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A screening study for COVID-19-associated pulmonary aspergillosis in critically ill patients during the third wave of the pandemic

Berrin Er[1] | Ahmet Görkem Er[2] | Dolunay Gülmez[3] | Taha Koray Şahin[4] | Burçin Halaçlı[1] | Gamze Durhan[5] | Ebru Ortaç Ersoy[1] | Alpaslan Alp[3] | Gökhan Metan[2] | Zeynep Saribas[3] | Sevtap Arikan-Akdagli[3] | Gülşen Hazırolan[3] | Seda Banu Akıncı[6] | Macit Arıyürek[5] | Arzu Topeli[1] | Ömrüm Uzun[2]

[1]Division of Intensive Care, Department of Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey

[2]Department of Infectious Diseases and Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

[3]Department of Medical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

[4]Department of Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey

[5]Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

[6]Division of Intensive Care, Department of Anaestesiology and Reanimation, Hacettepe University Faculty of Medicine, Ankara, Turkey

Correspondence
Berrin Er, Division of Intensive Care, Department of Internal Medicine, Hacettepe University Faculty of Medicine, Mutlukent, 3450 Street, No: A3, 06800, Cankaya, Ankara, Turkey.
Email: berriner.md@gmail.com

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Abstract

Background: COVID-19-associated pulmonary aspergillosis (CAPA) has been reported as an important cause of mortality in critically ill patients with an incidence rate ranging from 5% to 35% during the first and second pandemic waves.

Objectives: We aimed to evaluate the incidence, risk factors for CAPA by a screening protocol and outcome in the critically ill patients during the third wave of the pandemic.

Patients/Methods: This prospective cohort study was conducted in two intensive care units (ICU) designated for patients with COVID-19 in a tertiary care university hospital between 18 November 2020 and 24 April 2021. SARS-CoV-2 PCR-positive adult patients admitted to the ICU with respiratory failure were included in the study. Serum and respiratory samples were collected periodically from ICU admission up to CAPA diagnosis, patient discharge or death. ECMM/ISHAM consensus criteria were used to diagnose and classify CAPA cases.

Results: A total of 302 patients were admitted to the two ICUs during the study period, and 213 were included in the study. CAPA was diagnosed in 43 (20.1%) patients (12.2% probable, 7.9% possible). In regression analysis, male sex, higher SOFA scores at ICU admission, invasive mechanical ventilation and longer ICU stay were significantly associated with CAPA development. Overall ICU mortality rate was higher significantly in CAPA group compared to those with no CAPA (67.4% vs 29.4%, $p < .001$).

Conclusions: One fifth of critically ill patients in COVID-19 ICUs developed CAPA, and this was associated with a high mortality.

KEYWORDS
aspergillus, CAPA, COVID-19, critical care, incidence, intensive care, risk factors, screening

Footnote: BE's current work address is Ankara City Hospital, Intensive Care Unit, Ankara.

1 | INTRODUCTION

In the last two decades, patients with severe viral respiratory tract infections have been reported to develop invasive pulmonary aspergillosis (IPA).[1,2] Direct damage to the airway epithelium inflicted by the respiratory viruses and several immunological mechanisms have been described to explain the pathogenesis.[3] Cases of influenza-associated pulmonary aspergillosis (IAPA) were reported during the H1N1 pandemic, with an incidence rate as high as 19% and a 90 days mortality rate of 51% in a retrospective multicentre study.[4] Since the beginning of the SARS-CoV-2 pandemic, several case series and observational studies of COVID-19-associated pulmonary aspergillosis (CAPA) have been published.[5] Similar to IAPA, CAPA was also associated with a prolonged ICU stay, and high mortality. The incidence of CAPA varied considerably contingent on the study design and diagnostic criteria used. Some studies reported an incidence as low as 5%, whereas a few studies using a screening protocol found up to 34% of ICU patients had CAPA.[6–9] Concerns over aerosolisation of respiratory secretions have restricted diagnostic procedures such as bronchoalveolar lavage. On the contrary, the use of different criteria for diagnosis of CAPA has challenged interpretation of available data. A recent screening study performed during the first pandemic wave reported an IPA incidence of 15% in critically ill patients infected with COVID-19 based on the European Confederation for Medical Mycology and the International Society for Human and Animal Mycology (ECMM/ISHAM) proposed consensus criteria.[10,11]

Through the pandemic, corticosteroids and other immunosuppressive drugs became routine therapeutic regimens for severe COVID-19 infection.[12] In addition to direct damage caused by SARS-CoV-2 in the lungs, the impact of anti-inflammatory and immunesuppressive treatments on CAPA remains to be determined and in the literature, the incidence data of CAPA of the 3rd wave and later, in which the treatment regimen was changed, is limited.

