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STUDIES ON CLINICAL USE OF PANFUNGAL PCR AND CANDIDEMIA

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ACADEMIC DISSERTATION

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TIIVISTELMÄ

Tausta ja tavoitteet. Syvien sieni-infektioiden merkitys on lisääntynyt viime vuosikymmenien aikana, ja ne aiheuttavat merkittävää kuolleisuutta immuunipuutteisilla potilailla. Syville sieni-infektioille altistaa erityisesti elinsiirrot, syöpäsairaudet, HIV-infektio, immuunipuolustusta lamaavat hoidot sekä suolistoleikkaukset. *Candida*-hiivasieni on yleisin syvien sieni-infektioiden aiheuttaja, mutta muiden sienten kuten *Aspergilluksen* sekä harvinaisemipien sienten aiheuttamien infektioiden on kuvattu lisääntyneen. Viljely sekä mikroskopia ovat perinteisesti olleet tärkeimpiä diagnostisia tutkimuksia syvien sieni-infektioiden diagnostiikassa, mutta tehokkaampia ja tarkempia menetelmiä tarvitaan. Tämän tutkimuksen tarkoituksena oli selvittää geenimonistukseen (polymerase chain reaction, PCR) perustuvan sieni PCR -tutkimuksen merkitystä kliinisessä työssä. Lisäksi olemme selvittäneet kandidemioiden epidemiologiaa Helsingin ja Uudenmaan sairaanhoitopiirissä. Olemme tutkineet myös kandidemioiden kuolleisuuteen liittyviä riskitekijöitä sekä toistuvien ja pitkittyneiden kandidemioiden erityispiirteitä.

Menetelmät. Ensimmäisessä osajulkaisussa analysoimme retrospektiivisesti potilaita, joilla on tutkittu sieni PCR -tutkimus syvästä kudosnäytteestä vuosina 2013–2015. PCR-näytteitä oli 307. Vertasimme PCR-tuloksia viljelyn ja sieninatiivin tuloksiin. Syvän sieni-infektion todennäköisyyttä arvioitiin 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) laatimien kriteerien perusteella.

Kandidemiatutkimuksissa aineistona oli aikuispotilaat, joilla todettiin veriviljelypositiivinen *Candida*-lajin aiheuttama infektio 2007–2016 Helsingin ja Uudenmaan sairaanhoitopiirissä. Väitöskirjan 2. osajulkaisussa tutkimusjakso jaettiin kahteen viiden vuoden jaksoon, ja analysoimme muutoksia kandidemioiden epidemiologiassa tutkimusjakson aikana. 3. osajulkaisussa vertasimme kandidemia-potilaita, joilla todettiin pitkittynyt veriviljelypositiivisuus potilastapauksiin, joilla veriviljelypositiivisuus kesti alle viiden vuorokauden ajan. 4. osajulkaisussa tutkimme toistuvien kandidemioiden erityispiirteitä vertailemalla toistuvia kandidemioita sairastaneita potilaita potilaisiin, joilla todettiin vain yksittäinen infektioepisodi.

Tulokset. Sieni PCR oli positiivinen 48 (16%) potilaan näytteessä, ja näistä potilaista 23 todettiin varma tai todennäköinen syvä sieni-infektio. Viljelyn ja natiivitutkimuksen tulokset olivat yhtenevät sieni PCR -tutkimuksen kanssa >85% näytteistä. PCR-tutkimuksen herkkyys oli 61%, tarkkuus 92%, negatiivinen ennustearvo 93% ja positiivinen ennustearvo 54%.

Candida albicans oli yleisin kandidemioiden aiheuttaja. Eri *Candida*-lajien jakauman välillä ei todettu merkittävää muutosta tutkimusjakson aikana. 30

päivän kokonaiskuolleisuus oli 31%. Kuolleisuuden itsenäisiä riskitekijöitä olivat taustalla olevat sairaudet (McCabe luokitus 3), tehohoito infektion toteamishetkellä sekä yli 65 vuoden ikä. Tilastollisesti merkitsevää yhteyttä aikaisin aloitetun sienilääkityksen ja kokonaiskuolleisuuden välillä ei todettu, vaikkakin tehoavalla sienilääkityksellä oli suojaava vaikutus kokonaiskuolleisuutta vastaan. Pitkittynyt kandidemia todettiin 75 (21%) potilaalla. Syvät infektiopesäkkeet, keskuslaskimokatetri infektion toteamishetkellä ja empiirisesti aloitettu tehoton sienilääkitys olivat itsenäisiä riskitekijöitä pitkittyneeseen kandidemiaan. Toistuva kandidemia todettiin 6%:lla kaikista kandidemia-potilaista. Potilailla, joilla todettiin toistuva kandidemia, oli muita kandidemia-potilaita enemmän pitkäaikaisia suolistosairauksia sekä suonensisäistä huumeiden käyttöä.

Johtopäätökset. Syvien sieni-infektioiden diagnostiikka on vaativaa. Sieni PCR-tutkimus auttaa syvien sieni-infektioiden diagnostiikassa, mutta tutkimus on tärkeää yhdistää muiden diagnostisten menetelmien kanssa parhaan tuloksen saavuttamiseksi. Tutkimusjakson aikana sairaanhoitopiirissämme ei todettu merkittävää lisääntymistä non-*albicans Candida*-lajien aiheuttamissa kandidemioissa, vaikka muiden *Candida*-lajien kuin *C. albicansin* lisääntymistä on raportoitu toistuvasti muualta maailmasta. Sienilääkityksen aikaisella aloituksella ei vaikuta olevan yhtä suurta merkitystä kandidemioiden hoidossa kuin mikrobilääkityksen aloituksen ajankohdalla on osoitettu olevan bakteerien aiheuttamissa septisissä sokkitilanteissa. Kandidemia-potilailla pitäisi aktiivisesti etsiä ja tehokkaasti hoitaa mahdollisia syviä infektiopesäkkeitä sekä poistaa mahdollisimman aikaisin keskuslaskimokatetri, jotta pitkittyneiden kandidemioiden esiintymistä voidaan ehkäistä. Potilailla, joilla todettiin toistuvia kandidemioita, oli muita kandidemiapotilaita enemmän pitkäaikaisia suolistosairauksia sekä suonensisäistä huumeiden käyttöä.

ABSTRACT

Backgrounds and aims. Invasive fungal diseases (IFD) cause significant morbidity and mortality in immunocompromised and critically ill patients. These infections have gained greater importance in recent decades. The population at risk for IFDs in particular include those with haematologic malignancies, recipients of haematopoietic stem cell or solid organ transplantation, those with human immunodeficiency virus infection, immunosuppressive therapies, indwelling medical devices, and those who have undergone recent gastrointestinal surgery. *Candida* is the most frequent species that causes IFDs. However, other invasive mycoses such as aspergillosis and infections caused by other rarer fungal pathogens, have been reported to emerge. Diagnosis of IFDs is challenging. Culture and histopathology are the foundation of the diagnosis. However, more accurate and rapid diagnostic methods are needed. The purpose of this study was to assess the clinical use of panfungal polymerase chain reaction (PCR) in diagnosing IFD from deep tissue specimens. The study also aimed to provide recent epidemiological data for candidemia in the hospital district of Helsinki and Uusimaa and to analyse the risk factors for 30-day mortality in candidemia. The association between candidemia mortality and an early start of an effective antifungal treatment was evaluated and the risk factors for persistent and characteristics of recurrent candidemia were analysed.

Patients and methods. Study I was a retrospective cohort study that analysed the clinical use of panfugal PCR to diagnose IFD. We focused on specimens taken from normally sterile tissues and fluids. Bronchoalveolar fluid and blood samples were excluded. We compared results of panfungal PCR test to the results of culture and histopathology in 307 specimens. The likelihood of an IFD was evaluated with the criteria of 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).

Studies II-IV were retrospective, cohort studies. All patients with candidemia were identified from the microbiological database between 2007 and 2016. Patients <18 years were excluded. Study II included 350 patients with a positive blood culture results for *Candida* species. The study period was divided into two 5-year periods to analyse the changes in the epidemiology of candidemia. The main outcome mesure was 30-day overall mortality after the diagnosis of candidemia. In study III, we compared the patients with persistent candidemia (PC) with non-persistent cases. PC was defined as an isolation of the same *Candida* species from blood culture ≥5 days. In study IV, we compared patients with late recurrent (LR) candidemia and patients with a single candidemia episode to analyse the characteristics of LR candidemia. LR candidemia was defined as having at least two episodes of candidemia ≥30 days apart.

Results. Panfungal PCR was positive in 48 (16%) specimens, and 23 patients of these had a proven or probable IFD. The sensitivity and specificity of panfungal PCR in diagnosing IFD was 61% and 92%, respectively; the negative predictive value and positive predictive value were 93% and 54%, respectively. The concordance of PCR with culture and microscopy results was >85%.

C. albicans was the leading cause of candidemia and the distribution of *Candida* species showed no significant change during the study period. The overall 30-day mortality was 31%. McCabe score 3, ICU stay at the onset of candidemia and age >65 years were independent risk factors for 30-day mortality. An association between 30-day mortality and early start of effective antifungal treatment was not observed, although an effective antifungal treatment was a protective factor against mortality. PC was observed in 75 (21%) patients. Metastatic infection foci, presence of central venous catheter (CVC) and ineffective empirical antifungal therapy were independent risk factors for PC. LR candidemia was an uncommon event and diagnosed in 6% of all patients with candidemia. LR candidemia was associated with a history of intravenous drug use (IDU) and underlying gastrointestinal diseases.

Conclusions. Diagnosis of IFDs remains challenging. Our results show that panfungal PCR aids in the diagnosis of IFDs; however, it should be combined with other diagnostic methods. A significant shift to non-*albicans Candida* species causing candidemia was not observed in our hospital district during the study period. An early start of effective antifungal agent was not a protective factor against 30-day mortality. Effective antifungal treatment is beneficial in candidemia, but the early initiation of the medication seems not to be as crucial as it is in bacterial septic shock. Removal of CVC as early as possible, and search and treatment for metastatic infection foci are key elements for preventing PC. Underlying gastrointestinal diseases and a history of IDU were associated with LR candidemia.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Ala-Houhala M, Koukila-Kahkola P, Antikainen J, Valve J, Kirveskari J, Anttila VJ. Clinical use of fungal PCR from deep tissue samples in the diagnosis of invasive fungal diseases: a retrospective observational study. Clin Microbiol Infect. 2018;24(3):301-305.

II Ala-Houhala M, Valkonen M, Kolho E, Friberg N, Anttila VJ. Clinical and microbiological factors associated with mortality in candidemia in adult patients 2007–2016. Infect Dis (Lond). 2019;51(11-12):824-830.

III Ala-Houhala M, Anttila VJ. Persistent vs non-persistent candidaemia in adult patients in 2007–2016: A retrospective cohort study. Mycoses. 2020;63(6):617-624.

IV Ala-Houhala M, Anttila VJ. Characteristics of late recurrent candidemia in adult patients. Mycoses. 2021;64(5):503-510.

The publications are referred to in the text by their roman numerals.

ABBREVIATIONS

APACHE II	Acute Physiology And Chronic Health Evaluation II score
BAL	Bronchoalveolar fluid
BDG	β-D-glucan
BSI	Bloodstream infection
CDC	Centers for Disease Control and Prevention
CI	Confidental interval
CLSI	The Clinical and Laboratory Standards Institute
CSF	Cerebrospinal fluid
CVC	Central venous catheter
ECMM	European Confederation of Medical Mycology
EORTC/MSG	Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GI	Gastrointestinal
HSCT	Hematopoietic stem cell transplantation
HUS	Hospital district of Helsinki and Uusimaa
HUSLAB	Helsinki University Hospital Laboratory
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
IDU	Intravenous drug use
IFD	Invasive fungal disease
IQR	Interquartile range
ITS	Internal transcribed spacer
LR	Late recurrent
MALDI-TOF	Matrix-assisted laser desorption ionization-time of flight mass spectrometry
Mn/A-Mn	Mannan antigen and anti-mannan antibody
NPV	Negative predictive value
OR	Odds ratio
PAS	Periodic acid-Schiff
PC	Persistent candidemia
PCR	Polymerase chain rection
PPV	Positive predictive value

1 INTRODUCTION

Invasive fungal diseases (IFD) are an emerging problem worldwide. They represent an important infective complication for hospitalised patients and significantly contribute to morbidity and mortality (Brown et al. 2012). Immunocompromised and others critically ill patients are highly vulnerable to IFDs. The population at risk for IFDs are particularly patients with haematological malignancy, recepients of hematopoietic stem cell transplant (HSCT) and solid organ transplant, those with human immunodeficiency virus (HIV)/AIDS, and those on immunosuppressive medication. Recent gastrointestinal (GI) surgery, prolonged stay in an intensive care unit (ICU) and indwelling medical devices also increase the risk for IFDs (Pappas et al. 2018, Schmiedel and Zimmerli 2016, Lass-Flörl 2009).

There are millions of fungal species in the world, of which several hundred cause diseases in humans (Köhler et al. 2014, O'Brien et al. 2005), but most of the IFDs in humans are caused by *Candida*, *Aspergillus*, *Cryptococcus,* and *Pneumocystis* species (Brown et al. 2012). Even though, these four genera cause most of the IFDs, more rare fungi such as *Fusarium*, *Mucorales* and *Scedosporium* species have been increasingly reported (Marr et al. 2002, Caston-Osorio et al. 2008, Lass-Florl and Cuenca-Estrella 2017). Although the last 20 years we have witnessed the development of new antifungal agents and improvements in diagnostic procedures, the overall trend indicates that IFDs are increasing (Schmiedel and Zimmerli 2016).

Candida species remain the most common cause of IFDs (Montagna et al. 2013, Bitar et al. 2014). Invasive candidiasis consists of a spectrum of different clinical conditions, the most common of which is bloodstream infection (BSI), candidemia. Candidemia is associated with a high health-care costs, prolonged hospitalisation, and high mortality (Morgan et al. 2005, Zaoutis et al. 2005, Benedict et al. 2019); it is reported to be the seventh most frequent cause of BSI in Europe and the fourth in the US (Marchetti et al. 2004, Wisplinghoff et al. 2004). *Candida* species was also the fourth most common pathogen causing health-care associated infections in US in 2015 (Magill et al. 2018). The incidence of candidemia and the distribution of *Candida* species causing candidemia varies globally. *C. albicans* is the leading cause of candidemia worldwide, however, the proportion of infections caused by *Candida* species other than *C. albicans*, such as *C. parapsilosis and C. glabrata*, has grown in recent decades (Guinea 2014, Lockhart et al. 2012). In recent years, newly emerging species, such as *C. auris*, have become a concern. The non-*albicans Candida* species exhibit a higher level of resistance to antifungal agents, which is alarming (Pfaller[b] et al. 2010).

The diagnosis of IFDs is challenging. Many IFDs lack specific clinical findings. The symptoms are usually non-specific, such as fever, cough, dyspnea or confusion, which complicates the recognition of IFDs. Diagnosis of IFDs consists of

risk factors, clinical symptoms, and radiological and microbiological findings. Traditional methods, such as, histopathology and culture, are still important procedures for the diagnosis of IFDs. However, these methods have limitations, especially regarding sensitivity, and culture is time-consuming. Non-culture based methods are part of modern diagnostic methods and introduce the possibility to improve the diagnosis of IFDs. More accurate and rapid identification of causative pathogens is essential for the appropriate treatment of IFDs.

The purpose of this work was to evaluate the clinical utility of panfungal PCR for diagnosis of IFDs. We focused on deep tissue specimens. In this work, we also analysed the epidemiology of *Candida* BSIs in the hospital district of Helsinki and Uusimaa during a 10-year study period. We evaluated the risk factors for 30-day mortality in candidemia and the association between candidemia mortality and early start of an effective antifungal treatment. The aim was also to analyse the risk factors for persistent and characteristics of recurrent candidemia. Recent local epidemiological data for candidemia are essential for optimising the management of candidemia and for specifying the local prophylactic and empirical treatment guidelines.

2 REVIEW OF THE LITERATURE

2.1 OVERVIEW OF MYCOSES

Diseases caused by fungal species are called mycoses. Fungal are eukaryotic, which distinguish them from viruses and bacteria. Fungi are unicellular or multicellular organisms, and they reproduce by means of spores or conidia. Fungal species have a cell wall, which is a distinct difference from humans, bacteria and viruses. Fungi are ubiquitous and can be found in soil, water and air (Jorgensen and Pfaller 2015). Although, an estimated 1.5–5.0 million fungal species are known on planet Earth, only several hundred can cause disease in humans (Köhler et al. 2014, O'Brien et al., 2005). Clinically important fungi are divided into two main groups: yeasts and moulds. Additionally, some of the medically important fungi are dimorphic e.g. *Histoplasma capsulatum* or *Coccidioides immitis*. These fungi have two forms of growth, and have the ability to grow either as a yeast or as a mould during their lifecycle. Dimorphism is usually temperature-dependent, and these fungi are mostly endemic to specific geographical areas. (Jorgensen and Pfaller, 2015, Bennett et al. 2020)

Fungal pathogens can colonise their host and cause infections from mild superficial infections to severe, systemic, and life-threatening infections. Colonisation is defined as the presence of a mircoorganism on a host with growth, but without causing signs of an infection or immune response. Infection implies an invasion of the disease-causing microorganism in the tissues of a host, causing interaction between the microorganism and host leading to a subclinical or clinical reaction. Fungi that cause systemic infections can be divided into true pathogens and opportunistic fungi. True pathogens, such as *Blastomyces* or *Histoplasma*, are endemic to specific geographical areas and able to infect healthy, immunocompetent humans, and cause also life-threatening infections in immunocompromised persons. On the other hand, opportunistic fungal species, such as *Aspergillus* or *Candida*, cause systemic infections only in immunocompromised persons (Bennett et al. 2020).

2.2 DEFINITIONS

2.2.1 DEFINITION OF INVASIVE FUNGAL DISEASE

Fungi can cause superficial, mild, and life-threatening systemic infections. Invasive fungal disease (IFD) is a term that describes severe, systemic infections caused by yeast, moulds and other fungal species (De Pauw et al. 2008). The previous term for IFD was invasive fungal infections (Ascioglu et al. 2002).

