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Original article

RISK FACTORS ASSOCIATED WITH INVASIVE PULMONARY ASPERGILLOSIS IN SEVERE COVID-19 PATIENTS: A CASE-CONTROL STUDY

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Abstract

Introduction: Since the onset of the COVID-19 pandemic, cases of COVID-19-associated pulmonary aspergillosis (CAPA) have been described. Possible risk factors for the development of this condition have been proposed, although evidence in Latin American populations is limited. The objectives were to identify risk factors for the development of CAPA and describe the characteristics of this infection.

Materials and methods: A retrospective case-control study was conducted. The population consisted of adult patients with severe COVID-19, hospitalized in ICU and who had undergone diagnostic tests for invasive pulmonary aspergillosis.

Results: Seventy-five patients were evaluated, 21 in the case group and 54 in the control group. 64% were male, with an average age of 62.7 years. It was found that a history of diabetes (OR 3.3, CI 1.09-9.95, p=0.03), smoking (OR 3.47, CI 1.20-10, p=0.02), coronary artery disease (OR 5, CI 1.24-20.08, p=0.02), and a Charlson score equal to or greater than 5 (OR 1.27, CI 1-1.60, p=0.013) could be associated with the development of CAPA. Most cases were considered as possible CAPA (87.5%). The time between orotracheal intubation to the diagnosis of CAPA was 11.5 days. Fever was the most common symptom (90%), and only 24% of patients had compatible radiographic findings. Mortality in the case group was 61.9%.

Discussion: A history of diabetes, smoking, coronary artery disease, and a Charlson score equal to or greater than 5 may increase the risk of developing CAPA.

Key words: COVID-19, SARS-CoV-2, COVID-19-associated pulmonary aspergillosis, invasive pulmonary aspergillosis

Resumen

Factores de riesgo asociados a aspergilosis pulmonar invasiva en pacientes con COVID-19 grave: un estudio de casos y controles

Introducción: La evidencia sobre factores de riesgo para el desarrollo de aspergilosis pulmonar asociada a COVID-19 (CAPA), en poblaciones de Latinoamérica, es escasa. Los objetivos del presente estudio fueron identificar factores de riesgo para el desarrollo de CAPA en pacientes con COVID-19 grave y describir las características de la infección.

Materiales y métodos: Estudio retrospectivo de casos y controles. La población incluyó pacientes adultos, con COVID-19 grave, sometidos a pruebas diagnósticas para CAPA.

Resultados: Se evaluaron 75 pacientes, 21 casos y 54 controles (relación 1:2.6). El promedio de edad fue 62.7 años. El antecedente de diabetes (OR 3.3 IC 1.09 - 9.95,

p=0.03), tabaquismo (OR 3.47 IC 1.20-10, p=0.02), enfermedad coronaria (OR 5 IC 1.24-20.08, p=0.02) y *score* de Charlson ≥ 5 (OR 1.27, IC 1- 1.60, p=0.013) podrían asociarse al desarrollo de CAPA. El 87.5% de los casos se consideraron como CAPA posible. El tiempo entre la intubación orotraqueal y el diagnóstico de CAPA fue de 11.5 días. El síntoma más frecuente fue la fiebre (90%) y solo el 24% de los pacientes presentó hallazgos radiológicos compatibles con aspergilosis. La mortalidad en el grupo de casos fue de 61.9%.

Discusión: El antecedente de diabetes, tabaquismo, enfermedad coronaria y score de Charlson igual o mayor a 5 podría aumentar el riesgo de desarrollar CAPA en pacientes con COVID-19 grave.

Palabras clave: COVID-19, SARS-CoV-2, aspergilosis pulmonar asociada a COVID-19, aspergilosis pulmonar invasiva

KEY POINTS

- Cases of COVID-19 associated pulmonary aspergillosis (CAPA) have been described since the onset of the pandemic. Risk factors for the development of CAPA have been proposed: advanced age, severe disease, comorbidities (COPD, chronic liver and kidney disease, heart failure) and use of corticosteroids and tocilizumab. Nevertheless, the evidence is particularly scarce in Latin American populations.
- This single-centre case-control study found that having a Charlson score of 5 or higher and a history of diabetes, coronary artery disease, and smoking might increase the risk of developing CAPA. Most cases presented with fever and respiratory deterioration, and less than 25% showed radiological findings compatible with invasive pulmonary aspergillosis.

