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New insights into development and mortality of COVID-19-associated pulmonary aspergillosis in a homogenous cohort of 1161 intensive care patients

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Abstract

Background: COVID-19-associated pulmonary aspergillosis (CAPA) has been widely reported but homogenous large cohort studies are needed to gain real-world insights about the disease.

Methods: We collected clinical and laboratory data of 1161 patients hospitalised at our Institute from March 2020 to August 2021, defined their CAPA pathology, and analysed the data of CAPA/non-CAPA and deceased/survived CAPA patients using univariable and multivariable models.

Results: The overall prevalence and mortality of CAPA in our homogenous cohort of 1161 patients were 6.4% and 47.3%, respectively. The mortality of CAPA was higher than that of non-CAPA patients (hazard ratio: 1.8 [95% confidence interval: 1.1–2.8]). Diabetes (odds ratio [OR] 1.92 [1.15–3.21]); persistent fever (2.54 [1.17–5.53]); hemoptysis (7.91 [4.45–14.06]); and lung lesions of cavitation (8.78 [2.27–34.03]), consolidation (9.06 [2.03–40.39]), and nodules (8.26 [2.39–28.58]) were associated with development of CAPA by multivariable analysis. Acute respiratory distress syndrome (ARDS) (2.68 [1.09–6.55]), a high computed tomography score index (OR 1.18 [1.08–1.29]; $p < .001$), and pulse glucocorticoid treatment (HR 4.0 [1.3–9.2]) were associated with mortality of the disease. Whereas neutrophilic leukocytosis (development: 1.09 [1.03–1.15] and mortality: 1.17 [1.08–1.28]) and lymphopenia (development: 0.68 [0.51–0.91] and mortality: 0.40 [0.20–0.83]) were associated with the development as well as mortality of CAPA.

Conclusion: We observed a low but likely underestimated prevalence of CAPA in our study. CAPA is a disease with high mortality and diabetes is a significant factor for its development while ARDS and pulse glucocorticoid treatment are significant factors for its mortality. Cellular immune dysregulation may have a central role in CAPA from its development to mortality.

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KEYWORDS
acute respiratory distress syndrome, COVID-19-associated pulmonary aspergillosis, development, diabetes, glucocorticoids, mortality

1 | INTRODUCTION

The pandemic coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has triggered the emergence of a new entity of aspergillosis called COVID-19-associated pulmonary aspergillosis (CAPA). This novel fungal disease is defined as a co or superinfection of *Aspergillus* mould in patients with COVID-19 admitted to the intensive care unit (ICU). Pathologically, CAPA is characterised by the invasion of bronchial or lower airway tissues and a broad constellation of pulmonary complications including acute respiratory distress syndrome (ARDS) depending upon the host factors.[1–3]

The isolation of *Aspergillus flavus* from the respiratory tract from one of 99 patients with COVID-19 in Wuhan, China, in January 2020 was the first incidence of CAPA.[4] Thereafter, several letters, case series, and small cohort studies have increasingly reported CAPA from almost all parts of the world including Asia,[5–7] Europe,[3,8–16] Middle East,[17] North America,[1,18–20] and South America.[21–23] However, owing to their small sample size and use of different CAPA definitions, these studies could yield no adequate definitive information to improve the understanding and outcome of the disease. More recently, pooled reports in the form of multicenter or multinational studies and meta-analyses or systemic reviews have been published, reporting a summary effect by integrating the data from multiple research studies or sites to achieve a larger sample size for enhanced statistics.[24–31] However, the data of such studies are not robust enough to offer real-world information about CAPA because of the inherent clinical and methodological heterogeneity among the included studies. Thus, there is a compelling clinical need for homogenous large cohort studies based on CAPA-specific definitions and diagnostic criteria to precisely identify various clinical and laboratory factors associated with CAPA and develop novel strategies to improve the diagnosis, prevention, and treatment of the disease.

We have recently published practice guidelines for the rapid diagnosis of CAPA in the ICU setting.[32] Based on this diagnostic algorithm and univariable and multivariable analysis of the data, we report here the prevalence and outcome of CAPA and new insights about the factors involved in the emergence and mortality of the disease in a homogenous large cohort of 1161 patients admitted to the ICU of our institute during the two waves of the pandemic.

2 | PATIENTS AND METHODS

2.1 | Study design and ethics

This was a single-center ambispective observational study conducted from March 2020 to August 2021 at Rajdhani Corona Hospital, a 328-bed tertiary COVID-care hospital of SGPGIMS, Lucknow, Uttar Pradesh, India. The STROBE guidelines for observational studies were used for reporting. The study was approved by the Institutional Ethics Committee (IEC) (IEC Reference Code # 2021-190-IMP-EXP-41).

2.2 | Definitions

COVID-19 was defined as a positive real-time reverse-transcriptase polymerase chain reaction (PCR) for SARS-CoV-2 from nasopharyngeal swabs or respiratory samples. The diagnosis of the fungal infection/mycosis was defined according to the practice guidelines[32] as well as ECMM/ISHAM consensus criteria[33] for diagnosing CAPA using a combination of clinical, radiological, and mycological features of the disease. Non-CAPA was defined as COVID-19 patients with no *Aspergillus* infection. Neutrophilia was defined as an absolute neutrophil count of $>7.7\times10^3$ neutrophils/μl, lymphopenia as an absolute lymphocyte count (ALC) of $<1.0\times10^3$/μl, and thrombocytopenia as a platelet count $<150\times10^3$/μl.

2.3 | Data collection

Data from the first wave of the pandemic (10 March 2020 to 28 February 2021) were collected retrospectively. In the second wave (1 March to 28 August 2021), the data were collected prospectively. Clinical, laboratory, and microbiology data, treatment prescriptions, and outcomes of COVID-19 patients were obtained from electronic records of the patients available on the hospital information system of the Institute. Computed tomography (CT) scans of the chest were reviewed to record the radiological findings of the patients. COVID-19 patients with complete clinical and laboratory data from admission to discharge were included in the study. The data of all eligible patients were collected as part of their follow-up from admission to discharge or death.

2.4 | Mycological diagnostics

During the hospital stay, patients were screened for mycological testing at least once a week using respiratory (bronchoalveolar lavage [BAL]; nondirected BAL, and tracheal and bronchial aspirates) and serum samples as follows. Direct microscopy using 10% KOH mount, culture using Sabouraud-chloramphenicol dextrose agar plates (30°C; aerobiosis), and identification of cultured species using colony morphology, microscopic morphology, and MALDI-ToF mass spectrometry were performed on respiratory samples as routine tests at the institute. *Aspergillus* multiplex PCR was performed on available respiratory samples of the patients. DNA was extracted

from 1.0 ml of the respiratory sample using a QIAamp DNA mini kit (Qiagen). Purified DNA (10 μl) was amplified using Artus *Aspergillus* diff. RG PCR kit (Qiagen) according to the manufacturers' instructions. Galactomannan (GM) antigen and mannoprotein (MP) assays were performed on serum and BAL fluid using a Platelia *Aspergillus* Ag kit (Bio-Rad Laboratories) and AspLFD kit (OLM Diagnostics), respectively, according to the manufacturer's instructions. A GM index >0.5 were considered positive, while the MP results were provided as positive or negative.

2.5 | Statistical analysis

STATA17.0 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC), Statistical Package for Social Sciences, version 23 (SPSS-23, IBM), MedCalc® Statistical Software version 20.110 (MedCalc Software Ltd, Ostend, Belgium; GraphPad Prism version 9.4.0 for Windows, GraphPad Software, San Diego, California USA) software were used for data analysis. Continuous variables are presented as the median and interquartile range (IQR). Categorical variables were described as numbers and percentages. The Mann–Whitney *U* test was used to compare continuous variables. Fisher's exact test was used to compare categorical variables. Univariate analyses were performed for all the variables, and odds ratios (ORs) were calculated with 95% confidence intervals (CIs). To compare the mortality between various groups, Kaplan–Meier curves were plotted, and hazard ratios were calculated by the log-rank test. Multivariable analysis by binary logistic regression and Cox regression model was performed for variables found to be significant in univariable analysis. A two-sided *p*-value of <.05 was considered significant.

3 | RESULTS

In the first wave of the pandemic, a total of 2459 patients were hospitalised, of whom 1014 were admitted to the ICU, and 817 who fulfilled the eligibility criteria were enrolled in the study. In the second wave, a total of 1124 patients were hospitalised, of whom 478 were admitted to the ICU and 344 were included in the study (Figure 1).

