Diplomarbeit

The incidence of invasive aspergillosis and treatment with antifungal agents in immunosuppressed patients at a university hospital

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1 Abbreviation

ABPA= acute bronchopulmonary Aspergillosis

ALL= acute lymphatic leukemia

AML= acute myeloid leukemia

BAL= broncho alveolar lavage

BSI= blood stream infection

CFR= case fatality rate

CLL= chronic lymphoid leukemia

CRF= case report form

CSF= cerebrospinal fluid

CT= computed tomography

GM-CSF= granulocyte-macrophage colony-stimulating factor

HEPA= high efficiency particulate airfilter

HSCT= human stem cell transplantation

IA= invasive Aspergillosis

IFI= invasive fungal infection

IFNγ= interferon γ1b

IPA=invasive pulmonary Aspergillosis

LipoAMP=liposomal Amphotericin B

MDS= myelodysplastic syndrome

MEDOCS= Medical and nursing documentation network of Styria

MM= multiple myeloma

MRI= magnetic resonance imaging

NHL= non Hodgkin Lymphoma

rh= recombinant human

2 Summary

2.1 Aim

Invasive fungal infections in patients with hematologic malignancies are difficult to diagnose and outcome is often fatal. Therefore various numbers of antimycotic agents are prescribed, many of them preemptively. Because of the difficulties in diagnosis, the European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) established consensus definitions for defining opportunistic invasive fungal infections. Patients are classified of having “proven, probable or possible invasive fungal infection”. In this study we investigated the epidemiology of invasive fungal infections and the reason for administration of antifungal agents in patients with hematologic malignancies.

2.2 Methods

The study was conducted at the Department of Internal Medicine, Division of Hematology, Leopold-Auenbrugger Medical University Graz for a total of 7 months from May to December 2007.

In 117 patients receiving systemic antifungal treatment data were collected prospectively of underlying disease, agent, dosage, duration and reasons for receiving antifungal therapy. Fungal infections in study patients were retrospectively classified as possible, probable or proven according to the EORTC criteria (including clinical, radiologic and culture based approaches). Patients with possible, probable or proven invasive fungal infection were further screened for fungal species, risk factors, and outcome.

2.3 Results

22/117 patients (19%) had possible, 5/117 (4%) probable and 5/117 patients (4%) proven invasive fungal infection. 7/10 probable and proven infections were due to *Candida spp.*, 2/10 due to *Aspergillus* and 1/10 due to Zygomycetes.

153 courses of antifungal agents were prescribed during the study period. Caspofungin was prescribed in 72/117 (62%) of patients, followed by posaconazole in 38/117 (32%), itraconazole in 15/117 (13%), and voriconazole in 14/117 (12%). Fluconazole and amphotericin B were each prescribed in 6/117 (5%) of patients.

6/117 (5%) of patients received combination therapy. 52/117 (44%) of patients received antifungal prophylaxis, 81/117 (69%) of patients received preemptive, 5/117 (4%) of patients received directed antifungal treatment.

2.4 Conclusion

Proven invasive fungal infections are rare in patients with hematologic malignancies. Antifungal therapy in patients with hematologic malignancies is preemptive in most cases.

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3 Zusammenfassung

3.1 Ziel

Invasive Mykosen in Patienten mit hämatologisch maligner Grunderkrankung sind schwierig zu diagnostizieren und enden oft fatal. Deswegen wird eine große Zahl systemisch wirksamer antifungaler Therapeutika in diesem Patientenkollektiv verschrieben. Wegen der diagnostischen Schwierigkeiten, wurden von der European Organization for Research and Treatment of Cancer (EORTC) und der Mycoses Study Group (MSG) Konsensus Definitionen herausgegeben, welche opportunistische invasive Mykosen als bewiesene, wahrscheinliche oder mögliche invasive fungale Infektion definieren. In dieser Studie haben wir die Epidemiologie von invasiven Mykosen und die Ursache für die Gabe von Antimykotika bei Patienten mit maligner hämatologischer Grunderkrankung untersucht.

3.2 Methoden

Von Mai bis Dezember 2007 wurden 117 Patienten der Abteilung für Hämatologie, Universitätsklinik für Innere Medizin Graz mit hämatologisch maligner Grunderkrankung und systemischer antifungaler Therapie in die Studie eingeschlossen. Daten über antifungale Therapie, Dosierung, Dauer, Grund für die Verabreichung und Grunderkrankung wurden gesammelt. Fungale Infektionen in Studien-Patienten wurden nach den Kriterien der EORTC möglichen, wahrscheinlichen oder gesicherten invasiven Mykosen zugeteilt.

