Original article

Fungal colonization and infections in patients with COVID-19 in intensive care units: A real-life experience at a tertiary-care hospital

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ABSTRACT

Purpose: To evaluate the management of patients with COVID-19 in the intensive care units (ICUs) with fungal infection/colonization and to highlight diagnostic problems in these patients.
Methods: We included all patients with a COVID-19 diagnosis who were aged ≥18 years and followed in the ICU for the first 8 months. Patient data were obtained from medical records. We compared the risk factors, laboratory data, and outcomes of patients with fungal infection/colonization.
Results: A total of 118 patients (81 men and 37 women) were included. The mean age was 70.3 ± 14.8 (35−94) years. Of the patients, 79 (66.9%) patients were ≥65 years old. Fungal infection/colonization was detected in 39 (33.1%) patients. Fungi were isolated from 34 (28.8%) patients. Ten fungal species were isolated from 51 samples (the most common being *Candida albicans*). Three patients (2.5%) had proven candidemia. We observed two (1.7%) possible cases of COVID-19-associated pulmonary aspergillosis (CAPA). Eighteen patients (15.3%) underwent antifungal therapy. The risk of fungal infection/colonization increased as the duration of invasive mechanical ventilation increased. The fatality rate was 61.9% and increased with age and the use of mechanical ventilation. The fatality rate was 4.2-times-higher and the use of mechanical ventilation was 35.9-times-higher in the patients aged ≥65 years than in the patients aged <65 years. No relationship was found between fungal colonization/infection, antifungal treatment, and the fatality rate.
Conclusion: During the pandemic, approximately one-third of the patients in ICUs exhibited fungal infection/colonization. *Candida albicans* was the most common species of fungal infection as in the pre-pandemic area. Because of the cross-contamination risk, we did not performed diagnostic bronchoscopy and control thorax computed tomography during the ICU stay, and our patients mainly received empirical antifungal therapy.

1. Introduction

The clinical presentation of coronavirus disease 2019 (COVID-19) is highly variable. Most patients are asymptomatic or have mild illness, but 5%−30% of the patients are critically ill and require admission to an intensive care unit (ICU) [1,2]. Most of the patients who require ICU admission are of an advanced age and have multiple comorbidities [3]. These patients have a high risk of secondary bacterial and fungal infections due to their patient characteristics, immune dysfunction, and epithelial lung damage caused by COVID-19. Secondary fungal infections are associated with an increased fatality rate [4]. Owing to diagnostic limitations, it is difficult to diagnose invasive fungal infections (IFIs) during the pandemic era, especially in centers with limited facilities. An increased incidence of invasive candidiasis has been reported in patients with COVID-19 [5,6]. The incidence of IFIs in critically ill patients with COVID-19 was previously unknown. A recent multicenter study in France including 509 critically ill patients with COVID-19 demonstrated that 25% of these patients suffered from IFIs [7].

The new term COVID-19-associated pulmonary aspergillosis (CAPA) has emerged since the first wave of the pandemic and

describes an infection that differs from other IFIs [8]. While other IFIs are especially common in immunosuppressed patients with hematological malignancies, CAPA has been defined in patients with severe COVID-19-related pneumonia, regardless of whether they are immunosuppressed. In the literature, a few case reports or small case series of CAPA have been reported [4]. However, the lack of clinical awareness and inability to perform diagnostic interventions complicate the management of these patients. Therefore, in this retrospective, single-center, observational study, we evaluated the management of critically ill patients with COVID-19 who had fungal infection/colonization and underwent antifungal treatment in ICUs at a tertiary-care hospital during their follow-up and shared their real-life experience.

2. Methods

2.1. Study design and population

This retrospective observational study was conducted at a university hospital with 1100 beds (41 beds in the adult ICUs). We included all patients with new COVID-19 diagnoses who were ≥18 years of age and followed up in ICUs between March 1 and October 31, 2020. The COVID-19 diagnosis was determined using polymerase chain reaction (PCR) testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or chest computed tomography (CT) in patients with symptoms compatible with COVID-19. If the SARS-CoV-2 PCR test result in the nasopharyngeal specimen was negative, SARS-CoV-2 PCR testing of the deep tracheal aspirate (DTA) was repeated 2−4 times.

The demographic features, comorbidities, laboratory and radiologic findings, medical treatments, invasive procedures, clinical progress, and outcomes of the patients were extracted from their medical records. We compared the risk factors and outcomes between the patients with fungal infection/colonization and those without fungal infection/colonization.

2.2. Microbiological assessment

Peripheral blood, catheter blood, catheter tip, DTA, and urinary cultures were evaluated for fungal growth. None of the patients underwent diagnostic bronchoscopy because of concerns about SARS-CoV-2 infection spreading among healthcare workers and other patients. Serum or bronchial lavage galactomannan (GM) could not be performed in the ICUs.

Fungi were screened on clinical samples by direct microscopy (stained) and cultured on mycotic medium. The germ tube test, morphological appearance on cornmeal Tween 80 medium, chromogenic medium appearance, and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) were used to identify yeasts at the genus and species levels. Colony morphology, pigment formation, morphology in slide cultures, and MALDI-TOF MS were used for the mold identification [9].

2.3. Definitions

Proven candidemia: Isolation of *Candida* spp. from at least one peripheral and/or catheter blood culture with compatible clinical findings.

Suspected invasive candidiasis: Critically ill patients with risk factors for invasive candidiasis and/or *Candida* spp. growth in non-sterile sites and no other known cause of fever [10].