In 2002, a definition for invasive fungal infection in immunocompromised patients with cancer and hematopoietic stem cell transplants was published for clinical and epidemiological research purposes by a consensus group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) (Ascioglu et al. 2002). The criteria were revised in 2008 and 2019 (De Pauw et al. 2008, Donnelly et al. 2020). The definition published in 2008 classifies the probability of IFD diagnosis into the following three categories (Table 1): proven, probable, and possible (De Pauw et al. 2008). The definition of proven IFD requires the presence of moulds or yeasts identified by culture or histological analysis from a specimen taken from a disease site obtained by a sterile procedure. When *Cryptococcus neoformans* is considered, antigen detection in cerebrospinal fluid (CSF) samples or a positive result from an India ink preparation of CSF are also considered for proven IFD. The proven category applies to both immunocompromised and immunocompetent patients.

The categories for probable and possible IFDs are dependent on three elements: host factors, clinical signs and symptoms, and mycological evidence (Table 1). In the categories of probable and possible, the fungal element can be detected not only by microscopic analysis or culture, but also by indirect tests. However, nucleic acid-detection tests have lacked standardisation and validation and were not included in the criteria in 2008. The criteria for endemic mycoses are defined separately from moulds and yeasts, and the classification includes histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, sporotrichosis, and infection due to *Talaromyces marneffei* (De Pauw et al. 2008).

Table 1 *Criteria for proven, probable and possible invasive fungal disease except for endemic mycoses according to 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) (De Pauw et al. 2008).*

Category of IFD		Moulds	Yeasts
Proven	Proven IFD requires the presence of one of the mycological criterion		
	Microscopic analysis: sterile material	Hyphae or melanised yeast-like forms with evidence of associated tissue damage	Yeast cells
	Culture: sterile material*	Mould or "black yeast"**	Yeast
	Blood Culture	Mould e.g. *Fusarium* species***	Yeast or yeast-like fungi
	CSF: serology	Not applicable	Only for cryptococcosis: Antigen in CSF indicates disseminated cryptococcosis
Probable	Probable IFD requires the presence of a host factor, a clinical criterion, and a mycological criterion		
	Host factor	• Recent neutropenia • Receipt of an allogenic stem cell transplant • Prologned use of corticosteroids • Treatment with other T cell immunosuppressants • Inherited severe immunodeficiency	
	Clinical criterion	Lower respiratoy tract • 1 of the following signs on CT: Dense, well-circumscribed lesions with or without a halo sign, air-crescent sign, or cavity Tracheobronchitis • ulceration, nodule, pseudomemrane, plaque, or eschar seen on bronchoscopic analysis Sinonasal infection • Imiging showing sinusitis plus acute localized pain, nasal uncer with black eschar, or extention from paranasal sinus across bony barriers CNS infection on MRI or CT; focal lesions or meningeal enhancement Disseminated candidiasis	
	Mycological criterion: a direct or an indirect test	a) Direct test: cytology, direct microscopy or culture b) Indirect tests • Galactomannan antigen detected in serum/plasma, BAL or CSF, applicable for aspergillosis • β-D-glucan detected in serum (not applicable for cryptococcosis or zygomycoses) • Nucleic acid assays no applicable	
Possible	A host factor and a clinical criterion are present, but the mycological criteria are absent		

IFD, invasive fungal disease; CSF, cerebrospinal fluid; CT, computed tomography; CNS, central nervous system; BAL Broncoalveolar lavage. *Concurrent with an infectious disease process. **Bronchoalveolar fluid, a cranial sinus cavity specimen, and urine excluded. *** Recovery of *Aspergillus* species from blood cultures represents contamination.

2.2.2 DEFINITIONS AND TYPES OF CANDIDA INFECTIONS

Candida is a yeast fungus and a commensal organism of the human GI tract and skin (Nucci and Anaissie 2001, Bennett et al. 2020). *Candida* species are one of the most common fungal pathogens causing opportunistic infections in the world (Pfaller and Diekema, 2007, Bitar et al. 2014, Vallabhaneni et al. 2016). Clinical expression of diseases due to *Candida* species varies from superficial, mucosal infection to severe, life-threatening invasive candidiasis. Invasive candidiasis encompasses blood culture-positive *Candida* infections (candidemia) and deep-seated infections of tissue sites beneath mucosal surfaces. Deep-site infections includes visceral candidiasis, discitis, endocarditis, endophthalmitis, meningitis, and other deep-tissue involvements (Pappas et al. 2018, Kullberg and Arendrup, 2015). Chronic disseminated candidiasis (also called hepatosplenic candidiasis) is a rare infection due to *Candida* species that almost entirely occurs in patients with long-lasting neutropenia in haematological malignancies (Pagano et al. 2002, Cornely[b] et al. 2015).

Candidemia is defined as *Candida* species isolated from at least one blood culture; it is the most common form of invasive candidiasis (De Pauw et al. 2008, Cornely et al. 2012, Pappas et al. 2018). It is assumed that the most forms of invasive candidiasis (other than blood culture positive infections) originate from an earlier or undiagnosed candidemia (Kullberg and Arendrup 2015, Clancy and Nguyen 2013).

Persistent candidemia (PC) is defined as continued isolation of the same *Candida* species from blood culture in a candidemic patient. However, PC lacks a homogenous definition concerning the length of blood culture positivity. Despite appropriate antifungal treatment, *Candida* species take some time to clear from blood cultures. When candidemia was treated with micafungin or caspofungin therapy, the median time to obtaining negative blood cultures for *Candida* was 2–3 days (Pappas et al. 2007). The international guidelines for management of invasive candidiasis lack a definition for PC (Cornely et al. 2012, Pappas et al. 2016), and the definition varies widely in published studies. The length of blood culture positivity in PC is defined from 2–7 days in most studies (Nucci 2011).

Recurrent candidemia refers to a situation in which a patient has experienced more than one different episode of candidemia caused by same or different *Candida* species. The international guidelines for management of invasive candidiasis do not define recurrent candidemia either (Cornely et al. 2012, Pappas et al. 2016). In most clinical studies, recurrent candidemia is defined as an early recurrence if the second episode of candidemia is diagnosed less than 30 days after the first episode, and the candidemia episode is defined as late recurrent (LR) if the episodes are diagnosed more than ≥30 days apart (Munoz et al. 2016, Lai et al. 2019, Asmundsdottir et al. 2012).

2.3 EPIDEMIOLOGY

2.3.1 EPIDEMIOLOGY OF INVASIVE FUNGAL DISEASE

IFDs are an emerging problem worldwide. Diagnosis of IFD is challenging for clinicians, and the incidence of IFDs is likely underestimated mostly due to the absence of reliable diagnostics tests (Brown et al. 2012, Lass-Florl 2009). The immune system of a healthy individual has efficient mechanisms for preventing fungal infections. Most of the IFDs appear in immunocompromised patients (Brown et al. 2012).

IFDs caused by yeasts and moulds occur worldwide, but systemic endemic mycoses are mostly found in the Americas, Africa, and in Southeast Asia (Queiroz-Telles et al. 2017). Several hundred of fungi cause disease in humans (Köhler et al. 2014), but the most common pathogens that cause fungal disease in humas include *Candida, Aspergillus*, *Cryptococcus*, and *Pneumocystis*. Species belonging to these four genera have caused >90% of reported deaths due to fungal diseases (Brown et al. 2012). The overall incidence of IFD was 5.9/100 000 cases/year in France from 2001–2010, and the incidence increased over the study period in candidemia, invasive aspergillosis and mucormycosis (Bitar et al. 2014). However, the incidence rate of AIDS-associated *Pneumocystis* pneumonia and cryptococcosis decreased during the study period, which was expected due to the active use of effective antiretroviral therapy (Bitar et al. 2014).

The epidemiology of IFD has changed in recent decades (Nucci and Marr 2005, Richardson and Lass-Florl 2008). *Candida* is the most important cause of IFDs in the Western world, and *C. albican*s is the dominant *Candida* species (Guinea 2014), even though, a shift towards non-*albicans Candida* species has been observed in several population-based studies (Astvad et al. 2018, Puig-Asensio et al. 2014, Chapman et al. 2017, Lockhart et al. 2012). The epidemiology of candidemia will be discussed separately in chapters 2.3.2–2.3.5. On the other hand, *Aspergillus* and other moulds have become increasingly important pathogens causing IFDs in Europe in recent decades, and rare infections such as mucormycosis and fusariosis have emerged (Lass-Florl 2009).

Aspergillosis is the most common mould infection in humans. In a population-based study conducted in Spain, *Aspergillus* species was reported to cause >85% of invasive mould infections (Alastruey-Izquierdo et al. 2013). Most invasive aspergilloses are caused by *A. fumigatus,* followed by *A. flavus*, *A. terreus,* and *A. niger* (Binder and Lass-Flörl 2013, Taccone et al. 2015). There is evidence that non-*fumigatus Aspergillus* species are becoming increasingly common aetiologic agents (Marr et al. 2002, Baddley et al. 2001, Zanganeh et al. 2018). Many of patients with invasive aspergillosis have haematological malignancies, have received HSCT or solid organ transplantation or have severe lung diseases (Kontoyiannis et al. 2010,

Lass-Flörl and Cuenca-Estrella, 2017). Invasive aspergillosis is also associated with acute viral respiratory infections caused by respiratory syncytial virus, influenzavirus and adenovirus (Schauwvlieghe et al. 2018, Garcia-Vidal et al. 2014). In 2020, invasive pulmonary aspergillosis has been reported to occur in critically ill patients with coronavirus disease 2019 (COVID-19) treated in an ICU (van Arkel et al. 2020, Koehler et al. 2020, Alanio et al. 2020).

Cryptococcosis is one of the most predominant fatal fungal diseases worldwide, and *Cryptococcus* species are the second most common yeast after *Candida* species that cause opportunistic infections (Park et al. 2009, Brown et al. 2012, Castón-Osorio et al. 2008). *Cryptococcus neoformans* and *Cryptococcus gattii* cause most of cryptococcal infections in humans, and *C. neoformans* accounts for >90% of them (Maziarz and Perfect 2016). *Cryptococcus* species can cause disease in both immunocompromised and immunocompetent hosts. However, cryptococcal meningitis cases are most prevalent in middle- and low-income countries with HIV/AIDS patients (Schmiedel and Zimmerli 2016). The use of effective antiretroviral therapy has led to a remarkable decline in HIV-associated cryptococcal meningitis (Park et al. 2009, Sloan and Parris 2014).

Pneumocystis jirovecii also has a worldwide distribution. It is an opportunistic yeast that can cause life-threatening pneumonia in patients with immunosuppression (Ma et al. 2018). It is estimated that approximately 400 000 humans are affected by pneumonia caused by *P. jirovecii* every year, which is as many that are estimated to be affected by candidiasis caused by *C. albicans* (Brown et al. 2012*)*. In a recent study from France, *Pneumocystis* pneumonia was the second most common invasive fungal infection after candidemia with an annual incidence rate of 1.5/100 000 (Bitar et al. 2014). While HIV-associated *Pneumocystis* pneumonia is decreasing, prominent risk groups for *Pneumocystis* pneumonia are patients who are immunocompromised due to malignancy, transplantation or rheumatological diseases (Schmiedel and Zimmerli 2016).

Clinically, the most important non-*Aspergillus* moulds are *Mucorales*, *Fusarium* and *Scedosporium* species (Castón-Osorio et al. 2008). These fungi are rare, but geographical variation occurs. In a Spanish population-based study, *Fusarium* species was found in 1.2% of clinical mould isolates from deep tissue samples (Alastruey-Izquierdo et al. 2013). However, in a prospective multicentre study on haematological patients from Brazil, invasive fusariosis was reported to be even the leading IFD caused by moulds followed by invasive aspergillosis (Nucci[a] et al. 2013). These moulds exhibit a reduced susceptibility or some are even intrinsically resistant to antifungal agents and are therefore difficult to treat (Lass-Flörl and Cuenca-Estrella 2017). As use of posaconazole prophylaxis has reduced the incidence of invasive aspergillosis in haematological cancer patients, there have been observations of infections caused by these rarer moulds as breakthrough infections (Auberger et al. 2012, Michallet et al. 2011).

Available epidemiological data for IFDs from Finland are scarce. Fungi caused 4% of nosocomial BSIs in hospitals participating in the Finnish Hospital

Infection Program in Finland during 1999–2014 (Kontula et al. 2018). In 2015–2019, the Finnish National Infectious Diseases Register have reported annually 208–228 findings of yeasts in blood culture specimens in Finland, the highest rate in 2019 and the lowest in 2016. (Finnish National Infectious Diseases Register [Internet database 2020]). The average annual incidence of candidemia was 2.9/100 000 inhabitants in Finland between 2004 and 2007 (Poikonen et al. 2010). However, national data concerning the epidemiology of aspergillosis in Finland are lacking. The estimated incidence of infections caused by *Microascaceae* (including *Scedosporium* species) has been reported to be 0.8–1.7 cases per one million inhabitants per year in Finland (Issakainen et al. 2010).

2.3.2 CANDIDA SPECIES AND SPECIES DISTRIBUTION

Candida is ubiquitous. *Candida* species have been isolated from soil, plants, animals, humans, food, and hospital environments (Bennett et al. 2020). More than 200 species of *Candida* have been identified, and *Candida* is the largest genus of medically important yeast (Brandt and Lockhart 2012). At least 30 *Candida* species have been described as causing human infections (Miceli et al. 2011, Bradt and Lockhart 2012); however, >90% of human *Candida* infections are caused by *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. krusei* (Marchetti et al. 2004, Wisplinghoff et al. 2004, Toda et al. 2019, Pfaller et al. 2005). Although, *C. krusei* has been reported to be the fifth most common *Candida* species, the proportion of *C. krusei* isolates is relatively low, and has been reported to be only from 1–4% in most studies (Falagas et al. 2010). *C. krusei* has innate resistance to fluconazole and a reduced susceptibility to amphotericin B, which makes *C. krusei* more difficult to treat as a pathogen (Pfaller et al. 2008). Other non-*albicans Candida* species are emerging, and *C. dubliniensis, C. guilliermondii, C. lusitaniae, C. pelliculosa,* for example, have overtaken *C. krusei* as the fifth leading causative *Candida* species in some studies (Falagas et al. 2010). *C. glabrata* has an inherited decreased susceptibility to azoles and ability to rapidly acquire azole resistance (Schwarzmüller et al. 2014).

The emerging *Candida* species, *C. auris*, is causing growing concern worldwide. *C. auri*s within the ear camal was first reported in 2009 in Japan (Satoh et al. 2009), and the first invasive infections in South Korea (Lee et al. 2011). Since 2009, it has been reported in over 40 countries (CDC. Tracking Candida auris [Internet database 2020]). Previously, *C. auris* was occasionally phenotypically misidentified as *C. haemulonii, C. famata,* and other yeasts by commercially available identification systems (Girard et al. 2016, Kathuria et al. 2015, Chowdhary et al. 2016). Due to the failure of conventional diagnostic methods to accurately identify *C. auris,* the true incidence of *C. auris* is unknown (Navalkele et al. 2017, Spivak and Hanson 2018).

Genetic analysis using whole genome sequencing has revealed deep divergence within *C. auris* species. This divergence has led to the identification of four distinct geographic clades (I–IV): South Asian, South African, South American, and East Asian, with a possible fifth clade in Iran (Lockhart et al. 2017, Chow et al. 2019). *C. auris* has a tendency to spread rapidly in health-care settings, cause outbreaks (Schelenz et al. 2016, Ruiz-Gaitán et al. 2018, Forsberg et al. 2019, Govender et al. 2018), and it is associated with high mortality (Chowdhary et al. 2013, Lockhart et al. 2017). Outbreaks have been difficult to control as *C. auris* persists in hospital environments and is difficult to eradicate (Jeffery-Smith et al. 2017). Furthermore, *C. auris* has multidrug-resistant properties: most *C. auris* isolates are resistant to fluconazole, and the susceptibility to other azoles, amphotericin B and echinocandins varies (Sears and Schwartz 2017, Lockhart et al. 2017).

The candida species vary due to their unique properties and virulence factors. *C. parapsilosis* and *C. krusei* are less virulent than *C. albicans, C. tropicalis,* and *C. glabrata* (Arendrup et al. 2002). The virulence factors of *C. auris* are poorly understood, but it is considered to be highly virulent and even more virulent than *C. albicans* (Sherry et al. 2017). The five most common *Candida* species and *C. auris* can form biofilms (Cavalheiro and Teixeira, 2018, Short et al. 2019, Sherry et al. 2017).

The distribution of *Candida* species that cause candidemia has substantial geographical and centre-to-centre variability. Knowledge of the local epidemiology of the *Candida* species is important, as susceptibility to antifungal agents varies among the different species. *C. albicans* continues to be the leading *Candida* pathogen worldwide (Pfaller[a] et al. 2010, da Matta et al. 2017, Koehler et al. 2019). However, recent decades have witnessed an increase in proportion of non-*albicans Candida* species (Castanheira et al. 2016, Pfaller et al. 2011, Pfaller et al. 2019). The shift to the non-*albicans Candida* species has been reported in many parts of the world, mostly in Asia, southern Europe, and South and North America (Diekema et al. 2012, Matsumoto et al. 2014, Papadimitriou-Olivgeris et al. 2019, Lockhart et al. 2012, Puig-Asensio et al. 2014, Chapman et al. 2017, Zhou et al. 2016, da Matta et al. 2017).

C. glabrata is the most prevalent non-*albicans Candida* species in North and Central Europe and in the US (Lausch[a] et al. 2018, Hesstvedt et al. 2017, Khatib et al. 2016, Tsay et al. 2020). On the other hand, *C. parapsilosis* and *C. tropicalis* are dominat in Mediterranean areas, South America and Asia (Bassetti et al. 2013, Garnacho-Montero et al. 2010, Ben-Ami et al. 2012, Braga et al. 2018, Santolaya et al. 2019, Colombo et al. 2014, Nucci[b] et al. 2013 Jia et al. 2018, Morii et al. 2014). While, the increase of *C. glabrata* proportion is prominent in Europe and the US, a trend towards an increase in *C. glabrata* frequency has also been observed in studies conducted in Brazil, Argentina and Columbia (Falagas et al. 2010, da Matta et al. 2017). Although the frequency of candidemia caused by *C. auris* is unknown, single-centre studies have reported the proportion of *C. auris* to account for 27% of isolates in Pakistan and 38% in Kenya (Adam et al. 2019, Sayeed et al., 2020).