3.3 Resultate

22/117 Patienten (19%) hatten eine mögliche, 5/117 (4%) eine wahrscheinliche und 5/117 Patienten (4%) gesicherte invasive fungale Infektion. 7/10 wahrscheinlichen und gesicherten invasiven Mykosen wurde durch *Candida spp.*, 2/10 durch *Aspergillus* und 1/10 durch Zygomyceten verursacht.

153 antifungale Therapien wurden während der Studienlaufzeit verschrieben. Caspofungin wurde in 72/117 (62%) Patienten verschrieben, gefolgt von

Posaconazole in 38/117 (32%), Itraconazole in 15/117 (13%), und Voriconazole in 14/117 (12%). Fluconazole und Amphotericin B wurden je in 6/117 (5%) der Patienten verschrieben. 6/117 (5%) der Patienten erhielten Kombinations Therapie. 52/117 (44%) der Patienten bekamen Antifungale Prophylaxe, 81/117 (69%) der Patienten Präemptive und 5/117 (4%) der Patienten gezielte antifungale Therapie.

3.4 Zusammenfassung

Bewiesene Invasive fungale Infektionen in Patienten mit hämatologisch maligner Grunderkrankung sind selten.

Eine systemische antifungale Therapie wird in diesen Patienten meist präemptiv verschrieben.

4 Aspergillus and Candida species

4.1 Aspergillus species

The genus *Aspergillus* was first recognized in 1729 by Micheli, in Florence. Virchow published in 1856 the first complete microscopic descriptions of the organism. The genus *Aspergillus* is a group of molds and asexual forms of the family Trichocomaceae [17].

Aspergillus species are one of the most ubiquitous of the airborne saprophytic fungi and exposure to their spores is frequent. However disease due to tissue invasion with these fungi is uncommon and occurs primarily in immunocompromised hosts. Invasive aspergillosis is widely believed to occur as a consequence of exogenous acquisition of the conidia (spores) of the species. *Aspergillus* conidia are resilient and may survive for long periods in any substances that can absorb, retain, and transport infectious species, eg. woolen clothes or beding [16]. The airborne route is the most common route of transmission of *Aspergillus* infection, however, the sources of aspergillus may be broader, as waterborne transmission of *Aspergillus* conidia through contaminated aerosols has been suggested [18]. *Aspergillus fumigatus* is the most common *Aspergillus* species causing infection; other infection causing species include *A. flavus, A. terreus, A. niger*, and *A. lentulus*.

Table 1
Aspergillus species and their clinical significance (adapted from [16])

Aspergillus species	Mycological characteristics	Clinical significance	Mycoses
A. flavus	Olive to lime green colonies	Second most common species, produces aflatoxin	Sinusitis, cutaneous infection, pulmonary and disseminated disease
A. fumigatus	Smoky, blue- or gray green colonies, smooth conidia	Most common species causing invasive infection	Invasive pulmonary aspergillosis, disseminated infection, CNS, others
A. niger	Black colonies, radiate conidial head, rough conidia	Common cause of otomycosis, produces oxalate crystals	Otomycosis, cutaneous endophthalmitis, aspergilloma, less common invasive or disseminated disease
A. terreus	Beige to buff colonies	Increasing frequency, associated with soil, usually resistant to polyenes	Pulmonary, disseminated, cutaneous, keratitis, CNS
A. lentulus	Poorly sporulating variant of *A. fumigatus*	May be multidrug resistant and underdiagnosed	Invasive pulmonary, disseminated, other sites

4.1.1 Clinical manifestations

The spectrum of clinical syndromes associated with aspergillosis is diverse, ranging from allergic bronchopulmonary aspergillosis, which is an allergic response to the organism, asymptomatic colonization, superficial infection, to acute or subacute and chronic invasive disease. Generally the clinical presentation corresponds to the underlying immune defects, with greater immune suppression correlating with increased risk for invasive aspegillosis [16, 17]. Allergic bronchopulmonary aspergillosis is characterized by transient pulmonary infiltrates due to atelectasis. Aspergilloma, a pulmonary fungus ball due to *Aspergillus*, is a solid mass of hyphae, growing in a previous existing pulmonary cavity in patients with chronic lung disease. Chronic pulmonary aspergillosis includes three distinct syndromes, chronic cavitary pulmonary aspergillosis, chronic fibrosing aspergillosis and chronic necrotizing aspergillosis or subacute aspergillosis. Disseminated Aspergillosis involves the kidneys, liver, spleen and the central nervous system most frequently, followed by heart, bone, skin, and other organs. Invasive pulmonary aspergillosis is the most

common form of invasive aspergillosis and is described below. Other clinical manifestations include otomycosis, fungal sinusitis, where *Aspergillus flavus* is the most common cause, and endocarditis [12, 17].

