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Original Article

COVID-19-associated pulmonary aspergillosis is associated with increased in-hospital mortality and prolonged SARS-CoV-2 viral shedding

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Background/Purpose: Coronavirus disease 2019 (COVID-19)-associated pulmonary aspergillosis (CAPA) is common in critically ill patients with COVID-19 and is associated with worse outcomes. However, reports on CAPA and its impact on treatment outcomes in Asian populations are limited.

Methods: Patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse transcription polymerase chain reaction-confirmed COVID-19 admitted to intensive care units (ICUs) were retrospectively enrolled in this observational study. The incidence rate of CAPA during ICU admission was investigated. The clinical factors associated with CAPA, including corticosteroid exposure, were analyzed. The impact of CAPA on the treatment outcomes and SARS-CoV-2 viral shedding were explored.

Results: A total of 72 ICU-admitted patients with COVID-19 were included in the analysis. The incidence rate of CAPA was 15.3% (11/72) in all patients and 23% (11/48) in the mechanically ventilated patients. The median time from ICU admission to CAPA diagnosis was 15 days. A lower fibrinogen level (adjusted odds ratio [aOR], 0.983; 95% confidence interval [CI], 0.967–0.999) was independently associated with CAPA. The patients with CAPA had a higher in-hospital mortality rate (55% vs. 13%, $p = 0.001$) and a longer SARS-CoV-2 viral shedding time (22 days vs. 16 days, $p = 0.037$) than those without CAPA.

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Conclusion: Lower serum fibrinogen levels was independently associated with CAPA among the ICU-admitted patients with COVID-19. The patients with CAPA had a higher in-hospital mortality rate and a longer SARS-CoV-2 viral shedding time than those without CAPA.

Introduction

Invasive fungal infection has been described and defined comprehensively in immunocompromised patients[1] and has been increasingly investigated in critically ill patients.[2] In patients with acute respiratory distress syndrome (ARDS) resulting from influenza, pulmonary Aspergillus infection was highlighted as influenza-associated pulmonary aspergillosis (IAPA).[3] IAPA has been reported to occur in 11—23% of patients with severe influenza, with marked geographic differences.[4,5] The underlying mechanism of IAPA includes direct damage to the airway epithelium, enabling Aspergillus to invade the lung parenchyma and interfering with ciliary clearance, which consequently leads to immune dysfunction.[6]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus responsible for COVID-19, which has rapidly spread worldwide. Some distinctive immune responses, such as a decrease in T-cell populations[7] and ARDS resulting from massive production of cytokines,[8] are observed in patients with severe COVID-19. The pulmonary damage and enhanced inflammatory environment caused by SARS-CoV-2 increase the vulnerability of patients with COVID-19 to fungal infections.[9] COVID-19-associated pulmonary aspergillosis (CAPA) was initially described in early 2020 and is the predominant fungal disease in patients with COVID-19.[10] Previous studies have reported that the prevalence rate of CAPA ranges from 5.7% to 25% in patients with severe COVID-19 and that the mortality rate can be as high as 71%.[11—16] However, the definition of CAPA used in previous studies varied, which made it difficult to perform direct comparisons of treatment outcomes among different studies. The geographic variation in the incidence rate of pulmonary aspergillosis also highlights the importance of obtaining local epidemiological data in different geographic areas. The interaction between SARS-CoV-2 and Aspergillus also deserves further clarification. In this study, we investigated patients with severe COVID-19 admitted to intensive care units (ICUs) in Taiwan. The clinical factors associated with CAPA and treatment outcomes as well as the impact of CAPA on the SARS-CoV-2 viral shedding time were evaluated.

Patients and methods

Patients and settings

This retrospective observational study was conducted in Taipei Veterans General Hospital, a 2800-bed tertiary medical center in northern Taiwan. Patients with SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR)-confirmed COVID-19 admitted to ICUs from May 2021 to August 2021 were eligible for enrollment. Patients without SARS-CoV-2 RT-PCR confirmation and without ICU admission, those aged $<$18 years, and those with Human Immunodeficiency Virus (HIV) infection were excluded. The study protocol was approved by the Institutional Review Board (IRB) of Taipei Veterans General Hospital (IRB number: 2021-12-006BC), and the need for informed consent was waived.

