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Impact of physician awareness and microbiological examination on incidence of COVID-19-associated pulmonary aspergillosis: a retrospective study

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Abstract

Background The reported incidence of aspergillosis among COVID-19 patients has varied significantly, which can be partly attributed to differences in diagnostic approaches and levels of physicians' proficiency in diagnosing COVID-19-associated pulmonary aspergillosis (CAPA). Consequently, we conducted a retrospective study to investigate the potential reasons for these discrepancies and analyzed the risk factors for pulmonary aspergillosis in patients with COVID-19.

Method Data were retrospectively collected from December 1, 2022, to September 30, 2023, from patients who were admitted to the First Affiliated Hospital of Wenzhou Medical University. The research platform was used to screen patients with discharge diagnoses of COVID-19 pneumonia. CAPA was defined according to the 2020 ECMM/ISHAM criteria and the Chinese expert consensus. Clinical data that were collected included data about underlying diseases, laboratory examinations and microbiological detection. Analyses were conducted with R software, with continuous variables analyzed with t-tests, categorical variables analyzed with chi-square tests, and logistic regression and ROC curves used to assess risk factors for CAPA.

Results The incidence of CAPA was 13.4% in the general ward, 30.8% in the RICU, and 6.8% in other ICUs. The average time to CAPA diagnosis was 5.6 days in general wards, 3.7 days in the RICU, and 7.4 days in other ICUs. Diagnostic testing revealed the following sensitivities: 78% for BALF galactomannan (GM), 48% for serum GM, 52% for culture tests, and 71% for BALF mNGS. Risk factors for CAPA included chronic respiratory disease, chronic renal insufficiency, and diabetes. The primary *Aspergillus* species identified was *A. fumigatus*, followed by *A. flavus*.

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Conclusion Differences in incidence may arise from varying levels of physician awareness, which can influence the rate at which BALF and serum GM samples are submitted for testing. The sensitivity of BALF GM is higher than that of serum GM. Furthermore, BALF mNGS has the potential to enhance the clinical detection sensitivity of CAPA. Risk factors for CAPA include chronic respiratory disease, chronic renal insufficiency, and diabetes, which may aid in identifying at-risk patients. The primary *Aspergillus* species identified was *A. fumigatus*, followed by *A. flavus*, providing a reference for clinical empirical treatment.

Clinical trial number Not applicable.

Keywords CAPA, Incidence, RICU, General wards, Other ICUs, BALF GM, Serum GM, BALF mNGS, *A. fumigatus*

Introduction

The SARS-CoV-2 virus, which causes COVID-19 disease, led to the global pandemic [1]. As our understanding of COVID-19 continues to increase, it has become increasingly clear that in addition to the direct damage caused by the virus itself, secondary infections have also become major challenges in the treatment of patients [2]. In particular, aspergillosis, a common fungal infection, has raised widespread concern due to the severity and high mortality rate associated with this superinfection among severely and critically ill COVID-19 patients [3, 4].

The incidence of aspergillosis in COVID-19 patients varies significantly, ranging from 4 to 30% [5–8]. These differences may be partly attributed to the varying diagnostic testing methods and criteria, as well as the uneven distribution of medical resources, including healthcare facilities, equipment, and personnel [9]. Moreover, differences exist in the diagnostic capabilities and experiences of physicians from various departments concerning invasive pulmonary aspergillosis in COVID-19 patients, which may further influence the identification and reporting of such cases [10].

COVID-19-associated pulmonary aspergillosis (CAPA) has an extremely high mortality rate of 43-52%, and early identification and prompt therapeutic intervention are essential [11]. Mycological evidence plays a crucial role in the diagnosis of CAPA. The guidelines suggest various diagnostic techniques, such as microscopic examination of fungal samples, cultivation of fungi, molecular detection through polymerase chain reaction (PCR), measurement of the galactomannan (GM) antigen, and rapid testing via lateral flow assays [12]. PCR is recognized as a highly sensitive method for detecting *Aspergillus* infections [13]. However, we did not employ PCR since the detection methods are not broadly accessible in China, and our microbiology lab has not yet established a PCR test for *Aspergillus*. Metagenomic next-generation sequencing (mNGS), a newly developed approach in clinical microbiology, offers high sensitivity and rapid detection capabilities. It examines the nucleic acid sequences of microbial pathogens found in respiratory or blood samples from patients and is increasingly being utilized in clinical settings, thereby serving as a beneficial complement to CAPA diagnostics [14]. Furthermore, recognizing the clinical features and risk factors associated with CAPA is essential. Research has highlighted various independent risk factors for CAPA, including diabetes, chronic respiratory illnesses, chronic kidney disease, and conditions related to immunosuppression [15]. As a result, a comparison of similarities and difference may facilitate a deeper understanding of the clinical manifestations of CAPA.

To better understand the reasons for the differences in the incidence of aspergillosis and to analyse the risk factors for the development of pulmonary aspergillosis in COVID-19 patients with varying disease severity, we conducted a retrospective study. The aim of this research was to investigate the factors affecting the development of pulmonary aspergillosis and to evaluate the reliability of existing diagnostic techniques through the collection and analysis of clinical data from COVID-19 patients across various wards. We anticipate that this investigation will offer clinicians enhanced diagnostic insights and a scientific foundation for the prompt treatment of CAPA.

The Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (KY2024-R216) granted approval for this research, which was conducted in alignment with the principles outlined in the Declaration of Helsinki, as revised in 2013. We were unable to obtain informed consent from patients or their legal representatives due to the retrospective design of the study.

Methods

Study design and data collection

We gathered preliminary pertinent data on COVID-19 patients admitted to the First Affiliated Hospital of Wenzhou Medical University in Zhejiang Province, China, during the period from December 1, 2022, to September 30, 2023.

