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COVID-19-associated pulmonary aspergillosis (CAPA): Risk factors and development of a predictive score for critically ill COVID-19 patients

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Abstract

Background: COVID-19-associated pulmonary aspergillosis (CAPA) is a major complication of critically ill COVID-19 patients, with a high mortality rate and potentially preventable. Thus, identifying patients at high risk of CAPA would be of great interest. We intended to develop a clinical prediction score capable of stratifying patients according to the risk for CAPA at ICU admission.

Methods: Single centre retrospective case–control study. A case was defined as a patient diagnosed with CAPA according to 2020 ECMM/ISHAM consensus criteria. 2 controls were selected for each case among critically ill COVID-19 patients.

Results: 28 CAPA patients and 56-matched controls were included. Factors associated with CAPA included old age (68 years vs. 62, $p = .033$), active smoking (17.9% vs. 1.8%, $p = .014$), chronic respiratory diseases (48.1% vs. 26.3%, $p = .043$), chronic renal failure (25.0% vs. 3.6%, $p = .005$), chronic corticosteroid treatment (28.6% vs. 1.8%, $p < .001$), tocilizumab therapy (92.9% vs. 66.1%, $p = .008$) and high APACHE II at ICU admission (median 13 vs. 10 points, $p = .026$). A score was created including these variables, which showed an area under the receiver operator curve of 0.854 (95% CI 0.77-0.92). A punctuation below 6 had a negative predictive value of 99.6%. A punctuation of 10 or higher had a positive predictive value of 27.9%.

Conclusion: We present a clinical prediction score that allowed to stratify critically ill COVID-19 patients according to the risk for developing CAPA. This CAPA score would allow to target preventive measures. Further evaluation of the score, as well as the utility of these targeted preventive measures, is needed.

KEYWORDS
CAPA, COVID-19, critically ill, score

1 | INTRODUCTION

The coronavirus disease 19 (COVID-19) pandemic has affected more than 250 million people, with more than 5 million deaths due to this condition.[1] One third of hospitalised patients with COVID-19 will develop severe acute respiratory distress syndrome (ARDS), which usually leads to intensive care unit (ICU) admission and mechanical ventilation.[2]

COVID-19-associated pulmonary aspergillosis (CAPA) has recently been recognised as a major complication of critically ill COVID-19 patients.[3] It is estimated that 10%-20% of ICU-admitted COVID-19 patients eventually develop CAPA.[4,5] Recent studies have shown that cumulative incidence may vary from 5% or less to rates close to 40%, with higher incidence in patients needing mechanical ventilation.[6] Additionally, this entity is a life-threatening condition, with mortality rates usually exceeding 60% despite appropriate antifungal treatment.[5,7] Accordingly, some authors have proposed the use of antifungal prophylaxis[8,9] or the implantation of screening protocols along with pre-emptive treatment when *Aspergillus* spp. is isolated from respiratory samples.[10] Applying these special measures to all critically ill COVID-19 patients, including patients at low risk for CAPA, could result in patient's harm due to secondary effects of inappropriate antifungal therapy[11,12] and lack of efficiency. Thus, identifying patients at high risk of CAPA in order to target the aforementioned measures would be of great interest. However, to date, studies that attempted to identify risk factors for CAPA development are scarce and show contradictory results.[4,13,14] Furthermore, none of these studies has resulted in a score capable to predict CAPA development at ICU admission.

We intend to identify factors associated with the occurrence of CAPA among critically ill COVID-19 patients and to develop a clinical score capable of stratifying them according to the risk for CAPA at ICU admission.

2 | PATIENTS AND METHODS

We performed a single centre retrospective case–control study. Our hospital is a 613-bed tertiary teaching hospital in Madrid, with a catchment area of 550.000 inhabitants, with 22 ICU beds, that increased to 64 ICU beds during the first waves of the COVID-19 pandemic.

Cases were identified from a prospective cohort that includes all patients diagnosed with invasive fungal infection at our centre since January 2018. A case was defined as a patient diagnosed with CAPA between March 2020 and August 2021, which corresponded to the first 5 waves in our setting. CAPA was defined according to 2020 ECMM/ISHAM criteria.[15] Cases were classified either as possible, probable or proven CAPA.

Controls were selected from a prospective cohort including all ICU-admitted patients with ARDS due to COVID-19. Controls were matched to cases by admission date. 2 controls were selected for each case. As one of our main objectives was to identify risk factors for CAPA development, which could include patient's age, sex,

comorbidities and COVID-19 severity, we decided not to match controls according to these variables.

Data were collected from electronic medical records and managed using REDCap electronic capture tools,[16] with a license provided to Puerta de Hierro-Segovia de Arana Research Institute (IDISPHSA for its Spanish abbreviation).[17] Data collected using REDCap platform were anonymised and included demographics, comorbidities, microbiological data and outcomes.

The study was approved by the hospital ethics committee (protocol identification PI-10/22, reference 07/117290.9/22). Since this was a retrospective, non-interventional study and only required collection of previously generated and anonymised data, informed consent was not required.

