



COVID-19 and candiduria: an investigation of the risk factors and immunological aspects

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Abstract

Secondary fungal infections are frequently observed in COVID-19 patients. However, the occurrence of candiduria in these patients and its risk factors are underexplored. We evaluated the risk factors of candiduria in COVID-19 patients, including inflammatory mediators that could be used as prognostic markers. Clinical information, laboratory test results, and outcomes were collected from severely ill COVID-19 patients with and without candiduria. *Candida* species identification, antifungal susceptibility, and plasma inflammatory mediators' measurements were performed. Regression logistic and Cox regression model were used to evaluate the risk factors. A higher risk of longer hospitalization and mortality were observed in patients with candiduria compared to those with COVID-19 only. Candiduria was caused by *Candida albicans*, *C. glabrata*, and *C. tropicalis*. Isolates with intermediate susceptibility to voriconazole and resistant to caspofungin were identified. Classic factors such as the use of corticosteroids and antibacterials, the worsening of renal function, and hematological parameters (hemoglobin and platelets) were found to predispose to candiduria. The mediators IL-1 β , IL-1ra, IL-2, CXCL-8, IL-17, IFN- γ , basic FGF, and MIP-1 β were significantly increased in patients with COVID-19 and candiduria. Furthermore, IFN- γ , IL-1ra, and CXCL-8 were associated with the occurrence of candiduria in COVID-19 patients, whereas basic FGF, IL-1 β , and CXCL-8 were associated with the risk of death in these patients. Classical and immunological factors were associated with worse prognosis among patients with COVID-19 and candiduria. Some mediators, especially CXCL-8, can be a reliable biomarker of fungal coinfection and may guide the diagnostic and the treatment of these patients.

Keywords COVID-19 · Candiduria · Pro-inflammatory cytokines · CXCL-8 · Mortality

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects the lungs and invade epithelial cells and type II pneumocytes. It occurs through the binding of its surface spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor [1]. This interaction, followed by viral replication, damages not only the lungs, but also the heart, kidneys, and intestine. Furthermore, coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 triggers an inflammatory cascade that may be harmful to the host [2].

The severe tissue damage, the immunologic derangement resulting from SARS-CoV-2 infection, and the invasive procedures in the severe cases frequently predispose to secondary fungal infections [3, 4]. Previous studies have demonstrated a higher number of cases of COVID-19-associated

candidiasis (CAC), aspergillosis (CAPA), mucormycosis (CAM), and histoplasmosis in the different countries [5].

Candida species are major constituents of the human mycobiome and the leading cause of invasive fungal infections. Studies from Spain [6], India [7], Italy [4], UK [8], and China [9] reported occurrence rates of CAC of 0.7% (7/989), 2.5% (15/ 596), 8% (3/43), 12.6% (17/135), and 23.5% (4/17), respectively. Overall, due to neglected approach towards fungal infections, morbidity and mortality has been showed to worsen during the COVID-19 pandemic. For example, a recent systematic review showed that CAC doubled the mortality of critically ill COVID-19 patients [10]. In addition, a previous study from our group revealed that hospitalized patients with severe COVID-19 and candiduria have a 5-times higher risk of death when compared to patients without the coinfections [11].

Classical clinical factors such as prolonged intensive care unit (ICU) stays, central venous catheters, and broad-spectrum antibiotic use, may be key factors associated with candidiasis in COVID-19 patients. However, some reports suggest that the occurrence of candidiasis and the consequent increased mortality rate in COVID-19 patients can be related to impaired immune responses. It should further increase the awareness of clinicians for more effective diagnosis and treatment [12–14]. In this aspect, a study in Italy reported three cases of candidemia among patients with severe symptoms of COVID-19 and after treatment with tocilizumab, an IL-6 receptor blocker, suggesting that this interleukin may be related to fungal coinfection [15].

Although some studies have addressed the immune response in COVID-19 patients with candidemia, the immunological mechanisms involved in candiduria are poorly understood. Altogether, in this study, we aimed to verify the characteristics and the risk factors of candiduria in COVID-19 patients, including the evaluation of the plasma inflammatory mediators in these patients.

Methods

Patients' enrollment, clinical evaluation, and ethical aspects

Between May and November 2020, we enrolled patients with COVID-19 and candiduria coinfection ($n=18$) and patients with COVID-19 alone as a control group ($n=31$), admitted in the ICU of the Hospital Eduardo de Menezes, Belo Horizonte, Brazil. The control group was determined by randomly selecting patients with a diagnosis of COVID-19, which had no reported candidemia/candiduria during their ICU stay. However, they could have bacterial infections. COVID-19 diagnosis was confirmed by a positive real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 in

the nasopharyngeal swab, associated with suggestive signs, symptoms, and radiological findings. Candiduria was diagnosed by urine culture in CHROMagar® *Candida* differential medium. Plasma samples for the analysis of hematological, biochemical, and immunological parameters were collected on the same day of candiduria identification. Informed consent was obtained from all participants. This study was approved by the National Ethics Committee (Comissão Nacional de Ética em Pesquisa- CONEP) and the hospital's Ethics Committee (CAAE: 30627320.6.0000.0008).