Retrospective collection of clinical data was conducted for patients admitted to the Department of Pulmonary and Critical Care Medicine (PCCM), distinguishing between those diagnosed with CAPA and those without CAPA.The inclusion criteria were as follows: (1) Positive COVID-19 test confirmed by PCR throat swab or

bronchoalveolar lavage fluid (BALF); (2) Discharge diagnosis of "community-acquired pneumonia," "severe pneumonia," "viral pneumonia," or "COVID-19 pneumonia"; (3) Pulmonary imaging consistent with pneumonia; (4) Age over 18 years. The exclusion criteria were: (1) Cases of repeated hospitalization; (2) Incomplete clinical data; (3) Presence of comorbid *Aspergillus* infection prior to admission.

By analyzing hospitalization data, we determined the incidence of CAPA and the time to diagnosis of CAPA across different departments. We also assessed the performance of microbiological tests in diagnosing patients with CAPA. Furthermore, by comparing CAPA patients with non-CAPA patients in the Department of Pulmonary and Critical Care Medicine, we investigated the clinical characteristics and risk factors associated with CAPA.

Demographic information, clinical statistics, and results from microbiological examinations were gathered through the New Research Data Platform at the First Affiliated Hospital of Wenzhou Medical University. The data collection concluded on September 30, 2023, which marked the point when all patients' clinical outcomes were recorded.

Considering the variations in awareness of *Aspergillus* infections and the diagnostic methods employed by clinicians in different departments, we enrolled non-CAPA patients who were initially admitted to the Department of Pulmonary and Critical Care Medicine as the control group to minimize the impact of false-negative cases. The inclusion criteria were as follows: (1) COVID-19 positivity confirmed by PCR throat swab or bronchoalveolar lavage fluid (BALF); (2) Discharge diagnoses including "community-acquired pneumonia," "severe pneumonia," "viral pneumonia," or "COVID-19 pneumonia"; (3) Pulmonary imaging consistent with pneumonia; (4) Patients aged over 18 years. The exclusion criteria included: (1) Cases of repeated hospitalization; (2) Incomplete clinical data; (3) Comorbid *Aspergillus* infection prior to admission. 4.Concurrent other fungal infections. The control group included non-CAPA patients admitted during the same time period as the CAPA patients. Although we did not perform strict 1:1 matching, we ensured that the control group was representative of the general patient population during the study period.

Definition of COVID-19

The presence of the SARS-CoV-2 virus, confirmed by PCR positivity for the viral genome, was used to diagnose COVID-19 infection. Disease severity was categorized according to the World Health Organization's COVID-19 guidelines (WHO, 2022).

Definition of CAPA

Since our microbiology laboratory did not develop a polymerase chain reaction (PCR) test for *Aspergillus*, we did not include this method as a diagnostic criterion for this study. Additionally, metagenomic next-generation sequencing (mNGS) was incorporated.

The diagnostic criteria for CAPA are based on the definitions established in clinical studies, specifically the 2020 ECMM/ISHAM criteria and the Expert Consensus on the Diagnosis and Treatment of Severe CAPA patients [9, 16]. These criteria encompass proven, probable and possible CAPA. The proven CAPA meet at least one of the following criteria: (1) Invasive growth accompanied by associated tissue damage: histopathological or direct microscopic detection of fungal hyphae; (2) An infectious disease process: aspergillus recovered by culture or microscopy or histology obtained by a sterile aspiration or biopsy from a pulmonary site. The criteria for probable CAPA are as follows: (1) Imaging findings, preferably documented by chest computed tomography (CT), indicative of pulmonary infiltrates, which may present as one of the following patterns: dense, well-demarcated lesions with or without a halo, crescent air sign, cavity or wedge formations, or segmental or lobar consolidation; (2) Mycological tests that satisfy one of the following conditions: mNGS detection of *Aspergillus* in bronchoalveolar lavage fluid, a positive bronchoalveolar lavage culture, a positive bronchoalveolar lavage galactomannan test (BAL GM index ≥ 1.0), or a positive serum galactomannan test (serum GM index > 0.5). The criteria for possible CAPA are defined as follows: (1) Imaging findings that fulfill the criteria for lung infiltration; (2) Mycological assessments that satisfy at least one of these criteria include the microscopic observation of fungal components in sputum, which suggests the existence of a mold, or a positive culture obtained from non-bronchoscopic lavage (sputum). The final diagnosis of CAPA was established through a consensus agreement between two experienced respiratory physicians.

Statistical analysis

Variables that were continuous and met the equal variances assumption were examined through a t-test, with results shown as media alongside interquartile ranges. In cases where continuous variables did not conform to equal variances, Welch's t-test was utilized. For categorical variables, demographic data, underlying health conditions, and microbiological findings were reported as quantities and percentages, and analyzed via the chi-square test. To determine independent risk factors for CAPA, logistic regression analysis was conducted. Receiver operating characteristic (ROC) curves were created for the different risk factors. All statistical analyses were carried out using R version 4.3.2, which is available

as free software. The diagnostic times across various hospital departments were evaluated using one-way analysis of variance (ANOVA) via GraphPad Prism software, with outcomes displayed in a box plot format. A statistical significance threshold was established at $P < 0.05$.

Results

Overview of patients with COVID-19 pneumonia

A total of 871 patients admitted with confirmed COVID-19 pneumonia were screened. Of these, 109 were initially admitted to other Intensive Care Unit wards, including ICU and Emergency Intensive Care Unit (EICU), 98 to the Respiratory Intensive Care Unit (RICU), 238 to the general ward of the Department of Pulmonary and Critical Care Medicine, and 426 to other departments. During their hospital stay, some patients were transferred to other departments for treatment due to changes in their conditions, following consultations. Further analysis of patient transfer data revealed that 175 patients had been admitted to other ICUs at some point, and 162 had been admitted to the RICU (Fig. 1).

