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1 **Risk factors associated with Covid-19-associated pulmonary aspergillosis in ICU patients : a**
2 **French multicentric retrospective cohort**

3
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32 **Abstract**

33 **Objectives:** The main objective of this study was to determine invasive pulmonary aspergillosis (IPA)
34 incidence in the COVID-19 patients admitted to the intensive care unit (ICU), describe the patient
35 characteristics associated with its occurrence and evaluate the impact on prognosis.

36
37 **Methods:** We conducted a retrospective cohort study including all successive COVID-19 patients
38 hospitalized in four ICUs with secondary deterioration and ≥ 1 respiratory sample sent to the mycology
39 department. A strengthened IPA testing strategy including seven mycological criteria was used.
40 Patients were classified as probable IPA according to the EORTC/MSGERC classification if
41 immunocompromised and to the recent COVID-19-associated IPA classification otherwise.

42
43 **Results:** Probable IPA was diagnosed in 21 out of the 366 COVID-19 patients (5.7%) admitted to the
44 ICU and the 108 patients (19.4%) who underwent respiratory sampling for deterioration. No
45 significant differences were observed between patients with and without IPA regarding age, gender,
46 medical history and severity on admission and during hospitalization. Treatment with azithromycin for
47 ≥ 3 days was associated with the diagnosis of probable IPA (odds ratio, 3.1; 95%-confidence interval,
48 1.1-8.5; $p=0.02$). A trend was observed with high dose dexamethasone and the occurrence of IPA.
49 Overall mortality was higher in the IPA patients (15/21, 71.4% vs. 32/87, 36.8%; $p<0.01$).

50
51 **Conclusion:** IPA is a relatively frequent complication in severe COVID-19 patients responsible for
52 increased mortality. Azithromycin, known to have immunomodulatory properties, may contribute to
53 increase COVID-19 patient susceptibility to IPA.

54
55 **Keywords :** Aspergillus, COVID-19, azithromycin, SARS-CoV-2, Critical Care, Coronavirus,
56 Corticosteroids

57 Introduction

58 Although pulmonary invasive fungal disease is typically described in the immunocompromised host,
59 invasive pulmonary aspergillosis (IPA) has been increasingly reported in critically ill patients
60 including patients without classical risk factors of immunosuppression [1]. In acute respiratory distress
61 syndrome (ARDS) patients, ~12.5% of the patients had IPA as shown by random post-mortem
62 histopathological examination of lung tissue [2]. Coronavirus disease 2019 (COVID-19)-associated
63 pulmonary aspergillosis (CAPA) has been increasingly reported [3-5]. Whether the use of
64 immunomodulatory therapies such as corticosteroids prescribed to dampen detrimental inflammatory
65 response and antibiotics to treat and/or prevent bacterial superinfections is responsible for increased
66 susceptibility of COVID-19 patients to pulmonary invasive fungal disease remains incompletely
67 studied [6,7]. The aim of our study was to evaluate the incidence of IPA and the risk factors associated
68 with IPA in severe COVID-19 patients admitted to the intensive care unit (ICU), and evaluate the
69 impact of IPA on patient's outcome.

70 **Method**

71 We conducted a retrospective observational cohort study. All successive COVID-19 patients
72 admitted to the four ICUs of our two university hospitals between March 15th and May 1st 2020 with a
73 positive SARS-CoV-2 PCR (Cobas® SARS-CoV-2 Test, Roche) and ≥ 1 respiratory sample
74 (bronchoalveolar lavage (BAL), tracheal aspirate, sputum) sent to the mycology department were
75 included (Figure 1). Of note, the 27 first patients included were previously partially analyzed [3]. On
76 respiratory sample, culture, galactomannan (GM) (BAL only) and *Aspergillus* quantitative polymerase
77 chain reaction (qPCR) were systematically performed. In concomitantly received blood sample, GM,
78 β -D-glucan (BDG) and *Aspergillus* qPCR were performed on serum/plasma. Patients were classified
79 as probable IPA according to European Organization for Research and Treatment of Cancer (EORTC)
80 and the Mycoses Study Group Education and Research Consortium (MSGERC) consensus criteria in
81 immunocompromised patients [8] and according to the consensus case definition proposal for
82 influenza/COVID-19-associated pulmonary aspergillosis (CAPA) in ICU patients otherwise (Table
83 S1) [9]. An extensive list of clinical data was collected as part of the initial protocol (Table 1). The
84 cumulative dose of corticosteroids, azithromycin and β -lactams were determined as the total dose of
85 drug received prior to the day of sampling. Prescription of azithromycin $>1500\text{mg}$ and β -lactams >3
86 days were predefined as exposure variables. Azithromycin was systematically prescribed before or on
87 the day of admission to the ICU, except for 2 patients for whom it was introduced at day 4 and 5,
88 respectively. If no fungal infection was diagnosed the latest sample was used. Corticosteroids doses
89 were quantified as dexamethasone-equivalent [10].

