

Influenza- and COVID-19-associated pulmonary aspergillosis: are the pictures different?

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Research

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Abstract

Background

Invasive pulmonary aspergillosis (IPA) in intensive care unit patients is a major concern, in particular for those with acute respiratory distress syndrome (ARDS). As observed previously for influenza-associated ARDS, the SARS-CoV-2 pandemic has shown a high proportion of COVID-19 patients with ARDS to be at risk of developing invasive fungal diseases.

Methods

We used the new international definitions of influenza-associated pulmonary aspergillosis (IAPA) and COVID-19-associated pulmonary aspergillosis (CAPA) to compare the demographic, clinical, biological and radiological aspects of IAPA and CAPA in a monocentric retrospective study.

Results

Among the 120 ARDS patients included, we observed equivalent prevalence of IPA in Influenza and COVID-19 populations: 17 IAPA (23.9%) and 10 CAPA (20.4%). There were no significant differences in demographic or admission characteristics between patients with and without IPA. Kaplan-Meier curves showed significantly higher 90-day mortality for IPA patients overall ($p = 0.032$), whereas mortality did not differ between CAPA and IAPA. The duration of mechanical ventilation was higher for IPA patients (23 days [IQR 17–40]) than those without (17 days [IQR 9–25], $p = 0.038$). Patients with COVID-19 and influenza associated ARDS treated with corticosteroids were more likely to develop IPA. Radiological findings of IPA in both populations using the new criteria increased sensitivity but with still poor specificity. Nonetheless, they also showed interesting differences between IAPA and CAPA with a higher proportion of features suggestive of IPA in IAPA patients. Lastly, therapeutic drug monitoring also appeared challenging since a wide proportion of IPA patients had low plasma voriconazole concentrations, with a significant higher delay to reach voriconazole concentrations $> 2\text{mg/L}$ in CAPA versus IAPA patients ($p = 0.045$).

Conclusions

ICU patients presenting with ARDS during COVID-19 are very similar to those with severe influenza pneumonia in terms of prevalence of IPA and outcome, while CAPA is mainly favored by advanced age irrespective of the background. The dramatic consequences on the patients' prognosis emphasize the need for a better awareness in these particular populations. Larger prospective studies may help in designing the most well-adapted personalized management to prevent IPA, which represents a high burden of death in severe COVID-19 and *Influenza* pneumonia.

Introduction

Invasive pulmonary aspergillosis (IPA) has been mainly described in patients with severe neutrophil dysfunction, especially those with prolonged neutropenia (1). Increasing evidence shows that critically ill patients are at risk of IPA (2–4). Among them, the factors responsible for establishment of *Aspergillus* and

the development of IPA are heterogeneous. Lower respiratory tract impairment, prolonged mechanical ventilation, corticosteroid administration, or immunological dysfunction are often involved (2). The diversity of the patient backgrounds is reflected by their clinical and biological presentation and thus the criteria that should be used for case definition. Generic consensus definitions, such as those of the European Organization for Research and Treatment of Cancer/Mycosis Study Group Education and Research Consortium (EORTC/MSGERC) or the *AspICU* (1, 3, 5), are sometimes not adapted to specific groups of patients (6).

Influenza-associated pulmonary aspergillosis (IAPA) is an emerging complication of influenza infection, often associated with *Aspergillus* tracheobronchitis (7), that markedly increases influenza-associated mortality (8). Commonly recognized elements of IAPA include epithelial damage and the modulation of immune function directly due to the virus, although the pathogenesis is still unclear (7). IAPA cases exhibit atypical clinical features, which influence the results of diagnostic tests, such as broncho-alveolar lavage (BAL) and serum galactomannan (GM) or respiratory sample culture, as well as atypical radiological features (7). This led to the recent proposition of new criteria for IAPA case definition in intensive care unit (ICU) patients (7). Unlike IPA observed in immunocompromised patients, the radiological patterns of acute respiratory distress syndrome (ARDS) patients are much more difficult to interpret and the particularities of IPA imaging in these situations have thus far been only poorly evaluated.

The background of patients suffering from Coronavirus Disease 2019 (COVID-19) also appears to be highly compatible with the occurrence of IPA (9). ICU patients admitted for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus infections share specific features, commonly described to increase the risk for ventilator associated pneumonia (10) and particularly fungal diseases including ARDS, possible corticosteroid administration, and systemic dysregulation of immune function (11). The incidence of COVID-19-associated pulmonary aspergillosis (CAPA) in ICU patients varies according to the major national cohorts from the United Kingdom (14.1%), Italy (27.7%), Germany (26.3%), the Netherlands (19.4%), and France (National MY-CO-VID clinical trial: 21.8%) (12–16) and are similar to the rate of IAPA observed in ICU cohorts (19%) from Belgium and the Netherlands (8). Nevertheless, the situation appears to not be strictly transposable between the two co-infections, as, for example, *Aspergillus* tracheobronchitis is not commonly described in CAPA and serum GM is much less frequently positive in CAPA than IAPA (1, 7). However, a more accurate comparison between CAPA and IAPA has until recently been difficult due to the absence of a clear CAPA case definition, as highlighted by some authors (15).

The recently published CAPA case definition (17) has made it possible to compare patients suffering from IAPA and CAPA. This will improve our epidemiological knowledge concerning CAPA, which is still incomplete, and represents the first step towards improving the management of the COVID-19-associated fungal risk (18, 19). In this monocentric retrospective study, we aimed to compare the demographic, clinical, radiological, and biological features and outcomes of IAPA and CAPA cases in ICU applying the recently proposed case definitions of CAPA.