Candida colonization: Patients who had *Candida* spp. growth in one or more samples other than blood without clinical findings.

Mold colonization: Without radiologic and clinical findings of invasive pulmonary aspergillosis (IPA), mold growth on respiratory samples was considered to be indicative of colonization.

CAPA: The new European Confederation of Medical Mycology and International Society for Human and Animal Mycology (ECMM/ISHAM) consensus criteria for CAPA were retrospectively applied. Patients with COVID-19 requiring intensive care and who have compatible clinical features, radiological findings, and mycological evidence were diagnosed with proven, probable, or possible CAPA [11].

IPA: Patients with host factors (neutropenia, hematological and/or oncological malignancy, immunosuppressive therapy, etc.), clinical features, and radiological and mycological findings, and those who underwent antifungal therapy, were considered IPA [12].

2.4. Radiological assessment

Radiological evaluation for both COVID-19 and pulmonary aspergillosis was performed by an experienced radiologist. Initial chest CT images and chest radiographs (portable anterior-posterior radiographs obtained during the ICU stay) of the patients were assessed. Chest CT findings of COVID-19 are well-known and were defined as bilateral, patchy, rounded, or diffuse ground-glass opacities (GGO) with or without consolidation, interlobular septal thickening, crazy-paving pattern, and, less frequently, a reversed halo sign. Additional imaging features, such as vascular and bronchial dilatation in the peripheral parenchyma, adjacent pleural thickening, and an air-bubble sign, may also be present [13].

Radiological findings of pulmonary aspergillosis were defined as GGOs, nodular opacities, cavitating consolidations, and a tree-in-bud pattern. In this study, the presence of cavitating nodular infiltration on CT images or plain radiographs was accepted as a positive criterion for possible fungal infection [14,15].

2.5. Statistical analysis

The statistical analyses were performed using IBM SPSS (version 22.0; IBM Corp., Armonk, NY, USA). Descriptive data are presented as the numbers and percentages for the categorical variables. The mean and standard deviation (SD) were used for the continuous variables. To evaluate the relationship between the dependent and independent variables, the chi-square test was used for categorical variables, and the t-test was used for continuous variables. Statistical significance was set at $p < 0.05$. The risk factors and outcomes of patients with and without fungal infection/colonization were evaluated using univariate and multivariate logistic regression analyses (backward and conditional).

3. Results

3.1. Characteristics of the study population

We included 118 patients diagnosed with COVID-19 who were admitted to the ICUs during the study period. The algorithm used for the selection of the study population is shown in Fig. 1.

The mean ± SD age was 70.3 ± 14.8 years (range, 35−94 years) (median = 71 years). Eighty-one (68.6%) of the patients were male. Seventy-nine (66.9%) patients were ≥65 years old. Of these patients, 102 (86.4%) had at least one comorbidity. Fifty-four (52.9%) patients had one, 26 (25.5%) had two, and 22 (21.5%) had three and more comorbidities. The three most common comorbidities were hypertension (HT) ($n = 66$, 55.9%), diabetes mellitus (DM) ($n = 41$, 34.7%), and chronic cardiac disease ($n = 36$, 30.5%). Thirteen (11.0%) patients had immunosuppression, 11 (9.3%) of whom were undergoing chemotherapy for malignancies and two of whom were undergoing immunosuppressive therapy. All of the patients had severe or critical COVID-19 (≥6 score) according to the World Health Organization (WHO) severity definitions [16].
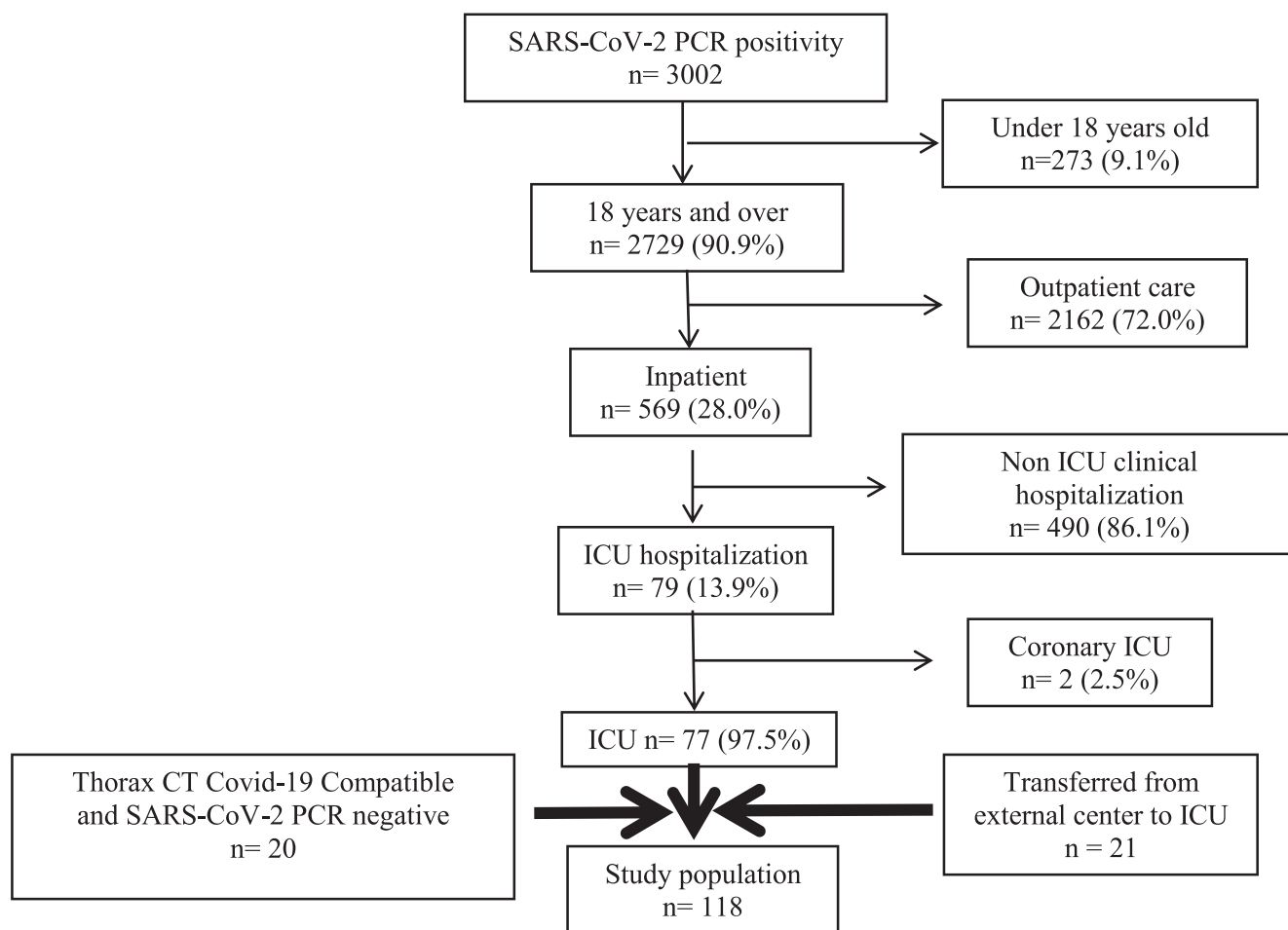
Fig. 1. Flowchart of the study inclusion criteria.