Coronavirus disease 2019 (COVID-19) has affected over 767 million people and caused 6 950 665 deaths worldwide as of July 2023[1]. With the availability of vaccines against SARS-CoV-2, the number of patients with severe disease requiring mechanical ventilatory support (MVS) has significantly decreased. However, groups of patients at risk of severe disease still exist, including those who are unvaccinated, elderly individuals, and those with comorbidities such as hypertension, diabetes, chronic lung, heart, liver or kidney disease, and active oncological or haematological disease, among others[2,3].

The presence of superinfections (bacterial and fungal) in severe COVID-19 patients is a significant concern. Since the beginning of the pandemic, cases of invasive pulmonary aspergillosis have been described in these patients, a condition known as COVID-19 Associated Pulmonary Aspergillosis (CAPA)[4–8]. The incidence of CAPA varies in reports, ranging from 2.5% to 35%[9]. In Latin America, there are few reports on the incidence of CAPA, with studies indicating an incidence of approximately 15-20% in Intensive Care Unit (ICU) hospitalised patients[10,11]. Mortality associated with CAPA reported in the literature is mostly around 50%[12].

Possible risk factors for the development of CAPA have been described, such as advanced age, a high Sequential Organ Failure Assessment (SOFA) score, comorbidities (COPD, chronic liver and kidney disease, heart failure), and the use of corticosteroids and tocilizumab[13–15].

The clinical and radiological manifestations of CAPA overlap with those of SARS-CoV-2 infection, making the diagnosis primarily based on microbiological evidence of fungal infection in respiratory samples obtained through various methods[16–21].

Currently, little is known about the possible risk factors for the development of CAPA in severe COVID-19 patients in Latin American populations. This knowledge would allow for an increased suspicion in certain patient groups, the implementation of appropriate diagnostic methods, and early initiation of treatment. Based on the aforementioned, the primary objective of this study is to identify possible risk factors associated with the development of CAPA in adult patients admitted to the ICU due to severe COVID-19 infection. As secondary objectives, we aim to describe the characteristics of this superinfection and define the mortality associated with CAPA in this population.

Materials and methods

A retrospective case-control study was conducted at a tertiary care hospital in Buenos Aires, Argentina.

Population

The study population consisted of individuals who met the following inclusion criteria: 1) adults aged 18 and older, 2) hospitalized in the ICU between March 1, 2020, and June 1, 2022, 3) with a confirmed diagnosis of COVID-19 (through polymerase chain reaction, antigen test, or anti-SARS-CoV-2 antibody testing), 4) requiring invasive mechanical ventilatory support (orotracheal intubation or tracheostomy), and 5) who underwent diagnostic testing for invasive pulmonary aspergillosis during their hospitalization. Patients with a prior history of aspergillosis and those who had mycological studies performed on samples other than tracheal aspirate or bronchoalveolar lavage were excluded.

Data collection

Data was collected through the Electronic Medical Records (EMR) between March 2020 and June 2022. Demographic data, clinical history, laboratory parameters, and mechanical ventilation details upon admission to the ICU, advanced life support measures, and details of microbiological studies for the diagnosis of invasive pulmonary aspergillosis requested during the ICU stay were recorded.

Definitions

Definitions for variables concerning clinical history, laboratory parameters, treatments received, and measures of advanced life support evaluated as risk factors for CAPA can be found in the Supplementary material. Respiratory samples for analysis were obtained through lung biopsy, bronchoalveolar lavage (BAL), and tracheal aspirate (TA). Diagnostic tests for *Aspergillus* detection included pulmonary biopsy histology, direct mycological examination, mycological culture on selective media (agar Sabouraud), and galactomannan detection by ELISA (Platelia *Aspergillus*, Bio-rad) in BAL, TA, and serum.

The case group included patients who developed proven, probable, and possible CAPA according to the criteria proposed by ECMM/ISHAM[22]. Additionally, patients with a direct mycological examination with compatible fungal elements, positive mycological culture for *Aspergillus*, and/or a galactomannan value in tracheal aspirate ≥ 2.0, were also considered as possible CAPA cases, according to the practices implemented in the institution where the research protocol was conducted. The control group consisted of patients who did not present microbiological evidence compatible with CAPA, following the previously mentioned criteria.

Statistical analysis

The distribution of variables was analysed graphically using histograms and analytically using the Shapiro-Wilks test. Variables with a normal distribution were expressed as mean and standard deviation (SD), while those with a non-normal distribution were expressed as median and interquartile range (IQR). Categorical data were presented as absolute frequency, percentage, and 95% confidence interval (CI 95%). Different comparative analyses between groups were conducted using the t-test or Mann-Whitney test for quantitative variables and the chi-squared test for categorical variables.