Methods

Populations

All patients who were admitted from September 20th, 2009 to February 8th, 2020 to the ICUs of the Rennes University Hospital for influenza-associated ARDS and underwent a mycological analysis of BAL, tracheal aspirate, or sputum were included in the study as "influenza patients" (n = 71). All patients who were admitted to the same unit for COVID-19 from March 3rd to September 9th, 2020 were included as "COVID-19 patients" (n = 49). The COVID-19 patients were strictly monitored for fungal infections twice weekly based on tracheal aspirates, as detailed in a previous publication (20). Influenza infection and COVID-19 were confirmed by RT-PCR of respiratory samples or nasopharyngeal swabs using the Influenza A/B r-gene™ (Argene®, bioMérieux, Marcy-l'Étoile, France) and TaqPath™ COVID-19 (Thermo Fisher Scientific, Illkirch-Grafenstaden, France) assays. ARDS was defined according to international guidelines (21). Epidemiological and clinical data were collected during hospitalization. A blood count and biochemical check-up, including the measurement of creatinine levels, were performed at the beginning of the hospitalization for each patient. For statistical analysis, patients were classified following the AspICU (3), IAPA (7), and CAPA (17) criteria when specified. Data presented in tables and figures were extracted from each patient's medical records. The simplified acute physiology score (SAPS) II was assessed within 24 hours following ICU admission and the Sepsis-Related Organ Failure Assessment (SOFA) score was calculated on days 1 and 5. This study conforms to the principles outlined in the Declaration of Helsinki and was approved by the institutional ethics board of Rennes University Hospital, France (N 20–56).

Detection of Aspergillus in respiratory samples by culture and PCR

Fungal culture was performed from respiratory samples in Sabouraud-Chloramphenicol media, inoculated with 100 µL of pellets and incubated for seven days at 30°C and 37°C. Isolated *Aspergillus* were identified at the section level based on microscopic features. *Aspergillus* fungi isolated from COVID-19 patients were then identified at the species level by MALDI-ToF mass spectrometry after fungal colony extraction (22) using a MALDI Biotyper device (Bruker France, Marne-la-Vallée, France) and the Mass Spectrometry Identification (MSI) database for the identification of fungi (23).

The molecular detection of *Aspergillus* was also performed on respiratory samples after DNA extraction. Briefly, 200 µL of a BAL pellet or other liquefied respiratory sample were first incubated overnight at 56°C with proteinase K (Qiagen France, Les Ulis, France). DNA was then extracted using the manual QIAamp DNA Mini Kit (Qiagen) or the automated EZ1 Advanced XL system (Qiagen) using the EZ1 DSP Virus Kit (Qiagen). *Aspergillus* qPCR assays were performed as previously described, targeting either an *Aspergillus* mitochondrial gene or an *Aspergillus* 28S rDNA region (23), depending on the period of inclusion.

Detection of Aspergillus galactomannan (GM) in blood and respiratory samples

GM measurement was performed in serum with an index cutoff > 0.5 and in BAL with an index cutoff ≥ 1 using the Platelia GM *Aspergillus* assay (Bio-Rad, Marnes-la-Coquette, France) following the manufacturer's recommendations. GM detection in non-bronchoscopic lavage respiratory samples was performed using the sōna *Aspergillus* lateral flow assay (LFA) (IMMY diagnostics, Oklahoma, USA), following the manufacturer's

recommendations, due to the biological hazard for laboratory workers. Quantitative results were obtained by reading the LFA with the sōna cube reader (IMMY diagnostics).

Imaging

Chest computed tomography (CT) scans of patients who developed IPA were analyzed by a senior radiologist who was blinded to the IAPA or CAPA status. The following items were categorized as absent or present as generally reported in the literature: diffuse reticular or alveolar opacities, wedge-shaped segmental or lobar consolidation, well-circumscribed nodules, halo signs, cavitation, air crescent signs, tree in bud, bronchial wall thickening, and pleural effusion (1, 3, 7).

Therapeutic drug monitoring

Among patients treated with voriconazole, therapeutic drug monitoring was performed after the initiation of this treatment. A plasmatic voriconazole trough concentration (VTC) target between 2 and 6 mg/L was recommended as voriconazole therapeutic range in ICU IPA patients (24).

Trough concentrations (= C_{min}), defined as concentrations measured 12h ± 2h after voriconazole administration, were measured from day 3 after the beginning of the treatment, after achievement of steady-state. Blood samples were centrifuged at 3200 G for 10 min, and then stored at -20°C until being assayed. Voriconazole plasma concentrations were determined using a validated liquid chromatography tandem mass spectrometry assay. The linearity range of the assay extends from 0.1 to 12 µg/mL.

Statistical analysis

Demographic and clinical characteristics of patients are presented as numbers and percentages for categorical variables and medians and interquartile ranges (IQR, 25%-75%) for continuous variables. The Mann-Whitney U test was used for quantitative data and qualitative data were compared using the Chi-square or Fisher test, as appropriate. Survival curves were constructed until day 90 from the diagnosis of ARDS using the Kaplan-Meier method and were compared using the log rank test. Two-sided tests were performed and reached statistical significance when the p-value was < 0.05.

All statistical analyses were performed using GraphPad Prism 8.4 (GraphPad Software, La Jolla, CA) and R Statistical Software 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Overall, 120 patients were included in the study, 71 admitted for severe influenza and 49 for severe COVID-19. Among them, 27 (22.5%) presented with COVID-19- (CAPA) and Influenza- (IAPA) associated pulmonary aspergillosis. Among the 10/49 (20.4%) CAPA patients, four were probable CAPA and six possible CAPA according to the most recent consensual definitions by Koehler et al. (17). Among the 17/71 (23.9%) IAPA patients, 13 fulfilled the definitions by Verweij et al. (7) and four the definitions by Blot et al. (3). A comparative analysis of these two groups of IAPA patients showed no significant differences in terms of background, severity, or outcome. Thus, we considered all 17 patients to constitute the same IAPA group during this study. The IAPA diagnosis was based on at least one positive GM result for the serum (n = 7/17,

41.1%) and/or BAL (n = 10/17 patients, 58.8%) and/or a fungal culture of *A. fumigatus* from respiratory samples (n = 15/17, 88.2%). The CAPA diagnosis was based on at least one positive GM result for serum (n = 3/10, 30%) and/or an *A. fumigatus*-positive culture from a non-bronchoscopic lavage (n = 9/10 patients, 90%) and/or combined positivity of GM and *A. fumigatus* PCR in non-bronchoscopic lavage (n = 1/10, 10%). Mycological arguments that allowed the IPA classification are presented for each patient in Table S1.

