORTC/MSG criteria for invasive aspergillosis, as there are no host-related risk factors nor specific clinical features.⁹ In addition, the more focused AspICU algorithm to diagnose 120 invasive pulmonary aspergillosis (IPA) in critically ill patients does not include new 121 diagnostic developments and appears to be ill adapted to COVID-19 patients.^{10, 11} 122 Furthermore, the typical radiological features of aspergillosis may be difficult to assess in 123 ARDS-associated COVID-19.¹² Finally, the sensitivity and specificity of current mycological 124 125 tests have still not been completely evaluated in this specific population.

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

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 (≥ 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.

The local investigators were requested to classify patients as being colonized or infected with proven or putative pulmonary aspergillosis according to the AspICU classification currently being used at the time of the study. During the analysis of data, the CAPA consensual definitions were published by Koehler et al. and were thus also used for the statistical 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 definitions.⁸ The characteristics of CAPA patients were compared to those of non-CAPA 180 181 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

An independent data monitoring committee provided an external medical and statistical review of the efficacy and safety data. Continuous variables are presented as means, with standard deviations, and medians (interquartile range) and categorical variables as numbers (percentage), with the corresponding 95% confidence interval. Subgroups (for example, positive and negative patients) were compared using standard statistical approaches: chi² tests 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

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

214

215 **Results**

Between February 29 and July 9, 2020, 576 mechanically ventilated COVID-19 patients, from 18 French ICUs, were enrolled in the MYCOVID study. The final analysis was carried out on 509 patients, as 11 were withdrawn/excluded and 56 did not undergo complete mycological screening or follow-up (<3 screening samples) (figure 1B). The mean number of samples (respiratory and serum) was $6 \cdot 6 \pm 3 \cdot 8$ (median $6 \cdot 0$ [95% CI $6 \cdot 3 - 7 \cdot 0$]) for the whole population. The number of included patients and the period of inclusion according to the center are presented in figure S1.

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- 224

225 Baseline characteristics

226 The mean duration of ICU stay was $32 \cdot 7 \pm 24 \cdot 1$ days (median 26.0, minimum 1, maximum 204). The baseline characteristics of the 509 patients included in the statistical analysis are 227 228 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 $27 \cdot 1 \pm 19 \cdot 8$ 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 (36.5% [32.3-40.9]). 235

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- 237

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

Demographic characteristics
Mean \pm SD, and 95% CI
109 (21.4% [17.9 - 25.2])
400 (78.6% [74.8 - 82.1])
59.4 ± 12.5
(58.1 - 60.4)
$86{\cdot}9\pm17{\cdot}6$
(85.3 - 88.5)
172.4 ± 9.1
(171.6 - 173.2)
$29 \cdot 3 \pm 5 \cdot 6$
$(28 \cdot 8 - 29 \cdot 8)$
Baseline characteristics
n (%, [95% CI])
62 (12.2% [9.5 - 15.3])
21 (4.1% [2.6 - 6.2])
167 (32.9% [28.8 - 37.1])
254 (50.1% [45.7 - 54.5])
34 (6.7% [4.7 - 9.2])
14 (2.8% [1.5 - 4.6])
323 (64·2% [59·9 - 68·4])
35 (6.9% [4.8 - 9.4])
6 (1.2% [0.4 - 2.6])
55 (10.8% [8.2 - 13.8])
111 (21.8% [20.7 - 33.9])
20 (3.9% [2.4 - 6.0])

