Elevated Levels of Interleukin 17A and Kynurenine in Candidemic Patients, Compared With Levels in Noncandidemic Patients in the Intensive Care Unit and Those in Healthy Controls

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Background. The interplay between Candida species and pattern recognition receptors, interleukins, kynurenine, and T cells has been studied in murine and ex vivo human studies, but data are lacking from patients with invasive fungal infections. Interleukin 17A (IL-17A) is considered an important component in host defense against Candida infections and is modulated by Candida-induced impairment of tryptophan-kynurenine metabolism.

Methods. Dectin-1, Toll-like receptor 2, and Toll-like receptor 4 expression; regulatory T cell (Treg) percentages; and interleukin 6, interleukin 10, IL-17A, interleukin 22, interleukin 23, interferon γ, kynurenine, and tryptophan levels were determined in candidemic patients and compared to levels in noncandidemic patients who are in the intensive care unit (ICU) and receiving antibiotic therapy and those in healthy controls, both with and without Candida colonization.

Results. Candidemic patients had significantly higher IL-17A and kynurenine levels, compared with noncandidemic patients, including Candida-colonized ICU patients and healthy controls. Within candidemic patients, time-dependent elevation of IL-17A and kynurenine levels was detected. IL-17A areas under the curve for differentiation between patients with early candidemia and those without candidemia (ICU patients, including Candida-colonized patients, and healthy controls) were between 0.94 (95% confidence interval [CI], 0.89–0.99) and 0.99 (95% CI, 0.99–1).

Conclusions. Candidemic patients had significantly higher IL-17A and kynurenine levels, compared with noncandidemic patients. The statistically significant association between IL-17A and kynurenine levels and candidemia suggests their potential as biomarkers for anticipation of invasive candidiasis.

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Keywords. candida; interleukins; kynurenine.
serve as the first line of defense by phagocytosis and direct killing of *Candida*. *Candida* species are mainly recognized by 2 membrane-bound pattern-recognition receptors, Toll-like receptor 2 (TLR-2), TLR-4, and C-type lectin receptors, such as dectin-1. Levels of the latter are increased in response to fungal sepsis in mouse leukocytes [1–4]. Dendritic cells, macrophages, and epithelial cells stimulate the differentiation of CD4⁺ T-helper cells and regulatory T cells (Tregs) in response to fungal contact by producing a distinct set of cytokines and other mediators [3]. The balance between T-cell subpopulations is crucial in regulating the course of *Candida* colonization and infection.

Besides Th1 and Th2 cell responses, Th17 cells have been described as an important T-helper cell subtype in regulating and conferring protection against fungal infections [3, 5]. The major cytokine secreted by Th17 cells, which are stimulated by interleukin 6 (IL-6), interleukin 23 (IL-23), and interleukin 1β (IL-1β), is interleukin 17A (IL-17A). IL-17A has multiple proinflammatory functions, such as recruitment of neutrophils, activation of neutrophil and macrophage phagocytosis, and activation of defensin release by epithelial cells [2, 3, 5–7]. Because of these pivotal properties, IL-17A is considered an important component in host defense against *Candida* infections. Against *Aspergillus* infections, however, it has been considered less important because *Aspergillus* is a poor inducer of IL-17A [8]. IL-17A production is stimulated in vitro by incubation of neutrophils with heat-killed *Candida albicans* or live *C. albicans* [6]. However, it has been reported that live *C. albicans* cells dampens host defense by downregulating IL-17A production in neutrophils stimulated by heat-killed *C. albicans*. The IL-17A–diminishing effect of live *C. albicans* is reached by secretion of soluble factors shifting tryptophan metabolism (by blocking indoleamine-2,3-dioxygenase [IDO] expression) away from kynurenines and toward 5-hydroxytryptophan metabolites. Increased 5-hydroxytryptophan levels subsequently inhibit host IL-17A production. This modulation of host response has been reported as a mechanism allowing *Candida* to colonize mucosal surfaces [6, 9]. Other important cytokines involved in host response to *Candida* species include interferon γ (IFN-γ), which is released from Th1 cells and leads to activation of phagocytes at sites of infection; interleukin 2 (IL-22), which is released from Th17 cells (and Th22 cells) and induces anticyclic proteins; and IL-10, which is released from Tregs, with the latter alleviating the immune response, reducing damage to the host but conversely causing immunosuppression [3, 10]. Th2 cells, activated by interleukin 10 (IL-10), also dampen the Th1 cell response.