In our study cohort, 74 patients were serum or BAL positive for GM or MP, and two had lung biopsy positive for *Aspergillus* by histopathology and culture, while seven, 34 and 10 patients had respiratory samples positive for *Aspergillus* by direct microscopy and culture, only culture, and multiplex PCR, respectively. According to the clinical, radiological, and mycological features of the diagnostic algorithm used, a total of 74 patients met the CAPA criteria and were classified as having proven (*n* = two), probable (*n* = 51), or possible (*n* = 21) CAPA. The *Aspergillus* species identified in the respiratory samples of proven and probable CAPA patients were *Aspergillus fumigatus* (*n* = 29), *A. flavus* (*n* = 08), *Aspergillus niger* (*n* =three); *Aspergillus terreus* (*n* =two), and two or more *A.* spp. (n = nine) (Table 1). The prevalence and mortality of CAPA in the first wave of the pandemic were 1.6% (13/817) and

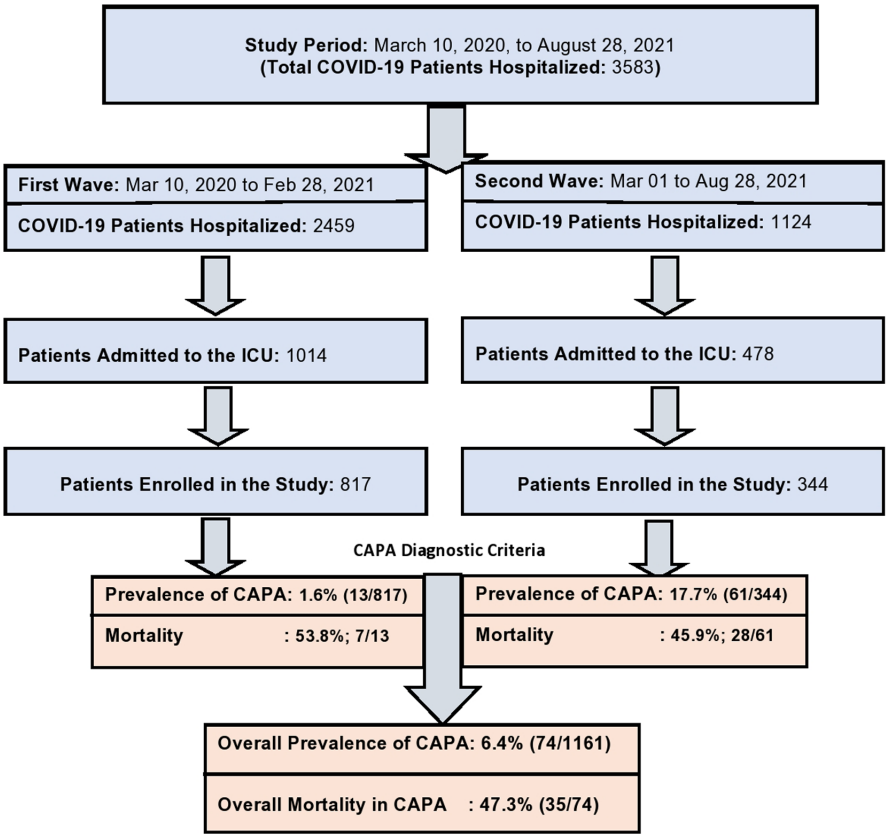

FIGURE 1 Prevalence and outcome of CAPA. A total of 3583 patients (2459 in the first wave and 1124 in the second wave of the pandemic) were hospitalised in RCH. A total of 1492 (first wave: 1014 and second wave: 478) patients were admitted to the ICU. Of these, 1161 (first wave: 817 and second wave: 344) eligible patients with complete clinical and laboratory records were included in the study. The prevalence and rate of mortality of CAPA in the first waves were 1.6% (13/817) and 53.8% (7/13), whereas in the second waves, they were 17.7% (61/344) and 45.9% (28/61), respectively. The overall prevalence and rate of mortality of CAPA during the entire pandemic remained at 6.4% (74/1661) and 47.3% (35/74), respectively. CAPA, COVID-19-associated pulmonary aspergillosis; COVID-19, coronavirus disease 2019; ICU, intensive care unit; RCH, Rajdhani Corona Hospital

TABLE 1 Diagnosis of CAPA patients ($n = 74$)

Clinical features	Radiological finding	Mycology biomarkers (GM/MP)	Mycology microscopy, culture or PCR	Asp species isolated
Proven CAPA ($n = 2$)				

• Symptoms
  • HTS & fever: 02
• Co-morbidities
  • DM + CPD: 01
  • DM + HTN: 01
	• Cavity, Consol, & Nodules: 01
	• Consol, GGO, & Nodules: 01
		• sGM & bMP (+): 01
		• sGM (+): 01
			• Asp(+) Biopsy Histopathology:02
			• Asp(+) Biopsy Culture: 02:
				Aspergillus fumigatus: 02

Probable CAPA ($n = 51$)				

• Symptoms
  • HTS: 07
  • Fever: 25
  • HTS & Fever: 06
  • Chest Pain & Fever: 05
  • HTS, Chest Pain & Fever: 08
• Co-morbidities
  • DM: 16
  • HTN: 06
  • CKD: 02
  • CPD: 04
  • DM & CLD: 01
  • DM & CPD: 03
  • DM & HTN: 10
  • HT & CAD: 01
  • DM, HTN & Bladder Ca: 01
  • DM, HTN & CLD: 01
  • DM, HT & CAD: 02
  • DM, CAD & CPD: 01
  • HTN, CLD & CPD: 01
  • HTN, CAD & CPD: 01
  • DM, HTN, CAD & CPD: 01
	• Consol: 01
	• Cavity & Consol: 02
	• Cavity & GGO: 01
	• Cavity & Nodule: 01
	• Consol & GGO: 11
	• GGO & Nodule: 04
	• Cavity, Consol & GGO: 03
	• Cavity, Consol & Nodule: 04
	• Console, GGO & Nodule: 08
	• Cavity, Consol, GGO & Nodule: 16
		• GM (+) ($n = 45$)
		  • sGM: 26
		  • bGM: 09
		  • sGM & bGM: 10
		• MP (+) ($n = 38$)
		  • sMP: 18
		  • bMP: 11
		  • sMP & bMP: 09
			• Asp(+) Microscopy& Culture: 07
			• Asp(+) Culture: 34
			• Asp (+) PCR: 10
				A. fumigatus: 29
				Aspergillus flavus: 08
				Aspergillus niger: 03
				Aspergillus terreus: 02
				*A.*multiple sp:09

Possible (n = 21)				

TABLE 1 (Continued)

Clinical features	Radiological finding	Mycology biomarkers (GM/MP)	Mycology microscopy, culture or PCR	Asp species isolated

• Symptoms:
  • HTS, FEVER & chest pain: 01
  • Fever: 20
• Co-morbidities:
  • DM: 06
  • HTN: 03
  • CPD: 01
  • DM + HTN: 03
  • DM + CPD: 01
  • HTN + CKD: 01
  • HTN, CKD & CPD: 01
  • HTN, CLD & CPD: 01
  • DM, HT & ALL: 01
  • DM, HTN & CPD: 02
  • CAD, CKD & CPD: 01
	• Consol & GGO: 09
	• Cavity, Console & GGO: 02
	• Cavity, Console & Nodule: 01
	• Consol, GGO & Nodule: 08
	• Cavity, Consol, GGO &Nodule: 01
		• GM (+) ($n = 21$)
		  • sGM: 21
		• MP (+): ($n = 16$)
		  • sMP: 16
			NA	NA

Note: Numbers mentioned against the clinical feature, HRCT finding, biomarkers, direct microscopy, Aspergillus culture, Aspergillus Multiplex PCR, and Aspergillus species show the number of patients having the given entity.

Abbreviations: ALL, acute lymphoblastic leukaemia; Asp, Aspergillus; b, bronchoalveolar lavage; Ca, cancer; CAD, coronary artery disease; CAPA, COVID-19-associated pulmonary aspergillosis; Cavity, cavitations; CKD, chronic kidney disease; CLD, chronic liver disease; Consol, consolidations; CPD, chronic pulmonary disease; DM, diabetes mellitus; GGO, ground glass opacities; GM, galactomannan; HRCT, high resolution computed tomography; HTN, hypertension; HTS, hemoptysis; MP, mannoprotein; NA, not available; PCR, polymerase chain reaction; s, serum.