A bivariate analysis was performed to identify factors associated with the development of invasive pulmonary aspergillosis. Variables that showed a significant association were included in a multivariable logistic regression model, along with clinically relevant variables. Odds ratios (OR) and their 95% confidence intervals (CI) were reported for both crude and adjusted models. Statistical significance was considered when $p < 0.05$. STATA 16.1 software was used for the analysis.

Ethical considerations

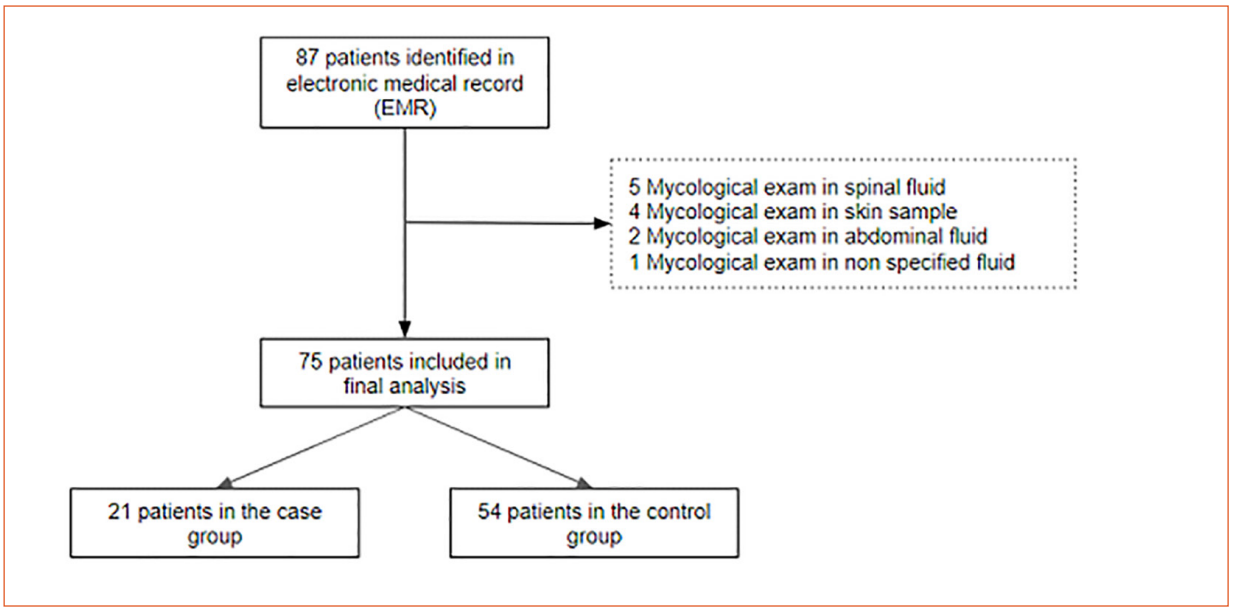
All stages of the study were carried out in compliance with current regulations and received approval from the institution's Research Protocol Ethics Committee.

Results

Population characteristics

A total of 87 patients were identified from the EMR. However, 12 individuals were excluded because they had mycological studies performed on samples other than respiratory ones. The participant flowchart is presented in Figure 1. The final population considered for analysis consisted of 75 patients, with 21 in the case group and 54 in the control group (ratio 1:2.6). The demographic and clinical characteristics of the population are summarized in Table 1. The average age was 67.2 years, and 64% of the patients were male. Regarding the presence of comorbidities, statistically significant differences were observed in the distribution of diabetes, coronary artery disease, and smoking history, which were more frequent in the case group than in the control group.

Figure 1 | Flowchart



COVID-19 infection characteristics

The characteristics related to COVID-19 infection in the studied population are summarized in Table 2. The median number of days from the onset of COVID-19 symptoms to ICU admission was 6 days in the case group and 8.5 days in the control group (p=0.19). Hospital mortality was slightly higher in the case group (61.9%) compared to the control group (55.6%), but this difference did not reach statistical significance (p=0.62). There were no differences between the groups regarding clinical, laboratory, and radiological parameters at the time of ICU admission (Supplementary material), treatments administered for SARS-CoV-2 infection, and the need for advanced life support measures.

Characteristics of COVID-19-Associated Pulmonary Aspergillosis (CAPA)

In the study population, 21 cases of CAPA were identified. Of these, 3 (14.2%) were considered probable cases, and 18 (85%) were possible cases, according to the criteria proposed by ECMM/ISHAM. Four lung biopsies were performed, but none of them showed evidence of invasive pulmonary aspergillosis. Most microbiological studies were conducted on respiratory samples obtained through a tracheal aspirate. The microbiological studies conducted on respiratory samples and their findings are summarized in Figure 2.

