Clinically Relevant Mycoses

A Practical Approach

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Preface

Introduction

Invasive fungal infections are rare but life-threatening disease for severely ill patients. Due to perpetually improving healthcare, there are life-saving and life-improving therapies for many hemato-oncological diseases, organ transplantation, advanced supportive intensive care, and new techniques making most complicated surgical interventions possible. However, all these patients are at risk for developing invasive fungal infections. Many efforts for better diagnosis and treatment of invasive fungal infections have been undertaken in the last 3 decades. A number of new antifungal agents have emerged during this period. Many clinical studies have been conducted to develop timely and efficient diagnosis and treatments focused on the patients particularly at risk.

Dermatomycoses are the most common fungal infections of mankind, never life-threatening but awesome and ugly. However, knowledge about these dermatomycoses and their treatment is waning.

Generally, medical students learn very little about invasive fungal infections because these are limited to a small patient population at risk. These patients are most frequently encountered in hospitals that focus on neoplastic and hematological diseases. However, many immunocompromised patients, e.g. organ recipients, are cared for in outpatients' clinics or general medicine offices and not specialized centers with a mycology lab service. Thus, the authors have agreed to write a book on fungal infections particularly meant to give a satisfactory overview and a solid background for caring, diagnosing, and treating these patients. Each author wrote a chapter using his and her particular expertise in the field of fungal infection. We thought that fungal infections although rare in the general practice are also of interest for doctors in training, doctors working in other fields than hematooncology or transplantation, and who come across patients being at risk of fungal infections or having fungal infections. Moreover, this book provides good information for senior medical students, nurses, or other highly specialized medical personal.

This book, *Clinically relevant mycoses: a practical approach*, aims to give a general overview on the clinical and scientific aspects of fungal infections. It should provide information on epidemiology, diagnostics, basics of antifungal therapy, and typical clinical syndromes like invasive Candida infection, aspergillosis, and mucormycoses, but also on special patient groups like pre-

mature neonates and children with hereditary immune defects or intensive care patients. It should be a basis for further study in the field of invasive fungal infections. The purpose of this book is to supply the basics and the evidence-based approach for the management of fungal infections.

Objective of This Book

The book provides an evidence-based practical approach to the most frequent fungal infections, diagnostics and treatment in a primary and secondary care hospitals. It gives an easy overview of basic medical and scientific background of fungal infections. Epidemiology, pathogenesis, clinical presentation, diagnostics, and treatment are carefully explained and discussed. The reader will acquire a good and clear perception of invasive fungal infection as well as the challenges in diagnostics and treatment. *Clinically relevant mycoses: a practical approach* will serve as a good tool for clinical management but also will provide the basis for putting further research questions and studies on this particular field. This book will be an invaluable companion for doctors, students of medicine and pharmacology, nurses, and other healthcare professionals.

The information contained in this book applies to all countries. It is the essential requirements for understanding fungal infections. However, different countries will have their different approach according to their specific needs, environment, incidence of fungal infection, and healthcare systems.

Anyone who needs more detailed information on invasive fungal infection and its management is recommended to contact specialized institutions dealing with high-risk patients like hemato-oncology or infectious diseases units and are referred to the high-quality textbooks and recent publications in this field.

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Elisabeth Presterl

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Part I

General

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Birgit Willinger

1.1 Epidemiology

More than 600 different fungi, yeasts and filamentous fungi, some of them are most commonly known as moulds and dermatophytes, have been reported to infect humans, ranging from common to very serious infections, including those of the mucosa, skin, hair and nails, and other ailments.

Particularly, invasive fungal infections (IFI) are found in patients at risk. Both yeasts and moulds are able to cause superficial, deep and invasive disseminated infections, whereas dermatophytes cause infections of the skin, nails and hair. Dermatophytoses are caused by the agents of the genera *Epidermophyton*, *Microsporum*, *Nannizia* and *Trichophyton*.