TABLE 2 Demographic, clinical, and laboratory data of CAPA and non-CAPA patients

Variable	CAPA ($n = 74$)	Non-CAPA ($n = 1087$)	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Demographics						
Age: years median (IQR)	55 (44.8, 64.3)	56 (45,65)	1.00 (0.99–1.01)	.968		
Female sex	25 (33.8%)	319 (29.3%)	1.22 (0.74–2.02)	.419		
ICU parameters						
Hospital stay: median (IQR)	18 (12.8, 29.0)	13 (9,19)	1.07 (1.04–1.09)	**<.001**	[a]	NS
IMV	26 (35.6%)	237 (21.8%)	1.98 (1.18–3.20)	**.009**	[a]	NS
NIV	18 (24.3%)	111 (10.2%)	2.70 (1.55–4.71)	**<.001**	[a]	NS
NVS	30 (40.5%)	750 (69.0%)	0.3 (0.20–0.50)	**<.001**	[a]	NS
Clinical symptoms						
Hemoptysis	25 (33.8%)	65 (6.0%)	8.02 (4.66–13.80)	**<.001**	7.91 (4.45–14.06)	<.001
Persistent fever	66 (78.4%)	789 (72.6%)	3.12 (1.48–6.56)	**.003**	2.54 (1.17–5.53)	.019
Chest pain	15 (20.3%)	50 (4.6%)	5.27 (2.78–9.94)	**<.001**	[a]	NS
ARDS	36 (48.6%)	566 (52.1%)	0.87 (0.54–1.40)	.473		
Comorbidities						
Diabetes mellitus	44 (60.3%)	436 (40.1%)	2.46 (1.52–3.99)	**.001**	1.92 (1.15–3.21)	.013
Hypertension	34 (45.9%)	455 (41.9%)	1.21 (0.72–1.90)	.543		
Chronic pulmonary disease	17 (23.0%)	164 (15.1%)	1.71 (0.92–3.01)	.095		
Chronic kidney disease	5 (7.7%)	149 (13.7%)	0.33 (0.17–1.07)	.109		
Chronic liver disease	4 (5.4%)	27 (1.9%)	2.34 (0.91–6.04)	.130		
Coronary artery disease	7 (7.7%)	121 (11.1%)	0.72 (0.35–1.66)	.484		
Malignancy	2 (2.7%)	23 (2.1%%)	1.43 (0.49–5.41)	.671		
Renal transplantation	2 (2.7%)	41 (6.6%)	0.73 (0.24–3.06)	1.000		
HIV syndrome	1 (1.4%)	5 (0.4%)	0.37 (.01–2.69)	.327		
CT findings[b]						
CTSI median (IQR)	13.5 (10.0,18.0)	12.0 (9.0, 14.0)	1.01 (1.10–1.19)	**.020**	[a]	NS
Cavitation	32 (43.2%)	4 (5.4%)	13.33 (4.41–40.36)	**<.001**	8.78 (2.27–34.03)	.002
Consolidation	68 (91.9%)	51 (68.9%)	5.11 (1.94–13.47)	**<.001**	9.06 (2.03–40.39)	.004
Nodules	45 (60.8%)	9 (12.2%)	11.21 (4.84–25.93)	**<.001**	8.26 (2.39–28.58)	.001
Laboratory biomarkers[b]						
TLC ($\times 10^3/\mu l$)	14 (7.5, 19.1)	10.9 (7.6, 14.5)	1.09 (1.03–1.15)	**.002**	6.61 (1.40–31.15)	.017
ANC ($\times 10^3/\mu l$)	10.8 (6.7, 17.5)	8.3 (6.1, 12.2)	1.09 (1.03–1.16)	**.002**	[a]	NS
ALC ($\times 10^3/\mu l$)	0.9 (0.5, 1.4)	1.3 (0.6, 2.7)	0.68 (0.51–0.91)	**.008**	0.56 (0.38–0.85)	.021
Platelets ($\times 10^3/\mu l$)	160.0 (103.3, 231.5)	222.0 (150.0, 296.0)	0.99 (0.98–1.0)	**.003**	[a]	NS
CRP (mg/L)	38.0 (10.6, 132.8)	7.0 (4.5, 15.9)	1.02 (1.01–1.03)	**<.001**	[a]	NS
D-Dimer (μg/ml)	2.0 (0.8, 5.2)	1.4 (0.6, 2.1)	1.32 (1.12–1.55)	**.001**	[a]	NS
Fibrinogen (mg/L)	499.0 (345.0, 608.0)	407.5 (310.5, 576.3)	1.0 (1.0–1.0)	**.015**	[a]	NS
Ferritin (ng/ml)	1235.5 (589.9, 2000.0)	338.2 (184.1, 842.1)	1.0 (1.0–1.0)	**.008**	[a]	NS
Anti-COVID-19 treatment						
Remdesivir	55 (74.3%)	856 (78.7%)	0.81 (0.51–1.34)	.371		
Hydroxychloroquine	22 (29.7%)	327 (30.0%)	1.03 (0.61–1.63)	.949		
Azithromycin	12 (16.2%)	127 (11.7%)	1.51 (0.81–2.87)	.787		

TABLE 2 (Continued)

Variable	CAPA ($n = 74$)	Non-CAPA ($n = 1087$)	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Dexamethasone	42 (56.8%)	651 (59.9%)	0.93 (0.51–1.45)	.595		
Methylprednisolone	20 (27.0%)	392 (36.1%)	0.74 (0.42–1.18)	.118		
Low MW heparin	62 (83.8%)	956 (87.9%)	0.78 (0.32–1.39)	.293		
Pulse steroids	17 (23.0%)	274 (25.2%)	0.90 (0.55–1.57)	.668		
Tocilizumab	06 (8.1%)	84 (7.7%)	1.17 (0.47–2.54)	.905		
Anti-fungal treatment						
Liposomal amphotericin-B	52 (70.3%)	NA	NA	NA	NA	NA
Voriconazole	24 (32.4%)	NA	NA	NA	NA	NA
Isavuconazole	08 (10.8%)	NA	NA	NA	NA	NA

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ARDS, acute respiratory distress syndrome; CAPA, COVID-19-associated pulmonary aspergillosis; CI, confidence interval; CRP, C-reactive protein; CT, computed tomography; CTSI, computed tomography severity index; HIV, human immunodeficiency virus; ICU, intensive care unit; IMV, invasive mechanical ventilation; IQR, interquartile range; MW, molecular weight; NA, not applicable; NIV, noninvasive ventilation; NVS, nonventilatory support; OR, odds ratio; SD, standard deviation; TLC, total leukocyte count.

[a]Included in multivariate analysis, found to be statistically nonsignificant.

[b]Non-CAPA patients ($n = 74$) similar in number and demographic features to CAPA patients were selected for CT findings and laboratory biomarkers. Laboratory biomarker values in the CAPA group on the day of diagnosis of CAPA and in the non-CAPA group on day 11 after admission (to match an average postadmission time of CAPA development) were taken.

The bold values are significanct in univarabe analysis.

