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Alterations of serum cytokine levels and their relation with inflammatory markers in candidemia

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Abstract

The roles of CRP, PCT, serum amyloid A (SAA), and cytokines in the diagnosis of fungal infections have not yet been clearly demonstrated. This study aims to measure the serum levels of interleukin (IL)-23, IL-17, IL-1β, tumor necrosis factor (TNF)-α, IL-10, transforming growth factor (TGF)-β, C-reactive protein (CRP), procalcitonin (PCT), and serum amyloid A (SAA) in cases of candidemia and to compare them with those observed in cases of bacteremia.

For this purpose, the serum cytokine levels from 50 patients with candidemia were compared with those of 14 patients with polymicrobial sepsis, 30 patients with bacteremia, and 27 healthy control subjects. The cytokine levels were studied using sandwich ELISAs according to the manufacturer protocol.

The serum levels of TGF-β, IL-23, and IL-17 were found to be significantly higher in the candidemia group in comparison with the samples from those with bacteremia and healthy controls. The PCT and SAA levels were higher in samples from the group with

bacteremia those from individuals with candidemia and the healthy control group. Assuming an IL-17 level threshold of >38.79 pg/ml, the sensitivity and specificity were 38% and 96.6%, respectively but considering an IL-23 threshold of >59.97 pg/ml, the sensitivity and specificity values were found to be 72% and 60%, respectively. The sensitivity and the specificity of the TGF-ß levels were found to be 85.71% and 53.33%, respectively, when the TGF-ß threshold is >560 pg/ml. PCT and SAA demonstrated a superior performance for the differentiation of candidemia and bacteremia.
Our study demonstrates that IL-17, IL-23, TGF-ß, PCT, and SAA levels could be a diagnostic marker for candidemia

Key words: candidemia, IL-17, IL-23, PCT, sepsis.

Introduction

In the last 25 years, the incidences of fungal infections have dramatically increased, for example, 19% of the infections documented in the intensive care units (ICU) were caused by fungi. More than 80% of the fungi that cause nosocomial infections are *Candida* species [1–3].

Based on the data obtained from the National Nosocomial Infections Surveillance (NNIS), USA, a 1.8–5.9-fold increase was detected in bloodstream infections caused by the *Candida* species between 1980 and 1990 depending on the hospital type. Another analysis of the data obtained within the same period revealed that the *Candida* species accounted for 85.6% of all nosocomial fungal infections [4–6].

In a more recent study, the mortality rate observed in adults attributed to candidemia was found to be 14.5% [7] and mortality in cases of invasive candidiasis may reach 40–50% [8].

Because delayed administration of the antifungal therapy is associated with an increase in mortality, early diagnosis and therapy is crucial for preventing invasive candidiasis [9–11].

There are no clinical characteristics that differentiates candidemia and bacteremia, but several studies have shown that C-reactive protein (CRP) and procalcitonin (PCT) had acceptable sensitivity in the diagnosis of the bacterial sepsis [12–15]. However, the roles of CRP, PCT, serum amyloid A (SAA), and cytokines in the diagnosis of fungal infections have not yet been clearly demonstrated.

This study aims to measure the serum levels of transforming growth factor (TGF)-β, interleukin (IL)-1β, IL-23, IL-17, IL-10, tumor necrosis factor (TNF)-α, CRP, PCT, and SAA in cases of candidemia and to compare them with those observed in the cases of bacteremia.

Materials and methods

Patients

A total of 64 patients with candidemia, including 50 patients with blood cultures that yielded *Candida* spp. alone and 14 patients in which bacteria and *Candida* spp. (polymicrobial sepsis group) were obtained in blood cultures, were studied in adult clinics of the Uludag University, Health Sciences Center between May 1, 2011, and February 28, 2013.

Thirty patients with bacteremia and 27 patients without any infections (20 healthy people without comorbidity who had been admitted to the center for blood transfusions as donor candidates and 7 people who had been hospitalized for elective surgery) were enrolled in the study to complete the comparative model. Venous blood samples obtained from patients and healthy control subjects were stored at −80°C until they were used in enzyme-linked immunosorbent assay (ELISA) analysis.

To determine the degree of the underlying disease, the patients from the wards were evaluated using the Charlson comorbidity index, and the severity of acute disease was evaluated using the Acute Physiology and Chronic Health Evaluation II (APACHE II) in ICU patients [16,17].

The definitions of nosocomial infections were established according to the definitions provided by the Center for Disease Control and Prevention (CDC) [18].

The mortality rates observed within the first 28 days after the development of candidemia were calculated.

The study was approved by Uludag University, Faculty of Medicine, Ethics Board, and all patients and control subjects enrolled in the study were informed about the study and provided their consent.

Blood Culture Procedure

Two or three blood samples for culture were taken from the patients based on the suspicion of sepsis as defined by the clinician with intervals of 5 to 30 min via different veins.

BACTEC PLUS (+) Aerobic/F (BD, Sparks, MD, ABD) blood culture bottles were used, which were each inoculated with 8–10 ml of blood.

Bacterial isolation from blood cultures was conducted in the Bacteriology Laboratory, Faculty of Medicine, Uludag University (BACTEC 9240 Becton Dickinson, INC, Sparks, MD, blood culture system).

Isolation and Characterization of the *Candida* Species

The yeasts obtained in pure cultures were identified to species using germination assays, the microscopic morphology on cornstarch-Tween 80 agar, the color of the colony in chromogenic broth, and the API-32C system kit (Bio-Merieux, France).

Measurement of the Serum Cytokine Levels

The serum cytokine levels were measured using an ELISA (human TGF-β1, Boster Immunoleader®; human IL-23, Omni Kine®; human IL-17, Boster Immunoleader®; human TNF-α, Assay Max®; human IL-1 β, Boster Immunoleader®; human IL-10, Boster Immunoleader). The cytokine levels were measured following the manufacturers' instructions, for which the detection limits were 15.6–1000 pg/ml for TGF-β1, 63–8000 pg/ml for IL-23, 31.2–2000 pg/ml for IL-17, 0.015 ng/ml for TNF-α, 3.9–250 pg/ml for IL-1 β, and 3.4–250 pg/ml for IL-10. Values below the detection limits were evaluated as zero.