Invasive infections encompass mainly immunocompromised patients, e.g. patients with the acquired immunodeficiency syndrome or immunosuppressed patients due to therapy for cancer and organ transplantation or undergoing major surgical procedures. As the patient population at risk continues to expand so also does the spectrum of opportunistic fungal pathogens infecting these patients. Invasive fungal infections may also be serious complications of traumatic injury characterized by fungal angioinvasion and resultant vessel thrombosis and tissue necrosis [1, 2]. In contrast to other settings, posttraumatic IFI occurs through direct inoculation of tissue with spores at the site of injury [3]. Both yeasts and moulds are able to cause superficial, deep and invasive disseminated infections, whereas dermatophytes cause infections of the skin, nails and hair.

1.1.1 Yeasts

Yeasts are fungi with a more or less ball-like shape. Yeasts multiply by budding but may form hyphae or pseudohyphae. Many infections are caused by yeasts with the *Candida* being the most common representative. In the last decades, the expansion of molecular phylogenetics has shown that some genera are polyphyletic, which means that some species are of different genetic origin and therefore unrelated. The genus *Candida* is now associated with at least ten different telemorphic genera including *Clavispora*, *Debaryomyces*, *Issatchenkia*, *Kluyveromyces* and *Pichia* [4]. More than 100 *Candida* species are known, whereas the majority of infections are caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* [5]. Other emerging species causing infections have been described. For example, *C. auris* is an emerging multidrug-resistant pathogen that is capable of causing invasive fungal infections, particularly among hospitalized patients with significant medical comorbidities [6].

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Other important genera are *Cryptococcus*, *Malassezia* and *Trichosporon*. Cryptococcal infections occur with a near worldwide distribution in immunosuppressed hosts. This infection is typically caused by *Cryptococcus neoformans*, an encapsulated yeast, and infection is acquired from the environment. *Cryptococcus neoformans* var. *grubii*, *C. neoformans* var. *neoformans* and *C. gattii* are the causes of opportunistic infections which are classified as AIDS-defining illness [7]. Non-*Cryptococcus neoformans* species, including *C. laurentii* and *C. albidus*, have historically been classified as exclusively saprophytic. However, recent studies have increasingly implicated these organisms as the causative agent of opportunistic infections in humans [8].

The lipid-dependent *Malassezia furfur* complex causes pityriasis versicolor, whereas the non-lipophilic *M. pachydermatis* is occasionally responsible for invasive infections in humans. *Trichosporon beigelii* used to be known as the principal human pathogen of the genus *Trichosporon*. Four newly delineated taxa (*T. asahii* and less frequently *T. mucoides*, *T. inkin* and *T. louberi*) are associated with systemic infections in man. *T. mycotoxinivorans* has been described recently as the cause of fatal infections in patients suffering from cystic fibrosis [4].

Saprochaete and *Geotrichum* spp. are rare emerging fungi causing invasive fungal diseases in immunosuppressed patients, mainly in patients with haematological malignancies, but also other non-haematological diseases as underlying disease have been reported [9]. The most important risk factor is profound and prolonged neutropenia [10].

Saccharomyces cerevisiae is a common food organism and can be recovered from mucosal surfaces, gastrointestinal tract and female genital tract of healthy persons. Occasionally, it causes vaginal infections and on very rare occasions invasive infections in immunocompromised and critically ill patients [4].

Rhodotorula species have traditionally been considered as one of common non-virulent environmental inhabitant. They have emerged as an opportunistic pathogen, particularly in immunocompromised hosts, and most infections have been associated with intravenous catheters in these patients. *Rhodotorula* spp. have also been reported to cause localized infections including meningeal, skin, ocular, peritoneal and prosthetic joint infections; however, these are not necessarily linked to the use of central venous catheters or immunosuppression [11].