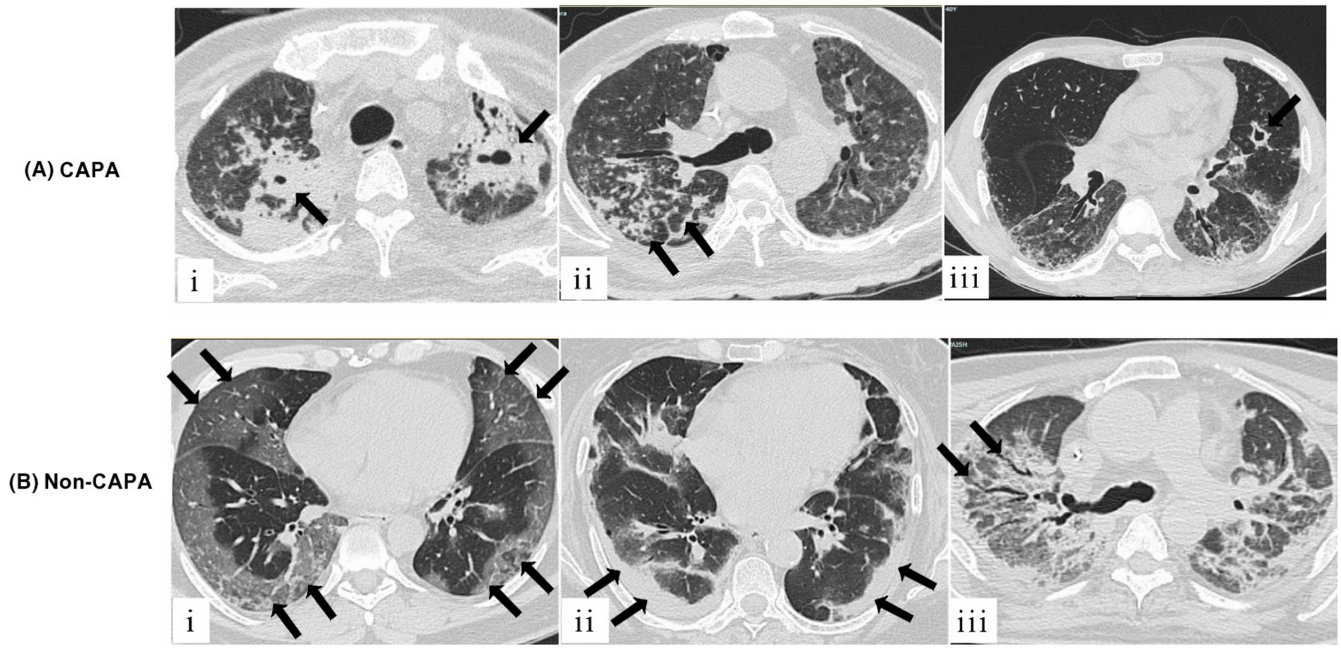

FIGURE 2 Representative chest CT images of (A) CAPA showing (i) large consolidations with cavitation (arrows), (ii) multiple nodular infiltrates (arrows), and (iii) small cavitary lesion (arrow) and (B) non-CAPA showing (i) diffuse subpleural ground glass and reticular opacities (arrows), (ii) organised peripheral consolidations (arrows), and (iii) combination of subpleural consolidation patches and reticular opacities with tractional bronchiectasis (arrows). CAPA, Covid-19-associated pulmonary aspergillosis; CT, computed tomography

53.8% (seven/13), while in the second wave, they were 17.7% (61/344) and 45.9% (28/61), respectively. The overall prevalence and mortality were 6.4% (74/1161) and 47.3% (35/74), respectively, during the entire period of the study (Figure 1).

The median age of CAPA patients was 55 (44.8–64.3), 33.8% (25/74) were female, and 63.5% (47/74) were under 60 years of age. Persistent fever, hemoptysis, and chest pain were present in 78.4%, 33.8%, and 20.3% of the patients, respectively. Among various co-morbidities, diabetes mellitus (DM) was the most common (60.3%) in the patients. In addition to standard anti-COVID treatment, pulse glucocorticoids (GC) (methylprednisolone or dexamethasone) were given to 17 (23%) CAPA patients, according to the severity of the

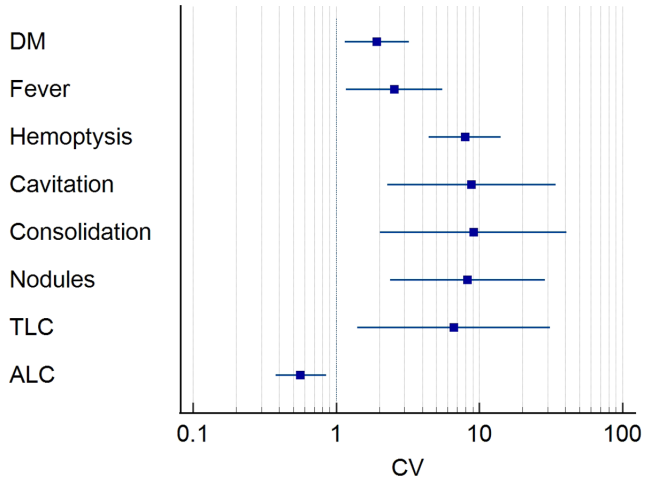

FIGURE 3 Multivariate analysis by binary logistic regression of factors associated with the development of CAPA. All factors found significant in the univariate analysis were analysed for the multivariate model, and the factors attaining statistical significance in the multivariate analysis are shown in this figure. ALC, absolute lymphocyte count; CAPA, COVID-19-associated pulmonary aspergillosis; CV, coefficient of variation; DM, diabetes mellitus; TLC, total leukocyte count

disease. Tocilizumab as an immunomodulator (IM) was given to six (8.1%) of the patients. Anti-fungal treatment comprising liposomal amphotericin-B, oral voriconazole, or oral isavuconazole was given to 52 (70.3%), 24 (32.4%), and 8 (10.8%) patients, respectively. Cases of suspected CAPA having no definitive mycological evidence of the disease were commonly treated with liposomal amphotericin-B alone (Table 2).

CAPA was diagnosed at a median of 11 days (IQR: (12.8–29.0) after ICU admission or diagnosis of COVID-19. A number of clinical, radiological, ICU, and laboratory factors were observed to have an association with the development of CAPA by univariable analysis (Table 2). Of these, the following factors were significant by multivariable analysis. DM (OR 1.92 [95% CI 1.15–3.21]; $p = .013$), hemoptysis (OR 7.91 [4.45–14.06]; $p < .001$), fever (OR 2.54 [1.17–5.53]; $p = .019$), CT findings of cavitation (OR 8.78 [2.27–34.03], $p = .002$), consolidation (OR 9.06 [2.03–40.39]; $p = .004$), nodules (OR 8.26 [2.39–28.58]; $p = .001$), high total leukocyte count (TLC) (OR 6.61 [95% CI: 1.4–31.15], $p = .017$); and low ALC (OR 0.56 [95% CI: 0.38–0.85], $p = .021$) (Table 2; Figures 2 and 3). There was no difference in the development of CAPA among pulse GC-treated vs untreated (5.8%, 17/291 vs 6.5%, 57/870; $p = .782$) and IM-treated vs untreated (6.7%, 6/90 vs 6.3%, 68/1071; $p = .823$) ICU patients (Table 2).

We observed an association of ICU-related, clinical, radiological, and laboratory factors with mortality in CAPA by univariable analysis (Table 3). Of these ARDS (OR: 2.68 [1.09–6.55]; $p = .031$), high CT Severity Index (CTSI) (OR 1.18 [1.08–1.29]; $p < .001$). High TLC (OR: 1.07 [1.02–1.12]; $p = .005$) and low ALC (OR 0.53 [0.29–0.97]; $p = .039$) were significant by multivariable analysis (Table 3; Figure 4).

Kaplan–Meier survival analysis along with log-rank test showed higher mortality in possible CAPA compared to proven/probable CAPA patients (HR 4.0 [I1.7–9.3]; $p = .001$) and in CAPA compared to non-CAPA patients (HR 1.8 [1.1, 2.8], p: .001) (Figure 5). The pulse GC-treated CAPA patients had significantly higher mortality than pulse GC untreated CAPA (HR 4.0 [1.3–9.2]; $p = .0001$) and than pulse GC-treated non-CAPA patients (HR 4.0 [1.3–11.9], $p = .0001$). The IM-treated than untreated non-CAPA patients had a higher rate of mortality (HR 1.7 [1.1–2.6], $p = .003$) (Figure 6).