Data collection and severity evaluation

Information regarding the demographic characteristics and underlying comorbidities was obtained via a hospital chart review. Results of laboratory tests on ICU admission were recorded. The disease severities were evaluated on the basis of the Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assessment (SOFA) score, and presence of shock (vasopressor use) on the day of ICU admission. Mechanical ventilation, renal replacement therapy, prone positioning, and extracorporeal membrane oxygenation (ECMO) were also recorded. The prescription of anti-viral agents, corticosteroids, tocilizumab, and anti-coagulants was based on the recommendations from Taiwan COVID-19 treatment guidelines.[17] Corticosteroid exposure was quantified as dexamethasone equivalents[18] and measured on the basis of the mean daily dosage of dexamethasone in milligrams from admission to CAPA diagnosis in CAPA cohort, and from admission to ICU discharge or death in non-CAPA cohort.

Diagnosis and definition of CAPA

CAPA was defined in accordance with the 2020 European Confederation for Medical Mycology and International Society for Human and Animal Mycology (ECMM/ISHAM) consensus criteria.[19] The diagnosis of CAPA was categorized into proven, probable, and possible based on a combination of microbiology, imaging, and clinical features. CAPA was diagnosed in accordance with the clinical judgment of the in-charge physicians liberally without a protocolized timeline. The level of galactomannan (GM) was measured using Platelia Aspergillus Ag assays (Bio-Rad Laboratories, Marnes-La-Coquette, France) following the manufacturer's instructions. The following diagnostic thresholds of the GM index were used: $>$0.5 in serum or $\geq$1.0 in BAL specimen for probable CAPA and $>$4.5 in non-bronchoscopic lavage (NBL)

specimen once, >1.2 in NBL specimen twice or more, or >1.2 in NBL specimen once plus another NBL mycology test positive for possible CAPA.

SARS-CoV-2 RT-PCR and viral shedding evaluation

SARS-CoV-2 RT-PCR was executed using Roche Cobas® 6800 system (Roche, Basel, Switzerland).[20] Selective amplification of the target nucleic acid from the sample was achieved with the use of target-specific forward and reverse primers for ORF1ab non-structural region, which is unique to SARS-CoV-2. Another conserved region in the structural protein envelope E-gene was selected for pan-sarbecovirus detection.

We reviewed the cycle threshold (Ct) values for both gene targets for all RT-PCR tests that were performed on NP swabs or endotracheal aspirates when the patients were mechanically ventilated, as described in a previous study.[21] The RT-PCR results were expressed in terms of the Ct value, and the samples were considered positive when the Ct value was ≤40. Viral dynamics were calculated on the basis of the Ct value of the SARS-CoV-2-specific target (ORF1ab). The infectious viral shedding time was defined as the time from symptom onset to the first day of obtainment of a Ct value beyond 30.[22,23] The patients without data on Ct values beyond 30 during their hospital stay were right-censored at the time of their last Ct value assessment.

Outcome evaluation

The outcomes evaluated in this study included the ICU stay, hospital stay, 28-day ventilation-free day, and all-cause mortality rate on day 30 and upon discharge. Differences in the treatment outcomes between the patients with COVID-19 with and without CAPA were analyzed accordingly. All patients were followed up from admission to death or discharge, with the last patient discharged on October 21, 2021.

Statistical analysis

The results were presented as medians (interquartile ranges [IQRs]), means (standard deviations), or numbers (%), as appropriate. The continuous variables were compared using an independent t-test or the Mann–Whitney U test and the categorical variables using Pearson's chi-square test or Fisher's exact test, as appropriate. The clinical variables showing between-group differences with p-values of <0.100 in the univariable logistic regression analyses were included in the multivariable logistic regression analyses using the enter method to determine factors that independently predict CAPA. Odds ratios and their 95% confidence intervals (CIs) were also calculated. Kaplan–Meier curves were constructed to evaluate the mortality rate and infectious viral shedding time of SARS-CoV-2. Two-sided tests were used, and p-values of <0.050 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows/Macintosh, version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

During the study period, a total of 296 patients with suspected COVID-19 who were admitted to ICUs were eligible for enrollment. After exclusion of 224 patients with negative SARS-CoV-2 RT-PCR reports for sampling at least twice, 72 patients with RT-PCR-confirmed COVID-19 were included in the analysis (Fig. 1). The demographic characteristics and clinical features of the enrolled patients are shown in Table 1.