The purpose of this study was to determine CAPA incidence according to ECMM/ISHAM criteria in critically ill patients with an intensive screening procedure, and to define the risk factors for CAPA and outcome during the third wave of the pandemic.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

We conducted this prospective cohort study in two independent tertiary intensive care units designated for patients with severe COVID-19 infection, and run by the Departments of Internal Medicine and Anaesthesiology and Reanimation of Hacettepe University, School of Medicine. The organisation and ICU bed capacity changed during the study period (maximum ICU bed capacity for COVID-19 patients was 38) between 18 November 2020 and 24 April 2021. Adult patients with confirmed molecular diagnosis of SARS-CoV-2 infection admitted to the ICU because of acute respiratory failure were included in this study.

Demographic data, body mass index, comorbidities (based on clinician diagnosis), host factors according to The European Organisation for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSG) consensus,[13] clinical severity scores (acute physiology and chronic health evaluation (APACHE-II), sequential organ failure assessment score (SOFA) at 24 hours of ICU admission), partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) at admission, drugs used to treat COVID-19 (favipiravir, remdesivir, corticosteroids, anti-IL-6, anti-IL-1 antagonists), mechanical ventilation support, vasopressor use, renal replacement therapy, bacterial ventilator-associated pneumonia, antifungal therapy, duration between PCR positivity, ICU admission and CAPA diagnosis, duration of ICU and hospital stay, and mortality data were recorded.

2.2 | Screening procedure

In addition to routine laboratory tests, galactomannan (GM) levels were monitored in serum in all patients, and in nondirected bronchial lavage (NBL) fluid in intubated patients together with NBL fungal cultures (Figure 1). The first screening tests were performed on the Day 7 of SARS-CoV-2 PCR positivity. If the time interval was more than 7 days since PCR positivity to ICU admission, they were performed in 48 h of ICU admission. Due to concerns over aerosolisation, NBL was performed with a closed suction system by giving 20 ml sterile saline in intubated patients as previously described.[14] The patients were screened until CAPA diagnosis, discharged from the hospital or death (whichever came first). Radiological investigations including computed tomography of the thorax were performed at clinicians' discretion. Chest radiograms and computed tomograms of the thorax obtained within 72 h of CAPA diagnosis were re-evaluated by an independent radiologist. Patients were categorised as probable, possible and

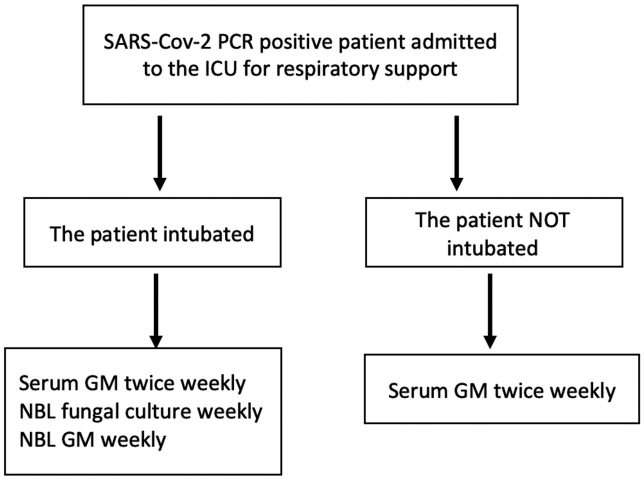

FIGURE 1 Screening protocol. The first samples were obtained on Day 7 of SARS-CoV-2 PCR positivity, or in 48 h of ICU admission if positivity was detected >7 days. ICU, intensive care unit; GM, galactomannan and NBL, nondirected bronchial lavage

non-CAPA according to ECMM/ISHAM criteria.[10] Clinical features of two groups (probable and possible CAPA vs non-CAPA) were compared.

This study was conducted in accordance with the amended Declaration of Helsinki. The study was approved by the Institutional Review Board of Hacettepe University (Date: 17 November 2020, GO 20/1121). Written informed consent was obtained from patients or their legal representatives.