Admission of patients and incidence of CAPA in different wards

A total of 91 patients diagnosed with CAPA were discharged from the Department of Pulmonary and Critical Care Medicine, comprising 14 proven CAPA cases, 61 probable CAPA cases and 16 possible CAPA cases (Table S1). Among these, 33 patients were diagnosed with CAPA following their admission to the respiratory intensive care unit (RICU) from the emergency department. Seventeen patients were initially admitted to the general ward and subsequently diagnosed with CAPA after being transferred to the RICU due to worsening conditions. Four patients were diagnosed with CAPA while in the general ward and were later transferred to the RICU for further treatment due to critical conditions. Additionally, 28 patients were diagnosed with CAPA in the general ward and were subsequently discharged after receiving treatment. 12 patients were diagnosed in other ICUs, and 9 of who were referred to the RICU after consultation. A comprehensive evaluation revealed that the incidence of CAPA was approximately 13.4% in the general ward (32/238), significantly higher at 30.8% in the RICU (50/162), and notably lower at 6.8% among patients admitted to other ICUs (12/175) (Fig. 2A).

Time from admission to CAPA diagnosis in different wards

The average time from hospitalization to the diagnosis of CAPA was 6.26 days. In contrast, the average time from admission to diagnosis of CAPA was approximately 7.4 days for patients admitted to other ICUs, 5.6 days for those admitted to the general ward, and notably shorter at 3.7 days for patients admitted to the RICU (Fig. 2B).

Laboratory diagnostics of invasive pulmonary aspergillosis

Among the 175 patients with COVID-19 pneumonia admitted to various ICUs (EICU or ICU), only 81 (46.28%) had serum GM specimens submitted for testing, while 10 (5.7%) had BALF GM specimens submitted. In contrast, of the 162 patients admitted to the RICU, 152 (94.3%) had serum GM specimens sent for testing, and 80 (49.7%) had alveolar lavage fluid GM specimens submitted. In the general ward, serum GM assays were conducted for 181 patients (76.05%), and BALF GM specimens were tested in 33 cases (13.8%).

Among the 91 patients diagnosed with CAPA, *Aspergillus* was identified in sputum cultures or smears from 36 patients, representing 39.56% of the cohort. The positive rate for bronchoalveolar lavage fluid (BALF) galactomannan (GM) testing was 78.00% (39 out of 50), while BALF culture yielded a positivity rate of 45.1% (23 out of 51). Additionally, 71.43% of patients (30 out of 42) tested positive using BALF mNGS. In serum GM testing,

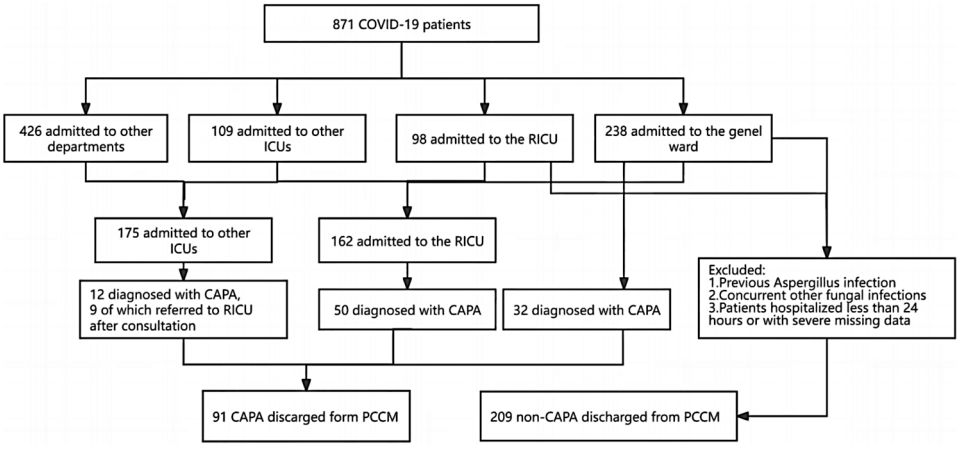

Fig. 1 Hospitalisation of COVID-19 patients and incidence of CAPA in different departments

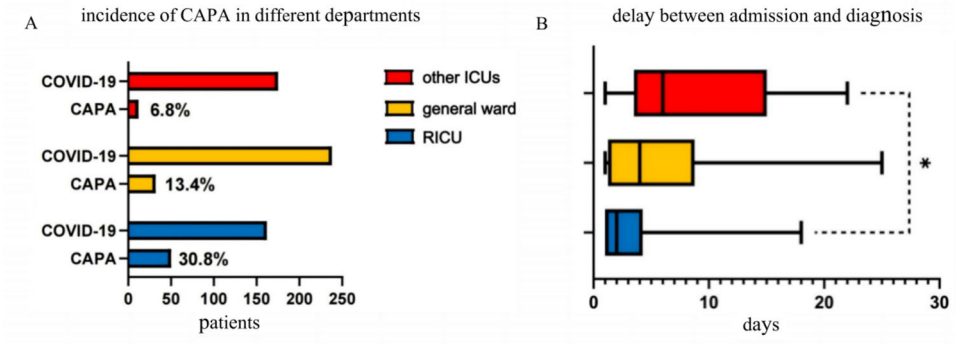

Fig. 2 (**A**)Incidence of CAPA in different departments. (**B**)Time to diagnosis of CAPA in different sections. *: The univariate contrast analysis shows statistical significance

Table 1 Diagnostic performance of serum GM, BALF GM, smear/culture, mNGS

	Count of sample	Count of positives	Sensitivity
BALF GM	50	39	78.00%
mNGS	42	30	71.43%
serum GM	86	42	48.84%
BALF culture	51	23	45.10%
sputum culture	91	36	39.56%

positivity was detected in 48.84% of patients (42 out of 86) (see Table 1). In total, 65 patients (71.4%) were found to be positive for *Aspergillus* species, which included 45 cases of *A. fumigatus*, 15 cases of *A. flavus*, 4 cases of *A. niger*, and 1 case of *A. terreus* (refer to Supplementary Material, Table S1).