Demographic and admission characteristics of the patients according to their aspergillosis status are summarized in Table 1. The median age was 59 years and 80 (66%) of 120 patients were men. There were no significant differences in demographic or admission characteristics between patients with and without IPA. CAPA patients were significantly older than IAPA patients (mean ages 72 and 58 years, $p = 0.036$). The proportion of immunosuppressed patients was numerically higher among patients with IPA (37%, 10/27) than those without (20%, 19/93), but the difference did not reach statistical significance ($p = 0.076$). Similarly, the frequency of immunosuppressed patients was lower among CAPA (20%), than IAPA patients (47.1%) without reaching statistical difference. Among recognized risk factors for IPA, solid cancers and hematological malignancies were observed for 25.9% of IPA patients and 12.9% of patients without IPA ($p = 0.13$). A summary of reported cases is presented in Table S2. The frequency of patients with neoplasia was lower in CAPA (10%) than IAPA patients (35.3%), without reaching statistical difference.

Table 1
Characteristics of patients according to aspergillosis status

	All patients (n = 120)	All aspergillosis patients (n = 27)	Non aspergillosis patients (n = 93)	P value	IAPA (n = 17)	CAPA (n = 10)	P value
Baseline characteristics							
Age (years)	59 (52–67)	60 (52–69)	59 (52–67)	0.54	58 (52–63)	72 (57–77)	0.036
Male sex	80 (66.4%)	17 (63%)	63 (67.7%)	0.21	11 (64.7%)	6 (60.0%)	> 0.99
Current smoking	31 (26%)	10 (37%)	21 (22.6%)	0.13	9 (52.9%)	1 (10.0%)	0.12
Obesity	20 (16.6%)	4 (15.4%)	30 (32.3%)	0.09	3 (17.6%)	1 (10.0%)	> 0.99
Diabetes	33 (27.5%)	4 (14.8%)	29 (31.2%)	0.14	2 (11.8%)	2 (20.0%)	0.61
Alcoholism	20 (16.6%)	6 (22.2%)	14 (15.1%)	0.39	5 (29.4%)	1 (10.0%)	0.36
Immunodepression (including neoplasia)	29 (24.1%)	10 (37%)	19 (20.4%)	0.12	8 (47.1%)	2 (20.0%)	0.23

Data are presented as medians (IQR: interquartiles) or n (%). P values comparing the invasive aspergillosis versus no aspergillosis groups and IAPA versus CAPA were calculated using Mann-Whitney (continuous variables) and Fisher or Chi2 tests when appropriate (categorical variables).

AKI: acute kidney injury, PaO₂: arterial oxygen partial pressure, SAPS II: Simplified Acute Physiology Score II, SOFA: Sequential Organ Failure Assessment, HM: hematological malignancies, COVID-19: Coronavirus Disease 2019, ECMO: extracorporeal membrane oxygenation; PaO₂: arterial oxygen tension; FiO₂: fraction of inspired oxygen; RRT: renal replacement therapy; ICU: intensive care unit.

	All patients (n = 120)	All aspergillosis patients (n = 27)	Non aspergillosis patients (n = 93)	P value	IAPA (n = 17)	CAPA (n = 10)	P value
Neoplasia	19 (15.8%)	7 (25.9%)	12 (12.9%)	0.13	6 (35.3%)	1 (10.0%)	0.2
- Solid cancer	3 (2.5%)	1 (3.7%)	2 (2.2%)	0.53	1 (5.9%)	0 (0.0%)	> 0.99
	16 (13.3%)				5 (29.4%)	1 (10.0%)	
- HM		6 (22.2%)	10 (10.7%)	0.19			0.36
Chronic obstructive pulmonary disease	17 (14.2%)	6 (22.2%)	11 (11.8%)	0.17	6 (35.3%)	0 (0.0%)	0.057
Chronic kidney disease	10 (8.3%)	2 (7.4%)	8 (8.6%)	> 0.99	1 (5.9%)	1 (10.0%)	> 0.99
Cirrhosis	8 (6.7%)	4 (14.8%)	4 (4.3%)	0.07	4 (23.5%)	0 (0.0%)	0.26
ARDS etiology	71 (59.2%)	17 (63%)	54 (58.1%)	0.65	NA	NA	NA
- Influenza		10 (37%)	39 (41.9%)				
- COVID-19	49 (40.8%)						
Clinical and biological admission ICU data							
Neutrophil (10 ⁹ /L)	6.9 (3.9–11.4)	8.2 (3.8–13.2)	6.9 (4.1–11)	0.67	8.0 (3.6–17.7)	8.5 (4.1–11.0)	0.72
Lymphocyte (10 ⁹ /L)	0.56 (0.32–0.87)	0.54 (0.36–0.72)	0.59 (0.32–0.93)	0.44	0.38 (0.29–0.55)	0.83 (0.72–0.92)	< 0.0001
Data are presented as medians (IQR: interquartiles) or n (%). P values comparing the invasive aspergillosis versus no aspergillosis groups and IAPA versus CAPA were calculated using Mann-Whitney (continuous variables) and Fisher or Chi2 tests when appropriate (categorical variables).							
AKI: acute kidney injury, PaO2: arterial oxygen partial pressure, SAPS II: Simplified Acute Physiology Score II, SOFA: Sequential Organ Failure Assessment, HM: hematological malignancies, COVID-19: Coronavirus Disease 2019, ECMO: extracorporeal membrane oxygenation; PaO2: arterial oxygen tension; FiO2: fraction of inspired oxygen; RRT: renal replacement therapy; ICU: intensive care unit.							