The following information was obtained from the medical records of patients: (1) patient characteristics: age, gender, use of antibiotics (number), and use of corticosteroids; (2) co-morbidities: diabetes, hypertension; (3) biochemical and hematological parameters: urea, creatinine, hemoglobin, platelet; (4) inflammatory parameters: leukocytes, C-reactive protein, lactate dehydrogenase; (5) outcome: length of hospital stay and mortality.

Fungal identification

The presumptive identification of *Candida* spp. was performed by culturing the collected urine in CHROMagar® *Candida* differential medium. For the definitive identification, yeast isolates were identified by polymerase chain reaction (PCR). Species-specific primers were used for *Candida albicans* (5'-TGTTGCTCTCTCGGGGCGGCCG-3'), *Candida glabrata* (5'-TGGGCTTGGACTCTCGCAGCTC-3'), *Candida tropicalis* (5'-TGGGCGGTAGGAGAATTGCGTTA-3'), and *Candida parapsilosis* (5'-GCATCAGTTGAGCGGTAGGATAAGC -3'). The universal reverse primer used was the NL4 (5'-CGTCCGTGTTTCAAGACGG-3'). Yeast isolates that were not identified by PCR, were then submitted to sequencing reactions using the Big Dye kit version 3.1 (Applied Biosystems, USA) in combination with the ABI 3730 automated system. The amplicons for sequencing reactions were obtained by PCR products using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and NL4 (5'-CGTCCGTGTTTCAAGACGG-3'). The DNA sequences obtained from the yeasts were compared with the sequences of type or reference species deposited in GenBank and belonging to international culture collections, using the BLASTn program (Basic Local Alignment Search Tool - version 2.215 of BLAST 2.0), available on the NCBI portal (<http://www.ncbi.nlm.nih.gov/blast/>).

Antifungal susceptibility

The susceptibility of *Candida* spp. isolates was evaluated according to the yeast broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) — document M27 A3 [16]. Itraconazole, voriconazole, and amphotericin B were tested at concentrations from

0.03 to 16 mg/L; fluconazole and caspofungin were used at concentrations from 0.125 to 64 and 0.015 to 8.0 mg/L, respectively. Plates were incubated at 37 °C for 24 h, and the reading to determine the minimum inhibitory concentration (MIC) was performed visually by observing the turbidity. *Candida albicans* ATCC 18804, *Candida tropicalis* ATCC 750, and *Candida glabrata* ATCC 2001 were used as quality controls as recommended by the CLSI. From the MIC values, the MIC50 and MIC90 (MIC values that inhibited 50 and 90% of the isolates, respectively) for each *Candida* spp. were calculated.

Analysis of the patients' immunological mediators

The quantification of 27 mediators, including cytokines, chemokines, and growth factors, was performed using the screening panel kit Bio-Plex Pro™ Human Cytokine 27-plex Assay (Bio-Rad, Hercules – CA, USA). Samples were read using the Bio-Plex 200 equipment, and the analyses were performed using the Manager software (Instituto René Rachou - Fiocruz Minas). The results were analyzed as the average of the fluorescence intensities and expressed in pg/dl.

Statistical analysis

To compare the patients' characteristics and outcomes between groups, categorical variables were summarized as numbers (percentages) and analyzed using the Fisher's exact test. Continuous variables were presented as mean \pm SD and analyzed using Mann–Whitney *U* or Student's *t* test; $p < 0.05$ was considered statistically significant. Mean levels of cytokines, chemokines, and growth factors were compared using Student's *t* test.

Conditional logistic regression was used to investigate associations of factors with candiduria in COVID-19 patients. Variables with a *P* value of < 0.1 in the univariate analysis were included in the multivariate model. The odds ratio (OR) was calculated to determine the strength of association. Correlations between the concentration of the immunological, hematological, and biochemical parameters were assessed by Spearman tests; and the heatmap was created using the GraphPad Prism Program, version 9.00 (GraphPad Software, San Diego, CA, USA).

Kaplan–Meier curves were built relatively to group status (death or discharged) in patients with COVID-19 and candiduria. Patients were categorized according to the median value, considering the (i) high levels of cytokines, chemokines, and growth factor ($>$ median) and (ii) low levels of biomarkers (\leq median). Cox model was first applied for comparative analysis of Kaplan–Meier curves for each cytokine, chemokine, and growth factor; and second with all biomarkers together and adjusted for age and sex as covariates. Hazard ratios (HR) were estimated via the Cox regression model.

Statistical analyzes were performed using the GraphPad Prism Program, version 9.00 (GraphPad Software, San Diego, CA, USA), and the Epi Info 3.5.3 program (Centers for Disease Control and Prevention, Atlanta, USA, available at <http://www.openepi.com>). For all tests, a *p* value < 0.05 was considered statistically significant.