In general, the median patient age was 66 (range, 58–74) years, and 47 (65%) were men. At ICU admission, the median SOFA score was 6 (IQR, 3–9), and the median APACHE II score was 13 (IQR, 8–22). The median duration of ICU and hospital stays was 17 (IQR, 7–32) and 31 (IQR, 16–59) days, respectively. During ICU admission, 48 (67%) patients received mechanical ventilator support; 6 (8%) received renal replacement therapy; and 4 (6%) received ECMO for ARDS. None of the included patients had immunocompromised status, including solid organ transplantation, stem cell transplantation, and prolonged use of systemic corticosteroid.

A total of 11 (15.3%) patients developed CAPA during ICU admission according to the ECMM/ISHAM consensus criteria, including 10 with probable CAPA and 1 with possible CAPA (Supplementary Table 1). The mean serum GM level at the diagnosis of CAPA was 1.05 (IQR, 0.69–1.22), and four affected patients had positive results on sputum culture for Aspergillus. A total of 37 (51%) patients received serum GM test, the median time from ICU admission to first serum GM test was 11 days (IQR, 7–15 days) (Supplementary Table 4). The median times from symptom onset to CAPA diagnosis and from ICU admission to CAPA diagnosis were 26 (IQR, 17–47) and 15 (IQR, 7–37) days, respectively (Table 1). The clinical information regarding the diagnoses and presentations in the 11 patients diagnosed with CAPA is shown in Supplementary Table 2. In the subgroup analysis of mechanically ventilated patients, incidence of CAPA was 23% (11/48).

As shown in Table 1, the demographic characteristics and underlying comorbidities were comparable between the patients with and without CAPA. The patients with CAPA had higher SOFA scores (9 vs. 5, $p = 0.031$), higher serum lactate levels (27 vs. 16 mg/dL, $p = 0.015$), and lower serum fibrinogen levels (312 vs. 440 mg/dL, $p = 0.047$) and received mechanical ventilation more frequently (100% vs. 61%, $p = 0.011$) than did the patients without CAPA. Meanwhile, the patients with CAPA were exposed to a higher dosage of corticosteroid, which was measured using the mean daily dexamethasone equivalent (10.9 vs. 4.5 mg, $p = 0.039$), than those without CAPA.

Clinical factors associated with CAPA

The univariable and multivariable analyses of the clinical factors associated with CAPA are shown in Table 2. In the univariable analysis, the clinical factors associated with CAPA included a lower serum level of fibrinogen and a higher

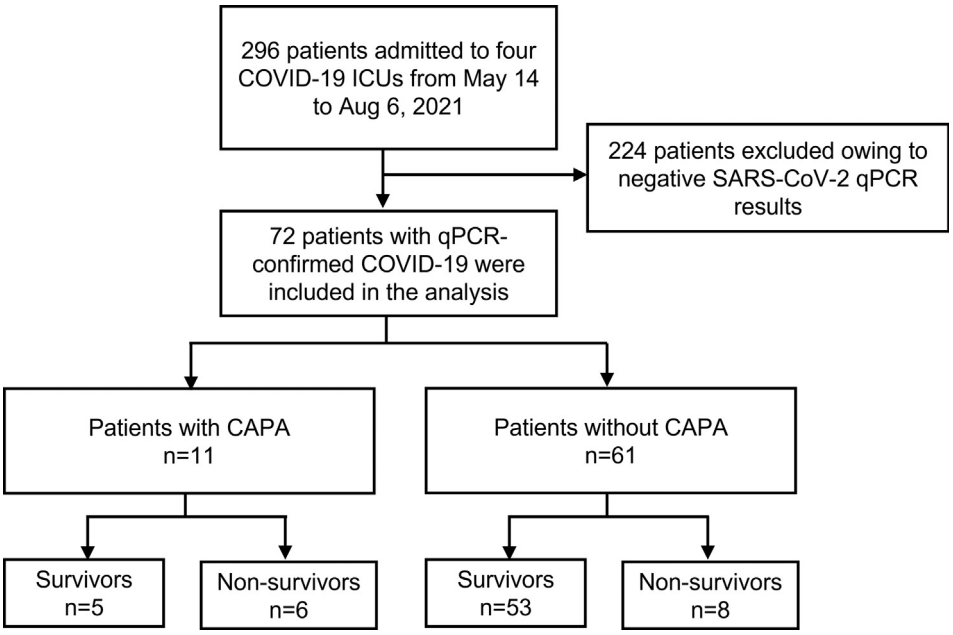

Figure 1 Study flowchart. CAPA, COVID-19-associated pulmonary aspergillosis; ICU, intensive care unit; qPCR, quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