2.1 | Laboratory and microbiological procedure

Galactomannan (GM) qualitative detection was performed by sandwich chemiluminescent immunoassay (CLIA) Aspergillus Galactomannan Ag VIRCLIA@ monotest (Vircell, Granada, Spain). According to manufacter's instructions, a result equal or greater than 0.20 was considered positive, both in serum and in bronchoalveolar lavage. This cut-off point has been validated against the well-established indexes of GM Platelia, BioRad.[18]

Respiratory samples for fungal cultures were grown in Sabouraud-gentamicin-chloramphenicol agar and antifungal susceptibility tests were performed by broth microdilution at the national reference centre (Carlos III Health Institute, National Microbiology Centre, Majadahonda, Spain). In some samples, direct visualisation by KOH stain was performed at the request of the attending physician.

All tests were performed according to manufacturer´s instructions.

No CAPA screening protocol was implemented during the study period and the tests were ordered at the discretion of the attending physician. Fibrobronchoscopy and bronchoalveolar lavage were performed under specific request of attending physician.

2.2 | Definitions

ARDS was defined according to Berlin's criteria.[19] The acute physiology and chronic health evaluation (APACHE II score[20]) punctuation was calculated at ICU admission except from patients not admitted to ICU, where APACHE II was calculated at the time of severe ARDS development. The waves' period was considered as follows: first wave from February to July 2020; second wave from August to November 2020; third wave from December 2020 to February 2021; fourth wave from March to May 2021; and fifth wave from June to August 2021.

2.3 | Primary and secondary objectives

Our primary objective was to identify risk factors for CAPA among critically ill COVID-19 patients. Our secondary objective was to

develop and validate a clinical prediction score for the occurrence of CAPA among critically ill COVID-19 patients based on data available at ICU admission. We also sought to describe characteristics of CAPA patients and factors associated with poor outcome.

2.4 | Data analysis

Data are presented as median and interquartile range (IQR) for quantitative variables, and as absolute and percentage value for qualitative variables. For the primary and secondary purposes, inferential statistical analysis was done using chi-square test and Fisher exact test (when necessary) for qualitative variables and Mann–Whitney's U for quantitative ones. A 180-day mortality analysis was performed by means of Cox regression and Kaplan–Meier curves. Hazard ratios (HR) with 95% confidence intervals (CI) are provided.

To create the score, variables clinically and statistically significant in the previous univariate analysis were included in a multivariate logistic regression model in order to estimate each patient's predicted probability of developing CAPA.[21,22] As we did not intend to seek for independent-associated factors, we did not establish a limit of covariate based on the number of cases.[22-25] If the variables included in showed to improve the predictive model, they were considered for the final predicted probability calculation. Afterwards, the predicted probability was transformed into a punctuation score based on the beta-coefficient from this model. The score was subsequently applied to all patients. Calibration of the score was tested by the Hosmer-Lemeshow goodness-to-fit test.[25] Discrimination of the score was measured by the area under the receiving operator curve (AUC-ROC) with the 95% CI. A bootstrap procedure was employed for internal validation. The score was applied to 1000 bootstrap samples, and the optimism was calculated. Then, the bootstrapped corrected AUC-ROC was computed. A value of AUC-ROC of 0.5 indicates random predictions, and a value of 1 indicates perfect discrimination. A model with an AUC-ROC roughly above 0.7 is considered to be useful to perform predictions of individual subjects.[25] Cut-off points along with sensitivity, specificity and likelihood ratios are provided. Negative and positive predictive values were calculated applying the score to a population with a prevalence similar to that of our ICU cohort.

Bilateral p values below .05 were considered statistically significant. All statistical analyses were performed using SPSS version 25 software (SPSS Inc, IBM, Chicago, Illinois, United States).

3 | RESULTS

Between March 2020 and August 2021, 28 CAPA patients were prospectively identified in our centre, including 22 ICU-admitted patients, representing 6.04% (22/364) patients admitted to ICU with severe ARDS due to COVID-19. In addition to the 22 that were at the ICU ward, 3 others were on non-invasive mechanical ventilation at an intensive respiratory care unit and the remaining 3 were severe

ARDS COVID-19 cases receiving the highest possible oxygen supplementation at conventional hospitalisation. Of note, all of the 6 patients not admitted to ICU had developed critical COVID-19 with severe ARDS and had criteria for ICU admission. Therefore, they fulfilled ECMM/ISHAM 2020 consensus criteria for CAPA. However, due to healthcare system overload (and in particular the lack of available ICU beds), as well as age and basal comorbidity of some patients, other patients with potentially better survival opportunities were prioritised over them for ICU admission. Six (21.4%) CAPA patients were diagnosed during the first, 6 (21.4%) during the second wave, 5 (17.9%) during the third, 6 (21.4%) during the fourth wave and 5 (17.9%) during the fifth wave. Accordingly, 56-matched controls were selected.

3.1 | CAPA characteristics

Characteristics of the 28 CAPA patients included in the study are shown in Table 1.

Median time from hospital admission to CAPA diagnosis was 21 days (IQR 11–41), and median time from ICU admission to CAPA was 11 days (IQR 6-42).