Results

Clinical characteristics of patients with COVID-19 and candiduria

Mean age and gender distribution were similar between patients with COVID-19 alone and those with COVID-19 with candiduria (Table 1). There was no difference regarding the use of corticosteroids and antibacterials between the groups. However, the number of prescribed antibacterial agents was statistically higher in patients with COVID-19 and candiduria compared to those with COVID-19 only (5.28 versus 2.24; $p < 0.0001$). The percentage of patients with diabetes did not differ between the groups. On the other hand, the percentage of patients with hypertension was higher in COVID-19 compared to COVID-19 and candiduria group (64.51 versus 22.22%, $p = 0.007$). Among the hematological, biochemical, and inflammatory parameters, the mean values of serum creatinine and urea, and C-reactive protein, were significantly higher in patients with candiduria compared to the group with COVID-19 alone (2.37 versus 1.27 mg/dL, $p = 0.002$; 125.00 versus 75.77 mg/dL, $p = 0.006$; 162.90 versus 94.48 mg/L, $p = 0.023$, respectively). Mean hemoglobin and platelet levels were significantly lower in patients with candiduria (10.02 versus 12.39 g/dL, $p = 0.001$; 215333 versus 273533/mm³, $p = 0.041$, respectively). Furthermore, there was no statistical difference of leukocytes counting between the analyzed groups. Interestingly, patients with COVID-19 and candiduria had a significantly longer length of hospital stay and mortality compared to those with COVID-19 only (34.44 versus 12.48 days, $p < 0.001$; 77.78 versus 29.03% of patients, $p = 0.001$, respectively).

Candida albicans and non-*albicans* presenting different antifungal susceptibility profiles caused candiduria in COVID-19 patients

Among the 18 patients with candiduria, eight was caused by *C. albicans*, five by *C. tropicalis* and five by *C. glabrata*. Regarding drug susceptibility, 50% and 80% of *C. albicans* and *C. tropicalis* isolates, respectively, presented intermediate susceptibility to voriconazole and 40% of *C. glabrata* isolates were resistant to caspofungin (Table 2).

Table 1 Clinical characteristics, hematological and biochemical parameters of COVID-19 patients with or without candiduria

	COVID-19 (n=31)	COVID-19 and candiduria (n=18)	P value
Patient characteristics			
Age (years)	62.87 ± 14.23	64.83 ± 13.03	0.634
Female gender	15 (48.38%)	10 (55.56%)	0.769
Use of corticosteroids	14 (45.16%)	13 (72.22%)	0.082
Use of antibacterials	27 (87.10%)	17 (94.44%)	0.639
Number of antibacterials	2.24 ± 1.28	5.28 ± 2.02	<0.0001
Co-morbidities			
Diabetes	13 (41.94%)	10 (55.56%)	0.390
Hypertension	20 (64.51%)	4 (22.22%)	0.007
Biochemical/hematological data			
Serum creatinine (mg/dL)	1.27 ± 0.99	2.37 ± 1.46	0.002
Urea (mg/dL)	75.77 ± 49.88	125.00 ± 51.34	0.006
Hemoglobin (g/dL)	12.39 ± 2.08	10.02 ± 2.73	0.001
Platelet count (/mm ³)	273533 ± 98859	215333 ± 132144	0.041
Inflammatory parameters			
Leukocytes count (/mm ³)	12803 ± 12419	13889 ± 9838	0.406
C-reactive protein (mg/L)	94.48 ± 90.19	162.90 ± 111.10	0.023
Lactate dehydrogenase (mM/L)	1.46 ± 0.45	1.55 ± 0.85	0.821
Outcome			
Length of hospital stay (days)	12.48 ± 5.82	34.44 ± 22.99	<0.001
In-hospital mortality	9 (29.03%)	14 (77.78%)	0.001

In bold, significantly different parameters between the two groups of patients

Table 2 Number of isolates and minimal inhibitory concentration (MIC) of antifungal agents for *Candida* spp. from patients with COVID-19 and candiduria

Species (number of isolates)	Antifungal	Range MIC	MIC ₅₀	MIC ₉₀	S (%)	I (%)	R (%)
<i>C. albicans</i> (n = 8)	Fluconazole	0.06–0.25	0.125	0.25	100	NA	0
	Itraconazole	<0.03–0.25	0.125	0.20	100*	NA	0
	Voriconazole	0.06–0.5	0.25	0.50	50	50	0
	Caspofungin	<0.015–0.125	0.06	0.10	100	0	0
	Amphotericin B	0.5–1.0	1.0	1.0	100*	NA	0
<i>C. tropicalis</i> (n = 5)	Fluconazole	0.25–0.50	0.5	0.5	100	0	0
	Itraconazole	<0.03–0.125	0.093	0.125	100*	NA	0
	Voriconazole	0.125–0.25	0.25	0.25	20	80	0
	Caspofungin	0.06–0.125	0.093	0.125	100	0	0
	Amphotericin B	0.25–2.0	1.0	2.0	100*	NA	0
<i>C. glabrata</i> (n = 5)	Fluconazole	0.50–8.0	2.0	5.6	100	NA	0
	Itraconazole	0.06–1.0	0.25	0.70	100*	NA	0
	Voriconazole	0.125–4.0	0.5	2.8	NA	NA	NA
	Caspofungin	0.03–0.5	0.5	0.5	60	0	40
	Amphotericin B	0.5–2.0	0.5	1.60	100*	NA	0

*Epidemiological cutoff (Pfaller et al., 2012). MIC Minimal inhibitory concentration; S susceptible; I intermediate; R resistant; NA not available. MIC is represented by µg/mL. Antifungal susceptibility (S, I, or R) is represented by the percentage of isolates for each agent