The characteristics of COVID-19-associated pulmonary aspergillosis in the population are summarized in Table 3. The median number of days from the onset of COVID-19 symptoms to the diagnosis of CAPA was 26 days, and the median number of days between orotracheal intubation and the diagnosis of CAPA was 11 days. The main clinical manifestations in patients with CAPA included fever (90.5%) and respiratory deterioration (71.4%). As for radiological manifestations, only 1 patient had a cavitated lesion on chest X-ray, while 5 patients (23.8%) had findings compatible with aspergillosis on chest computed tomography. In the case group, 71.5% of patients received antifungal treatment, with voriconazole being the drug of choice in almost all cases. Therapeutic drug monitoring was performed in 75% of cases, and the drug levels were within the therapeutic range in 8 out of 9 patients. The mortality in the CAPA case group was 61.9%. A statistically significant association for death was observed in patients with a history of active oncohematological disease (0 vs.

Tabla 1 | Demographic and clinical characteristics of the population. Risk factors for CAPA

Population characteristic	Cases (n = 21)	Controls (n = 54)	Crude OR	p-value
Age (years) median (IQR)	71 (64-75)	66 (60-77)	1 (0.96-1.05)	0.74
Male sex n (%) (95% CI)	12 (57.1) (34-78)	36 (66.7) (52-79)	0.66 (0.24-1.88)	0.44
Charlson score median (IQR)	5 (3-7)	4 (2-5)	1.27 (1.0-1.60)	0.013
BMI (kg/m^2) median (IQR)	30.1 (26.5-34.2)	29.9 (25.1-33.1)	1.02 (0.92-1.12)	0.71
Obesity (BMI > 30) n (%) (95% CI)	11 (52.4) (30-74)	26 (48.1) (34-62)		0.66
Hypertension n (%) (95% CI)	16 (76.2) (53-92)	33 (61.1) (47-74)	2.03 (0.65-6.39)	0.22
Diabetes n (%) (95% CI)	9 (42.9) (22-66)	10 (18.5) (9-31)	3.3 (1.09-9.95)	0.03
Chronic kidney disease on hemodialysis n (%) (95% CI)	0 (0) (0-16)	1 (1.8) (0-10)	1	1.00
Heart failure n (%) (95% CI)	2 (9.5) (1-30)	4 (7.4) (2-18)	1.31 (0.22-7.78)	1.00
Coronary artery disease n (%) (95% CI)	6 (28.6) (11-52)	4 (7.4) (2-18)	5 (1.24-20.08)	0.02
Asthma n (%) (95% CI)	0 (0) (0-16)	2 (3.7) (0-12)	1	1.00
Smoking n (%) (95% CI)	11 (52.4) (30-74)	13 (24.1) (13-37)	3.47 (1.20-10)	0.02
COPD n (%) (95% CI)	3 (14.3) (3-36)	2 (3.7) (0-12)	4.33 (0.67-28)	0.13
Congenital immunocompromise[1] n (%) (95% CI)	1 (4.8) (0-24)	0 (0) (0-6)	1	0.28
Solid organ transplant[2] n (%) (95% CI)	3 (14.3) (3-36)	4 (7.4) (2-18)	2 (0.42-10)	0.36
Bone marrow transplant[3] n (%) (95% CI)	1 (4.8) (0-24)	1 (1.8) (0-10)	2.65 (0.16-44.42)	0.48
Active oncologic disease[4] n (%) (95% CI)	3 (14.3) (3-36)	4 (7.4) (2-18)	2.08 (0.42-10.22)	0.36
Active oncohematological disease[4] n (%) (95% CI)	1 (4.8) (0-24)	3 (5.6) (1-15)	0.85 (0.08-8.66)	1.00
Chronic corticosteroid use[5] n (%) (95% CI)	4 (19) (5-42)	6 (11.1) (4-23)	1.88 (0.47-7.48)	0.36
Immunosuppressive drugs (T lymphocyte)[6] n (%) (95% CI)	3 (14.3) (3-36)	4 (7.4) (2-18)	2.08 (0.42-10.25)	0.36
Immunosuppressive drugs (B lymphocyte)[7] n (%) (95% CI)	0 (0) (0-16)	1 (1.8) (0-10)	1	1.00
COVID-19 vaccination (full scheme)[8] n (%) (95% CI)	9 (42.9) (22-66)	19 (35.2) (23-50)	0.82 (0.46-1.48)	0.54