Measurement of CRP, Serum Amyloid A (SAA), and PCT Levels

In patients and in the control group, the CRP and SAA levels were measured using the nephelometric method (Siemens BNII, Cardiophase, Germany), and the PCT levels were evaluated using an ELISA kit (BRAHMS PCT) on the VIDAS device (Bio Mérieux).

Statistical Analysis

In the study, continuous variables were expressed as the median (minimum-maximum), and categorical variables were expressed as the frequency and the corresponding percentage value. Intergroup comparisons of the continuous variables were performed using Kruskal Wallis and the Mann–Whitney *U* test, and intergroup comparisons of the categorical variables were performed using the Pearson chi-square test, Fisher's Exact Chi-Square test, and Fisher-Freeman-Halton Exact test. In order to determine the cut-off value for cytokine serum levels, an ROC analysis was performed and an area under the curve (AUC), relevant sensitivity, and specificity values were calculated. The relationship between the continuous variables was examined using a Spearman correlation coefficient. In this study, analyses were performed using SPSS v. 20 software, and $P < 0.05$ was considered to be statistically significant.

Results

The study included 37 female (39.30%) and 57 male (60.70%) subjects, whereas there were 5 women and 22 male control subjects. The median age was 60 (19–81) years of those in the candidemia group, 60 (24–92) years for individuals in the bacteremia group, 63 (18–87) years in the polymicrobial sepsis group, and 36 (20–79) years for the members in the healthy control group. The age was higher in the candidemia and bacteremia groups in comparison with the healthy control group ($P < 0.001$ for both comparisons).

Eleven of the 50 patients with candidemia, 11 of the 30 patients with bacteremia, and 8 of the 14 patients with polymicrobial sepsis died within the first 28 days of the study. The 28-day mortality was higher in patients with polymicrobial sepsis in comparison with the candidemia patients ($P = 0.019$). The demographic characteristics of the groups are summarized in Table 1.

For the patients with candidemia (2 patients had two different species of *Candida* in the same blood culture) who were enrolled in the study, *C. albicans* ($n = 20$) was the most common etiologic agent, followed by *C. parapsilosis* ($n = 12$), *C. glabrata* ($n = 10$), *C. tropicalis* ($n = 6$), *C. keyfr* ($n = 3$), and *C. dubliniensis* ($n = 1$).

For the patients with bacterial sepsis who were enrolled in the study, the most common isolated bacteria was *Acinetobacter baumannii* ($n = 11$) and of the 36 microorganisms isolated from the 30 patients, 19 were gram-negative bacteria, and 17 were gram-positive bacteria.

We found that the SAA levels were significantly higher in the bacterial sepsis group than those in the candidemia group and the healthy control group ($P = 0.007$ and $P < 0.001$, respectively; Tables 2 and 3, Fig. 1).

While no significant differences were found among the groups in terms of CRP levels, the PCT levels were significantly greater in the bacterial sepsis group and the polymicrobial sepsis group in comparison with samples from the

Table 1. Demographic characteristics of patients and control group*.

	Candidemia (n = 50)	Bacteremia (n = 30)	Polymicrobial (n = 14)	Healthy controls (n = 27)
Patients	50(41)	30(25)	14(12)	27(22)
Female	25(50)	6(20)	6(43)	5(19)
Male	25(50)	24(80)	8(57)	22(82)
Age	60 (19–81)	60 (24–92)	63 (19–87)	36 (20–79)
Mortality at 28th day	11(22)	11(36.70)	8(57.10)	–
Clinic in which the patients is hospitalized/ICU	22(44)	27(90)	9(64.30)	–
Clinic in which the patients is hospitalized/ Internal wards	16(32)	3(10)	4(29)	–
Clinic in which the patients is hospitalized/ Surgical wards	12(24)	0(0)	1(7.10)	–
Duration of hospital stay until the occurrence of septic episode (range)	17.50 (2–98)	11 (1–95)	17.50 (2–173)	–
Time to serum sample collection(days)**	3 (1–9)	***	3 (2–8)	–

Note. *Data were given as median(minimum-maximum) or *n* (%). **Time passed from first positive blood culture sampling to the collection of serum sample for cytokine measurement. ***Blood culture and serum sampling were concomitantly performed.

Table 2. Serum cytokine levels in patients and control group.

	Candidemia (*n* = 50)	Bacteremia (*n* = 30)	Polymicrobial Sepsis (*n* = 14)	Control (*n* = 27)
IL-23 (pg/ml)	99.90 (27.30–1593.80)	58.10 (2.90–637.30)	93 (16.9–2202.50)	0(0–6721)
IL-17 (pg/ml)	0 (0–611.60)	0 (0–103.80)	12.40 (0–263.10)	0 (0–157.40)
TNF-α (pg/ml)	0 (0–406)	0 (0–129)	0 (0–261)	0 (0–84)
IL-1β (pg/ml)	12.10 (0–78.50)	9.30 (1.90–60.40)	12.60 (0–59)	4.02 (0–15.60)
IL-10 (pg/ml)	55.07 (14.1–683.08)	46.10 (0–1232.20)	73.80 (7–575.30)	3.7 (0–55.90)
TGF-ß (pg/ml)	900.70 (258.20–2835.10)	552.50(118–2690.70)	829.80 (243.50–1706.60)	744.4 (298.80–1481.20)
SAA (mg/l)	222 (2–997)	419 (8–1240)	209.50 (60–852)	3 (0–128)
PCT (ng/ml)	0 (0–35)	3 (0–109)	7 (0–108)	0 (0)
CRP (mg/dl)	10 (2–20)	12.50 (1–33)	11.50 (2–28)	0 (0–3)

Note, Data are given as median (minimum-maximum).

Table 3. *P* values obtained from paired comparison of the parameters significant at cytokine levels given in Table 2.

