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Opportunistic *Candida* Infections in Critical COVID-19 Patients

MİNE ALTINKAYA ÇAVUŞ[1]* and HAFİZE SAV[2]

[1] Department of Intensive Care, University of Health Sciences, Kayseri City Hospital, Kayseri, Turkey
[2] Department of Mycology, University of Health Sciences, Kayseri City Hospital, Kayseri, Turkey

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Abstract

The frequency of opportunistic fungal infections in critically ill patients whose intensive care unit stays are prolonged due to coronavirus disease 2019 (COVID-19) is higher than in the period before COVID-19. We planned this study to improve the management of *Candida* infections by defining the *Candida* species, the etiology of infections caused by *Candida* species, and the antifungal susceptibility of the species. This retrospective study included patients older than 18 hospitalized in the intensive care unit (ICU) with a definitive diagnosis of COVID-19 for seven months (from March 2021 to September 2021). All study data that we recorded in a standard study form were analyzed with TURCOSA (Turcosa Analytics Ltd. Co., Turkey, www.turcosa.com.tr) statistical software. The patients were evaluated in four groups as group 1 (candidemia patients, n = 78), group 2 (candiduria patients, n = 189), group 3 (control patients, n = 57), and group 4 (patients with candidemia in urine cultures taken before *Candida* was detected in blood culture, n = 42). *Candida* species were identified using both conventional and VITEK®2 (BioMérieux, France) methods. The antifungal susceptibility of fungi was determined using the E test method. Of the 5,583 COVID-19 patients followed during the study period, 78 developed candidemia, and 189 developed candiduria. The incidence of candidemia (per 1,000 admissions) was determined to be 1.6. As a result of statistical analysis, we found that *Candida albicans* was the dominant strain in candidemia and candiduria, and there was no antifungal resistance except for naturally resistant strains. *Candida* strains grown in blood and urine were the same in 40 of 42 patients. Mortality was 69.2% for group 1, 60.4% for group 2, and 57.8% for group 3. Antifungals were used in 34 (43.5%) patients from group 1, and 95 (50.2%) from group 2. In the candidemia group without antifungal use, mortality was quite high (77.2%). Antifungal use reduced mortality in the group 2 ($p < 0.05$). Length of ICU stays, comorbidity, broad-spectrum antibiotics, and corticosteroids are independent risk factors for candidemia in critically ill COVID-19 patients. Our study contributes to the knowledge of risk factors for developing COVID-19-related candida infections. The effect of candiduria on the development of candidemia in critically ill COVID-19 patients should be supported by new studies.

K e y w o r d s: opportunistic *Candida* infections, COVID-19, critical care

Introduction

The 2019 global coronavirus (COVID-19) pandemic has led to a crisis in many health and health care areas. The clinical course of the disease ranges from mild upper respiratory tract disease to acute respiratory distress syndrome (ARDS), needing mechanical respiratory support and hospitalization in an intensive care unit (ICU) (Arastehfar et al. 2020a). Critically ill patients with COVID-19 admitted to the ICU become susceptible to bacterial and ICU fungal pathogens that cause hospital-acquired infections (da Silva et al. 2019). A high mortality rate for COVID-19 patients co-infected with pathogenic fungi has been reported (Arastehfar et al. 2020b).

Candida species cause 8–10% of all bloodstream infections (Wisplinghoff et al. 2004). Candidemia occurs in approximately 30–35% of critical care patients (Méan et al. 2008). High Acute Physiology and Chronic Health Assessment (APACHE) II score, use of broad-spectrum antibiotics, accompanying bacterial infection, parenteral nutrition, diabetes mellitus (DM), kidney failure, pancreatitis, hemodialysis, mechanical ventilation, a central vascular catheter (CVC), and immunosuppressive therapy are well-known risk factors for hospital candidemia (Mermutluoglu et al. 2016). There is growing evidence that the incidence of candidemia is higher after COVID-19 than before COVID-19 (Nucci et al. 2021). The mortality rate is high in COVID-19 patients with candidemia. In spite of

* Corresponding author: M. Altınkaya Çavuş, Department of Intensive Care, University of Health Sciences, Kayseri City Hospital, Kayseri, Turkey; **e-mail: minealtinkaya@yahoo.com**

anti-fungal treatment, mortality reached 83% (Villanueva-Lozano et al. 2021).