Comparison between CAPA and non-CAPA patients

Ninety-one patients diagnosed with CAPA were admitted to the Department of Pulmonary and Critical Care Medicine, comprising 14 proven CAPA cases, 61 probable CAPA cases and 16 possible CAPA cases. To account for variations in awareness of *Aspergillus* infections and diagnostic methods among clinicians in different departments, we included 209 patients without CAPA as a control group, all of whom were admitted to the Department of Pulmonary and Critical Care Medicine (see Fig. 1). The median age of the CAPA patients was 76 years, with 70.32% being male. The CAPA group exhibited a higher proportion of patients with chronic respiratory diseases (including COPD, interstitial pneumonia, and history of tuberculosis), diabetes mellitus, and chronic renal insufficiency. Additionally, this group had a greater proportion of solid organ transplant patients, malignant tumors, and the use of steroid in the previous 60 days and immunosuppressant (Table 2). Most routine laboratory tests indicated no significant differences between the two groups, except for elevated leukocyte levels (9.41×10^9/L vs. 6.88×10^9/L, $p < 0.01$) and neutrophil counts (7.77×10^9/L vs. 5.80×10^9/L, $p < 0.01$), decreased albumin levels (29.1 g/L vs. 31.4 g/L, $p < 0.01$), increased BUN levels (11.9 mmol/L vs. 7 mmol/L, $p < 0.01$), higher LDH levels (449 U/L vs. 345 U/L, $p = 0.03$), elevated ferritin levels (1369 ng/L vs. 923 ng/L, $p = 0.01$), and lower CD4 + T-lymphocyte counts (108.5 cells/μL vs. 168.5 cells/μL, $p = 0.04$). Regarding chest imaging (the first CT scan upon admission or first bedside X-ray), the CAPA group had a higher proportion of patients exhibiting interstitial exudative changes involving multiple lung lobes (85.51% vs. 73.13%, $p = 0.03$). In terms of clinical treatment and prognosis, the CAPA group was more likely to require mechanical ventilation (49.45% vs. 22%, $p < 0.01$), CRRT (18.68% vs. 2.39%, $p < 0.01$), and vasoactive drugs (29.67% vs. 5.26%, $p < 0.01$). With respect to hormone therapy (methylprednisolone), a greater proportion of patients in the CAPA group received a daily hormone dosage of ≥ 40 mg compared to the non-CAPA group (50.05% vs. 20.57%, $p < 0.01$), while the total hormone amount administered in the CAPA group was lower than that in the non-CAPA group (280 mg vs. 510 mg, $p < 0.01$), as the CAPA group only included dosage prior to the diagnosis of CAPA (up to the date of microbiological specimen collection for diagnosis).In terms of prognosis, the CAPA group exhibited a higher proportion of critical illnesses (53.84% vs. 26.79%, $p < 0.01$) and a greater incidence of deaths or patients opting to discharge themselves due to severe conditions (47.25% vs. 22.48%, $p < 0.01$). Consequently, this resulted in a relatively shorter hospital stay for the CAPA group (16 days vs. 19 days, $p = 0.01$), as the poorer outcomes and the critical nature of the illness prompted more patients or their families to choose discharge despite ongoing medical needs.

Multivariate logistic regression analysis and receiver operating characteristic of independent risk factors for CAPA

We included the history of smoking and drinking, underlying diseases (such as hypertension, diabetes, chronic respiratory diseases, chronic heart disease, chronic liver diseases, and chronic renal insufficiency), and immunosuppressive conditions (including solid malignant