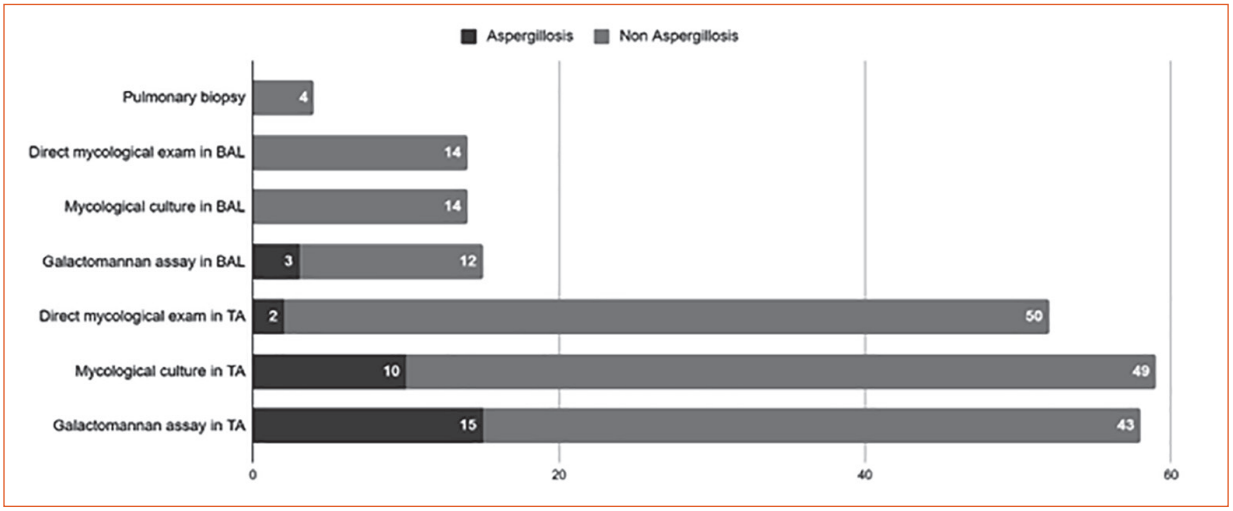
BMI: body mass index; COPD: chronic obstructive pulmonary disease
[1]Congenital immunocompromise: Common variable immunodeficiency (n=1). 2Solid organ transplant: Liver transplant (n=2), kidney transplant (n=3), heart transplant (n=1), and lung transplant (n=1). [3]Bone marrow transplant: Autologous transplant (n=1) and allogeneic transplant (n=1). [4]Active oncologic/oncohematological disease: Patients who received specific oncology treatment of any type in the last year (chemotherapy, immunotherapy, radiation therapy). [5]Chronic corticosteroid use: Chronic use of corticosteroids at a dose of meprednisone ≥ 4 mg/day or equivalent. [6]Immunosuppressive drugs (T lymphocyte): Tacrolimus, cyclosporine, mycophenolate. [7]Immunosuppressive drugs (B lymphocyte): Rituximab, ibrutinib. [8]COVID-19 Vaccination (full scheme): Patients who received at least two doses of an active vaccine with at least 21 days elapsed since the last dose before hospital admission.

Tabla 2 | Characteristics of COVID-19 infection

	Cases (n = 21)	Controls (n = 54)	p-value
Days from symptom onset to ICU admission	6	8.5	0.19
median (IQR)	(4-12)	(6-12)	
Days from ICU admission to endotracheal intubation	0	0	0.65
median (IQR)	(0-2)	(0-1)	
Total days of mechanical ventilation	23	25	0.42
median (IQR)	(20-36)	(15-36)	
Total days of ICU stay	27	29	0.71
median (IQR)	(21-51)	(19-47)	
Hospital length of stay	32	37.5	0.82
median (IQR)	(24-71)	(22-52)	
Hospital mortality	13 (61.9)	30 (55.6)	0.62
n (%) (95% CI)	(38-82)	(42-70)	
Treatment with dexamethasone	21 (100)	54 (100)	-
n (%) (95% CI)	(84-100)	(93-100)	
Treatment with meprednisone	2 (9.5)	14 (25.9)	0.12
n (%) (95% CI)	(1-30)	(15-40)	
Treatment with tocilizumab	3 (14.3)	14 (25.9)	0.28
n (%) (95% CI)	(3-36)	(15-40)	
Requirement of neuromuscular blockers	19 (90.5)	50 (92.6)	0.76
n (%) (95% CI)	(70-99)	(82-98)	
Requirement of proning maneuver	12 (57.1)	38 (70.8)	0.27
n (%) (95% CI)	(34-78)	(56-82)	
Requirement of venovenous ECMO	1 (4.8)	2 (3.7)	1
n (%) (95% CI)	(0-24)	(0-13)	

