Dissertation

Interleukin 17 pathway in invasive candidiasis

submitted by

Dr^{in}. med. univ. Stefanie WUNSCH

for the Academic Degree of

Doctor of Medical Science (Dr^{in}. scient. med.)

at the

Medical University of Graz

Division of Infectious Diseases and Tropical Medicine,

Department of Internal Medicine

under the supervision of

Univ.-Prof. Dr. med. univ. Robert KRAUSE

2021

Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz”.

Graz, 16.08.2021 Stefanie Wunsch eh.

Disclosures

Principal investigators

Dr.in med. univ. Stefanie Wunsch
Division of Infectious Diseases and Tropical Medicine
Department of Internal Medicine
Medical University of Graz
Auenbruggerplatz 15
8036 Graz
Austria
E-mail: stefanie.wunsch@medunigraz.at

Univ.-Prof. Dr. med. univ. Robert Krause
Division of Infectious Diseases and Tropical Medicine
Department of Internal Medicine
Medical University of Graz
Auenbruggerplatz 15
8036 Graz
Austria
E-mail: robert.krause@medunigraz.at

Co-Investigators

Assoz. Prof.in Priv.-Doz.in Dr.in med. univ. Ines Zollner-Schwetz

Dissertation committee member

Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Priv.-Doz.in Dr.in med. univ. Dr.in scient. med. Jasmin Rabensteiner

Dissertation committee member

Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

Priv.-Doz. Dr. med. univ. Gernot Schilcher

Dissertation committee member

Intensive Care Unit, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Dr. med. univ. Christoph Zurl

Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Division of General Paediatrics, Department of Paediatrics and Adolescent Medicine, Medical University of Graz, Graz, Austria

Mag. Dr. rer. nat. Heimo Strohmaier

Center for Medical Research, Medical University of Graz, Graz, Austria

Priv.-Doz. Mag. Dr. scient. med. Andreas Meinitzer

Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

Priv.-Doz. Mag. Dr. rer. nat. Wilfried Posch, PhD

Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

Univ.-Prof.in Dr.in med. univ. Cornelia Lass-Flörl

Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

Univ.-Prof. Dr. Oliver Cornely

Faculty of Medicine and University Hospital Cologne, Department I of Internal Medicine, University of Cologne, Cologne, Germany

Univ.-Ass.in Dipl.-Ing.in BSc Gudrun Pregartner

Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria

The present dissertation was conducted at the Doctoral School “Sustainable Health Research” (speaker: Univ.-Prof.in Andrea Berghold).

As part of the project “NOBICS – Novel Biomarker in Invasive Candidiasis/Candida Sepsis” this research was funded by the Austrian Science Fund (FWF) (project P 23037-B18).

Part of this thesis has been published in Wunsch, S.; Zurl, C.; Strohmaier, H.; Meinitzer, A.; Rabensteiner, J.; Posch, W.; Lass-Flörl, C.; Cornely, O.; Pregartner, G.; König, E.; Feierl, G.; Hoenigl, M.; Prattes, J.; Zollner-Schwetz, I.; Valentin, T.; Krause, R. Longitudinal Evaluation of Plasma Cytokine Levels in Patients with Invasive Candidiasis. *J. Fungi* 2021, *7*, 101.

All co-authors agree to the inclusion of their published data in the dissertation. Written statements are submitted together with the thesis. Permission to reproduce illustrations and figures from own or third-party publications has been granted.

I confirm that I have obtained permission to reproduce figures and/or tables published in the referenced journals from the respective copyright holders. Written permissions are submitted together with the thesis.

Acknowledgements

Foremost, I would like to express my sincere gratitude to my supervisor and mentor Prof. Robert Krause for his continuous support throughout thesis development and publication. His expertise and enthusiasm for the field of infectious diseases daily inspires and motivates me. Besides his position as a great researcher and physician, Prof. Krause is an exceptionally empathic, supportive, patient and kind head of our Division of Infectious Diseases and Tropical Medicine. It was (and is) a great privilege and honour to work under his guidance and to learn from him.

Furthermore, I would like to express my sincere thanks to my colleague and friend Dr. Christoph Zurl. Christoph not only helped me with collection and processing of blood samples, data acquisition, data analysis and technical issues, he also provided psychological support since he assured me that everything is going to be fine in the end.

Besides, I would like to thank the rest of my thesis committee, Prof.in Ines Zollner-Schwetz, Priv.-Doz.in Jasmin Rabensteiner, and Priv.-Doz. Gernot Schilcher, for their helpful advice and constructive feedback.

I would also like to thank our statistician Univ.-Ass.in Gudrun Pregartner. In fact, her statistical skills always seemed like a magical superpower to us.

Finally, I owe my deepest gratitude to my family and my partner Christopher. Thank you for your unconditional support and your love.

Contents

Abbreviations

A

AIRE	=	autoimmune regulator
A-Mn	=	anti-mannan antibody
AMP	=	antimicrobial peptide
AOLC	=	acridine orange leucocyte cytospin
APACHE	=	Acute Physiology and Chronic Health Evaluation
APC	=	antigen-presenting cell
APECED	=	autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy
APS-1	=	autoimmune polyendocrine syndrome type 1
ARDS	=	acute respiratory distress syndrome

B

BDG	=	1,3-beta-D Glucan
BSI	=	bloodstream infection

C

CAGTA	=	*C. albicans* germ tube antigen
C. albicans	=	*Candida albicans*
CARD9	=	caspase activation and recruitment domain-containing 9
CCL5	=	chemokine ligand 5
CDC	=	chronic disseminated candidiasis
CFU	=	colony-forming unit
CGD	=	chronic granulomatous disease
CLR	=	C-type lectin receptor
CMC	=	chronic mucocutaneous candidiasis
CRP	=	C-reactive protein

D

DC	=	dendritic cell
DC-SIGN	=	dendritic cell-specific ICAM3-grabbing non-integrin
DNA	=	deoxyribonucleic acid

E

EDTA	=	ethylenediaminetetraacetic acid
ELISA	=	enzyme-linked immunosorbent assay
EORTC	=	European Organisation for Research and Treatment of Cancer

EUCAST	=	European Committee of Antimicrobial Susceptibility Testing
F		
FDA	=	Food and Drug Administration
G		
GM-CSF	=	granulocyte-macrophage colony-stimulating factor
H		
HIES	=	hyper-IgE syndrome
HIV	=	human immunodeficiency virus
HPLC	=	high-performance liquid chromatography
I		
IC	=	invasive candidiasis
ICU	=	intensive care unit
IDO	=	indoleamine 2,3-dioxygenase
IFI	=	invasive fungal infection
IL	=	interleukin
IL-17RA	=	anti-IL-17 receptor A
IFN-ɣ	=	interferon-gamma
M		
MALDI-TOF-MS	=	Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry
MFI	=	median fluorescence intensity
MHC	=	major histocompatibility complex
MMP	=	matrix metalloproteinases
Mn	=	mannan antigen
MR	=	mannose receptor
MSG	=	Mycoses Study Group
N		
NET	=	neutrophil extracellular trap
NIV	=	non-invasive ventilation
NK	=	natural killer
NLR	=	NOD (nucleotide-binding oligomerization domain)-like receptor
NLRP3	=	NOD (nucleotide-binding oligomerization domain)-like receptor protein 3
NOD	=	nucleotide-binding oligomerization domain
NPV	=	negative predictive value

O

OI	=	oxygenation index
OPC	=	oropharyngeal candidiasis

P

PAMP	=	pathogen-associated molecular pattern
PBMC	=	peripheral blood mononuclear cell
PCT	=	procalcitonin
PNA FISH	=	Peptide Nucleic Acid Fluorescence In Situ Hybridisation
PPV	=	positive predictive value
PRR	=	pattern recognition receptor
PTX3	=	Pentraxin-related protein 3

R

RAR	=	retinoic acid receptor
RIG1	=	retinoid-inducible gene 1 protein
RLR	=	RIG-I-like receptor
RORγt	=	retinoic acid receptor (RAR)-related orphan receptor γt
ROS	=	reactive oxygen species

S

SAPS	=	Simplified Acute Physiology Score
SOFA	=	Sequential Organ Failure Assessment
SPP	=	species
STAT3	=	signal transducer and activator of transcription 3
SYK	=	spleen tyrosine kinase

T

TGF-β	=	transforming growth factor-β
Th cell	=	T helper cell
TIMM	=	Trends in Medical Mycology
TLR	=	toll-like receptor
TNF-α	=	tumor necrosis factor-alpha
TOF	=	time-of-flight
Treg cell	=	regulatory T cell

List of Figures

List of Tables

Zusammenfassung

Hintergrund

Interleukin (IL) 17A spielt eine entscheidende Rolle bei der Immunabwehr von *Candida* Infektionen. Rezente Daten zeigten signifikant erhöhte IL-17A Werte bei candidämischen Patient*innen verglichen mit nicht-candidämischen Patient*innen. Zusätzlich konnte ein zeitabhängiger Verlauf detektiert werden, mit signifikant höheren IL-17A Werten im frühen Stadium der Candidämie verglichen mit späteren Stadien.

Ziel der vorliegenden Studie war es, Plasmaspiegel von IL-17A sowie von anderen an der *Candida*-Immunabwehr beteiligten Zytokinen zu untersuchen, und ihren potentiellen Stellenwert als Biomarker für invasive *Candida* Infektionen zu evaluieren.

Methoden

Die Plasmaspiegel und zeitabhängigen Verläufe von IL-17A, Kynurenin, Tryptophan und anderen an der *Candida*-Immunantwort beteiligten Zytokinen (IL-6, IL-8, IL-10, IL-17F, IL-22, IL-23, Interferon-γ, Tumor-Nekrose-Faktor-α, Pentraxin-Related Protein 3, Transforming Growth Factor-β) wurden zwischen Patient*innen mit invasiver Candidiasis (IC (other), IC (true)), bakteriämischen Patient*innen (*Staphylococcus aureus*, *Escherichia coli*) und gesunden Kontrollen verglichen. Insgesamt erstreckten sich die seriellen Messungen über einen Zeitraum von 4 Tagen vor Abnahme der Indexkultur bis zu Tag 14 nach Abnahme der Indexkultur (-4; 14). Statistische Analysen wurden sowohl für die gesamte Studienpopulation (Hauptanalyse) als auch unter Ausschluss von immunsupprimierten Patient*innen und Patient*innen mit hämatologischen Erkrankungen durchgeführt (Sensitivitätsanalyse).

Resultate

Die IL-17A Plasmaspiegel waren in sämtlichen Patient*innengruppen in allen Zeitintervallen höher als in der gesunden Kontrollgruppe. Die höchsten IL-17A Werte zeigten Patient*innen mit invasiven *Candida* Infektionen um den Zeitraum der Abnahme der Indexkultur (-1; 2) [Median 8,8; IQR 5,4-17,3 pg/ml]. Candidämische Patient*innen wiesen in den Zeitintervallen (-1; 2) und (3; 7) signifikant höhere IL-17A Werte auf als Patient*innen mit IC (other). Der Vergleich von IL-17A Werten zwischen Patient*innen mit invasiver Candidiasis und bakteriämischen Patient*innen zeigte keine signifikanten Unterschiede. Interessanterweise wiesen Patient*innen mit IC (other) in den Zeitintervallen (-1; 2), (3; 7) und (8; 14) signifikant

höhere TGF-β-Werte auf als bakteriämische Patient*innen. Im Gegensatz dazu waren die TGF-β-Werte bei Patient*innen mit IC (true) nur im Vergleich zu Patient*innen mit *E. coli* Bakteriämie signifikant erhöht [für die Zeitintervalle (-4; -2), (-1; 2) und (3; 7)], nicht aber im Vergleich zu Patient*innen mit *S. aureus* Bakteriämie. Der Ausschluss von immunsupprimierten Patient*innen und Patient*innen mit hämatologischen Erkrankungen (Sensitivitätsanalyse) führte jedoch zu signifikant erhöhten TGF-β-Werten für Patient*innen mit IC (true) verglichen mit bakteriämischen Patient*innen (sowohl *S. aureus* als auch *E. coli*) für die Zeitintervalle (-4; -2), (-1; 2) und (3; 7).

Zusammenfassung

In der vorliegenden Studie konnte auf Basis der IL-17A Werte keine ausreichende Differenzierbarkeit zwischen Patient*innen mit IC und bakteriämischen Patient*innen gezeigt werden. Demzufolge könnte der Stellenwert von IL-17A vielmehr in der Diagnostik von Blutstrominfektionen im Allgemeinen liegen als in der Diagnostik invasiver *Candida* Infektionen. Die signifikant erhöhten TGF-β-Werte bei Patient*innen mit IC verglichen mit bakteriämischen Patienten weisen jedoch auf die potentielle Bedeutung von TGF-β in der Unterscheidung zwischen bakteriellen und *Candida*-Infektionen hin.

Abstract

Objectives

Interleukin (IL) 17A is one of the main cytokines related to *Candida*-specific immunity. Previous data indicated significantly elevated and time-dependent IL-17A levels in patients with candidemia compared to non-candidemic patients. The aim of the present study was the evaluation of levels and time courses of IL-17A, kynurenine, tryptophan, and other cytokines suggested to be involved in anti-*Candida* host defense as possible biomarkers for early identification of invasive candidiasis.

Methods

Plasma cytokine values (IL-6, IL-8, IL-10, Il-17A, IL-17F, IL-22, IL-23 (p19), IFN-ɣ, TNF-α, PTX3, TGF-β) were measured via performance of 11-Plex immunoassay. Kynurenine and tryptophan levels were determined by high-performance liquid chromatography. Serial cytokine measurements were carried out from previous 4 days up to day 14 relative to sampling of the index culture (−4; 14). For comparison of time-dependent courses of cytokine levels between the five study groups [invasive candidiasis (IC (other), IC (true), *Staphylococcus aureus* bacteremia, *Escherichia coli* bacteremia, healthy controls] specific time intervals (days) were defined (day 1 = day of index sampling): (-4; -2), (-1; 2), (3; 7), (8; 14). Statistical analyses were calculated for the total study population (i.e., main analysis), and after exclusion of immunocompromised patients and patients with hematologic malignancies (i.e., sensitivity analysis).

Results

IL-17A values were significantly increased in all patient groups compared to healthy controls. In patients with invasive *Candida* infections, the highest IL-17A levels were determined around the index sampling day (−1; 2) [median 8.8, IQR 5.4-17.3 pg/ml], whereas significantly lower values were measured prior and after index culture sampling. Patients with IC (other) had significantly lower IL-17A levels compared to IC (true) at time intervals (−1; 2) and (3; 7). However, the comparison of IL-17A values between candidemic and bacteremic patients did not reveal any statistically significant differences. Interestingly, TGF-β values were significantly increased in patients with IC (other) compared to bacteremic patients for time intervals (−1; 2), (3; 7) and (8; 14). On the contrary, the comparison of TGF-β levels between patients with IC (true) and bacteremic patients identified statistically significant differences solely for IC (true)

vs. *E. coli* bacteremia [IC (true) > *E. coli* for time intervals (−4; −2), (−1; 2) and (3; 7)], not for IC (true) vs. *S. aureus* bacteremia. However, exclusion of patients with immunosuppressive therapy and patients with hematologic malignancies (i.e., sensitivity analysis) demonstrated significantly higher TGF-β values for patients with IC (true) compared to bacteremic patients (both *S. aureus* and *E. coli*) for time intervals (−4; −2), (−1; 2) and (3; 7).

Conclusion

The time-dependent courses of IL-17A levels between patients with invasive *Candida* infections and bacteremic patients were not discriminative with respect to the etiology of either invasive *Candida* or bacterial infections. Accordingly, IL-17A may be valuable as a biomarker for either invasive *Candida* infections or bacterial blood stream infections rather than solely for invasive candidiasis. Besides, we identified significantly increased TGF-β levels in patients with invasive *Candida* infections compared to bacteremic patients, proposing a possible significance of TGF-β for differentiation between bacterial and *Candida* infections.

1 Introduction

1.1 *Candida* species

1.1.1 Brief history

The earliest reports of oral lesions potentially related to oral candidiasis date back to Hippocrates around 400 BC (1,2). In 1839, Langenbeck first detected fungi in mucosal lesions of a patient with typhoid fever at autopsy (3). A few years later, in 1843, Robin classified the fungus as *Oidium albicans* owing to the white colour of the fungal lesions. Throughout the following decades, more than 100 synonyms have been established. In 1890, Zopf named the fungus *Monilia albicans*, a genus only pathogenic for plants. More than a century later in 1923, Berkhout finally re-classified it under the current genus *Candida albicans (C. albicans)* (1,4). The first patient with invasive candidiasis (IC) was described by Zenker in 1861. After the discovery of penicillin in 1928, the widespread use of antibiotic therapy resulted in a significant increase in the incidence of *Candida* infections (1).

1.1.2 Microbiology

Candida species (spp.) are yeasts and therefore members of the fungal kingdom (1). Cell morphologies differ among different *Candida* spp.. Whereas *C. glabrata* grows as small yeasts, *C. krusei*, *C. parapsilosis*, and *C. auris* are dimorphic fungi and can present as pseudohyphal forms as well. *C. albicans* and *C. tropicalis* are polymorphic or pleomorphic fungi capable of exhibition of yeasts, pseudohyphae and true hyphae (5). Reproduction of *Candida* spp. has long been thought to occur solely asexually by a process called budding. However, in 2013, sexual reproduction of *C. albicans* was demonstrated (6). Macroscopically, *Candida* organisms predominantly appear as cream white to yellowish colonies that may resemble colonies of *Staphylococcus* spp. (1,7).

Up to date, the genus *Candida* comprises more than 200 spp. (7–9). Human pathogenic spp. include *C. albicans, C. glabrata, C. krusei, C. tropicalis, C. parapsilosis, C. lusitaniae, C. dubliniensis, C. guilliermondii, C. pseudotropicalis, C. inconspicua, C. rugosa, C. pelliculosa, C. kefyr, C. lipolytica, C. famata, and C. norvegensis* (1,10).

In 2009, *C. auris*, a novel *Candida* spp., was first cultured from an ear canal sample of a Japanese patient (11,12). *C. auris* is highly resistant to commonly used antifungals with a fluconazole resistance rate of 93%, a resistance rate to amphotericin B of 35%, and a resistance rate to echinocandins of 7%. Up to now, several outbreaks of *C. auris* infections, predominantly in health-care settings, have already been published (12,13). However, most of invasive *Candida* infections are caused by the following five spp.: *C. albicans, C. glabrata, C. krusei, C. tropicalis, C. parapsilosis* (9,12). Currently, *C. albicans* is responsible for approximately 50% of *Candida* infections (14). The unique microbiological features of *C. albicans* allow it to either colonize surfaces, e.g., the mucosa, and exist as commensals, or to become invasive causing *Candida* infection (2,8). Adherence of *Candida* spp. to host surfaces constitutes an important component of initial colonization and further progression to infection (8). Special surface properties of *C. albicans* promoting subsequent infection include biofilm formation, invasive growth, and thigmotropism (2).

1.1.3 Epidemiology

Candida spp. are ubiquitous fungi, which can be isolated from soil, animals, healthcare facilities, inorganic materials, and food. As constituents of normal human microbiota they colonize the skin, the female genital tract, the gastrointestinal tract, and the respiratory tract (1,2,7,8,15,16). Gastrointestinal colonization with *Candida* spp. is common. In one study, *Candida* was found in 80% of stool samples from healthy adults (17). *Candida* spp. are frequently recovered from the environment. Referring to its special adherence to inert polymeric surfaces, *Candida* is often detected on plastic material (2,8,18).

C. albicans accounts for most cases of IC, yet the incidence of infections caused by *Candida* spp. other than *albicans* is rising. Increased use of prophylactic or preemptive antifungals is leading to selective pressure and appears to be the main reason for this shift towards non-*albicans* spp. (5,7,9,12,14,15,19,20). Globally, the prevalence of non-*albicans Candida* spp. varies considerably. Whereas *C. glabrata* is the leading non-*albicans* spp. in the United States, Canada, northern Europe, and Australia, *C. parapsilosis* is the second most common *Candida* spp. in South America and Asia (14). Reasons for the geographic variability include regional antifungal usage patterns, individual patient risk factors and clonal outbreaks (12).

1.1.4 Colonization

Colonization with *Candida* spp., especially at multiple sites, is considered the main risk factor for invasive *Candida* infections in most studies (7,21–23). However, a minority of patients colonized actually develop IC (7,14,21). Therefore, the differentiation between *Candida* colonization and infection is often complicated, especially in severely ill patients (7).

In patients treated at intensive care units (ICUs) *Candida* colonization rate increases with the length of stay (7,24). Eggimann et al. (7) reported a *Candida* colonization rate of 5-15% of hospitalized patients at admission, rising to a colonization rate of 50-86% in critically ill patients during prolonged ICU stay. Additionally, *Candida* colonization of locations other than the gastrointestinal tract is increasing in ICU patients (25). Krause et al. (25) showed a shift to *Candida* spp. in the mycobiome of the lower respiratory tract of ICU patients with invasive ventilation regardless of previous antibiotic treatment.

1.1.5 Infection

1.1.5.1 Pathogenesis

Nucci et al. (26) performed a literature review to investigate the source of invasive *Candida* infections. Most likely *Candida* achieves access into the vascular system by one of two main routes: endogenous via the gastrointestinal tract, or exogenous via the skin (18,20,26). It was concluded that the endogenous route is responsible for most cases of IC as evidenced by both clinical and experimental studies (7,26). IC via the exogenous route, the skin, primarily occurs in patients with catheter-related candidemia with *C. parapsilosis* (26). *C. parapsilosis* colonizes the skin and forms biofilms on medical devices, thus it is often associated with nosocomial outbreaks (14).

In healthy adults, *Candida* spp. exist as commensals without causing disease. In case of impairment of host immunity or *Candida* overgrowth due to alterations in mucosal microbiota, *Candida* invasion with secondary hematogenous dissemination can occur (5,7,12). Key elements of the pathogenesis of IC comprise increased fungal burden in combination with a disruption of external barriers, e.g., skin and/or mucosa (16).

One of the three main risk factors, which are associated with subsequent invasive *Candida* infection, is prolonged exposure and/or re-exposure to broad-spectrum antibacterial agents.

Antibiotic therapy causes *Candida* overgrowth in the gut through a selective advantage of *Candida* spp. over bacteria. The second risk factor involves any disruption of either the gastrointestinal barriers by e.g., abdominal surgery, perforation or chemotherapy-induced mucositis or disruption of the skin barriers through e.g., central venous catheters leading to dissemination to the abdominal cavity and/or to invasion into the bloodstream. The third factor comprises impairment of the innate immune system through iatrogenic immunosuppression, e.g., cytotoxic therapy or corticosteroids (12).

1.1.5.2 Incidence

Infections due to *Candida* spp. are increasing worldwide (26). Between 2000 and 2010, a fivefold increase in candidemia incidence has been reported from European and US hospitals (27). The annual global burden of episodes of candidemia is approximately 400,000 cases, and most cases are reported from the industrialized countries (13). Potential reasons for rising incidence rates include increased survival of patients with severe diseases and neonates, rising numbers of surgeries (especially transplantations) and invasive procedures in general, as well as increasing usage of immunosuppressants and antibiotic therapies with broad-spectrum agents (19,27,28).
Worldwide, *Candida* spp. are described as the fourth leading cause of nosocomial bloodstream infections (BSI). Approximately 50% of those candidemic patients are found on the ICU (12,14,26,29). The reported incidence rates of candidemia depend on the patient population observed and the individual predisposing risk factors and vary between 1,2-25 cases per 100,000 people or 0,19-2,5 per 1,000 admissions (19). Further, the incidence rate is age-specific, with the highest rates observed in certain patient groups (<1 year of age and >65 years of age) (14,19). Currently, cases of community-acquired candidemia are reported more frequently, predominantly due to increased use of long-term intravenous access devices and parenteral outpatient antimicrobial therapy (12).

1.1.5.3 Clinical manifestations

Candida spp. are known to cause a broad spectrum of diseases including superficial skin and mucocutaneous infections, and potentially fatal invasive infections (7,12–14,30–32). The term “invasive candidiasis” comprises both BSIs with *Candida* spp., referred to as “candidemia”, and deep-seated tissue candidiasis. The latter includes intra-abdominal candidiasis (e.g., abscess, peritonitis) as well as involvement of other tissues or sites, e.g.,

muscles, bones, joints, and central nervous system (including the eyes) (12). Candidemia is the most common form of IC. Deep-seated candidiasis develops primarily from hematogenous dissemination (**Figure 1**) or, less frequently, from direct inoculation of *Candida* to a normally sterile site. *Candida* infections within tissues may remain restricted to the affected organs or enter the bloodstream, resulting in secondary candidemia (14).