Candiduria is common in inpatients, and the development of infection is due to colonization by perineal or catheter *Candida* species, and a cure is not required (Revankar et al. 2011). Treatment is recommended only in immunocompromised individuals and patients with the anatomical disease (Pappas et al. 2016). The simultaneous existence of *Candida* species in blood and urine may mean the diffusion of infection through the same entrance portal. However, it can also be two independent situations (Drogari-Apiranthitou et al. 2017).

Understanding the etiological factors and antifungal susceptibility of COVID-19 patients with candiduria and candidemia is crucial for the optimal management of COVID-19 patients. This study was conducted in the hope of improving patient outcomes. It was planned to determine the risk factors, *Candida* species antifungal susceptibility, and distribution of the species in COVID-19 patients who developed candiduria and candidemia.

Experimental

Materials and Methods

This study was performed in COVID-19 ICUs of a Health Sciences University Kayseri City Hospital and was approved by the Health Sciences University Kayseri City Hospital Ethics Committee. This retrospective study included patients older than 18 hospitalized in the intensive care unit (ICU) with a definitive diagnosis of COVID-19 for seven months (30 March 2021 to 30 September 2021).

In routine clinical follow-up, a standardized patient form was used to track COVID-19 patients, all laboratory and clinical characteristics of the patients, diurnal changes in their clinical condition, and treatments managed. Data for this study were recorded prospectively. Intensive care specialists followed all patients in ICU up diurnal until death or recovery.

The patient form included demographic characteristics (gender, age, weight, and height), and underlying comorbidities of the patients (diabetes mellitus, hypertension, coronary artery disease, chronic/acute renal failure, chronic obstructive pulmonary disease, neurological disease, malignancy, transplantation, goiter and other diseases), radiological information, and laboratory test results. Culture outcomes were monitored closely. Invasive procedures, including urinary catheter, invasive mechanical ventilation, a central venous catheter (CVC), total parenteral nutrition (TPN), and other risk factors for candidemia and candiduria, were recorded. In addition, antibiotics, corticosteroids, anti-cytokine therapy, and immunosuppressive drugs were recorded. Furthermore, length of ICU stay, length of hospital stay, Sequential Organ Failure Assessment (SOFA) score (Jones et al. 2009), Acute Physiology and Chronic Health Assessment (APACHE) II score (Knaus et al. 1985), mortality rates, and identification of *Candida* species, and their antifungal susceptibility were recorded.

Patients with *Candida* growth in urine and blood cultures during their hospitalization were identified by querying the pathogen database maintained by the Mycology Department. COVID-19 was described based on positive real-time polymerase chain reaction (PCR) (Bioeksen, Turkey) tests and computed tomography (CT) images for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Study definitions. Patients with both candidemia and candiduria were identified and diagnosed with COVID-19. Patients with at least one *Candida* strain isolated in their blood and urine cultures 48 hours after acceptance to the intensive care unit were included in the study. Candidemia was defined as a blood culture positive for *Candida* species; candiduria was defined as a urine culture positive for *Candida* species with ≥ 10,000 CFU/ml. If more than one of the same agents was isolated in the blood culture taken from a patient simultaneously, this was considered as a single growth. Patients with candiduria were identified and analyzed for study variables. Positive urine cultures within one week before the reproduction date of the patients with *Candida* growth in their blood cultures were recorded. Patients with candidemia were defined as group 1, with candiduria as group 2, while patients with no candidemia/candiduria during their hospital stay were reported as group 3. Patients with candidemia in the urine cultures taken before *Candida* species growth in the blood culture were defined as group 4.

Case/control matching. A control group was determined by randomly selecting one out of four ordinary patients with a diagnosis of COVID-19. They were accepted to a tertiary pandemic intensive care unit, had no reported candidemia/candiduria during their hospital stay, and were for whom complete clinical data was available (Group 3). Groups 1 and 2 were compared with group 3, the control group, in terms of all variables recorded for the study.

Mycological examination. Blood samples sent from various hospital departments to the microbiology laboratory were incubated in the BacT/Alert 3D Automation System (bioMérieux, France). When a positive signal was obtained from the BACTEC automatic blood culture system, inoculation was made from vials in which yeast cells from Sabouraud Dextrose Agar (SDA; Oxoid, England) culture media (without or with antibiotics) were seen by Gram-staining. The isolates were identified by the germ tube test and VITEK® 2 (bioMérieux, France), and morphological images were obtained

for the isolates grown on Tween-80-corn-meal agar. The guidelines for *in vitro* susceptibility of *Candida* species were taken from the Clinical and Laboratory Standards Institute document M27-A3 (CLSI 2008).

