New Microbiologica, 47, 1, 98-102, 2024, ISSN 1121-7138

SHORT COMMUNICATION

Analysis of cases with co-infection of COVID-19 and pulmonary aspergillosis

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SUMMARY

The objective of this study was to investigate the risk factors and diagnosis measure of COVID-19-associated pulmonary aspergillosis (CAPA). This study included 201 COVID-19 patients from December 1, 2022, to January 31, 2023; 7 (3.5%) were diagnosed with CAPA. The main risk factors were age, MV, ICU admission and COPD, and the presence of comorbidities such as ARDS and hypoproteinemia in COVID-19 patients, more susceptible to *Aspergillus* infection. In addition to specimen culture in the lower respiratory tract, the 1,3-β-D-glucan antigen test can serve as an important screening indicator for early CAPA diagnosis in non-granulocytopenia patients.

Received July 18, 2023 *Accepted* February 02, 2024

Severe coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global pandemic and caused countless deaths as a result of viral pneumonitis and its complications (Guan WJ *et al*., 2020). Severe/critical COVID-19 patients often have serious underlying diseases and are treated with measures such as corticosteroids, immunosuppressive agents, and mechanical ventilation (MV), which increase the risk of secondary fungal infections, most commonly *Aspergillus* (Gangneux JP *et al.*, 2020; Boyd S *et al*., 2022; Zhu X *et al*., 2020; Alanio A *et al*., 2020). There have been several reports of COVID-19-associated pulmonary aspergillosis (CAPA), raising concerns about this superinfection as an additional contributing factor to mortality (Chen N *et al*., 2020; Salmanton-Garcia J *et al*., 2021; Mitaka H *et al*., 2021; Lai CC and WL Yu, 2021). Upon the relaxation of epidemic prevention and control measures by China on December 13, 2022, a resurgence of the COVID-19 pandemic ensued, leading to a surge in cases, including those with *Aspergillus* infections (Cai J *et al*., 2023). The mortality rate of severe CAPA patients was unexpectedly high in our hospital. Therefore, in order to diagnose in time and reduce patient mortality due to CAPA, we explored the risk factors and diagnosis treatment measures.

Key words:
COVID-19, CAPA, infections, risk factors, diagnosis.

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All successive COVID-19 patients (admission to hospital due to COVID-19) at our hospitals from December 1, 2022 to January 31, 2023 with a positive PCR for SARS-CoV-2 and one or more samples (bronchoalveolar lavage fluid (BALF), sputum, Pleural effusions) sent to the mycology department were included, and culture and identification were systematically performed on these samples. In the concomitantly received blood sample, galactomannan (GM test) and 1,3-β-D-glucan (G test) were performed on serum/plasma. The agar plates for fungal isolation and cultivation were sourced from Zhengzhou Antu Biotechnology Co., Ltd. The Microflex LT/SH fully automatic bio-mass spectrometry detection system was used to identify fungi. 1,3-β-D-glucan detection utilized reagents from Beijing Gold Mountain River Technology Development Co., Ltd. (G test), and galactomannan antigen detection employed reagents from Dana BioTech Co., Ltd. (GM test).

The primary criteria evaluated for CAPA were the definitions from the European Confederation of Medical Mycology and International Society for Human and Animal Mycology (ECMM/ISHAM) published by Koehler and colleagues (Koehler P *et al*., 2021). Patients were classified as probable/possible CAPA. Statistical analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, IL). Counting data and metrological data are summarized as the n (%) and means±standard deviations (SDs), $P<0.05$ was considered a statistically significant difference. Research samples were collected in accordance with the relevant regulations on the management of human genetic resources in China, approved by the Ethical Examination and Approval Committee of the hospital (No.2023-003).