4 | DISCUSSION

To the best of our knowledge, this is the largest homogenous cohort study in the literature to date reporting prevalence, outcome, and factors associated with the emergence and mortality of the disease. Among a homogenous population of 1161 patients, we observed an overall prevalence of CAPA of 6.4%. However, this may not be the representative prevalence, as we had no clinical awareness of this new disease during the first wave of the pandemic, and hence, our retrospective data of this period yielded a substantially low prevalence (1.6%). In the second wave, being clinically alert, we systematically monitored all suspected patients for CAPA and observed a prevalence of 17.7%, which represents the actual prevalence of the disease at our center. There was no significant difference in the overall mortality in CAPA patients during the entire study period (47.3%) or in the first (53.8%) and second (45.9%) waves of the pandemic. The prevalence and mortality of CAPA patients in our study corroborate studies from Pakistan,[6] other research cohorts,[9,20] and systemic reviews.[27,30] As published in the literature, *A. fumigatus* was the most common mould species causing CAPA in our patients.[24,30]

We identified several important factors associated with the development of CAPA by multivariable analysis. The primary clinical risk factors associated with CAPA were hemoptysis and persistent fever, as reported by certain other studies.[15] The presence of DM was the only underlying comorbidity associated with CAPA in our patients, and it may be one of the root causes of the disease. DM causes structural and functional alterations in the lungs and weakens the immune system leading to a potential risk of pulmonary fungal infections, including aspergillosis.[34–36] A number of studies have shown DM to be common comorbidity of CAPA but they could not demonstrate its statistical association with the disease probably due to the small sample size.[15,30] One of the main hurdles associated with the differential diagnosis of lung lesions in CAPA is their nonspecific radiologic signs.[33] Different studies have reported different lung images in CAPA patients, and there is no consensus on their association with the disease.[1,15,37,38] Our study demonstrates a distinct and independent association of cavitation, consolidation, and nodules with CAPA, highlighting the role of these radiological signs as important risk factors for CAPA development. From laboratory factors, high TLC associated with increased absolute neutrophil count (neutrophilic leucocytosis) and reduced ALC (lymphopenia) were associated with CAPA by multivariable analysis. Neutrophilic leucocytosis

TABLE 3 Demographic, clinical, and laboratory data of died and survived CAPA patients

Variable	Died CAPA ($n = 35$)	Survived CAPA ($n = 39$)	Univariable OR (95 CI)	*p*-value	Multivariable OR (95% CI)	*p*-value
Demographics						
Age: years (median, IQR)	51.0 (44.0, 65.0)	55.0 (49.0, 64.0)	1.0 (1.0–1.0)	.072		
Female sex	9 (25.7%)	16 (41.0%)	2.0 (0.7–5.4)	.220		
ICU parameters						
Hospital stay: (median, IQR)	16 (12, 29)	19 (13,31)	1.1 (0.97–1.1)	.413		
IMV during first week	24 (68.6%)	2 (5.1%)	23.00 (5.85–90.42)	**<.001**	[a]	NS
NIV	8 (22.9%)	10 (25.6%)	1.19 (0.34–3.34)	1.000		
NVS at admission	3 (8.6%)	27 (69.2%)	0.04 (0.01–0.16)	**<.001**	[a]	NS
Clinical symptoms						
Hemoptysis	10 (28.6%)	15 (38.5%)	0.62 (0.21–1.72)	.462		
Persistent fever	33 (94.3%)	33 (84.6%)	3.0 (0.62–16.02)	.267		
Chest pain	7 (20.0%)	8 (20.5%)	1.09 (0.31–3.04)	1.000		
ARDS at admission	24 (68.6%)	11 (28.2%)	9.63 (3.33–27.88)	**<.001**	2.68 (1.09–6.55)	.031
Comorbidities						
Diabetes mellitus	21 (60.0%)	24 (61.5%)	0.91 (0.42–2.43)	1.000		
Hypertension	18 (51.4%)	16 (41.0%)	1.51 (0.62–3.83)	.484		
Chronic pulmonary disease	8 (22.9%)	9 (23.1%)	1.12 (0.67–2.13)	1.000		
Chronic kidney disease	2 (5.7%)	3 (7.7%)	1.30 (0.41–3.82)	1.000		
Chronic liver disease	3 (8.6%)	1 (2.6%)	0.65 (0.31–1.22)	.339		
Coronary artery disease	3 (8.6%)	4 (10.3%)	0.81 (0.22–3.91)	1.000		
Malignancy	1 (2.9%)	1 (2.6%)	1.00 (0.21–4.12)	.495		
Renal transplantation	0	2 (5.1%)	0.55 (0.41–0.69)	.495		
HIV syndrome	1 (2.9%)	0	0.51 (0.41–0.6)	1.000		
CT findings[b]						
CTSI (median, IQR)	18 (14, 20)	10 (8, 12)	1.69 (1.35–2.11)	**<.001**	1.18 (1.08–1.29)	<.001
Laboratory biomarkers[b]						
TLC ($\times 10^3/\mu l$)	19.1 (12.7, 26.8)	7.9 (6.9, 14.5)	1.17 (1.08–1.28)	**<.001**	1.07 (1.02–1.12)	.005
ANC ($\times 10^3/\mu l$)	17.5 (11.3, 24.7)	7.1 (5.7, 10.3)	1.24 (1.11–1.38)	**<.001**	[a]	NS
ALC ($\times 10^3/\mu l$)	0.7 (0.4, 1.3)	1.2 (0.6, 2.2)	0.40 (0.20–0.83)	**.013**	0.53 (0.29–0.90)	.039
Platelets ($\times 10^3/\mu l$)	89.0 (54.0, 135.0)	188.0 (156.0, 250.0)	0.98 (0.97–0.99)	**.01**	[a]	NS
CRP (mg/L)	86.0 (24.0, 152.0)	24.0 (5.0, 106.0)	1.00 (0.99,1.01)	.125		
D-dimer (μg/ml)	2.9 (1.3, 8.4)	1.1 (0.8, 2.1)	1.40 (1.15,1.70)	**.01**	[a]	NS
Fibrinogen (mg/L)	475.0 (390.0, 599.0)	499.0 (341.0, 618.0)	1.00 (1.00,1.00)	.375		
Ferritin (ng/ml)	2000.0 (978.2, 3490.7)	662.0 (378.0, 1287.8)	1.00 (1.00,1.00)	**.019**	[a]	NS
Anti-COVID-19 treatment						
Remdesivir	29 (82.9%)	26 (66.7%)	0.51 (0.13–1.81)	.266		
Hydroxychloroquine	10 (28.6%)	12 (30.8%)	1.9 (0.64–6.36)	.306		
Azithromycin	6 (17.1%)	6 (15.4%)	0.81 (0.22–39)	.685		
Dexamethasone	16 (45.7%)	26 (66.7)	2.1 (0.32–14.21)	.474		
Methylprednisolone	14 (40.0%)	6 (15.4%)	0.79 (0.11–6.34)	.777		

(Continues)

TABLE 3 (Continued)

Variable	Died CAPA ($n = 35$)	Survived CAPA ($n = 39$)	Univariable OR (95 CI)	p-value	Multivariable OR (95% CI)	p-value
Low MW heparin	30 (85.7%)	32 (82.1%)	0.62 (0.12–4.33)	.592		
Pulse steroids	12 (34.3%)	5 (12.8%)	0.34 (0.12–1.23)	.089		
Tocilizumab	3 (8.6)	2 (5.1%)	1.31 (0.25–8.10)	.804		
Anti-fungal treatment						
Liposomal amphotericin-B	27 (77.1)	25 (64.1)	0.91 (0.22–3.42)	.850		
Voriconazole	8 (22.9)	16 (41.0)	2.11 (0.61–7.72)	.249		
Isavuconazole	03 (8.6)	05 (12.8)	1.17 (0.21–5.92)	.868		

Abbreviations: ALC, absolute lymphocyte counts; ANC, absolute neutrophil counts; ARDS, acute respiratory distress syndrome; CAPA, COVID-19-associated pulmonary aspergillosis; CI, confidence interval; CI, confidence Interval; CRP, C-reactive protein; CT, computed tomography; CTSI, Computed Tomography Severity Index; HIV, human immunodeficiency virus; IMV, invasive mechanical ventilation; IQR, interquartile range; MW, molecular weight; NIV, noninvasive ventilation; NVS, nonventilatory support; OR, odds ratio; TLC, total leukocyte count.

[a]Included in multivariate analysis, found to be statistically nonsignificant.

[b]Non-CAPA patients ($n = 74$) similar in number and demographic features to CAPA patients were selected for radiologic findings and laboratory biomarkers. Laboratory biomarker values in died or survived CAPA were of the nearest day of death or discharge, respectively.

The bold values are significanct in univarabe analysis.