2.3.3 INCIDENCE OF CANDIDEMIA

The incidence of candidemia has changed over the recent decades, and the rates vary significantly between different countries and regions (Table 2). The incidence of candidemia increased five fold in the US during 1980–1990 (Banerjee et al. 1991, Gaynes et al. 1991). Use of more aggressive therapies (chemotherapy, transplantation, treatment in ICUs) and the increase in immunocompromised patient populations due to the more extensive use of immunosuppressive agents mostly influenced to the increase (Falagas et al. 2010). However, the incidence rate began to decrease in the middle of the 1990s and 2000s among low birthweight newborns, partly because of recommendations for fluconazole prophylaxis, and later on in the adult population as well (Fridkin et al. 2006, Benedict et al. 2018, Fisher et al. 2014). The incidence decreased significantly in Atlanta from 14.1 to 9.5/100 000 and in Baltimore from 30.0 to 14.4/100 000 person-years during 2008–2013 (Cleveland et al. 2015). A recent population-based laboratory surveillance study in 22 counties in four states in the US revealed the annual incidence of candidemia as 8.7/100 000 inhabitants from 2012–2016 (Toda et al. 2019). The burden of candidemia was evaluated in the US in 2017 and the overall estimated incidence was 7.0/100 000 inhabitants in the US (Tsay et al. 2020).

The changes in the incidence rates have been less apparent in Europe than in the US (Table 2). The rate increased from 4.3 to 8.1/100 000 inhabitants in Spain during the early 2000s (Almirante et al. 2005, Puig-Asensio et al. 2014). In Nordic countires, the situation has been more stable. The incidence has been low in Norway (3.9/100 000 from 2004–2012) (Hesstvedt et al. 2015). In Sweden, the incidence was 4.7/100 000 from 2015–2016 (Klingspor et al. 2018), and in Iceland 5.7/100 000 from 2000–2011 (Asmundsdottir et al. 2013). In Finland, the incidence of candidemia has been studied in two population-based studies in recent decades. The rates have also been low in Finland and were 1.9/100 000 inhabitants from 1995–1999 and 2.9/100 000 from 2004–2007 (Poikonen et al. 2003, Poikonen et al. 2010). However, Denmark differs from other Nordic countries, and the incidence of candidemia is closer to what of the US. In the early 1990s in Denmark, the incidence rate was 2/100 000 inhabitants, comparable with other Nordic countries, but rose more conspicuosly than in the neighbouring countries up to 10.4/100 000 over the period 1992–2004 (Arendrup et al. 2008). In recent reports after 2010, the incidence has also decreased modestly in Denmark (Astvad et al. 2018). The incidence rates reported from population-based studies in France, Scotland, Australia and Canada have been similar to those in Nordic countries (Bitar et al. 2014, Rajendran et al. 2016, Chen et al. 2006, Laupland et al. 2005). The overall, pooled incidence rate in Europe was 3.9/100 000 inhabitants calculated from population-based studies in a meta-analysis published 2019 (Koehler et al. 2019).

The incidence rates of candidemia are age-specific and are highest at the extremes, under 1-years and over 60-years old (Asmundsdottir et al. 2002). Candidemia is also more frequent in males than in females (Ericsson et al. 2013).

Table 2 *Summary of annual incidence rates of candidemia and proportions of C. albicans species reported in population-based studies published after the year 2000.*

Country/area	Time period	Incidence per 100 000 population	Propotion of C. albicans	Reference
Denmark	2003	11.0	63%	Arendrup et al. 2005
Denmark	2004–2006	10.4	60%	Arendrup et al. 2008
Denmark	2010–2011	9.4	52%	Arendrup et al. 2013
Denmark	2012–2015	8.4	48%	Astvad et al. 2018
Finland	1995–1999	1.9	70%	Poikonen et al. 2003
Finland	2004–2007	2.9	67%	Poikonen et al. 2010
Iceland	1980–1999	4.3	64%	Asmundsdottir et al. 2002
Iceland	2000–2011	5.7	56%	Asmundsdottir et al. 2013
Norway	1991–2003	2.4	70%	Sandven et al. 2006
Norway	2004–2012	3.9	68%	Hesstvedt et al. 2015
Sweden	2005–2006	4.2	61%	Ericsson et al. 2013
Sweden	2015–2016	4.7	55%	Klinsborg et al. 2018
France	2001–2010	2.5	NA	Bitar et al. 2014
Scotland	2005–2006	4.8	50%	Odds et al. 2007
Scotland	2012–2013	4.1	53%	Rajendran et al. 2016
Spain/Barcelona	2002–2003	4.3	51%	Almirante et al. 2005
Spain	2010–2011	8.1	45%	Puig-Asensio et al. 2014
USA/ Baltimore,	1998–2000	24.0	43%	Hajjeh et al. 2004
Connecticut		7.0	49%	
USA/Iowa	1998–2001	6.0	58%	Diekema et al. 2002
USA/Atlanta,	2008–2011	13.3	41%	Cleveland et al. 2012
Baltimore		26.2	34%	
USA/Atlanta,	2008–2013	14.1–9.5	40%	Cleveland et al. 2015
Baltimore		30.9–14.4	32%	
USA	2012–2016	8.7	39%	Toda et al. 2019
Canada	1999–2004	2.9	51%	Laupland et al. 2005
Australia	2001–2004	1.8	47%	Chen et al. 2006
Australia	2014–2015	2.4	44%	Chapman et al. 2017

2.3.4 BURDEN OF PERSISTENT AND RECURRENT CANDIDEMIA

Persistent candidemia (PC) lacks a homogenous definition. This is a significant problem when the incidence of PC is evaluated. The proportion of PC in patients with candidemia has been evaluated in some randomised, clinical trials, but mostly in retrospective cohort studies. Studies have been conducted in neonatal and child populations and in adult populations (Fu et al. 2018, Hammoud et al. 2013, Robinson et al. 2012, Levy et al. 2006, Agnelli et al. 2019). Persistently positive blood cultures were observed in 8–15% of patients with candidemia in randomised clinical trials (Pappas et al. 2007, Reboli et al. 2011, Queiroz-Telles et al. 2008, Mora-Duarte et al. 2002). The rate of PC among all patients with candidemia varied from 11–59% among adult patients (Agnelli et al. 2019, Kang et al. 2017, Li et al. 2018, Chen et al. 2012, Luzzati et al. 2005). In these studies, the definition of PC varied from >2 days to >7 days. In child and neonate populations the rates of PC ranged from 24–60% (Levy et al. 2006, Zaoutis et al. 2004, Fu et al. 2018, Robinson et al. 2012).

Some patients develop a recurrent episode of *Candida* BSI after surviving an initial episode. The time between the initial and the recurrent episode can range considerably, from weeks to years. LR candidemia is an uncommon finding, which complicates the evaluation of the incidence. A population-based study conducted in Iceland reported the occurrence of LR candidemia to be 4.4% of candidemic patients who survived the initial episode (Asmundsdottir et al. 2012). A nationwide study from Norway reported LR candidemia caused by the same *Candida* species in 2.4% of patients with candidemia (Sandven et al. 2006). In other studies, patients with LR candidemia have represented 1.5–9.2% of all patients with candidemia (Munoz et al. 2016, Nucci et al. 2010, Lai et al. 2019, Ghezzi et al. 2017, Antworth et al. 2013).

2.3.5 OUTCOME OF CANDIDEMIA

Candidemia is associated with considerably high mortality. Patients with candidemia are usually severely ill and may also die due to their underlying diseases.

The overal mortality of candidemia has been evaluated in Europe in a meta-analysis, and an increase in candidemia mortality was observed from 2000–2019 (Koehler et al. 2019). During the period, populations with immunosuppression and more complex surgical procedures have grown, which may have influenced mortality. On the other hand, the availability of effective and less toxic antifungal drugs have increased during the same period.

In population-based studies, the 30-day mortality of candidemia (Table 3) have been 30–44% in Europe and 28–36% in the US and in Australia

(Asmundsdottir et al. 2013, Lausch[b] et al. 2018, Hesstvedt et al. 2019, Poikonen et al. 2010, Almirante et al. 2005, Rajendran et al. 2016, Hajjeh et al. 2004, Cleveland et al. 2012, Chen et al. 2006). In a prospective, sequential, hospital population-based study from seven Euroepan countries, the 30-day overall mortality was 38% (Tortorano et al. 2004). In cohort studies, the 30-day fatality has been 35– 49% (Garnacho-Montero et al. 2013, Arendrup et al. 2011, Berdal et al. 2014, Luzzatti et al 2011, Diekema et al. 2012, Velasco and Bigni 2008). However, the mortality rate among patients treated in ICU have been reported to be even 50% or more (Lortholary et al. 2014, Colombo et al. 2014, Schroeder et al. 2020).

In 1988, a retrospective matched case-control study from the US reported attributable mortality of candidemia to be 38% (Wey et al. 1988). In 2003 and 2005, matched case-control studies reported attributable mortality of candidemia to be 49% and 19–24% (Morgan et al. 2005, Gudlaugsson et al. 2003). During these studies, standard treatment of candidemia was amphotericin B or fluconazole (Rex et al. 2000). After the introduction of echinocandins, a case-control study from Germany reported a still substantial attributable mortality of 26% in candidemia cases (Cornely et al. 2020).

Cause-specific mortality rates associated with different Candida species differ. Among the five most common *Candida* species, *C. albicans* is associated with the highest cause-specific mortality in adults, while *C. parapsilosis* with lower cause-specific mortality than the other *Candida* species (Pappas et al. 2003).

Table 3 *30-day case fatality in candidemia reported in population-based studies.*

Country/area	Time period	Case fatality within 30 days	Reference
Denmark	2010–2011	43%	Lausch[b] et al. 2018
Finland	1995–1999	35%	Poikonen et al. 2003
Finland	2004–2007	35%	Poikonen et al. 2010
Iceland	2000–2011	30%	Asmundsdottir et al. 2013
Norway	2008–2012	36%	Hesstvedt et al. 2019
Scotland	2012–2013	41%	Rajendran et al. 2016
Spain/Barcelona	2002–2003	44%	Almirante et al. 2005
Spain	2010–2011	31%	Puig-Asensio et al. 2014
USA/Baltimore and Connecticut	1998–2000	36%	Hajjeh et al. 2004
USA/Atlanta, Baltimore	2008–2011	29% 28%	Cleveland et al. 2012
Australia	2001–2004	28%	Chen et al. 2006

2.4 DIAGNOSTIC METHODS

The diagnosis of IFDs combines an assessment of patient-related risk factors, clinical symptoms and signs of the disease, and results from imaging procedures and laboratory tests. Traditional diagnostic methods, histopathology and culture, are still considered the gold standard for the diagnosis of IFD (Donnelly et al. 2020). However, these methods have limitations, particularly their low sensitivity, and the fact that cultures are time-consuming. Early and accurate identification of a causative fungus is crucial for appropriate management of IFDs. Newer, non-culture based methods present an opportunity for more accurately diagnoses, and to shorten the time to diagnosis of a fungal disease.

2.4.1 HISTOPATHOLOGY AND CULTURE

Histopathology

Histopathologic examination of a tissue specimen is a traditional tool for the diagnosis of IFDs. It requires a directed biopsy of an affected site, and the fungal elements are best visualised by specific stains. Basic stains for the identification of fungal morphologic characteristics are methenamine silver stain and Periodic acid-Schiff (PAS). PAS is a multistep method and requires several different reagents; it has been replaced in many laboratories by the procedure of the calcofluor white staining. The calcofluor white stain is a non-specific fluorochrome, and the procedure is rapid, when specimens can be observed. Giemsa stain can be used for the visualisation of intracellular forms of *Hisoplasma capsulatum* and colloidal carbon wet mounts for detection of encapsulated microorganisms, especially *Cryptococcus* species. There are also other special stains for identification of fungi (Jorgensen and Pfaller 2015).

The advantage of histopathologic examination is its ability to provide rapid, early presumptive diagnosis of the disease, and it is cost-effective. However, fungal classification by histopathology is difficult and may lead to diagnostic errors. Misclassification of the fungal organism occurs in histopathologic examination in at least 20% of cases (Guarner and Brandt 2011). Retrospective analyses, when correlating the results of culture and histopathologic examination, have reported that the overall accuracy for microscopic morphological techniques can vary from 20–80% (Sangoi et al. 2009, Schofield et al. 2007, Tarrand et al. 2003). Reliable identification of a fungal species based solely on morphological criteria in histopathology is generally very difficult, and identification requires highly skilled personnel. A

histopathologic examination can reveal invasion of tissues and vessels or an inflammatory reaction of the host to the fungus which can help to determine whether the fungus represents contamination, colonisation or a true infection (Sangoi et al. 2009, Guarner and Brandt 2011).

Culture

Culture is a key element in diagnosing IFDs. Like histopathology, a culture requires a directed sample from the infected site, which may be difficult to acquire. Reliable results can be obtained from normally sterile body fluids (e.g. blood, CSF, pleural effusion, synovial fluid) and biopsy materials. Detection of the fungal organism from culture depends on several variables, including fungal features, specimen volume, organism concentrations within the sample, and conditions during the culture procedure (Jorgensen and Pfaller 2015).

The diagnosis of IFD with blood and sterile-site cultures is limited by inadequate sensitivity (Clancy and Nguyen 2013). In general, yeast are easier to isolate from clinical samples than moulds (Jorgensen and Phaller 2015). Blood cultures are often negative in most medically important mould infections, e.g. *Aspergillus* (Ruhnke et al. 2018). In a retrospective analysis, blood cultures were positive only in 10% of cases with documented pulmonary aspergillosis (Girmenia et al. 2001). The sensitivity of blood cultures to diagnose invasive *Candida* infections is limited as well. Retrospective autopsy studies, conducted during time-period of 1984–2008, have demonstrated that the sensitivity of antemortem blood cultures from patients with autopsy-proven invasive disseminated candidiasis has ranged from 21% to 71% (Ness et al. 1989, Berenguer et al. 1993, van Burik et al. 1998, Kami et al. 2002, Thorn et al. 2010). In candidemia, blood cultures remain the most important method to detect the causative agent. In candidemia, the median time that blood cultures turn positive is 2–3 days, which may delay the diagnosis and complicates evaluation of the mycotic response to therapy (Pfeiffer et al. 2011, Arendrup et al. 2011). *C. glabrata* and *C. parapsilosis* are often associated with longer incubation times, and *C. tropicalis* and *C. krusei* with shorter times (Arendrup et al. 2011, Gokbolat et al. 2017).

2.4.3 NON-CULTURE BASED METHODS

β-D-Glucan detection

β-D-Glucan (BDG) is a cell wall polysaccharide that is found in most medically important fungi, with some exceptions (e.g. *Cryptococcus* species, *Mucorales* and *Blastomyces dermatitidis)* (Theel and Doern 2013). BDG is a panfungal marker for invasive fungal infections, but it cannot distinguish between different fungi, e.g. *Candida* and *Aspergillus* (Clancy and Nguyen 2018). The test is mostly performed from serum, which makes the specimen easily accessible. It has also been studied e.g. from CSF (Lyons et al. 2015). Several commercial assays have been developed. The major limitation of BDG testing is false positivity. Factors that may cause false-positive results include haemodialysis or haemofiltration, some Gram-positive bacteria, enteral nutrition, *Candida* and mould colonisation, blood products and immunoglobulins, surgical gauze, and certain β-lactam antimicrobials (Theel and Doern 2013). Many of these factors are common among hospitalised patients. Repeated measurements have been suggested to increase the diagnosis accuracy, where positivity is defined by two consecutive positive results rather than one (Hanson et al. 2012). However, the diagnostic benefit of BDG might be its high negative predictive value in a setting when the prevalence of a fungal infection is low (Antinori et al. 2016).

Polymerase chain reaction

Polymerase chain reaction (PCR) tests are molecular assays for the direct detection of fungal DNA in a clinical specimen (Kourkoumpetis et al. 2012). The DNA is first extracted from the sample, and the extracted DNA is detected with primers. PCR ampilifies the segments of DNA in a series of cycles of temperature changes, which is based on an enzymatic reaction. The repeated cycles of denaturation of the template DNA, annealing of the primers to their complementary sequences, and primer extension result in the exponential production of the specific target fragments. The PCR product is usually detected by electrophoresis or fluorescent probe techniques. (Barer et al. 2019).

The PCR assays can detect a broad range of fungi (panfungal) or they can be customised to detect specific genera or species. Panfungal assays detect fungal DNA in a clinical specimen via universal fungal primers. The most commonly used targets are one or more regions of the rRNA gene cluster (the international transcribed spacers 1 and 2 [ITS1 and ITS2] and the D1/D2 regions of the 28S rRNA gene) (Kidd et al. 2020, Kourkoumpetis et al. 2012). As PCR can provide species identification, PCR assays have a clear advantage over tests such as mannan antigen/anti-mannan antibody or BDG. PCR assays can be performed from different types of clinical

specimens, including blood, deep tissues and fluids (including fresh tissue and formalin-fixed paraffin embedded tissue samples), and also non-sterile samples e.g. bronchoalveolar fluid (BAL) (Kidd et al. 2020). These tests measure fungal DNA from viable and non-viable cells as well as from free-floating DNA (Kourkoumpetis et al. 2012). One advantage of PCR assays is their ability to detect and identify rare pathogens. PCR assays have a low threshold of fungal cell detection and their higher sensitivity can be an advantage, however, contamination or false positivity can also be a challenge (von Lilienfeld-Toal et al. 2009, Klingspor and Jalal 2006, McMullan et al. 2008). There are multiple commercial and in-house tests available. The heterogeneity of assays and study designs complicates the comparison of the tests.