Level of immunological mediators in patients with COVID-19 and candiduria

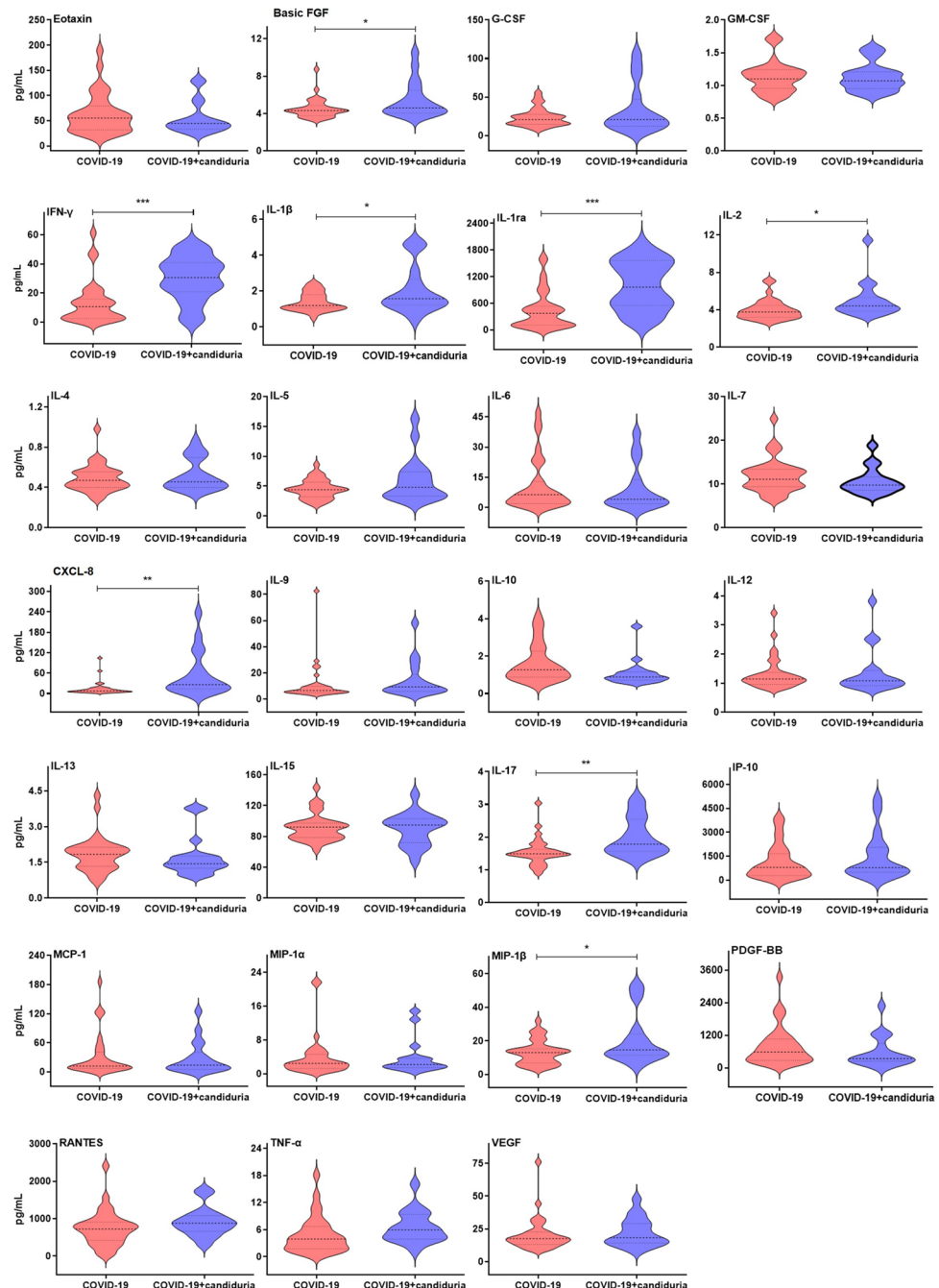
Subsequently, we analyzed and compared the serological immune response of COVID-19 patients with and without candiduria. The levels of IL-1 β ($p=0.0176$), IL-1ra ($p=0.0002$), IL-2 ($p=0.0241$), CXCL-8 ($p=0.0014$), IL-17 ($p=0.0023$), interferon γ (IFN- γ , $p=0.0005$), basic fibroblast growth factor (basic FGF, $p=0.0277$), and macrophage inflammatory protein 1 β (MIP-1 β ; $p=0.0251$) were

significantly increased in plasma from COVID-19 and candiduria patients compared to those with COVID-19 alone (Fig. 1).

Risk factors for candiduria in COVID-19 patients

Then, we investigated which patient’s characteristics would represent risk factors for candiduria in patients with COVID-19. Furthermore, we evaluated whether the increased levels of immunological mediators in the plasma of patients with

Fig. 1 Cytokines, chemokines and growth factors in plasma samples from patients with COVID-19 and candiduria (blue, $n = 18$), and COVID-19 only (red, $n = 31$). The horizontal lines in the inner box plot represent the median and interquartile range. Pairwise comparisons T-test show significantly higher levels of basic FGF, IL-1 β , IL-1ra, IL-2, CXCL-8, IL-17, IFN- γ , and MIP-1 β in patients with COVID-19 and candiduria compared to COVID-19 group (***: $p<0.001$, **: $p<0.01$, *: $p<0.05$)



COVID-19 and candiduria could be correlated with the coinfection and used as predictive biomarkers.