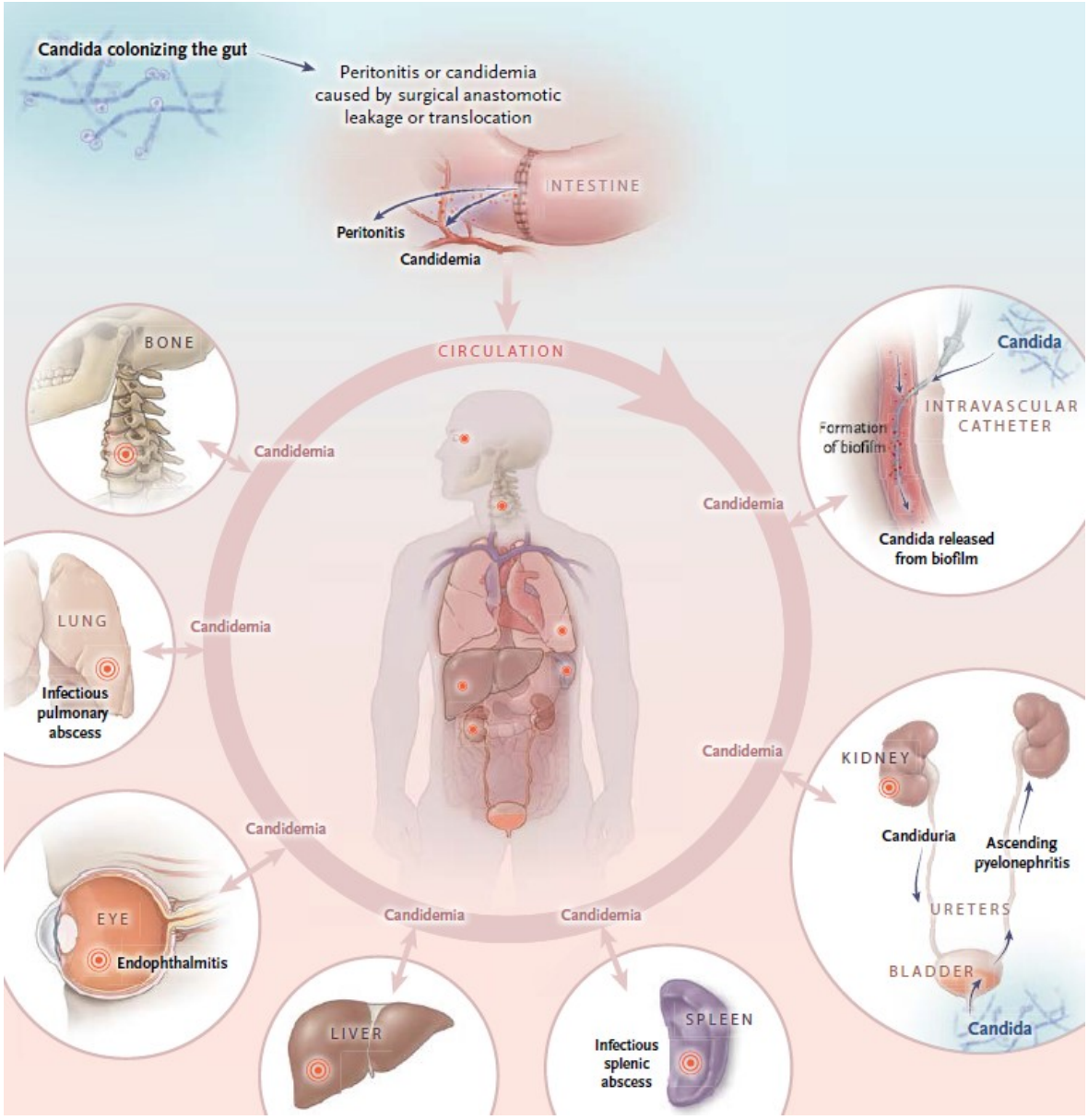

Figure 1. Clinical manifestations of invasive candidiasis.

Candida species colonizing the gastrointestinal tract invade through leakage of the mucosal barrier leading to either localized infection of distinct organs/tissues (e.g., peritonitis), or *Candida* bloodstream infection. In candidemic patients secondary, metastatic infections in the bones, lung, eyes, liver, spleen or kidneys can develop. Concerning patients with indwelling intravascular catheters, candidemia causes colonization of the catheter and biofilm formation.

The mortality of IC ranges from 5 to ≥70% (14,28,30,32,33). As invasive *Candida* infections primarily occur in severely ill patients, the differentiation between attributable mortality of candidemia and mortality owing to comorbidities is difficult to assess (28). According to Pappas et al. (12), a more accurate evaluation of candidemia-related mortality is probably 10-20%. Additional factors that contribute to mortality rates are age, chronic kidney disease, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, immunosuppressive therapy, venous catheter retention, infecting *Candida* spp. (e.g., lower virulence of *C. parapsilosis*), and the antifungal regimen (12).

Inadequate antiinfective therapy is an independent determinant of hospital mortality (33). Ibrahim et al. (33) identified the presence of *Candida* BSIs as risk factor for the administration of inadequate antimicrobial therapy. Garey et al. (29) conducted a study to relate mortality rates in candidemic patients to the first dose of fluconazole treatment, indicating that a delay in administration of antifungal therapy significantly affects mortality. Mortality rates were lowest for candidemic patients who received fluconazole on day 0 (15%) referring to the sampling of the index culture (i.e., culture date of the first blood sample positive for *Candida* spp.), compared to later initiation of fluconazole therapy after obtaining the index culture (day 1: 24%, day 2: 37%, day 3: 41%) (29).

Attributable costs of candidemia are reported to range between approximately 10 000 US$ and 40 000 US$ per patient (12,19).

1.1.5.4 Risk factors

Invasive *Candida* infections are predominantly associated with immunosuppression, but also occur in severely ill patients at the ICU without immunosuppressive therapy (7). Predisposing risk factors have been evaluated in multiple (mostly retrospective) studies including patients with and without immunosuppression. Several risk factors are directly related to the host (intrinsic risk factors), whereas others are linked to iatrogenic interventions (12) (**Table 1**).

In a prospective cohort study by Jordà-Marcos et al. (22) risk factors for candidemia in critically ill patients with an ICU stay ≥7 days included *Candida* spp. colonization, elective surgery, total parenteral nutrition and hemofiltration (22).

Table 1. Risk factors for invasive candidiasis.

General Risk Factors	
Candida colonization	**Intrinsic Risk Factors**
diabetes mellitus	
gastrointestinal bleeding or perforation	
necrotizing pancreatitis	
diarrhea	
sepsis	
severity of illness	
(extremes of) age	
birth weight (VLBW, ELBW)	
hematologic or solid-organ malignancy	
prior or concomitant administration of (broad-spectrum) antibiotics	**Iatrogenic Risk Factors**
intravascular devices	
ICU stay ≥7 days +/- assisted ventilation	
parenteral nutrition	
renal failure, including any type of dialysis	
immunosuppressive therapy	
any type of surgery	
burns	
treatment with H2-blockers	
multiple transfusion	
bladder catheter	
Additional Risk Factors in Immunocompromised Patients	
graft-versus-host disease	
mucositis	
severe neutropenia (absolute neutrophil count <500 cells/µl)	
solid organ transplant	
stem cell transplant	

VLBW = very low birth weight. ELBW = extremely low birth weight. ICU = intensive care unit.

Reproduced from (5,7,12,14,34,35).

1.1.6 Diagnostic approaches

Since invasive *Candida* infections are not associated with specific signs or symptoms, clinical diagnosis is often difficult (12,36,37). Further, no single test or decision rule is able to precisely distinguish between contamination, commensalism, colonization or infection. Therefore, the detection of *Candida* in patient samples must always be interpreted together with the clinical presentation and laboratory results (8). IC should be considered in high-risk patients with unexplained fever unresponsive to antibiotic treatment (12).

Since a delay of 1 to 2 days in initiation of adequate antifungal therapy is associated with doubled mortality rates in patients with invasive *Candida* infections (12), timely diagnosis and subsequent initiation of antifungals is essential for survival in those patients (28,30,35,36,38–40).

Presently available diagnostic tests include microscopy, histopathology, culture, antigen detection and polymerase chain reaction (PCR) (12,14,38). To increase sensitivity and specificity, the combined use of multiple diagnostic tests is recommended (14). Blood cultures are considered the diagnostic gold standard. However, disadvantages are a long turn-around time and limited sensitivity with a proposed missing rate of approximately 50% of cases (5,14,30,37,41,42). The strength of non-culture-based diagnostic approaches such as 1,3-beta-D Glucan (BDG) testing is its high negative predictive value (NPV), whereas positive predictive values (PPV) are low (42). Therefore, preemptive antifungal therapy is frequently initiated in severely ill patients after *Candida* spp. has been isolated from various non-sterile sites even without any sufficient evidence for invasive *Candida* infection (40). Since the INTENSE study (35) did not show any benefit, neither in preventing invasive *Candida* infections nor in reducing mortality rates, a preemptive antifungal strategy in high-risk ICU patients with intra-abdominal infections can not be supported by this study. Similarly, data from Ostrosky-Zeichner et al. (43) showed no statistically significant difference in terms of incidence of invasive *Candida* infections in ICU patients at risk receiving caspofungin prophylaxis.

Potential disadvantages of an empiric or preemptive approach include over- and undertreatment of patients, increased risk of antifungal resistance, increased costs and increased risk of adverse drug reactions (12,36,40). As a consequence, the development of valid diagnostic tests, which are optimally available on a daily basis, has frequently been called for in the literature (21,29,35,42,44).

1.1.6.1 Culture-based methods

IC leads to substantial morbidity and high mortality rates. One reason is the low sensitivity of culture-based methods (e.g., blood cultures, sterile-site cultures), which currently represent the diagnostic gold standard (12,30,37,41).

As described previously, IC comprises both candidemia as well as deep-seated candidiasis, whereas the latter may result from hematogenous spread or from direct inoculation of *Candida* spp. to a sterile site. Deep-seated candidiasis either remains localized or enters the bloodstream, resulting in secondary candidemia (only 5-20% of deep-seated candidiasis cases). Consequently, concerning the diagnosis of IC three entities have to be considered:

(isolated) candidemia, candidemia resulting from deep-seated candidiasis, and deep-seated candidiasis (30,42) (**Figure 2**).

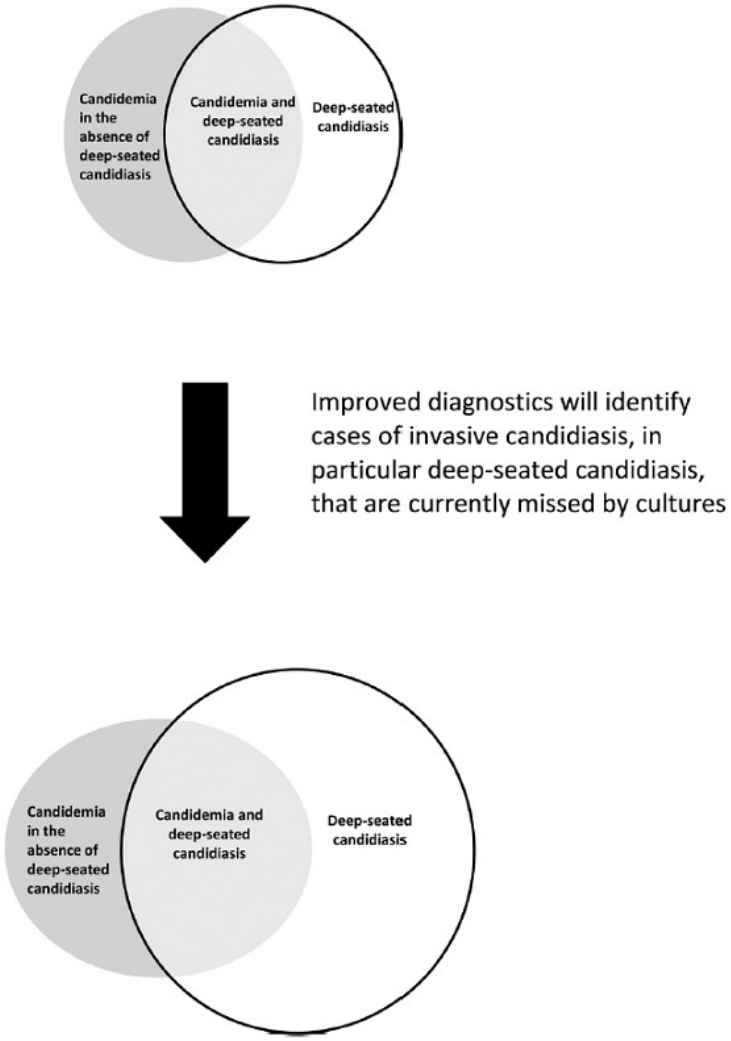

Figure 2. Diagnosis of different entities of invasive candidiasis: Impact of non-culture diagnostics.
In the top Venn diagram the three groups are almost similar in size according to recent data. As blood cultures have a poor sensitivity of approximately 50%, about half of the cases of group 2 (candidemia and deep-seated candidiasis) are missed, as well as all cases in group 3 (isolated deep-seated candidiasis). For patients in group 2 and 3, the relative impact of non-culture approaches concerning diagnosis of previously unrecognized invasive *Candida* infection is increased.
Reproduced from (30) with permission of Oxford University Press.

Epidemiology, management, and risk factors associated with death in ICU patients with invasive *Candida* infections were investigated in a prospective, multicenter, observational study including 271 patients (45). Approximately 40% of whom presented with isolated candidemia, 30% with candidemia and deep-seated candidiasis, and 30% with isolated deep-seated candidiasis (45).

Of course, blood cultures are only able to diagnose candidemia while *Candida* is present in the bloodstream (14). Rapid elimination of *Candida* spp. from the circulation, however, contributes to low sensitivity of blood cultures. In candidemic patients associated with deep-seated candidiasis, blood cultures often remain negative, because *Candida* has already been cleared from the bloodstream at the time of blood culture collection (14,30). Additionally, blood cultures only detect viable *Candida* cells. The median number of circulating *Candida* cells during an episode of candidemia is very low with 1 colony-forming unit (CFU)/ml, but up to 65% of *Candida* positive blood cultures have <1 CFU/ml (12,30). Antifungal prophylaxis further decreases the sensitivity of blood cultures (41). Due to slow growth compared to most bacteria, *Candida* spp. show a prolonged time to positivity for blood cultures (12,46). Time to positivity is usually 2-3 days (median) with a maximum of 8 days (30). Nevertheless, culture is still essential, as it represents the only diagnostic test that enables susceptibility testing (12,14).

The diagnosis of proven deep-seated candidiasis requires a *Candida* positive culture collected from a normally sterile site and/or positive histopathology (12,30,47). Possible limitations include the invasiveness of sampling (e.g., surgery, puncture) as well as poor sensitivity according to low burden of viable organisms (30,41).

1.1.6.1.1 Candida colonization index and Candida score

The *Candida* colonization index as well as the *Candida* score have been developed to support an early differentiation between *Candida* colonization and invasive *Candida* infection allowing timely initiation of empirical antifungal therapy.

The *Candida* colonization index was originally described in 1994 and is calculated as the ratio of the number of *Candida*-colonized body sites to the total number of sites sampled (48).

The *Candida* Score is based on defined risk factors including clinical symptoms of severe sepsis, surgery as the reason of ICU admission, multifocal colonization, and total parenteral nutrition. A score threshold >2,5 defines high risk for IC (36,49).

Both culture-based approaches lack sensitivity and specificity leading to over- as well as undertreatment of patients (36,40). Bruyère et al. (40) evaluated the performance of a *Candida* score-based strategy in ICU patients, and showed a sensitivity of 42.9%, a specificity of 64.7%, a NPV of 82.1%, and a PPV of 23.1% regarding the early identification of proven IC.

In summary, the advantage of both strategies lies primarily in evaluation of the dynamics of *Candida* colonization and associated risk stratification, especially for patients at very low

risk for IC (i.e., high NPV), or increasing risk in case of rising *Candida* colonization index or *Candida* Score (48).

1.1.6.2 Non-culture-based methods

With regard to the described limitations of culture-based diagnostic approaches, non-culture-based diagnostics are a pivotal component in the diagnostic strategy of IC. Non-culture-based strategies offer multiple advantages, though several weaknesses have to be considered (**Table 2**) (30).

Table 2. Potential advantages and weaknesses of non-culture-based diagnostics.

Reproduced from (30) with permission of Oxford University Press.

Potential Advantages	Potential Weaknesses
short turn-around time	no recovery of organisms
independent of viable organisms*	lack of species identification
positivity prior to culture	narrow spectrum (may solely detect *Candida* among multiple pathogens)
positivity despite antifungal therapy*	may need to be run in batch by laboratories due to restricted number of samples
quantitative data	low threshold for contamination
improved sensitivity (multicopy targets, amplification)	higher costs
detection of markers for drug resistance or other relevant phenotypes	

*The listed advantages may also be interpreted as potential disadvantages. For example, the independence of viable *Candida* cells may allow non-culture-based tests to diagnose deep-seated candidiasis if cell components are released into the bloodstream. At the same time, it poses a risk to detect dead organisms leading to false positive results.

1.1.6.2.1 Fungal antigens

1.1.6.2.1.1 Mannan antigen/anti-mannan antibodies

Mannan is a polysaccharide, which is a major cell wall component of *Candida* spp.. During invasive *Candida* infection it circulates in the bloodstream (37). Detection and quantification of mannan antigen (Mn) can be performed by latex agglutination test or sandwich enzyme-linked immunosorbent assay (ELISA). Its high immunogenicity contributes to rapid clearance from the bloodstream resulting in low sensitivity of corresponding diagnostic

approaches. The formation of immune complexes with circulating anti-mannan antibodies (A-Mn) further complicates the interpretation of test results (5,37). In order to increase sensitivity, combination tests for detection of both Mn and A-Mn are used (5,38). Several retrospective multicenter studies investigating the combination of tests for diagnosis of candidemia identified sensitivity rates of approximately 80%, and specificity rates of approximately 85%, respectively (38). Mikulska et al. (37) performed a literature review on the use of Mn and A-Mn in the diagnosis of IC. Beginning with the development of the first Mn/A-Mn ELISAs, 14 studies (thereof 13 retrospective) were included during a period of 10 years. Those 14 studies primarily included patients with malignant diseases, ICU patients and surgical patients with confirmed or suspected IC. Isolated Mn and A-Mn testing showed a sensitivity of approximately 60%, specificity was 80-90%. By combining Mn/A-Mn testing, sensitivity was increased to 83%. Further, the sensitivity of both Mn- and A-Mn tests was species-dependent, and was highest for *C. albicans*. In 73% of patients with candidemia Mn- or A-Mn testing was positive before the culture-based methods, resulting in a mean time advantage of 6-7 days (37,42).
Mn/A-Mn testing is currently recommended for the diagnosis of candidemia. However, owing to its high NPV (>85%) it is primarily used to exclude infection (38).

1.1.6.2.1.2 *Candida albicans* germ tube antigen

The second *Candida* antibody detection assay is the *Candida albicans* germ tube antigen (CAGTA) test. The test is primarily designed for *C. albicans*, yet it also detects invasive *Candida* infections with non-*albicans Candida* spp., although with lower sensitivity (12,42). The test has several weaknesses referring to the principle of antibody detection assays in general. Disadvantages of antibody detection tests are false-positive results in e.g., healthy individuals due to commensalism, and false-negative results, especially in patients with immunosuppressive therapy (weak antibody response) (5). Wei et al. (50) evaluated the diagnostic accuracy of the CAGTA assay and showed low sensitivity and specificity rates of 66% and 76%, respectively. Another study by Martínez-Jiménez et al. (51), detected a CAGTA sensitivity of 69% for patients suffering from candidemia and deep-seated candidiasis, in comparison to only 5% for patients with isolated candidemia.
In general, experience with the CAGTA assay is more restricted than with the Mn/A-Mn testing, therefore only combined use with other diagnostic approaches is recommended (5,12).

1.1.6.2.1.3 1,3-beta-D Glucan (BDG)

BDG is a polysaccharide, which is a cell wall component of most pathogenic fungi, including *Candida* spp., *Pneumocystis jirovecii*, *Aspergillus* spp. and other moulds (except Zygomycetes) (12,52–56). The cell wall of *Cryptococcus* spp. also contains BDG, yet only a small proportion is released during *Cryptococcus* infection. Accordingly, only 25% of patients with cryptococcosis present elevated BDG levels (52). BDG is a panfungal diagnostic marker, therefore not specific for invasive *Candida* infection (12).

There are several BDG assays available based on different pretreatment and measurement strategies, different horseshoe crab spp. and different types of beta-Glucan used as standards (55). To our knowledge, currently (commercially) available BDG tests include Fungitell® (Associates of Cape Cod, East Falmouth, MA, USA), Dynamiker® Fungus (1-3)-β-D-Glucan assay (Dynamiker Biotechnology Tianjin Co., Ltd., Tianjin, China), Wako β-glucan assay (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan), and Fungitec G Test MKII "Nissui" (Nissui Pharmaceutical Co., Ltd., Tokyo) (56–59). Up to now, Fungitell® is the only Food and Drug Administration (FDA)-approved BDG assay for the diagnosis of invasive fungal infections (IFI) (42,56). The Fungitell® Assay is based on the Limulus test. Through activation of factor G, a horseshoe crab coagulation enzyme, BDG leads to initiation of the coagulation cascade. The released activity is quantified by means of colorimetric methods (53,55,60,61).

Until recently, BDG testing was complicated by a labor-intensive test procedure and consequently long turnaround times. Additionally, the assay format was designed for parallel testing of 21 samples, making single-sample testing impracticable. In 2014, a modification of the conventional test procedure has been published allowing single sample testing in addition to large-scale testing with a considerably shortened turnaround time of approximately 45 minutes (62).

Karageorgopoulos et al. (55) performed a meta-analysis to assess the accuracy of BDG testing for diagnosis of IFIs. Appropriate literature was reviewed through 2010. Overall, the meta-analysis comprised 594 patients with proven or probable IFIs (primarily systemic *Candida* infections) from 16 studies. Pooled sensitivity and specificity rate were 77% and 85%, respectively. However, the authors highlighted marked statistical heterogeneity due to different study designs, patient population, type of pathogens, BDG assays, cut-off levels and prior antifungal therapies (55).

In a prospective single-center observational study from Posteraro et al. (54) an excellent NPV of 99% for BDG testing was reported, suggesting a major diagnostic benefit of BDG in ruling out IC. Additionally, high sensitivity and specificity rates were detected (93% and 91%, respectively). Further, BDG testing was associated with a time advantage of at least

24 to 72 hours compared to positive culture results in all proven invasive *Candida* infections (54).

Several studies have shown high sensitivity rates above 85% concerning the diagnosis of IC, though specificity was more divergent (12). Pappas et al. (12) reported specificity rates of 40-92%, similar to Kullberg et al. (14) with 31-79%.

Multiple potential sources of contamination have been identified causing falsely elevated BDG levels, including excessive *Candida* colonization without infection, immunoglobulins, albumin, antibiotic therapy with beta-lactam/beta-lactamase inhibitor combinations (e.g., piperacillin-tazobactam, amoxicillin-clavulanate), severe bacterial infections, severe mucositis, and surgical sponges and gauzes containing glucan (12,14,61). Recently, Szyszkowitz et al. (63) found that laparoscopic as well as open intestinal surgery led to increased BDG values (≥80 pg/mL) in 54% and 61% of patients, respectively. BDG results remained positive in up to 17% of patients without any clinical oder laboratory evidence of fungal infection or surgical complication (e.g., anastomotic leakage) even 4-5 days after surgery (63). Hemodialysis with cellulose membranes has frequently been reported to cause false-positive BDG results as well (12,14,61). Meanwhile, cellulose membranes have been replaced by synthetic membranes, which do not cause false positive BDG results anymore (64).

The afore-mentioned potential sources for contamination are commonly found in patients with high risk for IC (14). To decrease the risk for false positive results, repeated testing is recommended in case of a positive BDG value (42). Hanson et al. (65) observed increased specificity rates of BDG testing in the presence of at least two positive BDG values. Accordingly, the presence of at least two consecutively elevated BDG levels ≥80 ng/L (pg/mL) (Fungitell®) is included in the European Organisation for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria for diagnosis of probable IFIs in a defined risk population (66).

1.1.6.2.2 PCR-based tests

The development of PCR-based assays poses several challenges when it comes to fungal pathogens. Complicating factors include low numbers of circulating fungal cells, laborious deoxyribonucleic acid (DNA) extraction procedure, overlap of fungal and human DNA, and the existence of fungi in the environment and as human commensals (12). There are several commercial and in-house tests available (42). Commercial multiplex PCR assays cover the five most frequent pathogenic *Candida* spp. (*C. albicans, C. glabrata, C. krusei, C. tropicalis, C. parapsilosis*) (42). One of the available PCR-based commercial kits targeting

DNA sequences of fungi and bacteria is the LightCycler® Septi*Fast* system (Roche Diagnostics) (38). Although Septi*Fast* is the most commonly evaluated PCR technique, there were few numbers of patients with candidemia in published studies (38).
Avni et al. (67) performed a meta-analysis including 54 studies with nearly 5000 patients, of them 963 patients were diagnosed with proven, probable or possible IC. Sensitivity and specificity were 95% and 92%, respectively, for the diagnosis of candidemia in patients with suspicion of IC (67).
With exception of the T2Candida Panel test (T2 Biosystems, Lexington, MA, USA), PCR-based tests are not validated for detection of invasive *Candida* infections due to missing data (38,41,42).

1.1.6.2.2.1 T2Candida Panel

The T2Candida Panel is a new FDA-approved nanodiagnostic assay using manual application of T2 magnetic resonance for detection of *Candida* BSI. Through a fully automated instrument platform, the T2Candida Panel enables multiplex detection of the five most relevant *Candida* spp. (*C. albicans, C. glabrata, C. krusei, C. tropicalis, C. parapsilosis*) with a detection limit of 1-3 CFU/ml (12,42,46,66,68). T2Candida panel results are not available for single *Candida* spp. as they are grouped dependent on typical antifungal susceptibility patterns: *C. albicans/C. tropicalis, C. parapsilosis*, and *C. krusei/C. glabrata* (42,46).
Mylonakis et al. (46) conducted the first extensive clinical study evaluating T2 Magnetic Resonance Assay for fast detection of candidemia. In total, 1801 blood samples from hospitalized patients with routinely collected blood cultures were included. In addition, 250 samples were manually spiked with various concentrations of the five different *Candida* spp., and 50 samples were used as negative controls. Overall, sensitivity and specificity rates were high (91% and 99%, respectively). However, two candidemic patients with positive blood cultures were missed by the T2Candida Panel. Additionally, in 245 cases the T2Candida Panel displayed invalid results. In another study by Zurl et al. (68) the clinical value of T2Candida for diagnosis of candidemia was shown to be restricted due to missing blood culture positive cases. However, serial T2Candida testing led to increased positivity rates. In patients with suspected IC other than candidemia, T2Candida was considered as diagnostic add-on to sterile site cultures (68).
Recently, the presence of a positive T2Candida result has been added in the revised and updated EORTC/MSG criteria (66) as mycological evidence to support the diagnosis of candidemia.