	IL-23	IL-17	IL-1β	IL-10	TGF-ß	SAA	PCT
$Group_{1–2}$*	0.034	0.019	0.144	0.385	0.003	0.007	<0.001
$Group_{1–3}$*	0.626	0.768	0.498	0.508	0.408	0.760	0.006
$Group_{1–4}$*	<0.001	0.005	<0.001	<0.001	0.014	<0.001	ND**
$Group_{2–3}$*	0.182	0.024	0.668	0.326	0.096	0.078	0.416
$Group_{2–4}$*	<0.001	0.330	<0.001	<0.001	0.306	<0.001	ND**
$Group_{3–4}$*	<0.001	0.026	0.003	<0.001	0.257	<0.001	ND**

*$Group_{1–2}$: Candidemia-Bacteremia, $Group_{1–3}$: Candidemia-Polymicrobial sepsis, $Group_{1–4}$: Candidemia-Control, $Group_{2–3}$: Bacteremia-Polymicrobial sepsis, $Group_{2–4}$: Bacteremia-Control
$Group_{3–4}$: Polymicrobial sepsis-Control, **ND:** Not done
**As the serum levels were at an undetectable level in the healthy controls, the comparisons with control group could not be done.

candidemia group ($P < 0.001$ and $P = 0.006$, respectively; Tables 2 and 3).

The most interesting result of our investigation was the significantly higher levels of the Th17 type cytokine, IL-17, in the serum samples from patients with candidemia in comparison with samples from patients with bacterial sepsis and the healthy subjects (Tables 2 and 3) and that IL-17 levels were greater in the polymicrobial sepsis group than

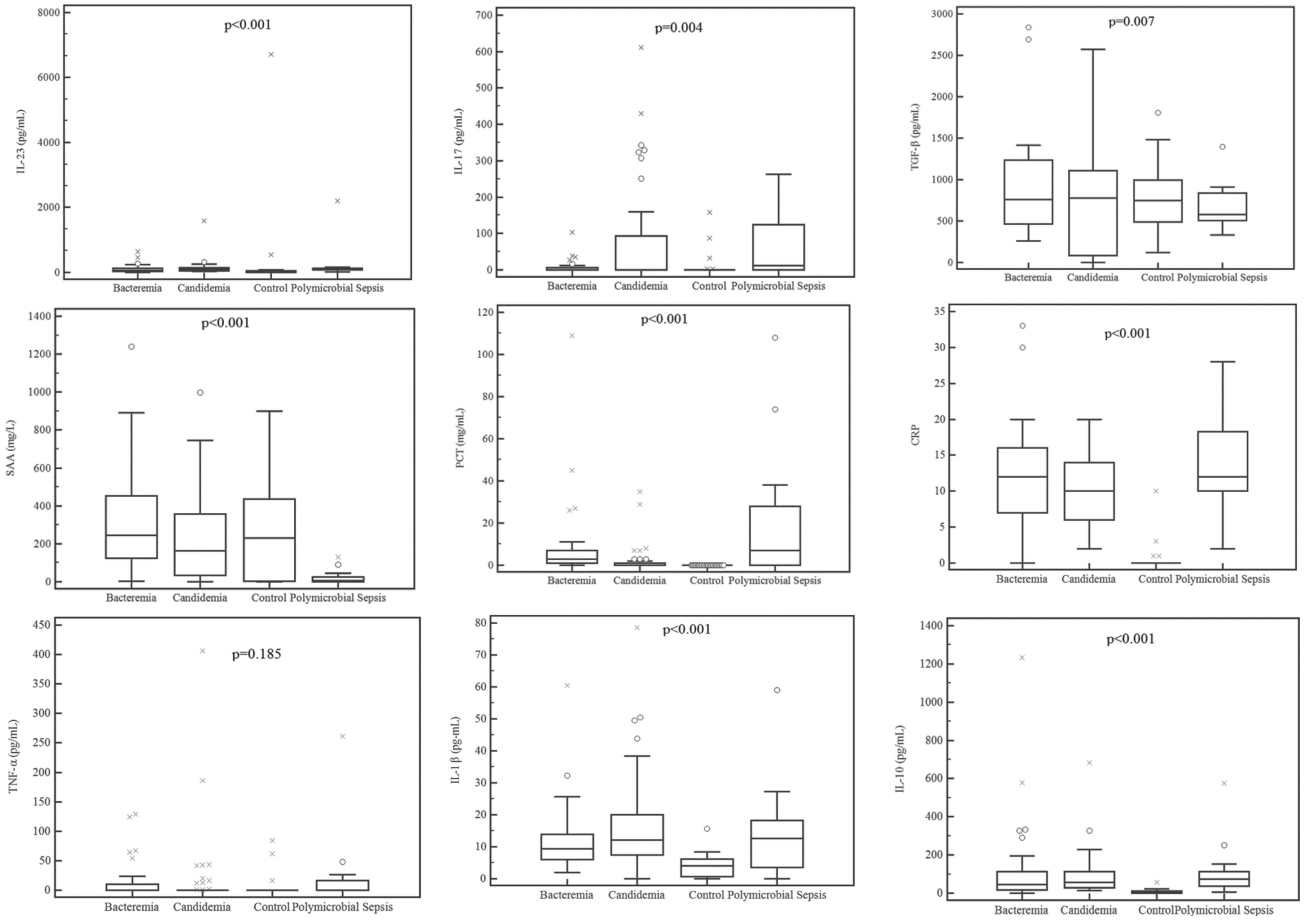

Figure 1. Serum cytokine levels in patients and control group.

those with bacterial sepsis ($P = 0.024$). The IL-23 levels were lower in the healthy control group in comparison with the bacterial sepsis, candidemia, and polymicrobial sepsis groups ($P < 0.001$).

When the proinflammatory cytokine levels were evaluated, it found that there were no significant differences between the study groups (Table 2). Conversely, the levels of the anti-inflammatory cytokine IL-10 were significantly elevated in the candidemia, bacterial sepsis, and polymicrobial sepsis groups in comparison with the healthy control group ($P < 0.001$ for all comparisons).

A statistically significant positive correlation was found for IL-17, IL-23, and TGF-β levels. Interestingly, the TGF-β levels were not correlated with any other cytokines, whereas IL-17 and IL-23 were positively correlated with most of the tested cytokines and inflammatory mediators (Table 4).