2.3 | Mycological culture and direct examination of respiratory specimens

NBL specimens were examined under microscope after Gram and calcoflour white staining to detect fungal elements. They were cultured on Sabouraud dextrose agar (Oxoid,), inhibitory mould agar (Himedia) and blood agar (BBL, France), and incubated at 35 ± 2°C and 30 ± 2°C for 7 days. In case of mould growth, colonies were identified using macroscopic and microscopic morphology as well as matrix-assisted laser desorption/ionisation mass spectrophotometry method using MBT Filamentous Fungi Library (Bruker,).[15] Species identification was accepted when the identification score was greater than 2. Antifungal susceptibilities of the isolated *Aspergillus* strains were tested according to Clinical and Laboratory Standards Institute guidelines.[16]

2.4 | Galactomannan antigen detection

NBL and serum samples were aliquoted and stored at −80°C. GM antigen was detected with enzyme-linked immunosorbent assay (ELISA) (Euroimmun Medizinische Labordiagnostika AG,) according to the manufacturer's recommendations. The results were evaluated according to ECMM/ISHAM 2020 consensus criteria.[10] Results with an optical density index (ODI) ≥ 0.5 and ≥4.5 for serum and respiratory samples, respectively, were accepted as positive.

2.5 | Statistical analysis

The statistical analysis was performed using the statistical software package SPSS 23.0.0.2 (SPSS,). Median (interquartile range [IQR]) for non-normally distributed data and percentage for categorical variables were used. Continuous variables were compared using Mann–Whitney *U*-test, and Fisher's exact test and chi-squared test for categorical comparisons. We aimed to enrol a minimum of 196 patients with a precision of estimation (95% CI) between 10% and 20%, with 80% power if the incidence was 15%. Statistical significance was set at 2-sided $p < .05$ for all above analysis. Binary logistic regression models were used to identify risk factors for IPA. Factors with a *p value* below .05 in univariate analysis were included in the regression models after performing the Hosmer–Lemeshow goodness-of-fit test. Survival at 60 days follow-up was calculated with Kaplan–Meier, and log-rank test was used for comparing CAPA/non-CAPA groups and groups treated with anti-mould therapy or not in CAPA cohort.

3 | RESULTS

Three hundred and two consecutive patients were admitted to the COVID-19 ICUs between 18 November 2020 and 24 April 2021, the period which corresponded to the third wave of the pandemic. Two hundred and thirteen patients were eligible for the study (Figure 2). The mean age was 65.9 ± 13.2 years, and 87 patients (40.8%) were female. Hypertension (50.2%), diabetes mellitus (31.9%) and coronary artery disease (25.4%) were the most common comorbidities.

A total of 1196 serum samples were obtained for GM index determination, and a total of 298 respiratory samples were collected for direct microscopic examination, fungal culture and GM index during the study. Collected samples for fungal cultures and GM tests were analysed after the study period; thus, the results did not affect the clinician's decision. Overall, 43 (20.1%) patients (12.6% probable, 7.5% possible) fulfilled the CAPA definition according to ECMM/ISHAM criteria (Table 1). Median time to CAPA diagnosis post-PCR positivity and post-admission to ICU were 17 days[11–25] and 10 days,[4–17] respectively.

Baseline demographic features (age, sex), body mass index, underlying comorbidities, drugs used to treat COVID-19 and initial Horowitz Index were similar in two groups. Corticosteroids were used in 198 (92.9%) patients. The median cumulative dose of corticosteroids converted to methylprednisolone in CAPA group was 360 mg (320–900 mg) compared to 256 mg (160–359 mg) in the non-CAPA cohort ($p = 0.001$); however, the median cumulative dose of corticosteroids up to CAPA diagnosis (320 mg, IQR: 160–448 mg) was similar to those who did not develop CAPA ($p = 0.165$). In CAPA cohort, APACHE-II (18 vs 15, $p < .001$) and admission SOFA (6 vs 4, $p < .001$) scores were higher than those in non-CAPA patients. High flow nasal cannula (HFNC) (60.5% vs. 45.3%, $p = 0.08$), invasive mechanical ventilation (IMV) (88.4% vs. 38.2%, $p < .001$) for mechanical ventilation support, the use of vasopressors (69.8% vs. 31.8%, $p < .001$) and renal replacement therapy (34.9% vs. 11.2%, $p = 0.08$) were more common in the CAPA cohort.

