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ORIGINAL ARTICLE

Comparison of influenza- and COVID-19-associated pulmonary aspergillosis in China

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Abstract

Purpose We conducted a monocentric retrospective study using the latest definitions to compare the demographic, clinical, and biological characteristics of influenza-associated pulmonary aspergillosis (IAPA) and COVID-19-associated pulmonary aspergillosis (CAPA).

Methods The study retrospectively enrolled 180 patients, including 70 influenza/IPA patients (with positive influenza A/B and *Aspergillus*) and 110 COVID-19/IPA patients (with positive SARS-CoV-2 and *Aspergillus*). Among them, 42 (60%) and 30 (27.3%) patients fulfilled the definitions of IAPA and CAPA, respectively.

Results The CAPA patients had significantly higher in-hospital mortality (13/31, 41.9%) than IAPA patients (8/42, 19%) with a *P*-value of 0.033. Kaplan–Meier survival curve also showed significantly higher 30-day mortality for CAPA patients ($P=0.025$). Additionally, the CAPA patients were older, though insignificantly, than IAPA patients (70 (60–80) vs. 62 (52–72), $P=0.075$). A lower percentage of chronic pulmonary disease (12.9 vs. 40.5%, $P=0.01$) but higher corticosteroids use 7 days before and after ICU admission (22.6% vs. 0%, $P=0.002$) were found in CAPA patients. Notably, there were no significant differences in the percentage of ICU admission or ICU mortality between the two groups. In addition, the time from observation to *Aspergillus* diagnosis was significantly longer in CAPA patients than in IAPA patients (7 (2–13) vs. 0 (0–4.5), $P=0.048$).

Conclusion Patients infected with SARS-CoV-2 and *Aspergillus* during the concentrated outbreak of COVID-19 in China had generally higher in-hospital mortality but a lower percentage of chronic pulmonary disease than those infected with influenza and *Aspergillus*. For influenza-infected patients who require hospitalization, close attention should be paid to the risk of invasive aspergillosis upfront.

Keywords COVID-19 · Influenza · Invasive pulmonary aspergillosis · CAPA · IAPA

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Introduction

Invasive pulmonary aspergillosis (IPA) is caused by *Aspergillus* species, which has been mainly described in immunocompromised patients, especially in severe and prolonged neutropenia, hematological malignancies, and solid organ transplantation [1, 2]. The risk factors for IPA development are heterogeneous and depend on the patient's underlying diseases and the type and duration of immunosuppressive therapy [3, 4]. In critically ill, non-immunosuppressed patients, severe influenza is recognized as a risk factor for IPA [3, 5, 6].

Influenza-associated pulmonary aspergillosis (IAPA) is an emerging complication of influenza infection that markedly increases influenza-associated mortality [3]. Nevertheless, the incidence of IAPA in hospitalized patients varies significantly between studies from different geographical regions, ranging from 0.2 to 23% [7–10]. The diagnosis of IPA is usually based on the positive culture of lower respiratory tract (LRT) specimens or galactomannan (GM) testing in serum or bronchoalveolar lavage fluid (BALF) [11, 12], as well as atypical radiological features [5]. A timely diagnosis of IAPA is necessary because of the poor outcomes observed despite early treatment with antiviral and antifungal therapy [3, 13, 14]. The newly proposed criteria for IAPA case definition in intensive care unit (ICU) patients may improve the management of IAPA patients [5].

Coronavirus disease-19 (COVID-19) is a worldwide pandemic. There have been several reports of COVID-19-associated pulmonary aspergillosis (CAPA), which raises concerns about the severity and mortality of this superinfection [15–17]. In a multinational observational cohort study of CAPA patients, the ICU mortality rates were significantly higher in CAPA patients (52%) than in CAPA-excluded patients (34%) [18], as shown in other studies [19, 20]. China implemented the "Ten New Measures" to optimize the prevention and control measures for COVID-19 in December 2022. Since then, a large number of patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have overwhelmed hospitals. However, there have been few reports of differences in the severity between patients with COVID-19 and influenza. The recently published CAPA case definition [21] has made it possible to compare patients suffering from IAPA and CAPA. Therefore, we set up this monocentric retrospective study to compare the demographic, clinical outcomes, and biological characteristics of IAPA and CAPA patients in China.

Methods

Study population and data collection

During the pandemic of SARS-CoV-2, the number of influenza patients was very small. Therefore, in this retrospective study, all patients who were admitted to China-Japan Friendship Hospital from October 2017 to March 2019 with PCR-confirmed influenza A/B were included. All patients who were admitted to the same hospital from 1 December 2022 to 15 January 2023 with PCR-confirmed SARS-CoV-2 were also included. Among them, patients with one or more positive mycological tests within 1 month of influenza/COVID-19 diagnosis were analyzed as potential cases. The collected test items included GM tests in serum and BALF, sputum culture, BALF culture, and BALF microscopy. The clinical data of these patients were retrospectively collected from the medical record system.