Whereas the interplay between *Candida* species and pattern-recognition receptors, interleukins, kynurenine, and T cells has been studied in murine models and in ex vivo human studies, few data are available from patients with invasive fungal infections, such as candidemic patients [6]. We therefore determined levels of IFN-γ, IL-6, IL-10, IL-17A, IL-22, and IL-23 in patients with candidemia; in noncandidemic patients in the intensive care unit (ICU), with or without *Candida* colonization; and in healthy individuals with or without *Candida* colonization. We additionally investigated the IDO-dependent tryptophan metabolite kynurenine in serum; determined dectin-1, TLR-2, and TLR-4 expression on human peripheral blood mononuclear cells (PBMCs); and determined Treg percentages.

**METHODS**

**Patients and Sampling**

This study was approved by the local ethical committee (19–322 ex 07/08) and was registered at Clinicaltrials.gov (clinicaltrials.gov identifier: NCT00786903). Individuals were prospectively screened for study inclusion, provided informed consent, and were assigned to one of the following 5 groups: group 1, healthy controls; group 2, patients in the ICU who were intubated, undergoing mechanical ventilation, and receiving antibiotic therapy because of extrapulmonary infection; group 3, patients in the ICU who were intubated, undergoing mechanical ventilation, and receiving antibiotic therapy because of pneumonia; and groups 4 and 5, patients with so-called late and early candidemia, respectively, as described below. With approval from the ethics committee of the Medical University of Graz, unconscious patients (ie, patients who were intubated and undergoing mechanical ventilation) were assigned to group 2 or 3 according to the presence or absence of pneumonia, respectively, and, after they became conscious, were asked to participate in the study. Healthy controls comprised subjects without underlying diseases who were undergoing general anesthesia for elective plastic surgery. This allowed access to the lower respiratory tract in these subjects. Group 4 comprised candidemic patients with blood samples obtained at the time when yeasts (ie, *Candida* species) were detected in blood culture. Because of this delay (mean duration, 4 days; range, 0–8 days) between collection of a blood specimen for culture and collection of a blood specimen for investigations described below, this group was referred to as the “late candidemia” group. To overcome this limitation (ie, the delay between collection of blood specimens for culture and for laboratory analyses in this study), we subsequently also included candidemic patients and corresponding serum samples from our recently established NOBIS (Novel Biomarker in Sepsis) cohort (ie, group 5 patients). In this cohort, serum samples subsequently used for study investigations were obtained simultaneously with blood specimens obtained for culture from patients with systemic inflammatory response syndrome, clinical suspicion of bacteremia/fungemia and order to obtain blood cultures by the attending physician. Serum samples were stored at −70°C until blood cultures showed *Candida* species, after which they were used for study investigations [11]. This candidemia group was referred to as the “early candidemia” group. In the remaining patients (ie, groups 1–3), blood
samples were obtained concomitantly with samples for microbiological analysis for determination of Candida colonization. Candida colonization was investigated by culture of oral swab specimens (for all patients in groups 1–3), perianal swab specimens (for all patients in groups 2 and 3), endobronchial secretions (for all patients in group 1), or bronchoalveolar lavage (for all patients in group 2 and 3) on Candida CHROM agar (Becton Dickinson, Heidelberg, Germany). For group 2–5, 3 pairs of blood specimens were obtained for culture by use of vacutainer systems and processed in automatic blood culture detection systems (Bactec FX, Becton Dickinson, Heidelberg, Germany). All isolated Candida species were identified with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as routinely performed. None of the patients had received antifungal therapy within 8 weeks prior to sampling. Patient characteristics were extracted from charts and computerized databases.

**Determination of Dectin-1, TLR-2, and TLR-4 Expression; Treg (CD4/CD25Foxp3) Percentages; and IFN-γ, IL-6, IL-10, IL-17A, IL-22, and IL-23 Levels**

Whole-blood specimens were collected at time points described above in ethylenediaminetetraacetic acid (EDTA)–containing tubes and serum sampling tubes (Greiner Bio-One, Kremsmünster, Austria). EDTA–whole-blood samples were immediately processed; serum was obtained after centrifugation at 1300 rcf (14.2 cm) and stored at −70°C until further analysis.