All patients had chest X-ray scans, and 17 patients had computed tomography of the thorax within 72 h of CAPA diagnosis. Radiological findings were nonspecific in all of them except cavitary lesions in three patients. In 43 patients with CAPA, serum GM ODI was above 0.5 in 27 (62.8%), and NBL GM index was above 4.5 in 11 (25.6%) patients. *Aspergillus* spp. grew in 20 (46.5%) patients (*Aspergillus fumigatus* in 13, *Aspergillus niger* in 6, *Aspergillus terreus* in 2, *Aspergillus flavus and Aspergillus lentulus* 1 each). Seventeen patients were categorised as probable CAPA based on serum GM criterion alone.

Antifungal therapy was initiated based on clinical decision and test results obtained from the routine hospital laboratory: Seventeen

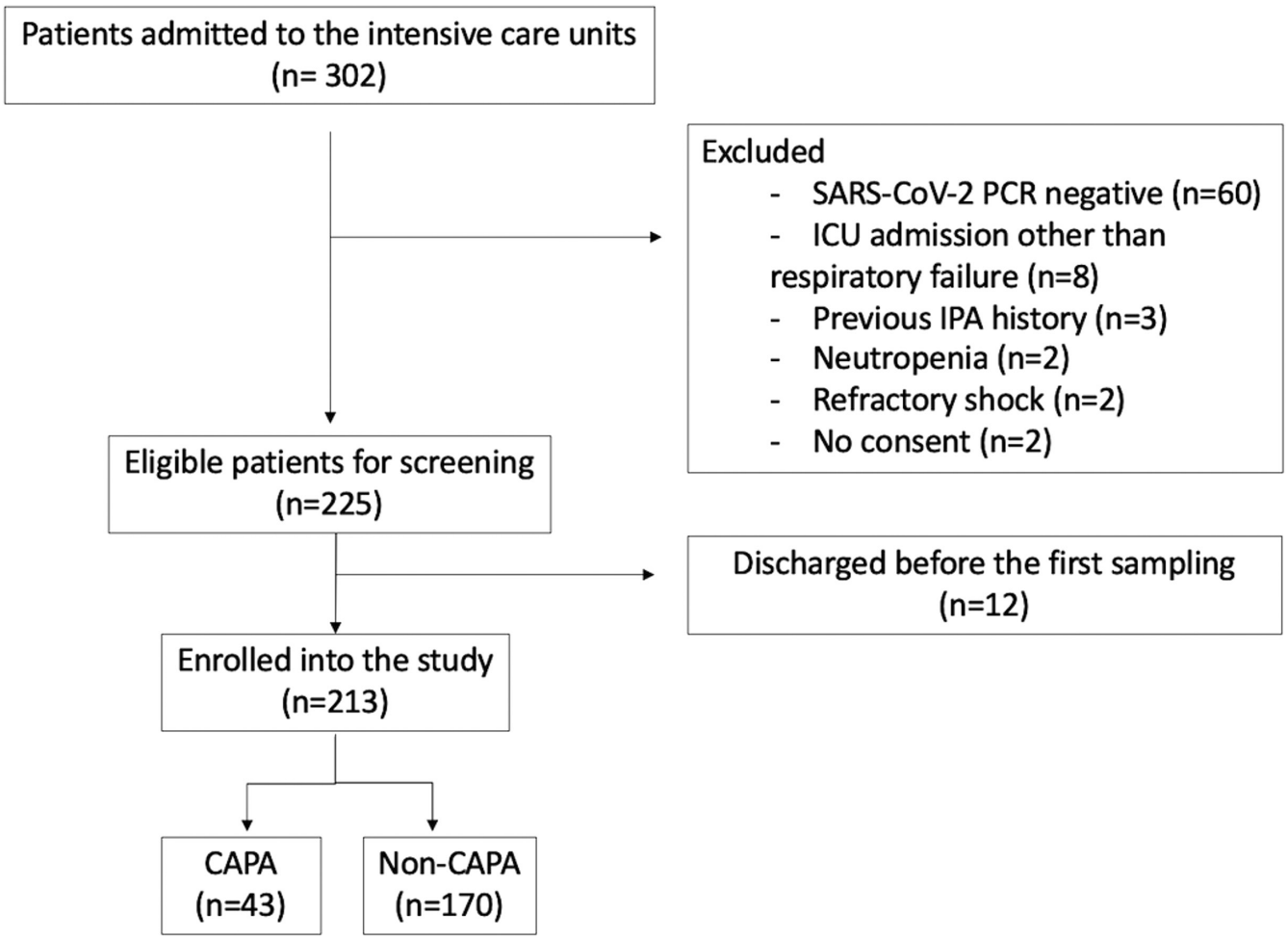

FIGURE 2 Flow chart of the study population. ICU, intensive care unit; IPA, invasive pulmonary aspergillosis and CAPA, COVID-19-associated pulmonary aspergillosis