Definitions

In this study, *Aspergillus* biologic positive was defined as positive for at least one of the following tests: sputum or BALF culture, BALF microscopy, and serum or BALF GM tests. *Aspergillus* isolates were identified at the species level based on microscopic and phenotypic features, and the identification of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS). Influenza/IPA patients were defined as those with positive influenza A/B and *Aspergillus*, and the COVID-19/IPA patients were defined as those with positive SARS-CoV-2 and *Aspergillus*. The proven aspergillosis cases, which need histology testing, were not included. The probable IAPA was defined according to the recent expert consensus, which had pulmonary infiltrate and at least one of the following: serum GM index $>$ 0.5 or BALF GM index $>$ 1.0 or positive *Aspergillus* BALF culture, or cavitating infiltrate (not attributed to another cause) and at least one of the following: positive sputum culture or positive tracheal aspirate culture [5]. The probable CAPA was defined according to the 2020 ECMM/ISHAM consensus criteria, including imaging findings of pulmonary infiltrate or cavitating infiltrate, refractory fever or pleural rub or chest pain or hemoptysis in clinical factors, and positive BALF culture or serum GM index $>$ 0.5 or BALF GM index $>$ 1.0 or positive *Aspergillus* BALF microscopy in microbiological tests [21].

Statistical analysis

Comparative analysis was conducted between influenza/IPA and COVID-19/IPA patients and IAPA and CAPA patients.

Demographic characteristics, underlying diseases, and clinical characteristics of patients were presented as numbers and percentages for categorical variables, and medians and interquartile ranges (IQR, 25–75%) for continuous variables. The qualitative data were compared using the chi-square or Fisher test as appropriate. The Mann–Whitney *U* test was used for quantitative data. Survival curves were constructed until day 30 from *Aspergillus* diagnosis 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 < 0.05. All statistical analyses were performed using GraphPad Prism 9.5.1 (GraphPad Software, La Jolla, CA, USA).

Results

Clinical characteristics and test results

Overall, 180 mycological-positive patients were included in the study, including 70 influenza/IPA patients and 110 COVID-9/IPA patients, with incidences of 12% (70/583) and 15% (110/733), respectively (Fig. 1). Among them, 42/70 (60%) and 31/110 (28.2%) patients fulfilled the definition of probable IAPA and CAPA, respectively. Demographic characteristics, underlying diseases, and clinical characteristics of the patients were summarized in Table 1. The median age of these 180 patients was 68 (58–76) years. A significant difference ($P = 0.007$) was found in age between influenza/IPA (63.5, 54.3–72.8) and COVID-19/IPA (70, 60–77.8) patients, but not IAPA and CAPA patients ((62 (52–72) vs. 70 (60–80), $P = 0.075$). No significant differences were found in the sex ratio.

Comparison between influenza/IPA and COVID-19/IPA patients

Among the underlying conditions, the COVID-19/IPA patients had significantly higher chronic kidney disease ($P = 0.029$) and solid organ transplantation ($P < 0.001$). Meanwhile, the proportion of chronic pulmonary disease was significantly lower in COVID-19/IPA patients (13.6%) than in influenza/IPA patients (41.4%) with $P < 0.001$. In addition, lymphocytes, $CD4^+$ T-cell, creatinine, and procalcitonin (PCT) at *Aspergillus* diagnosis were also significantly different between COVID-19/IPA and influenza/IPA patients (Table 1). ICU admission was significantly lower for COVID-19/IPA patients compared with influenza/IPA patients (38.2% vs. 57.1%, $P = 0.013$).