90 Culture of respiratory specimens were performed, as previously described [11]. For
91 *Aspergillus* qPCR, DNA was extracted from 1mL of plasma or from bead-beaten pellet of the
92 respiratory sample and resuspended in 1000 μL of DNA-free water using the Qiasymphony DSP
93 virus/Pathogen Mini kit (Qiagen,) and a QIASymphony apparatus (Qiagen). PCR assay was previously
94 reported [12]. GM and BDG detection were performed using Platelia Bio-Rad kit (BioRad
95 Laboratories) and Fungitell assay (Cape Cod Diagnostics) according to the manufacturer respectively.

96

97 *Statistics*

98 Data were reported in percentage, mean and standard deviation (SD) or median and
99 interquartiles [Q1-Q3] as appropriate. Univariate analyses were performed to assess an association
100 between clinical factors and IPA using Fisher's exact, Chi-2 and Wilcoxon tests as appropriate. Odds
101 ratios (OR) with 95%-confidence intervals (IC95%) were calculated for each significant variable
102 based on univariate logistic regression. All analyses were performed using R software, version 3.5.3
103 (<http://www.r-project.org>).

104

105 *Ethical statements*

106 Our institutional ethics committee approved the study (IDRCB, 2020-A00256-33; CPP, 11-
107 20-20.02.04.68737).

108

109 Results

110 A total of 366 patients with positive SARS-CoV2 qPCR were admitted to the four intensive
111 care units between March 15th and May 1st 2020 among which 246 were intubated and mechanically
112 ventilated (Figure 1). The mycology department received 193 respiratory samples from 108 patients,
113 whose conditions deteriorated despite appropriate initial care. Patient characteristics are described in
114 Table S2. Male/female sex ratio was 4.4 and median age was 61 years.

115 Twenty-one patients developed probable IPA according to CAPA criteria *stricto sensu* (n=19)
116 and EORTC/MSGERC definitions (n=2; one solid organ transplant recipient and one myeloma
117 patient). Overall, incidence was 5.7% (21/366) in severe COVID-19 patients admitted to the ICU and
118 8.5% (21/246) in those mechanically ventilated. IPA incidence in patients whose conditions worsened
119 despite appropriate care was 19.4% (21/108). The median times from symptom onset to IPA diagnosis
120 and from ICU admission to IPA diagnosis were 16 days (10-23) and 6 days (1-15), respectively.

121 When comparing patients who developed probable IPA (n=21) or not (n=87), no significant
122 differences were observed regarding general population characteristics and severity upon admission
123 (Table 1). Prescription of hydroxychloroquine (n=34) did not differ between both groups.
124 Administration of azithromycin for more than 3 days (cumulative dose ≥ 1500 mg) was associated with
125 probable IPA (OR, 3.1; IC95%, 1.1-8.5; p=0.025) (Figure S1). Of note, 34 patients received
126 azithromycin, which was prematurely discontinued on day 1 or day 2 in 8 patients because of QT
127 interval prolongation. Administration of high-dose corticosteroids was not significantly associated
128 with IPA (11.5 vs. 28.6%; p=0.08), although the cumulative dose ≥ 100 mg tended to be higher among
129 IPA patients (OR, 3.7; IC95%, 1.0-9.7). Details for the incidence of IPA among patients who received
130 azithromycin and/or corticosteroids is available in Table S3. Mortality was significantly higher in the
131 probable IPA group (15/21, 71.4% vs. 32/87, 36.8%; p<0.01). Further details on each IPA patients are
132 available in Table S4.

133

134 Discussion

135 In our study, the incidence of IPA in COVID-19 patients was 5.7% in all ICU patients and 8.5% in
136 those mechanically ventilated yet may be underestimated considering only patients with clinical
137 worsening were tested.

138 A cumulative azithromycin dose ≥ 1500 mg was associated with IPA. Azithromycin have *in vitro*
139 antiviral effect and is a broad spectrum antibiotic with immunomodulatory properties which could
140 have also both prevented bacterial superinfections and reduced inflammation [13]. A recent meta-
141 analysis found an increased mortality when hydroxychloroquine is associated to azithromycin [14].
142 Azithromycin-related impact on the risk of secondary infections have been only incompletely studied.
143 Azithromycin has been shown to decrease serum interleukin-6 and induce delayed down-regulation of
144 neutrophil oxidative burst and increased apoptosis up to 28 days after 3 doses of azithromycin (*i.e.*
145 1500 mg) in humans [15]. Neutrophils and oxidative burst represent the first and most important
146 immune system barrier against aspergillosis [16]. Furthermore, azithromycin may promote *Aspergillus*
147 colonization by altering the lung microbiome [17].