All isolates were cultured using SDA (Oxoid, United Kingdom). These isolates were tested for susceptibility against fluconazole (FLC), amphotericin B (AMB), caspofungin (CAS), and voriconazole (VRC) by the E-test (bioMérieux, France). Minimal inhibitory concentrations (MICs) of azole were the lowest concentrations providing an 80% reduction in growth. MICs of AMB were determined as the lowest concentration inhibiting any growth. MICs were also determined by the E-test method according to the manufacturer's guidelines. E-test strips of FLC (0.016–256 μg/ml), AMB (0.002–32 μg/ml), CAS (0.002–32 μg/ml), and VRC (0.002–32 μg/ml) were placed perpendicular to each other on an RPMI 1640 medium (Sigma Chemical Company, USA) plate. In both tests, quality control was performed by the CLSI document M27-A3, using *Candida parapsilosis* ATCC® 22019™ and *Candida krusei* ATTC® 6258™ (CLSI 2008).

Statistical analysis. Histogram, Q-Q plots, and Shapiro-Wilk's test were applied to assess the data normality. Levene's test was used to test variance homogeneity. To compare the demographic and clinical parameters among the study groups, one-way analysis of variance (ANOVA) or Kruskal-Wallis *H* tests were applied for continuous variables, while Pearson chi-square analysis or Fisher-Freeman-Halton test were used for categorical variables. Bonferroni adjusted Dunn's test, and Bonferroni adjusted *z* tests were performed for multiple comparison analysis. The risk factors of candidemia in Covid-19 patients were identified by univariate and multiple binary logistic regression analysis. Significant variables at $p < 0.25$ contingency level were included to the multiple models, and forward elimination was performed using Wald statistics to identify the independent risk factors of candidemia. The Hosmer-Lemeshow test and Nagelkerke's R^2 statistics assessed the model's goodness-of-fit. Analyses were conducted using the statistical software of TURCOSA (Turcosa Analytics Ltd Co, Turkey, www.turcosa.com.tr). A *p*-value less than 5% was considered statistically significant.

Results

Of the 5,583 COVID-19 patients followed during the study period, 78 developed candidemia, and 189 developed candiduria. The incidence of candidemia (per 1,000 admissions) was determined to be 1.6. Within the specified period, 78 COVID-19 patients with *Candida* growth in a total of 89 blood cultures were defined as group 1, and 189 COVID-19 patients with *Candida* growth in 209 urine cultures were defined as group 2. Randomly selected COVID-19 patients, who did not grow *Candida* in their cultures, formed a total of 57 patients in the control group, group 3. 42 candidemia patients with *Candida* growth in urine culture before *Candida* growth in blood culture were named as group 4.

There were significant differences in gender and age between group 1, group 2, and group 3. Male gender dominated in group 3, which had a lower mean age ($p < 0.05$). When group 1 and group 2 were compared with group 3 longer length of stay in hospital and ICU was statistically significant ($p < 0.05$). There was no difference between the groups concerning APACHE II, SOFA scores, and mortality. Central venous catheter use was higher in the *Candida* growing groups (especially femoral catheters). The use of catheters was statistically significant for both candidemia and candiduria and was determined to be a risk factor. In the candiduria and candidemia groups, HT, goiter, malignancy, and neurological disease were more common than in the control group ($p < 0.05$). Mortality in group 1 and group 2 was higher than in group 3. However, this difference was not statistically significant. Intubation was less in the control group than in the other groups ($p < 0.05$). The use of broad-spectrum antibiotics (BSA), corticosteroids, and total parenteral nutrition (TPN) were also higher in the *Candida* growing groups ($p < 0.05$). Interleukin 6 (IL 6) receptor inhibitor use was higher in the candidemia group, but this difference was not statistically significant. Antifungal use was found to be 34 (43.5%) and 95 (50.2%) in groups 1 and 2, respectively. In the candidemia group, mortality was found to be relatively high (77.2%) in patients who did not use antifungals. Antifungal use reduced mortality in the candiduria group (Group 2). This difference in mortality was statistically significant ($p < 0.05$) (Table I). While the *p*-value in the last column expressed the comparison between groups, the *p*-value in the bottom represented the comparisons of AF+/mortality and AF-/mortality for each group.