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]
	Clinical course data
	Mean \pm SD, and 95% CI
Duration of mechanical ventilation, days (508)	27·1 <u>±</u> 19·8
	(25.4 - 28.9]
SAPS II – admission (485)	44.1 ± 16.2
	$(42 \cdot 6 - 45 \cdot 5]$
SOFA – admission (398)	7.4 ± 3.9
	(7.0 - 7.8)
SOFA – day 7 (367)	8.7 ± 4.2
	(8.3 - 9.1)
SOFA – day 15 (261)	8.5 ± 4.6
	(7.9 - 9.0)
SOFA - discharge (285)	6.1 ± 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 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 prevalence of invasive aspergillosis using either the definition according to Koehler et al.⁸ 246 (i.e. proven/probable/possible CAPA) or that according to Blot et al.¹¹ (i.e. proven/putative 247 248 IPA). In total, 111/509 (21.8%) patients were classified as having CAPA according to 249 Koehler et al.;⁸ 87/509 (17.1%) fulfilled the criteria for proven/probable CAPA and 24/509 (4.7%) for possible CAPA. Use the AspICU classification published by Blot et al.¹¹ resulted 250 251 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

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
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])	
- 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])	201
	Duration of antifungal treatment (da	iys)
	Mean \pm SD (min, median, max)	
Fluconazole (n=30)	$19.2 \pm 20.4 (2, 14, 84)$	
Caspofungin (n=70)	$7.9 \pm 9.1 (1, 5, 60)$	
Liposomal amphotericin B (n=34)	$11.8 \pm 27.5 (1, 4, 156)$	
Voriconazole (n=73)	$15 \cdot 2 \pm 16 \cdot 1 \ (1, \ 10, \ 88)$	
Isavuconazole (n=15)	$12.3 \pm 6.1 (3, 12, 26)$	
		294

- 295 proven/putative
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297

298 Invasive aspergillosis

We used the case definition of CAPA according to Koehler et al.⁸ in all subsequent analyses. 299 300 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 ($62 \cdot 2 \pm 13 \cdot 3$ versus $58 \cdot 6 \pm 12 \cdot 2$, 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, showing a statistical difference between the CAPA group (15.3%) and non-CAPA group (5.3%, p=0.0016).

311 The mean duration of mechanical ventilation was 31.3±24.5 days for patients with CAPA 312 $(32.1\pm25.3 \text{ days for proven/probable CAPA and } 28.1\pm21.7 \text{ for possible CAPA})$ versus 313 26.0 ± 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 different between the three groups: 30.9%, 45.8%, and 59.8% for the non-CAPA, possible 315 316 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.

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

	pr/pb/pos CAPA*	Non-CAPA*	
	(n=111)	(n=398)	
Onset factors of CAPA* (n, %)	n, value or % (95% CI)	n, value or % (95% CI)	р
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 <u>+</u> SD, (95% CI)	$62 \cdot 2 \pm 13 \cdot 3 \ (59 \cdot 7 - 64 \cdot 7)$	$58.6 \pm 12.2 \ (57.4-59.8)$	
Weight, kg	104	387	0.07
Mean <u>+</u> SD, (95% CI)	$84 \cdot 1 \pm 18 \cdot 5 \ (80 \cdot 5 - 87 \cdot 7)$	87.3 ± 17.3 (85.9-89.4)	
BMI, kg/m^2	102	384	0.11
Mean <u>+</u> SD, (95% CI)	28.5 ± 5.4 (27.4-29.6)	29·5±5·7 (28·9-30·1)	
Obesity	9	53	0.14
(62, 12.2%)	8.1% (3.8-14.8)	13.3% (10.1-17.1)	
Diabetes mellitus	41	126	0.31
(167, 32.9%)	36.9% (28.0-46.6)	31.7% (27.2-36.6)	
High blood pressure	57	197	0.68
(254, 50.1%)	51.8% (42.1-61.4)	49.6% (44.6-54.7)	
COPD	11	23	0.12
(34, 6.7%)	9.9% (5.1-17.0)	5.8% (3.7-8.5)	
Asthma	5	9	0.20
(14, 2.8%)	4.5% (1.5-10.2)	2.3% (1.0-4.2)	
Transplantation	13	22	0.023
(35, 6.9%)	11.7% (6.4-19.2)	5.5% (3.5-8.2)	
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	$1 \cdot 0$
(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	$1 \cdot 0$
(16, 3.1%)	2.7% (0.6-7.7)	3.3% (1.8-5.5)	
Duration of mechanical	111	397	0.013
ventilation (508)	$31.3 \pm 24.5 \ (26.6-35.9)$	$26.0 \pm 18.1 \ (24.2-27.8)$	
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
$(485, 44 \cdot 1 \pm 16 \cdot 2)$	$47.3 \pm 17.5 \ (43.9-50.6)$	$43 \cdot 2 \pm 15 \cdot 7$ (41 · 6 - 44 · 8)	
SOFA – admission	100	298	0.22
$(398, 7.4 \pm 3.9)$	$7.8 \pm 3.7 (7.1 - 8.6)$	7.3 ± 4.0 (6.8-7.7)	
SOFA – day 7	93	273	0.010
$(367, 8.7 \pm 4.2)$	$9.6 \pm 3.7 \ (8.8-10.4)$	8.4 ± 4.4 (7.9-8.9)	
SOFA – day 15	78	183	0.075
$(261, 8.5 \pm 4.6)$	$9.2 \pm 4.2 \ (8.3-10.2)$	$8 \cdot 1 \pm 4 \cdot 8 \ (7 \cdot 4 - 8 \cdot 8)$	
SOFA - discharge	81	204	<0.0001
$(285, 6.1 \pm 6.0)$	$8.3 \pm 6.1 (7.0-9.7)$	$5.3 \pm 5.8 \ (4.5-6.1)$	