Table 2 Comparisons between CAPA and non-CAPA patient

	Total (*n* = 300)	CAPA(*n* = 91)	Non-CAPA(*n* = 209)	*P* value
Male	206 (68.67%)	64 (70.32%)	142 (67.94%)	0.783
Age, years	75 (65–81)	76 (65–81)	74 (65–81)	0.215
BMI, kg/m2	23.3 (20.9–25.6)	22.8 (20.9–25.6)	23.4 (21.0-25.6)	0.278
Smoke	78 (26.00%)	27 (29.67%)	51 (24.40%)	0.416
Drink	54 (18.00%)	17 (18.68%)	37 (17.70%)	0.968
Underlying disease				
Hypertension	168 (56.00%)	49 (53.84%)	119 (56.93%)	0.846
Diabetes	131 (43.67%)	51 (56.04%)	80 (38.27%)	0.006
Chronic respiratory diseases	34 (11.33%)	22 (24.17%)	12 (5.74%)	0.001
Chronic heart disease	37 (12.33%)	14 (15.38%)	23 (11.00%)	0.384
Chronic liver disesas	14 (46.67%)	10 (10.98%)	4 (1.91%)	0.002
Chronic renal insufficiency	53 (17.67%)	30 (32.96%)	23 (11.00%)	0.001
Immunosuppressive condition	94 (31.33%)	49 (53.84%)	45 (21.53%)	0.001
Solid Malignant Tumor	45 (15.00%)	26 (28.57%)	19 (9.09%)	0.001
Hematological malignancies	26 (8.67%)	11 (12.08%)	15 (7.17%)	0.243
Solid organ transplantation	16 (5.33%)	9 (9.89%)	7 (3.34%)	0.042
autoimmune disease	10 (3.33%)	4 (4.39%)	6 (2.87%)	0.744
Steroids in previous 60 days	19 (6.33%)	13 (14.28%)	6 (2.87%)	0.001
Immunosuppressants	18 (6.00%)	10 (10.98%)	8 (3.82%)	0.032
CT imaging				
consolidation	50 (16.67%)	20 (21.97%)	30 (14.35%)	0.144
multiple interstitial exudation	130 (43.33%)	76 (83.51%)	149 (73.13%)	0.001
Pleural effusion	106 (35.33%)	28 (30.76%)	78 (38.83%)	0.337
Laboratory examination				
Hemoglobin, g/L	114 (100–128)	113 (93–128) *n* = 91	115 (101–128) *n* = 201	0.378
Leukocyte count,10^9/L	7.63 (5.06–10.83)	9.41 (6.15–13.18) *n* = 91	6.88 (4.79–9.60) *n* = 201	0.001
Neutrophils,10^9/L	6.40 (3.98–9.32)	7.77 (5.28–11.93) *n* = 91	5.80 (3.72–8.38) *n* = 201	0.001
Lymphocytes,10^9/L	0.57 (0.35–0.94)	0.47 (0.30–0.81) *n* = 91	0.62 (0.39–0.97) *n* = 201	0.516
platelet count,10^9/L	169 (127–227)	169 (125–242) *n* = 91	169 (128–222) *n* = 201	0.813
APTT, s,	39.4 (35.8–46.6)	39 (33.7–45.8) *n* = 88	39.7 (36.5–46.9) *n* = 195	0.056
D-Dimer, mg/L,	1.36 (0.75–2.66)	1.96 (0.90–4.01) *n* = 88	1.21 (0.71–2.38) *n* = 195	0.115
ALT, U/L,	24.5(15.7–38.2)	25.0 (16.0–38.0) *n* = 89	23.0 (15.5–38.5) *n* = 199	0.111
AST, U/L,	34 (23–47)	30 (22–48) *n* = 89	34 (24–46) *n* = 199	0.148
TBIL,μmol/L	10 (8–14)	10 (8–14) *n* = 89	10 (8–13) *n* = 199	0.635
ALB, g/L	30.5 (27.8–33.6)	29.1(27.0-31.6) *n* = 91	31.4 (28.3–34.1) *n* = 199	0.001
GLO, g/L	27.9 (25.1–31.5)	28 (24.7–31.0) *n* = 91	27.9 (25.2–31.6) *n* = 199	0.491
Creatinine, mg/dL	78 (62–129)	99 (61–200) *n* = 91	76 (62–110) *n* = 199	0.008
BUN, mmol/L	8.2 (5.6–13.5)	11.9 (6.8–18.2) *n* = 91	7 (5.2–11.7) *n* = 199	0.001
Sodium, mmol/L	139 (136–142)	139 (134–142) *n* = 90	139 (136–142) *n* = 199	0.167
Blood glucose, mmol/L	9.20 (6.9–13.1)	10.0(6.77–15.27) *n* = 84	8.8 (6.9–12.5) *n* = 181	0.031
ProBNP, ng/L	629 (201–2244)	696 (314–2683) *n* = 81	561 (175–2121) *n* = 181	0.363
CRP, mg/L	66.5 (26.0-127.1)	59.4 (21.1-135.2) *n* = 89	68.6 (28.1-119.1) *n* = 194	0.631
ESR, mm/hr	26 (15–42)	21 (15–41) *n* = 35	26 (15–43) *n* = 76	0.845
LDH, IU/L	354 (274–506)	449 (324–582) *n* = 80	345 (258–455) *n* = 186	0.037
Fet, ng/ml	1018(593–2351)	1369 (709–2688) *n* = 82	923(522–2151) *n* = 154	0.019
CD4 + lymphocytes, cell/μL	145.5(82.5-248.8)	108(45–180) *n* = 76	168(95–277) *n* = 144	0.037
CD8 + lymphocytes, cell/μL	119.5 (63–200)	72.5 (37–159) *n* = 76	129 (80–209) *n* = 144	0.414
IL-2,pg/mL	0.81 (0.1–1.51)	0.6 (0.1–1.25) *n* = 76	0.87 (0.1–1.55) *n* = 166	0.096
IL-4,pg/mL	0.1 (0.1–0.73)	0.1 (0.1–0.99) *n* = 76	0.1 (0.1–0.63) *n* = 166	0.795
IL-6,pg/mL	52.11(10.9-179.1)	32.7 (10.1-209.4) *n* = 76	58.7 (11.6–177) *n* = 166	0.485
IL-10,pg/mL	4.5 (0.79–13.90)	7.15(2.0-16.2) *n* = 76	4.2 (0.2–12.1) *n* = 166	0.314
TNF-a, pg/mL	0.1 (0.1–0.96)	0.10 (0.1–1.24) *n* = 76	0.1 (0.1–0.9) *n* = 166	0.124
IFN-r, pg/mL	1.87 (0.1–3.74)	1.87 (0.1–3.04) *n* = 76	1.85 (0.1–3.92) *n* = 166	0.088

Table 2 (continued)

	Total ($n=300$)	CAPA($n=91$)	Non-CAPA($n=209$)	*P* value
IgG, g/L	10.71(8.5–12.9)	11.1 (9.1–13.2) $n=56$	10.4 (8.55–12.9) $n=128$	0.919
IgA, g/L	2.12 (1.5–3.05)	1.99 (1.43–2.98) $n=56$	2.17 (1.63–3.08) $n=128$	0.388
IgM, g/L	0.76 (0.52–1.07)	0.78 (0.55–1.15) $n=56$	0.74 (0.51–1.04) $n=128$	0.161
critical patients	105 (35.00%)	49 (53.84%)	56 (26.79%)	0.003
Treatments				
Biologic agent	49 (16.33%)	9 (9.89%)	40 (19.13%)	0.068
Mechanical Ventilation	91 (30.33%)	45 (49.45%)	46 (22.00%)	0.001
CRRT	22 (7.33%)	17 (18.68%)	5 (2.39%)	0.001
vasopressor	38 (12.67%)	27 (29.67%)	11 (5.26%)	0.001
methylprednisolone ≥ 40 mg/d	89 (29.66%)	46 (50.05%)	43 (20.57%)	0.001
methylprednisolone (mg)	280 (120–480)	200 (120–397)	510 (160–510)	0.005
Length of hospitalization	12 (7–20)	16 (10–21)	19 (7–19)	0.019
Death or Automatic discharge	90 (30.00%)	43 (47.25%)	47 (22.48%)	0.001