(39.5%) patients with probable/possible CAPA received anti-mould treatment compared to 5.1% with non-CAPA. The median time to initiation of treatment was 4 days later (range: 0.5 days to 9.5 days) compared to the screening results. The ICU mortality rate was similar in patients who received an effective anti-mould drug or did not (70.6% vs. 65.4%, $p > .99$) in the CAPA cohort.

Patients diagnosed with probable/possible CAPA had a longer ICU stay (23 days vs. 10.5 days, $p < .001$) and hospital stay (29 days vs 17 days, $p < .001$). Gender, APACHE-II, SOFA, IMV, use of vasopressor, renal replacement therapy, use of cumulative steroid before CAPA development, bacterial ventilator-associated pneumonia and length of ICU stay were included in binary logistic regression model. The p value for the Hosmer–Lemeshow test was 0.40. Logistic regression analysis revealed that male sex, higher SOFA score at ICU admission, IMV and longer ICU stay were independently associated with CAPA (Table 2).

The length of ICU and hospital stays, and 28- and 60 days mortality rates were higher in the CAPA cohort. Survival analysis of patients with and without CAPA and patients who received or did not receive anti-mould therapy in CAPA cohort are shown in Figures 3 and 4 (log-rank test; $p < .001$, $p = 0.68$, respectively).

4 | DISCUSSION

In this prospective cohort study, we found that CAPA incidence was 20.1% in critically ill patients with COVID-19 during the third wave of the pandemic, a period when corticosteroids were used widely after the RECOVERY trial.[12] Male sex, higher SOFA scores, IMV and longer ICU stay were risk factors for CAPA development. Overall mortality was higher in the CAPA group.

In the study by Lahmer et al. performed during the first wave of the pandemic, CAPA incidence was reported 34% in 32 mechanically ventilated patients who were screened since ICU admission.[7] In that study, modified Asp ICU criteria were used with a cut-off value of GM ODI >1 in NBL samples for CAPA diagnosis. The patient population (all on mechanical ventilation) in addition to the different diagnostic criteria used may explain higher incidence of CAPA compared to our study. In a large-scale study corresponding to the first wave, CAPA incidence was reported 15% in patients in whom at least three fungal samples (respiratory or serum) were analysed and ECMM/ISHAM criteria were used.[11]

Median time to CAPA diagnosis since ICU admission was 4 days in Lahmer's study, and 8 days in the study by Gagneux et al. compared