	All patients (n = 120)	All aspergillosis patients (n = 27)	Non aspergillosis patients (n = 93)	P value	IAPA (n = 17)	CAPA (n = 10)	P value
Ratio of PaO ₂ to FiO ₂ on day 1	98 (67–147)	98 (74–143)	105 (67–148)	0.96	86 (69–98)	143 (109–154)	0.01
SAPS II score on day 1	44 (35–61)	48 (36–64)	43 (34–60)	0.22	58 (42–64)	40 (34–68)	0.48
SOFA score on day 1	8 (5–10)	9 (5–12)	7 (4–10)	0.19	10 (7–13)	5 (2–8)	0.012
Clinical course data							
Duration of mechanical ventilation (days)	18 (11–27)	23 (17–40)	17 (9–25)	0.038	23 (16–49)	23 (19–30)	0.56
ECMO	45 (37.5%)	13 (48.1%)	32 (34.4%)	0.19	12 (70.6%)	1 (10.0%)	0.004
SOFA score on day 5	8 (6–12)	11 (7–14)	7 (5–11)	0.003	10 (6–14)	12 (8–13)	0.83
RRT use	37 (30.8%)	13 (48.1%)	24 (25.8%)	0.027	8 (47.1%)	5 (50.0%)	> 0.99
Corticosteroids use	55 (45.8%)	19 (70.4%)	36 (38.7%)	0.004	12 (70.6%)	7 (70.0%)	> 0.99
- before day 7	45 (81.8%)	16 (84.2%)	29 (80.6%)	0.008	12 (100%)	4 (57.1%)	0.22
- after day 7	10 (18.2%)	3 (15.8%)	7 (19.4%)	0.14	0 (0.0%)	3 (42.9%)	0.04
ICU length of stay (days)	22 (12–33)	25 (19–48)	19 (12–30)	0.044	29 (12–48)	24 (22–29)	0.97
Death in the ICU	28 (23.3%)	9 (33.3%)	19 (20.4%)	0.16	6 (35.3%)	3 (30.0%)	> 0.99
90-day survival	89 (74.2%)	16 (59.3%)	74 (79.6%)	0.032	9 (52.9%)	7 (70.0%)	0.44

Data are presented as medians (IQR: interquartiles) or n (%). P values comparing the invasive aspergillosis versus no aspergillosis groups and IAPA versus CAPA were calculated using Mann-Whitney (continuous variables) and Fisher or Chi² tests when appropriate (categorical variables).

AKI: acute kidney injury, PaO₂: arterial oxygen partial pressure, SAPS II: Simplified Acute Physiology Score II, SOFA: Sequential Organ Failure Assessment, HM: hematological malignancies, COVID-19: Coronavirus Disease 2019, ECMO: extracorporeal membrane oxygenation; PaO₂: arterial oxygen tension; FiO₂: fraction of inspired oxygen; RRT: renal replacement therapy; ICU: intensive care unit.

	All patients (n = 120)	All aspergillosis patients (n = 27)	Non aspergillosis patients (n = 93)	P value	IAPA (n = 17)	CAPA (n = 10)	P value
Death in hospital	31 (25.8%)	12 (44.4%)	19 (20.4%)	0.012	8 (47.1%)	4 (40.0%)	0.45
Data are presented as medians (IQR: interquartiles) or n (%). P values comparing the invasive aspergillosis versus no aspergillosis groups and IAPA versus CAPA were calculated using Mann-Whitney (continuous variables) and Fisher or Chi2 tests when appropriate (categorical variables).							
AKI: acute kidney injury, PaO2: arterial oxygen partial pressure, SAPS II: Simplified Acute Physiology Score II, SOFA: Sequential Organ Failure Assessment, HM: hematological malignancies, COVID-19: Coronavirus Disease 2019, ECMO: extracorporeal membrane oxygenation; PaO2: arterial oxygen tension; FiO2: fraction of inspired oxygen; RRT: renal replacement therapy; ICU: intensive care unit.							

The biological data at ICU admission are summarized in Table 1 and show that the CAPA patients were globally less severely ill than the IAPA patients. The SOFA score on day 1 was significantly lower for CAPA than IAPA patients ($p = 0.012$).

Survival analysis at day 90 showed higher mortality among all IPA patients ($p = 0.042$), whereas mortality did not differ between those with CAPA and IAPA (Figs. 1 and 2).

In this cohort, the duration of mechanical ventilation was higher for patients with IPA (23 days [IQR 17–40] than those without (17 days [IQR 9–25], $p = 0.038$) (Table 1). Renal replacement therapy was more frequent for IPA patients ($p = 0.027$) and supportive therapy by extracorporeal membrane oxygenation (ECMO) was less frequent for CAPA than IAPA patients ($p = 0.004$). Patients who developed IPA were more frequently treated by corticosteroids (70.4% versus 38.7%, $p = 0.004$) and the frequency of such treatment was similar for the CAPA and IAPA groups. The median ICU length of stay was longer for IPA patients (25 days [IQR 19–48] vs 19 days [IQR 12–30], $p = 0.04$). Finally, survival at 90 days after ICU admission was 59.3% for those with IPA and 79.6% for those without an *Aspergillus* infection ($p = 0.032$). Assessment of clinical and laboratory features at IPA diagnosis is presented in Table 2. Of note, a trend pointing towards a longer median interval between ICU admission and IPA diagnosis in CAPA (6 days [IQR 3–13]) was observed compared to IAPA patients (3 days [IQR 2–5]; $p = 0.14$). Among patients treated by voriconazole, CAPA patients experienced a trend towards a longer time to reach therapeutic range (VTC target: 2–6 mg/L) (7 days [IQR 6–32] vs 4 days [2–8], $p = 0.096$). Therapeutic drug monitoring of these patients showed a higher proportion of CAPA patients to get a delayed voriconazole therapeutic range since at day 5 after the initiation of voriconazole, 83.3% of CAPA patients and 33.3% of IAPA patients remained with voriconazole dosage under 2 mg/L ($p = 0.045$). Similarly VTC appeared lower in CAPA patients (2.2mg/L [IQR 1.1–4.4] versus 3.9mg/L [IQR 2–5.7]; $p = 0.01$). Chest CT scans were performed for 24 of 27 patients between eight days before and 12 days after IPA diagnosis. Lung parenchyma abnormalities were present in all patients (Table 3). A lower proportion of well-circumscribed nodules, tree-in bud, and bronchial wall thickening was observed for CAPA than IAPA patients (0% versus 42.9% [$p = 0.024$] for well-circumscribed nodules, 0% vs 50% [$p = 0.014$] for tree in bud, and 10% versus 57% [$p = 0.03$] for bronchial wall thickening). These different aspects are presented in Fig. 3.