Pneumocystis jirovecii (formerly known as *P. carinii*) is a unicellular, eukaryotic organism occurring in lungs of many mammals. *P. jirovecii* is a causative agent of *Pneumocystis* pneumonia. Although the incidence of *Pneumocystis* pneumonia (PCP) has decreased since the introduction of combination antiretroviral therapy, it remains an important cause of disease in both HIV-infected and non-HIV-infected immunosuppressed populations. The epidemiology of PCP has shifted over the course of the HIV epidemic both from changes in HIV and PCP treatment and prevention and from changes in critical care medicine. Although less common in non-HIV-infected immunosuppressed patients, PCP is now more frequently seen due to the increasing numbers of organ transplants and development of novel immunotherapies [12].

1.1.2 Filamentous Fungi

Filamentous fungi form colonies of different colours with a more or less woolly surface formed by the filamentous hyphae that may carry conidia (spores) that are disseminated easily via the air (asexual propagation). These fungi are generally perceived as moulds.

Although a wide variety of pathogens are associated with invasive mould diseases, *Aspergillus* spp. are counted among the most common causative organisms. Overall, the genus *Aspergillus* contains about 250 species divided into subgenera, which in turn are subdivided into several sections or species complexes. Of these, 40 species are known to cause diseases in humans. Most invasive infections are caused by members of the *A. fumigatus* species complex, followed by *A. flavus*, *A. terreus* and *A. niger* species complexes [13]. The *Aspergillus fumigatus* species complex remains the most common one in all

pulmonary syndromes, followed by *Aspergillus flavus* which is a common cause of allergic rhinosinusitis, postoperative aspergillosis and fungal keratitis. Lately, increased azole resistance in *A. fumigatus* has become a significant challenge in effective management of aspergillosis. The full extent of the problem is still unknown, but some studies suggest that resistance in *A. fumigatus* may be partially driven by the use of agricultural azoles, which protect grain from fungi [14]. Other species of *Aspergillus* may also be resistant to amphotericin B, including *A. lentulus*, *A. nidulans*, *A. ustus* and *A. versicolor*. Hence, the identification of unknown *Aspergillus* clinical isolates to species level may be important given that different species have variable susceptibilities to multiple antifungal drugs.

Mucormycosis is caused by fungi of the order *Mucorales*. Of fungi in the order *Mucorales*, species belonging to the family *Mucoraceae* are isolated more frequently from patients with mucormycosis than any other family. Among the *Mucoraceae*, *Rhizopus* is by far the most common genus causing infection, with *R. oryzae* (*R. arrhizus*) being the most common one [15, 16]. *Lichtheimia corymbifera*, *Rhizomucor* spp., *Mucor* spp. and *Cunninghamella* spp. are also known to cause jeopardizing infections. *Mucorales* are resistant to voriconazole and caspofungin in vitro and in vivo. The incidence of mucormycosis may be underestimated due to the low performance of diagnostic techniques based on conventional microbiological procedures, such as culture and microscopy. The most useful methods for detecting *Mucorales* are still microscopic examination of tissues and histopathology, which offer moderate sensitivity and specificity. Recent clinical studies have reported that mucormycosis is the cause of >10% of all invasive fungal infections when techniques based on DNA amplification by quantitative used to complement conventional methods [17].

Besides *Mucorales*, the emergence of other opportunistic pathogens, including *Fusarium* spp., *Paecilomyces* spp., *Scedosporium* spp. and the dematiaceous fungi (e.g. *Alternaria* spp.), became evident [5]. *Fusarium* spp., *Alternaria* spp. and *Scedosporium* spp. also account for mould infections among solid organ transplant recipients.

The genus *Fusarium* includes several fungal species complexes. These are ubiquitous soil saprophytes and pathogenic for plants [13]. Only a few species cause infections in humans [18]. Among these are the species complexes *F. solani*, *F. oxysporum*, *F. verticillioides* and *F. fujikuroi* [19]. *Fusarium* spp. have been involved in superficial and deep mycosis and are the leading causes of fungal keratitis in the world [18, 20]. Recently, these fungi have been identified as emerging and multiresistant pathogens causing opportunistic disseminated infections [21, 22].