A total of 201 patients with PCR-confirmed SARS-CoV-2 infection were included in this study. Out of these 201 patients, 7 cases (3.5%) were diagnosed with probable/possible CAPA, all of whom were severe/critically ill and older than those non-CAPA (79.86±5.21 vs 69.1±14.2, $P<0.05$). *Aspergillus* was cultured positive from the tracheal aspirate, BALF or pleural effusions samples of the 7 patients (5 cases from sputum, 2 cases from BALF and pleural effusions). Among the 7 patients co-infected with SARS-CoV-2 and *Aspergillus*, 5 patients were administered MV, 1 patient continuous renal replacement therapy (CRRT)-supported before *Aspergillus* infection occurrence. All patients had underlying diseases, with a statistically significant difference observed in patients with COPD ($P<0.001$). Patients undergoing treatment for COVID-19 who develop ARDS and hypoalbuminemia exhibited increased susceptibility to *Aspergillus* infection (refer to *Table 1*).

In order to further explore the diagnosis measures of CAPA patients, we conducted an analysis of various laboratory indicators. All 7 CAPA patients exhibited elevated neutrophil counts, alongside significantly decreased lymphocyte counts. Inflammatory markers, such as PCT and IL-6, demonstrated a 100% positivity rate. Notably, the positivity rate of G experiment surpassed that of GM experiment. Radiological investigations were performed at the clinicians' discretion. We found that chest CT of the 7 patients showed that the lesions were mainly multiple and involved both lungs. The primary CT findings included high-density shadows (71.4%), followed by lobar consolidation (57.1%), air bronchograms (42.9%), cavity (28.6%), accompanied by pleural effusion (57.1%). All 7 probable/possible CAPA patients received specialized treatment, with voriconazole being the recommended first-line therapy (85.7%), followed by amphotericin B and caspofungin. Following treatment, 2 patients improved and were discharged,

Table 1 - *Clinical features and selected laboratory indicators of CAPA and Non-CAPA.*

Variable	*CAPA (n=7)*	*Non-CAPA (n=194)*	*P value*
Gender, Male sex, no. (%)	6 (85.7)	96 (49.5)	0.060
Age, mean (SD), years	79.86 (5.21)	69.1 (14.2)	0.041
Type of specimen, no. (%)			
Sputum	5 (71.4)	188 (96.9)	0.001
BALF	1 (14.3)	0 (0)	<0.001
Pleural effusions	1 (14.3)	0 (0)	<0.001
Underlying disease, no. (%)			
Hypertension	5 (71.4)	74 (38.1)	0.077
Diabetes mellitus	1 (14.3)	35 (18.0)	0.799
CHD	2 (28.6)	24 (12.4)	0.210
Obesity	0 (0)	1 (0.5)	0.849
COPD	3 (42.9)	6 (3.1)	<0.001
Operation	0 (0)	18 (9.3)	0.398
Cerebral infarction	0 (0)	16 (8.2)	0.428
Complications, no. (%)			
ARDS	5 (71.4)	13 (6.7)	<0.001
Shock	1 (14.3)	2 (1.0)	0.004
Hypoalbuminemia	6 (85.7)	67 (34.5)	0.006
AKI	2 (28.6)	3 (1.5)	<0.001
Treatment, no. (%)			
MV	5 (71.4)	14 (7.2)	<0.001
ECMO	0 (0)	0 (0)	a
CRRT	1 (14.3)	0 (0)	<0.001
Corticosteroid treatment, no. (%)	3 (42.9)	51 (26.3)	0.331
ICU admission, no. (%)	4 (57.1)	8 (4.1)	<0.001

Abbreviations: BALF, Bronchoalveolar Lavage Fluid; ARDS, Acute Respiratory Distress Syndrome; CRRT, Continuous Renal Replacement Therapy; ECMO, Extracorporeal Membrane Oxygenation; AKI, Acute Kidney Injury; MV, Mechanical Ventilation; CHD, Coronary Heart Disease; "a" represents results with no meaningful value.

while 5 patients died. Details of patients' characteristics are provided in *Table 2*.