Table 2
Clinical and biological characteristics at CAPA and IAPA diagnosis

	All aspergillosis patients (n = 27)	IAPA (n = 17)	CAPA (n = 10)	P value
Temperature (°C)	38.2 (38.0–39.0)	38.0 (37.8–38.8)	38.9 (38.1–39.0)	0.19
Systolic pressure (mmHg)	92 (81–102)	92 (85–102)	90 (78–101)	0.95
Neutrophil count (10 ⁹ /L)	9.6 (4.5–16.5)	13.2 (5.5–19.5)	7.6 (4.0–10.3)	0.18
Lymphocyte count (10 ⁹ /L)	0.72 (0.51–1.03)	0.80 (0.50–1.22)	0.72 (0.55–0.80)	0.56
Ratio of PaO ₂ to FiO ₂	134 (102–179)	108 (86–165)	162 (147–208)	0.04
Septic shock	17 (63.0%)	12 (70.6%)	5 (50.0%)	0.41
Need for vasopressors	19 (70.4%)	12 (70.6%)	7 (70.0%)	> 0.99
Delay between admission and aspergillosis onset (days)	4 (2–8)	3 (2–5)	6 (3–13)	0.14
Mechanical ventilation duration after aspergillosis onset (days)	20 (9–36)	22 (8–46)	17 (11–23)	0.64
Time to VCZ therapeutic range (days) *	6 (4–9)	4 (2–8)	7 (6–32)	0.096
Delayed VCZ therapeutic range (> 5days) *	9/18 (50%)	4/12 (33.3%)	5/6 (83.3%)	0.045
VTC (mg/L) *	2.8 (1.5–5)	3.9 (2–5.7)	2.2 (1.1–4.4)	0.01
VTC min (mg/L) *	1.6 (0.5–3.8)	3.5 (1–5)	0.8 (0.2–0.8)	0.038
VTC max (mg/L) *	5.8 (4.5–7.2)	6.1 (5.4–7.6)	5.2 (3–7)	0.23

Data are presented as medians (IQR: interquartiles) or n (%). P values comparing IAPA versus CAPA were calculated using Mann-Whitney (continuous variables) and Fisher or Chi2 tests when appropriate (categorical variables).

IAPA: Influenza Associated Pulmonary Aspergillosis; CAPA: COVID-19 Associated Pulmonary Aspergillosis; PaO₂: arterial oxygen partial pressure; FiO₂: Fraction of inspired Oxygen; VCZ: Voriconazole; VTC: Voriconazole Trough Concentration

* Among patients treated by voriconazole that were performed a watchful therapeutic drug monitoring of voriconazole

	All aspergillosis patients (n = 27)	IAPA (n = 17)	CAPA (n = 10)	P value
Antifungal treatment duration (days)	42 (14–42)	17 (14–47)	42 (37–42)	0.49
Data are presented as medians (IQR: interquartiles) or n (%). P values comparing IAPA versus CAPA were calculated using Mann-Whitney (continuous variables) and Fisher or Chi2 tests when appropriate (categorical variables).				
IAPA: Influenza Associated Pulmonary Aspergillosis; CAPA: COVID-19 Associated Pulmonary Aspergillosis; PaO2: arterial oxygen partial pressure; FiO2: Fraction of inspired Oxygen; VCZ: Voriconazole; VTC: Voriconazole Trough Concentration				
* Among patients treated by voriconazole that were performed a watchful therapeutic drug monitoring of voriconazole				

Table 3
CT-scan analysis of IAPA and CAPA patients

	All aspergillosis patients (n = 24)	IAPA (n = 14)	CAPA (n = 10)	P value
Diffuse reticular or alveolar opacities	24 (100%)	14 (100%)	10 (100%)	0.99
Wedge-shaped segmental or lobar consolidation	17 (70.8%)	10 (71.4%)	7 (70.0%)	0.99
Well-circumscribed nodule(s)	6 (25.0%)	6 (42.9%)	0 (0.0%)	0.024
Halo sign	3 (12.5%)	2 (14.3%)	1 (10.0%)	0.68
Cavitation	5 (20.8%)	5 (35.7%)	0 (0.0%)	0.053
Air-crescent sign	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.99
Tree in bud	7 (29.2%)	7 (50.0%)	0 (0.0%)	0.019
Bronchial wall thickening	8 (33.3%)	8 (57.1%)	1 (10.0%)	0.03
Pleural effusion	9 (37.5%)	5 (35.7%)	4 (40.0%)	0.99
Data are presented as n (%). P values comparing influenza associated pulmonary aspergillosis (IAPA) versus COVID-19 associated pulmonary aspergillosis (CAPA). IAPA and CAPA groups were tested using Fisher's exact test (categorical variables). CT-scan: computerized-tomography scanner				