The genus *Scedosporium* has undergone a taxonomic reclassification. According to the new classification, the most common *Scedosporium* spp. involved in human infections are *S. apiospermum* (telemorphic state, *Pseudallescheria apiosperma*), *S. boydii* (*Pseudallescheria boydii*), *S. aurantiacum* and *S. prolificans* (*Lomentospora prolificans*). Owing to epidemiological reasons, most recent reports divide human infections by these species into mycoses caused by the *S. apiospermum* complex (which includes *S. apiospermum*, *S. boydii* and *S. aurantiacum*) and by *S. prolificans* [13].

Species belonging to the *S. apiospermum* complex are cosmopolitan, being ubiquitously present in the environment, but predominantly in temperate areas. They are commonly isolated from soil, sewage and polluted waters, composts and the manure of horses, dogs, cattle and fowl [23]. *S. prolificans* appears to have a more restricted geographical distribution, being found largely in hot and semiarid soils in southern Europe, Australia and California [24].

Table 1.1 shows the most common yeasts and moulds causing IFI.

1.1.2.1 Relevant Diagnostic Material for Diagnosis of Clinical Mycoses

For definite diagnosis of proven invasive fungal infections, histological and cultural evidence from biopsies, resection material or other specimens obtained from normally sterile body sites is required.

Table 1.1 Spectrum of opportunistic yeasts and moulds (exemplary, without claiming completeness)

Yeasts		Moulds	
Candida	*C. albicans* *C. glabrata* *C. parapsilosis complex* *C. tropicalis* *C. guilliermondii* *C. auris*	*Aspergillus* species complex	*A. fumigatus* *A. flavus* *A. terreus* *A. niger*
Cryptococcus	*C. neoformans* var. neoformans *C. neoformans* var. grubii *C. gattii*	*Mucorales*	*Rhizopus* spp. *Rhizomucor* spp. *Mucor* spp. *Lichtheimia corymbifera* *Cunninghamella* spp.
Trichosporon	*T. asahii* *T. mucoides* *T. inkin* *T. louberi* *T. mycotoxinivorans*	*Fusarium* species complexes	*F. solani* *F. oxysporum* *F. verticillioides* *F. fujikuroi*
Malassezia	*M. furfur* species complex *M. pachydermatis*	*Scedosporium*	*S. apiospermum* *S. boydii* *S. aurantiacum* *S. prolificans* = *Lomentospora prolificans*
Geotrichum and *Saprochaete*	*G. candidum* *S. capitate* *S. clavata*	*Paecilomyces*	*P. variotii*
Saccharomyces	*S. cerevisiae*	*Scopulariopsis*	*S. brevicaulis*
Rhodotorula	*R. rubra* *R. mucilaginosa* *R. glutinis* *R. minuta*	*Alternaria*	

Superficial samples like swabs, respiratory secretion, sputum or stools are not helpful for the diagnosis of invasive fungal infection as both yeasts and filamentous fungi easily colonize body surfaces.

1.1.2.2 Currently Available Diagnostic Methods

Currently, available laboratory methods for diagnosing invasive fungal infections include microscopic detection, isolation of the fungus, serologic detection of antibodies and antigen or histopathologic evidence of invasion [25]. Because of the limited sensitivity of all these diagnostic procedures, and concerns about specificity of some of them, a combination of various testing strategies is the hallmark of IFI diagnosis [17, 25].

1.1.2.3 Histopathology

Histopathology of excised human tissue samples is the cornerstone for diagnosing and identifying fungal pathogens. Direct examination for the presence of mycelial elements using appropriate staining (e.g. Grocott-Gomori methenamine silver, periodic acid-Schiff, potassium hydroxide-calcofluor white) should be performed on all clinical specimens, including respiratory secretions or any tissue sample [17].

However, identifying the specific pathogen based solely on morphological characteristics can be difficult or impossible, because several different organisms may have similar histopathological characteristics, e.g. *Fusarium* spp., and other filamentous fungi are indistinguishable from *Aspergillus* in tissue biopsies [26]. As *Aspergillus* is far more commonly encountered than the other pathogens mentioned, a pathologist often may describe an organism as *Aspergillus* or *Aspergillus*-like based upon morphological features alone. This can hinder diagnosis and may entail inappropriate therapy [27].