T2 magnetic resonance assay

T2 magnetic resonance assay is a nanodiagnostic method to diagnose candidemia. The test uses magnetic resonance to detect *Candida* species rapidly in a whole blood specimen; and the test does not require viable organisms from the specimen (Neely et al. 2013). The T2Candida panel (T2C; T2 Biosystems, Lexinton, MA, USA) detects the five most common *Candida* species (*C. albicans*/*C. tropicalis*, *C. glabrata*/*C. krusei* and *C. parapsilosis*) (Pfaller et al. 2016). Per-sample sensitivity and specificity of T2Candida was 91% and 99%, respectively, and the mean time to *Candida* detection and identification was 4.4±1.0 h in a multicentre trial (DIRECT) (Mylonakis et al. 2015). The benefit of this test is in its rapidity, but it only detects five *Candida* species. The test has also been studied to diagnose invasive candidiasis, especially intra-abdominal candidiasis, however, results were modest (Arendrup et al. 2019, Lamoth et al. 2020).

Mannan antigen and antimannan antibodies (Mn/A-Mn)

The combination of mannan antigen and anti-mannan antibodies (Mn/A-Mn) is a non-invasive, non-culture-based method for diagnosing invasive candidiasis. Mannan is a major component of the *Candida* cell wall and is one of the main *Candida* antigens that circulate during *Candida* infection (Mikulska et al. 2010). Different tests have been developed to detect mannan antigen or antimannan antibodies in serum. Low serum concentrations and rapid clearance from serum may limit the performance of *Candida* antigen tests (Ellepola and Morrison 2005). The combination of Mn/A-Mn has also been evaluated as a screening tool to diagnose invasive candidiasis in ICU and immunocompromised patients, with limited results (Duettmann et al. 2016, León et al. 2016). A meta-analysis of 14 studies revealed that Mn/A-Mn had sensitivities and specificities for invasive candidiasis of 58%/93%, and 59%/86% (Mikulska et al. 2010). In the meta-analysis, the sensitivity and specificity for the combination of Mn/A-Mn assay were 83% and 86%, respectively. Many people are colonised with

Candida species and may have low antibody levels even though they do not have a disease caused by *Candida* species (Jorgensen and Pfaller 2015). This may be problematic when the detection of antibodies specific to *Candida* are utilised as a diagnostic tool for infection.

Galactomannan aspergillus antigen

Galactomannan is a polysaccharide, which is a major component of the *Aspergillus* cell wall and is released by *Aspergillus* species during growth (Klont et al. 2004). A commercially available test is validated for use in serum and BAL specimens, but it is also used for detection of galactomannan from specimens of other body fluids, including CSF and urine. The method currently used is a double-sandwich ELISA using monoclonal antibody directed against galactomannan (Stynen et al. 1995). The test has been endorsed in microbiological criteria for the diagnosis of invasive aspergillosis in guidelines (De Pauw et al. 2008, Ullmann et al. 2018). The diagnosis of invasive aspergillosis is challenging and a tissue specimen for culture and histopathology examination is not always possible to achieve. Circulating galactomannan can be detected at a median time of 5–8 days before clinical signs and symptoms of invasive aspergillosis become visible (Verweij et al. 1997). The utility of galactomannan may be used to confirm a presumed diagnosis of invasive aspergillosis or to screen high risk patients to identify infection at an early stage of the disease (Miceli and Maertens 2015). It has been reported that the concentration of circulating galactomannan correlates with the burden of the disease. The test may be used to monitor treatment efficacy (Verweij et al. 1997, Hammarström et al. 2018).

2.4.4 IDENTIFICATION OF CANDIDA SPECIES AND ANTIFUNGAL SUSCEPTIBILITY TESTING

Identification of a *Candida* isolate recovered from clinical specimens is traditionally based on the biochemical, and morphological features of the yeast after growth in specialised media (Jorgensen and Pfaller 2015). However, phenotypic characteristics of a fungus may be difficult to identify and classification at the species level may be challenging. In recent decades, molecular methods have become a significant part of fungal identification. A method based on mass spectrometry is reliable and has become widely used (Cassagne et al. 2016). Matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) is a mass spectrometry method that identifies the genus and species of an organism. MALDI-TOF can identify a wide variety of both bacterial and fungal organisms as soon as an organism is detectable in a pure

culture (Croxatto et al. 2012). The identification of the microorganism is obtained by searching databases containing the MS spectra of proteins and peptides extracted from the organism of interest, and then uses scoring algorithms to compare the analysed spectra with a known reference (Cassagne et al. 2016). This method has significantly reduced the time from blood culture is draw to identification of the causative organism of BSI at the species level (Idelevich et al. 2014). A large two-centre study with 346 positive yeast blood cultures revealed that direct MALDI-TOF identification correctly identified of 96% of *C. albicans* and 87% of non- albicans *Candida* species compared with conventional culture-based methods (Spanu et al. 2012). The development of MALDI-TOF technology has been a remarkable advancement for patient care; species identification has become faster, and targeted treatment can be initiated earlier. It is an accurate, rapid, and cost-effective method of bacteria and yeast characterisation and identification (Wattal et al. 2017, van Veen et al. 2010).

Molecular methods based on PCR for species identification are also widely available. Molecular techniques are reliable and allow accurate identification of *Candida* species. A variety of next-generation sequencing technologies are also available. In particular, next-generation sequencing allows fungal genotyping, epidemiological outbreak investigations, and characterisation of drug resistance (Consortium OPATHY and Gabaldón 2019).

Susceptibility testing is used to determine the in vitro potency of antifungal agents. Phenotypic assays for the susceptibility of yeasts are standardized methods and are currently used (Cuenca-Estrella 2014). Molecular tools have good potential for providing more rapid results than phenotypic methods and have the advantage of determining the underlying genetic basis of antifungal resistance mechanisms. However, many of the molecular methods are not standardised (Perlin and Wiederhold 2017, Kidd et al. 2020).

The Clinical and Laboratory Standard Institute (CLSI) and European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) have both created a standardized method of antifungal susceptibility testing of yeasts based on broth microdilution methods (Clinical Laboratory Standards Institute 2008, Rodríguez-Tudela et al. 2010). Many clinical laboratories utilise several commercial, agar dilution and disc diffusion methods that correlate highly with reference procedures (Cuenca-Estrella 2014).

2.5 RISK FACTORS

2.5.1 RISK FACTORS FOR CANDIDEMIA

Candidemia is primarily a health-care associated entity in patients with several risk factors. The risk factors for candidemia have been comprehensively evaluated. Several prospective and retrospective case-control and cohort studies have been conducted. Some studies have also been conducted in a specific risk populations (e.g. ICU patients, neonates, neonate ICUs, and patients with haematological or solid organ malignancy). Colonisation with *Candida* species is a significant prerequisite for candidemia and the frequency of infection increases with an increased number of sites colonised (León et al. 2016, Voss et al. 1994). However, not all patients with *Candida* colonisation develop fungemia.

Patients at the highest risk for candidemia include those who are immunocompromised and receiving intensive care (Table 4). These patients undergo multiple invasive procedures and intensive medical and surgical treatments. Most candidemia patients have numerous simultaneous risk factors that predispose them to candidemia and they are typically critically ill (Pappas et al. 2003, Wey et al. 1989, Wenzel 1995). Malignancies, especially haematological malignancies, are common risk factors for the development of candidemia (Komshian et al. 1989, Tortorano et al. 2002, Laupland et al. 2005, Horn et al. 2009). Neutropenia and chemotherapy are associated with an increased risk of fungemia in patients with malignancies (Komshian et al. 1989, Horn et al. 2009, Karabinis et al. 1988). Recent GI surgery predisposes patients to candidemia, especially among patients who have anastomotic leakage or have had repeat laparotomies (Karabinis et al. 1988, Blumberg et al. 2001, Ortíz Ruiz et al. 2016, Ostrosky-Zeichner et al. 2007). Transplant recipients and premature newborns are also at increased risk (Laupland et al. 2005, Almirante et al. 2006, Krcmery and Barnes 2002).

In addition, many invasive procedures increase the risk for candidemia. Presence of a central venous catheter (CVC) and total parenteral nutrition have been identified as risk factors in several studies (Wey et al. 1989, Wenzel 1995, Falcone et al. 2017, Bross et al. 1989, Luzzati et al. 2013, Poissy et al. 2020). The use of broad-spectrum antimicrobials leading to overgrowth of *Candida* species in the GI tract, use of glucocorticoids and haemodialysis have also been identified as risk factors for candidemia (Wenzel 1995, Wey et al. 1989, Bross et al. 1989, Falcone et al. 2017, Poissy et al. 2020).

Intravenous drug use (IDU) is a more infrequent risk factor for candidemia, but the significance might be increasing (Poowanawittayakom et al. 2018). IDU has been reported to be an underlying condition in 10% of patients with candidemia (Zhang et al. 2020). Candidemia cases in patients with history of IDU are

more likely to be community acquired than patients with candidemia and no history of IDU (Zhang et al. 2020).

Table 4 *Risk factors for candidemia.*

Critical illness	Presence of central venous catheter
Gastrointestinal surgery	Use of broad-spectrum antimicrobials
Haematological malignancies	Use of glucocorticoids
Transplantation	Haemodialysis
Low birth weight neonates	

Certain risk factors are associated with candidemia due to different *Candida* species (Table 5). Two distinct risk factors for candidemia caused by non-*albicans Candida* species are exposure to fluconazole and the presence of CVC (Chow et al. 2008). Patient characteristics have considerable influence on the species distribution. The frequency of candidemia caused by *C. parapsilosis* decreases with age, and *C. parapsilosis* is associated with infections in neonates (Almirante et al. 2006, Pfaller[b] et al. 2002). *C. glabrata* is more common in the elderly (Hesstvedt et al. 2015, Astvad et al. 2018). Antifungal use has a wide impact on the distribution of *Candida* species. Previous use of fluconazole is particularly associated with *C. glabrata* and *C. krusei*, both of which have lower sensitivity to fluconazole (Lortholary et al. 2011, Hachem et al. 2008, Krcmery and Barnes 2002). Infections caused by *C. glabrata, C. krusei,* and *C. parapsilosis* are also associated with the recent use of caspofungin in a large study conducted in France (Lortholary et al. 2011). Patients with haematological malignancies and stem cell transplantation have more *C. krusei* and *C. tropicalis* infections than other *Candida* species (Lortholary et al. 2017, Horn et al. 2009, Cornely[a] et al. 2015). *C. glabrata* is more frequent in patients with the presence of CVC, recent GI surgery, diabetes mellitus and malignancies (Segireddy et al. 2011, Khatib et al. 2016, Cohen et al. 2010). *C. parapsilosis* is associated with the use of parenteral nutrition, nosocomial outbreaks, central catheters, and other implanted devices (Almirante et al. 2006, Krcmery and Barnes 2002). In Finland, candidemia outbreak caused by *C. parapsilosis* was reported in neonate ICU during a 12-year period (Saxen et al. 1995, Sarvikivi et al. 2005). Fluconazole prophylaxis was used to control the outbreak, however, development of fluconazole resistance of the causative clone was described during the outbreak (Sarvikivi et al. 2005). Understanding the differences between the *Candida* species is important to identify the patients at risk for candidemia and to improve the management of severe *Candida* infections.

Table 5 *Risk factors and clinical conditions that have been associated with different Candida species.*

***Candida* species**	**Risk factor**
C. glabrata	Previous use of fluconazole
	Older age
	Malignancies
	Diabetes mellitus
	Gastrointestinal surgery
C. krusei	Previous use of fluconazole
	Haematological malignancies
	Stem cell transplantation
C. parapsilosis	Neonate
	Central catheters
	Parenteral nutrition
C. tropicalis	Haematological malignancies

2.5.2 RISK FACTORS FOR PERSISTENT AND RECURRENT CANDIDEMIA

The risk factors for PC are partly different than those for overall candidemia. Established risk factors for candidemia, such as malignancies, transplantation, prior GI surgery, and exposure to antimicrobials or steroids, have not been associated with PC even though they have been reported as risk factors for candidemia (Kang et al. 2017, Agnelli et al. 2019). Initial treatment with a suboptimal dose of fluconazole appears to increase the risk of PC (Li et al. 2018). One of the most important risk factors for PC appears to be the presence of CVC during the diagnosis of candidemia (Li et al. 2018, Kang et al. 2017, Chen et al. 2012). Other major risk factors for PC are metastatic infection foci, such as thrombophlebitis, endocarditis, and deep tissue abscesses (Nucci and Perfect 2008).

Risk factors for recurrent candidemia have been analysed in some studies. Study from Iceland reported that patients with LR candidemia were younger and more likely to have underlying GI disease than patients with a single episode of candidemia (Asmundsdottir et al. 2012). An analysis conducted in Taiwan also revealed that patients with LR candidemia were younger (Lai et al. 2019). Patients with LR candidemia were more likely to require echinocandin treatment, and to have a higher rate of treatment failure. The group from Taiwan reported that 70% of the recurrent episodes were caused by non-*albicans Candida* species, and underlying GI disease

and neurological sequelae were independent risk factors for the development of LR candidemia (Lai et al. 2019). Munoz et al. evaluated LR candidemia in a matched case-control study (Munoz et al. 2016) and also observed that patients with recurrent candidemia were younger. Candidemia caused by *C. parapsilosis* and underlying GI disease were independent risk factors for LR candidemia in their study.

2.6 MANAGEMENT OF CANDIDEMIA

2.6.1 TREATMENT GUIDELINES FOR CANDIDEMIA

The recognition of risk factors for candidemia is crucial for the management as treatment is often initiated empirically before the diagnosis of candidemia is confirmed. Optimising the management of candidemia includes follow-up blood cultures, susceptibility testing, use of antifungal agents, and management of complications and indwelling devices. The first guideline from the Infectious Diseases Society of America (IDSA) for the management of candidiasis was published in 2000 and revised in 2009 as well as in 2016 (Rex et al. 2000, Pappas et al. 2009, Pappas et al. 2016). The European guideline for the diagnosis and management of *Candida* diseases was published in 2012 (Cornely et al. 2012).

The present text focuses on the treatment of candidemia in non-neutropenic adult patients. *Candida* species isolated from blood culture defines candidemia and should always be considered a relevant finding that requires treatment (De Pauw et al. 2008). Follow-up blood cultures are essential for determining resolution of candidemia. They guide the duration of antifungal treatment and detection of possible metastatic infection foci complicating candidemia. The ESCMID guideline recommends taking at least one control blood culture per day until culture results come back negative. The IDSA guideline recommends follow-up blood cultures to be performed every or every other day to identify the time point at which the candidemia has been cleared (Cornely et al. 2012, Pappas et al. 2016). Susceptibility testing is also an essential part of guiding candidemia treatment. It provides information on the local epidemiological situation and reveals possible increasing antifungal resistance. Susceptibility testing is a prerequisite for a possible step-down oral azole treatment.

Table 6 Choise of antifungal agent based on the *Candida* species finding during the management of candidemia.

Candida species	Fluconazole	Voriconazole	Echinocandins	Amphotericin B formulations
C. albicans	+++	++	+++	++
C. tropicalis	+++	++	+++	++
C. parapsilosis	+++	++	+++	++
C. glabrata	-/+	+	+++	++
C. krusei	-	+	+++	++

+++ preferred initial therapy, clinical active, ++ alternative therapy, clinical active, + less clinical activity, -/+ possible clinical activity, - no clinical activity.
(Cornely et al. 2012, Pappas et al. 2016).

The antifungal agents that are mostly used to treat candidemia (Table 6) include echinocandins (caspofungin, anidulafungin, and micafungin), azoles (fluconazole, voriconazole, posaconazole, isavuconazole), and polyenes (amphotericin B deoxycholate, liposomal amphotericin B, amphotericin B lipid complex). Echinocandins are the newest class of antifungal agents. Pharmacological characteristics, clinical efficacy, and safety profiles are similar among different echinocandins (Pappas et al. 2007, Suh et al. 2020, van der Geest[a] et al. 2016). They demonstrate fungicidal activity and good biofilm penetrations. They are also well tolerated, and have very few drug-drug interactions (Chandrasekar and Sobel 2006, Deresinski and Stevens 2003, Vazquez and Sobel 2006). However, echinocandins are only available as parenteral products, and do not reach therapeutic concentrations in the eye, central nervous system, and urine. Therefore, they are considered inappropriate antifungals for the treatment of infections at these sites (Antinori et al. 2016).

Azoles are widely used antifungals. However, azoles have less activity against *C. glabrata* and *C. krusei* than against other *Candida* species (Pappas et al. 2016). Fluconazole is the most commonly used azole for treatment of candidemia. Fluconazole has a high oral bioavailability and is also available for intravenous administration. Fluconazole has low plasma protein binding, and it circulates as an active drug. It is distributed evenly in tissues, including the central nervous system, the eyes, and urine. The tolerability of fluconazole is good, but it has some drug interactions (Eggimann et al. 2003, Debruyne and Ryckelynck 1993). Voriconazole is effective for candidemia; however, it has few advantages over fluconazole, and voriconazole is associated with greater toxicity than fluconazole (Kullberg et al. 2005). Isavuconazole is a broad-spectrum azole and the newest member of the azoles. It has an excellent in vitro activity against *Candida* species (Pfaller et al. 2013). However, when isavuconazole was compared with caspofungin in a large double-blind

trial in the treatment of candidemia and other invasive *Candida* infections, isavuconazole did not meet the criteria for noninferiority. All-cause mortality was similar in both groups (Kullberg et al. 2019). Voriconazole and isavuconazole are primary antifungal for mould infections.

Polyenes are broad-spectrum antifungals, and there is long-term clinical experience of polyenes for the treatment of candidemia. The main polyene is amphotericin B, which is fungicidal at high concentrations. Amphotericin B has more side-effects than other antifungals (Anaissie et al. 1996, Ullmann et al. 2006). Lipid formulation amphotericin B is an alternative for treatment of candidemia, especially if an azole- and echinocandin-resistant *Candida* infection is suspected (Pappas et al. 2016).