In our study, the incidence rate of *Aspergillus* was 3.5%, obviously lower than in other reports (Lai CC and WL Yu, 2021; Meawed TE *et al*., 2021; Erami M *et al*., 2023). This discrepancy may be attributed to the decline in severe cases of COVID-19 in the post-pandemic era. All 7 patients had underlying disease; elderly patients with underlying COPD should be vigilant against fungal infections (P<0.001). Patient admission to the ICU or undergoing MV therapy were significantly associated with *Aspergillus* infection. These results are concordant with studies in the literature (Lai CC and WL Yu, 2021; Delliere S *et al*., 2020). Previous research has indicated that up to 40% of COVID-19 hospitalized patients can develop ARDS (Wu C *et al.*, 2020), and thereby become susceptible to infections caused by *Aspergillus* spp (Dupont D *et al*., 2021). Our study revealed that ARDS and Hypoalbuminemia are the primary complications among COVID-19 patients (P<0.05), with an overall reported mortality rate of 100% for ARDS. Radiological and clinical signs of CAPA in non-neutropenic patients are mostly unspecific. Lung imaging findings included lobar consolidation, cavity, and air bronchograms, consistent with the microscopic findings in the literature (Wang J *et al*., 2020; Hashim Z *et al*., 2022). The presence of pleural effusion on CT scans indicates an increased incidence of *Aspergillus*. Hashim' s data shows that persistence or further deterioration of neutrophilic leukocytosis and lymphopenia leads to death, whereas their restoration improves the survival of CAPA patients (Hashim Z *et al*., 2022). All 7 patients with CAPA in our study did not have neutropenia, lymphocytes, PCT and IL-6 were non-specific. Diagnostic efforts through high index of suspicion with serological tests (GM test or G test) may be an effective supplementary method for diagnosing CAPA in a resource-limited setting (Marr KA *et al*., 2021). In

Table 2 - *Clinical characteristics of 7 patients with CAPA.*

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*	*Patient 6*	*Patient 7*
Gender / Age(y)	Male / 71	Male / 81	Male / 82	Male / 84	Female / 80	Male / 75	Male / 86
Type of specimen	Sputum	Sputum	Sputum	Sputum	BALF	Sputum	Pleural effusions
Underlying disease							
	Diabetes mellitus	Hypertension	COPD	Hypertension	Hypertension	Hypertension	Hypertension
	COPD	CHD	Heart disease			Cerebral infarction	
						COPD	
Symptom							
	Dyspnea	Fever	Dyspnea	Fever	Fever	Dyspnea	Fever
	Cough	Cough	Cough	Dyspnea	Vomiting	Cough	Dyspnea
		Dizzy		Cough	Fatigue		Cough
Complications							
	Hypo-albuminemia	ARDS	Hypo-albuminemia	ARDS	ARDS	ARDS	ARDS
				Hypo-albuminemia	AKI	Hypo-albuminemia	AKI
					Shock		Hypo-albuminemia
					Hypo-albuminemia		
CAPA Classification	Possible	Possible	Possible	Possible	Probable	Possible	Possible
Species	*Aspergillus spp*, culture from tracheal aspirate	*Aspergillus spp*, culture from tracheal aspirate	*Aspergillus spp*, culture from tracheal aspirate	*Aspergillus spp*, culture from tracheal aspirate	*Aspergillus spp*, culture from BALF	*Aspergillus spp*, culture from tracheal aspirate	*Aspergillus spp*, culture from Pleural effusions
Microscopic detection	Mycelium	Mycelium and Spores	-	Mycelium and Spores	-	Mycelium	Spores
Serum GM-test (<0.5 ug/L)	-	+	-	+	+	None	+