1.1.6.3 Limitations of currently available diagnostics

Culture-based diagnostic approaches such as blood cultures and sterile site cultures lack sensitivity and show long turn-around times leading to delayed initiation of antifungal therapy (30,42,46).

The *Candida* colonization index as well as the *Candida* score have low sensitivity and specificity rates and are therefore insufficient tools for timely recognition of invasive *Candida* infection (36,40).

Mannan antigen/anti-mannan antibody testing lacks data for deep-seated candidiasis, thus it is only recommended for diagnosis of *Candida* BSI and chronic disseminated candidiasis (38).

1,3-beta-D Glucan assays are non-specific for *Candida* spp. (panfungal marker), additionally they are predisposed to false positive results (12,14,46,61).

Molecular methods, predominantly PCR-based tests, are not sufficiently evaluated in patients with IC and are therefore (with exception of the T2Candida Panel test) not recommended (38,41,46).

In summary, currently no valid and at the same time rapid diagnostic test is available for early projection or diagnosis of invasive *Candida* infection. Owing to the lack of reliable diagnostic methods, antifungal treatment is often initiated in high-risk patients for IC after *Candida* spp. have been cultured from different non-sterile body sites (35,36,40,46). This approach leads to overtreatment, possible emergence of antifungal resistance, risk of adverse effects, and increased costs (46). Thus, the development of diagnostic tests, which allow reliable and rapid differentiation between *Candida* infection and colonization in critically ill patients is eagerly awaited (21,29,46).

According to Clancy et al. (30), an ideal diagnostic test for invasive *Candida* infection has high sensitivity and specificity, is minimally invasive, requires low volume sample, has a rapid turnaround time, is not labor-intensive, and provides susceptibility testing. Further, an ideal test should enable a timely diagnosis of patients with isolated deep-seated candidiasis, of patients with *Candida* BSI plus deep-seated candidiasis, and of patients with candidemia who presumably develop deep-seated candidiasis. Ideally it should also provide prognostic information in order to identify patients with increased risk for unfavorable outcome (30).

In 2015, Krause et al. (31) performed a prospective study to investigate levels of relevant cytokines involved in anti-*Candida* host defense. According to literature, *C. albicans* is able to diminish the immune response by alteration of tryptophan/kynurenine metabolic pathways, thus tryptophan and kynurenine levels were additionally measured. Candidemic

patients had higher levels of interleukin 17A (IL-17A), the main cytokine produced by T helper type 17 cells (Th17 cells), and kynurenine compared to non-candidemic patients. Additionally, values were significantly higher in the early course of candidemia compared to later stages. Therefore it was hypothesized that IL-17A may serve as biomarker for timely recognition of IC (31).
Similar results were shown in an observational, prospective trial, where IL-17A was significantly increased in three candidemic patients with septic shock (primarily abdominal focus) in comparison to non-candidemic septic patients regardless of *Candida* colonization status. These results further supported the potential of IL-17A as a biomarker for detection of invasive *Candida* infections (69).

1.2 Host defense against *Candida* infections

1.2.1 Immune tolerance versus *Candida* invasion

As a result of complex interactions with their hosts (e.g., plants, animals or humans), fungi are able to establish a wide spectrum of host-fungus relationships including symbiosis (e.g., commensalism), colonization or infection (70,71). Since fungi are able to adapt to changing environmental conditions, they can colonize virtually every niche within the human body (70). Despite a *C. albicans* colonization rate of approximately 30% in healthy individuals, the vast majority of patients does not develop invasive *Candida* infections unless disruption of external barriers or impairment of host immunity occurs (69,71,72). Colonization by *C. albicans* is tolerated through a balanced equilibrium of pro- and anti-inflammatory signals. The presence of *Candida* yeasts on the mucosa does not induce a strong inflammatory reaction, whereas the immune system is triggered during conversion of *Candida* yeasts to hyphae in case of tissue invasion (70,72). Up to now, the responsible mechanisms allowing differentiation between *Candida* colonization and tissue invasion are still poorly understood (72).

1.2.2 Host-fungus interaction

Fungal infections constitute a special entity in terms of immunology, since they can result from both lacking recognition, and from overwhelming inflammatory response (70,71). In fact, an early inflammatory response inhibits or restricts fungal infection, yet an uncontrolled

host reaction potentially complicates infection control (71). Accordingly, Romani et al. (70) defined two main components concerning host response to fungal infections. The term “resistance” comprises the ability to limit fungal burden, whereas “tolerance” is determined as the ability to alleviate host damage due to the immune reaction. More precisely, resistance is associated with decrease of the fungal burden via innate and adaptive immunity, while distinct tolerance mechanisms protect the host from overwhelming immune- or pathogen-associated damage (71).

Various immune cells and cell-signaling pathways are involved in the interaction between host, fungi, and microbiota (71). Innate immunity represents the first defense mechanism of the host leading to phagocytosis of *Candida* spp. by neutrophils and monocytes/macrophages (32,69,73). Additionally, cellular adaptive immunity – particularly respresented by Th cells – plays a crucial role in antifungal immune response (32,71). The bridge between innate and adaptive immunity is built through cytokines, which are produced by antigen-presenting cells (APCs) (74).

Besides common Th1 and Th2 cells, Th17 cells have been identified as pivotal T helper cell subgroup, which is involved in protection from fungal as well as bacterial infections. Interleukin 17A (IL-17A), the key cytokine produced by Th17 cells, has various proinflammatory functions and is considered as important part in anti-*Candida* host defense (31,32).

In order to balance effective elimination of fungi while limiting collateral destruction of tissue, an additional T helper cell subtype - regulatory T cells (T_{reg}) - are able to diminish host defense against *Candida* infections (32,69,70).

1.2.2.1 *Candida* recognition by the innate immune system

The initial step in host defense against *Candida* infections is the recognition of invading *Candida* spp. by pattern recognition receptors (PRRs), which are primarily expressed by myeloid phagocytes (12,75,76). There are four main classes of PRRs including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoid-inducible gene 1 protein (RIG1)-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). PRRs recognize conserved extra- or intracellular pathogen-associated molecular patterns (PAMPs) of invading *Candida* spp. and initiate downstream signaling leading to immune system activation and consecutive clearance of *Candida* (12,69,70,76,77) (**Figure 3**).

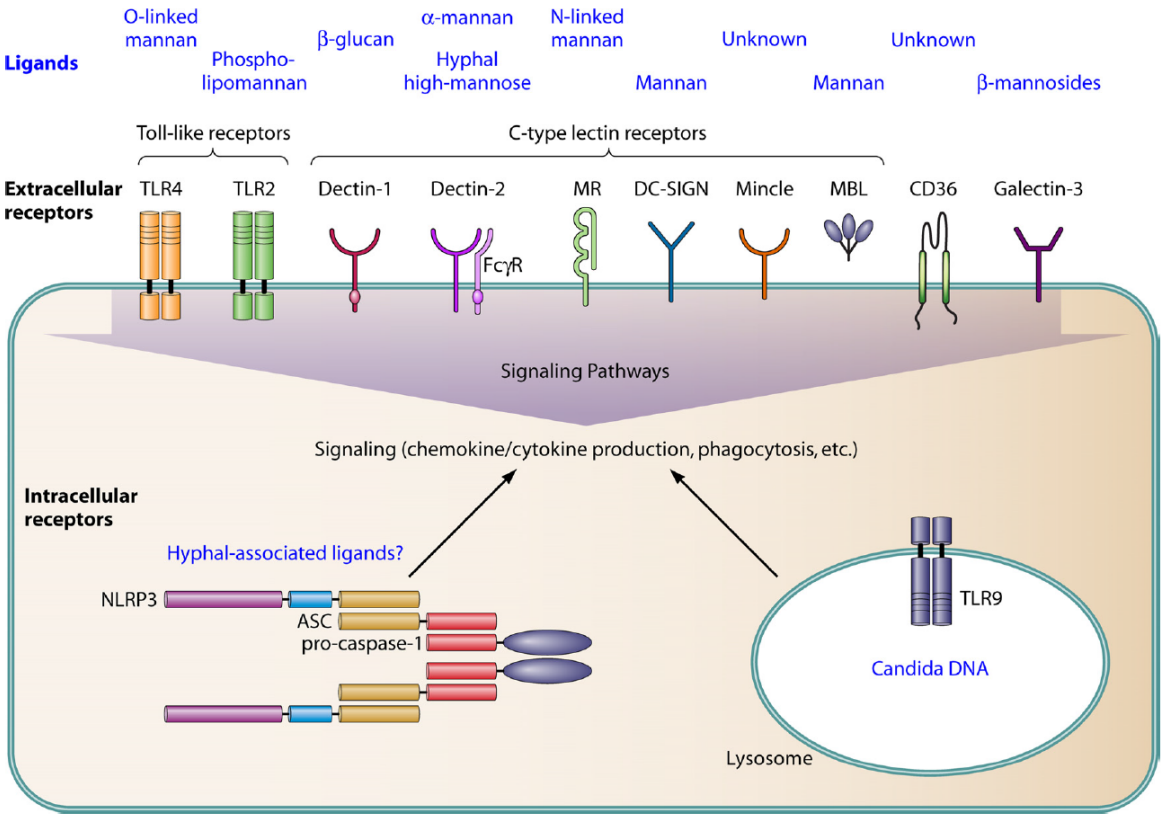

Figure 3. Recognition of pathogen-associated molecular patterns and consecutive signaling pathways in *Candida* infection.

After *Candida* recognition on the host cell surface by extracellular Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), downstream signaling is initiated leading to chemokine/cytokine production and phagocytosis. As soon as *Candida* is internalized, the fungal pathogen-associated molecular patterns can further activate intracellular receptors such as TLR9 or promote NLR family pyrin domain containing 3 (NLRP3) inflammasome activation.

Reproduced from (75) with permission of American Society for Microbiology.

The cell wall of *Candida* spp. consists of two different layers. The outer cell wall is largely composed of O- and N-linked glycoproteins, known as mannans. The inner layer consists of β-glucans (glucose polysaccharides), mainly β-(1,3)-glucan, and chitin (polymer of N-acetylglucosamine), which contribute to a strong cell wall integrity (70,75,76,78). The polysaccharide structures of the *Candida* cell wall constitute the main PAMPs recognized by PRRs during *Candida* infection (75,76).

TLRs were the first PRRs discovered and are either cell-membrane-associated (such as TLR2 or TLR4), or intracellular (e.g., TLR9) receptors. The two most important TLRs in terms of anti-*Candida* host defense are TLR2 and TLR4, whereas other TLRs (e.g., TLR1 and TLR6) play a secondary role (75,77–79).

The second major PRR family are CLRs, which comprise dectin-1, dectin-2, DC-specific ICAM3-grabbing non-integrin (DC-SIGN), mannose receptor (MR), MINCLE, langerin, and mannose-binding lectin (70,75). CLRs are primarily extracellular receptors (77). Dectin-1 is

the most relevant β-glucan receptor and promotes cytokine and chemokine production through activation of two different signalling pathways: the RAF pathway and the spleen tyrosine kinase (SYK)–caspase activation and recruitment domain-containing 9 (CARD9) pathway (SYK–CARD9 pathway) (70,76,79). The SYK–CARD9 pathway further activates the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome leading to activation of IL-1β and IL-18, two pro-inflammatory cytokines (70). MINCLE, a receptor expressed on monocytes and neutrophils, leads to induction of anti-*Candida* host defense through initiation of tumor necrosis factor (TNF) production (76). One function of dectin-2 is the recognition of mannose-rich structures, with lower affinity for yeast than hyphal forms (70,75,78). Additional functions of dectin-2 include the modulation of Th17 cell responses and direct killing of *C. glabrata* through phagocytosis (76).
DC-SIGN and MR both recognize *Candida* N-linked mannans, whereby the MR also takes part in antifungal Th17 cell responses (70,75–77).
Following the recognition of *Candida* PAMPs by the mentioned PRRs, a subsequent signaling pathway is initiated, with the objective of ultimate clearance of the invading *Candida* spp. (76).

1.2.2.1.1 Strategies of Candida to escape host defense mechanisms

Candida spp. have employed strategies to evade innate host defense, which contribute to fungal adaptation and opportunism (**Figure 4**) (70,75,76).
The most important evasion strategy of *C. albicans* involves the morphogenetic change from yeast- to hyphal form (75–77). Hyphal growth is necessary for piercing through phagocytes and tissue invasion, however, the presence of *Candida* as yeast cells allows free dissemination of *Candida* spp. in IC. *C. albicans* hyphae lack surface-exposed β-glucan, which is required for recognition by the immune system (77). Yeast- to hyphal-phase transition represents one of the key virulence factors of *C. albicans* (75).
Additionally, *C. albicans* is capable of downregulation of TLR4-expression of epithelial cells in order to prevent *Candida* recognition by epithelial cells (75).
Another mechanism comprises the shielding of important *Candida* PAMPs (such as β-glucans) from recognition by PRRs (e.g., dectin-1) (75,76).
Gow et al. (80) conducted a study to investigate the different cytokine production of human peripheral blood mononuclear cells (PBMCs) induced by contact with live and heat-killed *C. albicans* cells. Heat-killing of *C. albicans* was associated with cell wall disruption and exposure of β-glucans on the cell surface, resulting in significantly higher levels of cytokine production by PBMCs compared to live *C. albicans* (75,80).

Similarly, antifungal therapy leads to exposure of β-glucans, resulting in increased dectin-1 mediated production of proinflammatory cytokines (77). Furthermore, *C. albicans* is able to inhibit complement activation and opsonization of *Candida* spp. (75). *Candida* is also capable of inhibition of phagolysosome maturation, a formation which is crucial for killing of *Candida* spp. (75,76). Another escape strategy of *Candida* spp. is the active modulation of host cytokine secretion by soluble factors. In fact, *C. albicans* can specifically downregulate IFN-ɣ production and simultaneously upregulate IL-10 secretion, leading to a shift from a favourable Th1 response to a harmful Th2 response. Additionally, *C. albicans* is able to dampen host IL-17 production via modulation of the tryptophan metabolic pathway (75).

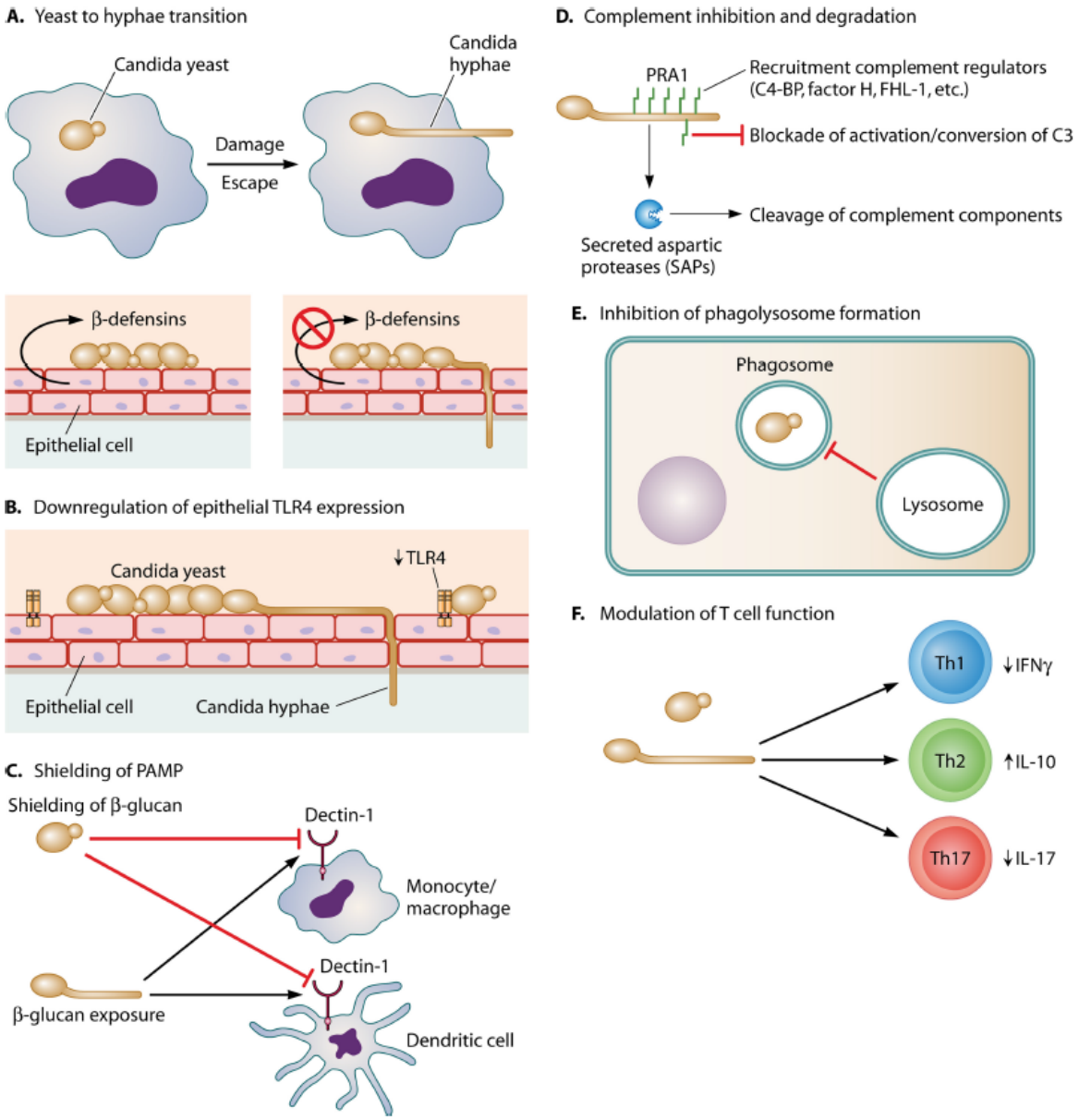

Figure 4. Strategies of *Candida* species for evasion of antifungal host defense.

Reproduced from (75) with permission of American Society for Microbiology.

1.2.2.2 Different cell types involved in anti-*Candida* host defense

1.2.2.2.1 Epithelial cells

Apart from the obvious function as passive mechanical barrier, epithelial cells are able to actively secrete antimicrobial peptides and mucins in order to prevent *Candida* tissue invasion (74–76).
Epithelial cells recognize invading *Candida* spp. through a TLR4-dependent mechanism (75,76). Upon recognition of the invading fungi, antimicrobial peptides such as direct candidacidal β-defensins are secreted to clear *Candida* infection (75,76). In addition, epithelial cells “raise the alarm” by induction of increased cytokine production resulting in immune system activation (74,75).

1.2.2.2.1.1 Monocytes and macrophages

Tissue-resident macrophages are important phagocytic cells for protection during invasive *Candida* infection. Via production of proinflammatory cytokines and chemokines, a signaling pathway is initiated, resulting in immune system activation and recruitment of other immune cells to the site of infection (12,74,76). Among others, blood monocytes migrate to the focus of infection where they differentiate into inflammatory macrophages (12,76).
Macrophages possess substantial anti-*Candida* killing capacity, especially within the first hours after infection (12,76). In a study from Qian et al. (81), macrophage-depletion in mice resulted in slower fungal clearance and increased mortality. Monocytes and macrophages are further involved in “trained immunity”, as they confer lymphocyte-independent protection via epigenetic reprogramming after undergone *Candida* infection (12).

1.2.2.2.2 Neutrophils

Neutrophils are essential for anti-*Candida* host defense (12,76). Following fungal tissue invasion, secretion of cytokines and chemokines by activated epithelial cells and tissue-resident macrophages results in recruitment of neutrophils to the site of infection (76). Neutrophil recruitment is crucial for clearance of *Candida* spp., thus neutropenia constitutes a major risk factor for IC. Further, neutrophils are the only cells which are able to prevent *Candida* yeast- to hyphal-phase transition (76).

In terms of effective killing of *Candida* spp., neutrophils are capable of both oxidative (reactive oxygen species (ROS)-dependent) pathways, and non-oxidative mechanisms including secretion of lysozyme, lactoferrin, elastase, β-defensin, gelatinase, and cathepsin G (12,74,76). While the ROS-dependent pathways are required for clearance of opsonized *Candida*, the ROS-independent pathways are involved in killing of non-opsonized *Candida* spp. (76).

Additionally, neutrophils are capable of neutrophil extracellular trap (NET) formation, known as NETosis. NETosis is associated with neutrophil degranulation and release of candidacidal factors such as cathelicidin. NET-formations trap and kill both *Candida* hyphae and yeast-forms (74,82,83).

1.2.2.2.3 Natural killer cells

Natural killer (NK) cells are innate lymphocytes, which originate from the bone marrow and constitute up to 15% of PBMCs (83,84). Together with monocytes/macrophages and neutrophils, NK cells represent the first line of immune defense against invasive *Candida* infections (76).

NK cells were initially classified as members of the innate immune system, although contributing to adaptive immunity as well (83). In most cases, an efficient innate immune response is enough to prevent disseminated *Candida* infection. In case of insufficient infection control through innate immunity, NK cells are able to activate the adaptive immunity in order to maximize killing capacity (76). Therefore, antifungal activity of NK cells comprises both direct antifungal effects via release of cytotoxic molecules (e.g., perforin), as well as indirect antifungal killing via secretion of cytokines (76,83).

NK cells are closely related to group 1 innate lymphoid cells. Through secretion of cytokines and chemokines, mainly interferon-gamma (IFN-ɣ), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-alpha (TNF-α), or chemokine ligand 5 (CCL5), NK cells influence the activity of the innate (e.g., neutrophils, dendritic cells (DCs)) as well of the adaptive immunity (Th cells). Further, NK cells are involved in establishing a long-lasting immunological memory, as shown in various animal models and also human studies (83–86).

1.2.2.2.4 Dendritic cells

DCs are capable of killing of *Candida* spp. through phagocytosis, though less efficient than macrophages (76). Upon fungal tissue invasion, phagocytosis of *Candida* spp. via receptors on the surface of DCs (DC-SIGN, MR) leads to *Candida* antigen processing and presentation to Th cells via major histocompatibility complex (MHC) class II molecules (75). DCs are essential for promotion of Th cell differentiation (71). In a study from Bozza et al. (87), it has been shown that pulmonary DCs, after internalization of fungal antigens, migrate to draining and peripheral lymphoid organs, where they induce selective Th differentiation. DCs regulate the adaptive immune response depending on *Candida* cell morphology. Ingestion of yeasts prompts Th1 differentiation, while ingestion of hyphae induces Th2 differentiation and concomitantly inhibits Th1 differentiation and secretion of IL-12 (75). Via secretion of different cytokines and chemokines, DCs have the unique capacity to either suppress or promote the adaptive antifungal immune response in order to avoid potentially harmful exaggerated inflammatory responses. There are multiple DC subsets, which are associated with different signalling pathways, contributing to the plasticity of the DC system and influencing the local Th/Treg balance (71).

1.2.2.2.5 Adaptive immunity

CD4+ effector T cells (also called Th cells) are divided into different subsets, each characterized by secretion of unique cytokines with specific functions (**Figure 5**). Balance of Th1 and Th17 cell-mediated immunity and their associated cytokines IFN-γ, TNF-α, IL-17A and IL-17F, is crucial for protective immunity against fungi (71).

Th cells		Cytokines	Functions
	Th1	IFN-γ/TNF-α	Fungal clearance Inflammation
	Th17	IL-17A/IL-17F	Defensins, neutrophil recruitment Inflammation
	Th22	IL-22	Defensins Tissue homeostasis
	Th2	IL-4/IL-13	Humoral response Allergy
	Th9	IL-9/IL-10	Tissue inflammation
	Treg	IL-10/TGF-β	Low inflammation Immunosuppression
	Tr1	IL-10	Low immunopathology

Figure 5. T helper cell subtypes in fungal infections.

Reproduced from (71) with permission of Frontiers.

1.2.2.2.5.1 Th1 cells

Following recognition of *Candida* antigens by TLRs and CLRs, DCs induce Th1 cell activation (70). Besides secretion of their signature cytokine IFN-γ, Th1 cells also produce other important proinflammatory cytokines such as TNF-α and GM-CSF (70,71,74). Th1 cells promote the production of opsonizing antibodies and activate neutrophils and macrophages at the site of infection (70,71,76).

The impact of Th1 cells on protective immunity against *Candida* infections has been displayed in various murine models and human studies (74). In a study from Netea et al. (88), IFN-γ-deficiency in mice resulted in augmented susceptibility to disseminated candidiasis. The same effect was demonstrated with mice defective in IL-18, a proinflammatory cytokine that induces Th1 cell responses (88).

Delsing et al. (89) evaluated the efficacy of adjunctive immunotherapy with IFN-γ in patients with IFIs (*Candida* and/or *Aspergillus* spp.). In this prospective, open-label pilot study, IFN-γ treatment was associated with increased proinflammatory cytokine responses, indicating enhanced anti-fungal immunity. However, larger studies are warranted to assess the clinical impact of IFN-γ immunotherapy (89).

1.2.2.2.5.2 Th2 cells

IL-4 and IL-13 are the main cytokines secreted by Th2 cells (**Figure 5**) (70,71). In contrast to Th1 and Th17 cell-mediated immunity, which is pivotal for protective antifungal immunity, the role of the Th2 cell lineage in fungal infections is still controversial (76). Since Th2 cells dampen beneficial Th1 cell responses and concomitantly stimulate alternatively activated macrophages, they favour fungal persistence, fungus-associated allergic responses, and disease relapse (70,71). Accordingly, in a mouse model, T-cell depletion and corresponding reduced IL-4 production was associated with increased resistance to disseminated candidiasis (90). Conversely, in a different study from the same research team (91), IL-4 deficient mice were shown to be more resistant in the early stage of IC, yet did not achieve sufficient infection control in the later stages.

1.2.2.2.5.3 Th17 cells

In 1986, Mosmann and Coffman (92) discovered two types of murine T helper cells which were characterized as Th1 and Th2 cells, dependent on their production of unique cytokines and distinct functions. About 20 years later, in 2005, studies from Harrington et al. (93) and Park et al. (94) reported about a new Th cell lineage, which did not correspond to the common Th1/Th2 classification. IL-17 was detected as main cytokine secreted by the newly discovered Th cell subset, thus the third type of effector T cells was named Th17 cells (73). The IL-17 family involves six different subforms, IL-17A to IL-17F (95). Besides production of their signature cytokine IL-17 (mainly IL-17A, to a lesser extent also IL-17F), Th17 cells release IL-21 and IL-22 (73,95).