4.2 Candida species and other mycoses

Candida are yeast-like fungi that can form true hyphae but also pseudohyphae. These yeasts are typically confined to human and animal reservoirs and are frequently recovered from the hospital environment. Only a few of more than 100 existing *Candida* species are recognized as causing diseases in humans. *Candida glabrata*, and *Candida albicans* account for 70-80% of yeasts isolated from patients with invasive candidiasis. Other clinically significant *Candida* species include *Candida krusei, Candida parapsilosis, Candida tropicalis, Candida lusitaniae, Candida guilliermondii, Candida kefyr* and *Candida dubliniensis* [19].

The most common *Candida* species causing disease is *Candida albicans*, which remains the predominant cause of candidemia worldwide. However, a shift towards other *Candida* species has been observed recently in some European centers [31]. *Candida glabrata* is now the second most important pathogen causing candidiasis; its incidence is increasing worldwide and it is intrinsically less susceptible to azoles. *Candida krusei* is clinically significant because of its intrinsic resistance to fluconazole and decreased susceptibility to all other antifungals, including ampotericin B [19]. The exact role of the increasing use of azoles in this epidemiological shift towards azole resistant non-*albicans Candida* species remains controversial [32].

Candida infections can present in a wide spectrum of clinical syndromes, depending on the degree of immunosuppression of the host and the site of infection. Clinical manifestations include cutaneous candidiasis syndromes, oropharyngeal candidiasis, esophageal candidiasis, respiratory tract candidiasis, vulvovaginal candidiasis, urinary tract candidiasis, abdominal candidiasis including peritonitis, candida osteomyelitis and arthritis, candidemia and disseminated candidiasis, CNS candidiasis, occular candidiasis, cardiac and endovascular candidiasis and chronic systemic candidiasis [19].

Zygomycetes encompass two orders of filamentous fungi, the Mucorales and the Entomophtorales, with members of the order Mucorales causing the majority of cases of zygomycosis (e.g. mucormycosis) in humans [20]. Clinical manifestations include rhinocerebral mucormycosis, pulmonary mucormycosis, cutaneous mucormycoses, disseminated mucormycoses or cerebral infection alone [12].

5 Clinical aspects of invasive fungal infection in the immunocompromised host

Over the last two decades, the incidence of invasive fungal infections has increased in all parts of the world [1]. In the United States, the incidence of fungal sepsis has increased three-fold between 1979 and 2000 [2] with the mortality due to mycoses increasing from 0.7 in 1980 to 2.4 deaths in 1997 per 100.000 population [6]. The etiology for this ongoing epidemiological development is not completely understood. However, the major contributing factor is the increasing size of the population at risk. The population at risk includes patients with hematologic malignacies, recipients of hematopoietic stem cell or solid organ transplants and persons with human immunodeficiency virus infection [1].

More intensive regimens among patients undergoing transplants or treatment for malignancies have resulted in more profound levels of immunosuppression that are sustained for longer periods. Likewise the survival of individuals with life-threatening illnesses has improved with the increasing use of invasive monitoring and aggressive therapeutic technologies in intensive care units. However, this aggressive treatment options have also contributed to an increase in the number of persons at risk for invasive fungal infections [1].

5.1 Infections in the immunocompromised host

Immunocompromised hosts are patients with leukemias, lymphomas, and solid tumors who are receiving chemotherapy, patients receiving bone marrow including stem cell transplants, all solid organ transplants, and patients using immunsuppressive agents and immune modulators for inflammatory disorders. The number of patients meeting these definitions is rising. Immunocompromised patients are at risk for infections by normally accepted human pathogens and human saprophytes, but also by environmental organisms of low intrinsic virulence [11].

Immuncompromised patients can be divided into three main types that predispose to different types of infections: (adapted from [11])

a.) Patients whose major defect is caused by cytotoxic therapy or irradiation and for whom the major defect is neutropenia (neutrophil count < 500 mm^3) and mucosal barrier damage

b.) Patients whose major defect is suppression of cell-mediated immunity due to truly immunosuppressive agents to control organ rejection or inflammation (patients receiving corticosteroids, organ transplants and antirejection therapy)

c.) Patients who show both forms of compromise (allogeneic bone marrow transplant recipients).