3.2. Microbiological assessment

During the ICU stay, blood cultures were obtained from 115 (95.5%) of the patients, DTA cultures from 83 (70.3%), and urinary cultures from 96 (81.3%). The mean number of blood culture sets per patient was 4.6 ± 3.9 (0−20) (median = 3), the mean number of DTA cultures was 2.8 ± 2.6 (0−14) (median = 2), and the mean number of urinary cultures was 2.9 ± 2.9 (0−14) (median = 2).

There was no fungal growth in 84 patients (71.2%). Fungi were isolated from 51 clinical samples of 34 (28.8%) patients. Ten fungal species were isolated from 26 (51.0%) urinary samples, 20 (39.2%) DTA samples, 3 (5.9%) peripheral blood samples, 1 (2.0%) catheter tip, 1 (2.0%) sputum sample. The distributions of the isolated fungi are shown in Table 1. The characteristics of the 18 patients who underwent antifungal therapy are summarized in Table 2. Other respiratory system infectious agents, such as *Pneumocystis* and *Zygomycetes*, could not be identified because bronchoalveolar lavage could not be performed.

Three patients (2.5%) were diagnosed with **proven candidemia**. Two of them were of an advanced age with multiple comorbidities (71 years old and 83 years old). The other patient was a 53-year- old man who had undergone renal transplantation and DM. They also demonstrated yeast growth in urinary or DTA samples, in addition to yeast growth in blood cultures. The patients received micafungin treatment and were discharged from the hospital. Thirteen (11.0%) patients were diagnosed with **suspected invasive candidiasis**. All patients received empirical antifungal treatment. No fungal growth was observed in three of these patients. The mortality rate was 69.2%

(9/13). Twenty patients (16.9%) exhibited **Candida colonization**. None of the patients had received antifungal therapy. The mortality rate was 71.4% (14/20). Of the patients with colonization, 85.0% (17/20) had growth from a single nonsterile body site.

Table 1
Fungal culture results and radiological findings of the ICU patients with COVID-19.

Test	n (%)
SARS-CoV-2 PCR (negative/positive)	20 (16.9) / 98 (83.1)
Fungi isolation	34 (28.8)
Isolated fungi	51 (100.0)
Candida spp.	47 (92.2)
Candida albicans	24 (47.1)
Candida glabrata	8 (15.7)
Candida parapsilosis	7 (13.7)
Candida tropicalis	5 (9.8)
Other[†]	3 (5.9)
Trichosporon spp.	2 (3.9)
Aspergillus flavus	1 (2.0)
Aspergillus fumigatus	1 (2.0)
Radiological findings	
Thorax CT: No	7 (5.9)
Thorax CT: Normal findings	4 (3.4)
Thorax CT: COVID-19-incompatible	14 (11.9)
Thorax CT: COVID-19-compatible	92 (78.0)
Thorax CT: Aspergillosis-compatible	1 (0.8)
Chest radiography: Aspergillosis-compatible	7 (5.9)

[†] Candida krusei (1), Candida lusitaniae (1), and Candida spp. (1).

Table 2
Characteristics of the patients with COVID-19 who underwent antifungal treatment.