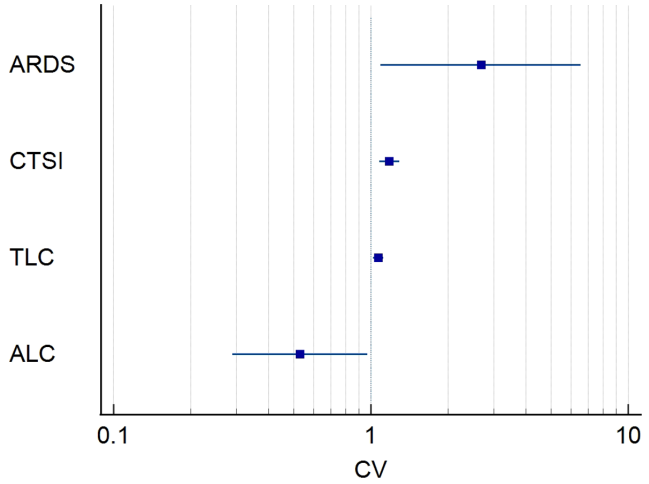

FIGURE 4 Multivariate analysis by binary logistic regression of factors associated with mortality in CAPA patients. All factors found significant in the univariate analysis were analysed for the multivariate model, and the factors attaining statistical significance in the multivariate analysis are shown in this figure. ALC, absolute lymphocyte count; ARDS, acute respiratory distress syndrome; CAPA, COVID-19-associated pulmonary aspergillosis; CTSI, computed tomography severity score; CV, coefficient of variation; TLC, total leukocyte count

reflects added infection with *Aspergillus* and may be due to altered immune homeostasis in patients. Although there is no clear previous data on the association of lymphopenia with CAPA in a comparative analysis of CAPA vs non-CAPA, one study in the literature has reported that the duration of lymphopenia is higher in CAPA than in non-CAPA, and its persistence for >10 days is associated with CAPA development.[15] Although, platelet counts and ferritin levels were significant in the univariable analysis only but they need to be included as laboratory risk factors due to their reported role in the development of CAPA.[25,39]

A concurrent diagnosis of CAPA with COVID-19 is rare, and it is diagnosed after ICU admission or even after discharge from the hospital.[15,25] We also diagnosed CAPA in our patients at a median of 11 days after ICU admission. It's timing of occurrence indicates that the ICU environment or other ICU factors may have some role in the development of CAPA, advocating hospitalisation of patients in the ICU with a HEPA filtered clean air environment. Severe COVID-19 patients with ARDS on invasive mechanical ventilation (IMV) are commonly suspected to have CAPA.[1,20,40,41] However, most of our patients had no ARDS or any ventilatory support showing that CAPA may also frequently occur in non-severe patients. Thus, COVID-19 in itself is an independent risk factor for CAPA, and all COVID-19 patients, whether severe or nonsevere, should be systematically screened on a regular basis for CAPA to have an early diagnosis and effective clinical management.

Alarmingly high mortality is the most crucial clinical issue in CAPA. We observed a significant association of ARDS with mortality in CAPA as reported by other studies from our region.[6] Due to high disease severity, almost all patients with ARDS are put on IMV. Despite being a life-saving intervention, IMV can cause severe complications such as acute kidney injury and sepsis, leading to multi-organ dysfunction syndrome.[42] Since IMV can contribute to high mortality in CAPA patients with ARDS; it is advisable to avoid invasive ventilation until inevitable and manage early-stage respiratory failure in the patients using a high-flow nasal cannula or NIV. A high CTSI is another risk factor for mortality in CAPA and it may be important for prognostic mentoring of the patients. Similar to their association with CAPA development as discussed above, neutrophilic leukocytosis and lymphopenia are also important risk factors for mortality of the disease by multivariable analysis. Our data also shows that persistence or further deterioration of neutrophilic leukocytosis and lymphopenia leads to death while their restoration improves the survival of CAPA patients. We found higher mortality in possible than in proven/probable CAPA patients probably due

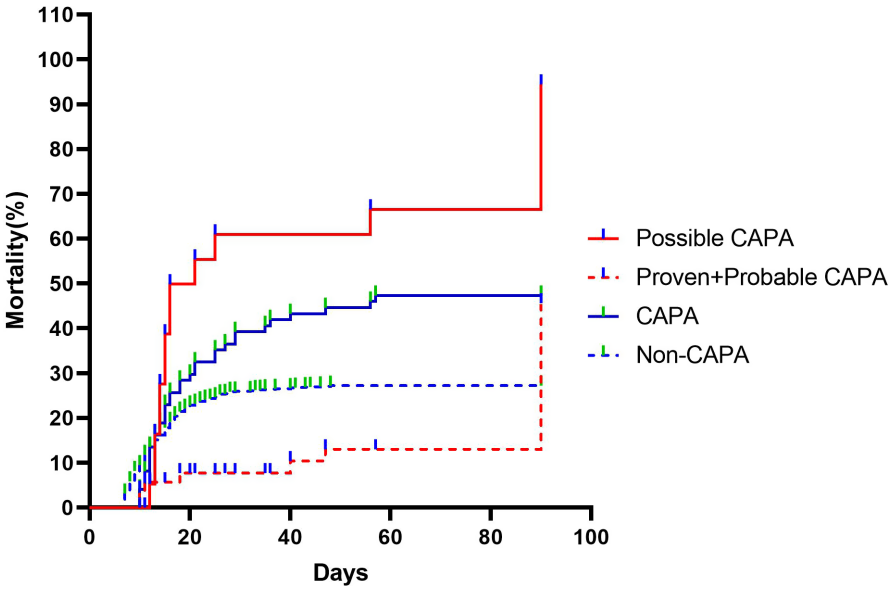

FIGURE 5 Kaplan–Meier curve showing the rate of mortality in possible CAPA (81%; 17/21) (solid red line) vs proven/probable CAPA (36.7%; 18/53) (dotted red line) (HR 4.0 [95% CI 1.7–9.3), $p = .001$); and CAPA (47.3%; 35/74) (solid blue line) and non-CAPA (27.2%; 296/1087) (dotted blue line) (HR 1.8 [95% CI 1.1–2.8], $p = .001$). CAPA, COVID-19-associated pulmonary aspergillosis; CI, confidence interval; HR, hazard ratio

to a less aggressive and nonspecific anti-fungal treatment given to patients with possible CAPA. Moreover, CAPA patients had significantly higher mortality than non-CAPA patients, suggesting that CAPA is a major cause of mortality in ICU patients. These results are concordant with studies in the literature, including a multicenter study showing CAPA to be responsible for >50% of mortality in ICU patients.[7,21]

The most important finding of this study is the substantially higher mortality in pulse GC-treated CAPA patients but significantly lower mortality in pulse GC-treated non-CAPA patients. The reports showing the promotion of growth and virulence of *Aspergillus* spp. by GC lend support to our observation.[43] During the two waves of the pandemic, we observed a beneficial effect of pulse GC treatment in some ICU patients with cytokine storms, while it had a detrimental effect in a subset of cases. As per the results of this study, patients benefitting from pulse GC treatment might be those having no Aspergillus infection, whereas patients having increased mortality after GC treatment might be having pre-existing acute or subacute CAPA. These observations provide a clinically crucial message for ICU physicians to exclude CAPA diagnosis before initiating pulse GC therapy in ICU patients and avoid any pulse GC treatment in CAPA patients as much as possible. We could not demonstrate a significant effect of IM on mortality in CAPA due to the very small number of IM-treated patients, but higher mortality was observed in IM-treated non-CAPA patients. A number of randomised clinical trials have been conducted on the efficacy of IM (tocilizumab), including one at our institute, but its benefit is not yet clear.[44] Similarly, a recent systematic review and meta-analysis of clinical trials reported no significant associations between treatment with tocilizumab and reductions in all-cause mortality in COVID-19 patients.[45] Our observations and these studies together suggest that IM treatment is avoidable in CAPA as well as non-CAPA patients.

Our study has certain limitations. First, the initial and major part of the study is retrospective. During this period, we had neither adequate knowledge about CAPA nor a well-defined protocol for accurate diagnostic work-up of the disease. Thus, many cases of CAPA are missed, resulting in underestimated disease prevalence in our study population. Second, for safety reasons, we could perform bronchoscopy only when the patients become COVID-19 negative and thus missed the early diagnosis of CAPA and factors involved in the emergence of the disease. Third, our study had only two definitive CAPA cases (i.e., cases with histopathological data showing tissue-invasive hyphae) for case–control analysis, and this restricted us to more accurately identifying the factors involved in the development and mortality of the disease. Further homogenous studies of prospective nature addressing these limitations are required to better define the incidence and risk factors involved in the development and mortality of the disease.