Several variables such as hypertension, number of antibacterial agents, serum creatinine, urea, hemoglobin level, cytokines IFN- γ , IL-1ra, and CXCL-8 were significantly associated with candiduria in COVID-19 patients in the univariate regression analysis (Table 3). However, in the multivariate conditional regression model, only the use of corticosteroids and number of administered antibacterials were risk factors independently associated with candiduria in COVID-19 patients.

We also evaluated the relationship between the occurrence of candiduria and the prognosis of patients with COVID-19. The analysis revealed an association between candiduria in COVID-19 patients with longer length of hospitalization and higher mortality rate (Table 3).

Searching for immune, hematological, and biochemical biomarkers for COVID-19 and candiduria

Next, we asked whether the levels of immunological mediators correlate with each other and with hematological and

biochemical parameters. The heatmap presented in Fig. 2 illustrates the significantly positive correlations among the pro-inflammatory cytokines, chemokine, and growth factor, except for CXCL-8, in patients with COVID-19 and candiduria. Basic FGF and CXCL-8 also demonstrated positive correlation with creatinine and urea, respectively. Negative correlations were observed for CXCL-8 with hemoglobin and platelet. The leukocyte, PCR, and lactate dehydrogenase did not show any significant correlations with the other biomarkers.

Immune mediators as biomarkers of the risk of death in COVID-19 and candiduria group

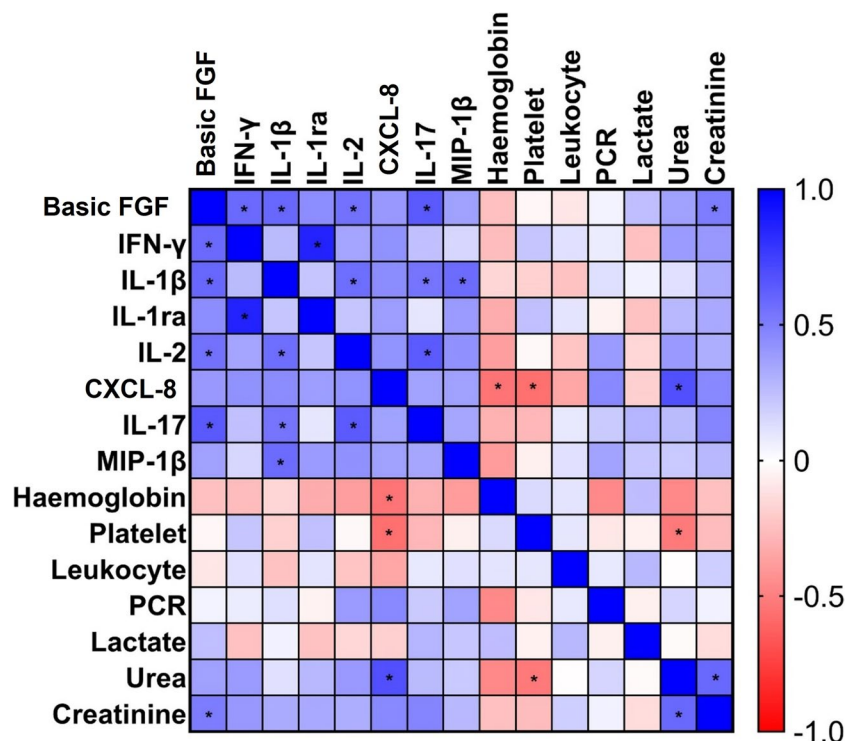
We also analyzed if the increased production of cytokines, chemokine, and growth factor influenced the patient's survival. We then stratified the patients according to the levels of immunological mediators, considering high versus low levels by using the median values of all analyzed patients in this study as cutoffs. We first evaluated how each mediator could predict the survival of the patients. The results from Cox regression showed that basic FGF was significantly associated with increased risk of death from patients with