Age Sex	Comorbid	IFI [†]	Fungal isolation	COVID-19 treatment	IFI risk factor	Antifungal	Outcome
71 F	HT[‡‡], DM[§§], dementia	PC[‡]	*Candida albicans* (urinary), *Candida glabrata* (urinary), *Candida parapsilosis* (blood, urinary)	FAV[††††] MP[¶¶¶¶] 40 mg 8 days	MV[‡‡‡‡‡], CVC[§§§§§], UC[¶¶¶¶¶], antibiotics, DM	Micafungin 11 days (9. day)	Survived
83 F	HT, AF[†††], dementia	PC	*Aspergillus flavus* (DTA) *Candida albicans* (urinary) *Candida parapsilosis* (blood)	FAV, immune plasma Dexamethasone 7 days	MV, CVC, UC, antibiotics	Fluconazole 10 days (17. day) Micafungin 21 days (46. day)	Survived
53 M	Kidney transplantation, HT, DM	PC	*Candida albicans* (blood, DTA[††])	FAV MP 40 mg 4 days	MV, CVC, UC, antibiotics, kidney transplantation	Micafungin 11 days (7. day)	Survived
70 M	HT, asthma, smoking	SIC[§]	–	FAV, [‡‡‡‡]HQN, AZI MP 250 mg 5 days	MV, CVC, UC, antibiotics	Caspofungin 1 day (17. day)	Died
70 F	HT, DM	SIC	*Candida glabrata* (urinary, DTA)	HQN, immune plasma MP 40 mg 6 days MP 80 mg 12 days	MV, CVC, UC, antibiotics, DM	Caspofungin 14 days (16. day)	Survived
78 M	HT	SIC	*Candida albicans* (urinary, DTA) *Trichosporon* spp. (urinary)	FAV, HQN, [§§§§]AZI immune plasma MP 40 mg 7 days	MV, CVC, UC, antibiotics	Micafungin 20 days (20. day)	Died
69 M	HT, CAD[¶¶]	SIC	–	FAV, immune plasma, Tocilizumab Dexamethasone 16 days	MV, CVC, UC, antibiotics	Anidulafungin 8 days (12. day)	Died
81 M	Pancreas ca	SIC	*Candida albicans* (urinary, DTA)	FAV, immune plasma, Tocilizumab MP 40 mg 3 days	MV, CVC, UC, antibiotics, malignancy	Caspofungin 6 days (4. day)	Died
60 M	No comorbidity	SIC	*Candida parapsilosis* (urinary)	FAV, immune plasma, Dexamethasone 8 days	MV, CVC, UC, antibiotics	Micafungin 9 days (26. day)	Survived
58 M	DM, CAD	SIC	*Candida parapsilosis* (urinary)	FAV, immune plasma, MP 40 mg 3 days	MV, CVC, UC, antibiotics, DM	Anidulafungin 9 days (18. day) Mikafungin 14 gün (44. gün)	Survived
46 M	HT, DM	SIC	*Candida albicans* (DTA)	FAV, HQN, immune plasma, ECMO MP 20 mg 3 days	MV, CVC, UC, antibiotics, DM, ECMO[††††]	Micafungin 12 days (12. day)	Died
70 M	HT	SIC	*Candida albicans* (urinary, DTA)	FAV, immune plasma MP 20 mg 3 days	MV, CVC, UC, antibiotics	Caspofungin 13 days (9. day)	Died
76 M	Smoking	SIC	*Candida krusei* (urinary)	FAV, immune plasma MP 40 mg 1 day	MV, CVC, UC, antibiotics	Fluconazole 3 days (3. day)	Died
36 M	No comorbidity	SIC	*Candida tropicalis* (urinary)	FAV, immune plasma, ECMO MP 40 mg 14 days	MV, CVC, UC, antibiotics, ECMO	Micafungin 10 days (7. day)	Died
61 M	No comorbidity	SIC	–	FAV MP 20 mg 1 days	MV, CVC, UC, antibiotics, abdominal surgery	Fluconazole 14 days (0. day)	Survived
74 M	DM, HT, RA[‡‡‡], Smoking	SIC	*Candida albicans* (urinary, DTA)	FAV, immune plasma Dexamethasone, 10 days	MV, CVC, UC, antibiotics, DM	Caspofungin 5 days (7. day)	Died
70 M	AML[¶¶¶]	IPA[¶]	–	HQN, MP, 40 mg, 3 days	AML, neutropenia, antibiotics	Caspofungin, 8 days; liposomal amphotericin B, 50 days	Died
62 F	DM, AML	IPA	–	FAV, HQN MP 80 mg 1 day	AML, neutropenia, antibiotics, DM	Caspofungin 1 day, liposomal amphotericin B 17 days	Died

[†]IFI: Invasive fungal infection; [‡]PC: Proven candidemia; [§]SIC: Suspected invasive candidiasis; [¶]IPA: Invasive pulmonary aspergillosis; [††]DTA: Deep tracheal aspirate.
[‡‡]HT: Hypertension; [§§]DM: Diabetes mellitus; [¶¶]CAD: Coronary artery disease; [†††]AF: Atrial fibrillation; [‡‡‡]RA: Rheumatoid arthritis; [¶¶¶]AML: Acute myeloid leukemia.
[††††]FAV: Favipravir; [‡‡‡‡]HQN: Hydroxychloroquine sulfate; [§§§§]AZI: Azithromycin; [¶¶¶¶]MP: Methylprednisolone; [††††]ECMO: Extracorporeal membrane oxygenation; [‡‡‡‡‡]MV: Mechanical ventilation; [§§§§§]CVC: Central venous catheter; [¶¶¶¶¶]UC: Urinary catheter.