Discussion

In this single-center study, 22.5% of patients admitted to the ICU for a severe viral infection, such as COVID-19 (20.4%) or *Influenza* pneumonia (23.9%), developed IPA. A similar prevalence of IPA in ICU patients was observed in the two major representative series of severe influenza (19.2% in a Dutch-Belgian multicenter study on 432 patients, (8)) and COVID-19 patients (21.8% in a French multicenter prospective study with 509 patients included (16)). Secondary fungal infections may have had an impact on the prognosis for these

patients, as the mortality rate, duration of mechanical ventilation, and ICU length of stay were associated with the occurrence of IPA. To date, only limited data are available on the comparison of IAPA and CAPA. In-hospital death occurred for 47.1% and 40% of patients with IAPA and CAPA, respectively, versus 20.4% for patients without aspergillosis. Such high mortality rate has already been observed for IAPA versus non IAPA ICU patients (51% versus 28%, respectively, (25)). Bartoletti et al., among others, have reported similar mortality rates of 44% for CAPA patients versus 19% for non-CAPA patients during the COVID-19 pandemic (13). Although the median age was significantly higher for CAPA than IAPA patients, other demographic data and characteristics of the patients at IPA diagnosis were comparable between the two populations. Among them, immunosuppression was not statistically significantly different, but 47.1% of IAPA patients were immunosuppressed versus 20% of CAPA patients. Concerning biological data, lymphopenia, the PaO₂ to FiO₂ ratio on day 1, and the SOFA score on day 1 were less severe for the CAPA than IAPA patients.

IPA diagnosis in non-neutropenic ICU patients is challenging, as the clinical and radiological features of IPA are not specific and can be affected by underlying conditions. Although the pathophysiology of pulmonary injury differs, influenza pneumonia and COVID-19-associated ARDS may lead to prolonged respiratory distress and inflammatory states and consequently to delayed explorations and treatment. In neutropenic patients, radiological findings may lead to a suspicion of fungal infections and guide microbiological investigations. The radiological criteria of pulmonary mold diseases have been recently revised in the consensus definitions for invasive fungal disease (1). The authors proposed to add a new more sensitive item, defined as wedge-shaped lobar or segmental consolidation, to the classical CT criteria (solid nodule, halo sign, cavitation, air-crescent sign). These updated criteria have been validated in a recent cohort analysis in which nodule and/or consolidation patterns were observed in more than 98% of IPA patients, irrespective of their neutrophil status (26). Nodule and consolidation patterns were present, alone or together, in our study in 83% of cases (93% of IAPA and 70% of CAPA). Of note, although these updated criteria increase the sensitivity of CT for the detection of aspergillosis, they concurrently decrease its specificity, and there is currently no CT sign that is both sensitive and specific for aspergillosis. In the present study, although radiological findings were generally considered to not be suggestive of IPA during ARDS, a meticulous analysis showed several interesting differences between the images, with well-circumscribed nodules, tree in bud, and bronchial wall thickening, which were observed significantly more frequently in IAPA than CAPA patients.

Because of these non-specific features, mycological testing is of great value for the screening of IPA in these patients. However, as observed in this and previous studies, serum GM is far less often positive in IAPA and CAPA cases than in classical immunosuppressed patients with hematological malignancies. Thus, the diagnosis relies mostly on respiratory samples. Compared to the AspICU classification, the main advance of the new consensual IAPA and CAPA definitions is to fine-tune criteria and tools for respiratory samples. In the IAPA definition according to Verweij et al., positive tracheal aspirates and even positive sputum cultures are criteria for probable IAPA, depending on the clinical presentation, with or without tracheobronchitis, which accounts for approximately 27% of all cases of IAPA (27), and pulmonary or cavitating infiltrates (7). Concerning CAPA, PCR and GM detection in respiratory samples are now included in the definition by Koehler et al. and although positive BAL is a criterion for probable CAPA, non-bronchoscopic respiratory

samples are criteria for possible CAPA. The severity and clinical outcome of IAPA and CAPA from this series relative to that of other ICU patients highlights the relevance of these new definitions.

These specific conditions suggest that IPA is likely to be underdiagnosed in ARDS populations (4), whereas recent evidence has suggested that critically ill patients, in particular both severe influenza and COVID-19 patients, are populations at risk of IPA. Several mechanisms that may facilitate fungal infection have been identified, such as sepsis-induced systemic immunosuppression due to severe influenza virus or SARS-CoV2 infection (28). Several recent studies reported profound lymphopenia in severe Covid-19 associated with the expansion of myeloid derived suppressor cells, which may promote the acquisition of secondary infections, as illustrated by the large proportion of COVID-19 patients that develop respiratory reactivation of Herpes virus (29). On the other hand, alveolar damage, dysfunction of mucociliary clearance, and local immune disorders due to COVID-19 or severe influenza pneumonia may also be key mechanisms involved in fungal invasion (8). Finally, recent therapeutic strategies have emerged worldwide during the first months of COVID-19 pandemic with the aim of reducing inpatient mortality, such as corticosteroids, new antiviral drugs or biotherapies. The recent RECOVERY trial (30) has positioned corticosteroids as first-line therapeutic agents, with a demonstrated improvement of patient prognosis. Although such improvement with low-dose corticosteroids (i.e., 6 mg of dexamethasone per day for 10 days) should be highlighted, we observed a significant association between corticosteroid use and the occurrence of IPA. Cohort studies have already demonstrated that corticosteroids increase the risk of IPA in severe influenza patients (11), emphasizing the need for enhanced awareness of IPA in these patients. Drug-drug interactions also arise in this context. Dexamethasone was widely used in CAPA patients and induces CYP2C9, which could decrease the VTC (31). Thus, therapeutic drug monitoring is a cornerstone for the patient management as we were faced in this series with low concentrations of voriconazole with a significant delay to reach the optimal therapeutic range in CAPA versus IAPA patients.

Our study had several limitations, including the sample size, which prevented multivariate analysis. Furthermore, during the first wave of the COVID-19 pandemic bronchoscopic explorations were considered as a source of exposure to SARS-CoV-2 for physicians due to the risk of the aerosolization during this procedure. Thus, we performed such investigations in COVID-19 patients less frequently while this risk has been debunked in recent papers (32). Hence, it was not always possible to identify bronchoscopic findings suggestive of *Aspergillus* tracheobronchitis, such as epithelial plaques, pseudomembranes, or ulcers.