329 330 *According to Koehler et al.⁸. SAPS II: Simplified Acute Physiology II, SOFA: Sequential Organ Failure Assessment, BMI: body-mass index, COPD: chronic obstructive pulmonary disease.

- 331
- 332
- 333 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).

336 Three host factors were significantly associated with overall mortality in the ICU: older age 337 (p=0.0001), immunosuppression (p=0.019), and, in particular, transplantation (p=0.0008), 338 table 4). Among the treatments and management measures administered in the ICU to the 339 patient population, the combination of lopinavir+ritonavir (Kaletra)(p=0.035) and anti-IL1 340 (p=0.036) were associated with death in the ICU, whereas cefotaxime was associated with 341 survival (p=0.0005, table 4). The duration of mechanical ventilation was longer for survivors 342 (28.9±21.2 [95% CI 26.5-31.2]) than for the patients who died (24.1±16.6 [21.7-26.5], table 343 4). Other treatment or management measures showed no significant statistical differences in 344 terms of mortality in the ICU (table 4).

345 Among secondary infections, only fungal co-infections were significantly associated with 346 death. In addition to the survival curves showing mortality to be significantly higher for 347 CAPA patients (figure 2), invasive aspergillosis was associated with death in the univariate 348 analysis (table 4), regardless of the case definition used: proven/probable/possible CAPA 349 (p < 0.0001), proven/probable CAPA (p < 0.0001), possible CAPA (p = 0.01), and 350 proven/putative IPA (p=0.011). The detection of Aspergillus in respiratory samples by culture 351 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 352 353 (voriconazole and/or isavuconazole) did not modify mortality (figure 3, p=0.86), nor did other 354 antifungal drugs increase survival.

355 Aside from invasive Aspergillus infections, among the other infections, only candidemia was 356 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 357 358 with death, which identified three significant factors: age >62 (HR 1.83 [95% CI 1.35-2.48]), 359 solid organ transplantation (HR 2.33 [1.43-3.79], and proven/probable CAPA (HR 1.77 360 [1.25-2.48], figure 4). Of note, possible CAPA and candidemia, although not significantly 361 associated with death, showed, however, a trend toward higher mortality (HR 1.37 [0.74-362 2.55] and 1.19 [0.71-2.01], respectively).

Table 4. Baseline characteristics, host factors, treatments, and secondary infections associated with death in the intensive care unit (ICU) of 509 mechanically ventilated COVID-19 patients.