Th17 cells play a significant role in immune defence against extracellular pathogens, additionally they are involved in the pathogenesis of autoimmune diseases and allergies (70). Further, Th17 cells contribute to fungus-specific T cell memory (70,71). The significance of Th17 cells in antifungal host defense is underlined in patients with loss of function mutations of signal transducer and activator of transcription 3 (STAT3) (i.e. Hyper-

IgE syndrome (HIES)), or defects in the IL-17 signaling axis, associated with frequent occurences of various fungal infections (74).

IL-1β is crucial for Th17 differentiation. Evolution of IL-1β first requires processing of the procytokine form by active caspase-1, which is strictly modulated by a special protein complex, the inflammasome. Among the different subsets of inflammasomes, the NOD-like receptor protein 3 (NLRP3) inflammasome is involved in anti-*Candida* host defense. Inflammasome activation is essential for discrimination between benign *Candida* spp. colonization and potentially harmful tissue invasion depending on morphological aspects. The yeast form of *C. albicans* has no effect on inflammasome activation, whereas *Candida* hyphae induce inflammasome activation after recognition by macrophages, leading to IL-1β production (72).

Contrary to Th1 cells, the differentiation of Th17 cells requires both activation of the transcription factor STAT3 by IL-6 and IL-21, and transforming growth factor-β (TGF-β)-driven induction of retinoic acid receptor (RAR)-related orphan receptor γt (RORγt) (74). Further, IL-23 is essential for stabilization of the Th17 phenotype, since it increases the secretion of IL-17 and IL-22, while suppressing IL-10 and IFN-γ release (73,74). IL-23 shares the p40 subunit with IL-12 and is therefore referred to as heterodimeric cytokine (73,96). In murine animal models, IL-23 deficiency correlated with a reduced incidence of autoimmune diseases, whereas the loss of IL-12 had no effect hereof. Accordingly, the IL-23/Th17 axis seems to be of major importance in the immunopathogenesis of autoimmune diseases (73).

Th17 cells appear early in the course of immune defence, albeit they influence a broad spectrum of Th1- and Th2-type immune responses. IL-17A-dependent neutrophil recruitment and release of defensins contribute to rapid and efficient infection control (70,71). However, the actual significance of the IL-17 pathway in fungal infections is still not fully understood. Factors potentially affecting the IL-17 pathway include stage and site of infection as well as environmental stimuli (70).

Fascinatingly, *C. albicans* is able to actively downregulate Th17 production via modulation of tryptophan metabolism. Possible consequences of downregulated IL-17 production comprise impaired infection control and chronic inflammation (32,70,71).

1.2.2.2.5.4 Treg cells

Treg cells constitute another Th cell subtype which is essential for balancing effective elimination of invading fungi and acceptable collateral tissue damage (69,70,74). Besides

their role in limiting excessive inflammatory response, Treg cells are also involved in long-term immunological memory (97).

Through secretion of their signature cytokine IL-10, Treg cells are able to dampen antifungal host response (69). Those immunosuppressive mechanisms are either cell contact dependent (inhibitory receptors) or independent (inhibitory cytokines and metabolites) (97). IL-10 is an inductor of Th2 cells, thus antagonizing the IFN-γ-driven proinflammatory effects of Th1 cell responses (69). In line with this, an inverse relation between IL-10 and IFN-γ in patients with IFIs was reported in several clinical studies (69,70). As a consequence, elevated IL-10 values have been directly related to enhanced susceptibility to IFIs and fungal persistence (69,70). However, Decker et al. (69) measured high IL-10 levels simultaneously with increased IFN-γ values both in patients colonized or infected. Following this, increased IL-10 secretion may be a consequence, rather than a cause of fungal infection in order to keep inflammation under control (69,70).

Contrary to the assumption that decreased Treg numbers result in enhanced clearance of IFIs, defective Treg development in mice led to pathogenesis of chronic mucocutaneous candidiasis (CMC) in autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED) patients (also known as autoimmune polyendocrine syndrome type 1 (APS-1)) (74).

Emerging data indicates a complex relation between Th17 and Treg responses, exemplified by murine models with *Candida* infections. Under specific conditions, an inversed relationship between Th17 and Treg responses has been demonstrated. During intraabdominal candidiasis, increased Treg activity and consecutive Th17 suppression leads to decreased *C. albicans* colonization. Conversely, Tregs are also able to stimulate Th17 responses and even to assume phenotypic characteristics of Th17 cells during oropharyngeal candidiasis (OPC) and disseminated candidiasis (97).

Since Treg cells are able to control innate and adaptive immunity, they may account for diverse effects, ranging from protective tolerance (corresponding high IFN-γ/IL-10 ratio) to actual immunosuppression (70,98). In fact, the microenvironment at the site of infection constitutes an important factor influencing the impact of the Treg/Th17 relationship on disease outcome (97).

1.2.3 Interleukin 17 and the Th17/IL-17 axis

1.2.3.1 Differentiation and function of interleukin 17

IL-17(A), the key cytokine produced by Th17 cells, has multiple proinflammatory features, as for example neutrophil recruitment, activation of phagocytosis by neutrophils and macrophages, and induction of antimicrobial peptides (AMP) (e.g., β-defensin) production via epithelial cells (32,70,72,76,78,97).

Secretion of IL-17 leads to a rapid inflammatory response that is dominated by neutrophils (73). Notwithstanding the potent effects of IL-17 on neutrophils, the actual significance of IL-17-mediated neutrophil chemotaxis in antifungal host defense is controversial. Although patients with chemotherapy-associated neutropenia are at higher risk for disseminated candidiasis, patients with isolated neutropenia or disorders of neutrophil function (e.g., chronic granulomatous disease (CGD)) are not exceedingly predisposed to *Candida* infections (78).

In a study by Huang et al. (99), IL-17AR knockout mice showed delayed and impaired neutrophil activation and migration into infected organs associated with increased mortality rates. The outcome of Th17-deficiency in humans, however, differs depending on the clinical syndrome. While patients with HIV infection or other Th17-deficiency such as HIES or APS-1 do not routinely develop potentially life threatening *Candida* infections, patients with homozygous mutations in CARD9 or patients with combined lymphopenia and neutropenia actually present with severe IC (78,100). However, patients with APS-1 show decreased IL-17 function due to IL-17 autoantibodies and develop severe mucocutaneous candidiasis (101,102) (see 1.2.4, **Figure 9**).

In any case, Th17-mediated immunity is crucial for *Candida*-specific immunity, especially at epithelial and mucosal surfaces (32,72,73,78). The significance of IL-17 regarding anti-*Candida* host defense has been demonstrated in patients with specific genetic defects of mucosal-antifungal immunity such as CMC or HIES, which presented significantly decreased IL-17 levels compared to healthy controls (32,72). Concerning the development of oral candidiasis, both murine and human studies identified IL-17 deficiency as important predisposing factor (78). Additionally, in patients with psoriasis and treatment with anti-IL-17A antibodies increased numbers of mild to moderate *Candida* infections have been reported (103). Following this, the IL-17 pathway appears to be of major importance for *Candida*-specific immunity (32).

IL-17 receptors are expressed in most parenchymal organs (73,104). Following IL-17 stimulation, secretion of proinflammatory cytokines/chemokines, primarily IL-1, IL-6, IL-8 (encoded by the CXCL8 gene), TNF, and matrix metalloproteinases (MMP), via IL-17 target-cells is induced (**Figure 6**) (73,97).

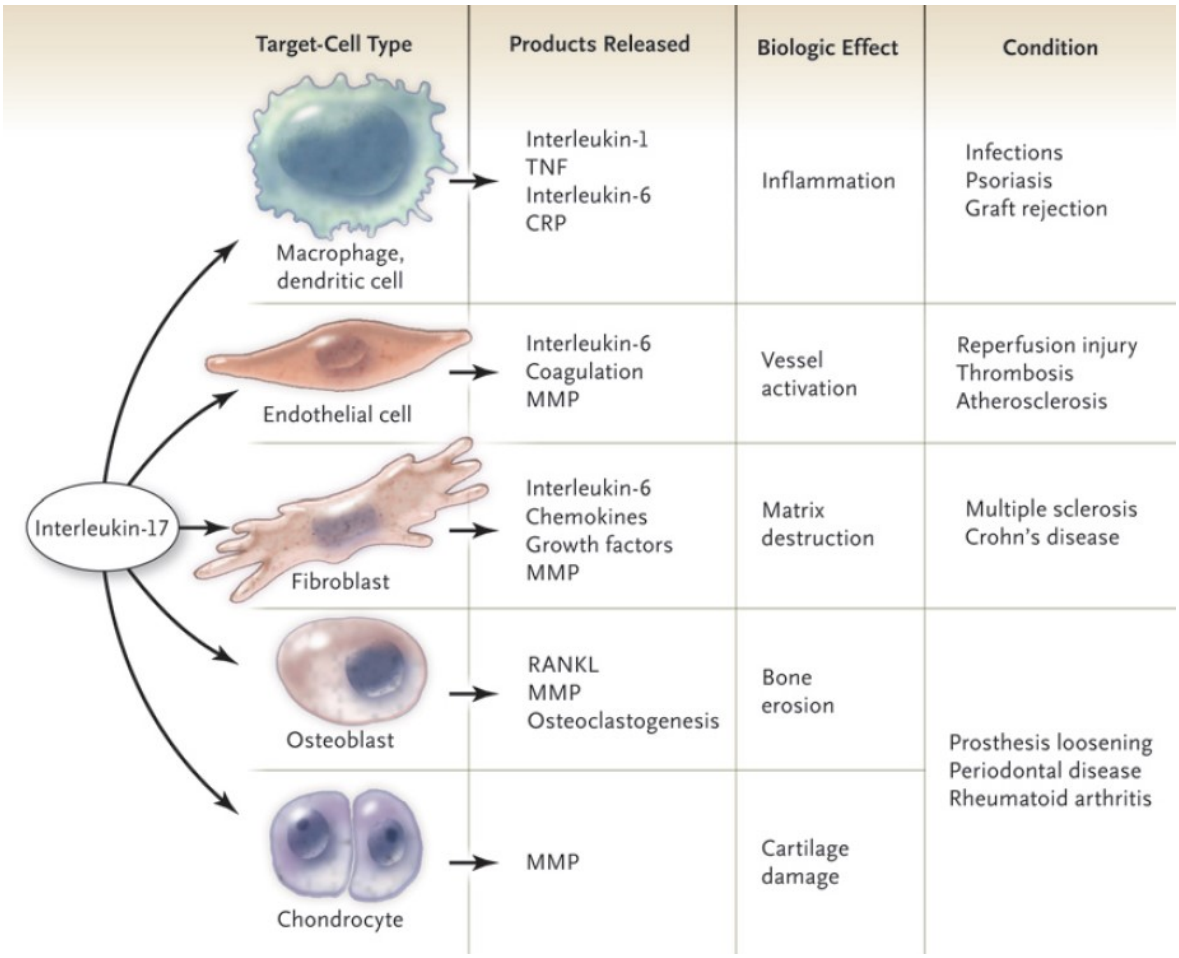

Figure 6. Effects of interleukin 17 on distinct target cells.

For each target-cell type the cytokines and chemokines secreted after stimulation with interleukin 17, the correlated physiologic effect, and the conditions associated are listed.

CRP = C-reactive protein. MMP = matrix metalloproteinase. RANKL = receptor activator of nuclear factor-κB ligand. TNF = tumor necrosis factor.

Reproduced with permission from (73), Copyright Massachusetts Medical Society.

Interestingly, IL-6 is a product released by IL-17 target-cell types as well as a differentiation factor for Th17 cells. Increased IL-6 release leads to IL-17 activation. Consequently, a positive feedback loop results in enhanced differentiation of naive T cells into Th17 cells. Additionally, increased chemokine production leads to migration of numerous effector T cells to the center of infection.

However, overproduction of IL-17 is associated with the pathogenesis of multiple autoimmune diseases such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, and multiple sclerosis (73,105).
Owing to its modulatory effects on neutrophil-mediated immune responses in fungal and bacterial infections, IL-17 presents one of the most important proinflammatory cytokines regarding host defense and autoimmunity (105).

1.2.3.2 Interplay between *Candida albicans* and interleukin 17 production

The capability of *C. albicans* to induce Th17-driven host response was reported in several studies (32,106).
However, in a study from Cheng et al. (32) investigating PBMCs in a co-culture with heat-killed and live *C. albicans*, it was shown that *C. albicans* is able to specifically downregulate IL-17 immune response as well. The IL-17-diminishing effect of live *C. albicans* is regulated by modulating effects on tryptophan/kynurenine metabolic pathways (31,32,75).
The potential of *C. albicans* to downregulate host immune response is crucial for enabling *Candida* colonization (31).

1.2.3.3 Tryptophan metabolism

The Tryptophan metabolism is regulated by two distinct pathways. One pathway involves the metabolic enzyme indoleamine 2,3-dioxygenase (IDO), which catalyzes the so-called kynurenine pathway by conversion of tryptophan into kynurenine (32,107). IDO is expressed in most human tissues, macrophages and DCs. Both stimulation by IFN-ɣ or other proinflammatory cytokines and direct activation through *Candida* hyphae (more than yeasts) lead to IDO induction (98,107).
IDO is able to precisely control the balance between Treg and Th17 cell responses in case of IFIs. Therefore, modulation of the IFN-γ-IDO axis may account for diverse effects, ranging from protective tolerance (i.e., equilibrium between resistance (control of fungal burden) and tolerance (control of inflammation)) to manifest immunosuppression (**Figure 7**) (70,98). According to its capacity to initiate differentiation of Treg cells while concomitantly inhibiting Th17 responses, IDO is crucial for suppression of acute inflammatory responses (70,71,108–110). Following this, IDO activation may serve as pathogen evasion strategy, supporting commensalism or chronic infection (70). Since IDO induction promotes a

tolerogenic environment, it may also be exploited by tumors as immune escape mechanism (109).

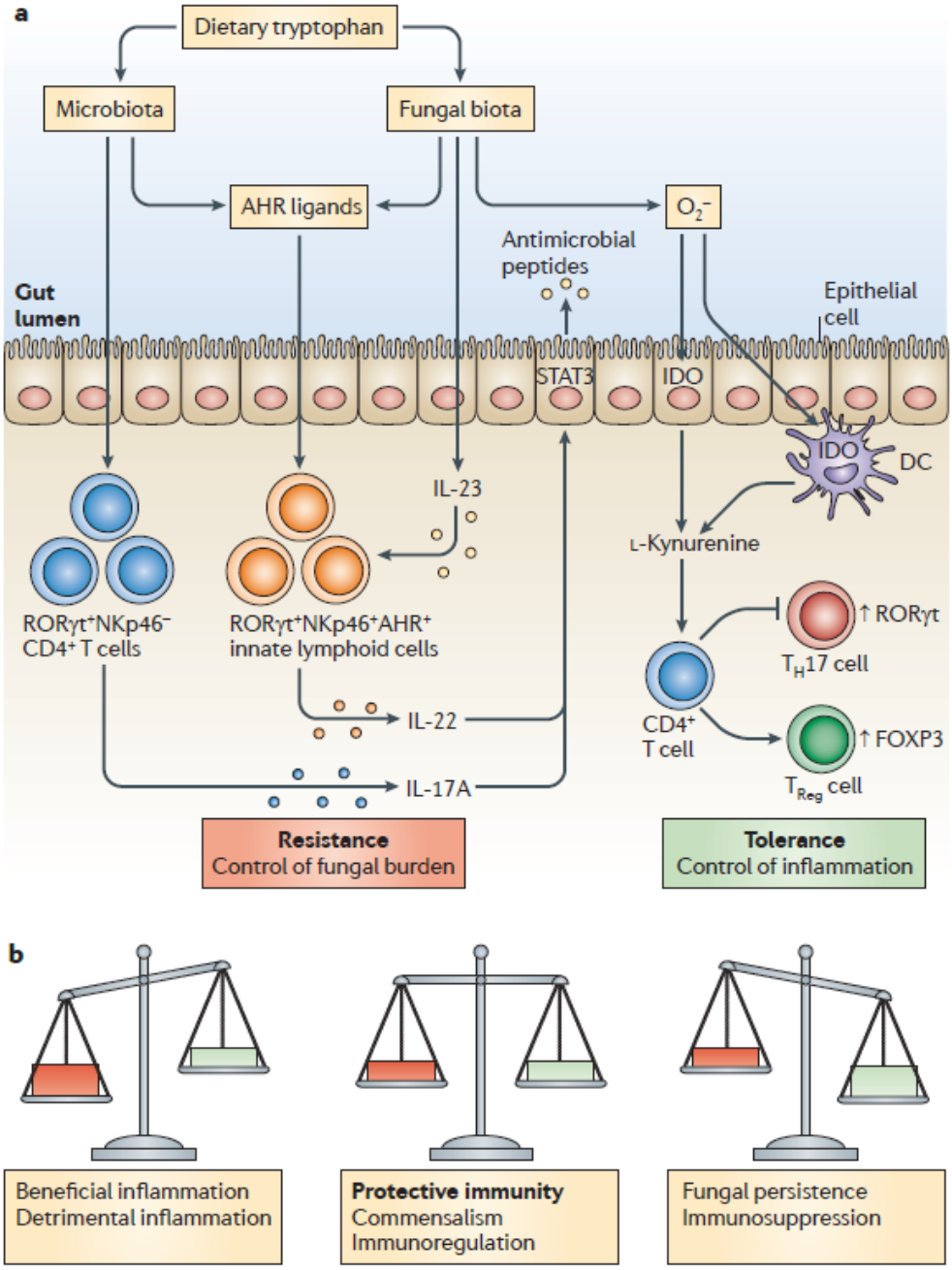

Figure 7. The tryptophan metabolism and its role in the Treg/Th17 cell balance during fungal infections.

The spectrum of host-fungus relationships ranges from (detrimental) inflammation to protective immunity and to fungal persistence and immunosuppression. Through exploitation of the IFN-γ-IDO axis, fungi have developed a strategy to modulate the immune response, and to either control fungal burden (i.e., resistance) or control exaggerated immune reaction (i.e., tolerance).

AHR = aryl hydrocarbon receptor. IL = interleukin. STAT3 = signal transducer and activator of transcription 3. IDO = indoleamine 2,3-dioxygenase. FOXP3 = forkhead box P3. RORγt = retinoic acid receptor-related orphan receptor-γt. Th17 cells = T helper type 17 cells. Treg cells = regulatory T cells. IFN = interferon.

Reproduced from (70) with permission of Springer Nature.

The second pathway of tryptophan metabolism comprises the hydroxylation of tryptophan into 5-hydroxytryptophan, catalyzed by the enzyme tryptophan hydroxylase. In the next step, 5-hydroxytryptophan is further metabolized into serotonin and melatonin (**Figure 8**) (32).

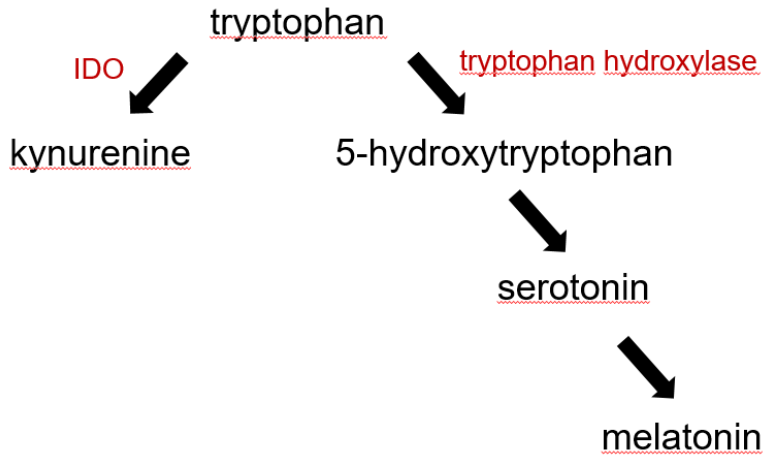

Figure 8. Tryptophan metabolism and related pathways.
IDO = indoleamine 2,3-dioxygenase.
Adapted from (32,107).

1.2.3.4 Therapeutic potential of interleukin 17

IL-17 is involved in the pathogenesis of various autoimmune diseases (111). Owing to its immunomodulatory effects on several cell types including lymphocytes, neutrophils, macrophages, dendritic cells, fibroblasts, keratinocytes, epithelial cells, and endothelial cells, monoclonal antibodies targeting IL-17 are supposed to treat a variety of inflammatory diseases (95).

Secukinumab was the first human IL-17A antagonist FDA-approved (2015) for first-line treatment of patients with moderate-to-severe plaque psoriasis, and for second-line treatment of psoriatic arthritis and ankylosing spondylitis (95,112). Ixekizumab (IL-17A antagonist) and Brodalumab (IL-17 receptor A (IL-17RA) antagonist) are other FDA-approved agents for treatment of moderate-to-severe plaque psoriasis (95).

Since IL-23 increases IL-17 secretion, IL-23 inhibition is another target to suppress autoimmune inflammation. Monoclonal antibodies against the p40 subunit of IL-12 and IL-23 (e.g., Ustekinumab) are effective treatment options in patients with psoriasis or Crohn's disease (73).

Antagonists of the transcription factor RORγt constitute another class of drugs targeting the IL-17 pathway. Together with other transcription factors such as STAT3, RORγt is necessary for production of IL-17A and IL-17F by Th17 cells. In murine psoriasis-like disease models RORγt inhibitors markedly inhibited the development of psoriatic skin lesions (95,113). Currently, several clinical trials are studying novel biologic treatment approaches targeting the Th17/IL-17 axis, therefore the pipeline contains a large number of drugs for various autoimmune disorders (114).

1.2.4 Genetic defects associated with *Candida* infections

The significance of IL-17 in antifungal host defense has been underlined in several murine models showing that the incidence of both systemic candidiasis and mucocutaneous *Candida* infections is significantly increased in IL-17 deficient mice. Though, patients with genetic polymorphisms of IL-17F, IL17RA, IL-17RC, dectin-1, STAT1 or STAT3 suffer from CMC, but not IC (76,97). CMC is marked by persistent or recurrent *Candida* infections of mucosal and epithelial membranes including the nails (97). In patients with APS-1, autoimmune regulator (AIRE) gene mutations result in auto-antibodies against IL-17A, IL-17F and IL-22 leading to CMC (**Figure 9**) (74,102).
That implies that Th17-mediated immunity is mainly required for humane anti-*Candida* host defense at epithelial and mucosal surfaces (76,97).

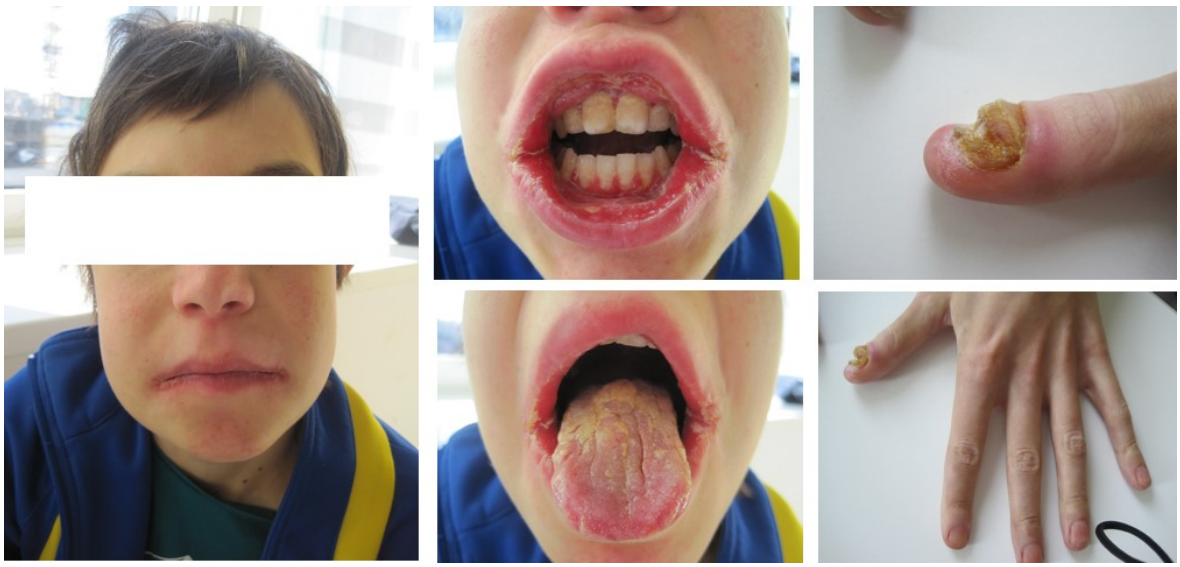

Figure 9. Chronic mucocutaneous candidiasis in a patient with autoimmune polyendocrine syndrome type I.
By courtesy of Prof. Christoph Högenauer, Division of Gastroenterology and Hepatology, Medical University of Graz.

In general, deficiencies in the TLR pathway do not severely dampen anti-*Candida* host defense (12). By contrast, CLRs are fundamental for *Candida* recognition and subsequent induction of innate and adaptive immune reactions (70). Thus, patients with a congenital defect in the CLR pathway adaptor protein CARD9 present significantly increased susceptibility to various fungal infections, whereas antibacterial and antiviral host response are not affected. Patients with CARD9 deficiency are predisposed to CMC, but also to severe IC (e.g., *Candida* meningitis), extrapulmonary aspergillosis and phaeohyphomycosis (70,76,78,97,100). Up to now, CARD9 deficiency is the only mutation linked to IC in humans (78).

Although dectin-1 belongs to the CLR family, dectin-1 polymorphisms, however, solely result in non-severe *Candida* infections such as recurrent vulvovaginal candidiasis or onychomycosis (76,115). In contrast, CARD9 mutation leads to a much severe phenotype, presumably because CARD9 also initiates downstream signaling of CLRs other than dectin-1 (12,76).