For cytokines and other inflammatory markers, the cut-off values for distinguishing between patients with candidemia and bacteremia are shown in Table 5. The AUC for distinguishing between candidemia and bacteremia using IL-17, IL-23, and TGF-ß were 0.640, 0.642, and 0.702, respectively ($P = 0.024$, $P = 0.022$, and $P < 0.001$, respectively). Using a cut-off level of greater than 38.79 pg/ml for the IL-17 levels yielded sensitivity and specificity values of 38.0% (95% CI = 24.7–52.8) and 96.6% (95% CI = 82.7–99.4), respectively (Fig. 2A). Cut-off values higher than 59.97 pg/ml for the IL-23 levels resulted in sensitivity and specificity values of 72.0% (95% CI = 57.5–83.6) and 60.0% (95% CI = 40.6–77.3), respectively (Fig. 2B). TGF-ß levels greater than 560 pg/ml resulted in sensitivity and specificity values of 85.7% (95% CI = 72.7–94.0) and 53.3% (95% CI = 34.3–71.6), respectively (Fig. 2C). In addition to these cytokines, PCT and SAA demonstrated superior performance in differentiating candidemia and bacteremia patients (data not shown), conversely, IL-1ß, IL-10, TNF-α, and CRP levels were not useful to distinguish these two infections (data not shown).

All possible combinations of cytokines and other biomarkers were evaluated for distinguishing between patients with candidemia and bacteremia, with the statistically significant results shown in Table 6.

Table 4. Correlations between Th17 type cytokines and the other examined cytokines and inflammatory markers in cases ($n = 120$).

	IL-17		IL-23		TGF	
	r	*P*	r	*P*	r	*P*
IL-17			0.345	<0.001	0.208	0.023
IL-23	0.345	<0.001			0.184	0.044
TGF-Beta	0.208	0.023	0.184	0.044		
IL-1	0.165	NS	0.456	<0.001	0.050	NS
IL-10	0.392	<0.001	0.486	<0.001	0.134	NS
TNF-Alpha	0.356	<0.001	0.204	0.025	0.014	NS
CRP	0.225	0.014	0.392	<0.001	−0.116	NS
SAA	0.012	NS	0.301	0.001	−0.178	NS
PCT	0.231	0.011	0.334	<0.001	0.087	NS

CRP: C-reactive protein, SAA: Serum Amyloid A, PCT: Procalcitonin, r: Spearman correlation coefficient, NS: Not significant.

Table 5. ROC analysis for serum cytokine levels in the patients with candidemia.

	Sens.(%)	Spec.(%)	PPV(%)	NPV(%)	Cut-off	AUC	*P*
PCT (ng/ml)	77.55	63.33	77.60	63.30	≤ 1	0.733	<0.001
SAA (mg/l)	67.35	66.67	76.70	55.60	≤ 315	0.681	0.005
IL-17 (pg/ml)	38	96.60	95	48.30	>38.70	0.640	0.024
IL-23 (pg/ml)	72	60	75	56.20	>59.90	0.642	0.022
TGF-β (pg/ml)	85.71	53.33	75	69.60	>560	0.702	<0.001

Sens.: Sensitivity, Spec.: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: Area under curve.

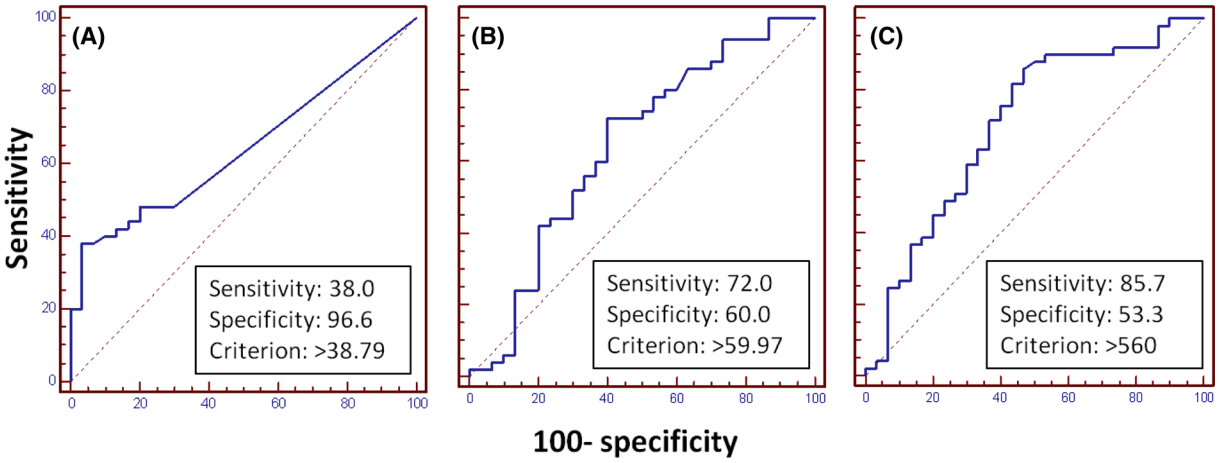

Figure 2. Receiver operating characteristics (ROC) curve for serum IL-17 (A) IL-23 (B) and TGF-β (C) levels in patients with candidemia and bacteremia. Chosen cut-off values for univariate analysis are indicated. This Figure is reproduced in color in the online version of *Medical Mycology*.

In all cases of sepsis, when the difference of the cytokine levels were examined among patients who died or those who survived as of day 28, it was found that the CRP, PCT, and IL-10 levels were higher in the patients who died ($P = 0.005$, $P < 0.001$, $P = 0.033$, respectively), and the IL-23 levels were higher in the survivors ($P = 0.043$; Table 7).

Regarding the mortality rate at day 28 for the candidemia group, it was found that only the PCT levels were higher in the patients who died ($P = 0.009$; data not shown, supplemental table 1).

After comparing the serum cytokine levels between the *C. albicans* and non-*C. albicans Candida* species in 48 patients with candidemia (2 patients were excluded from the analysis due to the growth of two different species of *Candida* in the same blood culture), only the IL-1β level was found to be higher in cases caused by *C. albicans* in comparison with the non-*C. albicans Candida* species ($P = 0.019$;

Table 6. All significant combinations for serum cytokine or inflammatory marker levels in the patients with candidemia.