	Death in the ICU (n=186)	Survival (n=323)	
Factors (n, %)	n, %, (95% CI)	n, %, (95% CI)	р
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 <u>±</u> 10·9	57.6 <u>±</u> 13.1	0.0001
Mean \pm SD, (95% CI)	(60.7-63.9)	$(56 \cdot 2 - 59 \cdot 1)$	
Weight, kg	86.8 ± 17.8	86.8 ± 17.5	0.93
Mean \pm SD, (95% CI)	(84.2-89.5)	(85.0-88.9)	
BMI, kg/m^2	29.2 ± 5.6	29.3 ± 5.7	0.77
Mean <u>+</u> SD, (95% CI)	(28.3-30.0)	(28.7-30.0)	
		Baseline characteristics	
		n (%, [95% CI])	
Obesity	17	45	0.11
(62, 12.2%)	9.1% (5.4-14.2)	13.9% (10.3-18.2)	
Dyslipidemia	8	13	0.88
(21, 4.1%)	4.3% (1.9-8.3)	4.0% (2.2-6.8)	
Diabetes mellitus	61	106	1.0
(167, 32.9%)	33.0% (26.3-40.3)	32.8% (27.7-38.2)	
High blood pressure	101	153	0.12
(254, 50.1%)	54.6% (47.1-61.9)	47.5% (42.0-53.1)	
COPD	17	17	0.09
(34, 6.7%)	9.1% (5.4-14.2)	5.3% (3.1-8.3)	
Asthma	8	6	0.10
(14, 2.8%)	4.3% (1.9-8.3)	1.9% (0.7-4.0)	
Initial lymphopenia	127	196	0.11
(323, 64.2%)	68.6% (61.4-75.3)	61.6% (56.0-67.0)	
Transplantation	22	13(4.0% (2.2-6.8)	0.0008
(35, 6.9%)	11.8% (7.6-17.4)		
Hematological malignancy	3	3	0.67
(6, 1.2%)	1.6% (0.3-4.7)	0.9% (0.2-2.7)	
All immunosuppression	28	27	0.019
(55, 10.8%)	15.1% (10.2-21.0)	8.4% (5.6-11.9)	
Lopinavir+Ritonavir (Kaletra)	50	61	0.035
(111, 21.8%)	26.9% (20.7-33.9)	18.9% (14.8-23.6)	
Remdisivir	7	13	0.88
(20, 3.9%)	3.8% (1.5-7.6)	4.0% (2.2-6.8)	
Oseltamivir	20	20	0.09
(40, 7.9%)	10.8% (6.7-16.1)	6.2% (3.8-9.4)	
Cefotaxime	1	24	0.0005
(25, 4.9%)	0.5% (0.0-3.0)	7.4% (4.8-10.9)	
Rovamycin	5	10	0.79
(15, 2.9%)	2.7% (0.9-6.2)	3.1% (1.5-5.6)	
Azithromycin	3	7	0.75
(10, 2.0%)	1.6% (0.3-4.6)	2.2% (0.9-4.4)	
Hydroxychloroquine	51	116	0.0502
(167, 32.9%)	27.4% (21.1-34.4)	36.0% (30.8-41.5)	
Dexamethasone	84	118	0.052
(202, 40.1%)	45.7% (38.3-53.1)	36.9% (31.6-42.4)	
Anti II 6	17	21	0.29
(38, 7.5%)	9.2% (5.4 14.3)	6.5% (4.1 0.8)	0.20
Anti-II 1	10	6	0.036
And-IL1	10	0	0.030

(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)	$322 \\ -0.6 \pm 5.6 (-1.2 - 0.00)$	0.11
Duration of mechanical ventilation (508)	$\frac{186}{24 \cdot 1 \pm 16 \cdot 6} (21 \cdot 7 - 26 \cdot 5)$	$322 \\ 28.9 \pm 21.2 \ (26.5-31.2)$	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% C	CD
SAPS II – admission	180	$\frac{1000}{305}$	0.004
$(485, 44.1 \pm 16.2)$	$46.8 \pm 16.1 (44.4-49.2)$	$42.5 \pm 16.1 \ (40.6-44.3)$	0 004
SOFA – admission (398, 7.4 ± 3.9)	$ 142 7.9 \pm 3.9 (7.2-8.5) $	256 $7.2 \pm 3.9 (6.7-7.6)$	0.08
SOFA - day 7 (366 8.7 ± 4.2)	$128 \\ 10.2 \pm 4.1 (9.410.9)$	239 7.9 ± 41 (7.4-8.4)	0.0001
SOFA - day 15 (261, 8.5 ± 4.6)	96 10·7 ± 4·1 (9·9-11·6)	165 $7.1 \pm 4.4 (6.5-7.8)$	0.0001
SOFA - discharge $(285, 6.1 \pm 6.0)$	$109 \\ 12.6 \pm 4.5 (11.7-13.4)$	176 $2.1 \pm 2.1 (1.8-2.4)$	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