The guidelines recommend an echinocandin as the initial antifungal agent for the treatment of candidemia (Cornely et al. 2012, Pappas et al. 2016). The IDSA guideline recommends fluconazole as an alternative to an echinocandin for initial treatment of candidemia in patients who are not critically ill and not infected by a resistant *Candida* species. The increasing prevalence of *Candida* species with decreased susceptibility to fluconazole is a significant issue worldwide and supports the recommendation of echinocandin as an initial treatment choice (Guinea 2014, Cleveland et al. 2012, Lortholary et al. 2011). A study comparing an echinocandin to fluconazole showed anidulafungin to be non-inferior and suggested a strong trend towards more favourable outcomes with anidulafungin (Reboli et al. 2007). A step-down strategy recommends switching echinocandin to fluconazole to simplify the treatment. This is a suitable option, when a patient is clinically stable, blood cultures have turned negative, and if the *Candida* species is susceptible to fluconazole. The ESCMID guideline recommends step-down after 10 days of intravenous treatment; the IDSA guideline recommends the transition within 5–7 days following the initiation of intravenous antifungal treatment (Cornely et al. 2012, Pappas et al. 2016). The feasibility of the step-down therapy has also been shown in studies conducted in critically ill patients (van der Geest[b] et al. 2016, Vazquez et al. 2014, Bailly et al. 2015).

The appropriate duration of antifungal treatment has not been studied extensively. There is a lack of randomised control trials. The duration of antifungal treatment depends on the diagnosed metastatic infection foci. Both the ESCMID and the IDSA guidelines recommend a therapy duration for uncomplicated candidemia of 14 days after the end of the blood culture positivity and symptoms related to candidemia have resolved (Cornely et al. 2012, Pappas et al. 2016). The recommendation is based more on consensus rather than analysed evidence. In a retrospective analysis, delayed complications of candidemia seemed not to be correlated with the duration of the antifungal treatment (Oude Lashof et al. 2003). Disseminated disease is usually diagnosed during the treatment, and is a reason for a longer treatment duration.

Treatment of candidemia includes procedures to diagnose deep organ involvements. Further clinical diagnostic workup is especially important when blood

culture positivity is persistent. Ocular involvements occured in 16% of candidemic patients in a prospective analysis (Oude Lashof et al. 2011), and in 20–22% of patients with candidemia in recent retrospective analyses (Son et al. 2019, Kato et al. 2018). Chorioretinitis (85% of cases with ocular involvements) was more common than endophthalmitis, and most of the patients with ocular lesions found at fundoscopy did not exhibit symptoms related to the ocular involvement (Oude Lashof et al. 2011). A prospective cohort study reported that 6% of candidemic patients had *Candida* endocarditis (Fernández-Cruz et al. 2015). The ESCMID guideline recommends transoesophageal echocardiography and fundoscopy as diagnostic procedures for patients with candidemia, while the IDSA guideline recommends a dilated ophthalmological examination for all nonneutropenic patients with candidemia (Cornely et al. 2012, Pappas et al. 2016). Additionally, if CVC or a peripherally inserted central catheter is present, the clinician should consider the possibility of a thrombus and search for thrombi as a complication of candidemia (Cornely et al. 2012). Early removal of CVC is strongly recommended as a part of candidemia treatment, especially when the source is presumed to be the CVC and the catheter can be removed safely (Cornely et al. 2012, Pappas et al. 2016). The decision on removal should be individualized for each patient.

2.6.2 ADHERENCE TO TREATMENT GUIDELINES

Guidelines aim to improve and facilitate the diagnosis and management of candidemia. However, routine clinical practice is often not straightforward. The complexity of guidelines might complicate the implementation of the recommendations in routine clinical practice. Adherence to the guidelines is not routinely monitored. In 2018, the European Confederation of Medical Mycology (ECMM) published a score to quantify clinical management of candidemia and measure adherence to guidelines (Mellinghoff[b] et al. 2018). The Equal Candida Score (ECMM QUALity of Clinical Candidemia Management Score) is a marker that measures the quality of diagnostic and therapeutic management of candidemia (Table 7), when treatment is intended to cure. The score also provides a tool for antifungal stewardship. It is based on the recommendations of the current ESCMID and IDSA guidelines (Cornely et al. 2012, Pappas et al. 2016). The score consists of the key factors of diagnostic, follow-up, and treatment procedures (Table 7). The maximum score is 19 for patients without CVC and 22 for patients with CVC.

Table 7 Equal Candida Score adapted from Mellinghoff[b] et al. 2018.

Diagnostic factors	**Patients with CVC**	**Patients without CVC**
Initial blood culture (40 mL)	3	3
Species identification	3	3
Susceptibility testing	2	2
Echocardiography	1	1
Ophthalmoscopy	1	1
Treatment factors		
Echinocandin treatment	3	3
Step down to fluconazole	2	2
Treatment for 14 days after first negative follow-up blood culture	2	2
Central venous catheter removal		
≤24 h from diagnosis	3	
24–72 h from diagnosis	2	
Follow-up factor		
Follow-up blood culture (at least one per day until negative)	2	2
Maximum score	22	19

CVC, central venous catheter.

3 AIMS OF THE STUDY

The objectives of this study were:

I To evaluate the clinical use of panfulgal PCR from deep tissue specimens in diagnosing invasive fungal diseases.

II To study *Candida* species distribution in HUS and risk factors associated with 30-day mortality in candidemia. We also evaluated the association between candidemia mortality and early start of an effective antifungal treatment.

III To analyse the risk factors for persistent candidemia in adult patients.

IV To analyse the characteristics of late recurrent candidemia in adult patients.

4 MATERIALS AND METHODS

4.1 OVERVIEW OF THE STUDY

The study consisted of two parts (Table 8). The first part (study I) evaluated the clinical use of panfungal PCR in diagnosing IFDs. The second part consisted of candidemia analyses (study II, III, IV). Both parts were conducted in HUS. Microbiological methods are described in original publications, however, this thesis is focused on the clinical aspects of IFDs and not on microbiological methods. The research board of the Inflammation Center at the Helsinki University Hospital has approved the study protocol.

Table 8 *Overview of the studies.*

	Study	Time period	Design		N
Part I	**I**	2013-2015	Cohort	Specimens	307
				Patients	296
Part II	**II**	2007-2016	Cohort	Patients	350
	III	2007-2016	Cohort	Patients with persistent candidemia	75
				Patients with non-persistent candidemia	151
	IV	2007-2016	Cohort	Patients with recurrent candidemia	20
				Patients with a single episode of candidemia	309

4.2 STUDY DESIGNS

4.2.1 DESIGN AND PATIENTS OF THE PANFUNGAL PCR STUDY (I)

Study I was a retrospective, observational cohort analysis. The study population consisted of all patients with a result from a panfungal PCR test analysed at Helsinki University Hospital Laboratory (HUSLAB) between January 2013 and December 2015. The patients were identified from the microbiological database. All age groups were included. We compared the PCR results with microscopy and culture results, which were the standard diagnostic methods. The likelihood of fungal infection was classified with the criteria of 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) (De Pauw et al. 2008).

HUSLAB analysed panfungal PCR results from every hospital district in Finland during the study period (Figure 1). In our study, we included patients who were treated at tertiary or secondary care hospitals in HUS. The hospitals included Aurora Hospital, Meilahti Tower and Triangle Hospitals, Children´s Hospital, Eye and Ear Hospital, Hyvinkää Hospital, Jorvi Hospital, Kätilöopisto Maternity Hospital, Lohja Hospital, Porvoo Hospital, Raasepori Hospital, Skin and Allergy Hospital, Surgical Hospital, Töölö Hospital, Women´s Hospital and Peijas Hospital. Many of the patients were treated in hospital districts other than HUS, and we did not have access to the patients' medical records. Patients were excluded if clinical data were unavailable (N=215).

Our aim was to evaluate the clinical use of panfungal PCR taken from sterile, deep tissue, or fluid specimens. We excluded BAL and blood culture specimens, and if the specimens were not obtained from a sterile site (Figure 1). The clinical data for the study were collected from electronic patient records. The collected data included patients's age, gender, immunosuppression, comorbidities, 3-month mortality, primary site of the specimen, microscopy and culture results, and the primary antifungal therapy. Data regarding the course of the fungal disease were collected to analyse the likelihood of IFD.

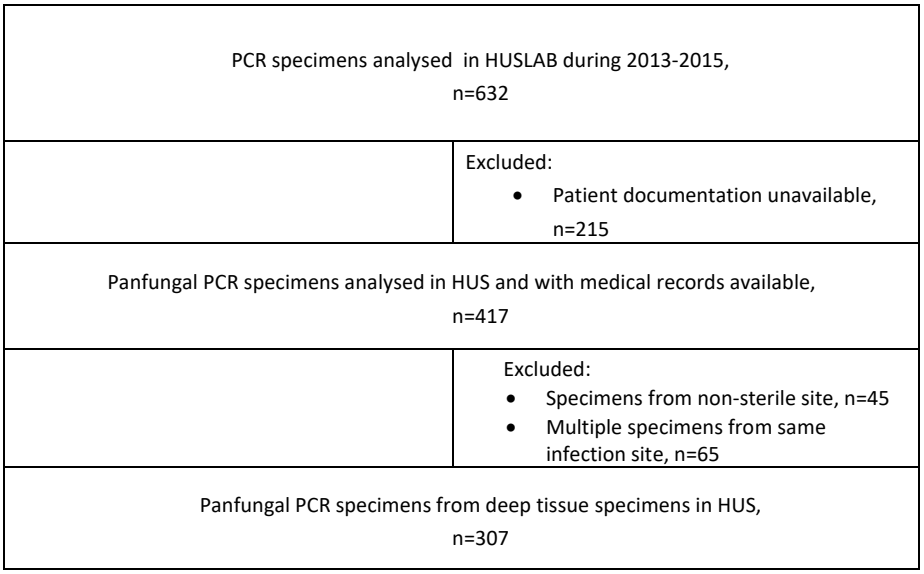

Figure 1 *Panfungal PCR study 2013-2015 (study I).*

4.2.2 DESIGN AND PATIENTS OF THE CANDIDEMIA STUDY (II-IV)

The candidemia studies (study II, III, IV) were retrospective, observational, cohort analyses. The study population included all patients with blood culture positive for *Candida* species in HUS between January 2007 and December 2016 (Figure 2). Patients with candidemia were identified from the microbiological database. The clinical data were collected from the electronic patient medical records from tertiary and secondary care hospitals. Patients <18 years were excluded (n=22). Twenty-one candidemia cases were diagnosed and treated only in primary care hospitals. The medical records from these patients were not available and these patients were excluded from the analysis. The information searched for the electronic database included patient age, gender, underlying co-morbidities and risk factors, the primary source of infection, complications, features of antimicrobial therapy, antifungal susceptibility data, and laboratory findings. The 30-day overall mortality was assessed in study II and III and 1-year mortality in study IV.

The incidence of candidemia in HUS was analysed in study II and the incidence of LR candidemia in study IV. The rates were calculated from the entire population of patients diagnosed with candidemia in HUS, also including patients <18 years and treated in primary care hospitals during the study period. In study II, the study period was divided into two periods (2007–2011 and 2012–2016) to analyse changes in the epidemiology of candidemia during the 10-year study period.

In study III, patients from the candidemia data were classified as PC or non-PC and compared to evaluate the risk factors and clinical outcome of PC. PC was classified as the same *Candida* species identified from blood culture taken 5 days or later after the first positive blood cultures were drawn. Non-PC included patients with candidemia when blood cultures were persistently positive <5 days and negative blood cultures were taken at least once. Patients were excluded if they died <5 days after the first positive blood culture was taken or if the patient was not treated with an antifungal agent. Patients were also excluded if no control blood cultures were performed.

<table>
<tr><td colspan="2">HUS 2007–2016
Episodes of candidemia in adults, n=374
Adult Patients, n=350</td></tr>
<tr><td></td><td>Excluded:
• Patient documentation not available, n=21</td></tr>
<tr><td colspan="2">Study II:
Adult patients with candidemia and clinical data available, n=329</td></tr>
<tr><td>Study III:
Persistent candidemia, n=75, versus non-persistent candidemia, n=151</td><td>Study IV:
Late recurrent candidemia, n=20, versus patients with a single candidemia episode, n=309</td></tr>
<tr><td>Excluded:
• No control blood culture, n=90
• Patients who died <5 days after the first positive blood culture was drawn, n=7
• Received no antifungal treatment, n=6</td><td></td></tr>
</table>

Figure 2 *Design of the candidemia study (study II, III, IV). Adult patients with candidemia in HUS 2007–2016.*

Study IV was also a cohort study. All adult patients with more than one episode of candidemia were identified from the candidemia data as recurrent candidemia cases. If the second episode was diagnosed ≤30 after the initial episode (early recurrence) the case was not considered as recurrent candidemia. We focused on late recurrence, which was defined as the time from the initial episode to recurrence >30 days. We compared patients with LR candidemia and patients with a single episode of candidemia to analyse the characteristics of LR candidemia.

4.3 MICROBIOLOGICAL METHODS

Study I: Tissue and fluid specimens were routine patient specimens (e.g. CSF, vitreous body, pleural effusion and tissue specimens). Microbiological analyses for culture and microscopy were performed using routine diagnostic methods at HUSLAB. Microscopy and culture were analysed from the same specimen as the panfungal PCR test. All clinical specimens were fresh specimens. The specimens were stored for a maximum of four days at +4ºC before PCR analysis, if necessary.

The method for PCR analysis is described in the original publication (Study I). The fungal PCR was designed to identify ribosomal DNA sequences of fungal chromosomes. The primers recognised two target gene regions, using the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information, Bethesda, MD, USA, https://blast.ncbi.nlm.nih.gov/Blast.cgi) for searching its database to predict secondary structures. Extracted DNA was detected with panfungal PCR using internal transcribed spacer, ITS_03 forward and reverse, and ITS_05 forward and reverse primers. PCR was performed using the DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA). Agarose gel electrophoresis was used for separation of PCR-amplified fragments; fragments were visualised under ultraviolet light. Amplified fragments were purified with ExoSAP Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) using both the forward and reverse primers and an ABI Prism 3100 genetic analyzer (Thermo Fisher Scientific). Analysis was then performed by BLAST. Each specimen was tested with an inhibitor control. A nontemplate control was tested with each run, and an extraction control was tested with each extraction lot. For each specimen, an empty reference container as a negative control was also used, which was opened at the sampling site.

For PCR analyses, fungal cells were collected in 650 µL of water and homogenised in Precellys (Bertin Instruments, Montigny-le-Bretonneux, France). The homogenised mixture was purified with the NucliSENS kit (bioMérieux, Marcy

l'Etoile, France) using the easyMAG automatic nucleic acid purification platform (bioMérieux), as described by the manufacturer. DNA was eluted into 100 µL, of which 1 µL was directly used for PCR amplification. The clinical specimens were homogenised in Precellys solution and purified using the Nordiag Arrow instrument (Isogen Life Science, Utrecht, the Netherlands) with a Viral NA Kit (DiaSorin, Saluggia, Italy) before PCR analysis.

Study II-IV: Blood culture specimens were routine patient specimens. Prior to 2012, *Candida* isolates were identified with traditional methods using biochemical and morphological features (ID 32 C®, bioMérieux, Marcy l'Etoile, France). Since 2012, *Candida* isolates are mostly identified by MALDI-TOF technology (Vitek MS, bioMérieux, Marcy l'Etoile, France) and on demand by sequencing of the ITS gene. Susceptibility testing was performed using the agar diffusion method (Etest®, bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Results interpretation is performed according to the EUCAST clinical breakpoint since 2011. The interpretation was performed by using the CLSI breakpoints prior to 2011 (Table 9).

Table 9 *Microbiological identification and susceptibility testing of Candida isolates from blood cultures in HUSLAB during 2007-2016.*

	Time period	Method
Identification		
	2007–2012	Morphological and biochemical features *
	2013–2016	mostly MALDI-TOF technology**
		Sequencing of ITS gene, if necessary
Interpretation of susceptibility test result		
	2006–2010	CLSI breakpoints
	2011–2016	EUCAST breakpoints

* ID 32 C®, bioMérieux, Marcy l'Etoile, France. ** Vitek MS, bioMérieux, Marcy l'Etoile, France.

CLSI, The Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing.

4.4 DEFINITIONS

Study I: The term deep tissue specimen included sterile specimens from tissues (e.g. liver, lung, lymph node or bone) and also fluid specimens if they were taken from a normally sterile body cavity (CSF, pleural effusion, ascites fluid, pericardial effusion

or vitreous body). An immunocompromised patient was defined as a patient with haematological malignancy, history of transplantation, HIV, genetic immunodeficiency, taking immunosuppressive medication or active cancer treated with chemotherapy in the previous 30 days before the panfungal PCR test was performed. Immunosuppressive medication included prednisolone (≥15 mg/day for >3 weeks), biological drug, tacrolimus mycophenolate, cyclosporine, methotrexate or azathioprine. The likelihood of fungal infection was evaluated with the criteria of the EORTC/MSG consensus group (De Pauw et al. 2008).

Study II-IV: Candidemia was defined as an infection caused by *Candida* species when a *Candida* species was isolated from a blood culture at least once. If a patient developed more than one episode of candidemia during the study period, the episodes were regarded as different episodes if the time between the episodes was >30 days. BSI caused by one or more than one (mixed infection) different *Candida* species were both included in the study population.

Candidemia was considered persistent if the blood cultures remained positive five days or longer after the first positive blood cultures were drawn. Candidemia was considered non-persistent if blood culture remained positive less than 5 days and at least one blood culture with a negative result was taken. Candidemia was considered late recurrent if a patient had two or more different episodes of candidemia during the study period and the second episode occurred >30 days after the initial episode.

Neutropenia was defined as neutrophil count $<0.5 \times 10^9$/L for two weeks before the onset of candidemia. Corticosteroid treatment was defined as prednisolone dose ≥15 mg/day taken longer than three weeks before the diagnosis of candidemia. Prior antimicrobial treatment was considered a broad-spectrum antibiotic with anaerobic coverage if a patient received it during the onset of candidemia.

The severity of underlying diseases was scored using the McCabe classification (Table 10) (McCabe and Jackson 1962). GI disease was considered as inflammatory bowel disease, short bowel syndrome, intestinal obstruction or active malignancy in the GI tract. Recent GI surgery was not included into the variable of GI disease. Prior GI surgery was evaluated as a separate variable, and considered when the surgery was performed less than 30 days before the onset of candidemia. Adherence to the guidelines of candidemia management was evaluated with the Equal Candida Score (Table 7) (Mellinghoff[b] et al. 2018).