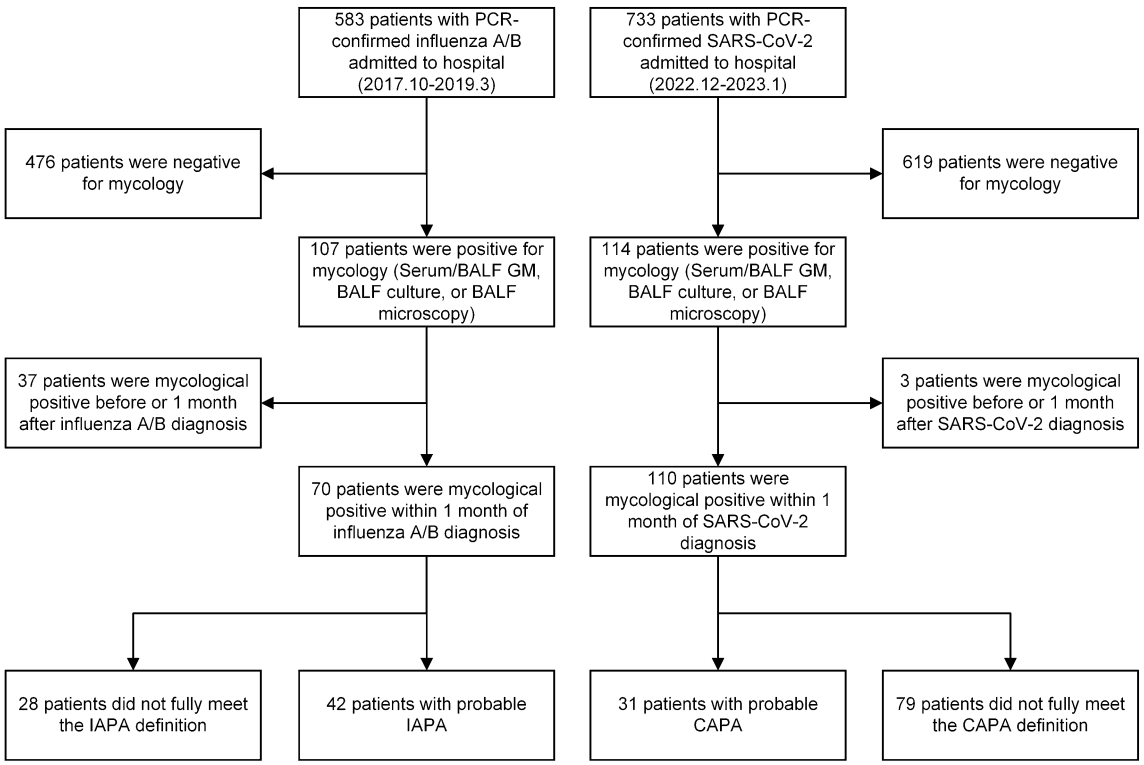

Fig. 1 Flowchart of the study including patients with IAPA and CAPA. IAPA, influenza-associated pulmonary aspergillosis; CAPA, coronavirus disease 2019-associated invasive pulmonary aspergillosis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Table 1 Characteristics of 180 patients with pulmonary aspergillosis and positive influenza A/B or SARS-CoV-2

	Total ($n=180$)	Influenza/IPA patients ($n=70$)	COVID-19/IPA patients ($n=110$)	*P*-value	IAPA ($n=42$)	CAPA ($n=31$)	*P*-value
Demographics							
Female	53 (29.4)	26 (37.1)	27 (24.5)	0.071	17 (40.5)	6 (19.4)	0.055
Age, median (IQR), years	68 (58–76)	63.5 (54.3–72.8)	70 (60–77.8)	**0.007**	62 (52–72)	70 (60–80)	0.075
BMI, median (IQR), kg/m^2	23.4 (20.8–26)	23.5 (19.7–26.6)	23.4 (21.3–25.9)	0.742	23.3 (19.1–27.5)	23.4 (21.1–25.9)	0.963
Underlying conditions							
Hypertension	90 (50)	30 (42.9)	60 (54.5)	0.126	18 (42.9)	15 (48.4)	0.639
Coronary disease	36 (20)	10 (14.3)	26 (23.6)	0.126	7 (16.7)	5 (16.1)	0.951
Cerebrovascular disease	41 (22.8)	18 (25.7)	23 (20.9)	0.454	12 (28.6)	9 (29)	0.966
Diabetes mellitus	67 (37.2)	22 (31.4)	45 (40.9)	0.2	11 (26.2)	13 (41.9)	0.157
Chronic kidney disease	32 (17.8)	7 (10)	25 (22.7)	**0.029**	4 (9.5)	4 (12.9)	0.716
Hemodialysis	7 (3.9)	5 (7.1)	2 (1.8)	0.111	2 (4.8)	0	0.505
Chronic liver disease	13 (7.2)	6 (8.6)	7 (6.4)	0.577	6 (14.3)	4 (12.9)	1
Liver cirrhosis	4 (2.2)	3 (4.3)	1 (0.9)	0.301	3 (7.1)	0	0.257
Autoimmune disease	18 (10)	8 (11.4)	10 (9.1)	0.61	4 (9.5)	1 (3.2)	0.387
AIDS	1 (0.6)	1 (1.4)	0	0.389	0	0	NA
Chronic pulmonary disease	44 (24.4)	29 (41.4)	15 (13.6)	**< 0.001**	17 (40.5)	4 (12.9)	**0.01**
COPD	15 (8.3)	10 (14.3)	5 (4.5)	**0.021**	6 (14.3)	2 (6.5)	0.454
Bronchiectasis	9 (5)	6 (8.6)	3 (2.7)	0.857	5 (11.9)	0	0.068
Asthma	2 (1.1)	1 (1.4)	1 (0.9)	1	1 (2.4)	0	1
Others	18 (10)	12 (17.1)	6 (5.5)	**0.011**	5 (11.9)	2 (6.5)	0.691
Hematological malignancy	4 (2.2)	1 (1.4)	3 (2.7)	1	0 (0)	1 (3.2)	0.425
Other hematological diseases	7 (3.9)	4 (5.7)	3 (2.7)	0.433	2 (4.8)	1 (3.2)	1
Solid organ malignancy	17 (9.4)	7 (10)	10 (9.1)	0.839	6 (14.3)	4 (12.9)	1
Solid organ transplantation	31 (17.2)	0	31 (28.2)	**< 0.001**	0	8 (25.8)	**0.001**
Kidney transplantation	16 (8.9)	0	16 (14.5)	**0.001**	0	3 (9.7)	0.072
Liver transplantation	1 (0.6)	0	1 (0.9)	1	0	0	NA
Lung transplantation	14 (7.8)	0	14 (12.7)	**0.002**	0	5 (16.1)	**0.011**
Laboratory tests at admission							
Leukocyte count, median (IQR), $10^9/L$	8.6 (5.4–12.5)	8.4 (5.7–13.8)	8.7 (5.3–12.3)	0.735	8.4 (5.5–13.2)	10.8 (6.5–16.6)	0.129
Neutrophils, median (IQR), $10^9/L$	7.4 (3.9–11.4)	7.2 (3.9–11.1)	7.5 (3.9–11.4)	0.934	7.1 (3.9–9.7)	9.1 (5.8–14.6)	0.056
Lymphocytes, median (IQR), $10^9/L$	0.8 (0.4–1.2)	0.9 (0.6–1.3)	0.7 (0.4–1.1)	**0.012**	0.9 (0.6–1.2)	0.7 (0.4–1)	0.141
CRP, median (IQR), mg/dL	47.1 (12.1–105.7)	64 (11–118.9)	40.6 (12.4–95.1)	0.508	56.3 (11–102.6)	43.3 (11.8–153.5)	0.703
Creatinine, median (IQR), mg/dL	78.4 (56.7–133.8)	66.6 (50.2–91.5)	98.3 (62.4–149.3)	**0.001**	63.5 (48.7–95.1)	88.6 (60.7–127.8)	0.052
LDH, median (IQR), IU/L	298.5 (222–494.3)	307 (224–504)	296 (214–494)	0.692	307 (225.5–504)	467 (255–561)	0.252