TABLE 1 Clinical characteristics of the patients

Variable	CAPA Probable+possible (*n*: 43)	No CAPA (*n*: 170)	*p* value
Mean age, years, (SD)	68.5 (12.5)	65.2 (13.3)	.12
Female sex, *n* (%)	12 (27.9)	75 (44.1)	.06
Mean body mass index, (SD)	27.8 (8.3)	27.9 (5.8)	.93
Comorbidities, *n* (%)			
Hypertension	20 (46.5)	87 (51.2)	.61
Diabetes mellitus	16 (37.2)	52 (30.6)	.46
Coronary artery disease	13 (30.2)	41 (24.1)	.43
Congestive heart failure	10 (23.3)	23 (13.5)	.15
Solid organ tumour	10 (23.3)	21 (12.4)	.09
Chronic renal failure	8 (18.6)	14 (8.2)	.08
Chronic obstructive pulmonary disease	4 (9.3)	12 (7.1)	.74
Asthma	2 (4.7)	8 (4.7)	>.99
Collagen tissue disease	1 (2.3)	4 (2.4)	>.99
Chronic hepatic failure	0	3 (1.8)	>.99
Solid organ transplantation	1 (2.3)	1 (0.6)	.36
Haematologic malignancy	0	1 (0.6)	>.99
EORTC/MSGERC host factors, any, *n* (%)	9 (20.9)	30 (17.6)	.66
APACHE-II scores, median (IQR)	18 (15–22)	15 (12–18)	<.001
SOFA scores, median (IQR)	6 (4–8)	4 (3–6)	<.001
PaO_2/FiO_2, median (IQR)	125 (89–175) N:41	135 (100–196) N:167	.33
COVID-19-specific therapies, *n* (%)			
Favipiravir	41 (95.3)	164 (96.5)	.66
Cumulative corticosteroid, mg, median (IQR)	360 (320–900)	256 (160–359)	<.001
Cumulative corticosteroid, mg, median, before CAPA diagnosis (IQR)	320 (160–448)	256 (160–359)	.16
Anti-IL-6	2 (4.7)	12 (7.1)	.74
Anti-IL-1	1 (2.3)	2 (1.2)	.49
Remdesivir	1 (2.3)	5 (2.9)	>.99
Mechanical ventilation support, *n* (%)			
NIV	32 (75.4)	104 (61.2)	.11
HFNC	26 (60.5)	77 (45.3)	.08
IMV	38 (88.4)	65 (38.2)	<.001
Vasopressor use, *n* (%)	30 (69.8)	54 (31.8)	<.001
Renal replacement therapy, *n* (%)	15 (34.9)	19 (11.2)	.001
Concomitant bacterial ventilator-associated pneumonia, *n* (%)	31 (72.1)	43 (25.3)	<.001
Antifungal treatment, *n* (%)	17 (39.5) - 14 voriconazole - 3 L-AmB	9 (5.3) - 8 voriconazole - 1 L-AmB	<.001
ICU LOS, days, median (IQR)	23 (13–40)	10.5 (6–19)	<.001
Hospital LOS, days, median (IQR)	29 (19–41)	17 (11–30)	<.001
Mortality, *n* (%)			
ICU mortality	29 (67.4)	50 (29.4)	.001
Hospital mortality	30 (69.8)	55 (31.8)	<.001
28-day mortality	20 (46.5)	42 (24.7)	.008
60-day mortality	30 (69.8)	52 (30.6)	<.001

Abbreviations: APACHE, acute physiology and chronic health evaluation; CAPA, COVID-19-associated invasive pulmonary aspergillosis; EORTC/MSGERC, The European Organisation for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium; FiO_2, fractionated oxygenl; HFNC, high flow nasal cannula; ICU, intensive care unit; IMV, invasive mechanical ventilation; IQR, interquartile range; L-AmB, liposomal amphotericin B; LOS, length of stay; n, number; NIV, noninvasive ventilation; PaO_2, partial pressure of oxygen; SD, standard deviation; SOFA, sequential organ failure assessment.

TABLE 2 Risk factors for CAPA

Variables	Odds ratio	95% Confidence interval	*p* value
Male sex	2.59	1.079–6.205	.033
APACHE-II	0.93	0.839–1.021	.12
SOFA	1.25	1.012–1.535	.039
Use of cumulative steroid before IPA	1.00	0.998–1.001	.32
Invasive mechanical ventilation	5.07	1.269–21.213	.022
Vasopressor use	1.12	0.363–3.461	.84
Renal replacement therapy	1.03	0.345–3.073	.96
Concomitant bacterial ventilator-associated pneumonia	2.03	0.761–5.405	.16
Length of ICU stay	1.03	1.004–1.053	.023

Abbreviations: APACHE, acute physiology and chronic health evaluation; CAPA, COVID-19-associated invasive pulmonary aspergillosis; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; SOFA, sequential organ failure assessment.