Response variable categories (0: Control/Group 3, 1: Candidemia/Group 1) were taken when performing logistic regression. In the multiple analyses, the OR (95% CI) of ICU stay, comorbidity, and BSA were 1.06 (1.01–1.11), 6.94 (2.01–23.90), and 90.68 (21.84–376.53), respectively. For the built multiple models, Nagelkerke's R^2 statistic was calculated as 0.731. ICU stay, comorbidity, and BSA variables in the multiple models predicted 74.5% of the variability of candidemia. The Hosmer-Lemeshow test resulted as $\chi^2 = 6.247$, $p = 0.620$. These results reveal the built multiple binary logistic regression model's appropriateness in predicting candidemia in Covid-19 patients (Table II).

Of the *Candida* species (n = 78), 43 (55.1%) were *C. albicans,* 14 (17.9%) were *C. parapsilosis,* 9 (11.5%) were *Candida tropicalis*, 7 (9%) were *Candida glabrata,*

Table I
Comparison of the demographic and clinical characteristics among the study groups.

Variable	Groups			$p^{\dagger}$
	Group 1 (n = 78)	Group 2 (n = 189)	Group 3 (n = 57)	
Gender (male)	54 (69.2)[a]	87 (46)[b]	29 (50.9)[b]	**0.002**
Age (year)	71.33 ± 13.67[ab]	73.42 ± 11.34[a]	68.51 ± 15.74[b]	**0.035**
APACHE II score	12 (7–19)	12 (7–19)	14 (9–19)	0.485
SOFA score	4 (2–6)	4 (2–6)	4 (3–6)	0.334
Hospital stay (day)	27 (18–43)[a]	22 (15–32)[b]	18 (13–26)[b]	**< 0.001**
ICU stay (day)	19 (10–27)[a]	14 (8–22)[ab]	10 (7–15)[b]	**< 0.001**
PCR (positive)	50 (64.1)	128 (67.7)	36(63.1)	0.889
Comorbidities	66 (84.6)[a]	162 (85.7)[a]	28(49.1)[b]	**< 0.001**
DM	23 (29.4)	70 (37)	16(28)	0.305
HT	30 (38.4)[ab]	97(49.7)[a]	17 (29.8)[b]	**0.008**
COPD	14 (17.9)[a]	35 (18.5)[a]	3 (5.2)[b]	**0.049**
CAD	23 (29.4)	40 (21.1)	8 (14)	0.093
Malignancy	10 (12.8)[a]	15 (7.9)[a]	0 (0.0)[b]	**0.022**
Goitre	6 (7.7)[a]	2 (1.0)[b]	1 (1.7)[ab]	**0.010**
Kidney transplantation	0 (0.0)	2 (1.0)	0 (0.0)	0.487
Immunological disease	3 (3.8)	2(1.0)	0 (0.0)	0.142
Neurological disease	13 (16.6)[a]	30 (15.8)[a]	2 (3.5)[b]	**0.044**
CKF/AKF	14 (17.9)	26 (13.7)	6 (10.5)	0.226
Mortality	54 (69.2)	133 (60.4)	33 (57.8)	0.208
Intubation	49 (62.8)[a]	107 (85.2)[a]	21 (36.8)[b]	**0.008**
Central Catheter	42 (53.9)[a]	58 (30.7)[b]	7 (12.2)[c]	**< 0.001**
Juguler	13 (30.9)	18 (31.0)	4 (57.1)	0.139
Femoral	23 (54.7)	31 (53.4)	2 (28.5)	**< 0.001**
Dialysis	6 (14.2)	9 (15.5)	1 (14.2)	0.286
BSA	75 (96.1)[a]	178 (94.1)[a]	12 (21.0)[b]	**< 0.001**
Corticosteroid	49 (62.8)[a]	142 (75.1)[b]	15 (26.3)[c]	**< 0.001**
IL-6 receptor inhibitors	7 (9.0)	7 (3.7)	3 (5.2)	0.214
TPN	24 (30.8)[a]	59 (31.2)[a]	5 (8.7)[b]	**0.003**
AF	34 (43.5)	95 (50.2)	–	0.192
AF+/mortality	20 (58.8)	56 (58.9)	–	0.511
AF–/mortality	34 (77.2)	76 (80.8)	–	0.803
$p^{\ddagger}$	0.080	**< 0.001**		