For determination of the percentages of cells expressing dectin-1, CD14+/TLR-2, and CD14+/TLR-4 and CD3/CD4/CD25high+ (ie, CD4+CD25highFoxp3+) Treg percentages, the EDTA-anticoagulated blood samples were used. After red cell lysis, staining of cells was performed using target-specific antibodies (Becton Dickinson, Heidelberg, Germany), and sorting was measured by flow cytometry on the FACSCalibur (Becton Dickinson, Heidelberg, Germany) or Navios (Beckman Coulter, Brea, CA) instruments, as described previously [12]. Gating strategies and further analysis of proportions of the subsets of cells were performed using Kaluza software (Beckman Coulter, Brea, CA).

IFN-γ, IL-6, IL-10, IL-17A, IL-22, and IL-23 serum levels were determined using the commercially available Bio-Plex Pro Human Th17 Cytokine 5-Plex assay on a Bioplex200 system (Biorad, Hercules, CA) according to the instructions of the manufacturer. The multiplex suspension bead array immunoassay allowed simultaneous measurement of IFN-γ, IL-6, IL-10, IL-17A, IL-22, and IL-23 serum levels according to the standard protocol. Standard curves for each analyte were generated using the reference analyte concentration supplied by the manufacturers. Each sample was measured at least twice on different 96-well plates. Cytokine concentrations were calculated using a 5-parameter standard curve derived from 3 measurements of reference cytokine concentrations supplied by the manufacturer. The measurement was performed using the Bio-Plex system in combination with Bio-Plex Manager software, version 4.1, with 5-parametric curve fitting (Bio-Rad).

**Determination of Tryptophan and Kynurenine Concentrations**

Tryptophan and kynurenine levels were measured in plasma samples by high-performance liquid chromatography (HPLC) with a simultaneous ultraviolet and fluorimetric detection system as described previously [13, 14]. In brief, a 100-µL plasma sample was deproteinized by adding of 100 µL of 5% (v/v) perchloric acid. After vortexing and 5 minutes of centrifugation at 11,000 ×g, 20 µL of the clear supernatant was injected in the chromatographic system. Separations were achieved on a Chromolith RP18e column (100 × 4.6 mm, 5 µm, Merck Darmstadt, Germany) at 30°C by isocratic elution with a mobile phase (pH 4.9), consisting of 50 mmol/L ammonium acetate, 250 mol/L zinc acetate, and 3% (v/v) acetonitrile, at a flow rate of 0.8 mL/min. Kynurenine and tryptophan were detected on a La Chrom UV-Detector Merck HITACHI L-7400 at 235 nm. Acquisition and processing of the chromatograms were performed using a Merck Hitachi LaChrom- D-7000 HPLC System Manager Software (VWR International/Scientific Instruments, Darmstadt, Germany). The concentrations were determined as the peak height measurement relative to external standards. All reagents were pro analysis grade and obtained from Merck (Darmstadt, Germany). Within-day coefficients of variation at different concentrations were in the range of 1.7% to 4.3% for kynurenine, and 0.7% to 2.9% for tryptophan. The between day coefficients of variation were 2.0% to 5.4% for kynurenine and 6.3% to 9.3% for tryptophan.

**Determination of Serum (1–3)-β-D Glucan Levels**

This investigation was performed according to recently described methods for all patients from groups 2–5 [15].

**Statistical Methods**

Statistical analysis was performed using SPSS, version 22 (SPSS, Chicago, IL), and nQuery Advisor 4.0 (Statistical Solutions, Cork, Ireland). Because of the explorative design of the study and the lack of preliminary in vivo data, a priori sample size calculation was not possible. We performed retrospective power analysis for the comparison of the 2 smallest groups (20 and 24 patients) at 80% for the Wilcoxon rank sum test. The sample size of our study groups was sufficient for the effect size that corresponded to an area under of the curve (AUC) of 0.756 in receiver operating characteristic (ROC) analysis or to a relative treatment effect of 0.9 SDs, assuming a normal distribution of data. Findings for all 5 patient groups were compared using the Kruskal–Wallis test, and differences between treatment groups were tested in a pairwise fashion. Pairs of groups were also compared using the Mann–Whitney U test. ROC curve analysis was performed for IL-17A and kynurenine. AUCs were calculated along with 95% confidence intervals (CIs). A P value of <.05 was considered statistically significant.
RESULTS

A total of 131 individuals were included in the study: 35 were in group 1, 20 were in group 2, 24 were in group 3, 27 were in group 4, and 25 were in group 5. Demographic data and Candida colonization rates in all groups are depicted in Table 1.