Metastatic infection complications were defined as endophthalmitis or chorioretinitis, endocarditis or pericarditis, vascular complications and dissemination to other solid organs. Antifungal therapy was defined as early if an effective antifungal agent according to the susceptibility results was started <48 hours after the first positive blood cultures were taken. CVC was removed early when it was performed within 48 hours after the first blood culture tested positive for *Candida* species was taken.

Table 10 *McCabe cassification, a grading for severity of underlying comorbidities.*

McCabe	Severity of comorbidities
1	Underlying comorbidities are likely to have non-fatal prognosis in ≥5 years
2	Underlying comorbidities have ultimately a fatal prognosis in 1–4 years
3	Underlying comorbidities have a rapidly fatal prognosis within one year

(McCabe and Jackson 1962).

4.5 STATISTICAL METHODS

Categorical variables were summarised using counts and percentages. Continuous variables with non-normal distribution were summarised as the median, first and third quartile (IQR, interquartile range), and in some cases minimum and maximum values.

Study I: The differences between categorical variables were analysed with two-tailed Chi-square test. *P*-values ≤0.05 were considered as statistically significant. Sensitivity and specificity for IFD diagnosis were calculated for panfungal PCR, culture and microscopy results. Positive predictive value (PPV) and negative predictive value (NPV) were also calculated. SPSS version 22 (SPSS Inc., Chicago, IL, USA) was used for all analyses.

Study II, III and IV: The differences between categorical variables were analysed with two-tailed Chi-square test or Fisher´s exact test, as appropriate. Continuous variables with non-normal distribution were compared with Mann-Whitney U-test. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. *P*-values ≤0.05 were considered statistically significant. Logistic regression analyses were performed. The variables were included into the multivariable regression analysis if the univariate *P*-value was <0.1 and variables were not multicollinear. SPSS versions 24 and 25 (SPSS Inc., Chicago, IL, USA) were used for analyses in the candidemia study.

5 RESULTS

5.1 RESULTS FROM PANFUNGAL PCR STUDY

5.1.1 PATIENT CHARACTERISTICS (STUDY I)

Overall, panfungal PCR was performed for 632 specimens in HUSLAB from 2013-2015 (Figure 1). All age groups were included. Patients were excluded, if the clinical data were unavailable or incomplete (n=215). The patients were also excluded, if the specimen was taken from a non-sterile infection site (n=45). If several specimens were taken from one patient at the same infection site, the repeated specimens were excluded (n=65). We included in our analysis 307 specimens from 296 patients with a panfungal PCR result performed on deep tissue specimens. Microscopy result was missing from five patients (one PCR positive and four PCR negative specimens). Culture result was missing from four patients (two positive PCR and two negative PCR specimens).

Male patients were 59% (182/307) of the patients, and the median age was 54.0 (range 1–87). Immunocompromised were 41% of all patients. The most frequent reason for immunosuppression was haematological malignancy (45% of all immunocomprimised patients), followed by transplantation (22%), immunosuppressive medication (11%), a solid organ malignancy treated with chemotherapy (9%) and other immunosuppressions (13%). Other immunosuppression included HIV and primary immunodeficiencies. The most frequent immunosuppressive agents were corticosteroids, biological drugs and azathioprine. The 3-month mortality was 7.5%.

5.1.2 TYPE OF SPECIMENS AND FUNGAL SPECIES IDENTIFIED WITH PCR

Overall, there were 307 specimens tested for panfungal PCR (Table 11). The panfungal PCR was positive in 48/307 (16%), culture in 21/303 (7%) and microscopy in 24/302 (8%) specimens. The most common type of specimen was cerebrospinal fluid 16%, followed by soft tissue abscess 13%, lung 12%, and pleural effusion 10%. Panfungal PCR was most frequently positive from lung specimens 32%, followed by pleural effusion 19%, liver 19%, and soft tissue abscesses 15%. Culture was most

frequently positive from liver 19% and lung 11% specimens and microscopy from lung 29% and liver 26% specimens.

The fungal species identified from PCR specimens were mostly *A. fumigatus* (n=7) and *Candida* species (n=9), when IFD was classified proven, probable or possible (Table 12). The panfungal PCR was also positive, when proven or probable infection was caused by *Histoplasma capsulatum, Hormographiella aspergillata, Scedosporium apiospermum, Phoma opuntiae, Cryptococcus alpidus, Cladosporium sphaerospermum* and two of *Rhizopus* species. If an IFD was not diagnosed according to the EORTC/MSG criteria, *Malassezia* species (N=11) was the most frequent species identified from PCR, followed by *Candida* species (n=5) and *Aspergillus* species (n=3).

Table 11 *Positive PCR, culture and microscopy results by different type of specimens.*

Type of specimen	n (%)	Positive PCR result, n	Positive culture result, n	Positive microscopy result, n	Proven. probable or possible IFD*, n
Cerebrospinal fluid	49 (16.0)	3	1	0	1
Soft tissue abscess	40 (13.0)	6	4	2	5
Lung	38 (12.4)	12	4	11	18
Pleural effusion	31 (10.1)	6	1	1	2
Liver	27 (8.8)	5	5	7	8
Bone	23 (7.5)	1	0	0	0
Vitreous body	16 (5.2)	2	1	0	3
Lymph node	15 (4.9)	2	0	0	0
Cerebral tissue	14 (4.6)	2	0	0	0
Other tissue	54 (17.6)	9	5	3	6
Total	307(100)	48 (15.6)	21(6.9)**	24(7.9)***	43 (14.0)

*According to the criteria of EORTC/MSG (De Pauw et al. 2008).

**Culture result was missing in 4 specimens and ***microscopy result was missing in 5 specimens.

Table 12 *Species identified from panfungal PCR, when the likelihood of IFD was evaluated as proven, probable or possible, or no IFD was diagnosed.*

Fungal species identified from PCR when IFD was proven, probable or possible*	Number of isolates	Fungal species identified from PCR without IFD*	Number of isolates
Aspergillus fumigatus	7	*Aspergillus fumigatus*	1
Candida albicans	5	*Aspergillus versicolor*	1
Candida krusei	1	*Aspergillus conigus*	1
Candida albicans/Candida glabrata	1	*Candida albicans*	2
Candida parapsilosis	1	*Candida parapsilosis*	1
Candida dubliniensis	1	*Candida sake*	1
Cryptococcus albidus	1	*Cryptococcus albidus*	1
Histoplasma capsulatum	1	*Cladosporium species*	2
Hormographiella aspergillata	1	*Malassezia species*	11
Phoma opuntiae	1	*Rhodotorula species*	1
Rhizopus microphorus/Rhizopus species	2		
Scedosporium apiospermum	1		
Cladosporium sphaerospermum	1		
Total	24	Total	22

*According to the criteria of EORTC/MSG (De Pauw et al. 2008).

5.1.3 PANFUNGAL PCR DIAGNOSING INVASIVE FUNGAL DISEASE

IFD was diagnosed according to the criteria of EORTC/MSG consensus group in 43 (14%) patients. Of these, 29 (67%) patients had proven, 10 (23%) had probable and 4 (9%) had possible IFD. Overall, there were 20/29 (69%) proven IFDs with positive PCR result, 18/29 (62%) proven IFDs with positive culture result and 19/29 (66%) proven IFDs with positive microscopy result. Probable IFD was considered in 3/10 patients with positive PCR result, 0/10 with positive culture result and 2/10 with positive microscopy result. Possible IFD was noticed in 1/4 patients with positive PCR result, and in 0/4 with positive culture or microscopy result.

The culture result was positive in 12/48 (25%) specimens that were positive for PCR and the microscopy was positive in 16/48 (33%) specimens with positive PCR result (Table 13). The concordance rate for PCR and culture results was 86% and for PCR and microscopy 87%.

The specificity, sensitivity, PPV and NPV of panfungal PCR were calculated according to the EORTC/MSG criteria evaluating the likelihood of IFD.

The specificity of PCR was 60.5% and the sensitivity 91.7%. The PPV of PCR was 54.2% and NPV 93.4%. PCR was positive in 22 specimens even though culture and microscopy results were negative. In 16 of the 22 specimens, the laboratory evaluated and reported to the clinician that the results were very likely contamination. In all of these 16 cases, the clinician agreed with the laboratory evaluation and there was no IFD according to the EORTC/MSG criteria. If these 16 specimens were excluded, the specificity of panfungal PCR was 97.6%. The specificity of culture for diagnosing IFD was 98.8% and sensitivity 43.9%. The PPV for culture was 85.7% and NPV 91.8%. The specificity of microscopy for diagnosing IFD was 98.8%, sensitivity 50.0%, PPV 87.5%, and NPV 92.4%.

Table 13 *Panfungal PCR compared with microscopy and culture results and with the likelihood of invasive fungal disease.*

		PCR positive N =48 n (%)	PCR negative N=259 n (%)	*P*
Microscopy	Positive N=24	16 (5)	8 (3)	<0.001
	Negative N=278	31 (10)	247 (82)	
Culture	Positive N=21	12 (4)	9 (3)	<0.001
	Negative N=282	34 (11)	248 (82)	
Likelihood of IFD*	Proven/probable/possible N=43	24 (8)	19 (6)	<0.001
	No IFD N=264	22 (7)	242 (79)	

*According to the criteria of EORTC/MSG (De Pauw et al. 2008).

**Culture result was missing in 4 specimens and ***microscopy result was missing in 5 specimens

5.2 RESULTS FROM CANDIDEMIA STUDY (II-IV)

5.2.1 PATIENTS CHARACTERISTICS IN CANDIDEMIA STUDY (II-IV)

Overall, 374 episodes of candidemia from 350 adult patients were diagnosed during 2007–2016; clinical data were available from 329 patients. Of the 350 patients, 75 had persistent and 20 had late recurrent candidemia. There were 24 recurrent episodes overall. The study period was divided in two periods (2007–2011 and 2012–2016; study II). The characteristics of the patients did not differ between the two 5-year periods. The only statistically significant differences between the 5-year periods were decrease in the presense of CVC (65.4% vs 43.3%, P = 0.018) and prior use of fluconazole at the onset of candidemia (21.3% vs 11.7%, P = 0.018).

The patients with candidemia were more male (61%), even though half of the patients with LR candidemia were female (Table 14). The median age of the entire study population was 65.0 (range 18–98). Patients with LR candidemia were a sligthly younger than patients in the whole candidemia population. The patients with candidemia had several comorbidities and 73% of them had McCabe classification 2 or 3. Solid tumor was recorded in 24% and haematological malignancy in 7% of the patients. In candidemia population, 4% had a history of transplantation. Neutropenia was evident in 7% and chemotherapy in 8% of the patients. Half of the patients had CVC at the diagnosis of candidemia, but was more frequent in PC (68 %) and LR candidemia (60%) patients than in the entire population. History of IDU was recorded in 25% of LR candidemia patients, and it was evident in 11% of all patients with candidemia. Half of the patients received broad-spectrum antibiotics at the onset of candidemia. Prior GI surgery was performed on 23 % of all patients, but was more frequent in PC (32%). Patients were most frequently treated in surgical wards (44%) at the onset of candidemia. Only 12% of the patients were treated in ICU and 5% in haematological or oncological departments at diagnosis. Patients were admitted to ICU due to the candidemia in 8% of cases, and previous ICU stay within 30 days before diagnosis of candidemia was observed in 25% of patients.

5.2.2 INCIDENCE OF CANDIDEMIA

The annual number of candidemia episodes varied between 27–53 cases per year, including all candidemia cases diagnosed in HUS (both children and adult patients). The average annual incidence rate of candidemia was 2.53 episodes per 100 000 inhabitants, being lowest (1.78 per 100 000 inhabitants) in 2009 and highest (3.42 per 100 000 inhabitants) in 2011.

Table 14 *Patient characteristics in candidemia study (studies II, III, IV) in 2007–2016.*

Characteristic	All candidemia patients N=329 n (%)	Persistent candidemia N=75 n (%)	Late recurrent candidemia N=20 n (%)
Male	199 (60.5)	43 (57.3)	10 (50.0)
Age, median (min-max)	65.0 (18–98)	61.0 (18–88)	59.5 (27–86)
McCabe			
1	89 (27.1)	20 (26.7)	4 (20.0)
2	158 (48.0)	40 (53.3)	13 (65.0)
3	82 (24.9)	15 (20.0)	3 (15.0)
Hemodialysis	28 (8.5)	10 (13.3)	2 (10.0)
Central venous catheter	120 (55.0)	51 (68.0)	12 (60.0)
Chemotherapy	25 (7.6)	6 (8.0)	0
Prior fluconazole	54 (16.4)	18 (24.3)	3 (15.0)
Broad-spectrum antimicrobials	163 (49.5)	39 (52.0))	7 (35.0))
Intravenous drug abuse	36 (10.9)	6 (8.0)	5 (25.0)
Prior gastrointestinal surgery	76 (23.1)	24 (32.0)	3 (15.0)
Neutropenia	23 (7.0)	5 (6.7)	0
Malignancy (solid organ)	80 (24.3)	17 (22.7)	3 (15.0)
Hematologic malignancy	23 (7.0)	6 (8.0)	1 (5.0)
Transplantation	14 (4.3)	4 (5.3)	1 (5.0)
Time from admission to candidemia			
Prior hospital stay 0–7 days	124 (37.7)	20 (26.7)	10 (50.0)
Prior hospital stay >7 days	205 (62.3)	55 (73.3)	10 (50.0)
Speciality at onset of candidemia			
Intensive care unit	38 (11.6)	12 (16.0)	2 (10.0)
Surgical	144 (43.8)	37 (48.3)	8 (40.0)
Medical	85 (25.8)	12 (16.0)	6 (30.0)
Haematology + oncology	17 (5.2)	5 (6.7)	1 (5.0)
Others	45 (13.7)	9 (12.0)	3 (15.0)

IQR, interquartile range.

5.2.3 CANDIDA SPECIES DISTRIBUTION AND SUSCEBTIBILITY RESULTS

Our study revealed no significant change in the species distribution in 2007–2011 vs. 2012–2016; *C. albicans* was the leading cause of candidemia in HUS during the entire study period (Figure 3). Although non-*albicans Candida* species increased slightly

from 36% to 43% when comparing the 5-year periods, the difference was not significant ($P = 0.118$). *C. albicans* accounted for 60% (233/386) of the isolates over the 10-year study period. The most frequent species isolated among non-*albicans Candida* was *C. glabrata (*22%, 83/386), followed by *C. parapsilosis* (5%, 20/386), *C. dubliniensis* (5%, 20/386), *C. tropicalis* (3%, 11/386), and *C. krusei* (2%, 8/386). The proportion of non-*albicans Candida* species ranged annually from 19–56% (Figure 4). The poportion of *C. glabrata* isolates increased from 19% to 24%, and the proportion of *C. parapsilosis* isolates from 4% to 7% comparing the 5-year periods, but the differences were not significant.

The susceptibility results in study II concerned fluconazole and anidulafungin. Resistance rates for fluconazole are presented from both five-year periods. However, results for anidulafungin are introduced only for the later period (2012–2016), as susceptibility testing for anidulafungin has been systematically performed in HUSLAB since 2011. *C. glabrata* resistance to fluconazole increased from 43% to 66% ($P = 0.048$) during the study period. On the other hand, *C. albicans* resistance to fluconazole was rare (0.9%). Overall, the anidulafingin resistance rate was low (2.1%) in our hospital district, and there were no isolates resistant to anidulfungin among *C. albicans* or *C. glabrata* isolates.

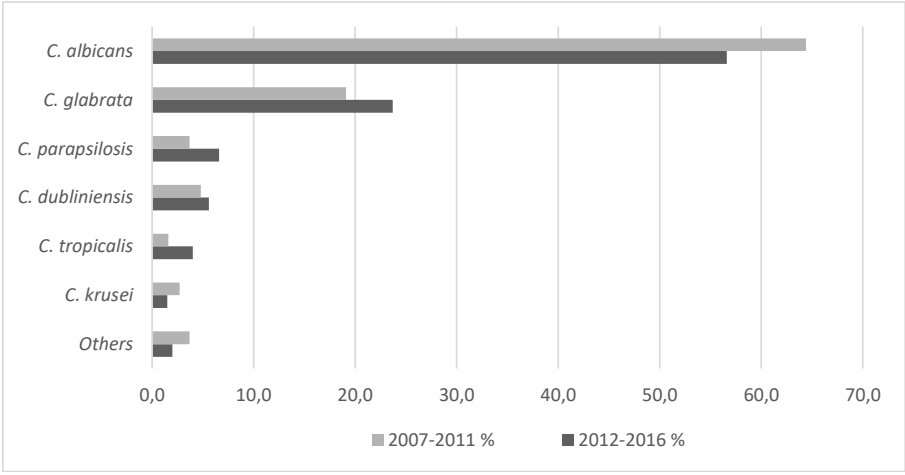

Figure 3 *Distribution of Candida species in HUS during 2007–2011 versus 2012–2016.*

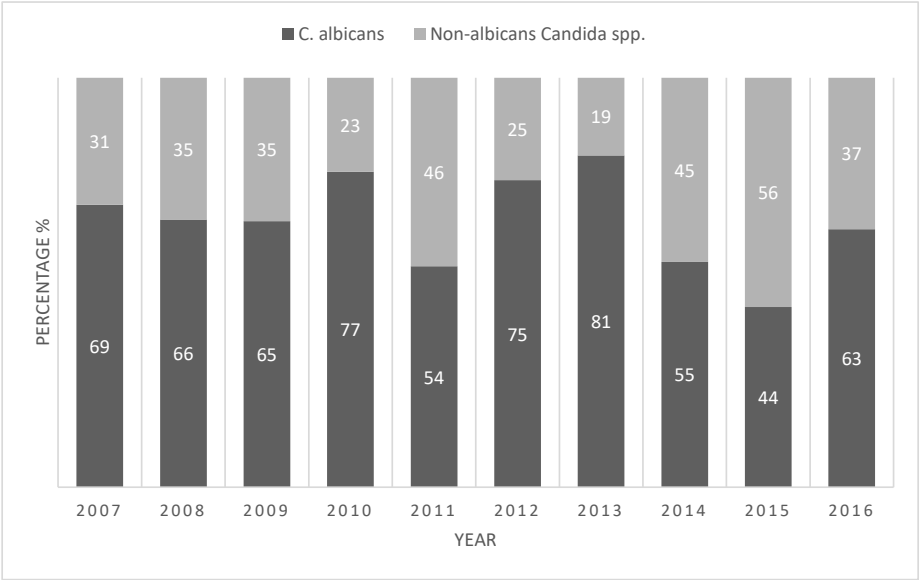

Figure 4 *Proportion of episodes caused by C. albicans versus non-albicans Candida species in HUS annually from 2007–2016.*

5.2.4 MORTALITY ANALYSIS (STUDY II)

The overall 7-day mortality of candidemia was 18% and 30-day mortality 31%. The 30-day mortality rate was only 9% among patients with no severe underlying illnesses (McCabe score 1). Some (n=46) of the patients received no or inappropriate antifungal treatment for candidemia. Many of these patients with poor prognosis died within a few days after candidemia diagnosis.We performed a mortality analysis with patients who received an effective antifungal treatment to analyse the significance of an early start of an effective antifungal. We excluded from the analysis the patients who received no or inappropriate antifungal treatment according to the susceptibility testing results. Patients who were treated with an effective antifungal were included (n=283). Among these patients, the 7-day and 30-day mortality rates were 10% and 23%, respectively.