2 Materials and Methods

Parts of the section “Material and Methods” have recently been published in Wunsch et al. (116).

2.1 Study objectives

The objective of the present study was the evaluation of IL-17A plasma levels and other cytokines involved in *Candida*-specific immunity as potential biomarkers for early anticipation of invasive *Candida* infection. Since previous research from our working group (31) suggested a time-dependent course of IL-17A levels in patients with IC, which has not been investigated further so far, we performed serial measurements of IL-17A, tryptophan, kynurenine and other cytokines. For comparative analysis, we also measured these cytokines in bacteremic patients and healthy controls.

Selection of cytokines was based on previous research from both murine models and clinical studies, showing an increase or decrease of these plasma or serum cytokine levels in patients with *Candida* infections (32,71,74,78,79,105,117–120). Investigated cytokines were: IL-6, IL-8, IL-10, IL-17A, IL-17F, IL-22, IL-23 (p19 subunit), IFN-ɣ, TNF-α, PTX3 and TGF-β.

2.2 Study design

The study was designed as prospective multicenter study, which was performed at the Medical University of Graz, Austria (center 1), the Medical University of Innsbruck, Austria (center 2), and the University Hospital of Cologne, Germany (center 3). Patients were recruited between September 2015 and December 2018.
The study was submitted for approval by the local ethics committee (Graz, protocol number 19-322 ex 07/08) and was executed according to the standards of “Good Scientific Practice”. Written informed consent was obtained from all patients qualified for study inclusion. Unconscious patients were informed and asked for their consent after their arousal.

2.3 Study cohort

Adult patients ≥18 years of age were prospectively screened for study inclusion and were assigned to groups 1-4 as described below (center 1). In center 2 and 3 only candidemic patients were included.

2.3.1 Group 1, patients with invasive candidiasis

Group 1 comprised patients with IC, which were further classified according to previously unpublished work from Bassetti et al. (EORTC/MSG definitions of invasive fungal diseases, proposed revisions and updates), presented at the “Trends in Medical Mycology” (TIMM) in Lisbon 2015. However, during the course of the study, revised and updated EORTC/MSG definitions (66) were published. Updated definitions primarily affect the diagnosis of probable IC (66). Diagnostic criteria for proven and probable IC used in our study are represented in **Table 3**.

In the presence of a host factor and typical clinical presentation but in the absence of mycological evidence, cases are defined as possible IC (66).

Table 3. Diagnostic criteria for invasive candidiasis used in this study.

Reproduced from (66) with permission of Oxford University Press, and from previously unpublished work of Bassetti et al., TIMM 2015.

Category	Host Factor	Clinical Presentation	Mycological Evidence
Proven IC[1]	not required	*Candida* species isolated from blood cultures or from other specimen obtained by a sterile procedure from a normally sterile site (including a freshly placed (<24 hours ago) drain) showing a clinical or radiological abnormality consistent with an infectious disease process	
Probable IC	At least one of the following: 1) impaired gut wall integrity[2] 2) impaired cutaneous barriers to bloodstream infection[3] 3) colonization with *Candida* species in ≥2 non-sterile sites[4] 4) recent history of neutropenia (<500 neutrophils/mm^3 for >10 days) temporally related to the onset of invasive fungal disease 5) hematologic malignancy 6) receipt of an allogeneic stem cell transplant *or* solid organ transplant 7) prolonged use of corticosteroids at a therapeutic dose of ≥0.3 mg/kg for ≥3 weeks in the past 60 days 8) treatment with other recognized T-cell immunosuppressants (e.g., calcineurin inhibitors, tumor necrosis factor-α blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues) during the past 90 days 9) inherited severe immunodeficiency (e.g., chronic granulomatous disease, STAT 3 deficiency, CARD9 deficiency, STAT-1 gain of function, or severe combined immunodeficiency) 10) acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids	clinical or radiological (non-pulmonary) abnormalities consistent with an infectious disease process that are otherwise unexplained	At least one of the following: 1) ß-D-glucan ≥80 pg/ml (Fungitell®) detected in at least 2 consecutive serum samples provided that other etiologies have been excluded 2) recovery of *Candida* in an adequate intra-abdominal specimen (obtained surgically or within 24h from external drainage)

[1]Since histopathological examinations require a certain time period, histopathological evidence of invasive candidiasis (IC) is usually retrospective in nature, making timely acquisition of blood samples unfeasible. Following this, histopathological evidence of IC was not included as diagnostic criterion of proven IC in our study.

[2]recent abdominal surgery, biliary tree abnormality, recurrent intestinal perforations, ascites, mucositis, severe pancreatitis, parenteral nutrition

[3]presence of central vascular access device, hemodialysis

[4]respiratory tract secretions, stool, skin, wound sites, urine, drains that have been in place for ≥24 hours

Although immunosuppression is included in the definition of probable IC (66), we initially intended not to recruit immunocompromised patients in order to prevent a possible influence of immunosuppression on investigated biomarker levels (e.g., cytokines). Therefore, exclusion criteria for patients in group 1 initially comprised immunosuppressive therapy (glucocorticoids with prednisone equivalent of ≥20mg/d, methotrexate, azathioprine, etc.), active hematooncological disease, human immunodeficiency virus (HIV) positivity, and/or antifungal therapy within 8 weeks prior to inclusion.
However, during patient recruitment and as described in previous literature it turned out early that a large number of patients with IC actually are immunosuppressed or have malignant diseases, many of them with concomitant antifungal prophylaxis. Additionally, recently revised and updated EORTC/MSG definitions (66) indeed include the presence of immunosuppression in the definition of probable IC. Consequently, we determined to enroll also immunocompromised patients, patients with malignancies and patients with recent or current antifungal therapy, and to perform subgroup analysis.

2.3.2 Group 2, patients with *Staphylococcus aureus* bacteremia

Patients with *Staphylococcus aureus* (*S. aureus*) positive blood cultures (i.e., *S. aureus* bacteremia) were assigned to group 2. The two bacteremia groups (group 2 and 3) served for comparative data analysis in order to test the specificity of obtained biomarker results for IC versus bacteremia. Inclusion criteria for patients in group 2 comprised age ≥18 years and ≥1 *S. aureus* positive blood culture(s).

2.3.3 Group 3, patients with *Escherichia coli* bacteremia

Group 3 comprised patients with *Escherichia coli* (*E. coli*) positive blood cultures (i.e. *E. coli* bacteremia). Inclusion criteria for patients in group 3 comprised age ≥18 years and ≥1 *E. coli* positive blood culture(s).

2.3.4 Group 4, healthy controls

Group 4 included individuals without any evidence of current or chronic infectious diseases.

Otherwise healthy patients with scheduled (mainly aesthetic) plastic surgery were recruited at the Division of Plastic Surgery, Department of Surgery at the Medical University of Graz. This group served as a control group and was used for comparative data analysis.

Exclusion criteria comprised:

1) clinical, laboratory or radiological evidence of current infectious disease (temperature >38°C, elevated C-reactive protein (CRP) >5mg/L, leukocytosis ≥11,4 10^9/L, elevated neutrophils)
2) antifungal therapy within 8 weeks prior to inclusion
3) immunosuppressive therapy (e.g., glucocorticoids, methotrexate, azathioprin, etc.)
4) active hematooncological disease
5) HIV positivity

2.4 Data acquisition

Demographic, clinical and laboratory data were extracted from charts and electronic medical records with the use of MEDOCS, the electronic medical documentation system at the University Hospital of Graz.

The following parameters were obtained in center 1:

- demographic data: gender, age
- cause of hospital admission
- cause of ICU admission
- ward (e.g., medical, surgical, ICU, other)
- duration of stay/ICU stay prior to sampling of index cultures
- SAPS2/3 Score
- non-invasive ventilation or invasive mechanical ventilation; if yes: duration
- oxygenation index
- recent intra-abdominal surgery
- recent other surgery
- antifungal therapy: prior to sampling of index cultures, on the sampling day and subsequently; if yes: duration
- antibiotic therapy: prior to sampling of index cultures, on the sampling day and subsequently; if yes: duration

- immunosuppressive therapy
- active hematooncological disease
- SOFA Score
- vasopressor therapy
- therapy with proton-pump inhibitors
- sedation
- morphine(s)
- parenteral or enteral nutrition; in case of parenteral nutrition: duration
- nasogastric tube
- number of central venous catheters
- femoral vascular access
- urinary catheter
- duration of fever prior to *Candida* spp./*S. aureus*/*E. coli* detection
- laboratory values: leukocytes, absolute neutrophil count, thrombocytes, CRP, procalcitonin (PCT), IL-6, 1,3-beta-D Glucan, galactomannan
- *Candida* species
- in case of candidemia: presence and result of follow-up blood cultures
- known *Candida* colonization; if yes: number of sites colonized
- bacteremia; if yes: bacterial genus/species
- other invasive bacterial infection
- viremia
- outcome; if death: intrahospital death, death within 30 days, death related to IC, death possibly related to IC

Due to limited personnel resources concerning data acquisition in center 2 and 3, a reduced data file was used for these centers. The reduced data file contained following variables:

- demographic data: gender, age
- cause of hospital admission
- cause of ICU admission
- duration of stay/ICU stay prior to sampling of index cultures
- SAPS2/3 Score
- recent intra-abdominal surgery
- recent other surgery
- antifungal therapy: prior to sampling of index cultures, on the sampling day and subsequently; if yes: duration

- antibiotic therapy: prior to sampling of index cultures, on the sampling day and subsequently; if yes: duration
- immunosuppressive therapy
- active hematooncological disease
- laboratory values: thrombocytes (10^9/L), CRP (mg/L), PCT (ng/mL), 1,3-beta-D Glucan (pg/mL)
- *Candida* species
- bacteremia; if yes: bacterial genus/species
- viremia
- outcome; if death: intrahospital death, death within 30 days, death related to IC, death possibly related to IC

2.5 Definitions

2.5.1 Data file variables

Patients with IC were classified into proven, probable and possible IC as mentioned above. According to the recently revised and updated EORTC/MSG definitions (66), recovery of *Candida* spp. from blood cultures (i.e., candidemia) or from a sterile site combined with clinical or radiological abnormality consistent with an infectious disease process was classified as proven IC. Definite candidemia cases (including only patients with *Candida* positive peripheral +/- central blood cultures) were labeled “true candidemia” (i.e., IC (true)). However, some of the study patients with candidemia presented *Candida* positive central blood cultures only but negative peripheral blood cultures, which potentially represents *Candida* colonization of central venous catheters rather than candidemia. Thus, differential time to positivity for determination of catheter-related BSI could not be calculated in these patients. Consequently, they could not be reliably classified as true candidemia and were classified as “IC (other)”.
As a consequence, the term “IC (other)” comprised patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC (116).

In order to evaluate the severity of illness in ICU patients, the Simplified Acute Physiology Score 3 (SAPS3) was applied. The SAPS3 Score serves as a mortality prediction model for

ICU patients and is based on patient characteristics collected within the first hour of ICU admission (e.g., demographics, comorbidities, circumstances of ICU admission, and biochemical and physiological disturbances) (121,122).
For ICU patients in center 1 and 2, the SAPS3 Score was automatically calculated through the electronic medical documentation system. For ICU patients in center 3, the SAPS3 Score was not available, therefore the SAPS2 Score was used instead.

The oxygenation index (OI) is defined as the ratio of arterial oxygen (PaO_2) to inspired oxygen concentration (FiO_2) (123). Acute respiratory distress syndrome (ARDS) is categorized in three severities based on the OI (Berlin definition). Mild ARDS is defined as 200mmHg < OI ≤ 300mmHg, moderate ARDS as 100mmHg < OI ≤ 200mmHg, and severe ARDS as OI ≤ 100mmHg (124).

In order to describe the severity of organ dysfunction in ICU patients, the Sequential Organ Failure Assessment (SOFA) Score was calculated. The SOFA Score is composed of scores from six organ systems (respiratory system, cardiovascular system, coagulation, liver, kidneys, central nervous system), graded from 0 to 4 according to the degree of organ dysfunction. Higher scores are associated with more severe organ dysfunction (125). Since calculation of the SOFA Score involves a number of different variables, the complete score could not be calculated for most patients. Following this, a partial SOFA Score was determined for patients in center 1, named “SOFA Score minimum”, including all variables available. Concerning center 2 and 3, determination of the SOFA Score was not possible due to incomplete data.

Recent (abdominal) surgery was defined as surgery within four weeks prior to inclusion.

Patients were classified as immunosuppressed in case of (a) receiving immunosuppressive therapy (e.g., glucocorticoids with prednisone equivalent of ≥20mg/d, methotrexate, purine analog, specific biological therapies, azathioprine, etc.), (b) active hematooncological disease, (c) HIV positivity, (d) status post solid organ transplantation, (e) primary immunodeficiencies.

Regarding the variable “duration of fever prior to *Candida* detection”, the threshold value of fever was defined as ≥38,3°C.

Concerning BDG testing, BDG values <15,38 pg/ml were defined as negative, values between 15,38 and 80 pg/ml were defined as intermediate and values ≥80 pg/ml were defined as positive.

Since swabs were performed at the discretion of the attending physician, no specific surveillance swabs concerning *Candida* colonization were obtainable. Therefore, the term "known *Candida* colonization" was applied, as *Candida* colonization could only be evaluated by use of the available swabs.

Outcome was specified as "death related to IC" or "death possibly related to IC". "Death related to IC" was defined as follows: (a) death in temporal connection with *Candida* positive blood cultures or sterile site specimens, (b) plus corresponding clinical symptoms, and (c) in the absence of other bacterial or viral infection or decompensated comorbidities. In most of these cases, an autopsy was performed confirming IC as underlying cause of death. If one of the afore-mentioned criteria could not be met, yet clinical assessment strongly suggested an association between IC and death, outcome was stated as "death possibly related to IC".

2.5.2 Sampling

The sampling day of (consecutively) *Candida* spp. positive blood or sterile site cultures, and *S. aureus or E. coli* positive blood cultures (i.e., index cultures) was defined as day 1. All of the obtained data referred to this date, unless otherwise stated. Positive follow-up cultures were not considered as new cases (116).
The detection date of yeasts on gram stain or *S. aureus* or *E. coli* with state of the art identification methods (e.g., Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF-MS)) was designated as "date of first record". The date of first record corresponded to the date of study inclusion and was dependent on laboratory operating times (116). If our automatic blood culture detection system BACTEC™ (BD, Heidelberg, Germany) indicated positive blood cultures beyond laboratory operating times (e.g., on weekends), further processing and eventual study inclusion was not possible until our microbiology laboratory was operating again.

Retained blood samples were collected as soon as *Candida* spp., *S. aureus* or *E. coli* was detected in blood cultures or sterile site specimens. These left-over samples are routinely drawn blood samples, which are analysed in our routine laboratories, and, after

determination of ordered lab tests, are stored at 4°C for up to 4 days in case that additional testing is requested (116).

2.6 Sampling schedule

Blood cultures, sterile site specimens and BDG testing were obtained as clinically indicated at the discretion of the treating physician. Routine blood cultures and sterile site specimens processed in our microbiology laboratories were monitored daily for the presence of *Candida* spp., *S. aureus* or *E. coli*. After blood culture positivity, routine staining and identification methods were performed (i.e., MALDI-TOF-MS; Bruker Maldi Biotyper®, Bruker, Vienna, Austria; Peptide Nucleic Acid Fluorescence In Situ Hybridisation (PNA FISH) test; Xpert MRSA test (GeneXpert system; Cepheid)) (116).

Following study inclusion, all available lithium heparin, ethylenediaminetetraacetic acid (EDTA) and serum tubes (Greiner Bio-One™, Kremsmünster, Austria) (i.e., retained blood samples) were collected. Retained blood samples from the sampling day of index cultures were defined as “day 1 samples”. Accordingly, blood samples from the day before were considered as “day -1 samples”, blood samples from the day after as “day 2 samples”, etc. (**Figure 10**) (116).

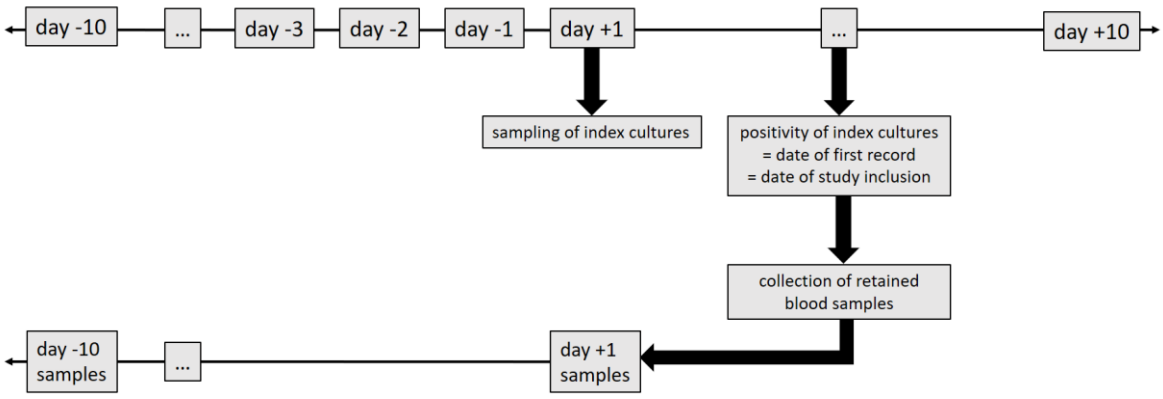

Figure 10. Sampling schedule.

With this sampling schedule investigation of biomarkers at earlier time points compared to previous studies was intended. Starting from the day of study inclusion, retained blood samples were collected on a daily basis until day 14. Therefore, we were able to investigate

blood samples obtained prior to sampling of index cultures as well as samples obtained afterwards (116).
In patients with candidemia or *S. aureus* bacteremia, control blood cultures were performed routinely on day 3 to 5 of adequate antifungal or antibiotic therapy to demonstrate clearance of microorganisms from bloodstream.

2.7 Processing

2.7.1 Blood samples

After processing of routinely drawn blood samples at the in-house clinical chemical laboratory unit, serum and lithium heparin tubes were centrifuged (3044rpm/2300g, 10 minutes) right before they were stored at 4°C for potential additional testing. EDTA tubes were not routinely centrifuged.
Following collection of retained blood samples, samples were aliquoted into sample tubes. Of the 1288 collected sample tubes, only 84 were other tubes than lithium heparin tubes (i.e., EDTA or serum). Accordingly, we decided to solely include lithium heparin tubes for cytokine measurements (11-Plex immunoassay) in order to guarantee comparability of results (i.e., 1204 tubes were analyzed).
Blood samples were stored at -80°C until further analysis to ensure stable cytokine concentrations for long-term storage periods (several months to years) (126).

2.7.2 Blood cultures

Blood cultures were drawn as clinically indicated at the discretion of the treating physician. Corresponding to our in-house recommendations (based on studies from Li et al. (127) and Lamy et al. (128)), one blood culture set for peripheral blood culture collection consisted of three aerobic and three anaerobic blood culture bottles (Bactec™, Becton Dickinson, Heidelberg, Germany). Blood culture bottles were incubated at 37°C in the Bactec™ FX system (Becton Dickinson, Heidelberg, Germany) for a duration of 5 days, unless otherwise requested. Positive blood culture bottles were processed by standard procedures, including gram staining and performance of fast identification methods as described above. Antimicrobial susceptibility testing was performed according to European Committee of Antimicrobial Susceptibility Testing (EUCAST) disk diffusion standards.

2.7.3 *Candida* cultures

Specimens for *Candida* cultures were processed by standard procedures (subculturing on CHROMagar™ *Candida* Medium (Becton Dickinson, Heidelberg, Germany); identification by MALDI-TOF-MS; antifungal susceptibility testing by E-test strips (AB Biotest, Solna, Sweden)) (116).

2.7.4 Serum (1-3)-beta-D Glucan

As previously published, serum BDG was determined by an adapted and automated protocol of the Fungitell® assay (positive ≥80 pg/ml) (62).

2.7.5 Cytokines (IL-6, IL-8, IL-10, Il-17A, IL-17F, IL-22, IL-23 (p19), IFN-ɣ, TNF-α, PTX3, TGF-β)

Cytokine values were determined using a customized 11-Plex immunoassay (assay ID: PPX-11-PTX3LAP; ThermoFisher Scientific Inc., Waltham, Massachusetts, USA) according to the instructions of the manufacturer. Detailed methods are described in Wunsch et. al (116).

2.7.6 Kynurenine and Tryptophan

Kynurenine and tryptophan concentrations were measured by high-performance liquid chromatography (HPLC) as described before (31). Due to limited testing capacities in one of the collaborating laboratories, kynurenine and tryptophan values were solely measured for randomly selected patients of study groups 1 to 3 from center 1 (Graz) (116).

2.8 Statistical analysis

Regarding descriptive analyses, medians and ranges (min-max) were calculated for continuous variables, whereas absolute and relative frequencies were used for categorical variables. Comparison of categorical variables was performed using the Chi-squared test,

for comparison of continuous variables between the groups the Kruskal-Wallis test was applied.

The number of cytokine measurements per patient varied and measurements were not usually available for each day, as blood samples were collected at the discretion of the treating physician. Accordingly, we compared interleukins, kynurenine/tryptophan, BDG, leukocytes, CRP, and PCT between the five study groups at pre-selected time intervals (days) defined as follows (day 1 corresponds to the day of index sampling): (-4; -2), (-1; 2), (3; 7), (8; 14). In case of multiple cytokine measurements per day, we used the first cytokine value of this particular study day. For healthy controls, only one cytokine level from the date of study inclusion was available, which was repeatedly used for each time interval. In order to consider repeated measurements per patient, linear mixed models with random intercept per patient were applied. For validation of model assumptions, residual plots were generated. Skewness in the outcome parameters of interest required a log transformation. Results are presented as estimates for group differences on the log-scale along with the 95% confidence interval and a corresponding *p*-value.

Analyses were calculated for the total study population (i.e., main analysis), and after exclusion of immunocompromised patients and patients with hematologic malignancies (i.e., sensitivity analysis). Data analysis was performed using SPSS, version 26 (SPSS, Chicago, IL), and R, version 3.6.1. P-values <.05 were considered statistically significant (116).

3 Results

Results from this study have recently been published in Wunsch et al. (116).

3.1 Study cohort

The study cohort comprised 101 patients with IC, 23 patients with *S. aureus* bacteremia, 28 patients with *E. coli* bacteremia, and 32 healthy controls (i.e., 184 patients in total). Demographic data and baseline characteristics of all study groups are depicted in **Table 4** (116).

Table 4. Demographic data and baseline characteristics.

Variable	invasive candidiasis	*S. aureus* bacteremia	*E. coli* bacteremia	healthy controls	*p*
No. of patients	101	23	28	32	
age, years; median (range)	64 (18-91)	69 (52-87)	79 (18-96)	46 (19-86)	**<.001**
sex					**.012**
male	64 (63.4)	17 (73.9)	10 (35.7)	15 (46.9)	
female	37 (36.3)	6 (26.1)	18 (64.3)	17 (53.1)	
cause of hospital admission					
elective surgery	24 (23.8)	2 (8.7)	0	32 (100)	
infectious disease	24 (23.8)	10 (43.5)	14 (50)		
gastrointestinal disease	20 (19.8)	1 (4.3)	7 (25)		
malignant disease	16 (15.8)	1 (4.3)	3 (10.7)		
cardiovascular disease	4 (4.0)	4 (17.4)	3 (10.7)		
other	13 (12.9)	5 (21.7)	1 (3.6)		
ward					
internal medicine	13 (12.9)	13 (56.5)	25 (89.3)		
surgery	27 (26.7)	4 (17.4)	1 (3.6)	32 (100)	
ICU	44 (43.6)	4 (17.4)	1 (3.6)		
other or unknown	17 (16.8)	2 (8.7)	1 (3.6)		
recent abdominal surgery	50 (49.5)	1 (4.3)	0	0	**<.001**
recent other surgery	42 (41.6)	5 (21.7)	5 (17.9)	0	
active hematooncological disease	41 (40.6)	2 (8.7)	5 (17.9)	0	**.001**

solid tumor	35/41 (85.4)	1/2 (50)	2/5 (40)		
hematologic malignancy	6/41 (14.6)	1/2 (50)	3/5 (60)		
immunosuppressive therapy	18 (17.8)	2 (8.7)	6 (21.4)	0	

Data are no. (%) of patients, unless otherwise indicated. ICU = intensive care unit.

In **Table 5** a detailed characterization of patients with immunosuppression is provided (116).

Table 5. Characteristics of patients with immunosuppression.

Variable	invasive candidiasis	*S. aureus* bacteremia	*E. coli* bacteremia
regimen			
single therapy	5/18 (27.8)	0	2/6 (33.3)
combination therapy	8/18 (44.4)	2/2 (100)	4/6 (66.7)
data not available	5/18 (27.8)	0	0
immunosuppressive agents			
corticosteroids at a dose of ≥0.3 mg/kg for ≥3 weeks in the past 60 days	7/18 (38.9)	2 (100)	0
calcineurin inhibitors	5/18 (27.8)	0	2/6 (33.3)
mTOR inhibitors	1/18 (5.6)	0	0
folate antagonists	0	0	1/6 (16.7)
topoisomerase inhibitors	1/18 (5.6)	0	2/6 (33.3)
pyrimidine antagonists	1/18 (5.6)	0	2/6 (33.3)
mycophenolate	4/18 (22.2)	0	2/6 (33.3)
alkylating agents	2/18 (11.1)	1/2 (50)	0
purine analogues	2/18 (11.1)	1/2 (50)	1/6 (16.7)
anti-CD20 antibody*	1/18 (5.6)	0	0
data not available	5/18 (27.8)	0	0

Data are no. (%) of patients, unless otherwise indicated.

mTOR = mechanistic target of rapamycin.