The median IL-17A level was significantly higher in group 2 (0.12 pg/mL; interquartile range [IQR], 0–0.28 pg/mL) and group 3 (0.12 pg/mL; IQR, 0–0.44 pg/mL), compared with that in group 1 (0 pg/mL; IQR, 0 pg/mL; \( P < .001 \) for both comparisons), and significantly higher in group 4 (0.73 pg/mL; IQR, 0.22–1.8 pg/mL) and group 5 (4.91 pg/mL; IQR, 2.58–27.55 pg/mL), compared with that in all other groups (\( P = .002 \) for comparison of group 4 vs group 3; \( P \leq .001 \) for all other comparisons). IL-17A levels in group 5 were significantly higher than those in group 4 (\( P < .001 \)). The difference between groups 4 and 5 was driven by a subset of 19 patients in group 4 who had blood samples obtained \( \geq 3 \) days after blood specimens were obtained for culture. After exclusion of those patients, no significant difference in IL-17A levels between individuals with early candidemia and those with so-called modified late
candidemia (defined as individuals with blood samples obtained ≤3 days after blood specimens were obtained for culture) was observed (data not shown).

Box plots of IL-17A values are depicted in Figure 1. The AUC for differentiation was 0.99 (95% CI, .99–1) between groups 5 and 1, 0.98 (95% CI, .94–1) between groups 5 and 2, and 0.94 (95% CI, .89–.99) between groups 5 and 3 (Table 2). The ROC curve for group 5, compared with cumulative data for groups 1–3, is shown in Figure 2. There was also a significant difference between groups 3 and 1, with an AUC of 0.78 (95% CI, .67–.89; Table 2).

### Table 2. Comparisons of Interleukin 17A (IL-17A) and Kynurenine Areas Under the Curve (AUCs) in Receiver Operating Characteristic Curve Analysis Obtained From Comparison of Different Patient Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-17A</th>
<th></th>
<th>Kynurenine</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>AUC (95% CI)</td>
<td>P Value</td>
<td>AUC (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>5 vs 1</td>
<td>0.99 (.99–1)</td>
<td>&lt;.001</td>
<td>0.91 (.82–.99)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5 vs 2</td>
<td>0.98 (.94–1)</td>
<td>&lt;.001</td>
<td>0.76 (.62–.91)</td>
<td>&lt;.003</td>
</tr>
<tr>
<td>5 vs 3</td>
<td>0.94 (.89–.99)</td>
<td>&lt;.001</td>
<td>0.78 (.66–.91)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5 vs 4</td>
<td>0.82 (.71–.99)</td>
<td>&lt;.001</td>
<td>0.65 (.49–.81)</td>
<td>NS</td>
</tr>
<tr>
<td>4 vs 1</td>
<td>0.91 (.83–.99)</td>
<td>&lt;.001</td>
<td>0.84 (.73–.96)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4 vs 2</td>
<td>0.79 (.66–.92)</td>
<td>.001</td>
<td>0.59 (.42–.76)</td>
<td>NS</td>
</tr>
<tr>
<td>4 vs 3</td>
<td>0.74 (.61–.87)</td>
<td>.002</td>
<td>0.64 (.49–.79)</td>
<td>NS</td>
</tr>
<tr>
<td>3 vs 1</td>
<td>0.78 (.67–.89)</td>
<td>&lt;.001</td>
<td>0.7 (.55–.84)</td>
<td>.01</td>
</tr>
<tr>
<td>3 vs 2</td>
<td>0.52 (.36–.67)</td>
<td>NS</td>
<td>0.44 (.28–.60)</td>
<td>NS</td>
</tr>
<tr>
<td>2 vs 1</td>
<td>0.82 (.69–.96)</td>
<td>&lt;.001</td>
<td>0.84 (.71–.97)</td>
<td>&lt;.001</td>
</tr>
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</table>