daily dosage of dexamethasone. In the multivariable analysis, the independent factors associated with CAPA included a lower level of fibrinogen (adjusted odds ratio [aOR], 0.983; 95% CI, 0.969—0.999). Higher daily dosage of dexamethasone has a trend of increased risk of CAPA (aOR, 1.531; 95% CI, 0.985—2.381), a dose—response effect of CAPA incidence could be observed in groups with incremental daily dosage of dexamethasone (Supplementary Fig. 1).

Impact of CAPA on the clinical outcomes

The comparisons of the treatment outcomes between the patients with and without CAPA are shown in Table 3. The patients with CAPA had longer ICU stays (34 vs. 15 days, $p = 0.008$), fewer 28-day ventilation-free days (0 vs. 6 days, $p = 0.009$), and a higher in-hospital all-cause mortality rate (55% vs. 13%, $p = 0.001$) than the patients without CAPA. The Kaplan—Meier curves demonstrated a significantly higher mortality rate in the patients with CAPA than in those without (log-rank $p = 0.024$); the curves separated early after ICU admission (Fig. 2a). In the subgroup analysis of the patients who received mechanical ventilator support, a trend of a higher mortality rate was observed in the patients with CAPA than in those without (log-rank $p = 0.065$) (Fig. 2b).

Association of CAPA with prolonged viral shedding

We further investigated the association between CAPA and the infectious viral shedding time of SARS-CoV-2. As shown in Supplementary Table 3, the initial Ct value at the diagnosis of COVID-19 was comparable between the patients with and without CAPA (23.7 vs. 22.3, $p = 0.476$). The comparison of the SARS-CoV-2 viral shedding time between the patients with and without CAPA is shown in Fig. 3a. The patients with CAPA had a longer viral shedding time than those without CAPA (22 vs. 16 days, $p = 0.037$). In the Kaplan—Meier analysis, the viral shedding time was significantly longer in the patients with CAPA than in those without (log-rank $p = 0.022$) (Fig. 3b).

Discussion

In this retrospective cohort study that enrolled critically ill patients with COVID-19, CAPA was diagnosed in 15.3%. Our findings are in line with recent reports demonstrating an incidence rate varying from 5% to 28%.[11—16,24] However, different CAPA definitions, including the updated EORTC/MSGERC consensus,[1] AspICU algorithm,[25] IAPA expert consensus,[26] and ECMM/ISHAM consensus criteria,[19] were applied in previous studies, making the performance of direct comparisons of the incidence rates among studies difficult. In our study, we used the ECMM/ISHAM definition of CAPA, which expanded diagnostic modalities and defined the cut-off value of the GM level for different specimen sites. This definition was used by subsequent large investigations. Based on the definition, we identified 10 probable CAPA cases and one possible CAPA case. The mycologic domain of the diagnostic criteria for CAPA in our patients was mostly based on a serum GM index of >0.5. Considering the poor sensitivity associated with the use of the serum GM level for diagnosing invasive pulmonary aspergillosis (IPA),[12] the incidence rate of CAPA in our study might have been underestimated. Reports on CAPA in Asian populations are limited. A case series study in China reported an incidence rate of CAPA of 7.7% in patients with COVID-19 according to the EORTC/MSGERC consensus definition.[27] Notably, the incidence rate of CAPA might have been underestimated because only 14% of the patients in this study were mechanically ventilated.