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	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*	*Patient 6*	*Patient 7*
G-Test (<60 pg/mL)	-	+	+	+	+	None	+
N (1.8-6.3x10^9/L)	7.43	10.25	7.26	12.41	9.13	15.55	10.18
L (1.1-3.2x10^9/L)	1	0.16	0.52	0.17	0.44	1.05	0.47
PCT (0-0.1ng/ml)	+	+	+	+	+	+	+
IL-6 (0-7pg/ml)	+	+	+	+	+	+	+
Chest CT							
	High-density shadows	High-density shadows	High-density shadows	High-density shadows	Ground-glass opacity	Ground-glass opacity with infiltration	High-density shadows
	Nodules	Lobar consolidation	Fiber line shadow	Cavity	Lobar consolidation	Air bronchograms	Lobar consolidation
	Cavity		Pleural effusions	Pleural effusions	Pleural effusions	Leaflets gridded	Pleural effusions
	Lobar consolidation				Air bronchograms		Balloon cavity
							Air bronchograms
Treatment							
MV	No	Yes	No	Yes	Yes	Yes	Yes
CRRT	No	No	No	No	Yes	No	No
Antifungal treatment	Voriconazole	Voriconazole	Voriconazole	Voriconazole	Voriconazole	Voriconazole	Fluconazole
		Caspofungin			Caspofungin		Amphotericin B
		Amphotericin B			Amphotericin B		
ICU	No	Yes	No	No	Yes	Yes	Yes
Outcome	Alive	Died	Alive	Died	Died	Died	Died

our cases, the positive rate of GM test was lower than that of G test, since serum GM is probably not a good marker for CAPA in these patients and in non-agranulocytosis patients (Meersseman W *et al.*, 2008; Kwak EJ *et al.*, 2004). Diagnosis of CAPA remains challenging, mainly because bronchoalveolar lavage fluid galactomannan testing and culture, which represent the most sensitive diagnostic tests for aspergillosis in the ICU, are hindered by the fact that bronchoscopies are rarely performed in COVID-19 patients due to the risk of disease transmission (Arastehfar A *et al.*, 2020; Verweij PE *et al.*, 2020; Wahidi MM *et al.*, 2020; Koehler P *et al.*, 2021). The use of systemic corticosteroids were not significantly associated with CAPA in our study. Whereas corticosteroids are a well-known risk factor for impaired neutrophil function and thus development of invasive fungal infections, it is now considered standard of care treatment in critically ill COVID-19 patients and therefore less likely to turn out as a significant predictor of CAPA (RECOVERY Collaborative Group HP, Lim WS, *et al.*, 2021).

In interpreting the findings, a number of limitations should be considered. First, our study pertains to the post-pandemic period, so there may be fewer cases of severe illness, which could predispose it to reporting bias. Second, because of aerosol production due to SARS-CoV2, a low percentage of patients in this study had mycologic evidence from BALF culture. However, despite these limitations, the current study highlights the risk of pulmonary aspergillosis in critically ill COVID-19 patients, adding to the growing literature on CAPA.

In conclusion, we observed here a low but probably underestimated prevalence of CAPA. Our study suggests that CAPA is a disease with high mortality, and older patients are at increased risk of developing *Aspergillus* infection. Hence, it is recommended to systematically perform screening for severe/critical COVID-19 patients on a weekly basis for early diagnosis and treatment to improve the outcome. The main risk factors were age, MV, ICU admission, COPD, and the presence of comorbidities such as ARDS and hypoproteinemia in COVID-19 patients,

who are more susceptible to *Aspergillus* infection. Neutrophilic leukocytosis and lymphopenia are centrally involved in CAPA pathology from its development to mortality and may serve as novel targets for developing new cellular or iron-depleting therapeutic modalities for the disease. The 1,3-β-D glucan antigen test can serve as an important screening indicator for the early diagnosis of CAPA in non-granulocytopenia patients. At the same time, close attention should be paid to the presence of pleural effusion on lung images. Our study would be helpful in the early diagnosis and effective clinical management of CAPA and of any other similar pandemic that we may face in the future.

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