Table 1 (continued)

	Total ($n=180$)	Influenza/IPA patients ($n=70$)	COVID-19/IPA patients ($n=110$)	*P*-value	IAPA ($n=42$)	CAPA ($n=31$)	*P*-value
CD4, median (IQR), cell/μL	274.5 (152.8–525.8)	439 (217.5–651)	193.5 (111.3–383)	**< 0.001**	345 (200–554)	271 (158–398)	0.128
CD8, median (IQR), cell/μL	189.5 (101.8–333)	208 (123.3–359.3)	171.5 (92–263.3)	0.124	223 (120–395)	171 (91–290)	0.292
NK, median (IQR), cell/μL	105 (56–240.3)	65.5 (47.3–207)	115 (58.8–238)	0.267	105 (60–280)	197 (70–323)	0.568
IL-6, median (IQR), pg/mL	44.1 (11.4–173.3)	22.8 (10–35.8)	49.2 (11.4–189.4)	0.235	33.4 (18.6–38.2)	113.5 (33–510.5)	0.078
PCT, median (IQR), ng/mL	0.3 (0.1–0.7)	0.4 (0.2–1.3)	0.2 (0.1–0.5)	**< 0.001**	0.4 (0.2–1.3)	0.2 (0.1–1)	0.163
ESR, median (IQR), mm/hr	44 (2**2**–**6**5.3)	42.5 (15.5–66.3)	44.5 (26.5–63)	0.399	41 (18–64.5)	37.5 (25–60.8)	0.788
ICU admission	82 (45.6)	40 (57.1)	42 (38.2)	0.013	28 (66.7)	19 (61.3)	0.635
Time from ICU admission to *Aspergillus* diagnosis, median (IQR), days	2 (1–6.5)	1 (1–2.3)	4 (1–7)	**0.028**	1 (1–2)	3.5 (1–7.3)	0.177
ICU length of stay, median (IQR), days	11.5 (6.3–20)	11.5 (7–19.3)	11.5 (6–20.8)	0.941	12 (8.8–23.3)	11 (6–16.5)	0.367
Mechanical ventilation, median (IQR), days	10.5 (5.3–20)	9.5 (5–19.3)	11 (6–20.8)	0.705	11 (5.8–23.3)	10 (6–20.5)	0.965
Corticosteroids use 7 days before and after ICU admission	32 (17.8)	1 (1.4)	31 (28.2)	**< 0.001**	0	7 (22.6)	**0.002**
Treatment							
Antifungal treatment	131 (72.8)	47 (67.1)	84 (76.4)	0.175	29 (69)	25 (80.1)	0.264
Antiviral treatment	140 (77.8)	66 (94.3)	74 (67.3)	**< 0.001**	40 (95.2)	26 (83.9)	0.265
Clinical outcome							
ICU mortality	30 (16.7)	10 (14.3)	20 (18.2)	0.494	7 (16.7)	9 (29)	0.207
In-hospital mortality	46 (25.6)	13 (18.6)	33 (30)	0.087	8 (19)	13 (41.9)	**0.033**
Time from observation to death, median (IQR), days	12.5 (8.3–18)	10 (7–10)	15 (9–18)	0.078	10 (7.8–10.5)	15 (10–21)	0.127
Time from observation to Aspergillus diagnosis, median (IQR), days	6 (1–10.8)	0 (0–4)	7 (5–11)	**0.008**	0 (0–4.5)	7 (2–13)	**0.048**
Time from Aspergillus diagnosis to death, median (IQR), days	6.5 (2–11)	8 (4–10)	6 (1–11)	0.769	9 (6.8–10)	6 (3–12)	0.561