Table 1 Demographic characteristics and disease severities of the ICU-admitted patients with COVID-19 with and without CAPA.[a]

	All patients (n = 72)	CAPA		p-value
		Yes, n = 11	No, n = 61	
Age	66 (58–74)	71 (62–77)	65 (55–74)	0.188
Male sex	47 (65)	7 (64)	40 (66)	>0.99
BMI	24.2 (22.4–28.4)	25.7 (22.3–28.6)	24.1 (22.4–27.0)	0.239
Comorbidities				
Diabetes	25 (35)	5 (45)	20 (33)	0.497
Cirrhosis	11 (15)	1 (9)	10 (16)	>0.99
Chronic airway diseases	10 (14)	0 (0)	10 (16)	0.342
Chronic kidney diseases	10 (14)	3 (27)	7 (12)	0.174
Malignancies	11 (15)	1 (9)	10 (16)	>0.99
Laboratory results at ICU admission				
White blood cell count, 10^9/L	6.0 (3.7–9.4)	7.3 (5.2–12.0)	5.7 (3.7–8.8)	0.270
Lymphocyte count, 10^9/L	0.63 (0.43–0.90)	0.71 (0.45–0.83)	0.62 (0.42–0.93)	0.919
Albumin level, g/dL	3.5 (3.2–3.9)	3.3 (3.1–3.6)	3.5 (3.2–4.0)	0.193
C-reactive protein level, mg/dL	8.3 (3.2–10.7)	7.6 (3.5–9.2)	8.5 (3.2–10.9)	0.747
Procalcitonin level, ng/mL	0.16 (0.08–0.45)	0.27 (0.11–0.69)	0.13 (0.08–0.44)	0.191
Ferritin level, ng/mL	1290 (657–2903)	1780 (798–3896)	1243 (578–2978)	0.570
LDH level, U/L	488 (312–577)	507 (354–979)	435 (295–561)	0.170
Lactate level, mg/dL	16 (12–23)	27 (15–34)	16 (12–19)	0.015*
D-dimer level, ug/mL	0.9 (0.5–3.8)	1.2 (0.7–17.3)	0.9 (0.5–2.2)	0.248
Fibrinogen level, mg/dL	440 (312–496)	312 (108–416)	440 (350–585)	0.047*
Clinical timeline				
Time from symptom onset to hospital admission	4 (2–9)	7 (5–11)	5 (3–9)	0.217
Time from symptom onset to CAPA diagnosis	N/A	26 (17–47)	N/A	N/A
Time from ICU admission to CAPA	N/A	15 (7–37)	N/A	N/A
Severities at ICU admission				
SOFA score	6 (3–9)	9 (5–10)	5 (2–8)	0.031*
APACHE II score	13 (8–22)	18 (12–28)	12 (8–20)	0.052
Vasopressor use	4 (6)	1 (9)	3 (5)	0.493
Treatment and events				
Mechanical ventilation	48 (67)	11 (100)	37 (61)	0.011*
Renal replacement therapy[b]	6 (8)	1 (9)	5 (8)	>0.99
Prone positioning	17 (24)	3 (27)	14 (23)	0.714
Extracorporeal membrane oxygenation	4 (6)	1 (9)	3 (5)	0.493
Mean daily dosage of dexamethasone[c]	5.3 (3.1–8.3)	10.9 (6.9–14.4)	4.5 (3.0–7.8)	0.039*
Tocilizumab use	40 (56)	7 (64)	33 (54)	0.558
Remdesivir use	44 (61)	6 (55)	48 (79)	0.128
Anticoagulant therapy	43 (60)	8 (73)	35 (57)	0.339

APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; CAPA, COVID-19-associated pulmonary aspergillosis; HIV, human immunodeficiency virus; ICU, intensive care unit; LDH, lactate dehydrogenase; SOFA, Sequential Organ Failure Assessment.
*$p < 0.050$.
[a] Data are presented as medians (interquartile ranges) and n (%), unless otherwise indicated.
[b] Including hemodialysis.
[c] Dosage of dexamethasone was counted from admission to CAPA diagnosis in CAPA cohort, and from admission to ICU discharge or death in non-CAPA cohort.

