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.
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,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 systematic 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 as well as ECMM/ISHAM consensus criteria33 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 × 10^9 neutrophils/μl, lymphopenia as an absolute lymphocyte count (ALC) of <1.0 × 10^9/μl, and thrombocytopenia as a platelet count <150 × 10^9/μl.

2.3 | Data collection

Data from the first wave of the pandemic (10 March 2020 to 28 February 2021) were collected prospectively. In the second wave (1 March to 28 August 2021), the data were collected retrospectively. 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 < 0.05 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
| Clinical features | Radiological finding | Mycology biomarkers (GM/MP) | Mycology microscopy, culture or PCR | Asp species isolated |
|-------------------|----------------------|-----------------------------|-----------------------------------|----------------------|
| **Proven CAPA (n = 2)** | | | | |
| Symptoms | • Symptoms | • Symptoms | | |
| • HTS & fever: 02 | • Cavity, Consol, & Nodules: 01 | • sGM & bMP (+): 01 | • Asp (+) Biopsy Histopathology: 02 | Aspergillus fumigatus: 02 |
| Co-morbidities | • DM + CPD: 01 | • sGM (+): 01 | • Asp (+) Biopsy Culture: 02: | |
| DM + HTN: 01 | • Cavity, GGO, & Nodules: 01 | | | |
| **Probable CAPA (n = 51)** | | | | |
| Symptoms | • Symptoms | • Symptoms | | |
| • HTS: 07 | • Consol: 01 | • GM (+) (n = 45) | | |
| • Fever: 25 | • Cavity & Consol: 02 | • sGM: 26 | | |
| • HTS & Fever: 06 | • Cavity & GGO: 01 | • bGM: 09 | | |
| • Chest Pain & Fever: 05 | • Cavity & Nodule: 01 | • sGM & bGM: 10 | | |
| • HTS, Chest Pain & Fever: 08 | • Consol & GGO: 11 | • MP (+) (n = 38) | | |
| Co-morbidities | • Co-morbidities | • Co-morbidities | | |
| • DM: 16 | • GGO & Nodule: 04 | • sMP: 18 | | |
| • HTN: 06 | • Cavity, Consol & GGO: 03 | • bMP: 11 | | |
| • CKD: 02 | • Cavity, Consol & Nodule: 04 | • sMP & bMP: 09 | | |
| • CPD: 04 | • Console, GGO & Nodule: 08 | | | |
| • DM & CLD: 01 | • Cavity, Consol, GGO & Nodule: 16 | | | |
| • DM & CPD: 03 | | | | |
| • DM & HTN: 10 | | | | |
| • HT & CAD: 01 | | | | |
| • DM, HTN & Bladder Ca: 01 | | | | |
| Possible (n = 21) | | | | |
| | | | | |
| | | | | |
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| Clinical features                  | Radiological finding                  | Mycology biomarkers (GM/MP) | Mycology microscopy, culture or PCR | Asp species isolated |
|-----------------------------------|---------------------------------------|-----------------------------|-----------------------------------|----------------------|
| Symptoms:                         | Consol & GGO: 09                      | GM (+) (n = 21)             | NA                                | NA                   |
| HTS, FEVER & chest pain: 01       | Cavity, Console & GGO: 02             | sGM: 21                     | NA                                | NA                   |
| Fever: 20                         | Cavity, Console & Nodule: 01          | MP (+): (n = 16)            | NA                                | NA                   |
| Co-morbidities:                   | Consol, GGO & Nodule: 08              | sMP: 16                     | NA                                | NA                   |
| DM: 06                            | Cavity, Consol, GGO & Nodule: 01      | NA                          | NA                                | NA                   |
| HTN: 03                           |                                      | NA                          | NA                                | NA                   |
| CPD: 01                           |                                      | NA                          | NA                                | NA                   |
| DM + HTN: 03                      |                                      | NA                          | NA                                | NA                   |
| DM + CPD: 01                      |                                      | NA                          | NA                                | NA                   |
| HTN + CKD: 01                     |                                      | NA                          | NA                                | NA                   |
| HTN, CKD & CPD: 01                |                                      | NA                          | NA                                | NA                   |
| HTN, CLD & CPD: 01                |                                      | NA                          | NA                                | NA                   |
| DM, HT & ALL: 01                  |                                      | NA                          | NA                                | NA                   |
| DM, HTN & CPD: 02                 |                                      | NA                          | NA                                | NA                   |
| CAD, CKD & CPD: 01                |                                      | NA                          | 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.
| 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   |                          |         |
| IMV                          | 26 (35.6%)    | 237 (21.8%)         | 1.98 (1.18-3.20)        | .009    |                          | NS      |
| NIV                          | 18 (24.3%)    | 111 (10.2%)         | 2.70 (1.55-4.71)        | <.001   |                          | NS      |
| NVS                          | 30 (40.5%)    | 750 (69.0%)         | 0.3 (0.20-0.50)         | <.001   |                          | 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   |                          | 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 (0.12-2.69)        | .327    |                          |         |
| **CT findings**              |               |                     |                         |         |                          |         |
| CTSI median (IQR)            | 13.5 (10.0,18.0)| 12.0 (9.0, 14.0)   | 1.01 (1.10-1.19)        | .020    |                          | 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**    |               |                     |                         |         |                          |         |
| TLC (x10^3/μl)               | 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 (x10^3/μl)               | 10.8 (6.7, 17.5)| 8.3 (6.1, 12.2)   | 1.09 (1.03-1.16)        | .002    |                          | NS      |
| ALC (x10^3/μl)               | 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 (x10^3/μl)         | 160.0 (103.3, 231.5)| 222.0 (150.0, 296.0)| 0.99 (0.98-1.0)        | .003    |                          | NS      |
| CRP (mg/L)                   | 38.0 (10.6, 132.8)| 7.0 (4.5, 15.9)  | 1.02 (1.01-1.03)        | <.001   |                          | NS      |
| D-Dimer (μg/ml)              | 2.0 (0.8, 5.2)| 1.4 (0.6, 2.1)     | 1.32 (1.12-1.55)        | .001    |                          | NS      |
| Fibrinogen (mg/L)            | 499.0 (345.0, 608.0)| 407.5 (310.5, 576.3)| 1.0 (1.0-1.0)           | .015    |                          | NS      |
| Ferritin (ng/ml)             | 1235.5 (589.9, 2000.0)| 338.2 (184.1, 842.1)| 1.0 (1.0-1.0)           | .008    |                          | NS      |