The mortality analysis is presented in Table 15. Age >65 years, McCabe score 3, prior corticosteroid treatment, dialysis and ICU stay at the time of candidemia diagnosis were associated with mortality in univariate analysis. Nevertheless, early initiation (<48 h after onset of candidemia) of effective antifungal treatment was not

associated with 30-day mortality (univariate OR 1.37, 95% CI 0.75–2.51). Multivariable logistic regression analysis revealed McCabe classification 3 (OR 20.55, 95% CI 5.98–70.60), age >65 years (OR 3.98, 95% CI 1.97–8.02), and ICU stay during the diagnosis of candidemia (OR 5.06, 95% CI 1.75–14.68) as independent risk factors for candidemia mortality.

Table 15 *Mortality analysis of candididemia patients who received effective antifungal treatment.*

	Alive N=218 n (%)	Dead N=65 n (%)	Univariate OR	95% CI	Multivariable OR	95% CI
Age >65 years	84 (38.5)	44 (67.7)	3.34	1.86–6.01	3.98	1.97–8.02
McCabe score 3	33 (15.1)	29 (44.6)	11.42	4.34–30.10	20.55	5.98–70.60
Malignancy	62 (28.4)	26 (40.0)	1.68	0.94–2.99	0.46	0.20–1.06
Corticosteroids	16 (7.3)	11 (16.9)	2.57	1.13–5.86	2.10	0.80–5.51
ICU at diagnosis	20 (9.2)	14 (21.5)	2.72	1.29–5.75	5.06	1.75–14.68
Dialysis	13 (6.0)	11 (16.9)	3.21	1.36–7.57	2.20	0.71–6.79

ICU, Intensive care unit; OR, Odds ratio; CI, Confidential interval.

5.2.5 RISK FACTORS FOR PERSISTENT CANDIDEMIA (STUDY III)

Persistent candidemia was identified in 75 (21%) and non-persistent in 151 (43%) patients. The number of PC ranged from two to 13 cases per year, with a mean of 7.5 cases annually. Blood cultures persisted positive for a median of 9.0 days (range 5–41) in PC. Median frequency of follow-up blood cultures was 5.0 (range 2–44). *C. albicans* was the most common cause of candidemia in both groups (63% vs. 65%, $P = 0.742$). The differences in species distributions between the groups were not significant.

No significant differences were observed between the groups regarding comorbidities (McCabe classification, diabetes, malignancies). Neutropenia, chemotherapy, dialysis and transplantation displayed neither statistically significant difference between patients with persistent and non-persistent candidemia. However, PC cases had more unexpected additional infection sites than non-PC cases (29% vs. 13%, $P = 0.003$). Ocular candidiasis, other solid organ complications and vascular complications were all more frequent with PC. Overall, 11 (5%)

endophthalmitis/reitinitis and six (3%) endocarditis was diagnosed during the study period.

In univariate analysis, an association with PC was observed with prior GI surgery (32% vs 19%, $P = 0.024$), presence of CVC (68% vs. 46%, $P = 0.002$), ineffective empirical antifungal agent (23% vs. 11%, $P = 0.011$), and metastatic infection foci (29% vs. 13%, $P = 0.003$). The length of hospital stay before the onset of candidemia was longer in PC vs. non-persistent cases (hospital stay >7 days; 73% vs. 60%, $P = 0.043$). In multivariable regression analysis, independent risk factors for PC were the presence of CVC (OR 2.71; 95% CI 1.31–5.59), metastatic infection foci (OR 3.60; 95% CI 1.66–7.79) and ineffective empirical treatment (OR 3.31; 95% CI 1.43–7.65) (Table 4 in the original publication of study III).

5.2.6 MANAGEMENT OF PERSISTENT CANDIDEMIA (STUDY III)

A half (48%) of the persistent cases were diagnosed in surgical wards, and 16% in ICU. Only 7% of PC was diagnosed in haematologic or oncologic wards. The primary source of the infection was more frequently catheter related in PC than in non-PC (43% vs. 19%; $P = 0.003$). After catheter-related infections, the next most common sources of infection among patients with PC were the GI (29%) and urinary tract (7%). The GI tract was the most common source (29%) in non-PC. The primary focus, which was applicable for source control, was identified in 137/226 (61%) patients, in 59 (79%) of persistent and 78 (52%) of non-persistent cases. Among these patients, the source control was performed less than 48 h after the onset of candidemia more often with non-PC than PC (PC 31% vs. non-PC 58%, $P = 0.002$). Fluconazole was used as first-line therapy most frequently in both groups (54% vs. 63%, $P = 0.202$). Echinocandins were started as an initial antifungal for 45% of the PC cases and for 35% of the non-PC cases ($P = 0.169$). Theere were no significant differences in 30-day mortality between the groups (PC 20% vs. non-PC 14%, $P = 0.239$), but the rates were lower than the overall 30-day mortality rate of candidemia (31%) in HUS during the 10-year study period.

Adherence to international guidelines for the management of candidemia was evaluated with the Equal Candida Score. Overall, the median score was 13.0 (IQR 11.0–15.0). The median score was 11.0 (IQR 11.0–14.0) for patients without CVC and 14.0 (IQR 11.0–16.0) for patients with CVC. The guideline adherence showed no significant difference between PC and non-PC groups (PC 13.0 vs. non-PC 12.0, $P = 0.068$). However, there was a significant difference when patients who survived 30 days after onset of candidemia were compared with non-survivors (13.0 vs. 11.0, $P = 0.004$). The median number of follow-up blood cultures was 5.0 (range 2-44), but daily follow-up blood cultures after diagnosis of candidemia were taken for only 10% of the

patients (22/226). Fundoscopy to exclude ocular involvement was performed for 53/226 (24%) and echocardiography for 120/226 (53%) of the patients. Treatment for 14 days after the first negative blood culture was completed in 183/226 (81%) of the patients. Initial echinocandin treatment was administered to 91/226 (40%) of patients, and step-down therapy from echinocandin to fluconazole was evident in 39/91 (43%) of those who received echinocandin as initial therapy.

5.2.7 CHARACTERISTICS OF LATE RECURRENT CANDIDEMIA (STUDY IV)

During the 10-year study period, LR candidemia was diagnosed in 20 patients, which accounted for 6% of all patients with candidemia. The annual incidence rate of LR candidemia was 0.13 per 100 000 inhabitants including recurrent candidemia also in paediatric population. The recurrence was caused by same *Candida* species in 12 patients and a different *Candida* species in eight patients. The median time between the initial and the recurrent episode of candidemia was 152 days (IQR 97.0–620.8). One patient had three episodes of recurrence, two had two and the remaining 17 patients had one recurrent episode. Only one recurrent episode and three of the first episodes of recurrent candidemia were community acquired. All the other episodes were nosocomial infections. The source of the infection for all of the community-acquired episodes was an emergency-situation of GI tract or IDU. *C. albicans*, *C. glabrata*, and *C. parapsilosis* caused 95% of the initial episodes in LR candidemia.

LR candidemia patients were more frequently 18–65 years old than patients with a single candidemia episode (LR candidemia 75% vs. single episode 52%, $P = 0.050$). Neutropenia, malignancies, transplantation, prior GI surgery or presence of CVC were not associated with LR candidemia. Metastatic infection foci had neither difference between the groups (LR candidemia 20% vs. single episode 15%, $P = 0.453$). However, underlying GI disease (40%, 8/20 vs. 10%, 32/309; $P = 0.001$), history of IDU (25%, 5/20 vs. 10%, 31/309; $P = 0.038$), and age of 18–65 years (75%, 15/20 vs. 52%, 162/309; $P = 0.050$) were associated with LR candidemia in univariate analysis. The underlying GI diseases were inflammatory bowel diseases, short bowel syndromes, strictures in the GI tract, intestinal pseudo-obstructions or active malignancies in the GI tract. In multivariable regression analysis, independent risk factors for LR candidemia were a history of IDU (OR 3.62, 95% CI 1.03–12.69) and underlying GI disease (OR 7.21, 95% CI 2.52–20.61).

6 DISCUSSION

6.1 CLINICAL USE OF PANFUNGAL PCR (STUDY I)

We evaluated panfungal PCR results from 307 specimens. The clinical sensitivity, specificity, PPV and NPV of panfungal PCR to diagnose invasive fungal infections were 61%, 92% and 54% and 93% (Table 16). Earlier in a smaller study, the clinical sensitivity of panfungal PCR among immunocomprimised patients was 69% and specificity 63%, while PPV and NPV were 86% and 38%, respectively (Babouee et al. 2013). In the Austrian study, sensitivity was 57% and specificity 97% for microscopy negative invasive fungal infections (Lass-Florl et al. 2013). PPV was 80% and NPV 92% in their analysis.

The concordance was 87% between panfungal PCR and microscopy and 86% between PCR and culture in our study. Concordance between panfungal PCR and conventional methods have been similar (>80%) to our results in other studies (Lass-Florl et al. 2013, Trubiano et al. 2016). The performance of panfungal PCR in diagnosing IFDs is comparable with the conventional methods and may increase the diagnostic yield.

Table 16 *Comparison of studies on the use of panfungal PCR from deep tissue specimens for diagnosis of IFD.*

Author	Published year	Number of specimens	Sensitivity %	Specificity %	PPV %	NPV %
Ala-Houhala et al.	2018	307	61	92	54	93
Lass-Flörl et al.	2013	206	57	97	80	92
Babouee et al.	2013	34	69	63	86	38

PPV, positive predictive value; NPV, negative predictive value.

Our study population was non-selected and only 41% of our patients were immunocompromised, which was quite low compared with other studies. The use of panfungal PCR has been mostly studied in immunocompromised patients (Babouee et al. 2013, Trubiano et al. 2016). Lass-Flörl et al. studied the utility of panfungal PCR in a non-selected patient population when microscopy results were negative (Lass-Florl et al. 2013). Studies evaluating PCR for *Aspergillus* and mucormycoses have been conducted mainly in patients with haematological

malignancies or with other immunosuppression (Hammond et al. 2011, Rickerts et al. 2006, Rickerts et al. 2007).

We focused on deep tissue specimens, which also included fluid specimens, if they were taken from a sterile body cavity. The most frequent specimen types were CSF (16%), soft tissue abscesses (13%) and lung (12%). PCR was mostly positive from lung and pleural effusion specimens, soft tissue abscesses, and liver specimens. Even though CSF specimens were the most frequent specimens, IFD was found according to EORTC/MSG criteria only in three patients, when PCR was analysed from CSF. IFD was mostly found from patients whose specimens were from lung (40%), liver (26%), vitreous body (19%), and soft tissue abscesses (13%). This might reflect the fact that lungs, liver and vitreous body are common infection sites for candidiasis and aspergillosis (Kullberg and Arendrup 2015, Latgé and Chamilos 2019). Trubiano et al. studied panfungal PCR from deep tissue specimens and from BAL specimens. They concluded that PCR testing on BAL specimens is challenging and the ability of panfungal PCR to identify potential pathogens from BAL specimens was limited. They recommended that panfungal PCR should be used only in tissue specimens obtained from sterile sites and not from BAL specimens (Trubiano et al. 2016).

The rate of positive results from panfungal PCR was 16% in our study. The relatively low number of positive results probably reflects the fact that our patient population was not selected and the probability of IFD was very low in many of the cases before the PCR specimen was taken. Proven or probable IFD was diagnosed in 48% of the patients with a positive panfungal PCR result. We found a positive correlation between the positive PCR results and proven or probable IFD (P <0.001). However, there were positive PCR results without IFD. In our analysis, PCR was positive in 22 specimens when culture and microscopy results were concurrently negative and diagnostic criteria of IFD was not fulfilled. Sixteen of these results were very likely contaminations according to the laboratory and clinicians evaluation. If these 16 specimens were excluded from the analysis, the specificity of panfungal PCR for diagnosing IFD would increase from 92% to 98%. The probability of an IFD before the tissue specimen was taken was very low in many of the cases in our study.

Suspicion of a fungal infection is a challenge for clinician. Panfungal PCR is a valuable addition to standard diagnostic methods for detection of invasive fungal pathogens in specimens taken from primary sterile body sites. It allows rapid detection and identification of a funus at the species level (Lass-Florl et al. 2013, Babouee et al. 2013). Atlhough, the panfungal PCR test supports the key diagnostic findings, it should be used together with other diagnostic tests. It has an ability to identify rare and unexpected pathogens. However, due to its universal character and sensitivity panfungal PCR assays are prone to contamination (Camp et al. 2020). The greatest value of the panfungal PCR test is realised when the preliminary suspicion of IFD is high, or if a difficult-to-culture pathogen is suspected. One advantage of the panfungal PCR test is objectivity. Thus is in contrast to histopathology, which requires high subjective diagnostic experience.

6.2 CANDIDA SPECIES AND ANTIFUNGAL SUSCEPTIBILITY (STUDY II)

C. albicans was the leading cause of candidemia in HUS from 2007–2016, and there were no significant changes in the proportion of *C. albicans* during the study period. *C. albicans* accounted for 64% of candidemias during the first 5-year period and 57% during the second period.

The dominance of *C. albicans* has also been reported in population-based studies conducted in other Nordic countries. *C. albicans* caused 55–68% of candidemias in Iceland, Sweden and Norway (Asmundsdottir et al. 2013, Hesstvedt et al. 2015, Klingspor et al. 2018). However, a decrease in the proportion of *C. albicans* isolates was reported in Denmark from 2004–2015 (Astvad et al. 2018). The reasons for the differences in candidemia epidemiology between Nordic countries have been investigated previously (Hesstvedt et. al 2017). The use of certain antibacterial drugs and use of fluconazole were significantly higher in Denmark compared to other Nordic countries, thus possibly contributing to the observed differences in the epidemiology of candidemia in Nordic countries.

The distribution of *Candida* species causing candidemia has been evaluated in two population-based studies in Finland in recent decades. *C. albicans* accounted for 70% of candidemias in Finland from 1995–1999 (Poikonen et al. 2003), and 67% from 2004–2007 (Poikonen et al. 2010). The earlier study also reported the distribution of *Candida* species causing candidemia in the Helsinki University Hospital during the same period, and *C. albicans* was also the leading (70%) cause of candidemia (Poikonen et al. 2003). Although, we observed no statistically significant sift to non-*albicans Candida* species it appears that the proportion of non-*albicans Candida* species is also slowly increasing in our hospital district. The Finnish National Infectious Diseases Register provides annual register data on the number of all *Candida* isolates identified in blood cultures in Finland. According to the register, 2018 was the first year in Finland when the proportion of *C. albicans* accounted for less than half (49%) of the all yeast isolates diagnosed in blood cultures annually (Finnish National Infectious Diseases Register [Internet database 2020]).

Although our study did not show a significant shift to non-*albicans Candida* species, such a shift has been observed on a global scale. A study from SENTRY surveillance program, including 135 medical centres in 39 countries, has reported an increase in the isolation of *C. glabrata* and *C. parapsilosis* and a decrease in the isolation of *C. albicans* during a 20-year period (Pfaller et al. 2019). The predominance of non-*albicans Candida* species has been reported particularly from the US, Australia, Southern Europe, Asia and South America (Toda et al. 2019, Cleveland et al. 2015, Chapman et al. 2017, Ben-Ami et al. 2012, Puig-Asensio et al. 2014, da Matta et al. 2017, Morii et al 2014, Santolaya et al. 2019).

C. glabrata caused 22% of candidemia and was the second leading cause of candidemia in our study. A high number of cases caused by *C. glabrata* has also been reported also from Denmark, Australia and the US (Arendrup et al. 2013, Chapman et al. 2017, Astvad et al. 2018, Horn et al. 2009, Diekema et al. 2012, Hajjeh et al. 2004). On the other hand, the proportion of *C. parapsilosis* was low in our study, which is consistent with other Nordic countries (Asmundsdottir et al. 2013, Hesstvedt et al. 2015, Lausch[a] et al. 2018). The number of cases due to *C. parapsilosis* is distinctly higher in studies conducted in southern Europe and Brazil (Bassetti et al., 2013, Colombo et al. 2014, Garnacho-Montero et al. 2013, Murri et al. 2018). No *C. auris* isolates were identified during our study. So far (before October 2020), no isolation of *C. auris* has been detected in Finland. Norway is the only Nordic country, where *C. auris* has been isolated before October 2020 (CDC. Tracking Candida auris [Internet database 2020]).