Data are presented as no. (%) or median (IQR) unless otherwise indicated. Bold indicated data with a significant difference. *IPA*, invasive pulmonary aspergillosis; *SARS-CoV-2*, severe acute respiratory syndrome coronavirus 2; *IAPA*, influenza-associated pulmonary aspergillosis; *COVID-19*, coronavirus disease-19; *CAPA*, COVID-19-associated pulmonary aspergillosis; *BMI*, body mass index; *AIDS*, acquired immune deficiency syndrome; *COPD*, chronic obstructive pulmonary disease; *CRP*, C-reaction protein; *LDH*, lactate dehydrogenase; *NK*, natural killer cell; *IL-6*, interleukin 6; *PCT*, procalcitonin; *ERS*, erythrocyte sedimentation rate

However, the ICU mortality and in-hospital mortality were numerically higher among COVID-19/IPA patients, although the difference did not reach statistical significance ($P=0.494$, 0.087).

Comparison between IAPA and CAPA patients

Different results were obtained for the IAPA and CAPA patients that fulfilled the latest definitions. The proportion

of solid organ transplantation (especially lung transplantation) was significantly higher in CAPA patients (25.8% vs. 0%, $P=0.001$), and chronic pulmonary disease was significantly lower in CAPA patients (12.9% vs. 40.5%, $P=0.01$). Interestingly, the CAPA patients had significantly higher in-hospital mortality ($P=0.033$) and corticosteroids use 7 days before and after ICU admission ($P=0.002$), and longer time from ICU admission to *Aspergillus* diagnosis ($P=0.028$). However, the percentage of ICU admission ($P=0.635$) and ICU mortality ($P=0.207$) between the two groups were not significantly different. Notably, 11 of 31 CAPA patients were treated with steroids for SARS-CoV-2, and the in-hospital mortality was 54.5% (6/11). At the same time, the remaining 20 patients did not use steroids, and the in-hospital mortality was 35%, with no significant difference between the two. No statistically significant differences were observed among other factors.

Clinical outcomes

A total of 46/180 (25.6%) patients died in hospital since influenza A/B or SARS-CoV-2 diagnosis, including 13/70 (18.6%) influenza/IPA patients and 33/110 (30%) COVID-19/IPA patients (Table 1). Among them, 8/42 (19%) and 13/31 (41.9%) patients fulfilled the definitions of IAPA and CAPA, respectively. Survival analysis at day 30 after *Aspergillus* diagnosis showed significantly higher mortality among CAPA patients ($P=0.025$), whereas mortality did not differ between influenza/IPA and COVID-19/IPA patients ($P=0.108$), as shown in Fig. 2. The time from observation to death was not statistically significant between CAPA patients and IAPA patients (15 (10–21) vs. 10 (7.8–10.5) days, $P=0.127$). However, the time from observation to *Aspergillus* diagnosis was significantly longer in CAPA patients than in IAPA patients (7 (2–13) vs. 0 (0–4.5), $P=0.048$).

Differences between survivors and non-survivors of the IAPA and CAPA patients were shown in Table S1. The results indicated that non-survivors had a significantly higher proportion of SARS-CoV-2 infection ($P=0.033$) and corticosteroids use 7 days before and after ICU admission ($P<0.001$), and a lower proportion of diabetes mellitus ($P=0.032$). Notably, solid organ transplantation did not greatly impact the survival of the patients, even fewer solid organ transplantation patients were found in the non-survivors group. In addition, several laboratory tests for *Aspergillus* diagnosis also had significant differences.

Bacterial infection analysis

Bacterial infections among these patients were analyzed. Of the 180 patients, 87 (48.3%) were positive for bacteria between influenza/SARS-CoV-2 diagnosis and 1 month after *Aspergillus* diagnosis. Meanwhile, 39 of the 73 patients who fulfilled the definitions of IAPA or CAPA were positive for bacteria, including 27 survivors and 12 non-survivors, which accounted for 51.9% and 57.1% of the total survivors and non-survivor, respectively. Additionally, the bacterial infection rates between influenza/IPA and COVID-19/IPA patients, and between IAPA and CAPA patients were not significantly different (Fig. 3A). A total of 169 non-repetitive clinical strains were isolated from the 87 patients (Fig. 3B), including *Enterobacteriales* ($n=40$), *Acinetobacter* spp. ($n=38$), *Pseudomonas* (N = 23), *Staphylococcus* spp. ($n=12$), and others ($n=56$). The resistant phenotypes of these strains were also analyzed. As shown in Fig. 3C, the carbapenem-resistant *Enterobacteriales* (CRE), carbapenem-resistant *A. baumannii* (CRAB), carbapenem-resistant *P. aeruginosa* (CRPA), and methicillin-resistant *Staphylococcus* (MRS) were not statistically significantly different between influenza and COVID-19 patients.