*i.e. Rituximab

Table 6 presents details of patients with invasive *Candida* infections (*n* = 101). Most of the patients were included in center 1 (85.1%), followed by center 2 (7.9%), and center 3 (6.9%). Proven IC was detected in 62 (61.4%) cases, 34 (33.7%) cases were classified as probable IC, and 5 (5%) cases as possible IC. A totel of 71 (70.3%) patients were candidemic, of these patients 56 (78.9%) were designated as true candidemia cases. The distribution of *Candida* spp. in patients with IC was as follows: *C. albicans* (69.3%), *C. glabrata* (12.9%), *C. parapsilosis* (7.9%), >1 *Candida* spp. (5.9%), and other *Candida* spp. (4%). In 40/56 (71.4%) patients with candidemia (total number) and 27/41 (65.9%) patients with true candidemia follow-up blood cultures were drawn, with a positivity rate of 17.4% (7/40) and 25.9% (7/27), respectively (data only available for center 1, Graz) (116). In patients with invasive *Candida* infections *Candida* colonization rate was 51%. The swabs used for culture and calculation of *Candida* colonization rates were up to the discretion of the treating physician, therefore specific surveillance swabs were not available for the complete study cohort.

Table 6. Characteristics of patients with invasive candidiasis.

Variable	No. (%) of patients
overall study cohort	101 (100)
Center 1 (Graz)	86 (85.1)
Center 2 (Innsbruck)	8 (7.9)
Center 3 (Cologne)	7 (6.9)
Category	
proven IC	62 (61.4)
probable IC	34 (33.7)
possible IC	5 (5.0)
candidemia	
total number	71 (70.3)
true candidemia[1]	56 (55.4)
***Candida* species**	
C. albicans	70 (69.3)
C. glabrata	13 (12.9)
C. parapsilosis	8 (7.9)
>1 *Candida* spp.	6 (5.9)
other *Candida* spp.	4 (4.0)
follow-up blood cultures[2]	

candidemia (total number)[3]	40/56 (71.4)
true candidemia[4]	27/41 (65.9)
positivity rate of follow-up blood cultures[2]	
candidemia (total number)[5]	7/40 (17.5)
true candidemia[6]	7/27 (25.9)
***Candida* colonization[7]**	44/86 (51.2)
number of sites colonized, median (range)	1 (0-5)

IC = invasive candidiasis.

[1]positive peripheral blood cultures +/- positive central blood cultures (exclusion of positive blood cultures from central venous catheters only)

[2]Data only available for center 1 (Graz).

[3]number of patients with *Candida* follow-up blood cultures performed relative to total number of candidemic patients

[4]number of patients with *Candida* follow-up blood cultures performed relative to number of patients with true candidemia

[5]number of patients with ≥1 positive follow-up blood culture(s) relative to total number of candidemic patients

[6]number of patients with ≥1 positive follow-up blood culture(s) relative to number of patients with true candidemia

[7]Swabs were performed at the discretion of the attending physician, therefore no specific surveillance swabs concerning *Candida* colonization were available. Data concerning *Candida* colonization only available for center 1.

Clinical characteristics of patients with IC, *S. aureus*, and *E. coli* bacteremia are displayed in **Table 7**.

The duration of hospital stay as well as the duration of ICU stay prior to sampling of index cultures were significantly longer in patients with invasive *Candida* infections compared to bacteremic patients ($p < .001$). Acute blood markers of inflammation (CRP, PCT) did not significantly differ among the study groups. BDG values were available for 55/86 (64.0%) IC patients from center 1 (determination was up to the treating physicians). The highest BDG values were measured on the index sampling day, albeit 4/13 patients with invasive *Candida* infections had positive BDG values (≥80 pg/mL) as early as one week prior to sampling of index cultures. Medication, devices and oxygenation strategies are depicted in **Table 7**. Briefly, in patients with IC the rate of intensified ICU treatment, ICU devices, and respiratory support (both invasive and non-invasive mechanical ventilation (NIV)) was significantly higher compared to bacteremic patients ($p < .001$). The median duration of antibiotic treatment prior to sampling of index cultures was 12 (range 0–145) days for patients with invasive *Candida* infections compared to 0 days in bacteremic patients (*S. aureus*: range 0-5, *E. coli*: range 0-51; $p < .001$ for both comparisons) (116).

Table 7. Clinical course of patients with invasive candidiasis, *S. aureus* bacteremia and *E. coli* bacteremia.

Variable	invasive candidiasis	*S. aureus* bacteremia	*E. coli* bacteremia	*p*
No. of patients	101	23	28	
duration of stay prior to index sampling, days	16 (0-98)	1 (0-24)	0 (0-52)	**<.001**
duration of ICU stay prior to index sampling, days	5 (0-121)	0 (0-5)	0 (0)	**<.001**
laboratory values on day of index sampling				
CRP (mg/L)	132 (3-521)	146 (5-472)	93 (1-399)	
PCT (ng/mL)	1.0 (0.1-109.8)	0.95 (0.2-358.8)	5.2 (0.2-84.9)	
positive BDG (≥80 pg/mL)*				
within 1 week prior to index sampling[1]	4/13 (30.8)	n.a.	n.a.	
on index sampling day[1]	9/15 (60)	n.a.	n.a.	
within 1 week after index sampling[1]	21/43 (48.8)	n.a.	n.a.	
vasopressors[2,*]	28/86 (32.6)	1 (4.3)	1 (3.6)	**<.001**
proton-pump inhibitors*	65/86 (75.6)	13 (56.5)	15 (53.6)	**.024**
sedation[3,*]	28/86 (32.6)	4 (17.4)	0	**<.001**
morphines*	45/86 (52.3)	5 (21.7)	6 (21.4)	**<.001**
parenteral nutrition*	38/86 (44.2)	0	0	**<.001**
duration of parenteral nutrition prior to index sampling, days	8 (0-65)	-	-	
nasogastric tube*	39/86 (45.3)	1 (4.3)	0	**<.001**
number of central venous catheters*	1 (0-3)	0 (0-2)	0 (0-1)	**<.001**
femoral vascular access*	12/86 (14)	0	0	**.016**
urinary catheter*	59/86 (68.6)	7 (30.4)	6 (21.4)	**<.001**
duration of fever prior to *Candida* spp./*S. aureus*/*E. coli* detection, days*	0 (0-16)	1 (0-3)	1 (0-7)	
oxygenation index*	253 (139-523)	280	n.a.	
respiratory support*				
invasive mechanical ventilation prior to index sampling	38/86 (44.2)	1 (4.3)	0	**<.001**
duration of mechanical ventilation prior to index sampling, days	7 (1-49)	1 (1)	-	
NIV prior to index sampling	23/86 (26.7)	0	0	**<.001**
duration of NIV prior to index sampling	3 (1-15)	-	-	

duration of mechanical ventilation starting from index sampling until end of ventilation, days	0 (0-72)	0 (0-28)	0 (0-2)	**.018**
severity of illness				
SAPS3 Score[4]	54 (19-98)	62 (31-72)	44 (34-53)	
SAPS2 Score[5]	29 (21-53)	-	-	
SOFA Score minimum[6,*]	2 (0-11)	2 (0-8)	2 (0-6)	
antibiotic therapy				
duration of antibiotic therapy prior to index sampling, days	12 (0-145)	0 (0-5)	0 (0-51)	**<.001**
antibiotic therapy during index sampling	85 (84.2)	6 (26.1)	3 (10.7)	**<.001**
antibiotic therapy after index sampling	96 (95)	23 (100)	28 (100)	
antifungal therapy				
antifungal therapy within 8 weeks prior to inclusion	15 (14.9)	0	0	
antifungal therapy during index sampling	11 (10.9)	0	2 (7.1)	
antifungal therapy after index sampling	91 (90.1)	0	0	
bacteremia	10 (9.9)	23 (100)	28 (100)	**<.001**
other invasive bacterial infection*	36/86 (41.9)	2 (8.7)	4 (14.3)	**.001**
viremia[7]	9/33 (27.3)	0/2	1/7 (14.3)	
outcome				
intrahospital death	29/101 (28.7)	9 (39.1)	1 (3.6)	**.008**
death related to IC	16/29 (55.2)	-	-	
death possibly related to IC	1/29 (3.4)	-	-	
death within 30 days after index culture	24/101 (23.8)	7 (30.4)	1 (3.6)	**.026**

Data are given as median (range) or no. (%). Characteristics were determined on day of index sampling, unless otherwise indicated. ICU = intensive care unit. CRP = C-reactive protein. PCT = procalcitonin. NIV = non-invasive ventilation. n.a. = not available/not performed. IC = invasive candidiasis. BDG = 1,3-beta-D Glucan.

*****Data only available for center 1 (Graz).

[1]number of positive BDG values (≥80 pg/mL) relative to number of BDG measurements

[2]norepinephrine, vasopressin

[3]propofol, benzodiazepine

[4]not available for candidemic patients from center 3 (Cologne)

[5]only for candidemic patients from center 3 (Cologne)

[6]Due to missing data, the complete SOFA Score could not be determined for most patients, therefore a partial SOFA Score was calculated including all variables available. “SOFA Score minimum” not available for candidemic patients from center 2 (Innsbruck) and 3 (Cologne).

[7]predominantly Herpes simplex virus and Cytomegalovirus viremia; performance of Virus-PCRs at the discretion of the attending physician

As shown in **Table 7**, 10 (9.9%) IC patients additionally had bacteremic episodes during the study period. In order to evaluate a potential influence of concomitant bacteremia on biomarker levels of patients with IC, further microbiological data were obtained (**Table 8**). Following clinical and microbiological evaluation, concomitant bacteremia of included IC patients from center 1 was classified as (most likely) bacterial contamination rather than true bacteremia. Due to limited clinical and microbiological data of patients from center 2 and 3, no reliable evaluation of clinical relevance of detected bacteremia was feasible for those patients.

Table 8. Characteristics of patients with invasive candidiasis and concomitant bacteremia.

Patient ID	Center	INVASIVE CANDIDIASIS			BACTEREMIA					
		Date of Index Sampling	Date of First Record	Definition	Date of BC Sampling	Date of BC Positivity	Venipuncture Site	No. of positive BC bottles	Bacterial genus/species	Evaluation
IC 38	1	22.09.16	26.09.16	candidemia	(1) 22.09.16 (2) 29.09.16	(1) 22.09.16 (2) 03.10.16	(1) central (2) n.d.	(1) n.d. (2) 1/6	(1) *S. epidermidis* (2) *S. capitis*	most likely contamination
IC 63	1	11.07.17	14.07.17	candidemia	11.07.17	12.07.17	peripheral	3/4	*S. hominis*	unclear*
IC 105	1	10.09.18	14.09.18	candidemia	10.09.18	12.09.18	peripheral	1/4	*S. haemolyticus*	most likely contamination
I3	2	02.04.17	04.04.17	candidemia	n.d.					
I16	2	16.02.18	17.02.18	candidemia						
I17	2	15.03.18	15.03.18	candidemia						
001-69358	3	12.08.15	13.08.15	candidemia	n.d.				*S. haemolyticus*	n.d.
001-59055	3	19.01.15	n.d.	candidemia					*S. haemolyticus*, *S. oralis*	
001-94840	3	19.02.16	20.02.15	candidemia					*Parvimonas micra*	
001-35437	3	14.02.15	17.02.15	candidemia					*E. faecalis*, *K. pneumoniae*	

BC = blood cultures. CNS = coagulase-negative staphylococci. n.d. = no data. *S.* = *Staphylococcus*. *E.* = *Enterococcus*. *K.* = *Klebsiella*.
*Growth of CNS solely in peripheral blood cultures, growth of *Candida* in simultaneously drawn central blood cultures. Except for central venous catheter, no indwelling foreign devices.

3.2 Cytokine measurements

3.2.1 Interleukin 17A

Figure 11 shows boxplots of IL-17A values of the five investigated study groups (IC (true), IC (other), *S. aureus* bacteremia, *E. coli* bacteremia, healthy controls) per time interval (116). Additionally, pairwise group comparisons between study groups for each time interval are given in **Table 9** (116).

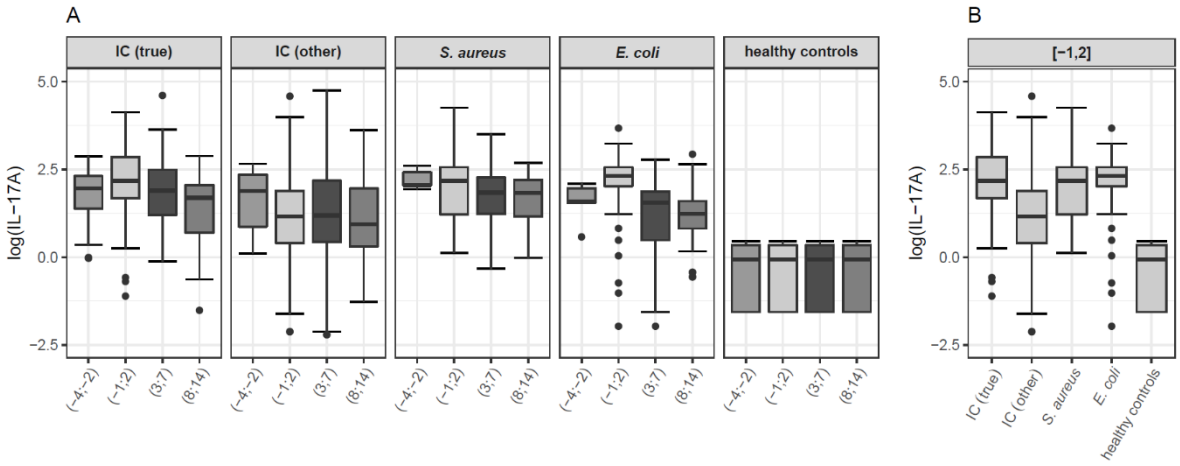

Figure 11. Boxplots of interleukin 17A (IL-17A) values of the investigated study groups for each time interval (A), and with focus on time interval (−1; 2) (B) for the total study population.

IL-17A values (pg/mL) are depicted on the logarithmic scale. In the boxplots, median values are depicted as bold line and the box spans from first to third quartile; whiskers extend to a maximum of 1.5 times the interquartile range (IQR, third minus first quartile) out from the respective box end; all remaining values are indicated as dots. Time intervals represent study days. Time interval (−1; 2) includes blood samples collected one day prior until up to two days after collection of the index sample. IC = invasive candidiasis. IC (true) = patients with true candidemia (*n* number of patients in boxplot B = 46, *n* number of samples in boxplot B = 97). IC (other) = patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC (*n* number of patients in boxplot B = 41, *n* number of samples in boxplot B = 81). *S. aureus* = patients with *S. aureus* bacteremia (*n* number of patients in boxplot B = 22, *n* number of samples in boxplot B = 45). *E. coli* = patients with *E. coli* bacteremia (*n* number of patients in boxplot B = 27, *n* number of samples in boxplot B = 48).

Table 9. Differences in interleukin 17A levels between study groups per time interval for the total study population.

Time interval (study days)	Contrast	Estimate (lower, upper CI)	*p*
(-4; -2)	IC (true) – IC (other)	0.39 (-0.22, 1.00)	.205
	IC (true) - *S. aureus*	-0.43 (-1.55, 0.68)	.436
	IC (true) - *E. coli*	0.38 (-0.46, 1.22)	.365
	IC (true) - healthy	2.30 (1.53, 3.07)	**<.001**
	IC (other) - *S. aureus*	-0.82 (-2.01, 0.37)	.169
	IC (other) - *E. coli*	-0.01 (-0.94, 0.92)	.986
	IC (other) - healthy	1.91 (1.05, 2.78)	**<.001**
	S. aureus - *E. coli*	0.81 (-0.51, 2.13)	.219
	S. aureus - healthy	2.73 (1.46, 4.01)	**<.001**
	E. coli - healthy	1.92 (0.88, 2.96)	**.001**
(-1; 2)	IC (true) - IC (other)	0.93 (0.49, 1.38)	**<.001**
	IC (true) - *S. aureus*	0.11 (-0.43, 0.64)	.694
	IC (true) - *E. coli*	-0.07 (-0.57, 0.43)	.785
	IC (true) - healthy	2.49 (1.46, 3.51)	**<.001**
	IC (other) - *S. aureus*	-0.83 (-1.37, -0.28)	**.003**
	IC (other) - *E. coli*	-1.00 (-1.52, -0.49)	**<.001**
	IC (other) - healthy	1.55 (0.52, 2.58)	**.003**
	S. aureus - *E. coli*	-0.18 (-0.77, 0.42)	.559
	S. aureus - healthy	2.38 (1.31, 3.45)	**<.001**
	E. coli - healthy	2.56 (1.50, 3.61)	**<.001**
(3; 7)	IC (true) - IC (other)	0.99 (0.52, 1.46)	**<.001**
	IC (true) - *S. aureus*	0.29 (-0.28, 0.86)	.317
	IC (true) - *E. coli*	0.75 (0.20, 1.30)	**.008**
	IC (true) - healthy	2.38 (1.34, 3.43)	**<.001**
	IC (other) - *S. aureus*	-0.70 (-1.28, -0.12)	**.019**
	IC (other) - *E. coli*	-0.24 (-0.80, 0.32)	.405
	IC (other) - healthy	1.39 (0.34, 2.44)	**.010**
	S. aureus - *E. coli*	0.46 (-0.18, 1.11)	.159
	S. aureus - healthy	2.09 (0.99, 3.19)	**<.001**
	E. coli - healthy	1.63 (0.54, 2.72)	**.004**
(8; 14)	IC (true) - IC (other)	0.33 (-0.27, 0.94)	.276
	IC (true) - *S. aureus*	-0.25 (-0.87, 0.37)	.425
	IC (true) - *E. coli*	-0.07 (-0.68, 0.54)	.821
	IC (true) - healthy	1.72 (0.76, 2.69)	**.001**
	IC (other) - *S. aureus*	-0.58 (-1.19, 0.03)	.061

IC (other) - *E. coli*	-0.40 (-1.01, 0.20)	.187
IC (other) - healthy	1.39 (0.43, 2.35)	**.005**
S. aureus - *E. coli*	0.18 (-0.44, 0.80)	.563
S. aureus - healthy	1.97 (1.01, 2.94)	**<.001**
E. coli - healthy	1.79 (0.83, 2.75)	**<.001**

Estimates are depicted on the logarithmic scale. CI = confidence interval. IC = invasive candidiasis. IC (true) = patients with true candidemia. IC (other) = patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC. *S. aureus* = patients with *S. aureus* bacteremia. *E. coli* = patients with *E. coli* bacteremia. healthy = healthy controls.

Descriptive statistics (mean, standard deviation, median, interquartile range) of IL-17A levels of the respective study groups for the defined time intervals are depicted in **Table 10** (116).

Table 10. Descriptive statistics of interleukin 17A levels.

Time interval	Study group	Mean	SD	Median	IQR
(-4; -2)	**IC (true)**	7.6	5.1	7.1	4.0-10.0
	IC (other)	6.7	4.6	6.6	2.4-10.6
	S. aureus	9.4	2.8	7.7	7.7-11.2
	E. coli	5.3	2.5	4.8	4.7-7.1
	healthy controls	0.9	0.6	0.9	0.2-1.4
(-1; 2)	**IC (true)**	11.9	9.9	8.8	5.4-17.3
	IC (other)	6.7	13.2	3.2	1.5-6.6
	S. aureus	11.6	13.1	8.8	3.4-13.0
	E. coli	10.8	7.1	10.1	7.5-13.0
	healthy controls	0.9	0.6	0.9	0.2-1.4
(3; 7)	**IC (true)**	10.3	12.2	6.6	3.3-12.0
	IC (other)	9.5	17.4	3.3	1.5-8.8
	S. aureus	6.8	4.9	6.3	3.4-9.7
	E. coli	4.7	3.4	4.7	1.6-6.5
	healthy controls	0.9	0.6	0.9	0.2-1.4
(8; 14)	**IC (true)**	5.6	4.2	5.4	2.0-7.8
	IC (other)	7.1	9.7	2.5	1.4-7.1
	S. aureus	6.3	3.3	6.2	3.2-9.1
	E. coli	4.6	4.1	3.4	2.3-4.9
	healthy controls	0.9	0.6	0.9	0.2-1.4

Values are given in pg/ml. Time intervals represent study days. SD = standard deviation. IQR = interquartile range. IC (true) = patients with true candidemia. IC (other) = patients with candidemia of unclear significance (positive blood cultures from

central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC. *S. aureus* = patients with *S. aureus* bacteremia. *E. coli* = patients with *E. coli* bacteremia.

Collectively, IL-17A values were significantly elevated in all patient groups (IC (true), IC (other), *S. aureus* bacteremia, *E. coli* bacteremia) compared to healthy controls for each of the defined time intervals. Further, IL-17A levels were significantly higher in patients with IC (true) compared to IC (other) for time intervals (−1; 2) and (3; 7) ($p < 0.001$ for both comparisons; see **Table 9** for details). Patients with IC (other) had lower IL-17A levels compared to patients with *S. aureus* bacteremia at time intervals (−1; 2) and (3; 7) ($p < 0.02$ for both comparisons), whereas there was no difference between IC (true) patients and *S. aureus* bacteremia for any of the investigated time intervals. At time interval (−1; 2), patients with IC (other) had significantly lower IL-17A levels compared to patients with *E. coli* bacteremia ($p < 0.001$). Regarding time interval (3; 7), true candidemic patients had higher IL-17A values compared to patients with *E. coli* bacteremia (p 0.008), whereas no significant differences could be determined for the other time intervals (see **Table 9** for details). Statistical analyses for the sensitivity population (i.e., calculations without immunosuppressed patients and patients with hematologic malignancies) did not lead to different findings (data not shown) (116).

Time-dependent courses of IL-17A levels within the study group IC (true) are depicted in **Table 11** (116). Briefly, IL-17A levels peaked around the date of index culture and decreased afterwards.

Table 11. Comparison of interleukin 17A levels between the different time intervals within the study group IC (true).

Comparison of time intervals	Estimate (lower, upper CI)	*p*
(-4; -2) vs. (-1; 2)	-0.50 (-0.74, -0.26)	**<.001**
(-4; -2) vs. (3; 7)	-0.45 (-0.71, -0.20)	**.001**
(-4; -2) vs. (8; 14)	-0.01 (-0.30, 0.28)	.961
(-1; 2) vs. (3; 7)	0.05 (-0.11, 0.21)	.553
(-1; 2) vs. (8; 14)	0.49 (0.28, 0.70)	**<.001**
(3; 7) vs. (8; 14)	0.44 (0.25, 0.63)	**<.001**

Estimates are depicted in logarithmic scale. Time intervals represent study days. IC = invasive candidiasis. IC (true) = patients with true candidemia. CI = confidence interval.

3.2.2 Other Cytokines (IL-6, IL-8, IL-10, IL-17F, IL-22, IL-23 (p19), IFN-γ, TNF-α, PTX3, TGF-β)

Cytokine levels of the different study groups around the date of index sampling (days −1; 2) are presented in **Figure 12** (116).

Table 12 compares values of above-mentioned cytokines other than IL-17A between all study groups at time interval (−1; 2) (116).

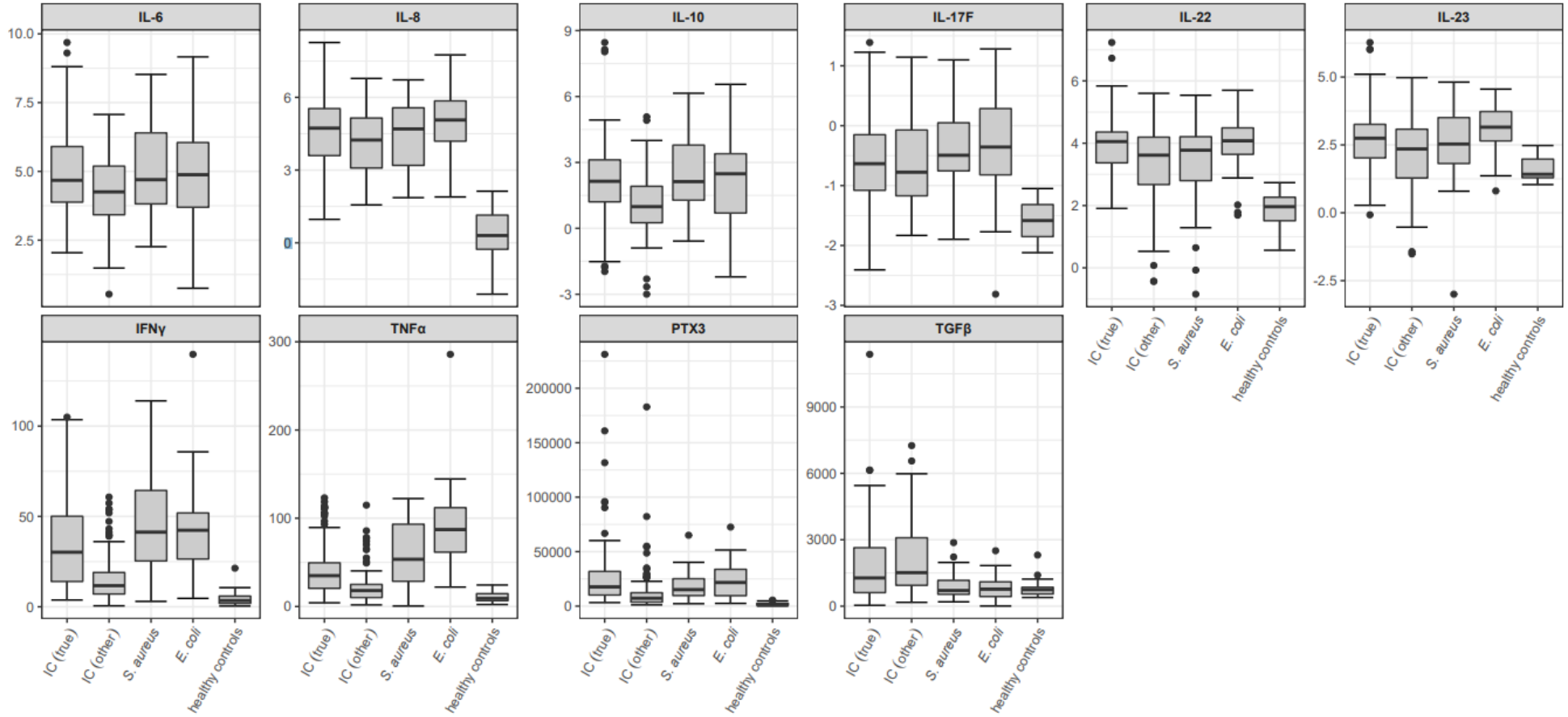

Figure 12. Boxplots of designated cytokine values (IL-6, IL-8, IL-10, IL-17F, IL-22, IL-23, IFN-γ, TNF-α, PTX3, TGF-β) for the total study population at time interval (−1; 2) relative to index sampling.