Two (1.7%) patients exhibited **mold infection/colonization**. The first patient was 75-year-old man who underwent routine hemodialysis. The patient had symptoms for 1 week and was admitted to the ICU the day after hospitalization. *Aspergillus fumigatus* was detected from the DTA sample obtained on the first day of ICU admission. The patient died on the second day of ICU admission. The second patient was 83-year-old man with dementia, atrial fibrillation, and HT. *Aspergillus flavus* was detected in the DTA samples, which were taken on the 13th and 17th days of the ICU stay. He also had proven candidemia with *Candida parapsilosis* on the 21st day. The patient received micafungin for 18 days. The patient survived and was discharged after 55 days in the ICU. These two patients were analyzed retrospectively according to the new ECMM/ISHAM criteria and considered to have possible CAPA (non-cavitary pulmonary infiltrates on chest CT,

refractory fever, and *Aspergillus* growth in non-bronchoscopic respiratory samples) [11].

Two (1.7%) patients were diagnosed with **IPA** according to the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC-MSG) criteria [12]. The patients received chemotherapy for acute myeloid leukemia in the hematology ward. The patients were already on caspofungin and liposomal amphotericin B therapy for IPA. The patients were transferred to the ICU after being diagnosed with a severe SARS-CoV 2 infection. Both patients died on the second day of the ICU stay.

Proven bacterial pneumonia (VAP and/or community acquired pneumonia) was detected in 33% (39/118) of patients. Eight (20.5%) of these patients had accompanying microbiologically confirmed bacterial pneumonia upon ICU admission. The isolated microorganisms

Table 3

Comparison of demographic characteristics, risk factors, and fatality rate between patients with fungal infection/colonization and those without fungal infection/colonization.

Criteria	Fungal infection/colonization No, n = 79 (66.9%)	Fungal infection/colonization Yes, n = 39 (33.1%)	p
Age (mean ± SD)	70.6 ± 15.9 (35−94)	69.8 ± 12.2 (36−87)	0.786
Sex (male/female)	56 (70.9) / 23 (29.1)	25 (64.1) / 14 (35.9)	0.455
Comorbid disease	68 (86.1)	34 (87.2)	1.000
ICU stay (d) (mean ± SD)	6.3 ± 5.5 (1−25)	20.9 ± 16.0 (1−79)	**0.0001**
Corticosteroids	49 (62.0)	32 (82.1)	**0.035**
Tocilizumab	16 (20.3)	3 (7.7)	0.111
Antibiotics	66 (83.5)	39 (100.0)	**0.005**
Invasive mechanical ventilation	54 (68.4)	39 (100.0)	**0.0001**
Duration (d) (mean ± SD)	5.7 ± 5.3 (1−24)	17.4 ± 14.0 (2−79)	**0.0001**
Central venous catheterization	55 (68.4)	39 (100.0)	**0.0001**
Mortality	47 (59.5)	26 (66.7)	0.450

included *Streptococcus pneumoniae* (*n* = 4), *Staphylococcus aureus* (methicillin-resistant, *n* = 2; methicillin-susceptible, *n* = 1), and *Neisseria meningitidis* (*n* = 1). In the follow-up of these patients, VAP was detected in four of them (4/8), and all of them had fungal colonization.

Thirty-five (29.6%) (35/118) patients had 41 ventilator-associated pneumonia (VAP) episodes. The most frequently isolated organism was extremely-drug resistant *Acinetobacter baumannii*, which was isolated in 56.0% (23/41) of the VAP episodes (12 with bacteremia). Other microorganisms included carbapenem-resistant *Klebsiella pneumoniae*, *Burkholderia cepacia*, and *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter kobei*, and *Enterobacter aerogenes*. Of these patients, 31.4% (11/35) had both VAP and suspected invasive candidiasis, 25.7% (9/35) had both VAP and fungal colonization, and 2.8% (1/35) had both VAP and proven candidemia.

3.3. Fungal infection/colonization

The demographic features, risk factors for fungal infection, and mortality rates of patients with and without fungal infection/colonization are shown in Table 3. Risk factors that were statistically significant in univariate analysis were evaluated using multivariate logistic regression analysis. Only the duration of invasive mechanical ventilation exhibited an increased the risk of the fungal infection/colonization, and the magnitude of this increase was 1.21-fold [*p* = 0.0001 (95% CI: 1.114−1.329)].

3.4. Radiological assessment

Initial chest CT scans of 111 patients were available, and six patients had also control chest CT scans. Seven patients had no chest CT scans. All patients underwent portable chest radiography during their ICU stay. Seven patients had radiological findings that were compatible with IPA. Cavitary nodules were detected on six chest radiographs and one chest CT scan. None of the patients who had radiological findings for IPA exhibited mycological evidence of CAPA. Two patients underwent antifungal therapy for suspected invasive candidiasis. Three of the seven patients died.

3.5. Management of ICU patients with COVID-19

The medical therapies and invasive procedures performed are shown in Table 4. Intravenous methylprednisolone was administered in 59 (50.0%) patients, and intravenous dexamethasone was administered in 22 (18.6%) patients.

Eighteen patients (15.3%) had antifungal therapy. The most commonly used antifungal agents were micafungin, caspofungin, and

fluconazole. The mean duration of the ICU stay was 11.1 ± 12.3 days (1−79 days) (median = 7 days). The distribution of patients with fungal growth who received antifungal therapy and died is presented in Fig. 2.

3.6. Outcomes

The fatality rate of the study population was 61.9% (*n* = 73). Of these patients who died, 58 (79.4%) were 65 years or older. Fatality was significantly higher in patients aged ≥ 65 years (*p* = 0.0001). The relationships between fatality and sex, comorbidity, corticosteroid, tocilizumab, immune plasma, antibiotics, invasive mechanical ventilation use and duration, fungal growth, fungal infection/colonization, and antifungals are shown in Table 5.