Sixteen patients (57.1%) were classified as probable CAPA and 12 (42.8%) as possible CAPA. Comparison between probable and possible cases is shown in Table S1. Bronchoalveolar lavage culture was only available in 2 out of 12 possible CAPA and none of them had GM performed in this sample. There were no cases of proven CAPA. Evidence of tracheobronchitis was noted in 4 out of 14 patients with available bronchoscopy (28.6%). In 23.1% (6/26) cases serum galactomannan was positive and 1 (3.6%) had extra-pulmonary organ involvement: the case consisted of a endogenous fungal endophthalmitis in the context of an extensive probable CAPA (without microbiological confirmation of the ocular involvement).

Aspergillus fumigatus complex was the most frequently isolated species (64.3%, n = 18), followed by *Aspergillus niger complex* (14.3%, n = 4). There were isolated cases of *Aspergillus terreus* and *Aspergillus flavus (3.6% each)*. In one case (3.6%), the *Aspergillus* species was not identified and in 3 cases (10.7%) there was not a culture growth. In 20 cases, an antifungigram was available: only 1 case (5.0%) was resistant to amphotericin B (*A terreus*, intrinsic resistance), and 1 case (5.0%) of voriconazole and isavuconazole resistance was detected in a patient with no species identification. CAPA patients had an in-hospital mortality rate of 60.7% (n = 15), while controls had an in-hospital mortality of 14.3% (n = 8) (p < .001).

3.2 | Factors associated with CAPA

When comparing patients with and without CAPA regarding baseline characteristics (Table 1), CAPA patients were older (median age 68 years (IQR 65–72) vs. 62 (52–71), p = .033) and more frequently active smokers (17.9% vs. 1.8%, p = .014). CAPA patients had more often chronic respiratory diseases (48.1% vs. 26.3%, p = .043),

TABLE 1 Factors associated with CAPA

Variable	CAPA (n = 28)	Control (n = 56)	p	Missing
Comorbidity				
Age (years)	68 (65–72)	62 (52–71)	.033	0
Sex (female)	21.4% (6)	30.4% (17)	0.446	0
Active smoking	17.9% (5)	1.8% (1)	.014	11
Arterial hypertension	64.3% (18)	44.6% (25)	0.108	0
Diabetes mellitus	39.3% (11)	17.9% (10)	.059	0
Chronic respiratory disease	48.1% (13)	26.3% (15)	.043	0
COPD	28.6% (8)	10.7% (6)	.060	0
Asthma	3.6% (1)	7.1% (4)	0.661	0
Other	21.4% (6)	10.7% (6)	0.202	0
Chronic cardiac failure	21.4% (6)	8.9% (5)	0.168	0
Ischaemic heart disease	21.4% (6)	5.4% (3)	.054	0
Chronic renal failure	25.0% (7)	3.6% (2)	.005	0
Liver cirrhosis	7.1% (2)	0	0.108	0
Solid malignancy	7.1% (2)	10.7% (6)	0.713	0
Prior immunocompromise				
Any IC condition	42.9% (12)	19.6% (11)	.037	0
Haematological malignancy	14.3% (4)	3.6% (2)	0.172	0
Solid organ transplantation	17.9% (5)	1.8% (1)	.014	0
Autoimmune disease	14.3% (4)	12.5% (7)	1.000	0
Previous chronic corticoid	28.6% (8)	1.8% (1)	<.001	0
Other previous IS treatments	28.6% (8)	10.7% (6)	.060	0
COVID-19 presentation and management prior to CAPA diagnosis				
Neutropenia	14.3% (4)	1.8% (1)	.042	0
Confirmed bacterial coinfection	57.1% (16)	44.6% (25)	0.356	0
Viral coinfection other than CMV	7.1% (2)	1.8% (1)	0.547	0
Renal replacement therapy	35.7% (10)	10.7% (6)	.008	0
Vasopressor drug therapy	42.9% (12)	39.3% (22)	0.816	0
APACHE II	13 (9–18)	10 (8–13)	.026	2
Any corticoid treatment	100%	100%	1.000	0
Corticoid pulses	46.4% (13)	28.6% (16)	.085	0
Tocilizumab	92.9% (26)	66.1% (37)	.008	0
1 dose	43.5% (10/23)	86.1% (31/36)	.001	4
2 or more doses	56.5% (13/23)	13.9% (5/36)		
Anakinra	10.7% (3)	8.9% (5)	1.000	0
Remdesivir	14.3% (4)	5.4% (3)	0.215	0
Antibiotics	96.4% (27)	91.1% (51)	0.658	0
Blood components transfusion	51.9% (14)	21.8% (12)	.011	0
Outcomes				
In-hospital mortality	60.7% (17)	14.3% (8)	<.001	0
CAPA-associated death	76.5% (13/17)	–	–	0
COVID-associated death	88.2% (15/17)	87.5% (7/8)	1.000	0
ICU length of stay	57 (28–85)	18 (13–38)	.010	27
Hospital length of stay	66 (43–88)	33 (22–58)	.003	25

Note: Qualitative variables are expressed as percentage (absolute number). Quantitative variables are expressed as median (interquartile range).