Values are expressed as n (%), mean ± SD or median (1^{st}–3^{rd} quartiles). Different superscripts among groups indicate a statistically significant difference between groups. Significant results are shown in bold. $p^{\dagger}$ – significance value for the between-group comparisons, $p^{\ddagger}$ – significance value for the within-group comparisons
TPN – total parenteral nutrition, BSA – broad-spectrum antibiotic, DM – diabetes mellitus, HT – hypertension, COPD – chronic obstructive pulmonary disease, CAD – coronary artery disease, CKF/AKF – chronic kidney failure/acute kidney failure, AF – antifungal, AF+ – antifungal use, AF– – no antifungal use, APACHE II score – acute physiology and chronic health evaluation score, SOFA – sequential organ failure assessment score, ICU – intensive care unit, IL-6 – interleukin-6

and 5 (6.4%) were other *non-albicans Candida* in group 1. Of the *Candida* species in group 2 (n = 189), 123 (65.1%) were *C. albicans*, 29 (15.3%) were *C. parapsilosis*, 22 (11.6%) were *C. tropicalis,* 9 (4.7%) were *C. glabrata*, and 6 (3.2%) were other *non-albicans Candida*. 53.8% (n = 42) were patients with *Candida* growth in their urine culture before *Candida* growth in the blood (Group 4). Of the *Candida* species in group 4 (n = 42), 26 (61.9%) were *C. albicans,* 10 (23.8%) were *C. parapsilosis*, 3 (7.1%) were *C. tropicalis*, and 3 (7.2%) were other *non-albicans Candida*. *Candida* strains grown in blood and urine were the same in 40 (95.2%) patients.

Fluconazole was the most commonly used antifungal in all groups (Table III). The Intensive Care Special-

Table II
Univariate and multiple binary logistic regression analysis in identifying candidemia in Covid-19 patients.

Variable	Univariate		Multiple	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Gender (female/male)	2.17 (1.07–4.41)	**0.032**	–	–
Age (year)	1.01 (0.99–1.04)	0.269	–	–
APACHE II score	0.97 (0.93–1.02)	0.181	–	–
SOFA score	0.93 (0.83–1.05)	0.224	–	–
Hospital stay (day)	1.04 (1.02–1.07)	**0.002**	–	–
ICU stay (day)	1.05 (1.02–1.09)	**0.002**	1.06 (1.01–1.11)	**0.025**
Comorbidities	5.70 (2.55–12.74)	**< 0.001**	6.94 (2.01–23.90)	**0.002**
DM	1.07 (0.50–2.28)	0.858	–	–
HT	1.47 (0.71–3.05)	0.299	–	–
COPD	3.94 (1.08–14.43)	**0.039**	–	–
CAD	2.56 (1.05–6.25)	**0.039**	–	–
Goitre	4.67 (0.55–39.89)	0.159	–	–
Neurological disease	4.52 (0.96–21.24)	0.056	–	–
CKF/AKF	1.86 (0.67–5.18)	0.235	–	–
Intubation	2.90 (1.43–5.88)	**0.003**	–	–
Central Catheter	8.33 (3.36–20.65)	**< 0.001**	–	–
BSA	93.75 (25.09–350.24)	**< 0.001**	90.68 (21.84–376.53)	**< 0.001**
Corticosteroid	4.73 (2.24–9.99)	**< 0.001**	–	–

Significant results are shown in bold.
TPN – total parenteral nutrition, BSA – broad-spectrum antibiotic, DM – diabetes mellitus,
HT – hypertension, COPD – chronic obstructive pulmonary disease, CAD – coronary artery disease,
CKF/AKF – chronic kidney failure/acute kidney failure,
APACHE II score – acute physiology and chronic health evaluation score,
SOFA – sequential organ failure assessment score, ICU – intensive care unit,
OR – Odds ratio, CI – Confidence interval

ist started all fluconazole treatment empirically. In our hospital, antifungals other than fluconazole are provided with the Infectious Diseases Specialist's report and added to the treatment according to fungus species from the culture.

The antifungal susceptibility tests for the 78 yeast isolates included in the study are summarized in Table IV, and the relevant MIC values for antifungal resistance were not found against *C. albicans*, *C. tropicalis*, or *C. parapsilosis* and low MICs levels were observed against all antifungal agents. Seven isolates of five *C. glabrata* specie isolates had dose-dependent sensitivity to fluconazole. Voriconazole was determined to be the most sensitive drug based on antifungal MIC 90 values (Table IV).

Discussion

This study aimed to determine the risk factors for diagnosed *Candida* infections in critically ill patients with COVID-19, as well as the epidemiology and antifungal sensitivity of isolated *Candida* species. This study has some limitations; first, it was a retrospective analysis of a single center, and, therefore, was subjected to the limitations of retrospective analysis.