Table 3 Conditional logistic regression for candiduria in COVID-19 patients

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
Age (< 65; \geq 65 years)	0.933	0.288–3.027	0.909			
Gender	1.328	0.419–4.209	0.630			
Diabetes	1.731	0.536–5.587	0.359			
Hypertension	0.157	0.042–0.596	0.007	0.026	0.000–1.624	0.084
Use of corticosteroids	3.152	0.903–11.006	0.072	71.300	1.011–5028.037	0.049
Use of antibacterials	2.508	0.259–24.303	0.428			
Number of antibacterials (< 3; \geq 3)	39.020	4.557–334.084	0.001	149.768	1.482–15136.982	0.033
Serum creatinine (mg/dL) (low/high)	0.082	0.018–0.362	0.001	0.011	0.000–5.917	0.160
Urea (mg/dL) (low/high)	0.104	0.024–0.457	0.003	66.978	0.056–80272.155	0.245
Hemoglobin (g/dL) (low/high)	4.491	1.260–16.006	0.021			
Platelet count (/mm ³) (low/high)	2.998	0.883–10.176	0.708	0.097	0.001–8.613	0.308
Leukocytes count (/mm ³) (low/high)	0.700	0.216–2.265	0.552			
C-reactive protein (mg/L) (low/high)	0.353	0.101–1.233	0.103	0.108	0.002–4.917	0.253
Lactate dehydrogenase (mM/L) (low/high)	0.700	0.177–2.771	0.611			
Basic FGF (low/high)	0.632	0.200–1.995	0.434	0.417	0.011–16.516	0.641
IFN- γ (low/high)	0.091	0.021–0.387	0.001			
IL-1 β (low/high)	0.502	0.157–1.610	0.247			
IL-1ra (low/high)	0.156	0.042–0.581	0.006	0.028	0.001–1.690	0.743
IL-2 (low/high)	0.347	0.107–1.130	0.079	0.087	0.001–10.849	0.087
CXCL-8 (low/high)	0.096	0.023–0.401	0.001	1.089	0.035–34.301	0.322
IL-17 (low/high)	0.310	0.093–1.028	0.056	1.858	0.046–75.843	0.962
MIP-1 β (low/high)	1.000	0.319–3.135	1.000			
Length of hospital stay (< 20; \geq 20 days)	8.423	2.072–54.234	0.003	10.887	0.357–332.411	0.171
In hospital mortality	8.556	2.207–33.170	0.002			

OR Odds ratio; CI confidence interval. In bold, significantly different parameters between the two groups of patients

Fig. 2 Spearman correlation matrix among immunological, hematological, and biochemical biomarkers in patients with COVID-19 and candiduria. Positive correlation coefficients are shown in shadows of blue, while negative correlations in shadows of red. The * represents significant correlations ($p < 0.05$)



COVID-19 and candiduria (Fig. 3). Subsequently, we considered all mediators together in the model (data not shown), for their relative independence, and adjusted for sex and age. Interestingly, the high levels of basic FGF (HR=37.150) and CXCL-8 (HR=16.020), and the low levels of IL-1 β (HR=0.021) were significantly associated with reduced survival ($p=0.008$, $p=0.003$, and $p=0.013$, respectively).

Discussion

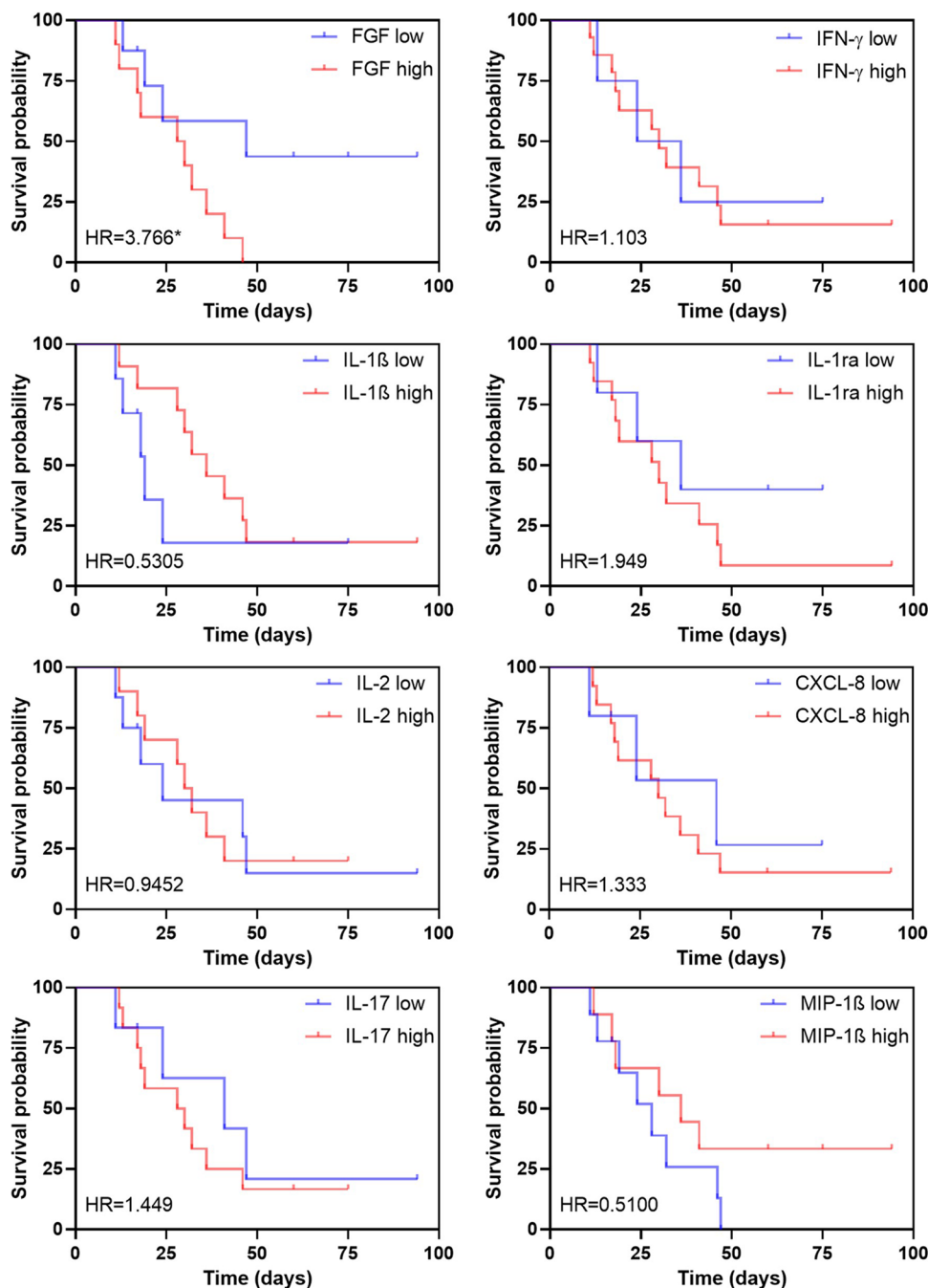
In this study, we evaluated the causes and consequences of candiduria in severely ill COVID-19 patients. *Candida* spp. urinary tract infections can be caused either by the ascending (via the urethra) or the hematogenous routes. In this aspect, candiduria can lead to candidemia, which is associated with high morbidity and mortality [17]. However, since most urinary tract infections are caused by bacteria, the diagnostic of yeast is often ignored and definition of candiduria is not standardized.