Continuous variables shown as media alongside interquartile ranges. chronic respiratory diseases including tuberculosis, asthma, chronic obstructive pulmonary disease, silicosis in study. Immunosuppressive condition including solid Malignant Tumor, hematological malignancies, solid organ transplantation, autoimmune disease, steroids or immunosuppressants in the past. CAPA: COVID-19-associated pulmonary aspergillosis; BMI: body mass index; APTT: Activated partial thromboplastin time; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBIL: total bilirubin; ALB: albumin; GLO: globulin; BUN: Blood Urea Nitrogen; CRP: C-reaction protein; LDH: lactate dehydrogenase; ERS: erythrocyte sedimentation rate; Fet: Ferritin; IL: interleukin; TNF: tumor necrosis factor; IFN: Interferon; CRRT: continuous renal replacement therapy

Table 3 Multivariate logistic regression analysis of independent risk factors for CAPA

Variable	Odds ratio	95%CI	*P* value
Smoke	1.48	0.65–3.37	0.352
Drink	0.92	0.36–2.32	0.862
Hypertension	0.47	0.23–0.93	0.031
Diabetes	2.95	1.51–5.76	0.002
Chronic respiratory diseases	4.3	1.75–10.54	0.001
Chronic heart disease	1.51	0.64–3.55	0.344
Chronic liver disesas	3.19	0.79–12.81	0.102
Chronic renal insufficiency	5.11	2.28–11.41	0.001
Any immunosuppressive status	1.87	0.32–10.8	0.484
Solid Malignant Tumor	2.62	0.49–13.86	0.259
Hematological malignancies	1.18	0.18–7.41	0.857
Solid organ transplantation	0.25	0.02–3.06	0.278
autoimmune disease	0.51	0.05–4.51	0.541
Steroids in previous 60 days	3.62	0.78–16.61	0.098
Immunosuppressants	1.79	0.22–14.36	0.582

tumors, hematological malignancies, solid organ transplantation, autoimmune diseases, and the use of corticosteroid in the previous 60 days or immunosuppressants in the multivariate logistic regression analysis (Table 3).

The analysis revealed that diabetes (OR 2.95; 95% CI 1.51–5.76; $P<0.01$), chronic respiratory diseases (OR 4.3; 95% CI 1.75–10.54; $P<0.01$), chronic renal insufficiency (OR 5.11; 95% CI 2.28–11.41; $P<0.01$) were independent risk factors for CAPA.

We also plotted receiver operating characteristic (ROC) curves to evaluate various risk factors (Fig. 3). The area under the curve (AUC) for diabetes was the highest (AUC: 0.589; CI: 0.528–0.650), whereas the AUCs for chronic respiratory diseases (AUC: 0.408; CI: 0.361–0.455) and chronic renal insufficiency (AUC: 0.390; CI: 0.337–0.443) were both below 0.5. Although individual factors did not achieve statistical significance, the AUC for the immunosuppressive condition (AUC: 0.662; CI: 0.603–0.720) and the combination of underlying diseases and immunosuppressive conditions (AUC: 0.768; CI: 0.708–0.827) were both above 0.5.

Discussion

Research on CAPA has focused primarily on COVID-19 patients in ICUs, with the prevalence of CAPA varying widely across centres, ranging from 4 to 30% [5–8, 17]. Few studies have focused on CAPA in COVID-19 patients who do not require ICU admission. However, COVID-19 patients who are not admitted to the ICU could also develop CAPA, and *Aspergillus* infection may be a cause of disease progression in these noncritical patients [18]. In our study, the incidence of CAPA among COVID-19 patients in the general ward was approximately 13.4%. This rate is notably lower than that reported in patients who are admitted to the RICU, where the incidence is 30.8%. However, this rate remains higher than the incidence reported in other ICUs, which is 6.8%. The main reason is that diagnosing CAPA is complex, requiring the integration of clinical presentations, imaging, and mycological tests, compounded by the additional challenge of potential diagnostic uncertainty in patients [19]. Another reason may be that physicians have varying levels of awareness about CAPA, leading to varied diagnostic strategies between departments, and systemic CAPA screening is rarely performed [20]. Indeed, the reported variation in the incidence of CAPA has partly been attributed to variations in diagnostic approaches [21]. Galactomannan antigenemia is a biomarker that is used for the diagnosis of invasive aspergillosis [22]. The