In conclusion, we observed here a low but likely underestimated prevalence of CAPA. Our study shows that CAPA is a disease with high mortality. Hence, all COVID-19 patients whether severe or non-severe should be systematically screened for CAPA on weekly basis for early diagnosis and treatment to improve the outcome. DM may be the root cause of CAPA development in ICU patients. ARDS, irrational use of pulse GC, and an unclear, delayed, or missed diagnosis are major factors of mortality in CAPA. Neutrophilic leukocytosis, lymphopenia, thrombocytopenia, and hyperferritinemia are centrally involved in CAPA pathology from its development to mortality and may serve as novel targets for developing new cellular or iron-depleting therapeutic modalities for the disease. Our study would be helpful in the early diagnosis and effective clinical management of the disease in the ongoing wave of COVID-19 and any other similar pandemic that we may face in the future.

AUTHOR CONTRIBUTIONS

Zia Hashim: Conceptualisation, Methodology, Software, Writing—Review & Editing, Format analysis, **Alok Nath:** Project

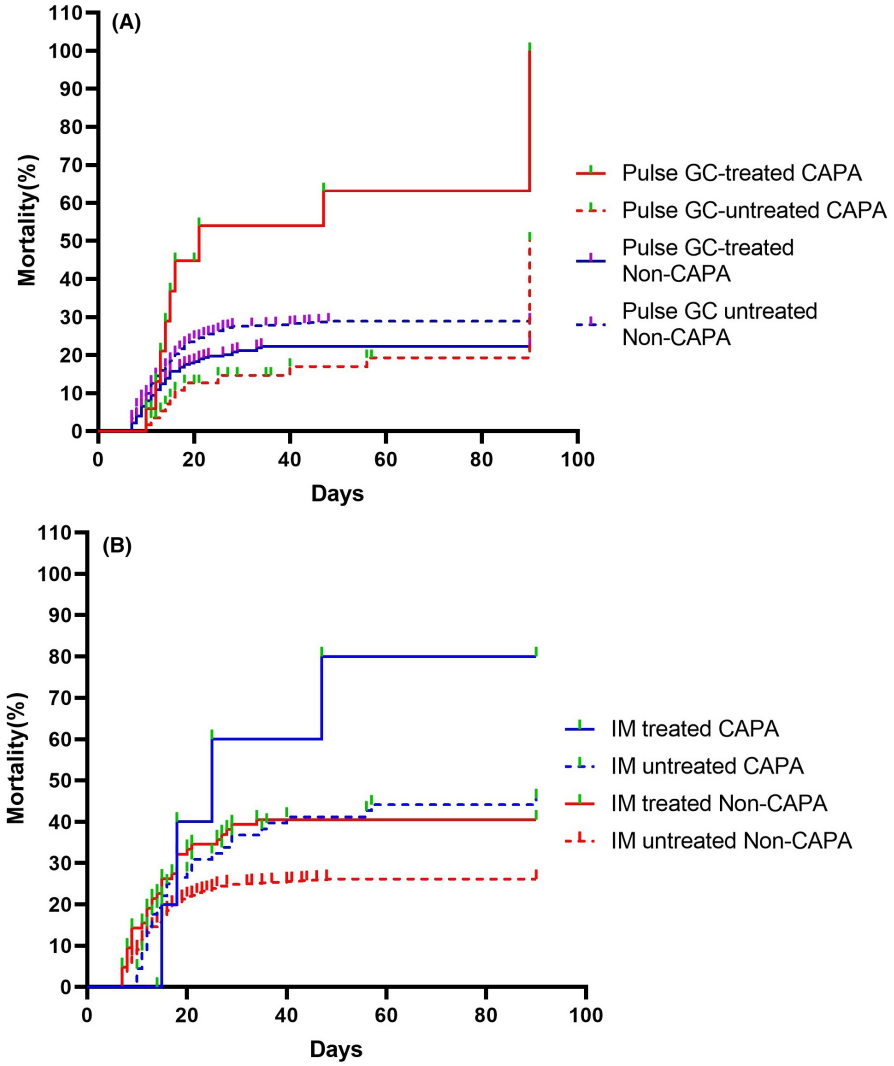

FIGURE 6 Kaplan–Meier curve showing the rate of mortality in (A) pulse GC-treated CAPA (70.6%; 12/17) (solid red line) vs pulse GC-untreated CAPA (40.4%; 23/57) patients (dotted red line), (HR 4.0 [CI: 1.3–9.2], $p = .0001$); pulse GC-treated CAPA (70.6%; 12/17) (solid red line) and pulse GC-treated non-CAPA patients (solid blue line) (28.9%, 235/813) patients (HR 4.0 [95% CI 1.3–11.9], $p = .0001$); and pulse GC-treated non-CAPA (22.3%; 61/274) (solid blue line) and pulse GC-untreated non-CAPA (28.9%, 235/813) patients (dotted blue line) (HR 0.7 [95% CI: 0.6–0.9], $p = .035$). (B) IM-treated CAPA (66.6%; 4/6) (solid blue line) vs IM-untreated CAPA (45.6%; 31/68) patients (dotted blue line), (HR 1.5 [95% CI: 0.4, 5.0], *$p = .413$); IM-treated CAPA (66.6%; 4/6) (solid blue line) vs IM-treated non-CAPA (40.5%; 34/84) patients (solid red line). (HR 1.5 [95% CI 0.4–5.3], *$p = .409$); and IM-treated non-CAPA (40.5%; 34/84) (solid red line) vs IM-untreated non-CAPA (26.1%, 262/1003) patients (dotted red line); (HR 1.7 [95% CI 1.1–2.6], $p = .003$). *A non-significant p value here may be due to the very small number of patients in the IM-treated CAPA group. CAPA, COVID-19-associated pulmonary aspergillosis; CI, confidence interval; GC, glucocorticoids; HR, hazard ratio; IM, immunomodulators