ICU: intensive care unit; ECMO: extracorporeal membrane oxygenation

Figure 2 | Microbiological studies for aspergillosis detection



BAL: bronchoalveolar lavage; TA: tracheal aspirate

Tabla 3 | Characteristics of COVID-19 associated pulmonary aspergillosis in the population (n = 21)

Classification according to ECMM / ISHAM criteria	
Proven	0
n (%) (95% CI)	
Probable	3 (14.3)
n (%) (95% CI)	(3-36)
Possible	18 (85.7)
n (%) (95% CI)	(64-97)
Clinical characteristics	
Days between symptom onset and CAPA diagnosis median (IQR)	26 (16-32)
Days between endotracheal intubation and CAPA diagnosis median (IQR)	11 (7-20)
Fever[1]	19 (90.5)
n (%) (95% CI)	(70-99)
Respiratory deterioration[2]	15 (71.4)
n (%) (95% CI)	(48-89)
Chest X-ray[3]	1 (4.8)
n (%) (95% CI)	(0-24)
Chest CT scan[4]	5 (23.8)
n (%) (95% CI)	(8-47)
Pharmacological treatment	
Antifungal treatment	15 (71.4)
n (%) (95% CI)	(48-88)
Voriconazole treatment	12 (57.1)
n (%) (95% CI)	(34-78)
Amphotericin B treatment	3 (14.3)
n (%) (95% CI)	3-36)

ECMM: European Confederation of Medical Mycology; ISHAM: International Society for Human and Animal Mycology

[1]Fever: Persistent fever or new febrile event within 72 hours before CAPA diagnosis. [2]Respiratory deterioration: Increased oxygen requirements, decreasing PAFI, or the need for neuromuscular blockers or nitric oxide within 72 hours before CAPA diagnosis. [3]Chest X-ray: Presence of new infiltrates, nodules, or cavitation on chest X-ray performed within 72 hours before CAPA diagnosis. [4]Chest CT scan: Presence of findings consistent with CAPA (bilateral ground-glass opacities, lobar consolidation, nodules, halo sign, reversed halo sign, crescent sign, tree-in-bud pattern, cavitation) on chest CT scan performed within 72 hours before CAPA diagnosis.

3 patients, p=0.042) and in those who had a longer duration of MVS (21 vs. 37.5 days, p=0.0023) (Supplementary material).

Risk factors for the development of CAPA

In the analysis of risk factors for the development of invasive pulmonary aspergillosis in severe COVID-19 patients, univariate analysis indicated a statistically significant increase in the risk for patients with a Charlson score of 5 or higher (OR 1.27, CI 1-1.6, p=0.013), a history of diabetes (OR 3.3 CI 1.1-9.9, p=0.03), coronary artery disease (OR 5 CI 1.2-20.1, p=0.02), and smoking (OR 3.47 CI 1.2-10, p=0.02). Due to the absence of other statistically significant associations, a multivariate analysis was not conducted.

Discussion

In this single-centre, retrospective, observational case-control study that assessed risk factors for the development of COVID-19-associated pulmonary aspergillosis, it was found that having a Charlson score of 5 or higher and a history of diabetes, coronary artery disease, and smoking

might increase the risk of developing this condition. Additionally, most patients presented with fever and respiratory deterioration as the most common clinical manifestations, while fewer than 25% of cases showed radiological findings compatible with invasive pulmonary aspergillosis. Previous studies have described risk factors for CAPA, including advanced age, the use of corticosteroids and tocilizumab, a history of COPD, asthma, chronic kidney or liver disease, a high SOFA score on admission, and prolonged duration of mechanical ventilation[11–13,23,24]. Diabetes has been previously described as a risk factor for COVID-19-associated mucormycosis[14], but its association with the development of CAPA has not been previously reported. However, there are case reports of aspergillosis in patients with diabetes as the only risk factor, outside the setting of viral infections[25,26]. It is worth noting that no statistically significant associations were found for some risk factors reported by prospective studies, such as the use of corticosteroids. However, chronic use of corticosteroids before the onset of viral infection showed a slight trend towards increased risk, although it was not statistically significant (OR 1.88, CI 0.5-7.5, p=0.36). Finally, in line with previous studies, no significant association was found between known risk factors for invasive pulmonary aspergillosis, such as a history of active oncohematological disease, solid organ transplantation, bone marrow transplantation, and the use of T or B cell immunosuppressive drugs. However, a history of active oncohematological disease was significantly associated with mortality in patients with CAPA (p=0.042).