Pathogens encountered in neutropenia and mucositis (group a) primarily arise from the skin, oral cavity and the GI tract. Bacteria include *Staphylococcus epidermididis, S. aureus, S. viridans, Enterococcus*, enteric Gram-negatives and *Pseudomonas*. Anaerobes are less common. Fungal infections usually develop after antibiotic therapy has reduced the bacterial flora for several days. *Candida* and *Aspergillus* species are most common [11].

Pathogens encountered in patients with suppression of T Cell functions include the same community-acquired pathogens as the normal host. Patients are at increased risk of bacterial infections with *Mycobacteria* species, *Listeria monocytogenes, Nocardia* species, and *Salmonella* species. Reactivation of old viral infections is a major concern. Fungal infection is often life threatening and may be difficult to diagnose; most common are *Cryptococcus*, *Aspergillus* species, *Fusarium* species, the *Mucor/Rhizopus* group, *Histoplasmosis* or *Coccidiomycosis* depending on geographical location and dematiaceous fungi or black mold [11].

5.2 Invasive fungal infection

During the past several decades, opportunistic invasive fungal infections in immunocompromised hosts have assumed a much greater importance, largely because of the increasing size of the population at risk. Clinical important fungi consist of yeast, molds, and dimorphic species, with each posing a different challenge to clinicians. The most important causative organisms of invasive fungal infection in patients with hematologic malignancies are *Candida* and *Aspergillus*,

followed by Zygomycetes; all of them are mainly healthcare-associated. In addition to infections acquired in hospitals and other healthcare settings, there has also been a marked increase of the community-acquired mycoses, that are endemic in the Americas [8]. Fungal infection is more difficult to diagnose compared with bacterial infections by conventional culture. Treatment is also difficult because of the limited range of antifungal agents [3]. At particularly high risk for difficult-to-treat and often fatal invasive fungal infections are patients with neutropenia (eg. resulting from chemotherapy for acute myelogenous leukemia or the myelodysplastic syndrome), and patients undergoing allogeneic HSCT [49]. Among 3228 patients who underwent HSCT during 1999-2003 in italy, IFI occurred in 3,7% of patients (7,8% in allogeneic HSCT recipients vs 1,2% among the autologous HSCT recipients). 2,8% of all IFIs were due to moulds (*Aspergillus* spp. in 94%), 0,9% due to *Candida* yeasts [54].

5.2.1 Invasive Aspergillosis

Aspergillus is the second major cause of invasive fungal infections, accounting for 10-20% of all invasive mycoses [4]. The trend of mortality associated with aspergillosis has demonstrated an exponential total increase of 357% from 1980 to 1997 [6]. Reasons are the use of more intensive cytotoxic anticancer chemotherapy and the introduction of novel immunosuppressive therapies for organ transplant recipients, both of which have prolonged the period of risk for many individuals, the increasing number of patients undergoing solid organ, bone marrow, and hematopoietic stem cell transplantation, but also the growing awareness of aspergillosis among clinicians and improved diagnostic approaches [16].

Invasive Aspergillosis most frequently originates via inhalation of *Aspergillus* conidia into the lungs, however, other routes of exposure such as inhalation of water aerosols contaminated with *Aspergillus* conidia have been suggested [18]. The inhaled small resting conidia enlarge and germinate in the absence of effective pulmonary host defenses, then they transform into hyphae with subsequent vascular invasion and eventual disseminated infection [16,17,46].

IA is extremely uncommon in immunocompetent hosts. Corticosteroids play a major role in increasing susceptibility to *Aspergillus* by increasing the linear growth rate by as much as 30% to 40% and cell synthesis by greater than 150%, and also by decreasing oxidative killing of the organism by pulmonary macrophages [46].

Prolonged and profound neutropenia is another key risk factor for the development of IA. Graft-versus-host disease, and cytomegalovirus infection are important risk factors for the recurrence and progression of *Aspergillus* infections after bone marrow recovery.

Invasive pulmonary aspergillosis, the most common form of invasive aspergillosis in immunocompromised patients, occurs after approximately 2 weeks of neutropenia or during graft-versus-host disease. Symptoms include fever, dry cough, shortness of breath, pleuritic chest pain, hemoptysis as well as pulmonary infiltrates [16].

Among allogeneic human stem cell tansplant (HSCT) recipients invasive aspergillosis has emerged as a leading cause of infection-related mortality [54]. However, assessing the incidence of invasive aspergillosis in this group is difficult because of the lack of a consistent case definition and the absence of effective surveillance mechanisms [1]. But the situation is improving, with adoption of an agreed set of consensus definitions for clinical trials of invasive fungal infections [5]. However these definitions require the use of aggressive diagnostic procedures which may not be performed in all cases. Because of this it is likely that incidence figures will still underestimate the true burden of disease [5].