The occurrence of candiduria, as well as the causative species, is underexplored in COVID-19 patients. In our study, *C. albicans* was the most isolated species from candiduria in COVID-19 patients, followed by *C. glabrata* and *C. tropicalis*. Similarly, previous study demonstrated that of the *Candida* species in patients with COVID-19 and candiduria, the most prevalent species were *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* [18]. Furthermore, most of *C. albicans* and *C. tropicalis* from our study presented

intermediate susceptibility to voriconazole and 40% of *C. glabrata* isolates were resistant to caspofungin. The first study [11] carried out in this regard was performed by our group with patients with COVID-19 and revealed that *Candida* spp. and *Aspergillus* spp. represented 98.2% (54) and 5.5% (3) of the fungal isolates, respectively. These isolates were obtained from different sites such as tracheal aspirate ($n = 33$), urine ($n = 25$), blood ($n = 4$), catheter tip ($n = 4$), sputum ($n = 3$), and mini-BAL ($n = 1$). Subsequently [19], we identified the following prevalence of fungal species in these patients: *C. albicans* (54%), *C. tropicalis* (23%), *C. glabrata* (14%), *C. parapsilosis* (3%), *C. kefyr* (3%), *C. lusitanae* (1%), *A. nomius* (1%), and *A. flavus* (1%). Regarding susceptibility profile, two isolates (5%) of *C. albicans* and two isolates (20%) of *C. glabrata* were resistant to caspofungin. In addition, one isolate (5.88%) of *C. tropicalis* and seven isolates (70%) of *C. glabrata* were resistant to voriconazole, and two isolates (20%) of *C. glabrata* resistant to fluconazole. These observations reinforce the importance of identification at the species level to provide an adequate selection of the antifungal therapy. It is of great significance, especially when considering that candiduria may be a sentinel of a disseminated infection, and that echinocandins are a first-line antifungal for the treatment of invasive candidiasis [20, 21].

The number of antibacterial agents administered was statistically higher in patients with COVID-19 and candiduria compared to those with COVID-19 alone. Furthermore, the multivariate conditional regression model showed the use of

Fig. 3 Survival curves based on the increased levels of cytokines, chemokine, and growth factor in patients with COVID-19 and candiduria. Cox regression model showing overall survival with hazard ratio (HR) for each mediator if high (red) or low (blue). The * represents the significant result ($p < 0.05$)



corticosteroids and the number of administered antibacterial agents as the only risk factors independently associated with candiduria in COVID-19 patients. The use of corticosteroids is recommended by the World Health Organization (WHO) for the treatment of patients with severe symptoms of COVID-19, and although they attenuate the inflammatory response generated by the virus, they cause immunosuppression and might therefore increase the risk for candidiasis [22]. COVID-19 patients in the ICU usually receive broad-spectrum antibiotics as empirical therapy, particularly during the first pandemic wave [19]. These drugs suppress

the bacterial microbiota of the gastrointestinal and genital system, which facilitate colonization by *Candida*, predisposing to candiduria [23]. In this aspect, a previous study demonstrated that ICU patients, who receive four different antibiotics and have colonization of clinical specimens such as urine, presented 80% increased risk of developing candidemia [24].

Patients with COVID-19 and candiduria had a significantly longer mean hospital length of stay and mortality compared to those with COVID-19 only. The univariate regression analysis also revealed an association between

candiduria in COVID-19 patients and longer hospitalization period and higher mortality rate. In a study from Spain, 22% of the patients who stayed more than 7 days in the ICU developed candiduria and approximately one-third of ICU patients with a positive *Candida* culture had a urinary catheter, which are common characteristics in severely ill COVID-19 patients in the first pandemic wave [24].