Combinations	p(LR model)	Sens.(%)	Spec.(%)	PPV(%)	NPV(%)	AUC	p(AUC)
PCT*SAA	0.003	79.59	73.33	83.00	68.70	0.77	<0.001
PCT*IL-17	0.045	81.63	56.67	75.50	65.40	0.71	<0.001
SAA*IL-23	0.009	34.69	86.67	81.00	44.80	0.61	0.040
IL-17*IL-23	<0.001	48.00	86.67	85.70	50.00	0.65	0.001
IL-17*TGF-β	0.014	85.71	56.67	76.40	70.80	0.71	<0.001
IL*23*TGF-β	<0.001	36.73	96.67	94.70	48.30	0.64	0.009
SAA*IL-17*IL-23	0.003	40.82	90.00	87.00	48.20	0.63	0.020
SAA*IL-23*TGF-β	0.001	36.73	93.33	90.00	47.50	0.62	0.038
IL-17*IL-23*TGF-β	<0.001	51.02	86.67	86.20	52.00	0.70	<0.001
SAA*IL-17*IL-23*TGF-β	<0.001	38.78	93.33	90.50	48.30	0.63	0.011

p(LR model): *P*-value of logistic regression model, p(AUC): *P*-value of area under ROC curve, Sens.: Sensitivity, Spec.:Specificity, PPV: Positive predictive value, NPV: Negative predictive value.

Table 7. Serum cytokine levels in the patients with sepsis who died or those who survived at 28th day.

	Survived (n = 64)	Died (n = 30)	*P*-value
IL-23 (pg/ml)	98.20(2.95–2202.50)	75.73(13.60–266.60)	0.043
IL-17 (pg/ml)	0(0–611.60)	11.30(0–328.9)	0.303
TNF-α (pg/ml)	0(0–406)	0(0–261)	0.416
IL-1β (pg/ml)	11.70(0–78.5)	9.50(0–60.40)	0.207
IL-10 (pg/ml)	51.30(0–683)	80.10(7–1232.20)	0.033
TGF-β (pg/ml)	842.70(243.80–2835)	776.20(118–2569)	0.805
SAA (mg/l)	287(8–997)	264(2–1240)	0.702
PCT (ng/ml)	1(0–38)	3.50(0–109)	<0.001
CRP (mg/dL)	10(1–30)	13.50(3–33)	0.005

Note, Data are given as median (minimum-maximum).

data not shown, supplemental table 2). The IL-1β level was found to be higher in *C. albicans* cases as compared with those involving *C. glabrata* species ($P < 0.001$).

Of the 50 patients with candidemia, 14 were treated with antifungal therapy before obtaining a serum sample for cytokine measurement. When the serum cytokine levels were compared among patients who received and those who did not receive antifungals, no significant difference was detected (data not shown, supplemental table 3).

Discussion

In our study, we investigated whether or not the levels of the acute phase proteins and proinflammatory cytokines would be useful for the diagnosis of candidemia.

As supported by the meta-analyses of relevant studies that have been collectively reviewed, the CRP and procalcitonin levels may be useful in the differential diagnosis of sepsis-SIRS in patients presenting systemic inflammatory response syndrome [19–22]. Another meta-analysis showed that PCT was more useful for the differential diagnosis of sepsis-SIRS compared than CRP [23]. It has been found that the serum CRP levels were lower in patients with candidemia than those with bacteremia [12, 24–26]. The role of the CRP and PCT serum levels in the diagnosis and prognosis of the invasive candida infections has not been fully explored or described in the literature.

In our study, we could not find any difference among patients with candidemia, bacteremia, and polymicrobial sepsis in terms of CRP levels. This may be attributed to the heterogeneity of the patients enrolled in the study and to the critically nature of the illness of some patients who formed a substantial part of the patients enrolled in our investigation.

Lower PCT levels were reported in the candidemia group of patients in comparison with the bacteremia group, with the sensitivity found to be between 60% and 100%, and the specificity between 69% and 100% with different cut-off values varying between 0.5–8.06 ng/ml [12, 24–26]. In our study, we found that the PCT levels were lower in the candidemia group compared with the bacteremia and polymicrobial sepsis groups. In the cases of candidemia, a PCT threshold value as $\leq$ 1 ng/ml was found to have a sensitivity of 77.6%, a specificity of 63.3%, a positive

predictive value of 77.6%, and a negative predictive value of 63.3% in differentiating from patients with bacteremia.

In the study performed by Fu et al., different threshold values of 2.12 and 8.06 were obtained for gram-positive and gram-negative bacteria, respectively [26]. As this differentiation cannot be made in our and in studies, the threshold values could have been affected. The differences among patient groups included in the study may have affected the threshold values [12,24,25].

In one study, the PCT levels were found to be significantly higher in gram-negative bacteremia compared with the same form of infections caused by gram-positive bacteria [27]. Furthermore, the PCT levels are lower in sepsis attacks that occur after the first sepsis attack [28].

In a previous study on patients with invasive fungal infections, it was demonstrated that the PCT levels tended to increase between the first day and the tenth day, that PCT and CRP levels at the first and third days were significantly lower compared with those with bacterial infections, and that the difference lost its significance at the fifth and tenth day [24]. In our study, unlike other studies, samples for PCT levels could not be collected concomitantly with the blood cultures, but the samples were collected a median of 3 days after the initial blood cultures, which were the basis for the candidemia diagnosis. Despite this difference, our study revealed lower PCT levels in the cases of candidemia.

Like CRP, SAA is an acute phase protein. Although it seems to have the same value as CRP, it was reported to be more sensitive in neonatal sepsis compared with CRP and PCT. Furthermore, another study conducted on late neonatal sepsis demonstrated a higher value for CRP [29–31]. We encountered only one study that addressed the role of SAA in the differentiation of adult invasive fungal infections from bacterial sepsis.

Although the SAA levels were higher in the cases of candidemia in the study conducted by Fu et al., no difference in terms of SAA levels was detected between bacterial sepsis and candidemia [26].