Laboratory diagnostics of invasive pulmonary aspergillosis

Laboratory diagnostics of invasive pulmonary aspergillosis included BALF or sputum culture (52.8%), BALF microscopy (20%), and GM tests in blood serum (52.8%) and BALF (35%) were shown in Table 2. The positive

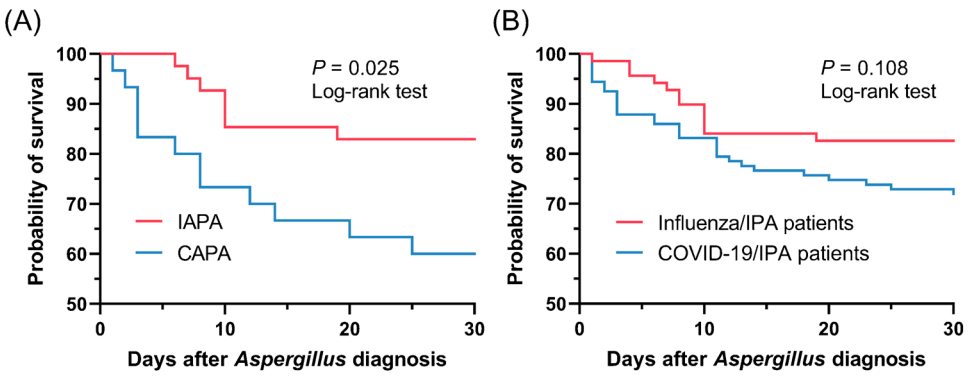

Fig. 2 Kaplan–Meier survival curves for 30-day mortality among IAPA and CAPA patients, and among influenza/IPA and COVID-19/IPA patients. IAPA, influenza-associated pulmonary aspergillosis; COVID-19, coronavirus disease-19; CAPA, COVID-19-associated pulmonary aspergillosis; IPA, invasive pulmonary aspergillosis