In our analysis, the serum fibrinogen level was independent factors associated with CAPA. Previous studies have reported controversial results regarding the association between the use of tocilizumab, a monoclonal antibody against interleukin-6 (IL-6) receptor, and CAPA.[11,12,15] In our study, we found no association between tocilizumab use and CAPA. Currently, low-dosage dexamethasone is considered the standard of care in critically ill patients with COVID-19 owing to the benefit in reducing mortality, which was demonstrated by the RECOVERY Collaborative Group.[28] However, the role of systemic corticosteroid use in the development of CAPA remains controversial, and previous studies have revealed inconsistent results.[12,14–16,24] Most of these studies recognized the use of corticosteroids by dichotomization, while the dosage-response effect of corticosteroids on CAPA deserves further investigation. In our study, dexamethasone was prescribed in 93% (67/72) of the enrolled patients, and we observed a trend of an increased incidence rate of CAPA in the patients with a higher exposure to corticosteroids (Supplementary Fig. 1). Although

Table 2 Univariable and multivariable analyses of the clinical factors associated with CAPA in the ICU-admitted patients with COVID-19.

Variables	Univariable[a]			Multivariable[a]		
	Odds ratio	95% confidence interval	p-value	Adjusted odds ratio	95% confidence interval	p-value
Age	1.027	0.979–1.078	0.276			
Body mass index	1.113	0.938–1.321	0.219			
Diabetes mellitus	1.708	0.456–6.279	0.420			
Lactate level	1.008	0.988–1.029	0.439			
Fibrinogen level	0.990	0.981–1.000	0.040*	0.983	0.967–0.999	0.035*
SOFA score	1.206	0.997–1.460	0.054	0.859	0.333–1.741	0.519
APACHE II score	1.066	0.989–1.048	0.095	1.017	0.875–1.362	0.436
Mean daily dosage of dexamethasone[b]	1.230	1.066–1.419	0.005*	1.531	0.985–2.381	0.059
Tocilizumab use	1.485	0.394–5.601	0.560			

APACHE, Acute Physiology and Chronic Health Evaluation; CAPA, COVID-19-associated pulmonary aspergillosis; ICU, intensive care unit; SOFA, Sequential Organ failure Assessment.
*$p < 0.050$.
[a] Odds ratios and 95% confidence intervals were derived from logistic regression analysis.
[b] Dosage of dexamethasone was counted from admission to CAPA diagnosis in CAPA cohort, and from admission to ICU discharge or death in non-CAPA cohort.

Table 3 Treatment outcomes of the ICU-admitted patients with COVID-19 with and without CAPA.[a]

	All patients (n = 72)	CAPA		p-value
		Yes, n = 11	No, n = 61	
Treatment outcomes				
ICU stay, day	17 (7–32)	34 (17–74)	15 (6.5–28)	0.008*
Hospital stay, day	31 (16–59)	45 (24–92)	28 (15–57)	0.125
28-day ventilation-free days	0 (0–12)	0 (0–0)	6 (0–13)	0.009*
30-day mortality from ICU admission	11 (15)	3 (27)	8 (13)	0.356
In-hospital mortality	14 (19)	6 (55)	8 (13)	0.001*

CAPA, COVID-19-associated pulmonary aspergillosis; ICU, intensive care unit.
*$p < 0.050$.
[a] Data are presented as medians (interquartile ranges) and n (%).

low-dosage dexamethasone may reduce mortality in patients with severe COVID-19, clinicians should be aware of the potential risk of corticosteroid use for secondary infections, especially CAPA, and avoid unnecessary corticosteroid prescription. For critically ill patients with COVID-19 prescribed with corticosteroids for medical purposes, protocolized investigation of CAPA is encouraged.

Herein, the fibrinogen level was elevated in most patients with COVID-19, with a median level of 455 mg/dL. The degree of elevation of the fibrinogen level strongly correlates with the IL-6 level, and progressive decreases in the fibrinogen level are associated with mortality in the late stage of COVID-19,[29] which can be related to disseminated intravascular coagulation. In this study, a lower level of fibrinogen at ICU admission was identified as a clinical factor associated with CAPA. Conversely, one study demonstrated a distinct finding that IPA was accompanied by a significant increase in the fibrinogen level in patients with leukemia.[30] These inconsistent results need further investigation to establish the association between the fibrinogen level and CAPA and determine the underlying pathogenesis.