Demographic results, laboratory values, and risk factors for COVID-19 patients who acquired *Candida* infections in the intensive care unit were examined. A study published 2021 reported that the incidence of candidemia in the COVID-19 period increased two times compared to the pre-COVID-19 period (Kayaaslan et al. 2021). In our study, the incidence of candidemia (per 1,000 admissions) was higher (1.6 in 2021) during the pandemic period than (0.61 in 2019) before. The incidence of candidemia was reported as 2.34 (Omrani et al. 2021), 4.4 (Kayaaslan et al. 2022) episodes per 1,000 ICU days in COVID-19 patients.

When all three groups were compared, and only candidemia cases were evaluated, it was determined that male gender, intensive care and hospital length of stay, intubation, TPN, and central venous catheter use were statistically significant ($p < 0.05$). It was previously known that endogenous colonization and invasive procedures, such as intubation and catheter use that develop after a prolonged stay in hospital increase, the

Table III
Candida species distribution and frequency of antifungal use in the study groups.

	Total	FLC	CAS	ANI	MIC	AMB
Group 1						
n	78	12	11	3	3	5
C. albicans	43 (55.1)	6 (50.0)	5 (45.5)	0 (0.0)	1 (33.3)	3 (60.0)
C. dubliniensi	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. glabrata	7 (9.0)	1 (8.3)	1 (9.1)	1 (33.3)	0 (0.0)	0 (0.0)
C. krusei	3 (3.8)	1 (8.3)	0 (0.0)	0 (0.0)	2 (66.7)	1 (20.0)
C. lusitaniae	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. parapsilosis	14 (17.9)	3 (25.0)	3 (27.3)	1 (33.3)	0 (0.0)	0 (0.0)
C. tropicalis	9 (11.5)	1 (8.3)	2 (18.2)	1 (33.3)	0 (0.0)	1 (20.0)
Group 2						
n	189	46	32	9	4	4
C. albicans	123 (65.1)	34 (73.9)	22 (68.8)	7 (77.8)	3 (75.0)	0 (0.0)
C. glabrata	9 (4.7)	1 (2.2)	1 (3.1)	1 (11.1)	1 (25.0)	1 (25.0)
C. kefyr	2 (1.1)	2 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
C. krusei	2 (1.1)	0 (0.0)	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
C. lusitaniae	1 (0.5)	0 (0.0)	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
C. parapsilosis	29 (15.3)	4 (8.7)	3 (9.4)	0 (0.0)	0 (0.0)	1 (25.0)
C. tropicalis	22 (11.6)	5 (10.9)	4 (12.5)	1 (11.1)	0 (0.0)	1 (25.0)
Group 4 (53.8% of candidemia)						
n	42	9	6	1	0	3
C. albicans	26 (61.9)	5 (55.6)	4 (66.7)	0 (0.0)	0 (0.0)	3 (100.0)
C. parapsilosis	10 (23.8)	3 (33.3)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
C. glabrata	1 (2.4)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
C. tropicalis	3 (7.1)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. krusei	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. duplensie	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Values are expressed as n (%).
FLC – fluconazole, CAS – caspofungin, ANI – anidulafungin, MIC – micafungin, AMB – amphotericin B

Table IV
Antifungal results against Candida species, reproduced in blood.

Species	VRC µg/ml			FLC µg/ml			CAS µg/ml			AMB µg/ml		
	GM	MIC_{50}	MIC_{90}	GM	MIC_{50}	MIC_{90}	GM	MIC_{50}	MIC_{90}	GM	MIC_{50}	MIC_{90}
C. albicans (n = 42)	0.42	0.25	0.75	2.42	2	4	0.74	0.75	1	0.59	0.25	1.5
C. parapsilosis (n = 13)	0.39	0.25	0.5	4.07	4	8	1.46	1	2	0.84	0.75	1.5
C. glabrata (n = 7)	0.42	0.47	0.5	22.5	16	48	1.02	1.5	1.5	0.53	0.5	0.75
C. tropicalis (n = 9)	0.35	0.38	0.5	3.22	2	4	0.75	0.5	2	0.91	0.38	2
C. krusei (n = 3)	0.21	0.25	0.38	74.6	64	128	0.75	0.75	1	0.59	0.75	1

VRC – voriconazole, FLC – fluconazole, CAS – caspofungin, AMB – amphotericin B,
GM – geometric mean, MIC – minimal inhibitory concentration

risk of candidemia (Eggimann et al. 2015). In studies on COVID-19 patients, 70–90% of patients received antimicrobial therapy, and only 10% had fungal or bacterial infections (Lai et al. 2020; Rawson et al. 2020). It was concluded that long-term use of broad-spectrum antibiotics used in intensive care patients with a diagnosis of COVID-19 is a significant risk factor for fungal development (Coşkun and Durmaz 2021). In our study, antibiotic treatment was initiated empirically for each patient, and combined broad-spectrum antibiotics were used. The use of BSA was found to be an independent risk factor for the development of candidemia.