Abbreviations: CAPA, COVID-associated pulmonary aspergillosis; COPD, Chronic obstructive pulmonary disease; IC, Immunocompromised.

TABLE 2 Multivariate logistic regression model for developing of the CAPA risk score

Variable	OR	95% CI	Beta-coefficient	Points
Age (per 5 years)	1.35	0.60–3.00	0.493	64–69 years: 2
				>/= 70 years: 3
Active smoking	3.58	1.67–7.70	1.26	3
Chronic respiratory disease	1.26	0.33–4.78	0.23	2
Chronic renal failure	2.67	0.35–20.31	1.98	4
Prior chronic corticoid	49.61	5.41–149.1	5.00	5
Tocilizumab	20.96	1.56–278.9	3.04	4
APACHE II (per 3 points)	1.64	0.71–3.76	0.297	10–12:1
				>/=13:2

Note: All 7 variables included in the regression model improved the predictive model, so all of them were considered to calculate the score punctuation. In order to calculate the score punctuation, beta-coefficients of categorical variables were transformed into points using the following rule: beta-coefficient lower than 0.5:2 points; beta-coefficient 0.5-1.5:3 points; beta-coefficient 1.6-4:4 points; beta-coefficient greater than 4:5 points. In the case of non-categorical variables (age and APACHE II), punctuation was divided in 3 groups, the first with no punctuation: a second group with a low punctuation and a third with high punctuation. Due to higher beta-coefficient for age compared to APACHE II, age was given more weight to the score.

TABLE 3 CAPA risk score punctuation

Variable		Points
Years	64–69 years	2
	>/= 70 years	3
Active smoking		3
Chronic respiratory disease		2
Chronic renal failure		4
Chronic corticoid treatment		5
Tocilizumab treatment		4
APACHE II at ICU admission	10–12	1
	>/= 13	2
Total		0–23

FIGURE 1 CAPA risk score receiver operator curve. The AUC was 0.861 (95% CI 0.78–0.93, *p* < .001)

chronic renal failure (25.0% vs. 3.6%, *p* = .005) and prior immune-compromise (42.9% vs. 19.6%, *p* = .037). Specifically, they were more frequently under chronic corticosteroid treatment prior to hospital admission (28.6% vs. 1.8%, *p* < .001).

With respect to COVID-19 complications and management, CAPA patients had received more frequently tocilizumab (92.9% vs. 66.1%, *p* = .008) and had a higher APACHE II at ICU admission (median 13 (IQR 9–18) vs. 10 (8–13), *p* = .026).

3.3 | CAPA prediction score development and validation

Among factors associated with CAPA, age, active smoking, chronic respiratory diseases, chronic renal failure, previous chronic corticosteroid treatment, tocilizumab therapy for COVID-19 and APACHE II at ICU admission were selected for the multivariate logistic
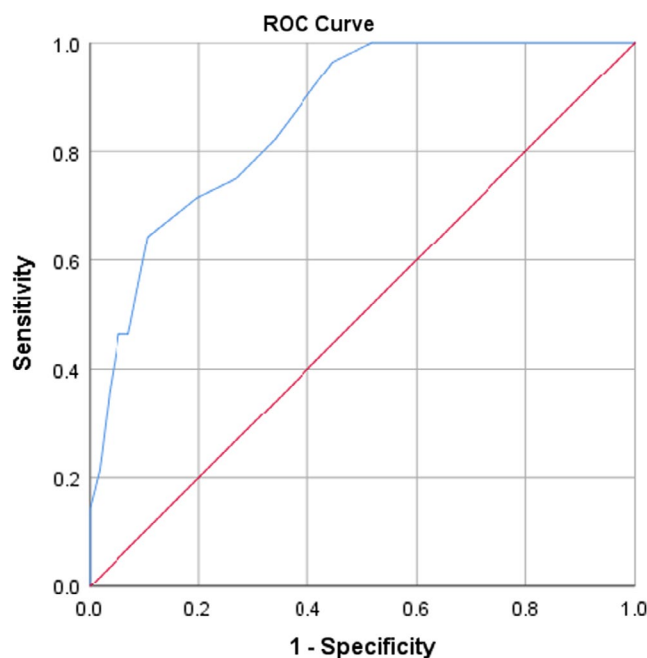
regression model. The model is summarised in Table 2. The calculated score is shown in Table 3.

The score presented a good calibration, with a *p*-value in the goodness-to-fit Hosmer-Lemeshow test of 0.565. The area under ROC was 0.861 (95% CI 0.78–0.93, *p* < .001, Figure 1). In the internal validation, after 1000 bootstrap samples, the optimism estimated was 0.047, with a bootstrapped corrected area under ROC was 0.854 (95% CI 0.77–0.92). Figure 2 shows bootstrap samples area under ROC values histogram.