1.1.2.4 Microscopy

Direct microscopy is most useful in the diagnosis of superficial and subcutaneous fungal infections and, in those settings, should always be performed together with culture.

Recognition of fungal elements can provide a reliable and rapid indication of the mycosis involved. Various methods can be used: unstained wet-mount preparations can be examined by light-field, dark-field or phase contrast illumination [28]. Because yeast and moulds can stain variably with the Gram stain, a more specific fungal stain is recommended [29].

Microscopy may help to discern whether an infection is caused by yeast or moulds. The presence of pseudohyphae and optionally blastoconidia indicates the presence of yeast, whereas moulds are most commonly seen as hyaline hyphomycetes, generally characterized by parallel cell walls, septation (cross wall formation in hyphae), lack of pigmentation and progressive dichotomous branching as in *Aspergillus*, *Fusarium* or *Scedosporium* species [30]. However, it is impossible to differentiate between the respective genera of the mentioned fungi. It is important to look for septate and nonseptate hyphae, thus allowing to distinguish between *Aspergillus* sp. and members of the *Mucorales*. Mucoraceous moulds have large ribbon-like, multinucleated hyphal cells with non-parallel walls and infrequent septa. The branching is irregular and sometimes at right angles. Hyphae can appear distorted with swollen cells, or compressed, twisted and folded [30]. Another group of moulds causing tissue invasion with a distinctive appearance is the agents of phaeohyphomycosis, such as *Alternaria* and *Curvularia*. These fungi have melanin in their cell walls and appear as pigmented, septate hyphae [31]. The detection of fungal hyphae and/or arthrospores in skin, nail or hair samples may indicate the presence of dermatophytes but give no special hint as to the species involved.

The most common direct microscopic procedure relies on the use of 10–20% potassium hydroxide (KOH), which degrades the proteinaceous components of specimens while leaving the fungal cell wall intact, thus allowing their visualization [30].

The visibility of fungi within clinical specimens can be further enhanced by the addition of calcofluor white or blankophores. These are fluorophores, which are members of a group of compounds known as fluorescent brighteners or optical brighteners or "whitening agents" and bind to beta 1–3 and beta 1–4 polysaccharides, such as found in cellulose and chitin. When excited with ultraviolet or violet radiation, these substances will fluoresce with an intense blueish/white colour [25]. Optical brightener methods have been shown to be more sensitive than KOH wet mount [31]. Filamentous fungi like aspergilli, which stain poorly by the Gram procedure, may be unveiled on gram-stained microscopic mounts after removal of immersion oil by subsequent Blankophor staining [32]. As optical brighteners provide a rapid and sensitive method for the detection of most fungi, their use is encouraged for respiratory samples, pus, tissue samples and fluids from sterile sites when a fluorescence microscope is available [33].

Also, lactophenol cotton blue is easy to handle and often used for the detection and identification of fungi. Other stains are frequently used in direct microscopy, such as the India ink wet mount, which is useful for visualization of encapsulated fungi, particularly *Cryptococcus neoformans*. Although a negative direct examination cannot rule out fungal disease, visualization of fungal elements in specimens can often secure initial information helpful in the selection of empirical antifungal therapy [32].

For detection of *P. jirovecii*, special staining as, for example, direct immunofluorescent staining is required. Sputum induction and BAL are the most commonly used, although non-HIV-infected patients with PCP may require lung biopsy for diagnosis. Standard staining methods include methenamine silver, toluidine blue-O or Giemsa stain. Monoclonal antibodies can be used to detect *Pneumocystis* with a rapid, sensitive and easy-to-perform immunofluorescence assay [12].

1.1.2.5 Culture

Culture remains one of the key methods for diagnosing fungal infection. Though often slow, sometimes insensitive and sometimes confusing with respect to contamination, culture may yield