Species distribution has geographical differences, but the reasons for which are partly unclear. Azole use is one key aspect. Use of fluconazole has been reported to increase candidemia caused by non-*albicans Candida* species (Chow et al. 2008, Lortholary et al. 2011). On the other hand, the incidence of non-*albicans Candida* species has been reported to decline after reduction in fluconazole use (Bassetti et al. 2009). An increase of *C. glabrata* cases was observed concomitantly with an increase in azole use and with increasing age in a study conducted in Denmark (Astvad et al. 2018). Patient age has a notable impact on species distribution in every geographical area (Pfaller[a] and Diekema 2002). *C. glabrata* is associated with advanced age and *C. parapsilosis* with neonatal and pediatric age groups (Hesstvedt et al. 2015). *C. parapsilosis* is also associated with the hospital environment, prolonged stay in ICU, and parenteral nutrition (Montagna et al. 2013). Furthermore, the use of broad-specturm antimicrobials reflect the species distribution (Hebert et al., 2010). Health-care workers play a key role in the transmission of microbes (Parry et al. 2001). Infection control policies, reduction of fluconazole overuse, antimicrobial stewardship, and overall hospital environment are most likely key elements in preventing the increase of non-*albicans Candida* species.

Antifungal resistance to fluconazole among *C. albicans* isolates was rare in HUS. Less than 1% of the tested isolates were resistant to fluconazole. No *C. albicans* or *C. glabrata* isolates were resistant to anidulafungin. However, the rate of fluconazole resistance among *C. glabrata* isolates was high. Overall, 24% of *Candida* bloodstream isolates in HUS had potentially acquired resistance or were intrinsically (*C. glabrata* and *C. krusei*) resistant to fluconazole during the study period. However, fluconazole was the most frequent initially started antifungal in our study. The guidelines for the management of invasive candidiasis have changed during the study period. The IDSA guideline published in 2009 recommended fluconazole or echinocandin as an initial treatment for candidemia (Pappas et al. 2009), but the expert panel favoured an echinocandin treatment if the patient is more severally ill or has been exposed recently to azoles. The guideline published in 2016 recommends echinocandin as the initial antifungal agent in management of candidemia (Pappas et

al. 2016). Despite the recommendations, fluconazole was the most frequently (50%) used first-line antifungal also during the later 5-year period (2011–2016) in our study. Hesstvedt et al. evaluated the epidemiology of candidemia in Nordic countries in 2011 (Hesstvedt et al. 2017). They observed that even though Denmark had more *C. glabrata* candidemias than Norway, Sweden or Finland, the Danish *C. glabrata* isolates had considerably lower resistance to fluconazole than isolates in Norway, Sweden or Finland. Finland had the highest rate. There was not a clear reason for this result, but number of cases was lower in Finland and Sweden than Denmark in their study, which might have affected the results. In fact, Denmark had the highest use of antifungals, including fluconazole, among the Nordic countries (Hesstvedt et al. 2017). Differences in susceptibility methods might have some influence on the susceptibility results, but the tests in general have demonstrated an acceptable rate of concordance (Arendrup et al. 2001).

Similarly to our study, fluconazole resistance among *C. albicans* was uncommon during 20-year surveillance study (Pfaller et al. 2019). The surveillance study reported a steady increase in fluconazole resistance of *C. glabrata* isolates over 20 years in the US. Globally, fluconazole and echinocandin resistance remain uncommon among the four most prevalent *Candida* species. However, slow and steady emergence of resistance to fluconazole and echinocandins have been noticed in isolates of *C. tropicalis* and *C. glabrata* (Pfaller et al. 2019).

The incidence of candidemia in our hospital district was low, and it remained steady during the study period. Similarly, the incidence has been low in population-based studies conducted in Finland, Sweden, Norway and Iceland (Poikonen et al. 2003, Poikonen et al. 2010, Klingspor et al. 2018, Hesstvedt et al. 2015, Asmundsdottir et al. 2013).

6.3 CHARACTERISTICS OF CANDIDEMIA (STUDY II)

The patients with candidemia were severily ill and have several co-morbidities. Most (73%) patients in our analysis had McCabe score 2 or 3. In a Danish nationwide study, 74% of the patients with candidemia had multi-morbidity, including a third with ≥3 underlying co-morbidities (Lausch[b] et al. 2018).

Neutropenia, haematologic malignancies and HSCT have been identified as well-known risk factors for candidemia in previous studies (Fridkin and Jarvis 1996, Komshian et al. 1989, Wenzel 1995). However, these factors were present in only a small proportion (<7% each of them) of candidemia cases in our study. This finding was also observed in a recent population-based study from the US (Toda et al. 2019). In their study, only <5% of patients with candidemia had these underlying

conditions. HSCT was reported in only 1% and haematological malignancy in 9% of candidemia cases in a Norwegian study (Hesstvedt et al. 2019). The systematic use of antifungal prophylactics among patients with HSCT and hematologic malignancies propably has influenced on these factors (Pagano et al. 2017, Segal et al. 2007, Cornely[a] et al. 2015).

Almost half of the patients (44%) were treated in surgical wards at the diagnosis of candidemia in our study, which is consistent with other studies (Tortorano et al. 2004, Hesstvedt et al. 2019, Poissy et al. 2020). This suggests the importance of surgical procedures as contributing factors to candidemia. Only 12% of the patients were in the ICU at the time of diagnosis and 8% of the patients were admitted to the ICU because of candidemia after the diagnosis in our study. During the 5-year periods, no change was observed regarding the hospital departments involved at candidemia onset. The proportion of patients who were in the ICU during the diagnosis has been reported to be 20–45% in many studies, which is higher than in our study (Berdal et al. 2014, Garnacho-Montero et al. 2010, Klingspor et al. 2018, Lindberg et al. 2019, Lausch[b] et al. 2018, Lortholary et al. 2014, Hajjeh et al. 2004). Candidemia emerges primarily with intermittent fever and more seldom with septic shock. Randomized clinical trials of adjunctive therapies for severe sepsis or septic shock indicated that fungi are responsible only in ≤6% of all cases with severe sepsis or septic shock (Annane et al. 2018, Ranieri et al. 2012, Opal et al. 2013).

Most of candidemia cases are health-care associated infections (Toda et al. 2019). In our study, 92% of candidemia cases were nosocomial. The proportion of patients with candidemia and a history of IDU was 11% in our analysis, consistent with recent studies conducted in the US (Toda et al. 2019, Zhang et al. 2020). Most of these cases with a history of IDU were community-acquired similar to an earlier study (Zhang et al. 2020). Single-centre studies have reported an increase in *Candida* BSIs related to IDU in the last decade (Poowanawittayakom et al. 2018, Rossow et al. 2020). The proportion of IDU was especially high (36%) in patients with candidemia aged 19–44 years (Zhang et al. 2020). Candidemia patients with IDU are younger, the mortality rate is lower and these patients lack of traditional risk factors for candidemia, such as malignancies, GI surgery, and transplantation (Zhang et al. 2020, Rossow et al. 2020). In Finland, the number of people who use drugs, and the number of patients who have been treated as inpatients for drug related illnesses have increased in recent deacedes (Kankaanpää et al. 2016, Rönkä and Markkula 2020). Trends in IDU should be monitored closely and clinicians should be aware of IDU as an emerging risk factor for candidemia.

6.4 MORTALITY ANALYSIS (STUDY II)

A strong correlation has been observed between an early start of antimicrobial treatment and reduction of mortality in patients with bacterial septic shock (Seymour et al. 2017, Kumar et al. 2006). However, results from studies concerning a potential benefit of early antifungal therapy in patients with candidemia are conflicting. Some investigators have suggested the importance of early treatment for candidemia (Morrell et al. 2005, Garey et al. 2006). Nevertheless, we found no association with the early initiation of an effective antifungal and 30-day mortality of candidemia. Our study revealed the association between comorbidities and mortality in candidemia.

The studies that have reported an associatation between an early initiation of an antifungal treatment and mortality are retrospective, cohort analyses (Morrell et al. 2005, Garey et al. 2006, Patel et al. 2009). A connecting factor for these studies is the lack of routine antifungal susceptibility testing and certainty of targeted treatment. Furthermore, the number of cases was low (n=9) in the group that received antifungal treatment within 12 h after blood cultures (Morrell et al. 2005), or only fluconazole therapy (without newer antifungals) were analysed (Garey et al. 2006). However, all of these studies still reported an association with comorbidities (APACHE II score) and mortality of candidemia, which is in concordance with our results (Morrell et al. 2005, Garey et al. 2006, Patel et al. 2009).

More recent studies have reported results that were similar to our study. Early initiation of an antifungal treatment was not associated with decreased mortality (Arendrup et al. 2011, Parkins et al. 2007, Kludze-Forson et al. 2010, Klevay et al. 2008, Paiva et al. 2016). Although, we did not observe a statistically significant association between timing of treatment and mortality, treatment with an effective antifungal was a protective factor against mortality. The same observation of a beneficial association between appropriate antifungal treatment and mortality was also reported in an another study (Parkins et al. 2007). While effective antifungal treatment is beneficial in candidemia, early timing of the antifungal treatment does not to appear to be as critical as in bacterial septic shock.

We observed an overall 30-day mortality rate of 31%, which is similar to earlier studies (Hesstvedt et al. 2019, Arendrup et al. 2011, Lausch[b] et al. 2018, Puig-Asensio et al. 2014,). We excluded from the mortality analysis the patients with candidemia for whom the effective antifungal treatment was not started at all due to the general poor prognosis. When these patients were excluded, the 30-day mortality was 23%. The 30-day mortality rate was only 9% among patients with no severe underlying illnesses (McCabe score 1). Candidemia mortality is driven to a large extent by the underlying diseases (Grim et al. 2012, Murri et al. 2016, Hesstvedt et al. 2019, Kludze-Forson et al. 2010). This explains why the mortality of candidemia has not decreased in the last 20 years despite advances in antifungal therapy. To improve outcomes, it is crucial to recognise early the patients who are at risk for candidemia.

6.5 PERSISTENT CANDIDEMIA (STUDY III)

Candidemia persisted in 21% of patients in our hospital district. In a Spanish single centre cohort study the proportion of PC was 14% (Agnelli et al. 2019). Although critical illness and underlying comorbidities (e.g. cancer) correlate with mortality in candidemia they don't correlate with its persistence. Comorbities or host factors were not associated with PC in this study or in others (Agnelli et al. 2019, Kang et al. 2017). Instead, proper treatment of candidemia does correlate with persistence, as our study revealed that the presence of CVC, metastatic infection foci and ineffective empirical antifungal therapy were independent risk factors for PC. Other studies have also revealed the presence of CVC as a risk factor for PC (Kang et al. 2017, Li et al. 2018, Ortega et al. 2011), although a recent study did not find this association (Agnelli et al. 2019). Biofilms are formed on prosthetic devises (Ramage et al. 2006), which may be a logical explanation.

Unexpected infection sites, including ocular candidiasis, other solid organ complications and vascular complication were more frequent among patients with PC. Metastatic infection sites were independently associated with PC in a recent study (Agnelli et al. 2019). Son et al. studied ocular involvements in candidemia and reported that 21% of candidemic patients have chorioretinitis or endophthalmitis (Son et al. 2019). PC was also independently associated with ocular involvements in their study. Oude Lashof et al. studied ocular manifestations of candidemia and reported the duration of candidemia was significantly longer in patients with ocular involvements than in patients without ocular involvements (Oude Lashof et al. 2011). Our study revealed that early source control was associated with non-PC in a subgroup analysis, which further supports the evidence that active search and treatment of infection foci is crucial to prevent persistence of blood culture positivity.

We analysed adherence to international guidelines with the Equal Candida Score (Mellinghoff[b] et al. 2018). Our study did not reveal a statistically significant association with the score and persistence of candidemia. In recent studies, guideline adherence was higher in survivors vs. non-survivors, which is consistent with our results (Cornely et al. 2020, Huang et al. 2020). We evaluated the score in a selected patient population (patients with no follow-up blood cultures were excluded from our cohort) and regardless of that the median score was only 14 (IQR 11–16) for patients with CVC and 11 (IQR 11–14) for patients without CVC. The results are clearly less than the maximum score. In other studies, the mean score was between 8–11 (Mellinghoff[a] et al. 2018, Nemer et al. 2019, Huang et al. 2020). Cornely et al. reported that surviving patients had a median score of 17 (IQR 16–19) and those that did not survive had a median score of 16 (14–18) (Cornely et al. 2020).

In our cohort, every patient had at least one follow-up blood culture taken. However, daily follow-up blood cultures were performed only for 10% of the patients. Echocardiography was performed for 53% of patients and ophthalmoscopy

for 24% of patients. Treatment for 14 days after the first negative blood culture was completed in 81% of the patients. Initial echinocandin treatment was administered to 40% of patients, and 43% of those who received echinocandin as initial therapy had step down therapy from echinocandin to fluconazole. The adherence to guidelines should be better than it was in our study especially to considering the fact that ineffective initial antifungal treatment and metastatic infection foci were risk factors for PC. Systematic consultation of an infectious disease specialist is an approach to increase guideline adherence.

6.6 LATE RECURRENT CANDIDEMIA (STUDY IV)

Our study revealed that LR candidemia is an uncommon finding. The annual incidence rate of LR candidemia was 0.13/100 000 inhabitants in the Hospital District of Helsinki and Uusimaa. The proportion of LR candidemia cases was 6% of all candidemia patients during the 10-year study period. Our result supports the findings of earlier studies. In 2017, a study from the US identified 1226 episodes of candidemia in 1140 patients and reported a proportion of recurrent candidemia in 7% of all candidemic patients (Tsay et al. 2020). In other studies, the proportion of LR candidemia has been reported to range from 2–9% (Munoz et al. 2016, Lai et al. 2019, Asmundsdottir et al, 2012).

In our study, underlying GI disease and a history of IDU were associated with LR candidemia. The association between chronic GI diseases and LR candidemia is in concordance with earlier reports (Asmundsdottir et al. 2012, Munoz et al. 2016). Clancy et al. also reported underlying GI diseases and abdominal surgery as common prior finding in recurrent candidemia (Clancy et al. 2000). The GI tract is a major site for *Candida* colonization (Nucci and Anaissie 2001, MacFie et al. 1999, Miranda et al. 2009). The GI tract may be a continuous source of infection in patients with chronic, non-life-threatening GI diseases. Long-term disruption of the barrier function of the GI mucosa may predispose these patients to recurrent *Candida* infections.

A history of IDU was another risk factor for LR candidemia in our study. The patients with history of IDU and candidemia are younger and do not have traditional risk fators (e.g. malignancies, transplantation) for candidemia (Zhang et al. 2020, Rossow et al. 2020). IDU causes several health problems and may predispose to candidemia and other infections. IDU may also favor emergence of antifungal resistance (Grosset et al. 2016).

Our candidemia study supports the finding that LR candidemia appears to be a different entity than PC. Recurrent candidemia was not associated with metastatic infection foci or presence of CVC in our study, similarly to earlier reports (Agnelli et

al. 2019, Kang et al. 2017). In our study, unexpected infection foci were as common with LR candidemia as with single candidemia episodes. The patients with LR candidemia are younger and less frequently had life-threatening underlying co-morbidities than candidemia patients overall.

6.7 LIMITATIONS OF STUDIES I-IV

Study I: The retrospective nature is a major limitation. The data are available only if it is documented in the patient's records. The clinical data were collected only in one hospital district, even though it is the largest hospital district in Finland. The real-life design aimed to evaluate the utility of panfungal PCR in clinical work. However, when PCR specimens were collected during routine clinical care, some specimens were from patients with a very low probability of IFD, which may have influenced to the results.

Study II-IV : The retrospective study design was also a limitation of the candidemia study. Follow-up blood cultures are an essential part of candidemia management. However, daily follow-up cultures are not performed in clinical practice regularly and this was a limitation especially when we analysed PC. Monitoring of the infection clearance with blood cultures is often forgotten or neglected if the patient has a poor prognosis or if the patient responds very well to treatment. Only a small prorpotion of the patients had follow-up blood cultures taken daily. We excluded patients from the study III if no control blood cltures was performed. It was a significant proportion of the entire patient population, and may have reflected to the results. Recurrent candidemia is a rare event. Although our study provided information from a long time period (10 years), the number of recurrent candidemia patients was nevertheless low. Our study evaluated the clinical aspects and characteristics of recurrent candidemia, but our study had the limitation of lacking the genotyping results of the recurrent episodes.

7 CONCLUSIONS

In conclusion, panfungal PCR from deep tissue specimens is helpful for diagnosis of IFDs, but should be combined with other microbiological diagnostic methods. The optimal approach for diagnosis of IFDs relies on the combination of host factors, clinical findings, and multiple diagnostic strategies, None of the currently available diagnostic tests provides sufficient sensitivity and specificity alone. Panfungal PCR may be significant when an infection is suspected to be caused by pathogen, which is difficult to culture.

Overall, the incidence of candidemia in the Hospial district of Helsinki and Uusimaa was low, and we observed no significant shift to non-*albicans Candida* species. *C. albicans* caused more than 50% of the candidemia episodes during the 10-year study period. Haematologic malignancies, neuropenia and bone marrow transplantation are well known risk factors for candidemia, however, only a small proportion of cases were in patients with these underlying conditions. Our result supports findings in other studies from recent years. Treatment with an effective antifungal was a protective factor against mortality, however, our study did not demonstrate an association between 30-day mortality and an early start of antifungal treatment. Candidemia mortality is driven significantly by the underlying diseases.

Our study established that inadequate management of candidemia correlates with persistance of blood culture positivity. PC was associated with metastatic infection foci, presence of CVC, and ineffective empirical treatment. Active search and treatment of metastatic infection foci and early removal of CVC are key elements for preventing persistence of blood culture positivity in candidemia. The Equal Candida Score is a helpful tool to monitor guidelines adherence and assists clinicians optimising candidemia management. In our study, adherence to international guidelines was less than it should be. Consultation of infectious specialist is important in management of candidemia, and should be systematic. LR candidemia was a rare event. It was associated with underlying GI disease and history of IDU. Clinicians should be aware of these factors as possible risk factors for recurrent candidemia.

This doctoral study was focused on the underlying clinical factors associated with candidemia and provided recent epidemiological data from the hospital district of Helsinki and Uusimaa. In future studies, it will be important to continue the monitoring of the burden of candidemia and to conduct a population-based study concerning epidemiological data from the whole country.

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