371 Anti-IL6 treatment

372 Among the 38 patients who received anti-IL6 for COVID-19, 15.3% were in the proven/probable/possible CAPA group versus 5.3% in the non-CAPA group (p=0.0016, table 373 374 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 >62 was significantly associated with the absence of anti-IL6 treatment (0.25 [0.11-383 0.58]).

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Table 5. Uni- and multivariate analysis of factors associated with anti-IL6 treatment in
 509 intubated and mechanically ventilated COVID-19 patients.

	Anti-IL6 treatment	No anti-IL6	Univariate	Multivariate
Factors	n (% [95% CI])	n (% [95% CI])	Р	OR
				(95% CL)
Age, years	38	468	0.001	0.25
Mean \pm SD (95% CI]	$53{\cdot}1\pm12{\cdot}8$	$59{\cdot}9\pm12{\cdot}3$		(0.11-0.58) for
	(48.9 - 57.3)	(58.8 - 61.0)		age ≥ 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
Yes	29 (76·3% [59·8 - 88·6])	173 (37.3% [32.9 - 41.9])		(2.89-15.23)
No	9 (23.7% [11.4 - 40.2])	291 (62.7% [58.1 - 67.1])		
Aspergillus culture in			0.040	-
respiratory samples	38	468		
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			0.002	5.01
antigenemia	38 (0)	468 (0)		(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])		

394 **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 study, the rigorous monitoring dedicated to fungal diagnosis, the role of previous 402 403 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. By contrast, we also used the recent CAPA definitions and included only the most severe 406 407 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,⁸ 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 than 80 patients.¹³⁻²² Such varying prevalence may be related to variable definitions of IPA, 413 414 the monocentric nature of most of the studies, and the relatively low number (<150) of 415 patients included in 8 of the10 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.

We identified several factors associated with CAPA. Patients with CAPA were significantly older, their disease was more severe based on the SOFA score on day 7, and they were more frequently under immunosuppressive therapy, particularly for organ transplantation. They also more frequently received anti-IL6 treatment, which was, furthermore, independently associated with CAPA by multivariate analysis. The benefit/risk ratio of anti-IL6 treatment needs to be carefully evaluated due to the conflicting results concerning its use and clinical interest for COVID-19 patients.^{23, 24} Other risk factors, not investigated in this study, could be linked to environmental exposure. Indeed, several teams have modified their existing air
treatment to protect healthcare workers from aerosolization by creating negative pressure
zones, which could potentially increase the risk of *Aspergillus* exposure.

429 Certain other respiratory viruses, such as influenza, also predispose patients to IPA.¹ 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,¹ 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 BAL, as also suggested by van Grootveld.²⁵ In the MYCOVID study, all positive diagnostic 451 452 procedures on respiratory and/or blood samples were statistically linked to increased mortality 453 and should be considered.

Aside from aspergillosis, the MYCOVID study highlights the occurrence of other fungal infections, with a high prevalence of 7.5%, of which the most frequent was candidemia in 6.3% of patients. Several authors have noted a global increase in the incidence of candidemia in COVID-19 patients, even candidemia due to *Candida auris*.^{26, 27} Among them, a recent 458 review reported an overall incidence varying from 0.7% to 23.5%, of which most occurred in the ICU.²⁶ The reasons for such an increased incidence in this specific population is as yet 459 460 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 studies have suggested that direct disruption of the intestinal barrier caused by COVID-19 462 may be an additional risk factor for candidemia.^{21, 28} Indeed, it is known that enterocytes are 463 infected by SARS-CoV-2, suggesting a possible link with *Candida* gut translocation.²⁹ 464 465 Recently, a pilot study found enrichment of the fecal mycobiome with Candida and Aspergillus during the hospitalization of 30 patients with COVID-19 relative to controls.³⁰ 466 467 However, more studies are needed to determine whether such alterations can explain the observed increased incidence of candidemia. The high mortality of patients with candidemia 468 469 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.