Review of the literature from 1995 until 1999 by Lin et al showed an overall case-fatality-rate (CFR) associated with invasive aspergillosis of 58%, despite aggressive treatment with Amphotericin B deoxycholate and lipid formulations. The CFR was highest for bone marrow transplant recipients (86.7%) and for patients with central nervous system or disseminated aspergillosis (88.1%) [7]. Marr et al reviewed 5589 recipients of hematopoietic stem cells and found an increase of the incidence of invasive aspergillosis from 6% in 1992 to 12% in 1999 in allograft recipients, and from 1,1% in 1992 to 5,3% in 1999 in autograft recipients, respectively [9].

5.2.2 Invasive Candidiasis and other invasive mycoses

Candida is causing 70-90% of all invasive mycoses [4]. In the United states *Candida* species are ranked as the fourth most common cause of hospital acquired blood stream infection, representing approximately 8% to 10% of all BSIs, with an incidence of 1,5 cases per 10.000 patient days [28]. In comparable European studies *Candida* were a less common cause of bloodstream infections. In a 10 year

restrospective study carried out in Switzerland *Candida* species ranked number seven accounting for 3% of all bloodstream infections, with an incidence of 0,5-0,7 case per 10.000 patient days. In this survey, one third of all episodes of candidemia occurred in intensive care units, one third in hematology-oncology units and other medical wards, and one third in surgical and pediatric wards [29].

Critically ill and severely immunocompromised patients are at particularly high risk of invasive candidiasis. Candidemia, one of the most frequent clinical manifestations of candidiasis, is often associated with disease outside the bloodstream. In many cases the infection spreads from *Candida* colonization in the gastrointestinal tract or other organs, after immunosuppression and mucosal damage caused by cytotoxic therapy; this helps to explain why *Candida* blood stream infection is such a devastating disease. Morgan *et al*, estimated the attributeable mortality rate of candidemia to be 19-24%, depending on the patients age and receiving adequate treatment [30].

Host defects play a major role in the development of candidemia. Disruption of the skin barrier from burns, wounds or also indwelling intravascular devices (which account for at least 20% of candidemias) in combination with the absence of an effective host defense system predispose for disseminated, often life-threatening candidal infections [19]. Clinical presentation of candidemia varies from fever alone and absence of any organ-specific manifestations, to fulminant sepsis. Asymptomatic disseminated infection is also possible.

Important other invasive mycoses include Cryptococcosis, Zygomycosis (Mucormycosis), Fusariosis, Scedosporiosis, Blastomycosis, Histoplasmosis, Coccidiomycosis and Paracoccidioidomycosis [47]. Most infections with *Cryptococcus neoformans* occur in AIDS patients with meningitis being the most common clinical presentation [12]. Zygomycosis, which was formerly known as mucormycosis is a rapidly progressive disease characterized by angioinvasion, thrombosis, tissue necrosis, and dissemination in the immunosuppressed susceptible host [20]. The incidence of serious invasive zygomycosis in immunosuppressed patients has increased which has been attributed to extensive prophylactic voriconazole use, to which these organisms are intrinsically resistant [21]. Prior exposure to voriconazole increased the risk for zygomycosis 20-fold, while patients with fungal sinusitis had a 80-fold higher probability of zygomycosis [21] Histoplasmosis, Coccidiomycosis and Blastomycosis are endemic mycoses in the

United States, Mexico and many other countries of the world [48]. They present a major health threat especially to HIV infected or immunosupressed individuals in these countries, however in Europe they are rare, with the large majority of cases being imported from countries where these fungals are endemic [8].

5.3 Diagnosis of Invasive Fungal Infection

The inability to consistently make a convincing and early diagnosis of invasive fungal infection (IFIs) remains one of the central problems. The signs and symptoms are often nonspecific and microbiological cultures are usually negative. Histopathological diagnosis requires invasive procedures to obtain specimens, which is often hindered by the grave conditions in these patients. Invasive fungal infections have a high mortality, which has contributed to the difficulties in establishing timely diagnosis and initiating prompt antifungal therapy.