Abbreviations: CI, confidence interval; NS, not significant.
Median kynurenine levels were significantly higher in group 5 (6.92 μmol/L; IQR, 4.11–9.1 μmol/L), compared with all other noncandidemic groups (group 1, 2.26 μmol/L [IQR, 1.93–2.64 μmol/L]; group 2, 3.87 μmol/L [IQR, 2.91–4.63 μmol/L]; and group 3, 3.08 μmol/L [IQR, 2.23–4.77 μmol/L]; P values are specified in Table 2). There was a trend but no statistically significant difference in kynurenine levels between groups 4 and 5. Since kynurenine is a metabolite of the essential amino acid tryptophan, we also calculated a kynurenine/tryptophan ratio, to exclude dietary influence (ie, low kynurenine levels due to low tryptophan levels); results were similar in this calculation (data not shown).

Median IL-6 levels were significantly elevated in all groups, compared with those in group 1. Significant differences were also observed among groups 2–5, with a higher median value in group 3 (100.4 pg/L; IQR, 34.25–301.6 pg/L), compared with group 2 (35.41 pg/L; IQR, 28.49–49.23 pg/L) and group 4 (35.78 pg/L; IQR, 14.28–131.5 pg/L; P < .05 for both comparisons). The median IL-10 level was lower in group 1 (no elevation above the detection limit of 1.4 pg/mL), compared with the other groups. Median IL-23 levels were not elevated in candidemic patients (no elevation above the detection limit of 1.55 pg/mL), and there were no differences in median IFN-γ and IL-22 levels (no elevation above the detection limits of 9.32 pg/mL and 7.24 pg/mL, respectively) between all groups. There was no difference in the median percentage of PBMCs expressing decentin-1 (≤0.1% cells in all groups), TLR-2 (group 1, 1.6%; group 2, 6.3%; group 3, 5.1%; and group 4, 4.7%), and TLR-4 (group 1, 4.6%; group 2, 9.9%; group 3, 5.9%; and group 4, 4.9%) and the median Treg percentage (≤0.6% in all groups), as determined by fluorescence-activated cell-sorter analysis between groups 1–3 (between groups 2 and 3 for TLR-2) and group 4.

Five patients in group 3 had elevated IL-17A levels, of whom 2 had Candida colonization. No invasive candidiasis could be detected by culture-based methods, but all 5 patients had elevated (1–3)-β-D glucan test results. Three patients in group 3 and 3 patients in group 2 with blood cultures growing Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae (group 3), Staphylococcus aureus, and Staphylococcus epidermidis (group 2) did not have elevated IL-17A levels (Table 1). Thirty-two percent of patients (17 of 52) in groups 4 and 5 had malignant solid and/or hematologic underlying diseases, whereas malignant diseases were absent in all other groups. Results did not change after exclusion of patients with malignancies (data not shown).

**DISCUSSION**

Our main finding was that candidemic patients had significantly higher IL-17A and kynurenine levels, compared with noncandidemic patients.