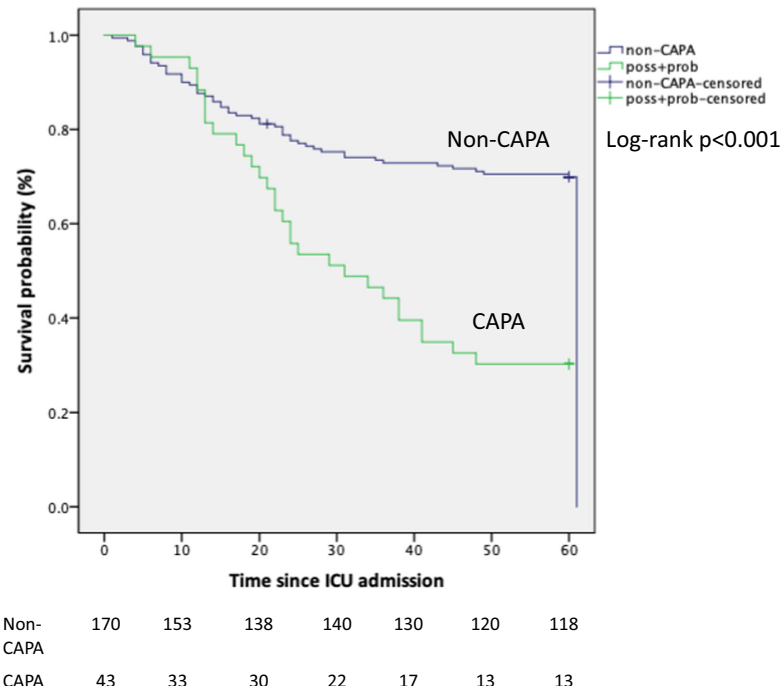

FIGURE 3 Survival at 60 days follow-up according to CAPA. CAPA: COVID-19-associated pulmonary aspergillosis, Pb: probable, poss: possible and ICU: intensive care unit

to 12 days and 20 days in retrospective studies.[8,17] In our study, median time to CAPA diagnosis was 10 days. This may be due to the study design—we performed the first fungal sampling on Day 7 of SARS-CoV-2 PCR positivity, as well as inclusion of patients not on mechanical ventilation which proved to be difficult to obtain respiratory samples.

It was emphasised that mortality can be reduced with early diagnosis and treatment of CAPA with a screening program in patients with COVID-19 in the ICU.[18] Defining the risk factors is important to predict vulnerable patients for CAPA earlier in time. It may enable to prioritise the use of anti-mould prophylaxis or intensive screening protocols in appropriate patients.[19] After excluding the patients with neutropenia, allogeneic stem cell transplantation and grade 3 or 4 acute graft-versus-host disease, we found no difference in host factors according to EORTC/MSG criteria. In our patient population, male sex, higher SOFA scores, invasive ventilation and longer ICU stay were independently associated with CAPA development.

To our knowledge, this is the first screening study during the third wave of the pandemic when corticosteroids and other immunosuppressive agents have become almost a routine clinical practice in critical patients in English literature. Corticosteroids were administered in the majority of our patients (92.9%). In a retrospective study by Fekkar et al, IPA incidence was 4.8% when 16.7% of ICU patients received corticosteroids for treatment of COVID-19.[6] According to the study by White et al, a strong association was detected between high-dose corticosteroid administration and multiple *Aspergillus* positive results.[18] In a multicentre retrospective cohort, administration of higher cumulative doses corticosteroid tended to be higher in CAPA group (OR 3.7, 95% CI 1.0–9.7).[20] In concordance with these results, the total incidence