5.3.1 Approaches to diagnosis

Probable IFIs can be identified by one of four different approaches:

(a) culture-based analysis;

(b) radiographic determination of a disease compatible with the diagnosis of IFI;

(c) enzymatic immunoassays that detect fungal antigen galactomannan or ß-D-glucan

(d) DNA-amplification assays using polymerase chain reaction technology

5.3.1.1 Microscopy and Culture-based analysis

Culture-based approaches, although arguably the most convincing tool, are limited due to lack of accessibility, lack of sensitivity, and lack of speed. Histopathology is the most reliable diagnostic method for invasive aspergillosis, although a similar appearance is seen with *Fusarium* and *Scedosporium.* In acute bronchopulmonary aspergillosis but not in invasive disease microscopic sputum examination is helpful. Microscopy of BAL is sometimes helpful in invasive disease. Culture provides the definitive diagnostic method in IA, although interpretation is difficult. Isolation of

Aspergillus may come from sputum and BAL; it can also often be isolated from sinus washings or biopsies but is seldom isolated from blood, urine or CSF [12].

Histopathology provides definitive evidence of infection with *Candida* species. Microscopy and culture of normally sterile body fluids reveals yeast cells with or without filament production, and growth of yeast colonies after 24 to 48 hours. The species should be identified of the isolates and, if appropiate, antifungal susceptibility testing should be carried out. Candidemia may be diagnosed by blood culture. However, this may be line-associated and transient, or indicative for disseminated disease [12]. The presence of broad, mostly non-septate hyphae with rightangled branching in specimens from necrotic lesions, sputum or BAL is highly significant for mucormycosis [12].

5.3.1.2 Radiographic determination of an IFI

Radiographic approaches are also not satisfactory, although for example the “halo sign” seen on high resolution computed tomography is clinically significant during the acute/early phase of invasive pulmonary aspergillosis [15]. Other diagnostic signs for IPA are the appearance of crescent-shaped air pockets, cavitation, nodules, alveolar infiltrates and interstitial infiltrates [44]. CT and MRI scans have enhanced the early diagnosis of IA and invasive candidiasis, as lesions are often visible earlier than on a chest-X-ray [12].

In chronic disseminated candidiasis CT and MRI are helpful for diagnosis and determining the extent of brain involvement, while a normal liver scan has a highly negative predictive value. Fundoscopic examination may reveal opacities [12].

Table 2
Abnormalities commonly seen in pulmonary imaging of fungal infections (adapted from [44])

Radiologic findings	Fungus
Alveolar infiltrates	Aspergillus, Blastomyces, Candida, Coccidioides, Cryptococcus, Histoplasma, Pneumocystis, zygomycetes
Interstitial infiltrates	Aspergillus, Coccidioides, Cryptococcus, Histoplasma. Paracoccidioides, Penicillium, Pneumocystis
Nodules	Aspergillus/ zygomycetes (halo sign), Candida, Coccidioides, Cryptococcus, Histoplasma, Paracoccidioides, Pneumocystis
Masses	Aspergillus, Blastomyces, Coccidioides, Cryptococcus, zygomycetes
Cavitation	Aspergillus/zygomycetes (air crescent sign), Blastomyces, Coccidioides, Cryptococcus, Histoplasma, Paracoccidioides, Pneumocystis
Abscesses	Candida, Pseudallescheria (Scedosporium), zygomycetes
Adenopathy	Coccidioides, Cryptococcus, Histoplasma
Pleural effusion	Candida, Coccidioides, Cryptococcus, Histoplasma, Pneumocystis

5.3.1.3 Enzymatic Immunoassays and Fungal DNA detection by PCR

Early diagnosis of an IFI remains a great challenge with the methods above. For this reason non–culture-based approaches that are based on the detection of specific fungal antigens, fungal metabolites, or fungal DNA have been the focus of continued study. So far, all these methods have more or less a low sensitivity and specificity. Therefore most fungal infections can not be diagnosed definitively without tissue biopsies [14].

Antigen testing, such as ELISA for galactomannan is useful in invasive aspergillosis, as is the detection of 1-3-β-D-glucan in serum. PCR may be used for the detection of fungal genomic sequences, but this approach is still experimental. In acute bronchopulmonary aspergillosis and aspergilloma precipitin testing is useful [12]. Serologic antibody testing may be useful for aspergilloma, but is not recommended for invasive disease because the immuncompromised patients most at risk are less likely to mount a sufficient response [16].

Serology is useful in immunocompetent individuals with *Candida* infection; high or rising antibody titres (1:8 or greater) are considered indicative of active infection. Quantitative determination of anti-Candida mannan IgG is useful, as is detection of mannoprotein antigen by ELISA [12].

5.3.2 Diagnosis of IFI in patients with hematologic malignancies

Diagnosis of fungal infection in severely immunosuppressed patients with hematologic malignancies remains a serious challenge, especially without tissue biopsy. IFIs in patients with hematologic malignacies like AML are often presumed or probable fungal infections that are treated empirically. Antifungal treatment even may be started preemptively in high risk AML patients with prolonged severe neutropenia. Most preemptive treatment is given to recipients of allogeneic stem cell transplants, especially those recipients with a high risk for developing IFIs. Because these patients very often receive antifungal agents, either prophylactically or as treatment, the sensitivity of enzymatic immunoassays drops sharply [14].