The significant risk factors in the univariate analysis were evaluated using multivariate logistic regression analysis; the fatality rate increased 1.05-fold [*p* = 0.002 (95% CI: 1.020−1.090)] with age and 29.6-fold [*p* = 0.0001 (95% CI: 6.233−140.601)] in the presence of mechanical ventilation. When subgroup analysis was performed for patients aged ≥65 years, the fatality rate was 4.2-times higher (95%

Table 4

Medical treatments and invasive procedures of the patients with COVID-19 in the ICU.

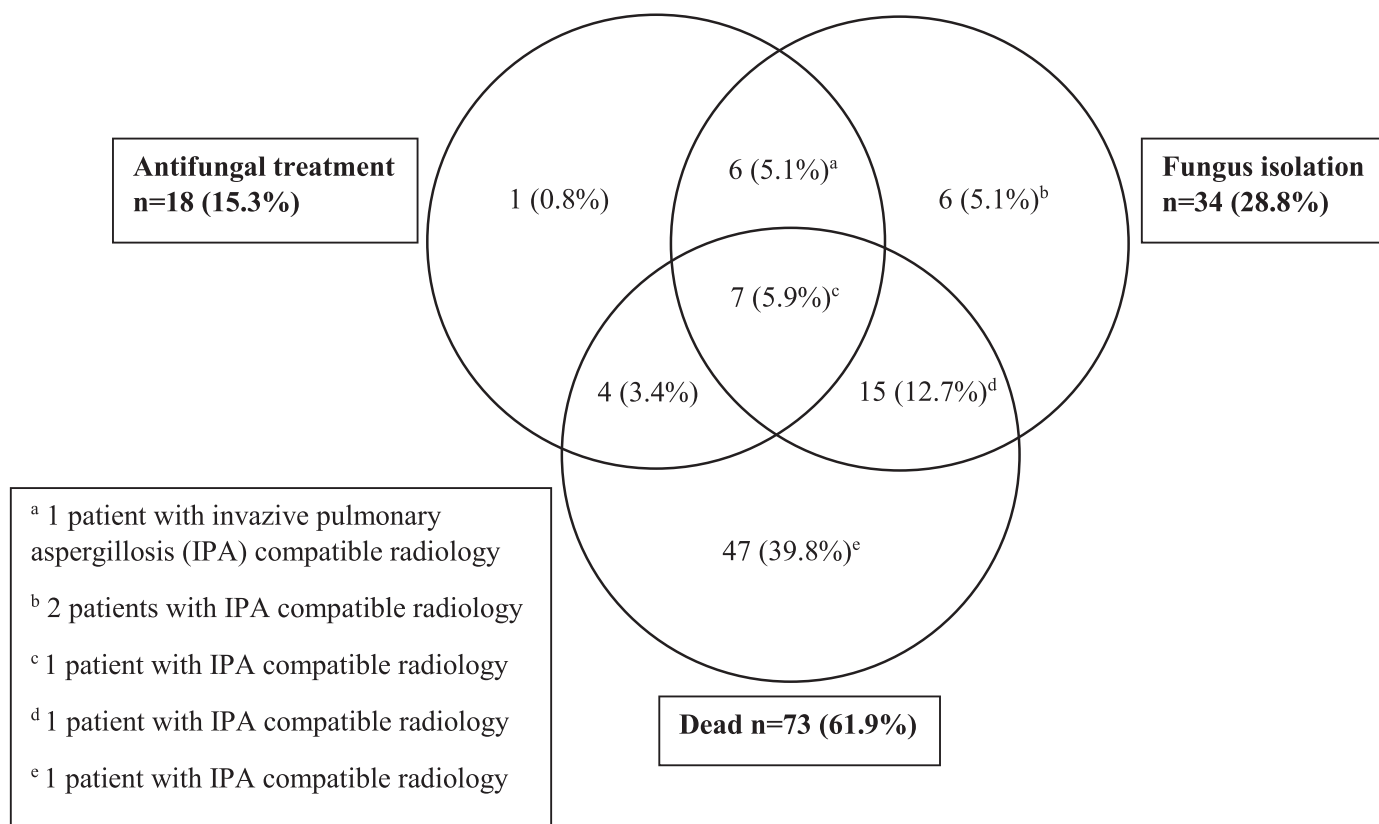| Treatment	n (%)
COVID-19 treatment	
No	2 (1.7)
Hydroxychloroquine sulfate	16 (13.6)
Hydroxychloroquine sulfate + Azithromycin	3 (2.5)
Favipiravir	49 (41.5)
Favipiravir+ Hydroxychloroquine sulfate	36 (30.5)
Favipiravir + Hydroxychloroquine sulfate + Azithromycin	12 (10.2)
Immune plasma	52 (44.1)
Tocilizumab	19 (16.1)
Corticosteroids	81 (68.6)
Initial time (d) (mean ± SD)	6.3 ± 7.2 (0−50)
Duration of treatment (d) (mean ± SD)	6.3 ± 4.8 (1−27)
Antibiotics	105 (89.0)
Antifungals	18 (15.3)
Initial time (d) (mean ± SD)	10.2 ± 7.5 (0−26)
Duration of treatment (d) (mean ± SD)	9.9 ± 6.5 (1−23)
Invasive procedures	
Invasive mechanical ventilation	93 (78.8)
Duration (d) (mean ± SD)	10.6 ± 11.4 (1−79)
Central venous catheterization	94 (79.7)
Urinary catheterization	117 (99.2)

Fig. 2. Patients with antifungal treatment, fungus isolation, and fatality.