Cut-off point	S (%)	E (%)	+LR	−LR	PPV	VPN
Score >/= 5 points	100%	48.2%	1.96	0	11.0%	100%
Score >/=6 points	96.4%	55.4%	2.16	0.06	12.2%	99.6%
Score >/= 10 points	64.3%	89.3%	6.01	0.40	27.9%	97.5%
Score >/= 12 points	46.4%	94.6%	8.67	0.57	35.6%	96.5%

TABLE 4 Proposed cut-off points for CAPA risk stratification based on the score

Note: Abbreviations: +LR, positive likelihood ratio; E, specificity; −LR, negative likelihood ratio; PPV, positive predictive value; PV, negative predictive value; S, sensitivity.
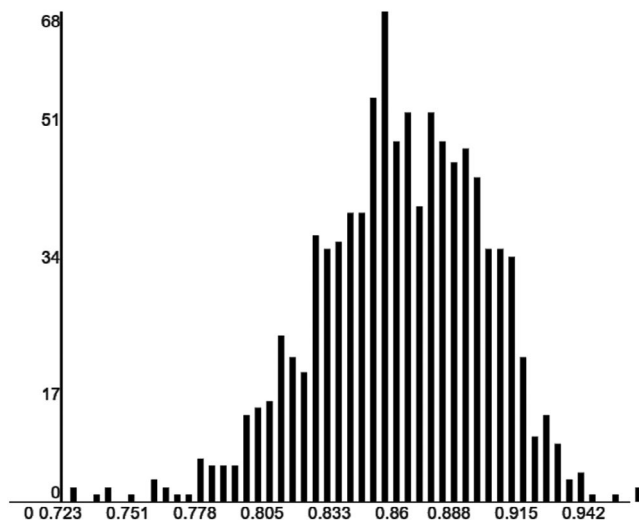

FIGURE 2 Histogram of distribution of area under the receiver operator curve (AUC) of 1000 bootstrapped samples. The optimism estimated was 0.047, with a corrected AUC of 0.854 (95% CI 0.77–0.92)

Cut-off points are provided in Table 4. A score below 6 points had a sensitivity of 96.4% (95% CI 89.1%–100%) and specificity of 55.4% (95% CI 41.9%–68.8%). The negative likelihood ratio (NLR) was 0.06, and the positive likelihood ratio (PLR) was 2.16. Applying this cut-off to a population with a prevalence similar to ours would result in a negative predictive value (NPV) of 99.6% and a positive predictive value (PPV) of 12.2%.

A score equal or over 10 points had a sensitivity of 64.3% (95% CI 45.4%–83.2%) and specificity of 89.3% (95% CI 80.9%–97.6%). The PLR was 6.01 and NLR was 0.40. Applying this cut-off to a population with a prevalence similar to ours would result in a PPV of 27.9% and a NPV of 97.5%.

Hence, when applying this score to critically ill COVID-19 patients in our population, we would be able to identify 3 groups of patients according to CAPA risk: 1- Very low risk for CAPA: patients with a score inferior to 6 (predicted risk lower to 0.5%); 2- Intermediate risk for CAPA: patients with a score between 6 and 9 (predicted risk 5%-10%); 3- High risk for CAPA: patients with a score equal or greater than 10 (predicted risk greater than 25%). However, if the same score is applied in other population with a high prevalence of CAPA (ie, 15%), we would identify 2 groups of patients: 1- Very low risk for CAPA: patients with score inferior to 6 (predicted risk lower than 1%); 2- High risk for CAPA: patients with score equal or greater than 6 (predicted risk greater than 30%).

3.4 | Factors associated with mortality among CAPA patients

Table 5 summarises baseline characteristics, COVID-19 complications, management and CAPA characteristics between survivors and non-survivors. Classification as probable CAPA according to ECMM/ISHAM criteria (vs. possible CAPA) was associated with higher in-hospital mortality (81.3% vs. 33.3%, respectively, $p = .019$). Moreover, among patients with probable CAPA, those with positive serum GM had a higher 180-day mortality than those with a negative value (HR 3.88, 95% CI 1.16–12.82). Thus, incorporating serum GM to ECMM/ISHAM classification allowed to discriminate patients according to mortality risk. Survival analysis is shown in Figure 3.

Other factors such as age, prior immune-compromise, chronic respiratory diseases and radiological findings were not associated with mortality in CAPA patients. All patients received antifungal treatment, except for one patient who died 24 h after CAPA diagnosis. No specific antifungal drug (ie, voriconazole, isavuconazole or amphotericin B) was associated with improved outcomes. Antifungal combination (vs. monotherapy) was not associated with an inferior mortality (63.6% vs. 58.8%, $p = 1.000$).

4 | DISCUSSION

In the present study, several baseline characteristics determined at ICU admission such as age, active smoking, chronic respiratory diseases, prior immuno-compromise, chronic corticosteroid, APACHE II and tocilizumab treatment were associated with the development of CAPA in COVID-19 patients. A clinical prediction score based on these characteristics was developed that allowed to stratify critically ill COVID-19 patients in low, intermediate or high risk for CAPA at the time of ICU admission. CAPA mortality in the present series was associated with CAPA classification according to ECMM/ISHAM consensus criteria (possible vs. probable CAPA) and serum GM levels.