Most CAPA cases in this series were classified as possible aspergillosis (87.5%) based on microbiological studies performed on tracheal aspirate samples, taking into account the restrictions on performing procedures with a high risk of aerosolization such as LBA, especially at the beginning of the pandemic. Regarding the microbiological test performed, 10 positive mycological cultures were obtained from tracheal aspirates, with the recovery of *Aspergillus flavus* in 50% of cases, highlighting the importance of this agent as a cause of aspergillosis. It is estimated that *Aspergillus flavus* may have greater virulence than *Aspergillus fumigatus,* requiring a smaller inoculum to cause invasive infections and exhibiting natural resistance to amphotericin B, although it has low resistance to azoles[27]. It is also suggested that the larger size of *A. flavus* spores may be associated with a greater tendency to develop tracheobronchial involvement rather than pulmonary involvement[28]. In 15 cases, galactomannan levels in tracheal aspirate were greater than 2.0, a cutoff point suggested by Roman Montes et al. for the diagnosis of possible CAPA, with a specificity of 81.7%[16]. Finally, no galactomannan testing was performed on blood samples in any case, which could have helped strengthen the diagnosis of invasive aspergillosis in our population. However, the evidence regarding the use of this test in non-neutropenic patients is contradictory, with studies reporting a sensitivity of 22%, specificity of 84%, and possible cross-reactivity with beta-lactam antibiotics in this group of individuals[29]. Nevertheless, in a case series of CAPA, up to 50% of patients had galactomannan levels in serum greater than 0.5[30], suggesting that this method could contribute to the diagnosis of the disease.

Regarding the clinical evolution of the disease, the median number of days between endotracheal intubation and the diagnosis of CAPA was 11.5 days, slightly higher than what has been reported in the literature (8-8.5 days) [31]. This could partly be explained by the absence of systematic screening for CAPA in the included patients. The majority of patients presented with persistent fever or recurrent fever (90.5%) and deterioration in respiratory status (71.5%) as the primary clinical manifestation. Only 23.8% of the patients had a chest CT scan with findings compatible with pulmonary aspergillosis, in contrast to another series[11], where up to 60% of patients had suggestive radiological findings, such as bilateral ground-glass opacities, lobar consolidation, nodules, halo sign, reversed halo sign, crescent sign, tree-in-bud pattern or pulmonary cavitation. This may be due to potential overdiagnosis of CAPA cases (based on respiratory samples other than bronchoalveolar lavage) or the possibility of greater tracheobronchial involvement than pulmonary involvement in the participants.

The presented work has several limitations. Firstly, the diagnosis of most CAPA cases was based on respiratory samples other than lung biopsies and bronchoalveolar lavage, making it

difficult in many cases to distinguish between colonization and infection. This is a common limitation in many similar studies, highlighting the need for further research into appropriate diagnostic methods for detecting this condition. Secondly, the temporality of the collected data may pose difficulties in generalizing the findings, as with the advent of SARS-CoV-2 vaccines and new treatments, we may observe different profiles of patients with severe COVID-19 in the future, with potential implications for CAPA development. Lastly, the single-centre design of the study may also limit the generalizability of the findings to other similar populations.

The strengths of the study are first place, it represents a large cohort of patients with severe COVID-19 requiring mechanical ventilation evaluated for CAPA in Latin America, and second, it provides a detailed characterization of clinical manifestations and radiological findings of probable and possible CAPA, adding to the growing body of evidence, that contribute to the understanding and managing of this condition in patients with COVID-19.

In summary, this single-centre, retrospective, observational, case-control study has contributed to the understanding of COVID-19-associated pulmonary aspergillosis (CAPA) by identifying relevant risk factors and clinical characteristics. A Charlson score equal to or greater than 5 and a history of diabetes, coronary artery disease, and smoking stand out as potential predictors for the development of this condition in patients with severe COVID-19.