The main strengths of this study included the standardized management of ARDS and mycological testing of all patient samples, allowing an exhaustive laboratory data set. Our expert laboratory accredited by the French Committee of Accreditation and applying to become an ECMM Excellence Center in mycology, implemented fungal culturomic and genomic tools, as well as *Aspergillus* antigen detection, to allow easy classification of cases. Furthermore, this is one of the first studies to apply the new consensual criteria for both IAPA and CAPA, whereas questions have been recently raised concerning the relevance of other classifications in determining the true burden of disease (6).

Conclusion

ICU patients presenting with ARDS during COVID-19 are very similar to those with severe influenza pneumonia in terms of the prevalence of IPA and outcome. It is now possible to draw the archetype of such patients using the new clinical and biological case definitions of IAPA and CAPA. Radiological findings of IPA in both populations using the new criteria increased the sensitivity but still lack specificity. Nevertheless, they also showed interesting differences between IAPA and CAPA. IAPA typically occurs in a more immunosuppressed and/or chronic respiratory disease background than CAPA, which is mainly favored by advanced age, irrespective of the medical background. Finally, reaching voriconazole trough concentrations remains challenging in CAPA patients and emphasizes the importance of therapeutic drug monitoring. Future larger prospective studies may help in designing the most well-adapted personalized management to prevent IPA, which represents a high burden of death in severe COVID-19 and *Influenza* pneumonia.

Declarations

Ethics approval and consent to participate

This study conforms to the principles outlined in the Declaration of Helsinki and was approved by the institutional ethics board of Rennes University Hospital, France (N 20-56).

Consent for publication

Not applicable

Availability of supporting data

The datasets from this study are available from the corresponding author on request.

Competing interests

The authors report no conflict of interest related to this work

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Contribution of authors

Conception and design was provided by FR, KP, MLed, AG, JMT, AM and JPG;

Data analysis and interpretation were carried out by FR, KP, BA, HG, DLP, CBK, AB, BLD, YL, MLes, BP, CC, AM, FRG, SB and JPG; CT scans analysis were performed by MLed; Drafting and revision of the manuscript was carried out by FR, MLed, YL, YLT and JPG with all authors providing critical feedback and edits to subsequent revisions.

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Figures

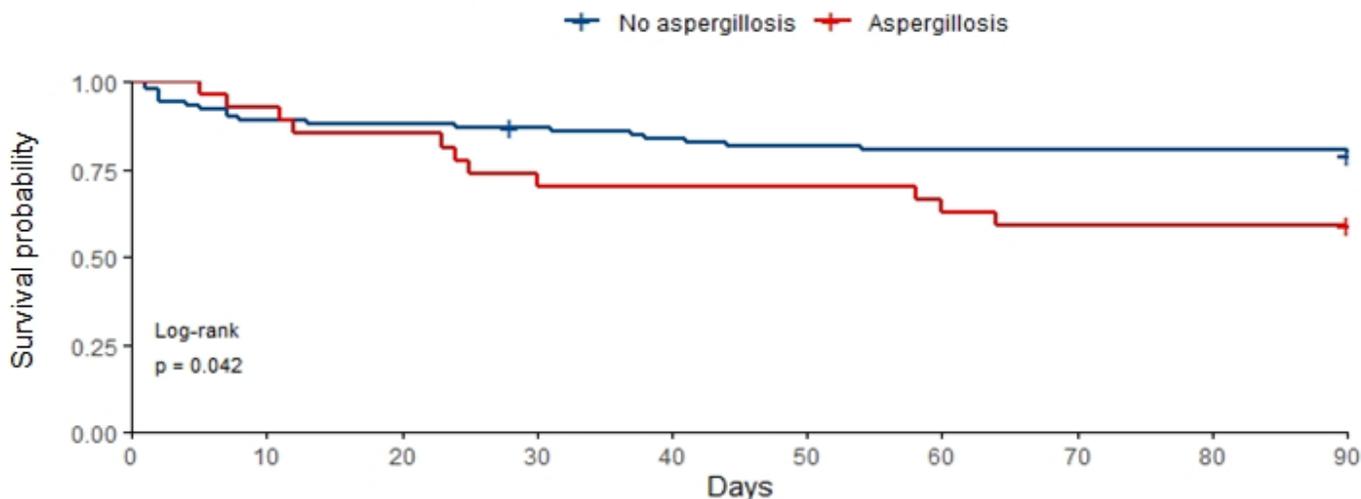


Figure 1

Cumulative 90-day mortality from admission to the intensive care unit in the whole population