Previous studies have identified risk factors for CAPA, including age, prior respiratory diseases, chronic renal failure, chronic corticosteroid use, neutropenia, COVID-19 severity and treatment of the COVID-19 episode with corticosteroid or tocilizumab.[4,13,26-28] In the present study, we could validate most of these factors. However, we could not corroborate the association between corticosteroid treatment for COVID-19 and CAPA, since, due to local protocols, corticosteroids were extensively used in COVID-19 patients very early in the pandemic. Additionally, we found associations that had not

TABLE 5 Factors associated with in-hospital mortality among patients with COVID-associated pulmonary aspergillosis

Variable		Total (n = 28)	Survivor (n = 11)	Non-survivor (n = 17)	p
Comorbidity					
Age (years)		68 (65–72)	71 (64–74)	68 (56–72)	.161
Sex (female)		21.4% (6)	27.3% (3)	17.6% (3)	.653
Active smoking		17.9% (5)	27.3% (3)	11.8% (2)	.738
Chronic respiratory disease		48.1% (13)	45.5% (5)	47.1% (8)	1.000
Chronic cardiac failure		21.4% (6)	27.3% (3)	17.6% (3)	.653
Chronic renal failure		25.0% (7)	36.4% (4)	17.6% (3)	.381
Haematological cancer		14.3% (4)	18.2% (2)	11.8% (2)	1.000
Solid organ transplant		17.9% (5)	9.1% (1)	23.5% (4)	.329
Chronic corticoid		28.6% (8)	18.2% (2)	35.3% (6)	.419
Other IS treatments		28.6% (8)	18.2% (2)	35.3% (6)	.419
COVID-19 presentation and management prior to CAPA diagnosis					
Bacterial coinfection		57.1% (16)	45.5% (5)	64.7% (11)	.441
RRT		35.7% (10)	18.2% (2)	47.1% (8)	.226
Vasopressor drug		42.9% (12)	27.3% (3)	52.9% (9)	.172
APACHE II		13 (9–18)	9 (7–13)	15 (11–20)	.017
Corticoid pulses		46.4% (13)	36.4% (4)	52.9% (9)	.460
Tocilizumab		92.9% (26)	100% (11)	88.2% (15)	.505
Blood transfusion		51.9% (14)	54.5% (6)	50.0% (8)	1.000
Aspergillosis radiology and clinical presentation					
Days from admission		21 (11–41)	23 (10–57)	19 (12–38)	.280
Respiratory worsening		85.7% (24)	90.9% (10)	82.4% (14)	.635
Refractory fever		17.9% (5)	27.3% (3)	11.8% (2)	.353
Haemoptysis		28.6% (8)	18.2% (2)	35.3% (6)	.419
Tracheobronchitis		20.0% (4/12)	60.0% (3/5)	14.3% (1/7)	.031
Solitary nodule		14.3% (4)	9.1% (1)	17.6% (3)	.635
Multiple nodules		25.0% (7)	18.2% (2)	29.4% (5)	.668
Cavitary nodule (s)		25.0% (7)	27.3% (3)	23.5% (4)	1.000
Alveolar infiltrate		67.9% (19)	81.8% (9)	58.8% (10)	.197
Positive serum GM		23.1% (6/26)	0% (0/11)	40.0% (6/15)	.018
Aspergillosis microbiology					
A fumigatus complex		64.3% (18)	63.6% (7)	64.7% (11)	.963
A niger complex		14.3% (4)	18.2% (2)	11.8% (2)	
Other species		10.7% (3)	0	17.7% (3)	
No culture growth		10.7% (3)	18.2% (2)	5.9% (1)	
Aspergillosis classification					
ECMM/ISHAM	*Probable*	57.1% (16)	27.3% (3)	76.5% (13)	.019
	Possible	42.9% (12)	72.7% (8)	23.5% (4)	
Treatment and outcomes					
Combination therapy		39.3% (11)	36.4% (4)	41.2% (7)	1.000
Voriconazole		50.0% (14)	36.4% (4)	41.2% (7)	1.000
Isavuconazole		39.3% (14)	54.5% (6)	47.1% (8)	1.000
Amphotericin B		35.7% (10)	27.3% (3)	41.2% (7)	.368

Note: Abbreviations: GM, galactomannan; IC, immunosuppressive; RRT, Renal replacement therapy.

previously been described between immune-compromise or solid organ transplantation and CAPA development. Our institution cares for a high percentage of patients with these conditions, compared to other cohorts,[4,29,30] which may have facilitated to unveil these associations.