In our study, we found that the SAA levels were higher in the bacterial sepsis group compared with the candidemia group and the healthy control group. The fact that the collection of serum samples from the patients with candidemia was delayed by a median of 3 days compared with the blood cultures may explain the low SAA levels in our patients with candidemia to some extant; however, the SAA levels were not different between the bacteremia group and the polymicrobial sepsis group in which the time of collection for the blood culture was not different from that observed in candidemia group.

During candida infections, pattern-recognition receptors located on the surfaces of the professional phagocytes, especially Dectin-1, recognize both mannans and mannoproteins of the fungus as well as the other structures on the fungus, such as beta-glucan and chitin. The cytokines released vary by the pattern that is recognized and by the pattern-recognition receptor. While the releases of TNF-α, IL-6, IL-12, and IL-1β are stimulated via several pathways, the secretion of IL-23 and IL-17 depend on the stimulation by IL-23 rather than through lectin receptors. Th17 cells secrete special cytokines, including IL-17A (IL-17), IL-17F, IL-21, and IL-22. The cytokines IL-1β, IL-6, and TGF-β induce the development of Th17 cells, while IL-23 is an essential cytokine for the maintenance of Th17 cells [32]. Our study demonstrates that the level of the Th17 type cytokine IL-17 is elevated in the serum samples of the patients with candidemia in comparison with both patients with bacterial sepsis and the healthy control subjects. Among the above mentioned cytokines, the serum TGF-β and IL-23 levels were found to be increased and to be able to differentiate the patients with candidemia from both the patients with bacteremia and the healthy control subjects, whereas the IL1-β levels were only higher in comparison with the healthy control subjects. The protective role of Th17 responses in the antifungal host defense was first established in IL-17 receptor-deficient (IL-17RA) mice that showed increased susceptibility to a disseminated *C. albicans* infection [33]. Another similar study demonstrated that IL-17RA mice showed increased mortality and higher fungal loads in the kidneys in a model of disseminated candidiasis, which was partially caused by a lower neutrophil recruitment in the IL-17RA mice [34]; however, in one study of IL-17RA mice, it was suggested that IL-22 mediates protection, whereas IL-17A contributes to disease susceptibility [35].

In addition, an oropharyngeal candidiasis model in mice showed that an IL-23p19 or IL-17 deficiency led to severe oropharyngeal candidiasis, whereas it did not cause severe oropharyngeal candidiasis in IL-12p35 knock-out mice (a model for investigating IL-12 response) [36]. In addition, patients with impaired Candida-specific Th17 responses, such as patients with hyper immunoglobulin E (IgE) syndrome or chronic mucocutaneous candidiasis (CMC), are highly susceptibility to mucosal *C. albicans* infections [37,38]. All of these observations strongly indicate that Th17 responses are important for not only systemic but also mucosal host defenses against candida infections in humans.

Our results show that IL-17, IL-23, and TGF-β serum levels were significantly increased in candidemia when compared with bacteremia. Conversely, the specificity of IL-17 is higher than the specificities of IL-23 and TGF-β in the patients with candidemia. Several studies showed that

IL-23 and IL-17 play an important role in the development of the inflammatory response against the infectious agent [39].

In addition to their important roles in immunopathogenesis, the diagnostic utility of serum IL-17 and IL-23 levels were found to be promising in our study. Our study is the first to provide insight into both the role in immunopathogenesis and the potential diagnostic use of IL-17 and IL-23.

On the other hand, when we reviewed the previous studies for the early diagnosis of candidemia based on a combination of risk factors, colonization index, and serum β-glucan levels, we observed a high negative predictive value (97–100%) and a low positive predictive value (9–66%). In our study, we found that a higher positive predictive value and a lower negative predictive value than previous studies. We found the highest positive predictive value for IL-17. We believe that the measurement of these cytokine levels and/or biomarkers in addition to the risk factors and the colonization index could help in the early diagnosis of candidemia [40–43].

In our study, we also measured the serum levels of TNF-α and IL-1β, both of which are important proinflammatory cytokines. The TNF-α and IL-1β levels were not significantly different among the candidemia, bacterial sepsis, polymicrobial sepsis, and healthy control groups.

Presterl et al. examined the serum levels of TNF-α at the first, seventh, and fourteenth days in 20 patients with candidemia, 20 patients with bacteremia, and 20 control subjects without an infection. The researchers found that the TNF-α levels were elevated and not different between the candidemia and the bacteremia groups on the first day. The TNF-α levels decreased in the bacteremia group in the following days, but the TNF-α levels remained elevated in the candidemia group, prompting a recommendation for an increased duration of antifungal therapy in cases of candidemia [44].

In the Rosentul et al. study on 93 patients with candidemia, the serum IL-1β levels were reported to be very low [45].

In previous studies, both proinflammatory and anti-inflammatory cytokines were shown to be elevated in the patients with severe sepsis and septic shock [46,47].

In our study, we suspected that the absence of the intergroup differences of TNF-α and IL-1β levels may be explained by the fact that the serum sample was not obtained at the onset of the sepsis in the patients with candidemia, by the short half-life of these two cytokines, or by their low serum levels [48,49]; however, animal studies show that *Candida albicans* is not as strong of an inducer of TNF-α in comparison with bacteria [50].

In another animal study, TNF-α was shown to not play a major role in candida sepsis in terms of the progression to lung injury and multiple organ failure [51]. The results of these two animal studies led us to question the role of TNF-α in invasive candida infections.

IL-10 inhibits the synthesis of the proinflammatory cytokines, has an anti-inflammatory effect, and plays an important role in the control of the immune response during the course of the systemic infections [52].

In our study, the serum IL-10 levels observed in the candidemia group were not different from those observed in the bacterial sepsis and polymicrobial sepsis groups; however, when the levels of cytokines between all deceased and surviving patients with candidemia and bacteremia were compared at the twenty-eighth day, CRP, PCT, and IL-10 levels were found to be higher in the deceased patients. Again, in the studies conducted on the patients with sepsis, the IL-10 levels were found to be higher in deceased patients [46,53]. In both bacterial sepsis and candidemia, the persistence of the anti-inflammatory effect adversely affects the prognosis.