Anti-COVID-19 treatment

| Treatment                  | CAPA (n = 74) | Non-CAPA (n = 1087) | Univariable OR (95% CI) | p-value | Multivariable OR (95% CI) | p-value |
|----------------------------|---------------|---------------------|-------------------------|---------|--------------------------|---------|
| 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    |                          |         |

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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.

*Included in multivariate analysis, found to be statistically nonsignificant.

*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 significant in univariate 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
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 [1.7–9.3]; 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 systemically 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, other research cohorts, and systemic reviews. As published in the literature, *A. fumigatus* was the most common mould species causing CAPA in our patients.

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. 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. 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. One of the main hurdles associated with the differential diagnosis of lung lesions in CAPA is their nonspecific radiologic signs. Different studies have reported different lung images in CAPA patients, and there is no consensus on their association with the disease. 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...
| 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   | 1.18 (1.08–1.29)         | <.001   |
| 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   | 2.68 (1.09–6.55)         | .031    |
| **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**                |                   |                        |                        |         |                          |         |
| 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**       |                   |                        |                        |         |                          |         |
| TLC (x10³/μl)                  | 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 (x10³/μl)                  | 17.5 (11.3, 24.7) | 7.1 (5.7, 10.3)        | 1.24 (1.11–1.38)       | <.001   | a                        | NS      |
| ALC (x10³/μl)                  | 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 (x10³/μl)            | 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    |                          |         |
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. 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. A concurrent diagnosis of CAPA with COVID-19 is rare, and it is diagnosed after ICU admission or even after discharge from the hospital. 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. 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 non-severe, 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. 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. 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 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. 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. A concurrent diagnosis of CAPA with COVID-19 is rare, and it is diagnosed after ICU admission or even after discharge from the hospital. We also diagnosed CAPA in our patients at a median of 11 days after ICU admission. 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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.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 treatment 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.

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 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.
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

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.
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