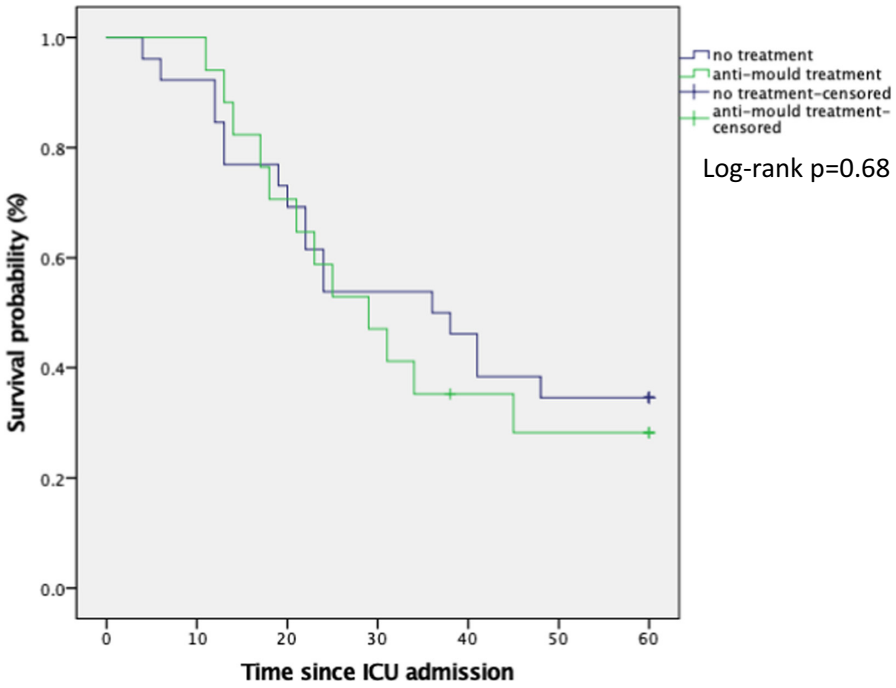

FIGURE 4 Survival at ICU discharge according to anti-mould treatment

of CAPA was reported as 9% in patients followed in the ICU in 2020, whereas there was an increase in the number of CAPA cases after dexamethasone was introduced as standard for early treatment.[21] The steroid effect may not have been shown since our study was conducted at a time when steroids were more frequently utilised and we did not have a structured control group in which steroids were not used. However, overall higher incidence compared to the studies in which the data of the first wave were shared may be related to widely used corticosteroids. In contrast to the previous multicentre studies, anti-IL-6 therapy was not detected as a risk factor in our study.[11,22]

Radiological findings were nonspecific in most of our patients in the CAPA cohort. As described in a recent review, the role of imaging for diagnosing CAPA is limited; however, computed tomography can contribute to identify other reasons for deterioration. New nodules with cavitation or halo sign, or consolidations can trigger a diagnostic work-up for CAPA.[23]

Fungal samples were analysed after the study period; therefore, they were not available to the clinician for patient management. The ICU team and consulting infectious diseases physicians started antifungal treatment based on clinical judgement and routine mycological tests ordered. Antifungal therapy was initiated in approximately 40% of our CAPA cohort. The mortality rates were similar in patients with probable/possible CAPA who received or did not receive antifungal drug effective against *Aspergillus* species. This lack of improved outcome was also reported in a recent screening study.[11] This might be related to late initiation of treatment in clinical practice when the results of screening were not available in real-time. In our study, there was a median of 4 days of delay (range: 0.5 days to 9.5 days) in real practice. Alternatively, the severity of the clinical conditions of patients with CAPA might have precluded a favourable effect of antifungal treatment on the overall outcome. The lengths of ICU and hospital stays, and overall mortality were higher in CAPA group compatible with the previous several studies.[4,24]

There are some limitations in our study. First, we used NBL for respiratory sampling as a useful and easy technique which showed good concordance with BAL in CAPA population previously.[25] However, ECMM/ISHAM consensus definitions require BAL, not NBL for the 'probable' category, patients with NBL positivity are directly included in the possible group. In addition, there are limited data to support the cut-off values recommended by the ECMM/ISHAM for GM in NBL to diagnose 'possible' cases. This may result in underestimation of the probable group in our study. Second, because the screening results were not available in the management of patients, we could not evaluate the efficacy of early diagnosis and treatment. This was beyond the scope of this study. Similarly, we did not have a control group of non-COVID-19 patients; therefore, we did not evaluate the impact of SARS-CoV-2 on development of IPA. Finally, this study was performed during the third wave. Viral evolution, emerging new variants and treatment modalities might cause different results in different time periods.

In conclusion, CAPA incidence is quite high in critically ill patients. Male sex, higher SOFA scores, invasive ventilation and longer ICU stay are risk factors for CAPA development. CAPA is associated with a higher mortality rate in critically ill patients. Further studies should be conducted to evaluate the impact of early diagnosis and treatment on patient outcome as well as prophylactic measures in SARS-CoV-2-infected patients at high risk for developing CAPA.

AUTHOR CONTRIBUTIONS

BE, AGE, OU, GM, SAA, AT and SBA designed the study. BE, AGE and TKS collected the data. SAA, DG, ZS, AA and GH performed the

microbiological analysis. GD and MA evaluated computed tomography scans and chest X-rays. BE and AGE performed the analysis. BH, EOE, OU, GM, SAA, AT, SBA, DG, ZS, AA, GH, GD and MA helped supervise the project. BE and AGE wrote the draft, and OU was the major contributor in writing the manuscript. All authors contributed to interpretation and writing the manuscript. All authors read and approved the final manuscript. BE and AGE should be considered joint first author.

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CONFLICT OF INTEREST

GM gave payment for presentations from Gilead, Pfizer, Merck, Sharp, and Dohme (MSD), 3 M, support for attending meetings from Gilead, Pfizer. SAA gave payment for presentation from Gilead, support for attending meetings Astellas. SBA gave multiple honoraria for lectures unrelated to the manuscript from own university. All other authors have nothing to disclose.

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.

ORCID

Berrin Er https://orcid.org/0000-0002-2256-3619
Dolunay Gülmez https://orcid.org/0000-0001-9021-0439
Gülşen Hazırolan https://orcid.org/0000-0003-4546-9729

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