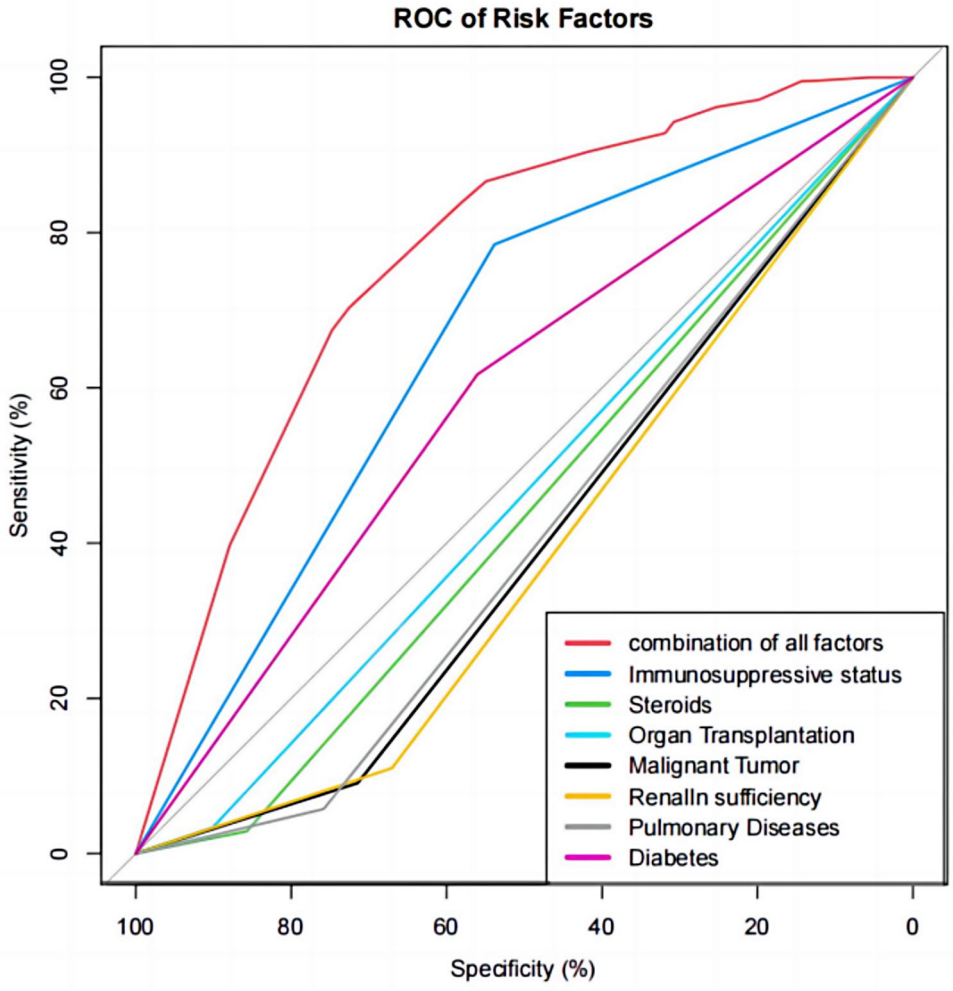

Fig. 3 Receiver operating characteristic (ROC) curves to evaluate different risk factors of CAPA. Immunosuppressive stasus including solid Malignant Tumor, hematological malignancies, solid organ transplantation, autoimmune disease, steroids or immunosuppressants

appropriate specimen type for GM testing varies across populations with different immune statuses. The BALF GM test is recommended for non-granulocyte-deficient patients, whereas the serum GM test is recommended for granulocyte-deficient patients [23, 24]. In our study, among patients who were admitted to other ICUs, serum GM levels were available in less than half of the patients 46.28%(81/175), whereas a mere 5.7%(10 /175) had BALF GM samples tested. In contrast, within the RICU, a significantly greater percentage of patients (94.3%) were tested for serum GM, and 49.7% were tested for BALF GM. Furthermore, in the general ward, the rate of serum GM testing was 76.05% (181/238), and the rate of BALF GM testing was 13.8% (33/238). These figures highlight the notable differences in GM testing practices across various wards. Feys S et al. emphasized the importance of including BALF sampling rates in the accurate reporting of CAPA incidence [25]. They revealed that CAPA is often underdiagnosed in studies because of the incorrect assumption that patients who are not sampled are free of CAPA, leading to an inaccurate denominator. This study also suggests that the underdiagnosis of CAPA is likely prevalent in routine clinical practice, not just in research-based settings.

Previous reports have documented a median time frame of 2–4 days for progression from influenza pneumonia to influenza-associated pulmonary aspergillosis (IAPA). In comparison, the diagnosis of CAPA following a positive test for COVID-19 has shown a wider span, typically falling between 8 and 16 days [26, 27]. In our retrospective study, the mean interval from hospital admission to the identification of CAPA was 6.26 days. For patients who were admitted to other ICUs, the average time to CAPA diagnosis was approximately 7.4 days. In contrast, the average time required to establish the diagnosis upon admission to a general ward was approximately 5.6 days. Notably, the mean time to confirm CAPA diagnosis in the RICU, as verified mainly by alveolar lavage, was significantly shorter, at approximately 3.7 days. The principal reasons for the observed differences

may be attributed to the heavy reliance on microbiological evidence for CAPA diagnosis [16, 28]. Our retrospective study revealed that the diagnosis of CAPA in patients in other ICUs was chiefly based on sputum cultures and serum GM assays. In the general wards, the diagnosis predominantly hinged on similar sputum culture and serum GM findings, complemented by BALF tests for some patients. Moreover, the diagnosis in the RICU is largely confirmed by alveolar lavage examination. However, diagnosing CAPA predominantly depends on BALF analysis, as serum GM tests have limited sensitivity [29]. Shadrivova O et al. discovered that the GM assay in BALF had the highest positivity rate among CAPA patients, at 56% (25 positive out of 45 tested). Culture tests identified 31% of the cases (14/45), whereas serum GM tests had the lowest positivity, detecting only 7% (3/45) [30]. Zhou X et al. reported in their study that the diagnostic sensitivity of BALF GM testing was the highest, at 84.9%, followed by mNGS at 65.5% and serum GM at 40.7% [15]. Research from earlier investigations indicates that the sensitivity of the bronchoalveolar lavage fluid (BALF) GM (ranging from 42 to 100%) surpasses that of serum GM (between 3% and 50%) in individuals who possess relatively normal immune functions [31]. In our study, 78% (39/50) of patients had positive BALF galactomannan test results. Serum GM tests were positive in 48% (42/86) of the patients. Cultures, including sputum and BALF, were positive in 48 patients (52%). The high rate of positive serum GM tests may be attributed to the inclusion of both immunocompetent and immunocompromised patients in our study. Additionally, due to the limited number of BALF examinations, the diagnosis of some cases relies excessively on the results of serum GM tests. A study on plasma mNGS in patients with CAPA reported a sensitivity of 67% and a specificity of 97% for *Aspergillus* detection [32]. In our study, 42 CAPA patients underwent BALF mNGS testing, with a 71.34% positive rate, suggesting that mNGS enhances the clinical detection sensitivity of CAPA and may also shorten the diagnostic interval [33].