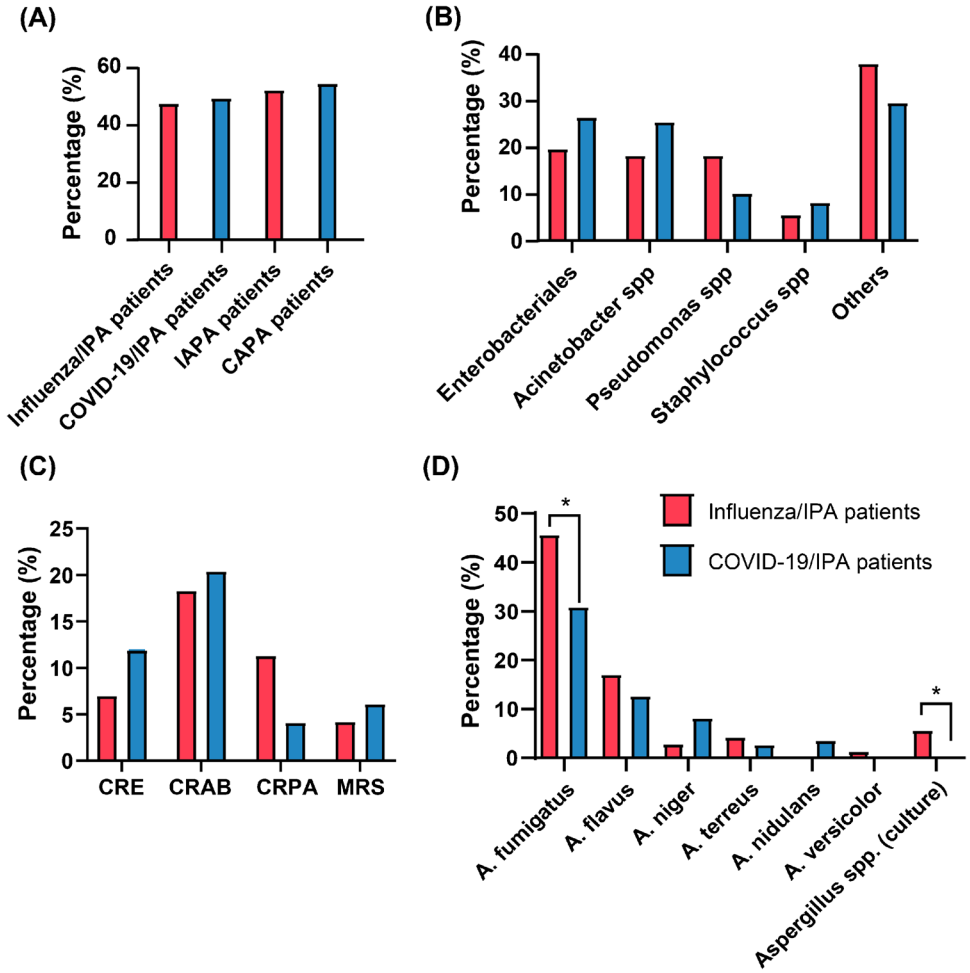

Fig. 3 Bacteria and *Aspergillus* that were isolated from the 180 enrolled patients. **A** The positive rate of bacteria in influenza/IPA patients, COVID-19/IPA patients, IAPA patients, and CAPA patients. **B**, **C** Distribution of bacteria among influenza/IPA patients and COVID-19/IPA patients. CRE, carbapenem-resistant *Enterobacteriales*; CRAB, carbapenem-resistant *A. baumannii*; CRPA, carbapenem-resistant *P. aeruginosa*; MRS, methicillin-resistant *Staphylococcus*. **D** *Aspergillus* isolated from IAPA and CAPA patients. IAPA, influenza-associated pulmonary aspergillosis; CAPA, coronavirus disease 2019-associated invasive pulmonary aspergillosis; IPA, invasive pulmonary aspergillosis. The significance level was as follows: $*P < 0.05$

Table 2 Laboratory diagnostics of *Aspergillus* of the 180 enrolled patients

	Total ($n=180$)	Influenza/IPA patients ($n=70$)	COVID-19/IPA patients ($n=110$)	*P*-value	IAPA ($n=42$)	CAPA ($n=31$)	*P*-value
Culture (+)	95 (52.8)	45 (64.3)	50 (45.5)	**0.014**	18 (42.9)	6 (19.4)	**0.035**
Microscopy (+)	36 (20)	10 (14.3)	26 (23.6)	0.126	8 (19)	6 (19.4)	0.974
Serum GM (+)	95 (52.8)	41 (58.6)	54 (49.1)	0.214	31 (73.8)	23 (74.2)	0.971
BALF GM (+)	63 (35)	24 (34.3)	39 (35.5)	0.873	15 (35.7)	13 (41.9)	0.589

Data are presented as no. (%). Bold indicated data with a significant difference. *IPA*, invasive pulmonary aspergillosis; *IAPA*, influenza-associated pulmonary aspergillosis; *COVID-19*, coronavirus disease-19; *CAPA*, COVID-19-associated pulmonary aspergillosis; *GM*, galactomannan; *BALF*, bronchoalveolar lavage fluid

rate of the serum GM test was 72% (95/132) for all the 180 enrolled patients, and the positive rate of BALF GM test was 37.5% (36/96). In addition, the median time from observation to BALF GM positivity was significantly shorter than that to serum GM positivity (2 (0–5.5) vs. 3 (0–10), $P=0.03$). Histology testing was not included in this study. IAPA patients had a significantly higher positive rate of culture. In addition, the positive rates of GM test in serum and BALF were not statistically different between CAPA patients and IAPA patients.

Distribution of Aspergillus

Aspergillus culture was positive in 95 cases, accounting for 52.8% of these 180 patients. Among them, 118 *Aspergillus* were identified, including 66 *A. fumigatus*, 26 *A. flavus*, 11 *A. niger*, 6 *A. terreus*, 4 *A. nidulans*, and 1 *A. versicolor* (Fig. 3D). The *A. fumigatus* was identified the most among influenza and COVID-19 patients (45.7% vs. 30.9%), and a significant difference was observed between them ($P=0.044$). COVID-19 patients had a higher percentage of *Aspergillus* other than *A.*

fumigatus compared with influenza patients. Notably, all 4 *A. nidulans* were identified in COVID-19 patients, and patients infected with *A. fumigatus* were not admitted to ICU and survived during the observation period.

Discussion

To date, limited data are available on the comparison of IAPA and CAPA patients. Previous studies have reported that IPA may affect up to 30% of intubated patients with COVID-19 admitted to the ICU [15, 22], with similar results observed in influenza patients [3, 23]. In the current retrospective study, among 733 hospitalized patients with COVID-19, 110 (15%) developed invasive aspergillosis, while 70 (12%) of 583 influenza patients developed invasive aspergillosis. There was no obvious difference in the incidence between the two groups, and the slightly lower incidence in the influenza cohort may be explained by the different pathogenesis of SARS-CoV-2 infection [3]. However, the incidences were all lower than those of the above reports. One reason may be that we recruited all patients infected with influenza A/B or SARS-CoV-2, regardless of ICU admission. Nevertheless, our results suggested that patients with influenza or COVID-19 are prone to developing IPA.

In previous reports, the median time between diagnosis of influenza pneumonia and IAPA was 2–4 days, and the time from COVID-19 positivity to CAPA diagnosis spanned from 8 to 16 days [24, 25]. Similar results were found in this retrospective study. Compared with CAPA patients, the time window between observation to IPA diagnosis was significantly shorter in IAPA patients. The reasons for the difference may include different timing and methods of IPA diagnosis. Nevertheless, this result highlights the importance of early diagnosis and treatment of IPA in hospitalized patients with influenza.