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Conflict of interest: None to declare

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SUPPLEMENTARY MATERIAL

Definitions

- Coronary artery disease: was considered for those patients who had experienced an acute myocardial infarction or suffered from stable angina pectoris.
- Smoking: patients who were active smokers were considered smokers.
- Congenital immunocompromise: only patients with an immunocompromise that could increase the risk of aspergillosis (such as common variable immunodeficiency) were included.
- Oncological/hemato-oncological disease: individuals who received specific treatment for their condition (chemotherapy, immunotherapy, radiotherapy) within 6 months prior to admission.
- Chronic corticosteroid use: patients who used corticosteroids at a dose equivalent to 4 mg or more of prednisone daily for more than 3 months prior to admission.
- Respiratory status deterioration: increase in oxygen requirements, a decrease in the arterial oxygen pressure/fraction of inspired oxygen ratio, or the need for neuromuscular blockers or nitric oxide within 72 hours prior to the diagnosis of CAPA.

Supplementary Material - Risk factors for mortality in patients with COVID-19-associated pulmonary aspergillosis (CAPA)

Population characteristics	Died (n = 13)	Survived (n = 8)	p-value
Age	71	70.5	0.66
median (ICI)	(64-78)	(64-73)	
Male sex	8	4	0.67
n (%)	(61.5)	(50)	
Charlson score	5	6	0.15
median (ICI)	(3-6)	(5-7.5)	
Hypertension	10	6	1
n (%)	(76.9)	(75)	
Coronary artery disease	3	3	0.63
n (%)	(23.1)	(37.5)	
Diabetes	5	4	0.67
n (%)	(38.46%)	(50%)	
Smoking	6	5	0.66
n (%)	(46.1)	(62.5)	
COPD	2	1	1
n (%)	(15.4)	(12.5)	
Bone marrow transplant	1	0	1
n (%)	(7.7)		
Oncohematological disease	0	3	0.042
n (%)		(37.5)	
Chronic corticosteroid use	3	1	1
n (%)	(23.1)	(12.5)	
Immunosuppressive drugs (T lymphocyte)	2	1	1
n (%)	(15.3)	(12.5)	
Neutrophil count/mm^3	8640	4829	0.25
median (ICI)	(5976-10771)	(2994-9578)	
Lymphocyte count/mm^3	562	630	0.83
median (ICI)	(337-862)	(453-853)	
Treatment with tocilizumab	2	1	1
n (%)	(15.3)	(12.5)	
Treatment with meprednisone	0	2	0.13
n (%)		(25)	
Total days of MVS	21	37.5	0.0023
median (ICI)	(17-23)	(33.5-88.5)	

COPD: chronic obstructive pulmonary disease; MVS: mechanical ventilatory support

Material - Definition of COVID-19-associated pulmonary aspergillosis (CAPA) cases proposed by ECMM (European Confederation of Medical Mycology) / ISHAM (International Society for Human and Animal Mycology)

CAPA classification	Entry criterion	Imaging criterion	Microbiological criterion
Proven Tracheobronchitis or other pulmonary forms	COVID-19 patients requiring intensive care and a temporal relationship1		At least one of the following: Detection by histopathology or direct microscopy of fungal hyphae, with invasive growth associated with tissue damage or Aspergillus detected in culture, direct microscopy, histology, or PCR for Aspergillus from material obtained by sterile aspiration puncture or biopsy of a pulmonary site affected by an infectious process.
Probable Other pulmonary forms	COVID-19 patients requiring intensive care and a temporal relationship1	Pulmonary infiltrates or nodules, preferably documented by chest CT or cavitation (not attributable to another cause)	At least one of the following: Detection of fungal elements by microscopy from BAL, positive culture in BAL, galactomannan in BAL ≥ 1.0, galactomannan in serum ≥ 0.5; 2 or more PCRs for Aspergillus in blood; positive PCR in BAL (<36 cycles); positive PCR in blood and positive PCR in BAL with any cycle threshold allowed.
Possible Other pulmonary forms	COVID-19 patients requiring intensive care and a temporal relationship[1]	Pulmonary infiltrates or nodules, preferably documented by chest CT or cavitation (not attributable to another cause)	At least one of the following: Detection of fungal elements by microscopy from NBL, positive culture in NBL, galactomannan in NBL ≥ 4.5; 2 galactomannan in NBL ≥ 1.2; galactomannan in NBL ≥ 1.2 plus another positive test in NBL (PCR or LFA).

PCR: polymerase chain reaction; CT: computed tomography; BAL: bronchoalveolar lavage; NBL: non-bronchoscopic lavage; LFA: lateral flow assays

[1]*Entry Criterion: Real-time PCR for SARS-CoV-2 positive at any time during the 2 weeks between hospital admission and ICU admission or within 72-96 hours after ICU admission*