The multivariate analysis in our study revealed that chronic renal insufficiency (OR 5.11) and chronic respiratory disease (OR 4.3) were independent risk factors for CAPA, similar to the findings presented in other studies [34]. Chronic respiratory diseases can lead to ongoing inflammation and damage to airways and lung tissues, creating opportunities for *Aspergillus spp.* to invade [35]. Compared with premature ageing of the immune system, chronic renal insufficiency in patients with CAPA is more prevalent because of weakened immune function, which is the result of metabolic dysregulation [36]. Diabetes (OR 2.95) emerged as an additional risk factor in our research, aligning with the results of a global observational study conducted by Juergen Prattes and colleagues [14]. Patients with diabetes mellitus experience metabolic disorders that result in compromised immune cell functions, including those of macrophages and dendrite cells, which are essential for both innate and adaptive immune responses. Additionally, a hyperglycaemic environment alters the metabolic pathways of these immune cells, leading to a decline in immune function. In addition, patients with diabetes may not be able to appropriately increase their levels of important cytokines and adhesion molecules to combat pathogens after infection [37]. The *Aspergillus* species identified in this study were primarily *A. fumigatus*, followed by *A. flavus* (Supplementary material, Table S1), which was associated with a higher prevalence of this species, as other studies reported [6, 35]. Lung infections caused by *A. fumigatus* result from the inhalation of airborne conidia, which are widely distributed across diverse environments [38]. When *A. fumigatus* colonizes the respiratory tract, it can progress to invasive disease under conditions such as immunosuppression or alterations in the pulmonary microenvironment. Moreover, the multigenic virulence factors of *A. fumigatus* creates obstacles that impede the host's ability to eliminate fungal propagules [39].

This study represents a large-scale clinical investigation, examining the incidence of CAPA across intensive care units and general wards and identifying risk factors for CAPA as well as the role of microbiological examinations. A significant strength of our study was the comprehensive collection of patients with COVID-19 pneumonia from various departments. Nevertheless, this research faced multiple limitations. First, the design being confined to a single center, along with a limited sample size, restricted both the generalizability and accuracy of our results. Second, the retrospective nature of our study meant that we could not ensure the collection of adequate lower respiratory tract specimens such as BALF GM, BALF mNGS for all patients, potentially leading to missed diagnoses of false-negatives. Additionally, the lower number of BALF sample tests compared to compared to serum GM testing may have introduced bias in the reported sensitivity of diagnostic tests. Third, several patients tested positive for serum GM or sputum culture alone, which does not definitively rule out the possibility of false-positives. Furthermore, excluding CAPA patients who were not treated by physicians in PCCM may limit the generalizability of our analysize of comparison between CAPA and non-CAPA patients. In future research, we plan to address this limitation by conducting a more multicenter studies or prospective trials to validate the diagnostic and risk factor findings.

Conclusion

The diagnosis of CAPA is complex, leading to varied diagnosis rates among hospitalized patients, particularly in intensive care units. Our study revealed quicker CAPA diagnoses in the RICU, emphasizing the importance of microbiological evidence. The sensitivity of BALF GM is greater than that of serum GM. Moreover, BALF mNGS has the potential to enhance clinical detection of *Aspergillus*. Chronic renal insufficiency and respiratory diseases, along with diabetes, increase the risk of CAPA in COVID-19 patients. *A. fumigatus* was the most commonly detected *Aspergillus* species and can be used as a reference for clinical empirical treatment. To improve the diagnosis of CAPA in clinical settings, it is crucial to enhance physicians' awareness and to promptly perform BALF sampling for GM and mNGS tests in COVID-19 patients with risk factors. Additionally, the introduction of *Aspergillus* PCR tests may further enhance detection of CAPA.

Abbreviations

CAPA	COVID-19 associated pulmonary aspergillosis
IAPA	Influenza-associated pulmonary aspergillosis
ECMM/ISHAM	European Confederation of Medical Mycology (ECMM) and the International Society for Human and Animal Mycology (ISHAM)
RICU	Respiratory Intensive Care Unit
other ICUs	Including ICU and EICU
ICU	Intensive Care Unit
EICU	Emergency Intensive Care Unit
General ward	The general ward of the Department of Respiratory and Critical Care Medicine
BALF	Bronchoalveolar lavage fluid
GM	Galactomannan
mNGS	Metagenomic next-generation sequencing
ROC	Receiver operating characteristic curves
AUC	Area under the curve
OR	Odds ratio
CI	Confidence interval
p	p-value
PCR	Polymerase chain reaction
BMI	Body mass index
APTT	Activated partial thromboplastin time
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
TBIL	Total bilirubin
ALB	Albumin
GLO	Globulin
BUN	Blood Urea Nitrogen
CRP	C-reaction protein
LDH	Lactate dehydrogenase
ERS	Erythrocyte sedimentation rate
Fet	Ferritin
IL	Interleukin
TNF	Tumor necrosis factor
IFN	Interferon
CRRT	Continuous renal replacement therapy

Supplementary Information

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Supplementary Material 1

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Author contributions
This study was designed by YZ and YPL and the data was collected and assembled by LJX, TTH, XLL and XBC. Analysis and interpretation of data was done by PCL, SSS, and LY. The final manuscript was approved by all authors.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This retrospective study was conducted in accordance with the Declaration of Helsinki (revised in 2013), approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (KY2024-R216) and the informed consent was waived.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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