Based on the aforementioned risk factors, we constructed a clinical prediction score, the CAPA score that reliably stratified critically ill COVID-19 patients according to their risk for CAPA development. To the best of our knowledge, this is the first published score effective to predict CAPA risk. In our population, a patient with a CAPA
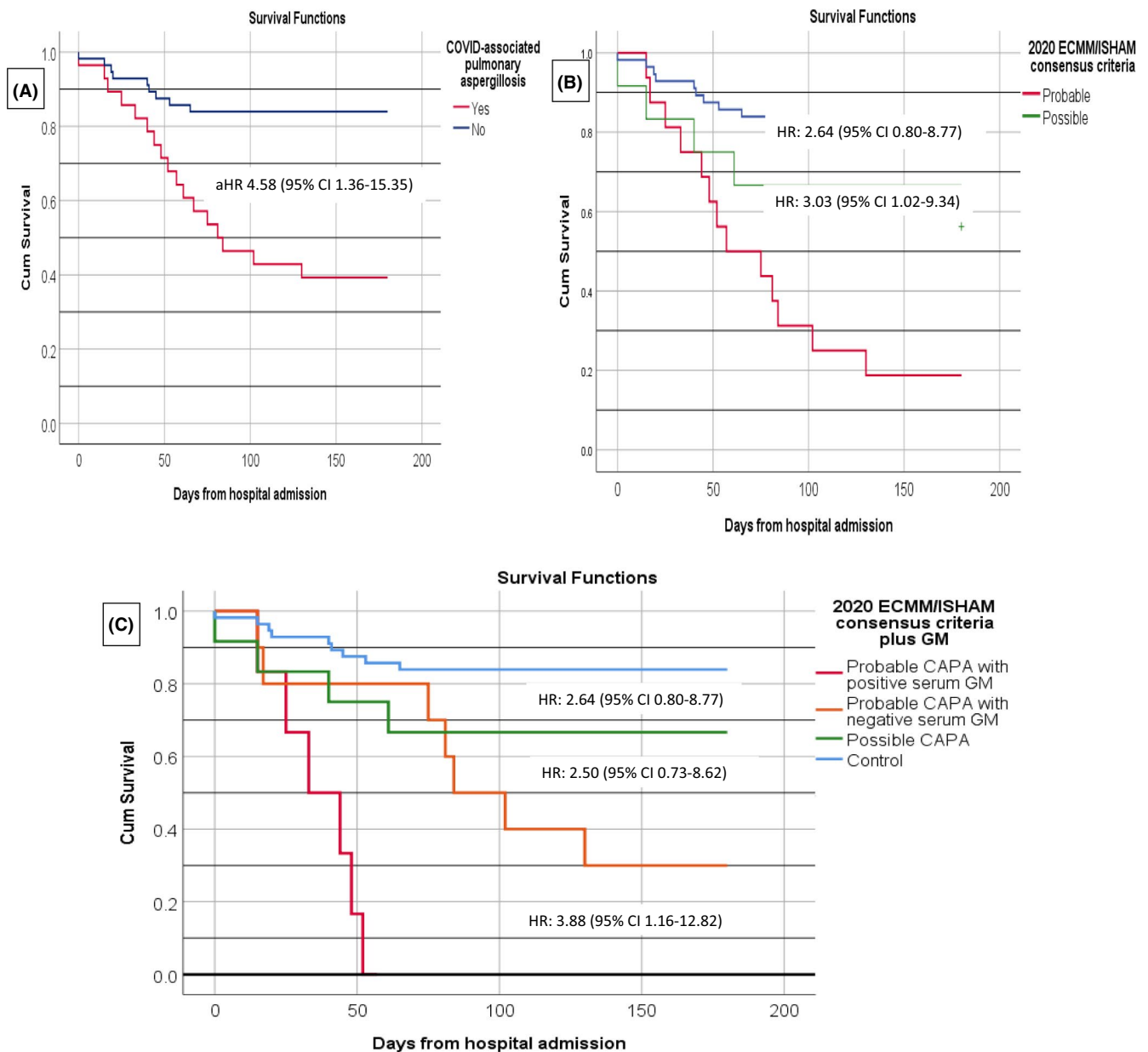
FIGURE 3 Kaplan–Meier survival curves of 180-day mortality among different population of COVID-associated pulmonary aspergillosis (CAPA) and controls. Survival analysis was made by means of Cox regression. Hazards ratios (HR) with their 95% confidence interval (CI) are presented. Figure 1A represents survival curve among patients with CAPA and ICU controls. Adjusted HR (aHR) was obtained after adjusting for age, smoking, chronic respiratory disease, immunocompromised status, prior chronic corticoid treatment, chronic renal failure, renal replacement therapy, APACHE II at ICU admission and blood component transfusion. Figure 1B represents survival curve according to 2020 ECMM/ISHAM consensus criteria CAPA classification and Figure 1C survival curve according to 2020 ECMM/ISHAM consensus criteria plus serum galactomannan (GM). Only patients with probable CAPA had positive serum GM

score below 6 points would have a risk for CAPA inferior to 0.5%. Additionally, a patient with a CAPA score value equal or greater than 10 would present a CAPA risk over 25%.

Although the prevalence of CAPA in our ICU-admitted patients (6.04%) is similar to other works[27] and is consistent with studies in our setting[31] and autopsy studies,[32] some other authors have noted a higher prevalence,[4,12,28,33] up to 30%.[34] These differences could be related to regional variations in incidence.[35] Using the same cut-off of 6 points in a high prevalence population to identify patients at low

risk would remain adequate, as it would identify patients with a risk of developing CAPA of approximately 1%. Of note, in populations with a higher prevalence of CAPA, the cut-off of 6 points would be enough to identify patients at high risk, since patients with 6 or more points would present a risk greater than 40%.