The in-hospital mortality of COVID-19/IPA patients (30%) was higher than that of influenza/IPA patients (18.6%), but this difference was not statistically significant, consistent with a previous study, in which the incidence of in-hospital deaths was 47.1% and 40% ($P = 0.45$), respectively [26]. However, a population-based retrospective cohort study in France showed that the in-hospital death among patients in ICU was significantly higher ($P < 0.001$) in COVID-19 patients (3949/14,585, 27.1%) than those in 2018–2019 seasonal influenza patients (885/4,926, 18%) [27]. The population type and sample size could partially explain the different results. We then compared the in-hospital mortality between IAPA and CAPA patients. Interestingly, a significant difference was observed ($P = 0.033$) although with a relatively smaller sample size for CAPA (13/31, 41.9%) and IAPA (8/42, 19%) patients. The survival curve showed similar results.

When examining the effect factors of survival, SARS-CoV-2 infection was significantly higher in the non-survivor group. Age was also an important factor related to the clinical outcomes. According to a previous study, ICU-admitted COVID-19 patients were significantly older than influenza patients (72 (57–77) vs. 58 (52–63), $P = 0.036$) [26], and our study showed similar results (70 (60–77.8) vs. 63.5 (54.3–72.8), $P = 0.007$). Summarily, CAPA patients had higher in-hospital mortality than IAPA patients, and the reasons need to be further explored.

In addition to the in-hospital mortality, the ICU admission and ICU mortality in IAPA and CAPA patients were also analyzed, but the difference was not statistically significant, which was consistent with the previous study [26]. Nevertheless, the proportion of ICU admissions for COVID-19/IPA patients was significantly lower than that of influenza/IPA patients ($P = 0.013$), although the former had a higher ICU mortality rate. The reason for the lower ICU admission for COVID-19/IPA patients included that the sudden appearance of COVID-19 put an overwhelming strain on the medical resources, resulting in some patients not being successfully admitted to the ICU.

IPA was laboratory diagnosed in 180 patients using a variety of methods. The positive rate of culture was similar to that of serum GM test in influenza/IPA and COVID-19/IPA groups, and was significantly higher in influenza/IPA patients. While in IAPA and CAPA groups, the positive rate of serum GM was significantly higher than that of culture, which was speculated to be related to the high false positive rate of the GM test. In addition, in this study, the positive rate of BALF GM test was lower than the serum GM test, which was inconsistent with a previous study [28]. In that study, the GM test in BALF showed the highest positive rate (25/45, 56%) in CAPA patients, followed by culture (14/45, 31%), microscopy (11/45, 24%), GM in the blood (3/45, 7%), and histology (3/45, 7%) [28]. The reasons for this result include limited specificity of serum GM test, and the relatively high false positive result on a single test, especially in the ICU [29]. The most detected *Aspergillus* species in this study was *A. fumigatus*, which was associated with a higher prevalence of this species [20, 30]. *Aspergillus nidulans* isolates have mainly been studied in immunodeficient patients with the chronic granulomatous disease, and are more virulent than the commonly *A. fumigatus*, increasing the chance of mortality against this organism [31, 32]. However, in our study, all 4 COVID-19/IPA patients infected with *A. nidulans* were not admitted to ICU and survived during the observation, indicating that *A. nidulans* were not directly relevant to the outcomes of COVID-19/IPA patients.

The current study had several limitations. First, this study was a single-center study with a small sample size, which hindered the precise interpretation of the results. Second, as this study was retrospective, we could not retrieve the

necessary clinical details of all patients, especially the proof of *Aspergillus* tracheobronchitis, such as epithelial plaques, pseudomembrane, eschar, or ulcers through medical records query, which resulted in an underestimation of the population fulfilling the criteria of IAPA and CAPA. Third, the CAPA patients were enrolled during the Omicron era, the findings in this study may not apply to other variants. Another potential limitation was that some patients were only positive for serum GM test, which could not rule out some false positive cases.

In conclusion, IPA represents a high burden of death in severe COVID-19 and influenza infections. Patients infected with SARS-CoV-2 and *Aspergillus* during the concentrated outbreak of COVID-19 in China had generally higher in-hospital mortality and age than those infected with influenza and *Aspergillus*. Larger prospective studies in the future may help design the most appropriate strategies to prevent IPA.

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Author contribution BC and BL contributed to the study conception and design. Material preparation, data collection, and analysis were performed by JZ, XZ, and DP. The first draft of the manuscript was written by JZ. JZ, XZ, BL, and BC commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are not publicly available due to individual privacy but are available from the corresponding author on reasonable request.

Declarations

Ethics approval The study was performed in line with the principles of the Ethics Committee of China-Japan Friendship Hospital (2022-KY-052).

Competing interests The authors declare no competing interests.

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