Values of interleukin parameters are depicted on the logarithmic scale; other values are given in pg/mL. In the boxplots, median values are depicted as bold line and the box spans from first to third quartile; whiskers extend to a maximum of 1.5 times the interquartile range (IQR, third minus first quartile) out from the respective box end; all remaining values are indicated as dots. IL = interleukin. IFN-γ = interferon-γ. PTX3 = Pentraxin-related protein 3. TNF-α = tumor necrosis factor-α. TGF-β = transforming growth factor-β. IC = invasive candidiasis. IC (true) = patients with true candidemia. IC (other) = patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC. *S. aureus* = patients with *S. aureus* bacteremia. *E. coli* = patients with *E. coli* bacteremia.

Table 12. Comparison of cytokine values other than interleukin 17A (IL-6, IL-8, IL-10, IL-17F, IL-22, IL-23, IFN-γ, TNF-α, PTX3, TGF-β) between the different study groups at time interval (−1; 2) (*p*-values).

Contrast	IL-6	IL-8	IL-10	IL-17F	IL-22	IL-23	IFN-ɣ	TNF-α	PTX3	TGF-β
IC (true) vs. IC (other)	.134	**.048** **IC (true) >**	**.025** **IC (true) >**	.991	**.019** **IC (true) >**	.064	**<.001** **IC (true) >**	**<.001** **IC (true) >**	**<.001** **IC (true) >**	.070
IC (true) vs. *S. aureus*	.520	.509	.359	.340	**.013** **IC (true) >**	.470	.107	.209	.561	.079
IC (true) vs. *E. coli*	.354	.241	.862	.698	.857	.090	.060	**<.001** ***E. coli* >**	.436	**.010** **IC (true) >**
IC (true) vs. healthy	n.d.	**<.001** **IC (true) >**	n.d.	0.109	**<.001** **IC (true) >**	.053	**<.001** **IC (true) >**	**<.001** **IC (true) >**	**<.001** **IC (true) >**	**.027** **IC (true) >**
IC (other) vs. *S. aureus*	.069	.335	**.008** ***S. aureus* >**	.413	.648	.379	**<.001** ***S. aureus* >**	**<.001** ***S. aureus* >**	**.012** ***S. aureus* >**	**.002** **IC (other) >**
IC (other) vs. *E. coli*	**.030** ***E. coli* >**	**.005** ***E. coli* >**	**.030** ***E. coli* >**	.748	**.022** ***E. coli* >**	**.001** ***E. coli* >**	**<.001** ***E. coli* >**	**<.001** ***E. coli* >**	**<.001** ***E. coli* >**	**<.001** **IC (other) >**
IC (other) vs. healthy	n.d.	**<.001** **IC (other) >**	n.d.	.123	**.002** **IC (other) >**	.330	**<.001** **IC (other) >**	**.001** **IC (other) >**	**<.001** **IC (other) >**	**<.001** **IC (other) >**
S. aureus* vs. *E. coli	.843	.114	.479	.556	**.014** ***E. coli* >**	**.035** ***E. coli* >**	.899	**.003** ***E. coli* >**	.239	.641
***S. aureus* vs. healthy**	n.d.	**<.001** ***S. aureus* >**	n.d.	.054	**.006** ***S. aureus* >**	.149	**<.001** ***S. aureus* >**	**<.001** ***S. aureus* >**	**<.001** ***S. aureus* >**	.869
***E. coli* vs. healthy**	n.d.	**<.001** ***E. coli* >**	n.d.	.086	**<.001** ***E. coli* >**	**.006** ***E. coli* >**	**<.001** ***E. coli* >**	**<.001** ***E. coli* >**	**<.001** ***E. coli* >**	.737

Statistical data analysis was performed for the total study population. Bold *p*-values indicate statistically significant differences with a *p*-value less than 0.05. IC = invasive candidiasis. IC (true) = patients with true candidemia. IC (other) = patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC. *S. aureus* = patients with *S. aureus* bacteremia. *E. coli* = patients with *E. coli* bacteremia. healthy = healthy controls. IL = interleukin. IFN-γ = interferon-γ. PTX3 = Pentraxin-related protein 3. TNF-α = tumor necrosis factor-α. TGF-β = transforming growth factor-β. n.d. = no data.

Interestingly, both patients with IC (true) and IC (other) showed significantly higher TGF-β levels compared to bacteremic patients, with the exception of IC (true) compared to *S. aureus* bacteremia. However, performance of the sensitivity analysis (i.e., total population minus patients with immunosuppressive therapy and hematologic malignancies), revealed significantly higher TGF-β levels for patients with IC (true) compared to bacteremic patients (both *S. aureus* and *E. coli*) for time intervals (−4; −2), (−1; 2) and (3; 7) (116).

3.2.3 Kynurenine and Tryptophan Concentrations

Figure 13 displays kynurenine and tryptophan values together with the respective kynurenine/tryptophan ratios for time interval (−1; 2) (total study population) (116).

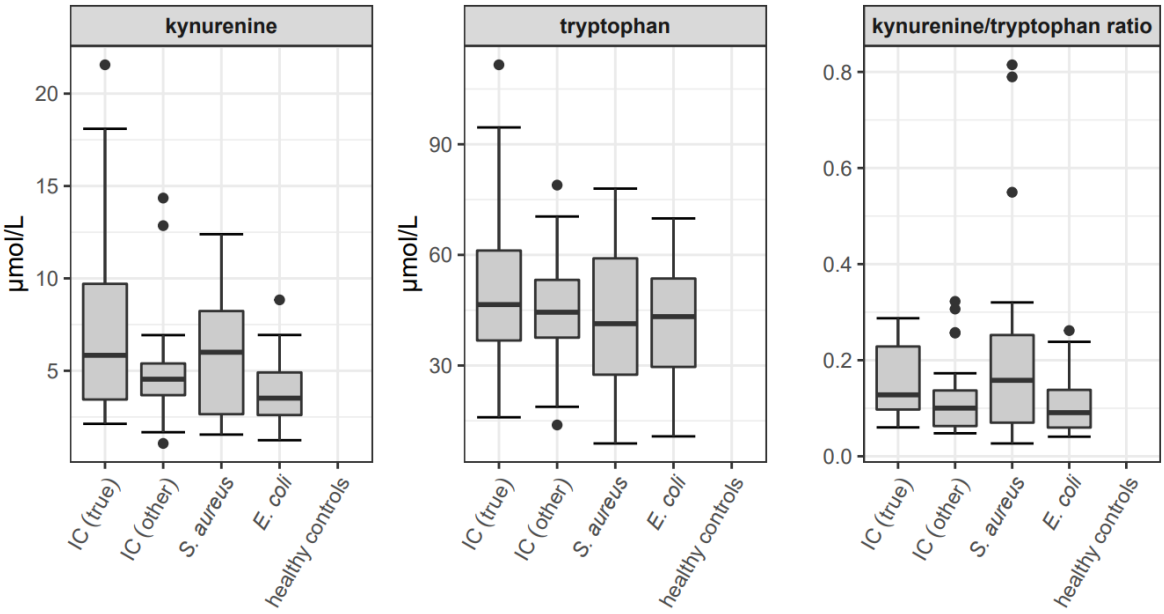

Figure 13. Boxplots of kynurenine and tryptophan concentrations as well as the kynurenine/tryptophan ratio for the total study population at time interval (−1;2) (relative to index sampling).

Except for the ratio, values are given in µmol/L. In the boxplots, median values are depicted as bold line and the box spans from first to third quartile; whiskers extend to a maximum of 1.5 times the interquartile range (IQR, third minus first quartile) out from the respective box end; all remaining values are indicated as dots. IC = invasive candidiasis. IC (true) = patients with true candidemia. IC (other) = patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC. *S. aureus* = patients with *S. aureus* bacteremia. *E. coli* = patients with *E. coli* bacteremia.

Interestingly, the only statistically significant difference was observed regarding the comparison of kynurenine levels between IC (true) patients (median 5.8, range 2.1–21.6

pg/mL) and patients with *E. coli* bacteremia (median 3.5, range 1.2–8.8 pg/mL), with significantly higher kynurenine levels in true candidemic patients (*p* 0.023) (116).

4 Discussion

Currently available diagnostic tests for invasive *Candida* infections either lack sensitivity or specificity or have long turn-around times (30,42,46). Since survival of patients with IC is determined by timely initiation of antifungal therapy (28,30,35,36,38–40), the development of valid and rapid diagnostic tests is urgently needed.

In our recent study (31), we detected significantly increased IL-17A values in candidemic patients compared to ICU patients with pneumonia, ICU patients with extrapulmonary infections, and healthy controls. The potential value of IL-17A as biomarker for discrimination between *Candida*-infected and non-infected patients was further highlighted in an clinical observational study by Decker et al. (69), in which patients with septic shock due to *Candida* spp. had significantly higher IL-17A levels than patients with septic shock and negative fungal cultures as well as patients with septic shock and *Candida* colonization.

Unlike previous studies, which performed cytokine measurements earliest from the time of clinical suspicion of infection (69,119,120), we aspired to obtain cytokine levels from earlier measurements, ideally even before index sampling. With this strategy, we aimed to assess IL-17A as well as other cytokines involved in *Candida* immune defense as possible biomarkers for early detection of invasive *Candida* infections. In fact, the analysis of cytokine values over a longer time period, in particular even before clinical suspicion of sepsis, is considered one of the strenghts of our study.

In sum, IL-17A values were significantly elevated in all patient groups (IC (true), IC (other), *S. aureus* bacteremia, *E. coli* bacteremia) compared to healthy controls for each defined time interval. Additionally, patients with IC (true) had significantly higher IL-17A levels than patients with IC (other) at time intervals (−1; 2) and (3; 7). Comparisons of IL-17A levels between patients with IC (true) and *S. aureus* bacteremia did not show any statistically significant differences for each investigated study interval. Except for study interval (3; 7) (IC (true) > *E. coli*), IL-17A levels did not significantly differ between patients with IC (true) and *E. coli* bacteremia. Referring to these observations, the comparison of IL-17A values between patients with candidemia and bacteremia did not reveal a discriminative competence of IL-17A (116). Interestingly, calculations without immunosuppressed patients and patients with hematologic malignancies (i.e., sensitivity analysis) yielded identical differences of IL-17A levels between the different study groups for each time interval (116).

These results did not confirm those from our previous study (31), which also comprised patients with either candidemia or bacterial infections, however only a few patients were bacteremic at the time of sample collection. Actually, concomitant bacteremia occurred in solely 3/20 patients with extrapulmonary infection and 3/24 patients with pneumonia (31). In contrast, only patients with bacteremia (*S. aureus*, *E. coli*) were used for data comparison in our current study (116).

In a study from Li et al. (119), IL-17A levels were significantly elevated in patients with candidemia compared to patients with gram-positive bacteremia, while, as also shown by our data, no significant differences could be observed for the comparison of patients with candidemia vs. gram-negative bacteremia. Contrary to this, data from Akin et al. (120) revealed significantly increased IL-17 values in patients with candidemia compared to bacterial sepsis irrespective of the underlying organism.
In fact, certain (primarily gram-negative) bacteria such as *Klebsiella pneumonia, E. coli*, *Citrobacter rodentium*, *Salmonella enteritidis, Bacteroides* spp., Borrelia *burgdorferi, Cutibacterium* acnes, and *Mycobacterium tuberculosis* are known to stimulate Th17 responses. However, multiple studies identified *C. albicans* as the by far strongest inducer of IL-17 production (73,105). *Aspergillus fumigatus*, on the other hand, mainly promotes Th1 responses rather than IL-17, and is therefore considered a weak inducer of IL-17 (129). In a study by Akin et al. (120), the highest IL-17 levels were detected in patients with polymicrobial sepsis (i.e., *Candida* and bacterial growth in blood cultures), though sample size was rather small (n = 11). Equivalent to our study, patients with candidemia had a broad range of IL-17 values (minimum 0, maximum 611.6 pg/mL) compared to a considerably smaller range for patients with bacteremia (minimum 0, maximum 103.8 pg/mL). However, cytokine measurements for the respective study groups were performed at different time points. In detail, index sampling and sample collection for cytokine measurements were simultaneously performed for bacteremic patients, whereas serum samples were obtained three days (median) after index sampling for candidemic patients (120). Li et al. (119) conducted blood sample collection in a comparable manner for patients with *Candida* and bacterial BSI, though only samples within 48 hours after hospital admission were included.

Overall, most of the measured cytokines were significantly higher in patients with IC (true) compared to IC (other). As mentioned before, some of the candidemic patients showed *Candida* growth in central blood cultures only. Since these cases could not be reliably classified as catheter-related BSIs, due to e.g., missing peripheral blood cultures or missing AOLC (acridine orange leucocyte cytospin) tests, those patients had to be considered as

“IC (other)”. Accordingly, the study group IC (other) involved patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC (116).

Longitudinal evaluation of IL-17A values within the study group IC (true) revealed significantly lower IL-17A levels in the earliest time frame (−4; −2) (i.e., 4 to 2 days before index sampling) compared to the time interval around the day of index sampling (−1; 2); and compared to the time interval after index sampling (3; 7), respectively. However, comparative data analysis showed no significant differences between the earliest (−4; −2) and the latest (8; 14) investigated time intervals; and for time intervals (−1; 2) and (3; 7). IL-17A levels declined in later time intervals. In line with this, IL-17A levels were lower in the latest study interval (8; 14) in comparison to (−1; 2); and (3; 7), respectively. However, it has to be mentioned that the number of samples significantly differed among the investigated time intervals. The highest number of samples was available for time intervals (3; 7) (n = 112) and (−1; 2) (n = 97), respectively, whereas significantly fewer samples were available for early (−4; −2) and late (8; 14) study days (n = 31, and n = 55, respectively). Previous data from Van de Veerdonk et al. (105) showed a steadily increasing IL-17 secretion of human PBMCs after stimulation with *C. albicans* over a period of 7 days. Evaluation of kinetics of the other investigated cytokines revealed maximum IL-23 secretion within the first 24 hours; maximum IL-10 production in the first 48 hours, afterwards decrease to undetectable values at day 3; presence of IFN-γ from 48 hours, reaching its maximum at day 4 to 5 (105).

Especially the comparison of TGF-β levels between patients with invasive *Candida* infections and bacteremia showed notable results. TGF-β is a cytokine of both the innate and adaptive immune system, which is produced by almost all cell types including leukocytes. Interestingly, TGF-β primarily suppresses immune responses, however, when stimulated with IL-1 or IL-6, it promotes the differentiation of Th17 cells (120,130–133). In a study from Letterio et al. (134), it was shown that TGF-β is an crucial component of immune response to invasive *Candida* infections. Liver biopsy specimens from patients with chronic disseminated candidiasis (CDC) showed local production of TGF-β within inflammatory granulomas and surrounding hepatocytes. Also, co-culture of human PBMCs with *C. albicans* resulted in significantly increased TGF-β production. Since TGF-β has inhibitory effects on phagocytic response, the release of active TGF-β in immunocompromised patients with CDC is associated with suppressed cell-mediated immunity (134). Letterio et al. (134) inferred that TGF-β production is at least a consequence of invasive *Candida*

infection and could further contribute to disease progression in immunocompromised patients.

In our study, TGF-β values were significantly increased in patients with IC (other) compared to patients with bacteremia for time frames (−1; 2), (3; 7) and (8; 14). Contrary, patients with IC (true) only had significantly higher TGF-β values when compared to patients with *E. coli* bacteremia [for study intervals (−4; −2), (−1; 2) and (3; 7)], but not *S. aureus* bacteremia. Interestingly, exclusion of immunosuppressed patients and patients with hematologic malignancies (i.e., sensitivity analysis) led to significantly higher TGF-β levels in patients with true candidemia compared to bacteremia for time intervals (−4; −2), (−1; 2) and (3; 7) (116). This finding is consistent with previous data demonstrating significantly increased TGF-β values in patients with candidemia compared to bacteremia and healthy controls at unspecified time points (120).

C. albicans is able to downregulate immune response via modulation of tryptophan/kynurenine metabolic pathways in order to allow fungal colonization (31,32,75). Several studies have reported a crucial role for the tryptophan/kynurenine pathway in the induction of protective tolerance. In a study from Bozza et al. (107), IDO inhibition was associated with exaggerated inflammatory response and detrimental inflammation. Based on these results, Cheng et al. (32) presumed that modulation of the tryptophan metabolism might contribute to the IL-17-diminishing effect of live *C. albicans*. Consequently, Cheng and colleagues (32) conducted a study in which they stimulated PBMCs with heat-killed and/or live *C. albicans*. In order to avoid outgrowth of *C. albicans* accompanied by increasing cell death, a transwell system was applied. The transwell system prevented direct interaction between live *C. albicans* and PBMCs while still allowing free diffusion of the secreted soluble factors. For assessment of the impact of tryptophan metabolites on IL-17 secretion, PBMCs were stimulated with different concentrations of tryptophan, 5-hydroxytryptophan and kynurenine. The stimulation of PBMCs with heat-killed *C. albicans* resulted in upregulated IDO expression concomitant with significantly increased kynurenine levels, whereas 5-hydroxytryptophan was non-detectable. Exactly the opposite was detected (kynurenine ↓, 5-hydroxytryptophan ↑) when PBMCs were stimulated with live *C. albicans* or both live and heat-killed *C. albicans* (32).

Interestingly, live *C. albicans* by itself only induces marginal IDO activation, whereas live *C. albicans* in co-culture with heat-killed *C. albicans* can actually downregulate IDO expression by up to 67% (32). Via blockade of IDO expression, live *C. albicans* actively shifts tryptophan metabolism away from kynurenines and toward 5-hydroxytryptophan metabolites (75). Increased presence of 5-hydroxytryptophan, however, subsequently inhibits host IL-17 production (31,32,75).

Since IFN-γ is necessary for induction of IDO expression, Cheng et al. (32) additionally measured IFN-γ production in the co-culture of heat-killed and live *C. albicans*. As expected, live *C. albicans* significantly reduced IFN-γ secretion by heat-killed *C. albicans*. This finding further contributes to the assumption that live *C. albicans* is able to actively manipulate the tryptophan pathways in order to obtain immunomodulatory effects on host defense (32). Unlike IDO, the expression of tryptophan hydroxylase, which hydroxlates tryptophan to hydroxytryptophan, is not manipulated by either heat-killed or live *C. albicans* and is therefore not involved in interaction of *Candida* and tryptophan metabolism (32).

Based on these in vitro data, we inferred increased values of both IL-17A and kynurenine in patients with invasive *Candida* infections. Consistently, candidemic patients had significantly elevated IL-17A and kynurenine values compared to non-candidemic patients in our previous study (31). In our present study, however, we only observed higher kynurenine levels for candidemic patients compared to *E. coli* bacteremia around the time of index sampling (−1; 2). Since kynurenine and tryptophan values were exclusively available for patient groups (IC, *S. aureus*, *E. coli*) in our present study, no comparative calculations involving healthy controls could be performed. As kynurenine is a metabolite of the essential amino acid tryptophan, we calculated kynurenine/tryptophan ratios to account for possible dietary influences of tryptophan levels (135). The kynurenine/tryptophan calculations revealed no additional significant differences.
Previous data from Huttunen et al. (136) showed three times higher maximum kynurenine/tryptophan ratios in bacteremic patients (*S. aureus*, *Streptococcus pneumoniae*, β-hemolytic streptococcae, *E. coli*) compared to healthy Finnish blood donors. Following this, elevated kynurenine values as a result of high IDO activity may not only be specific for invasive *Candida* infections, yet may be associated with serious infections in general.

4.1 Limitations

Our study has some limitations. In fact, we aimed to constitute clearly defined study groups and a well-defined control group to guarantee comparability of results. Concerning age, however, patients of the study groups (IC, *S. aureus*, *E. coli*) were significantly older than healthy controls. This fact is mainly due to the circumstance that age is a major risk factor for both invasive *Candida* as well as bacterial infections (14,19,137).

Another limiting factor affects the classification of patients with invasive *Candida* infections.

The wide range of observed IL-17A levels in our study, including several outliers as well as many comparatively low IL-17A values, may, at least partially, correspond to the heterogeneity of patient groups with invasive *Candida* infections (i.e., IC (true), IC (other)). As described earlier, definite candidemia cases were characterized as IC (true), whereas the patient group IC (other) included patients with candidemia of unclear significance (positive blood cultures from central venous catheters only), proven IC other than candidemia, and patients with probable and possible IC. Whereas the detection of *Candida* spp. from (peripheral) blood cultures unambigously defines proven IC, definition criteria for both proven and probable IC also include less clear requirements such as detection of *Candida* spp. from a normally sterile site or from an adequate intra-abdominal specimen (66). According to the afore-mentioned EORTC criteria (66), patients with e.g., a single detection of *Candida* spp. from a normally sterile site were defined as proven IC, once a clinical or radiological hint indicating an infectious disease process was present. However, the significance of *Candida* spp. recovery was inconclusive for some of our study patients as several patients with probable and even proven IC fully improved without any antifungal therapy.

Further, we had to change exclusion criteria during patient recruitment. As mentioned before, we initially intended not to include immunocompromised patients as well as patients with active hematooncological diseases in order to prevent a possible influence on investigated cytokine levels. However, during patient recruitment we identified a large number of patients with IC and concomitant immunosuppressive therapy or malignant diseases. Also, recently revised and updated EORTC/MSG definitions (66) actually include the presence of immunosuppression in the definition of probable IC. Consequently, we determined to additionally enroll immunocompromised patients and patients with hematologic malignancies and to perform subgroup analysis.

One limiting factor potentially complicating interpretation of cytokine values is the presence of concomitant bacteremia in patients with IC. Most of the patients with IC have long durations of hospital stay (median 16 days in our study) leading to an increased risk for nosocomial BSIs. To overcome any confounding effects, further clinical and microbiological data were obtained in order to investigate the actual clinical significance of detected bacteremia (data only available for center 1; for further details see **Table 8**). Of the three patients with candidemia and concomitant bacteremia, contamination was most likely in at least two patients (growth of CNS in 1/4 and 1/6 peripheral blood culture bottles, respectively).

Another pitfall in determination of blood inflammatory markers affects blood sample handling and storage. Ideally, blood samples should be centrifuged immediately after venipuncture and subsequently frozen, since it has been shown that plasma IL-17 concentrations substantially increase already within a storage time of 4 hours at 4 °C (138). As opposed to this, in a study from Kenis et. al (126), which investigated the stability of several inflammatory markers in serum samples, it was found that IL-6 and IL-10 concentrations remained stable at 4°C over at least 21 days.
In our study setting, however, immediate centrifugation of obtained blood samples was not feasible, since we analysed retained blood samples from routine laboratory investigations collected from our collaborating laboratory. Although blood samples were centrifuged right before they were stored at 4°C, several hours may have passed from venipuncture to arrival of some of the blood samples at the laboratory unit.

4.2 Conclusion and Outlook

We did not detect a discriminative competence of both IL-17A and kynurenine for differentiation between *Candida* and bacterial BSI in this study. Accordingly, IL-17A may be valuable as a biomarker for either invasive *Candida* infections or bacterial blood stream infections rather than solely for IC. Besides, we identified significantly increased TGF-β levels in patients with invasive *Candida* infections compared to bacteremic patients, proposing a possible significance of TGF-β for differentiation between bacterial and *Candida* infections. However, larger studies are necessary to further investigate this finding.