Some studies suggest the efficacy of antifungal prophylaxis in preventing CAPA,[8,9] while other authors consider systematic screening in all patients.[10] However, all those studies have failed to demonstrate a benefit of these measures, in terms of survival,

in an unselected population. This could potentially be related to adverse effects of antifungal treatment[10,23-25] in patients with low risk of CAPA. Applying the CAPA score developed in the present study would allow to obviate these measures in patients with low risk for CAPA (ie, CAPA score below 6), and select patients at a higher risk who could benefit most from them (ie, patients with CAPA score equal or greater than 10 in a setting with low CAPA incidence, or CAPA score equal or greater than 6 in settings with high CAPA incidence). Nevertheless, the CAPA score needs external validation in other populations and further evaluation in order to assess the efficacy of the targeted approach based on the stratification in reducing CAPA cases and improving survival in critically ill COVID-19 patients. Additionally, other risk factors outside the score should be taken into account, such as CMV replication.[37]

Moreover, we intended to identify factors associated with mortality in CAPA patients. We found that the classification according to ECMM/ISHAM consensus criteria along with serum galactomannan allows to distinguish patients with a different mortality risk: possible CAPA, probable CAPA with negative serum GM and probable CAPA with positive serum GM. The prognostic value of serum GM among probable CAPA (according to ECMM/ISHAM classification) was noted in a previous study.[38] Our results support the hypothesis that the term CAPA encompasses a complex entity with various phases of invasion and damage[3,15,36,39] and that different biomarker profiles may correspond to different stages of the disease.[38]

Ours is a single centre retrospective study and has the inherent limitations of this design. One of the major limitations of our study is the relative small sample size, which may have limited the power of the statistical analysis. Another limitation is that there was no CAPA screening protocol in our institution, and, consequently, a respiratory fungal culture was not available for every control patient, so it is not possible to exclude that a small proportion of the controls could actually have CAPA. However, the prevalence of CAPA in our institution is similar to that found in similar settings[31] as well as in recent systematic reviews,[5,26] suggesting that the majority of CAPA cases were identified. Additionally, in the same way that other studies on CAPA, it is difficult to distinguish between *Aspergillus* spp. colonisation and invasive infection in critically COVID-19 patients, given that histologic samples are rarely available and clinical-radiological features are often overlapping and non-specific. Though, we tried to mitigate this limitation by systematically applying 2020 ECMM/ISHAM consensus criteria for CAPA diagnosis and classification. Another limitation is that not all cases were admitted to the ICU, with 6 CAPA patients outside ICU unit. However, all of them were critical COVID-19 with severe ARDS and fulfilled ICU admission criteria, though unfortunately were not admitted to ICU due to healthcare system overload. Finally, our score needs validation in external, prospective and larger samples. In spite of these limitations, we believe that our real-life results are of interest and can inspire further studies with larger figures.

In conclusion, we have developed and internally validated a clinical prediction score that allowed to stratify critically ill COVID-19 patients according to the risk for developing CAPA. Accordingly, at ICU admission, patients could be classified as low risk (score inferior to 6), intermediate risk (score between 6 and 9) and high risk (score equal or greater than 10). This CAPA score would enable targeting preventive measures such as periodic screening or antifungal prophylaxis to patients at high risk who could benefit most from them, while avoiding these measures in patients at low risk. Further evaluation of the score, as well as the usefulness of preventive measures in patients at high risk, is warranted.

CONFLICTS OF INTEREST

AFC declares personal fees for lectures/presentations/educational events outside the present manuscript. All other authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Jorge Calderon Parra: Conceptualization (lead); Data curation (equal); Formal analysis (equal); Methodology (equal); Project administration (equal); Software (lead); Writing – original draft (equal); Writing – review & editing (lead). **Ana Fernández-Cruz:** Conceptualization (lead); Formal analysis (equal); Investigation (equal); Methodology (lead); Project administration (lead); Supervision (lead); Validation (lead); Writing – original draft (lead); Writing – review & editing (lead). **Patricia Mills-Sanchez:** Conceptualization (equal); Data curation (equal); Investigation (equal). **Victor Moreno-Torres:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Writing – review & editing (equal). **Sandra Tejhado-Bravo:** Writing – review & editing (equal). **Isabel Sánchez-Romero:** Methodology (equal); Writing – review & editing (equal). **Barbara Balandín-Moreno:** Supervision (equal); Writing – review & editing (equal). **Marina Calvo-Salvador:** Writing – review & editing (equal). **Francisca Portero-Azorín:** Methodology (equal); Writing – review & editing (equal). **Sarela García-Masedo:** Methodology (equal); Writing – review & editing (equal). **Elena Muñez Rubio:** Writing – review & editing (equal). **Antonio Ramos-Martinez:** Methodology (equal); Supervision (equal); Validation (equal); Writing – review & editing (equal).

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