Antifungal treatment n=18 (15.3%)

Fungus isolation n=34 (28.8%)

1 (0.8%)

6 (5.1%)[a]

6 (5.1%)[b]

7 (5.9%)[c]

4 (3.4%)

15 (12.7%)[d]

47 (39.8%)[e]

Dead n=73 (61.9%)

[a] 1 patient with invazive pulmonary aspergillosis (IPA) compatible radiology

[b] 2 patients with IPA compatible radiology

[c] 1 patient with IPA compatible radiology

[d] 1 patient with IPA compatible radiology

[e] 1 patient with IPA compatible radiology

CI: 1.58−11.13), and mechanical ventilation exhibited a 35.9-times-higher risk of fatality (95% CI: 7.54−170.98).

4. Discussion

In this study, fungal infection/colonization was detected in 33.1% of critically ill patients with COVID-19. We detected proven candidemia in 2.5%, suspected invasive candidiasis in 11%, and *Candida* colonization in 17.7% of the patients. An increased incidence of invasive candidiasis has been reported in critically ill patients with COVID-19 [4−6]. The incidence of candidemia in ICUs during the pandemic was reported as 2.5% (15/596) in India, 5.1% (12/536) and 8.9% (8/89) in the USA, 10.8% (11/ND) in Spain, and 14% (7/50) in Greece [17−21]. Candidemia was detected in 66 (6.1%) of 1076 cases followed up in ICUs of our center over a 5-year period in the pre-pandemic period [22]. In a study from Turkey, researchers compared the incidence of candidemia in ICUs between the pre-pandemic and pandemic periods and demonstrated a two-fold increase in the incidence of candidemia in patients with COVID-19 [23]. These data demonstrate that different rates can be determined in different countries and even at different centers according to the structure and facilities of the ICUs.

The most commonly isolated fungi were *C. albicans* (45.5%), *C. glabrata* (15.9%), and *C. parapsilosis* (13.6%). Kayaaslan et al. also demonstrated that *C. albicans* was the most commonly isolated yeast in patients with COVID-19 with candidemia [23]. Arastehfar et al. reported that the most common fungi were *C. albicans* (44.1%) and *C. auris* [24]. Recently, an increasing number of *C. auris*-related invasive candidiasis and colonization cases have been reported in ICU patients with COVID-19. *C. auris* infection and colonization have not yet been identified in our center. The fact that *C. auris* causes epidemics and has a high antifungal resistance will make patient management more difficult during the pandemic period [18,25].

In our center, the only option for analyzing respiratory samples of patients with COVID-19 in ICUs is DTA. Only two (1.6%) patients had *Aspergillus* spp. growth in DTA samples. The GM test could not be performed, and there were non-cavitary pulmonary infiltrates revealed on chest CT. One of the patients died on the second day of the ICU stay before being diagnosed with IPA and undergoing antifungal therapy. The other patient had *Aspergillus* spp. growth on DTA samples twice. The patient underwent micafungin therapy for 3 weeks for concomitant candidemia. The patient survived and was discharged from ICU. These two patients were considered to have CAPA. Unfortunately, this diagnostic approach is inadequate for establishing a definite diagnosis of proven CAPA.

The diagnosis of IPA in the ICU is challenging, and a combination of host factors, radiological findings, and mycological results are needed. Previously, influenza-associated pulmonary aspergillosis

Table 5
Comparison of the risk factors for fatality in ICU patients with COVID-19.

Criteria	Survived, n = 45 (38.1%)	Died, n = 73 (61.9%)	p
Age (mean ± SD)	62.6 ± 13.1	75.0 ± 13.8	**0.0001**
Sex (male/female)	31 (68.9) / 14 (31.1)	50 (68.5) / 23 (31.5)	0.964
Comorbid disease	34 (75.6)	68 (93.2)	**0.011**
ICU stay (d) (mean ± SD)	13.1 ± 13.2	9.9 ± 11.6	0.177
Corticosteroids	32 (71.1)	49 (67.1)	0.650
Tocilizumab	9 (20.0)	10 (13.7)	0.442
Immune plasma	23 (51.1)	29 (39.7)	0.226
Antibiotics	40 (88.9)	65 (89.0)	1.000
Mechanical ventilation	22 (48.9)	71 (97.3)	**0.0001**
Duration (d) (mean ± SD)	13.7 ± 10.1	9.7 ± 11.7	0.150
Fungi isolation	12 (26.7)	22 (30.1)	0.686
Fungal infection/ colonization	13 (28.9)	26 (35.6)	0.456
Antifungals	7 (15.6)	11 (15.1)	1.000

(IAPA) was well-described in critically ill influenza patients followed up in ICUs and was associated with increased mortality [25]. Patients with IAPA and CAPA often do not exhibit host factors, the sensitivity of serum GM is low, and it is difficult to distinguish the typical radiological findings of IPA [26,27]. In this group, the EORTC-MSG or AspICU criteria missed most cases of CAPA [11,12]. Owing to the risk of transmission to healthcare workers and other patients, the use of bronchoscopy for microbiological diagnosis is limited [28]. It is difficult to differentiate colonization/infection when growth is detected in upper respiratory tract samples, such as those of the sputum and DTA. Therefore, recent CAPA definitions have been developed by ECMM/ISHAM based on clinical, radiological, and mycological examinations in critically ill patients with COVID-19. Culture, GM, and PCR examinations in BAL or NBL and serum GM were used as mycological diagnostic criteria [11].