Several studies have reported that patients with CAPA had poor treatment outcomes, including increased overall and ICU mortality rates[12,13,15,16,24] and prolonged intubation.[11] In our study, the patients with CAPA had longer ICU stays, fewer 28-day ventilation-free days, and higher in-hospital mortality rates than those without CAPA. However, the patients with CAPA had higher SOFA scores, higher serum levels of lactate, and lower serum levels of fibrinogen and were more likely to receive mechanical ventilation, which indicates a higher disease severity, than those without CAPA. These differences made it difficult to clarify whether CAPA per se is a risk factor associated with worse outcomes, especially mortality. However, in our subgroup analysis of patients who received mechanical ventilator support, we found that the patients with CAPA still had a higher mortality rate than those without CAPA (Fig. 2b).

It remains uncertain whether protocolized screening of CAPA is helpful in patients with severe COVID-19 for the early detection of CAPA and improvement of the treatment outcomes. With protocolized screening strategies, the PREDICO study reported a duration of 4 days from intubation to CAPA diagnosis, while the MYCOVID study reported a

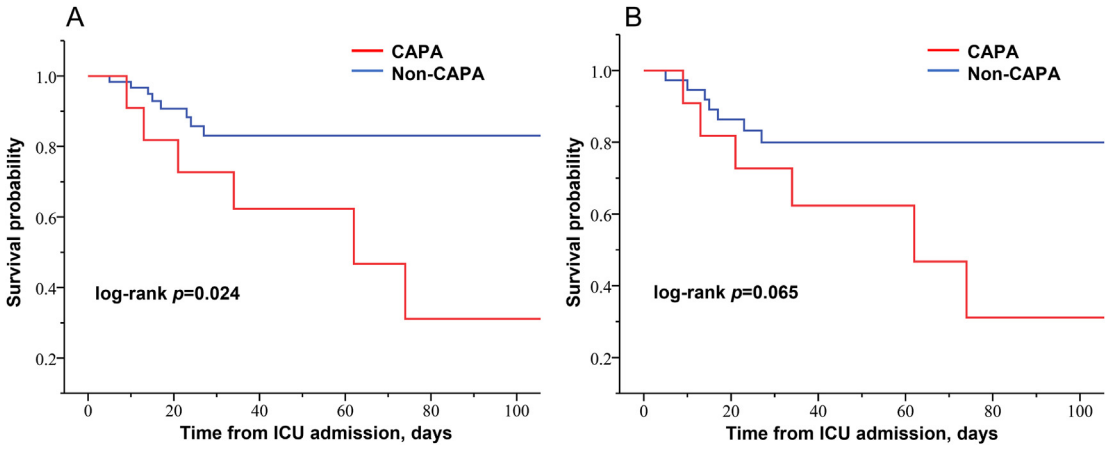

Figure 2 Kaplan–Meier curves of (a) all patients (n = 72) (log-rank p = 0.024) and (b) the mechanically ventilated patients (log-rank p = 0.065) with and without CAPA. CAPA, COVID-19-associated pulmonary aspergillosis; ICU, intensive care unit.