Some studies identified various comorbid conditions and risk factors for the development of candidemia

in COVID-19 patients. A study in Brazil revealed that it was associated with chronic obstructive pulmonary disease and candidemia in patients with COVID-19 (Bastos et al. 2020). In another study, the prevalence of candidemia and diabetes mellitus were reported to be important in patients with COVID-19 (Chowdhary et al. 2020). In this study, chronic obstructive pulmonary disease, hypertension, chronic arterial disease, goiter, and malignancy were associated with candidemia and candiduria ($p < 0.05$). Systemic corticosteroid therapy reduces mortality and improves clinical outcomes in hospitalized patients with COVID-19 (van Paassen et al. 2020). Interleukin-6 receptor antagonists have been shown to be effective in treating patients with COVID-19 with mild and severe cytokine syndrome (Zhang et al. 2020). Corticosteroids are currently the norm of care for patients hospitalized due to COVID-19 and other immunosuppressive agents (e.g., tocilizumab, etanercept (Vallabhaneni and Chiller 2016)) are used in certain groups of subjects, potentially increasing the risk of opportunistic fungal infection (Saha et al. 2020; Segrelles-Calvo et al. 2021; Seagle et al. 2022).

Immunosuppressive agents have been demonstrated to allow the development of viral infections in treated patients, as reported for oncogenic viruses. Indeed, this notion corroborates that the immunosuppressive agents increased the risk of opportunistic fungal infection (Rotondo et al. 2017).

Our study identified corticosteroid use as a risk factor for candidemia and candiduria in patients with COVID-19. Concurrently, we examined the effects of interleukin-6 receptor antagonist (tocilizumab) on the development of fungal infections in our patients. Fourteen patients used tocilizumab, and no relationship was identified between tocilizumab and *Candida* infections.

Sepsis, including fungal sepsis, is generally defined as life-threatening organ dysfunction caused by dysregulated systemic host inflammatory responses to microbial infection (Esposito et al. 2017). Opportunistic *Candida* infections activate the inflammasome system. The inflammasome is a large cytoplasmic complex within the innate immune system. It performs the stimulation of inflammatory caspase 1–5. The formation of an inflammasome triggers inflammation. Inflammasome activation also plays a role in the development of programmed lytic cell death (pyronecrosis/pyroptosis). Phagocyte damage may benefit the fungus by host cell lysis (Kasper et al. 2018). Since our study was planned retrospectively, we could not obtain information about the pathogenesis of *Candida* infections.

Candida species colonize or develop infections in ICU patients when urinary tract catheters are inserted. The simultaneous presence of *Candida* in blood and urine may mean spreading infection through the same portal of entry (Drogari-Apiranthitou et al. 2017). Candiduria is not a trigger for candidiasis in many ICU patients, but multivariate analyses have confirmed that it is a risk factor for invasive disease (Kauffman 2005; Binelli et al. 2006). In this retrospective study, concurrent candiduria was present in 42 (53.8%) of candidemia. In 40 of 42 cases with positive *Candida* cultures in the bloodstream and urine, the species involved were the same, but we cannot presume that the portal of infection was the same. The second limitation in our study is the lack of genetic analysis of *Candida* species isolated from blood and urine.

C. albicans was the most frequently isolated species in Turkey, Greece, and Iran (Arastehfar et al. 2021; Kayaaslan et al. 2021; Kokkoris et al. 2021). A study in India reported that *Candida auris* species were also encountered in addition to the common species of *Candida* (Niyas et al. 2021). A study conducted in the United States determined that the most frequently isolated species was the *non-albicans Candida* (Bishburg et al. 2021). In our study, *C. albicans* appeared to be the most frequently isolated species in candidemia and candiduria. In particular, three *C. krusei* and seven *C. glabrata* were part of the species responsible for candidemia. Epidemiological data on candidemia in COVID-19 patients may differ between countries. Although this could not be fully explained, it was thought that the treatment protocols used by clinics and patient sub-diseases could be effective.