Due to the difficulty of CAPA diagnosis and the fact that the actual incidence of the disease is unknown, prospective or national multicenter studies have been conducted to investigate the incidence [28−30]. In these studies, the new CAPA criteria were demonstrated to be superior to the AspICU criteria. It has been reported that CAPA is associated with mortality and changes in the course of COVID-19 [27, 29]. In a recent study by Gangneux et al., 25% mechanically ventilated patients with COVID-19 had IFIs; the most common IFI was CAPA (15%) [7].

In the multivariate analysis, the duration of invasive mechanical ventilation was associated with a higher risk of fungal colonization/infection. Bishburg et al. reported that patients with candidemia had a longer ICU stay and longer duration of mechanical ventilation [20]. Central venous catheter use, antibiotic use, and corticosteroid use were previously reported as the most common risk factors for invasive candidiasis [24]. Some studies have demonstrated that high-dose steroid therapy increases the risk of CAPA and candidiasis in patients with COVID-19 [29,31]. However, in some studies, steroid use was not found to be a risk factor for invasive candidiasis in patients with COVID-19 [4,21]. In a study by Gangneux et al., combination therapy with dexamethasone and anti-IL-6 was independently associated with CAPA, but the number of cases was low [7]. In our study, there was no statistically significant relationship observed between steroid use and fungal infection/colonization. Prospective and large-scale studies may reveal the relationship between CAPA, steroids, and the pathophysiology of COVID-19.

The fatality rate in our study was 61.9%. In a multicenter study in Turkey, the crude COVID-19 fatality rate in ICUs was 64.9% [32]. The systematic review by Quah et al. evaluated 15 studies from Asia, Europe, and North America and observed an overall mortality rate of 25.7% (range, 8.0% and 41.2%) [33]. In an Italian study, Grasselli et al. reported a mortality rate of 44.3% in 3988 patients in the ICU. An advanced age, male sex, COPD, HL, and DM are associated with mortality [34]. In our study, an advanced age and invasive mechanical ventilation were associated with fatality, and the crude fatality rate in our study was higher than those reported in previous studies. However, the fatality rate was similar in the studies conducted in our country. This observation might be related to the patient characteristics and admission criteria to the ICU of our center. Since our center is a tertiary care referral hospital, the median age of the patients was high, comorbid diseases were common, and all ICU patients had severe or critical COVID-19 according to the WHO severity definitions. In our center, a COVID-19 Pandemic Action Plan was prepared to ensure that all patients could undergo the necessary intervention as soon as possible and be transferred to the ICU without delay [35].

There was no statistically significant relationship observed between fungal infection/colonization and fatality. All three patients with proven candidemia received appropriate antifungal therapy and survived. Patients who underwent antifungal therapy for suspected invasive candidiasis had similar fatality rates to that of patients with Candida colonization who did not undergo antifungal therapy. It is

difficult to differentiate infection and colonization in patients with fungal growth in non-blood samples from ICUs. It is even more difficult to distinguish this in critically ill patients with COVID-19. Appropriate antifungal initiation is delayed because of inadequate mycological examinations and diagnostic difficulties. This situation adversely affects fatalities. In our cohort, the patients exhibited a high fatality rate related to severe/critical COVID-19 and probably led to an underestimation of the prevalence of CAPA because of diagnostic limitations. White et al. reported higher mortality in patients with COVID-19 with invasive fungal infections (38% vs. 52.8%). The mortality rate was 38.5% in patients with IFI who received appropriate antifungals and 90.0% in those who did not [28]. The mortality rate was 46.0% in patients with COVID-19 with invasive candidiasis [23]. In prospective studies in ICU patients with COVID-19, a significant relationship was found between CAPA and mortality, and the mortality rate in patients with CAPA was between 36.0% and 61.8% [7,26,28,29].

Our study has several limitations; it has a small, single-center, and retrospective design. Patients who were diagnosed with probable COVID-19 based on their clinical and radiological findings and negative SARS-CoV-2 PCR results were also included in the study. This might have led to heterogeneity in the cohort. Because of the risk of cross-contamination, we did not perform diagnostic bronchoscopy or control chest CT during the ICU stay. The high burden of laboratory work and pandemic shifts caused the cancelation of serum galactomannan tests like most of the microbiologic laboratory tests. No additional PCR tests were performed for other respiratory viruses for the same reason. Since only patients with COVID-19 were followed up in this ICU, there was no control group (non-COVID-19 group). To determine the effect of fungal infection and colonization on the outcome of ICU patients with COVID-19, yeast and mold infections were evaluated together. On the other hand, our study is the first study in Turkey in which all fungal infections were evaluated in critically ill patients with COVID-19.

During the pandemic, at least one-third (33.1%) of the cases in ICUs exhibited fungal infection/colonization. The most common species (Candida spp.), which was the same as in the pre-pandemic area. However, the diagnosis of CAPA cannot be proven due to limitations in the diagnostic processes and the inability to perform laboratory tests (we observed two possible CAPA cases). There is a need for larger prospective studies on this subject. Real-life data can reflect the incidence, diagnostic problems, and fatality of invasive fungal infections in critically ill patients with COVID-19 and can be used as an example for other ICUs with patients with COVID-19.

Authors' contributions

VAO and MC were involved in planning the study, conducting the study, and evaluating the results of the study. VAO supervised the research. All authors contributed to the writing of the final manuscript. All authors approved the final version of the manuscript.

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Statement of ethics

The study was approved by the xxx University Medical Faculty Noninvasive Medical Research Ethical Committee (08.02.2021;2021/04−08). This study was also granted permission from the Ministry of Health.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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