This study highlights the high prevalence and mortality of invasive aspergillosis and
candidiasis in ARDS-associated COVID-19 patients relative to that due to bacterial and viral
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 if480 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**
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- 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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507 **References**

- Schauwvlieghe A, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients
 admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med* 2018; 6: 782-92.
- Verweij PE, Rijnders BJA, Bruggemann RJM, et al. Review of influenza-associated
 pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert
 opinion. *Intensive Care Med* 2020; 46: 1524-35.
- 5143Bassetti M, Kollef MH, Timsit JF. Bacterial and fungal superinfections in critically ill515patients with COVID-19. Intensive Care Med 2020; 46: 2071-4.
- 516 4 Lai CC, Yu WL. COVID-19 associated with pulmonary aspergillosis: a literature 517 review. *J Microbiol Immunol Infect* 2021; **54**: 46-53.
- 518 5 Apostolopoulou A, Esquer Garrigos Z, Vijayvargiya P, Lerner AH, Farmakiotis D.
 519 Invasive pulmonary aspergillosis in patients with SARS-CoV-2 infection: a systematic
 520 review of the literature. *Diagnostics (Basel)* 2020; **10**: 807.
- Arastehfar A, Carvalho A, van de Veerdonk FL, et al. COVID-19 associated pulmonary
 aspergillosis (CAPA)-from immunology to treatment. *J Fungi (Basel)* 2020; 6: 91.
- Marr KA, Platt A, Tornheim JA, et al. Aspergillosis complicating severe coronavirus
 disease. *Emerg Infect Dis* 2021; 27: 18-25.
- Koehler P, Bassetti M, Chakrabarti A, et al. Defining and managing COVID-19associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for
 research and clinical guidance. *Lancet Infect Dis* 2020; published online Dec 14.
 https://doi.org/10.1016/s1473-3099(20)30847-1.
- Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis* 2020; **71**: 1367-76.
- Bassetti M, Giacobbe DR, Grecchi C, et al. Performance of existing definitions and tests
 for the diagnosis of invasive aspergillosis in critically ill, adult patients: a systematic
 review with qualitative evidence synthesis. *J Infect* 2020; **81**: 131-46.
- 536 11 Blot SI, Taccone FS, Van den Abeele AM, et al. A clinical algorithm to diagnose
 537 invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med*538 2012; **186**: 56-64.
- Machado M, Valerio M, Alvarez-Uria A, et al. Invasive pulmonary aspergillosis in the
 COVID-19 era: an expected new entity. *Mycoses* 2021; 64: 132-43.
- 541 13 Bartoletti M, Pascale R, Cricca M, et al. Epidemiology of invasive pulmonary
 542 aspergillosis among COVID-19 intubated patients: a prospective study. *Clin Infect Dis*543 2020; published online Jul 28. https://doi.org/10.1093/cid/ciaa1065.
- 544 Dellière S, Dudoignon E, Fodil S, et al. Risk factors associated with COVID-19-14 545 associated pulmonary aspergillosis in ICU patients: a French multicentric retrospective 546 Clin Microbiol Infect 2020; published online Dec cohort. 13. 547 https://doi.org/10.1016/j.cmi.2020.12.005.
- 548 15 Dupont D, Menotti J, Turc J, et al. Pulmonary aspergillosis in critically ill patients with
 549 Coronavirus Disease 2019 (COVID-19). *Med Mycol* 2021; **59**: 110-4.
- Fekkar A, Lampros A, Mayaux J, et al. Occurrence of invasive pulmonary fungal
 infections in patients with severe COVID-19 admitted to the ICU. Am J Respir Crit *Care Med* 2021; 203: 307-17.