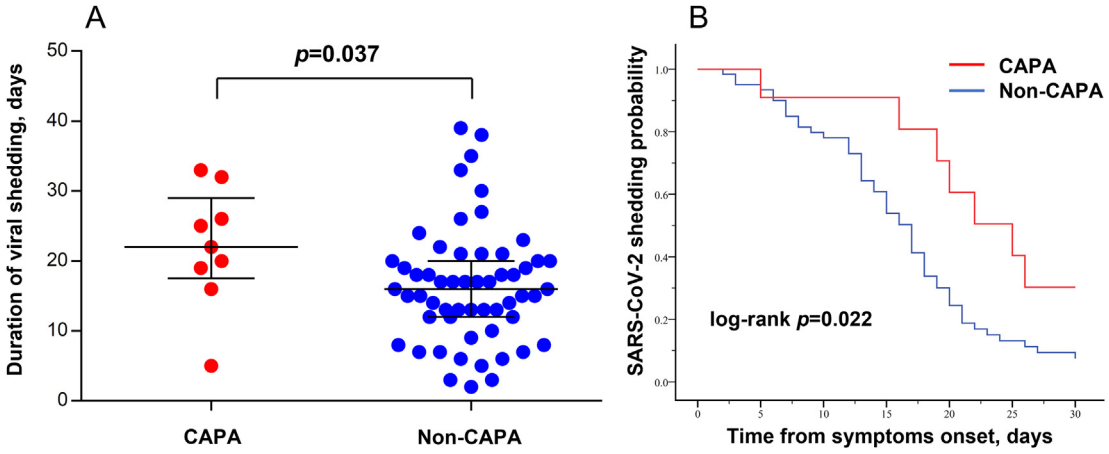

Figure 3 SARS-CoV-2 viral shedding time between the patients with and without CAPA. (a) The distribution of the SARS-CoV-2 viral shedding time is presented in dot plots (Mann–Whitney U test, p = 0.037). (b) Kaplan–Meier curves (log-rank p = 0.022). CAPA, COVID-19-associated pulmonary aspergillosis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

duration of 8 days from ICU admission to CAPA diagnosis.[12,13] In our study, the work-up for CAPA was based on the clinical judgement of the in-charge physicians. We observed a duration of 15 days from ICU admission to CAPA diagnosis, which is longer than previous findings. Considering the adverse impact of CAPA on the outcomes of patients with COVID-19, we believe that a protocolized screening strategy should be applied to patients with risk factors associated with CAPA to achieve early diagnosis and treatment and improve the treatment outcomes of patients with CAPA.

The Ct values on SARS-CoV-2 RT-PCR are inversely correlated with the viral load and work as a semi-quantitative evaluation marker. A higher viral load of SARS-CoV-2 was reported as an independent factor associated with higher severity and worse prognosis.[31–33] Prolonged viral shedding has been reported in ICU-admitted patients[34] and was associated with worse survival status.[21] However, the characteristics of the viral shedding of SARS-CoV-2 in patients with CAPA have rarely been evaluated. In our study, the infectious viral shedding time was defined as the time from symptom onset to the first day of obtainment of a Ct value of >30, considering that no virus has been previously isolated from samples with a Ct value of >30.[22] Despite the comparable Ct value on SARS-CoV-2 RT-PCR upon diagnosis, the patients with CAPA in our study had a longer viral shedding time than those without CAPA. To the best of our knowledge, this is the first study to describe the association between CAPA and prolonged viral shedding. Although the exact interaction between viral and fungal infections remains uncertain, we speculate that prolonged viral shedding represents a compromised host immunity,[35] which increases the vulnerability to Aspergillus infections. Our findings also indicate that patients with prolonged viral shedding should undergo aggressive screening for CAPA.

This study has several limitations. First, this retrospective, single-center study included only 11 CAPA and 61 non-CAPA cases in the analysis. Nevertheless, all CAPA cases were diagnosed following the ECMM/ISHAM consensus criteria. The small sample size might have underestimated the impact of CAPA on the treatment outcomes. Second,

there was no protocolized screening strategy for CAPA in our institution during the study period, along with limited bronchoscopy use. Therefore, there might have been delayed CAPA diagnoses or underdiagnoses in our patients with COVID-19. Third, repeat testing of SARS-CoV-2 RT-PCR was based on the clinical judgment of the in-charge physicians, rather than on a protocolized procedure. The viral shedding time might have been overestimated in the enrolled patients. Fourth, although Kaplan—Meier curves demonstrated increased mortality in CAPA group, the definite association between CAPA and mortality couldn't be confirmed in multivariable analysis. Considering the small case numbers in the present study, further study with a large sample size would be needed to verify the issue. Finally, none of the enrolled patients received vaccination against SARS-CoV-2 infection. Therefore, the findings of the study cannot be applied to patients who have received vaccination.

In conclusion, this is the first study to describe CAPA in Taiwan, with an incidence rate of 15.3% among ICU-admitted patients with COVID-19. The patients with CAPA had longer ICU stays and higher in-hospital mortality rates than those without. Lower serum fibrinogen levels was independently associated with CAPA while mean daily dosage of dexamethasone showed the marginal effect. Specifically, the patients with CAPA had prolonged shedding of SARS-CoV-2 compared with those without CAPA. Our study demonstrated the adverse impact of CAPA on the treatment outcomes of ICU-admitted patients with COVID-19. Physicians should be aware of the clinical features and diagnostic criteria of CAPA. A protocolized screening strategy should be applied to this patient population, especially those with potential risk factors, to achieve early diagnosis and prompt initiation of anti-fungal therapy.

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Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfma.2022.07.006.

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