administration, Supervision, **Ajmal Khan:** Resources, Validation, **Zafar Neyaz:** Writing—Original Draft,Investigation, **Rungmei SK Marak:** Investigation, **Prasant Areekkara:** Data Curation, **Atul Tiwari:** Data Curation, **Shivani Srivastava:** Data Curation, **Vikas Agarwal:** Writing—Review & Editing, **Swati Saxena:** Data Curation, **Nidhi Tripathy:** Data Curation, **Afzal Azim:** Format analysis, **Mansi Gupta:** Format analysis, **Durga Prasanna Mishra:** Format analysis, **Prabhakar Mishra:** Format analysis, **Ratender Kumar Singh:** Project administration, **Devender Gupta:** Investigation, **Anshul Gupta:** Investigation, **Om Prakash Sanjeev:** Investigation, **Tanmoy Ghatak:** Resources, **Ujjala Ghoshal:** Resources, **Radha Krishan Dhiman:** Supervision, Resources, **Naresh Kumar Tripathy:** Conceptualisation and study design, Format analysis, Writing—Original Draft, Review and editing, Investigation, Validation, Funding acquisition.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Koehler P, Cornely OA, Bottiger BW, et al. COVID-19 associated pulmonary aspergillosis. *Mycoses*. 2020;63(6):528-534.
2. Machado M, Valerio M, Alvarez-Uria A, et al. Invasive pulmonary aspergillosis in the COVID-19 era: an expected new entity. *Mycoses*. 2021;64(2):132-143.
3. Ranhel D, Ribeiro A, Batista J, et al. COVID-19-associated invasive pulmonary aspergillosis in the intensive care unit: a case series in a Portuguese hospital. *J Fungi (Basel)*. 2021;7(10):881.
4. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507-513.
5. Wang J, Yang Q, Zhang P, Sheng J, Zhou J, Qu T. Clinical characteristics of invasive pulmonary aspergillosis in patients with COVID-19 in Zhejiang, China: a retrospective case series. *Crit Care*. 2020;24(1):299.
6. Nasir N, Farooqi J, Mahmood SF, Jabeen K. COVID-19-associated pulmonary aspergillosis (CAPA) in patients admitted with severe COVID-19 pneumonia: an observational study from Pakistan. *Mycoses*. 2020;63(8):766-770.
7. Tio SY, Williams E, Worth LJ, et al. Invasive pulmonary aspergillosis in critically ill patients with COVID-19 in Australia: implications for screening and treatment. *Intern Med J*. 2021;51(12):2129-2132.
8. Dupont D, Menotti J, Turc J, et al. Pulmonary aspergillosis in critically ill patients with coronavirus disease 2019 (COVID-19). *Med Mycol*. 2021;59(1):110-114.
9. Borman AM, Palmer MD, Fraser M, et al. COVID-19-associated invasive aspergillosis: data from the UK national mycology reference laboratory. *J Clin Microbiol*. 2020;59(1):e02136-20.
10. Paramythiotou E, Dimopoulos G, Koliakos N, et al. Epidemiology and incidence of COVID-19-associated pulmonary aspergillosis (CAPA) in a Greek tertiary care academic reference hospital. *Infect Dis Ther*. 2021;10(3):1779-1792.
11. Helleberg M, Steensen M, Arendrup MC. Invasive aspergillosis in patients with severe COVID-19 pneumonia. *Clin Microbiol Infect*. 2021;27(1):147-148.
12. Falces-Romero I, Ruiz-Bastian M, Diaz-Pollan B, Maseda E, Garcia-Rodriguez J, Group SA-C-W. Isolation of *Aspergillus* spp. in respiratory samples of patients with COVID-19 in a Spanish Tertiary Care Hospital. *Mycoses*. 2020;63:1144-1148.
13. Meijer EFJ, Dofferhoff ASM, Hoiting O, Meis JF. COVID-19-associated pulmonary aspergillosis: a prospective single-center dual case series. *Mycoses*. 2021;64(4):457-464.
14. Lamoth F, Glampedakis E, Boillat-Blanco N, Oddo M, Pagani JL. Incidence of invasive pulmonary aspergillosis among critically ill COVID-19 patients. *Clin Microbiol Infect*. 2020;26(12):1706-1708.
15. Shadrivova O, Gusev D, Vashukova M, et al. COVID-19-associated pulmonary aspergillosis in Russia. *J Fungi (Basel)*. 2021;7(12):1059.
16. Rutsaert L, Steinfort N, Van Hunsel T, et al. COVID-19-associated invasive pulmonary aspergillosis. *Ann Intensive Care*. 2020;10(1):71.
17. Ghazanfari M, Arastehfar A, Davoodi L, et al. Pervasive but neglected: a perspective on COVID-19-associated pulmonary mold infections among mechanically ventilated COVID-19 patients. *Front Med (Lausanne)*. 2021;8:649675.
18. Wasylyshyn AI, Wasylyshyn GR, Linder KA, Miceli MH. COVID-19-associated pulmonary aspergillosis at an academic medical center in the Midwestern United States. *Mycopathologia*. 2021;186(4):499-505.
19. Lahmer T, Kriescher S, Herner A, et al. Invasive pulmonary aspergillosis in critically ill patients with severe COVID-19 pneumonia: results from the prospective AspCOVID-19 study. *PLoS One*. 2021;16(3):e0238825.
20. Bartoletti M, Pascale R, Cricca M, et al. Epidemiology of invasive pulmonary aspergillosis among intubated patients with COVID-19: a prospective study. *Clin Infect Dis*. 2021;73(11):e3606-e3614.
21. Velez Pintado M, Camiro-Zuniga A, Aguilar Soto M, et al. COVID-19-associated invasive pulmonary aspergillosis in a tertiary care center in Mexico City. *Med Mycol*. 2021;59(8):828-833.
22. Benedetti MF, Alava KH, Sagardia J, et al. COVID-19 associated pulmonary aspergillosis in ICU patients: report of five cases from Argentina. *Med Mycol Case Rep*. 2021;31:24-28.
23. Farias ME, Santana MF, Ferreira L, et al. COVID-19-associated pulmonary aspergillosis in a series of complete autopsies from the Brazilian Amazon. *Am J Trop Med Hyg*. 2022;106:571-573.
24. Delliere S, Dudoignon E, Fodil S, et al. Risk factors associated with COVID-19-associated pulmonary aspergillosis in ICU patients: a French multicentric retrospective cohort. *Clin Microbiol Infect*. 2020;27(5):790.e1-790.e5.
25. Xu J, Yang X, Lv Z, et al. Risk factors for invasive aspergillosis in patients admitted to the intensive care unit with coronavirus disease 2019: a multicenter retrospective study. *Front Med (Lausanne)*. 2021;8:753659.
26. Prattes J, Wauters J, Giacobbe DR, et al. Risk factors and outcome of pulmonary aspergillosis in critically ill coronavirus disease 2019 patients-a multinational observational study by the European Confederation of Medical Mycology. *Clin Microbiol Infect*. 2022;28:580-587.
27. Feys S, Almyroudi MP, Braspenning R, et al. A Visual and comprehensive review on COVID-19-associated pulmonary aspergillosis (CAPA). *J Fungi (Basel)*. 2021;7(12):1067.
28. Mitaka H, Kuno T, Takagi H, Patrawalla P. Incidence and mortality of COVID-19-associated pulmonary aspergillosis: a systematic review and meta-analysis. *Mycoses*. 2021;64(9):993-1001.
29. Singh S, Verma N, Kanaujia R, Chakrabarti A, Rudramurthy SM. Mortality in critically ill patients with coronavirus disease 2019-associated pulmonary aspergillosis: a systematic review and meta-analysis. *Mycoses*. 2021;64(9):1015-1027.
30. Chong WH, Neu KP. Incidence, diagnosis and outcomes of COVID-19-associated pulmonary aspergillosis (CAPA): a systematic review. *J Hosp Infect*. 2021;113:115-129.
31. Salmanton-Garcia J, Sprute R, Stemler J, et al. COVID-19-associated pulmonary aspergillosis, March-August 2020. *Emerg Infect Dis*. 2021;27(4):1077-1086.
32. Hashim Z, Neyaz Z, Marak RSK, Nath A, Nityanand S, Tripathy NK. Practice guidelines for the diagnosis of COVID-19-associated pulmonary aspergillosis in an intensive care setting. *J Intensive Care Med*. 2021;22:8850666211047166.
33. Koehler P, Bassetti M, Chakrabarti A, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. *Lancet Infect Dis*. 2021;21(6):e149-e162.
34. Garcia-Vidal C, Sanjuan G, Moreno-García E, et al. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study. *Clin Microbiol Infect*. 2021;27(1):83-88.
35. Ye Y, Chen Y, Sun J, et al. Hyperglycemia suppresses the regulatory effect of hypoxia-inducible factor-1α in pulmonary *Aspergillus fumigatus* infection. *Pathog Dis*. 2020;78(5):1-10.

36. Ghanaat F, Tayek JA. Weight loss and diabetes are new risk factors for the development of invasive aspergillosis infection in non-immunocompromized humans. *Clin Pract (Lond)*. 2017;14(5 Spec Iss):296-301.
37. Baratella E, Roman-Pognuz E, Zerbato V, et al. Potential links between COVID-19-associated pulmonary aspergillosis and bronchiectasis as detected by high resolution computed tomography. *Front Biosci (Landmark Ed)*. 2021;26(12):1607-1612.
38. Verweij PE, Brüggemann RJM, Azoulay E, et al. Taskforce report on the diagnosis and clinical management of COVID-19 associated pulmonary aspergillosis. *Intensive Care Med*. 2021;47(8):819-834.
39. Matthaiou EI, Sass G, Stevens DA, Hsu JL. Iron: an essential nutrient for *Aspergillus fumigatus* and a fulcrum for pathogenesis. *Curr Opin Infect Dis*. 2018;31(6):506-511.
40. Permpalung N, Chiang TP, Massie AB, et al. COVID-19 associated pulmonary aspergillosis in mechanically ventilated patients. *Clin Infect Dis*. 2022;74(1):83-91.
41. Gangneux JP, Dannaoui E, Fekkar A, et al. Fungal infections in mechanically ventilated patients with COVID-19 during the first wave: the French multicentre MYCOVID study. *Lancet Respir Med*. 2022;10(2):180-190.
42. Hua J, Qian C, Luo Z, Li Q, Wang F. Invasive mechanical ventilation in COVID-19 patient management: the experience with 469 patients in Wuhan. *Crit Care*. 2020;24(1):348.
43. Ng TT, Robson GD, Denning DW. Hydrocortisone-enhanced growth of *Aspergillus* spp.: implications for pathogenesis. *Microbiology (Reading)*. 1994;140(Pt 9):2475-2479.
44. Soin AS, Kumar K, Choudhary NS, et al. Tocilizumab plus standard care versus standard care in patients in India with moderate to severe COVID-19-associated cytokine release syndrome (COVINTOC): an open-label, multicentre, randomised, controlled, phase 3 trial. *Lancet Respir Med*. 2021;9(5):511-521.
45. Peng J, She X, Mei H, et al. Association between tocilizumab treatment and clinical outcomes of COVID-19 patients: a systematic review and meta-analysis. *Aging (Albany NY)*. 2022;14(2):557-571.

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