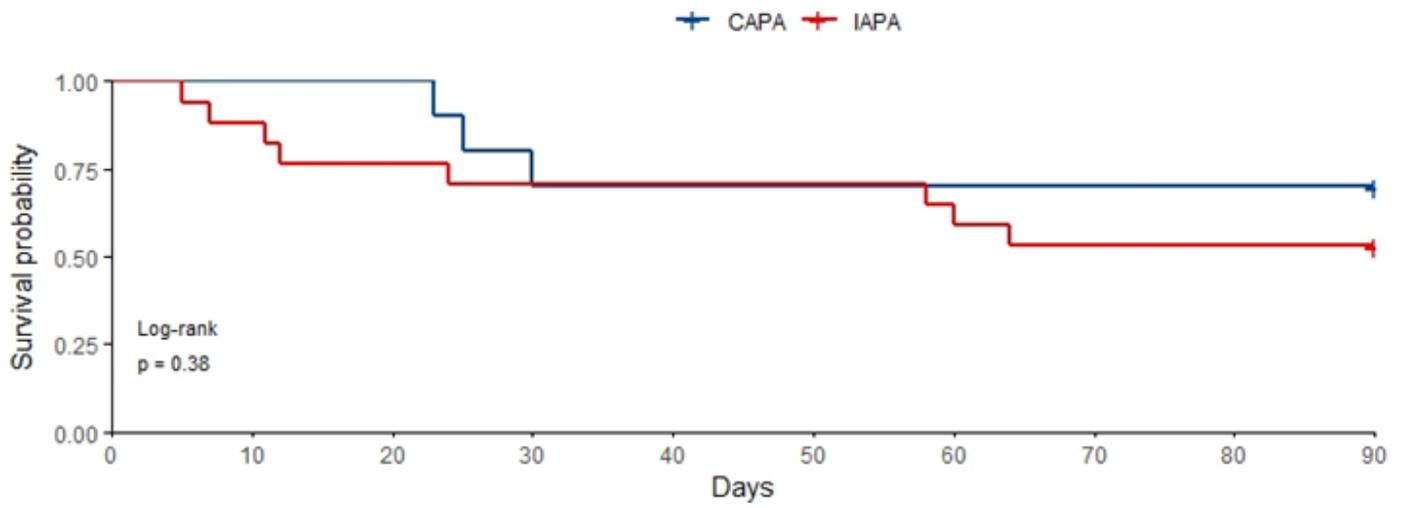


Figure 2

Cumulative 90-day mortality from admission to the intensive care unit among CAPA and IAPA patients

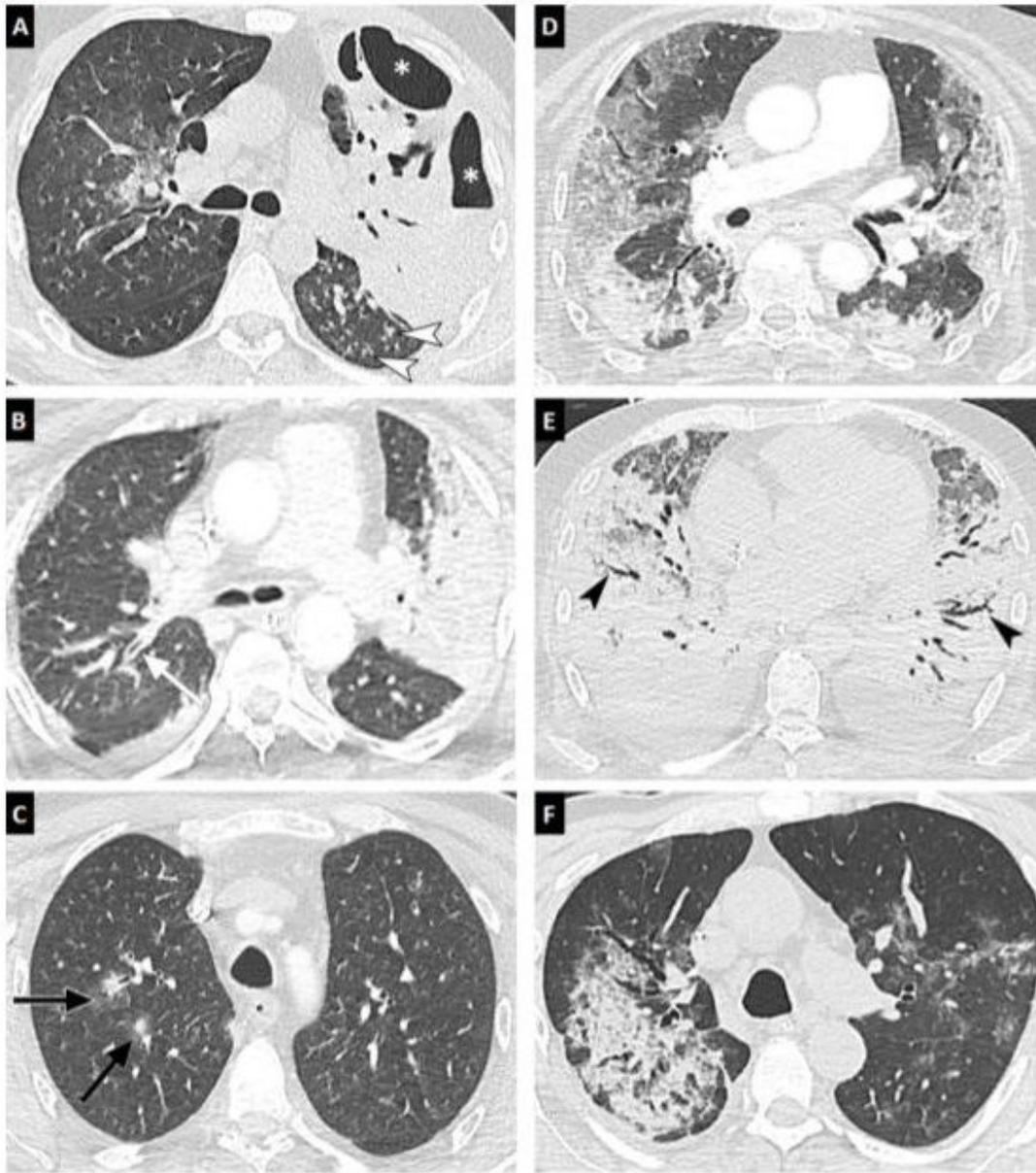


Figure 3

Chest CT-scan of IAPA and CAPA patients. Chest CT scans in the axial plane (lung window: WIPOO/L-500 HU) of three patients with IAEA (A, B, C) and three patients with CAPA (D, E, F). Typical CT findings in IAPA are unilateral or bilateral areas of consolidation with air bronchogram (A), cavitary formation (asterisks), tree in bud (white arrowheads), bronchial wall thickening (white arrow), or occasionally nodules with halo signs (C, black arrows). Patients with CAPA may exhibit non-specific CT findings, such as bilateral areas of ground-glass opacity and/or crazy paving (E), extensive consolidation areas associated with peripheral traction bronchiectasis (E, black arrowheads), or, more rarely, unilateral consolidation areas (F).

Supplementary Files

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