- Lamoth F, Glampedakis E, Boillat-Blanco N, Oddo M, Pagani JL. Incidence of invasive
 pulmonary aspergillosis among critically ill COVID-19 patients. *Clin Microbiol Infect*2020; 26: 1706-8.
- 556 18 Maes M, Higginson E, Pereira-Dias J, et al. Ventilator-associated pneumonia in 557 critically ill patients with COVID-19. *Crit Care* 2021; **25**: 25.
- Razazi K, Arrestier R, Haudebourg AF, et al. Risks of ventilator-associated pneumonia
 and invasive pulmonary aspergillosis in patients with viral acute respiratory distress
 syndrome related or not to Coronavirus 19 disease. *Crit Care* 2020; 24: 699.
- Ripa M, Galli L, Poli A, et al. Secondary infections in patients hospitalized with
 COVID-19: incidence and predictive factors. *Clin Microbiol Infect* 2021; 27: 451-7.
- White PL, Dhillon R, Cordey A, et al. A national strategy to diagnose COVID-19
 associated invasive fungal disease in the ICU. *Clin Infect Dis* 2020; published online
 Aug 29. https://doi.org/10.1093/cid/ciaa1298.
- Permpalung N, Chiang TP, Massie AB, et al. COVID-19 associated pulmonary
 aspergillosis in mechanically ventilated patients. *Clin Infect Dis* 2021; published online
 Mar 9. https://doi.org/10.1093/cid/ciab223.
- 569 23 Stone JH, Frigault MJ, Serling-Boyd NJ, et al. Efficacy of tocilizumab in patients
 570 hospitalized with Covid-19. *N Engl J Med* 2020; **383**: 2333-44.
- 571 24 Horby PW, Pessoa-Amorim G, Peto L, et al. Tocilizumab in patients admitted to
 572 hospital with COVID-19 (RECOVERY): preliminary results of a randomised,
 573 controlled, open-label, platform trial. *medRxiv* 2021: 2021.02.11.21249258.
- van Grootveld R, van Paassen J, de Boer MGJ, et al. Systematic screening for COVIDassociated invasive aspergillosis in ICU patients by culture and PCR on tracheal
 aspirate. *Mycoses* 2021; published online Feb 19. https://doi.org/10.1111/myc.13259.
- 577 26 Nucci M, Barreiros G, Guimarães LF, Deriquehem VAS, Castiñeiras AC, Nouér SA.
 578 Increased incidence of candidemia in a tertiary care hospital with the COVID-19
 579 pandemic. *Mycoses* 2021; 64: 152-6.
- 580 27 Prestel C, Anderson E, Forsberg K, et al. *Candida auris* outbreak in a COVID-19
 581 specialty care unit Florida, July-August 2020. *MMWR Morb Mortal Wkly Rep* 2021;
 582 **70**: 56-7.
- 583 28 Mastrangelo A, Germinario BN, Ferrante M, et al. Candidemia in COVID-19 patients: 584 incidence and characteristics in a prospective cohort compared to historical non-585 COVID-19 published controls. Clin Infect Dis 2020; online Oct 30. 586 https://doi.org/10.1093/cid/ciaa1594.
- 587 29 Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for gastrointestinal infection
 588 of SARS-CoV-2. *Gastroenterology* 2020; **158**: 1831-3 e3.
- S89 30 Zuo T, Zhan H, Zhang F, et al. Alterations in fecal fungal microbiome of patients with
 COVID-19 during time of hospitalization until discharge. *Gastroenterology* 2020; 159:
 1302-10.e5.
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- 596 597

- 599600 Koehler Philip
- 601 MARR Kierien, University of Seattle
- 602 Benoit Misset

- 603 Frédéric Lamoth
- 604 Stijn Blot, University of Gant
- 605 Vanderdonk
- 606 D Dennning, 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 Beiruth
- 613
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