As a consequence of the difficulties to diagnose invasive fungal infections, it is very difficult to determine the overall burden of infection. Surveillance efforts usually concentrate on determining just the burden of severe disease. In addition healthcare providers often feel, since no immediate public health action needs to be taken, that there is no need to report fungal infections. As a result of these factors, fungal infections are not only under-diagnosed, but also under-reported [1].

Established risk factors in patients with hematologic malignancies receiving myelotoxic chemotherapy are previous fungal infection, neutropenia exceeding 10 days, older age, active cancer, corticosteroid therapy, administration of broad spectrum antibiotics, allogeneic HSCT, central venous catheter and organ dysfunction [67].

Because of the difficulties in diagnosis of IFIs, the European Organization for Research and Treatment of Cancer (EORTC) Invasive Fungal Infections Cooperative Group and the Mycoses Study Group (MSG) of the National Institute of Allergy and Infectious Disease established consensus definitions for defining opportunistic invasive fungal infections. Patients are classified of having proven, probable or possible invasive fungal infection.

5.4 Treatment of Invasive fungal infections

5.4.1 Therapeutic Agents

Antifungal agents in use for IFIs include polyenes, azoles and echinocandins.

5.4.1.1 Polyenes (Amphotericin B)

Amphotericin B **deoxycholate** is a fungicidal agent with broad-spectrum antifungal activity that acts on ergosterol of the fungal membrane. It has been used for decades as the gold standard treatment for IA and invasive candidiasis. It is usually used at a dose ranging between 0,6 and 1mg/kg i.v. However, amphotericin B deoxycholate is often poorly tolerated being associated with nephrotoxicity (decreased glomerular filtration and tubular waisting of potassium, natrium and bicarbonate), and with infusion -related acute reactions (eg chills, fever, rigor, nausea, hypoxaemia, and hypotension), especially when administered over a short period of time (ie 4 to 6 hours). Underdosing and treatment interruptions due to infusion-related and renal toxicity may affect the efficiacy of amphotericin B deoxycholate therapy [45]. The administration of amphotericin B deoxycholate as a continuous infusion over 24 hours with saline loading has been shown to reduce infusion-related reactions and renal impairment [35]. A multivariate analysis on the epidemiology of renal toxicity in 494 patients receiving amphotericin B deoxycholate showed that male gender, body weight ≥90kg, chronic renal diseae, treatment with aminoglycosides or cyclosporin and doses of amphotericin B ≥35 mg/day were independent risk factors for nephrotoxicity, suggesting that alternative therapy might be appropiate in patients with 2 or more risk factors [34].

Lipid formulation of *amphotericin B* (colloidal dispersion, lipid-complex and liposomal) were developed to decrease toxicity and allow administration of higher doses of drug [16]. They are better tolerated than amphotericin B deoxycholate and have been in use mainly in patients intolerant to conventional amphotericin B or unlikely to tolerate it. In Austria mainly liposomal amphotericin B (AmBisome®) is used. It is usually used in a dose ranging between 3-5 mg/kg/day [12]. To date there are few randomised studies comparing the efficiacy of amphotericin B deoxycholate with that of lipid formulations. In a randomised double-blind study in 174 immunocompromised patients with IA similar success rates were obtained with

amphotericin B colloidal dispersion (35%) and amphotericin B deoxycholate (35%) [36]. Another study comparing a 1 to a 4 mg/kg/d dose of liposomal amphotericin B of first-line therapy in 87 patients with IA yielded similar survival rates (43 to 37%) [37]. Overall, all the available data suggest that lipid formulations are at least as efficacious as and better tolerated than amphotericin B deoxycholate. However, high costs, a relative paucity of clinical data and the existence of alternative antifungal therapies explain why lipid formulations have been generally used as second-line therapy in patients with IFIs [33]. Both innate and emergent resistance to amphotericin B are rare, but there is a lack of good methods of detecting; innate resistant species contain *Aspergillus terreus*, some isolates of C*andida* lusitaniae, some isolates of *Scedosporium* spp. and *Trichosporon* spp. [12].