In our study, an E-test was performed for *Candida* species. The relevant MIC values for antifungal resistance were not found against *C. albicans*, *C. parapsilosis*, or *C. tropicalis,* and low MICs levels were observed against all antifungal agents. Seven isolates of five *C. glabrata* species isolates had dose-dependent sensitivity to fluconazole. A significant antifungal resistance profile was not found based on the MIC values. Antifungal use was found to be 34 (43.5%), and 95 (50.2%) in groups 1 and 2, respectively. Fluconazole was used as an *in vivo* antifungal in both groups. In the candidemia group, mortality was found to be relatively high (77.2%) in patients, who did not use antifungals. Antifungal use reduced mortality in the candiduria group (Group 2). This difference in mortality was statistically significant ($p < 0.05$).

The mortality rate was 80% in patients, who developed candidemia and were followed up in the tertiary ICU due to COVID-19 (Coşkun and Durmaz 2021). According to reports from Italy, 50% mortality has been reported in COVID-19 patients with candidemia (Mastrangelo et al. 2021). It has been reported that mortality can be reduced when COVID-19 treatment is combined with specific antifungals against *Candida* species in severe COVID-19 patients taking immunosuppressant (Segrelles-Calvo et al. 2021). In our study, mortality was 69.2% in group 1, 60.4% in group 2, and 57.8% in group 3. In addition, when we looked at the

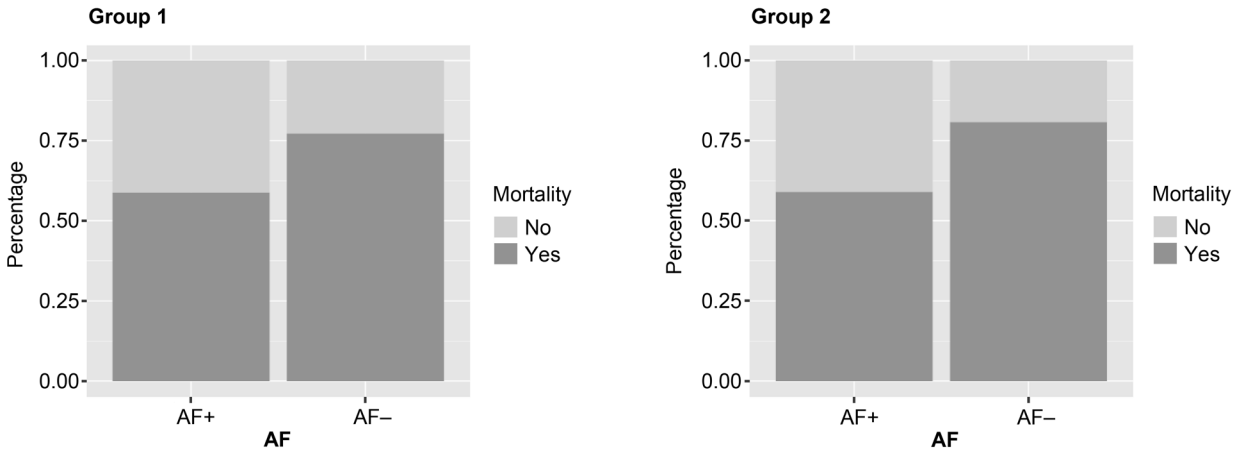

Fig. 1. Antifungal (AF) use/mortality graph.

effect of antifungal use on the groups on mortality, we saw that antifungals reduced mortality (Fig. 1). Especially in group 2, the decrease in mortality was statistically significant ($p < 0.05$).

In conclusion, our analysis found that *Candida* infections in patients with COVID-19 are associated with multifactorial risks. Besides the above risk factors, a certain number of candiduria was found in these patients before the candidemia diagnosis. Based on this result, although we believe that candiduria does not trigger candidemia, we still think that clinicians should keep this in mind, particularly in critical situations. With respect to epidemiological data, *C. albicans* was the most common species for candidemia and candiduria. Multiple-drug resistance was not found. Ongoing surveillance of *Candida* infections will be essential to inform changes in epidemiological characteristics and antifungal susceptibility in countries.

ORCID
Mine Altınkaya Çavuş https://orcid.org/0000-0003-2584-0463
Hafize Sav https://orcid.org/0000-0001-8435-396X

Ethical statement

Ethical approval was obtained from the Health Sciences University Kayseri City Hospital Ethics Committee on March 4, 2020 with decision number 321.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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