148 Corticosteroids are known to increase susceptibility to invasive fungal disease due to complex
149 quantitative and qualitative immune deregulation [10]. High dose corticosteroid, although not
150 precisely defined, was previously found to be associated with CAPA [4,5]. Although not
151 significantly associated to IPA in our study, probably because of insufficient statistical power, a trend
152 was observed after a cumulative dose of ≥ 100 mg dexamethasone-equivalent (OR, 3.7; IC95, 1.0-9.7),
153 Recent studies such as the RECOVERY trial and various meta-analyses showed that corticosteroid
154 administration is beneficial in COVID-19 patients requiring hospitalization [18]. Interestingly, the
155 cumulative dose of dexamethasone using the RECOVERY trial regimen does not exceed 60 mg.

156 Although no severity score or variables, including oxygenation parameters, were found to be
157 associated to IPA occurrence, we cannot rule out the extension of the lesion to be an associated risk
158 factor. Indeed, the extension of lung lesions quantified by computed tomography was a predictor of
159 COVID-19 severity and early death [19].

160 Susceptibility to IPA in previously immunocompetent critically ill patients is most likely
161 multifactorial. In ARDS patients, epithelial damage, impaired mucocilliary clearance and temporary
162 immune deregulation, starting with excess release of danger-associated molecular patterns (DAMPs)

163 secondary to COVID-19 damages, may be initiating factors [20]. The addition of known or suspected
164 risk factors, such as corticosteroid or azithromycin, further inhibiting neutrophils and innate immune
165 response may tilt the balance in favor of IPA development. The risk of IPA associated with
166 corticosteroids compellingly depends on its cumulative dose although cut-offs are not clearly defined
167 and depends on the underlying host factor [8]. Our findings raise questions regarding the possible
168 connection between azithromycin use and the observed increased susceptibility to IPA, which needs to
169 be further explored.

170

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177 **Conflict of interest**

178 The authors declare no conflict of interest related to the content of the present study

179 **Authors contribution**

180 *Writing-Original draft:* SD and AA. *Writing-Review & Editing:* All. *Conceptualization:* SD, AA and
181 SB. *Investigation:* SD, ED, SF, SV, MC. *Data curation:* SD, ED, SF, SV, MC, TFG. *Formal analysis:*
182 PAO, MS. *Visualization:* SD, AA, BM, EA, AM. *Supervision:* AA, BM, EA, AM.

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251 **Table 1.** Comparison of severe COVID-19 patients with and without probable invasive pulmonary
 252 aspergillosis
 253

	Total (n=108)	Without IPA (n=87)	With IPA (n=21)	OR	CI95%	p-value
Male n (%)	88 (81.5)	72 (82.8)	16 (76.2)	0.7	0.2-2.1	-
Age median [Q1-Q3]	62 [56-68]	62 [56-68]	63 [56.75-68.25]	-	-	0.63 ^o
Mechanical ventilation n (%)	105 (97.2)	85 (97.7)	20 (95.2)	0.5	0.04-5.3	
COVID risk factors						
HTA n (%)	64 (59.3)	50 (57.5)	14 (66.7)	1.5	0.5-4.0	-
Diabetes n (%)	40 (37.0)	31 (35.6)	9 (42.9)	1.4	0.5-3.6	-
Obesity n (%)	35 (32.4)	31 (35.6)	4 (19.0)	0.4	0.1-1.3	-
Coronary disease n (%)	15 (13.9)	13 (14.9)	2 (9.5)	0.6	0.1-2.9	-
BMI median [Q1-Q3]	28 [25-31]	28 [26-32]	28 [25-29]	-	-	0.70 ^o
Other patient characteristics						
Asthma n (%)	5 (4.6)	3 (3.4)	2 (9.5)	2.9	0.5-18.9	-
COPD n (%)	2 (1.9)	2 (2.3)	0 (0.0)	0.8	0.04-17.2	-
Immunocompromised patient n (%)	10 (9.3)	8 (9.2)	2 (9.5)	0.6	0.1-2.9	-
Long term corticosteroids n (%)	11 (10.2)	8 (9.2)	3 (14.3)	1.6	0.4-6.8	-
Severity at admission						
PaO₂/FiO₂ mean (SD)	173.47 (123.19)	169.63 (125.85)	187.79 (114.74)	-	-	0.34 ^o
Vasopressors in first	65 (60.2)	52 (59.8)	13 (61.9)	1.1	0.4-2.9	