IL-17A is considered an important component in host defense against Candida infections and is induced in response to fungal recognition of dendritic cells, macrophages, and epithelial cells [3]. Whereas IL-17A production is stimulated in vitro by incubation of neutrophils with heat-killed C. albicans or live C. albicans, the coculture of neutrophils with dead and live C. albicans dampens IL-17A production [6]. C. albicans shifts tryptophan metabolism away from kynurenines and toward 5-hydroxytryptophan metabolites, resulting in lower IL-17A levels and allowing Candida to colonize mucosal surfaces. These in vitro data suggest that invasive candidiasis should be associated with increased IL-17A levels and increased kynurenine levels. In accordance with this, in our candidemic patients, IL-17A levels were significantly higher than those in patients without invasive candidiasis. Furthermore, IL-17A levels were significantly higher in the early stages of candidemia, compared with late candidemia, suggesting a time-dependent course of IL-17A levels. When patients with an interval of >3 days between collection of blood specimens for culture and collection of serum specimens were excluded in group 4, no significant difference was seen between groups 4 and 5. Kynurenine levels corresponded to IL-17A levels, with higher values in early candidemic patients, compared with healthy controls or ICU patients without invasive candidiasis. IL-17A levels detected in our patients were lower than those in previous ex vivo studies, in which 7-day incubation of human neutrophils and 10⁶ colony-forming units/mL of C. albicans was used [9]. In vivo, it is very unlikely that certain neutrophil populations interact for 7 days with predefined and stable amounts of C. albicans; hence, the variation might reflect the different approach between the ex vivo study and our in vivo study. Whereas kynurenine levels were low in previously described patients with Aspergillus infections, kynurenine levels were increased in our candidemic patients. This corresponds to the observation that Aspergillus is a poor inducer of IL-17A [8]. One murine study showed that IL-22 had a more crucial role than IL-17 in mucosal host defense to C. albicans [16]. This was in contrast to other studies [6], and we also did not observe a difference in IL-22 levels between investigated groups. In late candidemic patients, IL-6 levels were lower than those in noncandidemic group 3 patients, and IL-23 levels were not elevated. IL-6 levels showed no difference between patients with early candidemia and patients in group 3. Thus, we could not confirm whether systemic IL-6 and IL-23 were fungus-specific stimulators of IL-17A–producing Th17 cells [2,3,5–7]. In patients with pneumonia (group 3), IL-6 values were significantly higher than those in group 2 patients, who had various extrapulmonary infections.

IL-17A has previously not been used for diagnostic purpose (ie, to discriminate between candidemic and Candida-colonized patients). The discriminating power of the IL-17A level between candidemic patients and healthy controls, as well as patients with pulmonary and extrapulmonary infections who were intubated and undergoing mechanical ventilation, was high, as demonstrated by ROC curve analysis (Table 2). Inflammatory
parameters used in differentiation between bacteremic and nonbacteremic patients with systemic inflammatory response syndrome showed AUCs of 0.73 for IL-6, procalcitonin, or suPAR in ROC curve analysis [17]. In contrast, calculation of AUCs in IL-17A ROC curve analysis revealed values of 0.94, 0.98, and 0.99 for comparisons of candidemic patients to healthy controls or ICU patients, suggesting that IL-17A is a potential biomarker for differentiation between Candida-infected and noninfected patients. Although there was a significant difference between groups 3 and 1, the AUC in ROC curve analysis was 0.78 (95% CI, 0.67–0.89), and therefore the discriminative power was weaker. Five patients in group 3, including 2 Candida-colonized patients, had elevated IL-17A levels. We did not find invasive candidiasis by culture-based methods, but all 5 patients had elevated (1–3)-β-D glucan test results. Therefore, invasive candidiasis might be present in these patients, leading to elevated IL-17A levels. There were no patients with an elevated (1–3)-β-D glucan level but a nonelevated IL-17A level. IL-17A levels were not elevated in all 6 patients in groups 2 and 3 with sepsis and bacteremia.

There was no difference in dectin-1, TLR-2, and TLR-4 expression on human PBMCs and in Treg counts, as determined by flow cytometry, between groups 1–3 (between groups 2 and 3 for TLR-2) and the late candidemia group. Unfortunately, patients with early candidemia could not be included in this investigation because candidemia was not predictable and human PBMCs cannot be stored for dectin-1, TLR-2, and TLR-4 investigation because candidemia was not predictable and human PBMCs cannot be stored for dectin-1, TLR-2, and TLR-4 investigation. It was therefore only possible to obtain and investigate blood cells after detection of Candida and investigate blood cells after detection of Candida in blood cultures, resulting in the described delay. Conflicting results regarding the presence of dectin-1 on human PBMCs in vitro and animal Candida infection models were reported [4, 18]. Increased dectin-1 expression was demonstrated in animal fungal sepsis models, and we might have missed this increase because of delayed sampling [4]. In contrast, in vitro dectin-1 expression on cell surfaces diminished because of internalization of glucan and the corresponding dectin-1 receptors [18]. Dectin-1 investigation in our candidemic patients might resemble both phenomena, resulting in overall unaltered dectin-1 expression.

In summary, candidemic patients had significantly higher IL-17A and kynurenine levels, compared with noncandidemic patients, which is in line with previous in vitro studies. The statistically significant association between IL-17A and kynurenine levels and candidemia suggests their potential as biomarkers for anticipation of invasive candidiasis, which should be investigated in further studies.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References