The immunological response in candidemia has been previously studied. In CAC, immunological factors have been recently added to all well-known risk factors such as mechanical ventilation, parenteral nutrition, broad spectrum anti-bacterial treatment, indwelling central venous or bladder catheters, older age, comorbidities, lymphopenia, and corticosteroids [15]. However, the mechanisms involved in the immunological defense related to candiduria are not completely understood [25]. More specifically, there is a knowledge gap about how an immune response contributes to the pathologies in candiduria in COVID-19 patients. A previous study performed an *ex vivo* experiment, in which blood samples from COVID-19 patients were exposed to the *C. albicans* lysate, resulting in a lower production of IL-6, TNF, IL-1 α , IL-1 β , IL-10, and IFN- γ compared to control — blood from a healthy donor exposed to the fungus [26]. However, we pioneered in describing the cytokine profile in blood samples from patients with COVID-19 and candiduria. Our results demonstrated that COVID-19 and candiduria induced an increase in the level of cytokines IL-1 β , IL-1ra, IL-2, CXCL8, IL-17, basic FGF, IFN- γ ; and of chemokine CCL4/MIP-1 β in the plasma of patients. We also observed significantly positive correlations among most of the eight cytokines, chemokine, and growth factor analyzed in patients with COVID-19 and candiduria. These cytokines (except IL-1ra) primarily play a pro-inflammatory role and are part of the cytokines release syndrome (CRS) or macrophage activation syndrome (MAS) in severely ill COVID-19 patients [2, 27].

Next, we evaluated whether these elevated cytokines were related to the occurrence of candiduria and increased risk of death in patients with COVID-19. IFN- γ and IL-1ra were significantly associated with the occurrence of candiduria in COVID-19 patients, whereas basic FGF and IL-1 β were significantly associated with the risk of death in the SARS-CoV-2 and candiduria cases. The role of the cytokines evaluated in our study has been described for both SARS-CoV-2 and *Candida* infections. For example, the IL-1 family members, the pro-inflammatory IL-1 β and the IL-1 receptor antagonist (IL-1ra, anti-inflammatory), are associated with the “cytokine storm” seen in patients with severe COVID-19 [28, 29]. In this context, the use of IL-1 β signaling antagonists, such as the anti-IL-1 β monoclonal antibody canakinumab or the IL-1 receptor antagonist anakinra were able to reduce the need for supplemental oxygen therapy, fever, time in the ICU, and mortality in COVID-19 patients

[30, 31]. Likewise, IL-1 β is activated by neutrophils during the early stages and of *Candida* spp. infection and by the inflammasome in the later stages. In addition, fungal cell wall β -glucan induces the IL-1ra expression [32]. Furthermore, pro-inflammatory cytokines and chemokines induce several pathological factors including hypercoagulation and pathological angiogenesis. Endotheliopathy could give rise to hypercoagulation by altering the level of different factors such as the von Willebrand factor. Regarding the pathogenesis of angiogenesis, proangiogenic factors such as the vascular endothelial growth factor (VEGF), basic FGF or placental growth factors (PlGF) were upregulated in the plasma or lung biopsies of COVID-19 patients [33, 34].

Importantly, we found that CXCL8 was one of the most robust biomarkers of candiduria in COVID-19 patients, since it was associated with the diagnosis of fungal infection or colonization, and with the risk of death of the patients. CXCL8 is a potent pro-inflammatory cytokine that plays a key role in the recruitment and activation of neutrophils during inflammation and is commonly increased in patients with COVID-19. Given the frequent neutrophilia seen in patients infected with SARS-CoV-2, it is possible that CXCL8 contributes to the pathophysiology of COVID-19 [35, 36]. CXCL8 is also the main mediator released at sites of *Candida* spp. infection, where it exhibits potent anti-*Candida* activity by recruiting and activating neutrophils and monocytes that recognize and eliminate fungal elements [32].

In conclusion, our results suggest that immunomodulation induced or increased by SARS-CoV-2 and *Candida* spp. is not trivial. Thus, the early measurements of inflammatory mediators can be reliable predictors of fungal coinfection and may help in the diagnostic and in the treatment of COVID-19-associated candidiasis.

The relatively small sample size and the conduction of a single-center study could lead a limitation of our analyses and further investigations on SARS-CoV-2-*Candida* coinfecting patients are needed. However, this study also has the strength to provide insights into the immune pathogenesis in patients with COVID-19 and candiduria and could be of value for the rational development of therapeutic strategies for the effective treatment of these coinfecting patients.

Author contributions All authors contributed to the study conception and design. Vanessa C.R. Magalhães, Rachel B. Caligorne, Alexandre S. Moura, Ana Raquel O. Santos, Tatiani Fereguetti, Juliana C. Martins, Lívia F. Rabelo, and Ana C. Lyon contributed to the patient enrollment, sample and data collection. Junya L. Singulani, Danielle L. Silva, Caroline M. Lima, and Vanessa C.R. Magalhães were responsible for the microbiological identification and antimicrobial susceptibility tests. Junya L. Singulani, Olindo A. Martins-Filho, and Jordana G. A. Coelho dos Reis were responsible for the analysis of inflammatory mediators. Daniel A. Santos performed conceptualization, funding acquisition, project administration, resources and supervision. The first draft of the manuscript was written by Junya L. Singulani, and all authors

commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are not publicly available to protect our participants' sensitive data but are available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was approved by the National Ethics Committee (Comissão Nacional de Ética em Pesquisa- CONEP) and the hospital's Ethics Committee (CAAE: 30627320.6.0000.0008).

Consent to participate Each participant signed written informed participatory consent.

Competing interest The authors declare no competing interests.

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