The serum levels of some other cytokines, such as IL-6, IL-8, and IFN-γ, which were found to be involved in candidemia in previous studies, were not investigated in this study [45]. The Th1 and IFN-γ responses are very important in the host defense mechanism against candida infections. Moreover, it was recently reported that adjunctive immunotherapy with IFN-γ can restore immune function in fungal sepsis patients, including candida sepsis [54], but recent observations also implicate the Th17 cells in the immune response to intracellular bacteria and fungi [55]. Whether or not the Th17 cells are involved in essential antimicrobial mechanisms or tissue destruction in these contexts is still unclear. Therefore, we focused primarily on Th17-related cytokines, which include IL-17 and IL-23. In addition, the serum levels of the proinflammatory cytokines TNF-α and IL-1β and the antiinflammatory cytokine IL-10 were also examined.

Consequently, we consider IL-17 and IL-23 to be proinflammatory cytokines; TGF-β promotes the development of a proinflammatory T cell group when stimulated with IL-6, although it is an anti-inflammatory cytokine. SAA and PCT, which are acute phase reactants, may be used as markers in the differentiation of candidemia and bacteremia. Given the threshold values of IL-17 and IL-23 obtained from candidemia serum samples and the recent introduction of procalcitonin in clinical practices, these tests may be useful for the diagnosis of candidemia. However, more extensive studies with a greater number of patients are warranted. Our study may offer insights into multicenter, prospective studies that will be conducted on a larger scale in the future.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Supplementary material

Supplementary material is available at *Medical Mycology* online (http://www.mmy.oxfordjournals.org/).

References

1. Eggimann P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis* 2003; **3**:685–702.
2. Lipsett PA. Surgical critical care: fungal infections in surgical patients. *Crit Care Med* 2006; **34**(9): 215–224.
3. Vincent JL, Rello J, Marshall J et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009; **302**: 2323–2329.
4. Banerjee SN, Emori TG, Culver DH et al. Secular trends in nosocomial primary bloodstream infections in the United States, 1980–1989. National Nosocomial Infections Surveillance System. *Am J Med* 1991; **91**(3B): 86S-89S.
5. Fisher-Hoch SP, Hutwagner L. Opportunistic candidiasis: an epidemic of the 1980s. *Clin Infect Dis* 1995; **21**(4): 897–904.
6. Hobson RP. The global epidemiology of invasive *Candida* infections-is the tide turning? *J Hosp Infect* 2003; **55**(3): 159–168.
7. Zaoutis TE, Argon J, Chu J et al. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. *Clin Infect Dis* 2005; **41**(9): 1232–1239.
8. Ostrosky-Zeichner L, Pappas PG. Invasive candidiasis in the intensive care unit. *Crit Care Med* 2006; **34**(3): 857–863.
9. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of *Candida* bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. *Antimicrob Agents Chemother* 2005; **49**(9): 3640–3645.
10. Garey KW, Rege M, Pai MP et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. *Clin Infect Dis* 2006; **43**(1): 25–31.
11. Grim SA, Berger K, Teng C et al. Timing of susceptibility-based antifungal drug administration in patients with Candida bloodstream infection: correlation with outcomes. *J Antimicrob Chemother* 2012; **67**(3): 707–714.
12. Martini A, Gottin L, Menestrina N et al. Procalcitonin levels in surgical patients at risk of candidemia. *J Infect* 2010; **60**(6): 425–430.
13. Rau BM, Frigerio I, Buchler MW et al. Evaluation of procalcitonin for predicting septic multiorgan failure and overall prognosis in secondary peritonitis: a prospective, international multicenter study. *Arch Surg* 2007; **142**(2): 134–142.
14. Meisner M, Tschaikowsky K, Palmaers T et al. Comparison of procalcitonin (PCT) and C-reactive protein (CRP) plasma concentrations at different SOFA scores during the course of sepsis and MODS. *Crit Care* 1999; **3**(1): 45–50.
15. Rau BM, Kemppainen EA, Gumbs AA et al. Early assessment of pancreatic infections and overall prognosis in severe acute pancreatitis by procalcitonin (PCT): a prospective international multicenter study. *Ann Surg* 2007; **245**(5): 745–754.
16. Hall WH, Ramachandran R, Narayan S et al. An electronic application for rapidly calculating Charlson co-morbidity score. *BMC Cancer* 2004; **4**: 94.
17. Goffi L, Saba V, Ghiselli R et al. Preoperative APACHE II and ASA Scores in patients having major general surgical operations: prognostic value and potential clinical applications. *Eur J Surg* 1999; **165**(8): 730–735.
18. Horan TC, Gaynes RP. Surveillance of nosocomial infections. In: Mayhall CG (ed.). *Hospital Epidemiology and Infection Control*, 3rd. ed. Philadelphia: Lippincott Williams & Wilkins, 2004: 1659–1702.
19. Povoa P. C-reactive protein: a valuablemarker of sepsis. *Intensive Care Med* 2002; **28**(3): 235–243.
20. Sierra R, Rello J, Bailen MA et al. C-reactive protein used as an early indicator of infection in patients with systemic inflammatory response syndrome. *Intensive Care Med* 2004; **30**(11): 2038–2045.
21. Uzzan B, Cohen R, Nicolas P et al. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or travma: a systematic review and meta-analysis. *Crit Care Med* 2006; **34**(7): 1996–2003.
22. Tang BM, Eslick GD, Craig JC et al. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. *Lancet Infect Dis* 2007; **7**(3): 210–217.
23. Simon L, Gauvin F, Amre DK et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systemic review and meta-analysis. *Clin Infect Dis* 2004; **39**(2): 206–217.
24. Petrikkos G, Christofilopoulou S, Tentolouris NK et al. Value of measuring serum procalcitonin, C-reactive protein, and mannan antigens to distinguish fungal from bacterial infections. *Eur J Clin Microbiol Infect Dis* 2005; **24**(4): 272–275.
25. Charles PE, Dalle F, Aho S et al. Serum procalcitonin measurement contribution to the early diagnosis of candidemia in critically ill patients. *Intensive Care Med* 2006; **32**(10): 1577–1583.
26. Fu Y, Chen J, Cai B et al. The use of PCT, CRP, IL-6 and SAA in critically ill patients for an early distinction between candidemia and gram positive/negative bacteremia. *J Infect* 2012; **64**(4): 438–440.
27. Charles PE, Ladoire S, Aho S et al. Serum procalcitonin elevation in critically ill patients at the onset of bacteremia caused by either gram negative or gram positive bacteria. *BMC Infect Dis* 2008; **8**: 38.
28. Charles EP, Ladoire S, Snauwaer A et al. Impact of previous sepsis on the accuracy of procalcitonin for the early diagnosis of