48H n (%)						
Creatininemia (mg/dL) mean (SD)	103.34 (74.01)	92.64 (47.19)	149.85 (132.96)	-	-	0.08°
D-dimer median [Q1- Q3]	2395 [1193- 4635]	2325 [1163- 4563]	2515 [1610- 10917]	-	-	0.63°
LDH mean (SD)	755.11 (312.15)	759.49 (303.61)	740.20 (350.52)	-	-	0.80°
SAPS2 mean (SD)	39.93 (14.40)	40.4 (14.6)	38.1 (13.8)	-	-	0.58°
SOFA mean (SD)	6.02 (3.79)	5.8 (3.6)	7.1 (4.5)	-	-	0.28°
Severity during hospitalization						
Nadir PaO2/FiO2 mean (SD)	79.75 (37.21)	81.54 (39.01)	72.50 (28.40)	-	-	0.50°
ECMO n (%)	10 (9.3)	9 (10.3)	1 (4.8)	0.4	0.1-3.6	-
Renal replacement therapy n (%)	38 (35.2)	30 (34.5)	8 (38.1)	1.2	0.4-3.1	-
Vasopressors n (%)	89 (82.4)	70 (80.5)	19 (90.5)	2.3	0.5-10.9	-
Specific COVID therapy						
Lopinavir-ritonavir n(%)	16 (14.8)	10 (11.5)	6 (28.6)	3.1	0.9-9.8	
Hydroxychloroquine n(%)	34 (31.5)	27 (31.0)	7 (33.3)	1.1	0.4-3.1	
Azithromycin + Hydroxychloroquine n(%)	29 (26.9)	22 (25.3)	7 (33.3)	1.4	0.5-4.1	
Immunoglobulins n(%)	3 (2.8)	3 (3.4)	0 (0.0)	0.6	0.03-11.3	
Sarilumab n(%)	1 (0.9)	1 (1.1)	0 (0.0)	4.3	0.3-71.8	

Ecuzumab <i>n</i>(%)	6 (5.6)	4 (4.6)	2 (9.5)	2.2	0.4-12.8	
Tocilizumab <i>n</i>(%)	4 (3.7)	2 (2.3)	2 (9.5)	4.5	0.6-33.8	
Therapy with cumulative dose before sampling						
Azithromycin >1500 mg total dose <i>n</i> (%)	26 (24.1)	17 (19.5)	9 (42.9)	3.1	1.1-8.5	
Dexamethasone >1000mg <i>n</i> (%)	16 (14.8)	10 (11.5)	6 (28.6)	3.1	1.0-9.8	
Any β-lactam > 3 days <i>n</i> (%)	90 (83.3)	74 (85.1)	16 (76.2)	0.6	0.2-1.8	
Respiratory sample characteristics						
PaO₂/FiO₂ at sampling mean (SD)	173.69 (91.70)	173.18 (96.05)	175.66 (74.40)	-	-	0.612 ^o
% BAL macrophages mean (SD)	31.23 (21.94)	31.00 (22.76)	32.17 (20.21)	-	-	0.836 ^o
% BAL PMN mean (SD)	47.37 (30.92)	47.08 (31.99)	48.50 (28.89)	-	-	0.959 ^o
% BAL Lymphocytes mean (SD)	20.63 (18.94)	21.17 (19.10)	18.50 (19.92)	-	-	0.795 ^o
Outcome						
Mortality <i>n</i> (%)	47 (43.5)	32 (36.8)	15 (71.4)	4.3	1.5-12.1	<0.01 ^o
LOS days mean (SD)	24.33 (18.88)	25.13 (19.18)	21.05 (17.60)	-	-	0.313 ^o

254 BAL: Bronchoalveolar lavage; BMI: body mass index; CI95%: 95% confidence interval; IPA: invasive

255 pulmonary aspergillosis; LOS: length of stay; OR: odd ratio; PMN: polymorphonuclear; SAPSII, Simplified

256 Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; ^oWilcoxon test

257

258

259 **Figure 1.** Study flowchart.

260 Direct examination of respiratory samples were performed only on samples collected after the March
261 27th as initial data regarding the contamination risk of lab technicians were not available. *as
262 recommended by the manufacturer. BAL: bronchoalveolar lavage; BDG: β -D-glucans; GM:
263 galactomannan; ICU: intensive care unit; qPCR: quantitative polymerase chain reaction.

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