Bibliography

1. Edwards JE. Candida Species. In: Principles and Practice of Infectious Diseases. 7th ed. Philadelphia: Elsevier Churchill Livingstone; 2010. p. 3225–40.
2. Kumamoto CA, Vinces MD. ALTERNATIVE CANDIDA ALBICANS LIFESTYLES: Growth on Surfaces . Annu Rev Microbiol. 2005 Oct;59(1):113–33.
3. Langenbeck B. Auffingung von Pilzen aus der Schleimhaut der Speiserohre einer Typhus-Leiche. Neue Not Geb Natur Heilk. 1839;12:145–7.
4. Rippon J. Candidiasis and the pathogenic yeasts. In: Medical Mycology, The Pathogenic Fungi and the Pathogenic Actinomycetes. Philadelphia: WB Saunders; 1988. p. 532–81.
5. Pitarch A, Nombela C, Gil C. Diagnosis of Invasive Candidiasis: From Gold Standard Methods to Promising Leading-edge Technologies. Curr Top Med Chem. 2018 Nov 26;18(16):1375–92.
6. Hickman MA, Zeng G, Forche A, Hirakawa MP, Abbey D, Harrison BD, et al. The “obligate diploid” Candida albicans forms mating-competent haploids. Nature. 2013 Feb 7;494(7435):55–9.
7. Eggimann P, Garbino J, Pittet D. Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. Lancet Infect Dis. 2003 Dec;3(11):685–702.
8. Pendleton KM, Huffnagle GB, Dickson RP. The significance of Candida in the human respiratory tract: Our evolving understanding. Vol. 75, Pathogens and Disease. Oxford University Press; 2017.
9. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Vol. 20, Clinical Microbiology Reviews. 2007. p. 133–63.
10. Yapar N. Epidemiology and risk factors for invasive candidiasis. Vol. 10, Therapeutics and Clinical Risk Management. 2014. p. 95–105.
11. Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. Candida auris sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiol Immunol. 2009 Jan;53(1):41–4.
12. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Rev Dis Prim. 2018 May 11;4.
13. Chowdhary A, Sharma C, Meis JF. Candida auris: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog. 2017;13(5):e1006290.
14. Kullberg BJ, Arendrup MC. Invasive candidiasis. Vol. 373, New England Journal of

Medicine. Massachussetts Medical Society; 2015. p. 1445–56.
15. Ostrosky-Zeichner L, Al-Obaidi M. Invasive Fungal Infections in the Intensive Care Unit. Infect Dis Clin North Am. 2017 Sep;31(3):475–87.
16. McCarty TP, Pappas PG. Invasive Candidiasis. Vol. 30, Infectious Disease Clinics of North America. W.B. Saunders; 2016. p. 103–24.
17. Gorbach SL, Nahas L, Lerner PI, Weinstein L. Studies of intestinal microflora. I. Effects of diet, age, and periodic sampling on numbers of fecal microorganisms in man. Gastroenterology. 1967 Dec;53(6):845–55.
18. Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of Candida species to plastic surfaces. Infect Immun. 1985 Oct;50(1):97–101.
19. Arendrup MC. Epidemiology of invasive candidiasis. Vol. 16, Current Opinion in Critical Care. 2010. p. 445–52.
20. Méan M, Marchetti O, Calandra T. Bench-to-bedside review: Candida infections in the intensive care unit. Vol. 12, Critical Care. 2008.
21. Eggimann P, Calandra T, Fluckiger U, Bille J, Garbino J, Glauser MP, et al. Invasive candidiasis: Comparison of management choices by infectious disease and critical care specialists. Intensive Care Med. 2005 Nov;31(11):1514–21.
22. Jordà-Marcos R, Álvarez-Lerma F, Jurado M, Palomar M, Nolla-Salas J, León MA, et al. Risk factors for candidaemia in critically ill patients: A prospective surveillance study. Mycoses. 2007 Jul;50(4):302–10.
23. Caggiano G, Puntillo F, Coretti C, Giglio M, Alicino I, Manca F, et al. Candida colonization index in patients admitted to an ICU. Int J Mol Sci. 2011 Oct;12(10):7038–47.
24. Vincent J-L, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International Study of the Prevalence and Outcomes of Infection in Intensive Care Units. JAMA. 2009 Dec 2;302(21):2323.
25. Krause R, Moissl-Eichinger C, Halwachs B, Gorkiewicz G, Berg G, Valentin T, et al. Mycobiome in the lower respiratory tract - a clinical perspective. Vol. 7, Frontiers in Microbiology. Frontiers Media S.A.; 2017.
26. Nucci M, Anaissie E. Revisiting the source of candidemia: skin or gut? Clin Infect Dis. 2001 Dec 15;33(12):1959–67.
27. Bassetti M, Righi E, Ansaldi F, Merelli M, Cecilia T, De Pascale G, et al. A multicenter study of septic shock due to candidemia: outcomes and predictors of mortality. Intensive Care Med. 2014 Jun 8;40(6):839–45.
28. Falagas ME, Apostolou KE, Pappas VD. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. Eur J Clin Microbiol Infect Dis. 2006 Jul 14;25(7):419–25.

29. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis. 2006 Jul 1;43(1):25–31.
30. Clancy CJ, Nguyen MH. Finding the missing 50% of invasive candidiasis: How nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013 May;56(9):1284–92.
31. Krause R, Zollner-Schwetz I, Salzer HJF, Valentin T, Rabensteiner J, Prüller F, et al. 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. J Infect Dis. 2015 Feb 1;211(3):445–51.
32. Cheng S-C, van de Veerdonk F, Smeekens S, Joosten LAB, van der Meer JWM, Kullberg B-J, et al. Candida albicans dampens host defense by downregulating IL-17 production. J Immunol. 2010 Aug 15;185(4):2450–7.
33. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest. 2000;118(1):146–55.
34. 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 Sep 1;49(9):3640–5.
35. Knitsch W, Vincent J-L, Utzolino S, François B, Dinya T, Dimopoulos G, et al. A randomized, placebo-controlled trial of preemptive antifungal therapy for the prevention of invasive candidiasis following gastrointestinal surgery for intra-abdominal infections. Clin Infect Dis. 2015 Dec 1;61(11):1671–8.
36. León C, Ruiz-Santana S, Saavedra P, Galván B, Blanco A, Castro C, 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 May;37(5).
37. Mikulska M, Calandra T, Sanguinetti M, Poulain D, Viscoli C, Third European Conference on Infections in Leukemia Group. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. Crit Care. 2010;14(6):R222.
38. Cuenca-Estrella M, Verweij PE, Arendrup MC, Arikan-Akdagli S, Bille J, Donnelly JP, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. Clin Microbiol Infect. 2012 Dec;18 Suppl 7:9–18.
39. Reiss E, Obayashi T, Orle K, Yoshida M, Zancopé-Oliveira RM. Non-culture based

diagnostic tests for mycotic infections. Med Mycol. 2000;38 Suppl 1:147–59.

40. Bruyère R, Quenot J-P, Prin S, Dalle F, Vigneron C, Aho S, et al. Empirical antifungal therapy with an echinocandin in critically-ill patients: prospective evaluation of a pragmatic Candida score-based strategy in one medical ICU. BMC Infect Dis. 2014 Jul 11;14:385.
41. Nguyen MH, Wissel MC, Shields RK, Salomoni MA, Hao B, Press EG, et al. Performance of Candida real-time polymerase chain reaction, β-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. Clin Infect Dis. 2012 May;54(9):1240–8.
42. Clancy CJ, Nguyen MH. Diagnosing Invasive Candidiasis. Kraft CS, editor. J Clin Microbiol. 2018 Feb 14;56(5):e01909-17.
43. Ostrosky-Zeichner L, Shoham S, Vazquez J, Reboli A, Betts R, Barron MA, et al. MSG-01: A Randomized, Double-Blind, Placebo-Controlled Trial of Caspofungin Prophylaxis Followed by Preemptive Therapy for Invasive Candidiasis in High-Risk Adults in the Critical Care Setting. Clin Infect Dis. 2014 May 1;58(9):1219–26.
44. Montravers P, Dupont H, Eggimann P. Intra-abdominal candidiasis: the guidelines-forgotten non-candidemic invasive candidiasis. Intensive Care Med. 2013 Dec;39(12):2226–30.
45. Leroy O, Gangneux J-P, Montravers P, Mira J-P, Gouin F, Sollet J-P, et al. Epidemiology, management, and risk factors for death of invasive Candida infections in critical care: a multicenter, prospective, observational study in France (2005-2006). Crit Care Med. 2009 May;37(5):1612–8.
46. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, et al. T2 Magnetic Resonance Assay for the Rapid Diagnosis of Candidemia in Whole Blood: A Clinical Trial. Clin Infect Dis. 2015 Mar 15;60(6):892–9.
47. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) C. Clin Infect Dis. 2008;46(12):1813–21.
48. Eggimann P, Pittet D. Candida colonization index and subsequent infection in critically ill surgical patients: 20 years later. Intensive Care Med. 2014 Sep 26;40(10):1429–48.
49. León C, Ruiz-Santana S, Saavedra P, Almirante B, Nolla-Salas J, Álvarez-Lerma F, et al. A bedside scoring system (“Candida score”) for early antifungal treatment in nonneutropenic critically ill patients with Candida colonization. Crit Care Med. 2006 Mar;34(3):730–7.

50. Wei S, Wu T, Wu Y, Ming D, Zhu X. Diagnostic accuracy of Candida albicans germ tube antibody for invasive candidiasis: systematic review and meta-analysis. Diagn Microbiol Infect Dis. 2019 Apr 1;93(4):339–45.
51. Martínez-Jiménez MC, Muñoz P, Guinea J, Valerio M, Alonso R, Escribano P, et al. Potential role of Candida albicans germ tube antibody in the diagnosis of deep-seated candidemia. Med Mycol. 2014 Apr;52(3):270–5.
52. Mennink-Kersten MASH, Verweij PE. Non-culture-based diagnostics for opportunistic fungi. Infect Dis Clin North Am. 2006 Sep;20(3):711–27, viii.
53. Obayashi T, Kawai T, Yoshida M, Mori T, Goto H, Yasuoka A, et al. Plasma (1→3)-β-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet. 1995 Jan 7;345(8941):17–20.
54. Posteraro B, De Pascale G, Tumbarello M, Torelli R, Pennisi M, Bello G, 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.
55. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. β-D-glucan assay for the diagnosis of invasive fungal infections: A meta-analysis. Clin Infect Dis. 2011 Mar 15;52(6):750–70.
56. Wright WF, Overman SB, Ribes JA. (1-3)-β-D-Glucan Assay: A Review of its Laboratory and Clinical Application. Lab Med. 2011;42(11):679–85.
57. Mercier T, Guldentops E, Patteet S, Beuselinck K, Lagrou K, Maertens J. Beta-D-glucan for diagnosing Pneumocystis pneumonia: A direct comparison between the Wako -glucan assay and the fungitell assay. J Clin Microbiol. 2019 Jun 1;57(6).
58. Dichtl K, Seybold U, Wagenera J. Evaluation of a turbidimetric β-D-glucan test for detection of pneumocystis jirovecii pneumonia. J Clin Microbiol. 2018 Jul 1;56(7).
59. Obayashi T, Sugimoto A, Takigawa C, Sekiya N, Nagasawa J, Ozaki Y. Validation of a new (1→ 3)-β-D-glucan test kit, fungitec G test MKⅡ “Nissui.” Japanese J Med Mycol. 2015;56(2):J73–9.
60. Miyazaki T, Kohno S, Mitsutake K, Maesaki S, Tanaka KI, Ishikawa N, et al. Plasma (1→3)-β-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. J Clin Microbiol. 1995;33(12):3115–8.
61. Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge RJ, et al. -D-Glucan as a Diagnostic Adjunct for Invasive Fungal Infections: Validation, Cutoff Development, and Performance in Patients with Acute Myelogenous Leukemia and Myelodysplastic Syndrome. Clin Infect Dis. 2004 Jul 15;39(2):199–205.
62. Prüller F, Wagner J, Raggam RB, Hoenigl M, Kessler HH, Truschnig-Wilders M, et al. Automation of serum (1→3)-beta-D-glucan testing allows reliable and rapid

discrimination of patients with and without candidemia. Med Mycol. 2014 Jul;52(5):455–61.

63. Szyszkowitz A, Zurl C, Herzeg A, Berger A, Gemes G, Mitteregger M, et al. Serum 1,3-beta-D-glucan values during and after laparoscopic and open intestinal surgery. Open Forum Infect Dis. 2018 Dec;5(12):ofy296.
64. Prattes J, Schneditz D, Prüller F, Jaindl E, Sauseng N, Hoenigl M, et al. 1,3-ß-d-Glucan testing is highly specific in patients undergoing dialysis treatment. J Infect. 2017;74(1):72–80.
65. Hanson KE, Pfeiffer CD, Lease ED, Balch AH, Zaas AK, Perfect JR, et al. β-D-glucan surveillance with preemptive anidulafungin for invasive candidiasis in intensive care unit patients: a randomized pilot study. PLoS One. 2012;7(8):e42282.
66. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis. 2019 Dec 5;
67. Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: Systematic review and meta-analysis. J Clin Microbiol. 2011 Feb;49(2):665–70.
68. Zurl C, Prattes J, Zollner-Schwetz I, Valentin T, Rabensteiner J, Wunsch S, et al. T2Candida magnetic resonance in patients with invasive candidiasis: Strengths and limitations. Med Mycol. 2019 Oct 15;
69. Decker S, Sigl A, Grumaz C, Stevens P, Vainshtein Y, Zimmermann S, et al. Immune-Response Patterns and Next Generation Sequencing Diagnostics for the Detection of Mycoses in Patients with Septic Shock—Results of a Combined Clinical and Experimental Investigation. Int J Mol Sci. 2017 Aug 18;18(8):1796.
70. Romani L. Immunity to fungal infections. Nat Rev Immunol. 2011 Apr;11(4):275–88.
71. Borghi M, Renga G, Puccetti M, Oikonomou V, Palmieri M, Galosi C, et al. Antifungal Th Immunity: Growing up in Family. Front Immunol. 2014 Oct 15;5:506.
72. Cheng S-C, van de Veerdonk FL, Lenardon M, Stoffels M, Plantinga T, Smeekens S, et al. The dectin-1/inflammasome pathway is responsible for the induction of protective T-helper 17 responses that discriminate between yeasts and hyphae of Candida albicans. J Leukoc Biol. 2011 Aug;90(2):357–66.
73. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. N Engl J Med. 2009 Aug 27;361(9):888–98.
74. Dambuza IM, Levitz SM, Netea MG, Brown GD. Fungal Recognition and Host Defense Mechanisms. Microbiol Spectr. 2017 Jul 14;5(4).
75. Cheng S-C, Joosten LAB, Kullberg B-J, Netea MG. Interplay between Candida albicans and the mammalian innate host defense. Infect Immun. 2012

Apr;80(4):1304–13.

76. Netea MG, Joosten LAB, Van Der Meer JWM, Kullberg BJ, Van De Veerdonk FL. Immune defence against Candida fungal infections. Vol. 15, Nature Reviews Immunology. Nature Publishing Group; 2015. p. 630–42.
77. Netea MG, Brown GD, Kullberg BJ, Gow NAR. An integrated model of the recognition of Candida albicans by the innate immune system. Nat Rev Microbiol. 2008 Jan;6(1):67–78.
78. Hernández-Santos N, Gaffen SL, Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, et al. Th17 cells in immunity to Candida albicans. Cell Host Microbe. 2012 May 17;11(5):425–35.
79. Ferwerda G, Meyer-Wentrup F, Kullberg B-J, Netea MG, Adema GJ. Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. Cell Microbiol. 2008 Oct;10(10):2058–66.
80. Gow NAR, Netea MG, Munro CA, Ferwerda G, Bates S, Mora-Montes HM, et al. Immune Recognition of Candida albicans β-glucan by Dectin-1. J Infect Dis. 2007 Nov 15;196(10):1565–71.
81. Qian Q, Jutila MA, Van Rooijen N, Cutler JE. Elimination of mouse splenic macrophages correlates with increased susceptibility to experimental disseminated candidiasis. J Immunol. 1994 May 15;152(10):5000–8.
82. Urban CF, Reichard U, Brinkmann V, Zychlinsky A. Neutrophil extracellular traps capture and kill Candida albicans and hyphal forms. Cell Microbiol. 2006 Apr;8(4):668–76.
83. Schmidt S, Tramsen L, Lehrnbecher T. Natural killer cells in antifungal immunity. Vol. 8, Frontiers in Immunology. Frontiers Media S.A.; 2017.
84. Lam VC, Lanier LL. NK cells in host responses to viral infections. Curr Opin Immunol. 2017 Feb;44:43–51.
85. Kielczewska A, Kim H-S, Lanier LL, Dimasi N, Vidal SM. Critical residues at the Ly49 natural killer receptor's homodimer interface determine functional recognition of m157, a mouse cytomegalovirus MHC class I-like protein. J Immunol. 2007 Jan 1;178(1):369–77.
86. Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. Science (80-). 2002 May 17;296(5571):1323–6.
87. Bozza S, Gaziano R, Spreca A, Bacci A, Montagnoli C, di Francesco P, et al. Dendritic Cells Transport Conidia and Hyphae of Aspergillus fumigatus from the Airways to the Draining Lymph Nodes and Initiate Disparate Th Responses to the Fungus . J Immunol. 2002 Feb 1;168(3):1362–71.

88. Netea MG, Vonk AG, van den Hoven M, Verschueren I, Joosten LA, van Krieken JH, et al. Differential role of IL-18 and IL-12 in the host defense against disseminated Candida albicans infection. Eur J Immunol. 2003 Dec;33(12):3409–17.
89. Delsing CE, Gresnigt MS, Leentjens J, Preijers F, Frager FA, Kox M, et al. Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series. BMC Infect Dis. 2014 Mar 26;14(1):166.
90. Mencacci A, Perruccio K, Bacci A, Cenci E, Benedetti R, Martelli MF, et al. Defective antifungal T-helper 1 (TH1) immunity in a murine model of allogeneic T-cell-depleted bone marrow transplantation and its restoration by treatment with TH2 cytokine antagonists. Blood. 2001 Mar 1;97(5):1483–90.
91. Mencacci A, Del Sero G, Cenci E, D'Ostiani CF, Bacci A, Montagnoli C, et al. Endogenous interleukin 4 is required for development of protective CD4+ T helper type 1 cell responses to Candida albicans. J Exp Med. 1998 Feb 2;187(3):307–17.
92. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986 Apr 1;136(7):2348–57.
93. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005 Nov;6(11):1123–32.
94. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang Y-H, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005 Nov;6(11):1133–41.
95. Kurschus FC, Moos S. IL-17 for therapy. Vol. 87, Journal of Dermatological Science. Elsevier Ireland Ltd; 2017. p. 221–7.
96. Trinchieri G, Pflanz S, Kastelein RA. The IL-12 family of heterodimeric cytokines: New players in the regulation of T cell responses. Vol. 19, Immunity. Cell Press; 2003. p. 641–4.
97. Whibley N, Gaffen SL. Brothers in arms: Th17 and Treg responses in Candida albicans immunity. Heitman J, editor. PLoS Pathog. 2014 Dec 4;10(12):e1004456.
98. Romani L, Puccetti P. Protective tolerance to fungi: the role of IL-10 and tryptophan catabolism. Vol. 14, Trends in Microbiology. 2006. p. 183–9.
99. Huang W, Na L, Fidel PL, Schwarzenberger P. Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J Infect Dis. 2004 Aug 1;190(3):624–31.
100. Glocker E-O, Hennigs A, Nabavi M, Schäffer AA, Woellner C, Salzer U, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N Engl J Med. 2009 Oct 29;361(18):1727–35.

101. Glocker EO, Grimbacher B. Mucosal antifungal defence: IL-17 signalling takes centre stage. Vol. 89, Immunology and Cell Biology. Immunol Cell Biol; 2011. p. 823–5.
102. Puel A, Döffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med. 2010 Feb 15;207(2):291–7.
103. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CEM, Papp K, et al. Secukinumab in plaque psoriasis - Results of two phase 3 trials. N Engl J Med. 2014;371(4):326–38.
104. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Vol. 21, Immunity. 2004. p. 467–76.
105. van de Veerdonk FL, Marijnissen RJ, Kullberg BJ, Koenen HJPM, Cheng S-C, Joosten I, et al. The Macrophage Mannose Receptor Induces IL-17 in Response to Candida albicans. Cell Host Microbe. 2009 Apr;5(4):329–40.
106. Matsuzaki G, Umemura M. Interleukin-17 as an effector molecule of innate and acquired immunity against infections. Microbiology and Immunology Center for Academic Publications Japan; 2007 p. 1139–47.
107. Bozza S, Fallarino F, Pitzurra L, Zelante T, Montagnoli C, Bellocchio S, et al. A Crucial Role for Tryptophan Catabolism at the Host/ Candida albicans Interface . J Immunol. 2005 Mar 1;174(5):2910–8.
108. Zelante T, De Luca A, D'Angelo C, Moretti S, Romani L. IL-17/Th17 in anti-fungal immunity: What's new? Eur J Immunol. 2009 Mar;39(3):645–8.
109. Zelante T, Fallarino F, Bistoni F, Puccetti P, Romani L. Indoleamine 2,3-dioxygenase in infection: the paradox of an evasive strategy that benefits the host. Vol. 11, Microbes and Infection. 2009. p. 133–41.
110. Romani L, Zelante T, De Luca A, Fallarino F, Puccetti P. IL-17 and therapeutic kynurenines in pathogenic inflammation to fungi. J Immunol. 2008 Apr 15;180(8):5157–62.
111. Hohenberger M, Cardwell LA, Oussedik E, Feldman SR. Interleukin-17 inhibition: role in psoriasis and inflammatory bowel disease. Vol. 29, Journal of Dermatological Treatment. Taylor and Francis Ltd; 2018. p. 13–8.
112. Fala L. Cosentyx (Secukinumab): First IL-17A Antagonist Receives FDA Approval for Moderate-to-Severe Plaque Psoriasis. Am Heal drug benefits. 2016 Mar;9(Spec Feature):60–3.
113. Imura C, Ueyama A, Sasaki Y, Shimizu M, Furue Y, Tai N, et al. A novel RORγt inhibitor is a potential therapeutic agent for the topical treatment of psoriasis with low risk of thymic aberrations. J Dermatol Sci. 2019 Mar 1;93(3):176–85.

114. Bartlett HS, Million RP. Targeting the IL-17-TH17 pathway. Vol. 14, Nature Reviews Drug Discovery. Nature Publishing Group; 2014. p. 11–2.
115. Ferwerda B, Ferwerda G, Plantinga TS, Willment JA, van Spriel AB, Venselaar H, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med. 2009 Oct 29;361(18):1760–7.
116. Wunsch S, Zurl C, Strohmaier H, Meinitzer A, Rabensteiner J, Posch W, et al. Longitudinal evaluation of plasma cytokine levels in patients with invasive candidiasis. J Fungi. 2021 Jan 1;7(2):1–20.
117. Gaffen SL, Hernández-Santos N, Peterson AC. IL-17 signaling in host defense against Candida albicans. Immunol Res. 2011 Aug;50(2–3):181–7.
118. De Luca A, Zelante T, D'Angelo C, Zagarella S, Fallarino F, Spreca A, et al. IL-22 defines a novel immune pathway of antifungal resistance. Mucosal Immunol. 2010 Jul;3(4):361–73.
119. Li X, Yuan X, Wang C, Muthu SE. The clinical value of IL-3, IL-4, IL-12p70, IL17A, IFN-γ, MIP-1β, NLR, P-selectin, and TNF-α in differentiating bloodstream infections caused by gram-negative, gram-positive bacteria and fungi in hospitalized patients: An Observational Study. Med (United States). 2019 Sep 1;98(38).
120. Akin H, Akalin H, Budak F, Ener B, Ocakoğlu G, Gürcüoğlu E, et al. Alterations of serum cytokine levels and their relation with inflammatory markers in candidemia. Med Mycol. 2015;53(3):258–68.
121. Moreno RP, Metnitz PGH, Almeida E, Jordan B, Bauer P, Campos RA, et al. SAPS 3 - From evaluation of the patient to evaluation of the intensive care unit. Part 2: Development of a prognostic model for hospital mortality at ICU admission. Intensive Care Med. 2005;31(10):1345–55.
122. Huseini T, Manners D, Jones S, Piccolo F. External validation of the SAPS3-CNIV score to predict hospital mortality following noninvasive ventilation: a retrospective single-centre study. ERJ open Res. 2019 Apr;5(2):00232–2018.
123. Covelli HD, Nessan VJ, Tuttle WK. Oxygen derived variables in acute respiratory failure. Crit Care Med. 1983 Aug;11(8):646–9.
124. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: The Berlin definition. JAMA - J Am Med Assoc. 2012 Jun 13;307(23):2526–33.
125. Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med. 1998 Nov;26(11):1793–800.

126. Kenis G, Teunissen C, De Jongh R, Bosmans E, Steinbusch H, Maes M. Stability of interleukin 6, soluble interleukin 6 receptor, interleukin 10 and CC16 in human serum. Cytokine. 2002;19(5):228–35.
127. Li J, Plorde JJ, Carlson LG. Effects of volume and periodicity on blood cultures. Vol. 32, Journal of Clinical Microbiology. 1994. p. 2829–31.
128. Lamy B, Roy P, Carret G, Flandrois J-P, Delignette-Muller ML. What is the relevance of obtaining multiple blood samples for culture? A comprehensive model to optimize the strategy for diagnosing bacteremia. Clin Infect Dis. 2002 Oct 1;35(7):842–50.
129. Chai LYA, van de Veerdonk F, Marijnissen RJ, Cheng S-C, Khoo AL, Hectors M, et al. Anti-Aspergillus human host defence relies on type 1 T helper (Th1), rather than type 17 T helper (Th17), cellular immunity. Immunology. 2010 May;130(1):46–54.
130. Allen SJ, Mott KR, Wechsler SL, Flavell RA, Town T, Ghiasi H. Adaptive and Innate Transforming Growth Factor Signaling Impact Herpes Simplex Virus 1 Latency and Reactivation. J Virol. 2011 Nov 1;85(21):11448–56.
131. Li MO, Wan YY, Sanjabi S, Robertson AKL, Flavell RA. Transforming growth factor-β regulation of immune responses. Vol. 24, Annual Review of Immunology. Annu Rev Immunol; 2006. p. 99–146.
132. Wahl SM. Transforming growth factor-β: innately bipolar. Vol. 19, Current Opinion in Immunology. Curr Opin Immunol; 2007. p. 55–62.
133. Wahl SM, Wen J, Moutsopoulos N. TGF-β: A mobile purveyor of immune privilege. Vol. 213, Immunological Reviews. Immunol Rev; 2006. p. 213–27.
134. Letterio JJ, Lehrnbecher T, Pollack G, Walsh TJ, Chanock SJ. Invasive candidiasis stimulates hepatocyte and monocyte production of active transforming growth factor β. Infect Immun. 2001;69(8):5115–20.
135. Costantini C, Bellet MM, Renga G, Stincardini C, Borghi M, Pariano M, et al. Tryptophan Co-Metabolism at the Host-Pathogen Interface. Front Immunol. 2020 Jan 29;11.
136. Huttunen R, Syrjänen J, Aittoniemi J, Oja SS, Raitala A, Laine J, et al. High activity of indoleamine 2,3 dioxygenase enzyme predicts disease severity and case fatality in bacteremic patients. Shock. 2010 Feb;33(2):149–54.
137. Martin GS, Mannino DM, Moss M. The effect of age on the development and outcome of adult sepsis. Crit Care Med. 2006;34(1):15–21.
138. Skogstrand K, Ekelund CK, Thorsen P, Vogel I, Jacobsson B, Nørgaard-Pedersen B, et al. Effects of blood sample handling procedures on measurable inflammatory markers in plasma, serum and dried blood spot samples. J Immunol Methods. 2008;336(1):78–84.