blood stream infection in critically ill patients. *BMC Infect Dis* 2008; **8**: 163.
29. Cicarelli DD, Vieira JE, Bensenor FE. Comparison of C-reactive protein and serum amyloid A protein in septic shock patients. *Mediators Inflamm* 2008; **2008**: 631414.
30. Çetinkaya M, Özkan H, Köksal N et al. Comparison of serum amyloid A concentrations with those of C-reactive protein and procalcitonin in diagnosis and follow-up of neonatal sepsis in premature infants. *J Perinatol* 2009; **29**(3): 225–231.
31. Uçar B, Yıldız B, Akşit MA et al. Serum amyloid A, procalcitonin, tumor necrosis factor-alpha,and interleukin-1 beta levels in neonatal late-onset sepsis. *Mediators Inflamm* 2008; **2008**: 737141.
32. Locksley RM. Nine lives: Plasticity among T helper cell subsets. *J Experimental Med* 2009; **206**(8): 1643–1646.
33. Huang W, Na L, Fidel PL et al. Requirement of interleukin-17a for systemic anti-candida albicans host defense in mice. *J Infect Dis* 2004; **190**(3): 624–631.
34. van de Veerdonk FL, Kullberg BJ, Verschueren IC et al. Differential effects of IL-17 pathway in disseminated candidiasis and zymosan-induced multiple organ failure. *Shock* 2010; **34**(4): 407–411.
35. De Luca A, Zelante T, D'Angelo C et al. IL-22 defines a novel immune pathway of antifungal resistance. *Mucosal Immunol* 2010; **3**(4): 361–373.
36. Conti HR, Shen F, Nayyar N et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Experimental Med* 2009; **206**(2): 299–311.
37. Grimbacher B, Holland SM, Gallin JI et al. Hyper-IgE syndrome with recurrent infections–an autosomal dominant multisystem disorder. *N Engl J Med* 1999; **340**(9): 692–702.
38. Eyerich K, Foerster S, Rombold S et al. Patients with chronic mucocutaneous candidiasis exhibit reduced production of Th17-associated cytokines IL-17 and IL-22. *J Invest Dermatol* 2008; **128**(11): 2640–2645.
39. Bosmann M, Ward PA. Therapeutic potential of targeting IL-17 and IL-23 in sepsis. *Clin Transl Med* 2012; **1**(1): 4.
40. Pittet D, Monod M, Suter PM et al. *Candida* colonization and subsequent infections in critically ill surgical patients. *Ann Surg* 1994; **220**(6): 751–758.
41. Leon C, RuizSantana S, Saavedra P et al. Usefulness of the "*Candida* score" for discriminating between *Candida* colonization and invasive candidiasis in non-neutropenic critically ill patients: a prospective multicenter study. *Crit Care Med* 2009; **37**(5): 1624–1633.
42. Ostrosky-Zeichner L, Sable C, Sobel J et al. Multicenter prospective development and validation of a clinical prediction rule for nosocomial invasive candidiasis in the intensive care setting. *Eur J Clin Microbiol Infect Dis* 2007; **26**: 271–276.
43. Posteraro B, De Pascale G, Tumbarello M et al. Early diagnosis of candidemia in intensive care unit patients with sepsis: a prospective comparison of(1–3)-β-D-glucan assay, *Candida* score, and colonization index. *Crit Care* 2011; **15**(5): R249.
44. Presterl E, Lassnigg A, Mueller-Uri P et al. Cytokines in sepsis due to *Candida albicans* and in bacterial sepsis. *Eur Cytokine Netw* 1999; **10**(3): 423–430.
45. Rosentul DC, Plantinga TS, Scott WK et al. The impact of caspase-12 on susceptibility to candidemia. *Eur J Clin Microbiol Infect Dis* 2012; **31**(3): 277–280.
46. Gogos CA, Drosou E, Bassaris HP et al. Pro-versus anti-inflammatory cytokine profile in patients with severe sepsis. a marker for prognosis and future therapeutic options. *J Infect Dis* 2000; **181**(1): 176–180.
47. Bozza FA, Salluh JI, Japiassu AM et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. *Critical Care* 2007; **11**(2): R49.
48. Dinarello CA, Gelfand JA, Wolff SM. Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. *JAMA* 1993; **269**(14): 1829–1835.
49. Dinarello CA. Interleukin-1. *Cytokine Growth Factors Rev* 1997; **8**(4): 253–265.
50. Matuschak GM, Munoz C, Epperly NA et al. TNF-alpha and IL-6 expression in perfused rat liver after intraportal candidemia vs. *E. coli* or *S.aureus* bacteremia. *Am J Physiol* 1994; **267**(2): R446–54.
51. Matuschak GM, Lechner AJ. The yeast to hyphal transition following hematogenous candidiasis induces shock and organ injury independent of circulating tumor necrosis factor-alpha. *Crit Care Med* 1997; **25**(1): 111–120.
52. Cyktor JC, Turner J. Interleukin-10 and immunity against prokaryotic and eukaryotic intracellular pathogens. *Infect Immun* 2011; **79**(8): 2964–2973.
53. Heper Y, Akalın H, Mıstık R et al. Evaluation of serum C-reactive protein, procalcitonin, tumor necrosis factor alpha, and interleukin-10 levels as diagnostic and prognostic parameters in patients with community-acquired sepsis, severe sepsis, and septic shock. *Eur J Clin Microbiol Infect Dis* 2006; **25**(8): 481–491.
54. Delsing CE, Gresnigt MS, Leentjens J et al. Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series. *BMC Infect Dis* 2014; **14**: 166.
55. Peck A, Mellins ED. Precarious balance: Th17 cells in host defense. *Infect Immun* 2010; **78**(1): 32–38.