5.4.1.2 Azoles

Azoles inhibit the synthesis of ergosterol of the fungal cell membrane. The azole family of antifungals can be classified into two groups: the imidazoles (clotrimazole, ketoconazole, miconazole) and the triazoles (fluconazole, itraconazole, voriconazole, posaconazole). In the late 1980s triazoles became standard therapy for invasive candidiasis. Several clinical studies have compared the efficiacy and safety of azoles with that of amphotericin B deoxycholate for IFIs in non-neutropenic patients, but only few data are available on the treatment of neutropenic patients. Overally triazoles are more easily tolerated, but are primarily considered fungistatic as opposed to fungicidal (lethal) drugs [45]. Innate resistance of certain yeast species and moulds to various azole agents is predictable and there is not necessarily cross-resistance among the azoles. *Candida krusei* is innate resistant to fluconazole, *Candida glabrata* and *Candida tropicalis* are often azole resistant, as are most zygomycetes species and some *Scedosporium* species. Emergent resistance is currently rare, but has been documented in *Candida albicans* and *Candida glabrata* [12].

Fluconazole **(Diflucan®)** is available as intravenous and oral formulations. Oral fluconazole is well absorbed and the bioavailability is not influenced by H2 blocking agents. Fluconazole is clearly the best tolerated agent among the triazole antifungals [45]. In a multicentre study of 206 non-neutropenic patients with candidemia, fluconazole was found to be better tolerated than and as efficious as amphotericin B deoxycholate with success rates of 72% vs 79%, respectively [39]. Fluconazole is not used for IA. Given the broad use of fluconazole, an increased incidence of infections

due to non-*albicans Candida* species with reduced, dose-dependent susceptibility or intrinsinc resistance to azoles has been reported in the late 1990s [31]. The usual dose of fluconazole is ranging from 10-15mg/kg body weight/day for disseminated candidiasis, and from 100-400 mg/day for prophylactic treatment [12, 43]. A loading dose of twice the daily dose is recommended. In patients with creatinine clearance of less than 50ml/min the daily dose should be reduced by half; if less than 20ml/min by 75% [10].

Itraconazole **(Sporanox®)** is available as oral and intravenous formulations. The poor bioavailability of its original oral formulation has limited its use and an intravenous formulation has become available. However, the intravenous formulation has only recently been approved for up to 2 weeks of clinical use [16]. Itraconazole has been used as first-line treatment of IA in few small studies. In a study of 31 patients with IA who were treated with itraconazole a response rate of 48% was reported [38]. However, given the highly variable bioavailability of oral itraconazole and risk of drug-drug interactions (eg with immnosuppressive drugs, rifampicin, macrolides, anticonvulsants), which are serious drawbacks for the treatment of patients with life-threatening infections, monitoring itraconazole blood levels during therapy is mandatory [10]. For these reason, itraconazole is more frequently used in less immunosuppressed patients who are able to take oral therapy and for use as sequential oral therapy [16]. Itraconazole is approved for use as salvage therapy for aspergillosis. Dosage for IPA is 400-600mg/ day for 4 days, then 200mg twice daily; for prophylactic use 100-400mg/day [12].

Voriconazole **(V-fend®)** is a potent, broad spectrum triazol and is available as oral (bioavailability 60 to 100%) and intravenous formulation. Voriconazole is a methylated analogue of fluconazole with enhanced activity against yeast as well as important opportunistic moulds including *Aspergillus* and *Fusarium* [45]. It is metabolised in the liver via the P-450 system and has therefore the potential for multiple drug interactions (eg with rifampicin, anticonvulsants, sirolimus, tacrolimus, cyclosporin, oral anticoagulants, statins, protease inhibitors, omeprazole) [10]. Therefore close clinical monitoring is needed. The main side effects include transient and fully reversible, non sight-threatening visual disturbances in about 30 to 40% of patients, liver function abnormailities in 10%-15%, skin rash in 6%, and hallucinations [16, 45]. Voriconazole presently is the treatment of first choice for IA. In the largest prospective, multicenter, randomised, comparative study ever performed with 277

patients, the efficiacy and safety of upfront therapy with voriconazole was compared to that of amphotericin B deoxycholate. After 12 weeks of protocol therapy success and survival rates were significantly better in the voriconazole group than in the amphotericin B group (53% versus 32% and 71% versus 58%, respectively). Moreover fewer severe side effects occurred in patients treated with voriconazole than in those treated with amphotericin B [13]. In a randomized, comparative multi-center trial containing 422 patients with invasive *Candida* infections, of whom >95% had candidemia, voriconazole was shown to be at least as effective as a regimen of amphotericin B deoxycholate followed by intravenous or oral fluconazole [40]. Voriconazole has a cidal activity against many *Aspergillus spp.* inclunding *A. terreus*, and has become the recommended primary therapy for most patients with invasive aspergillosis [16]. Initial dosage for IPA is 6mg/kg i.v. b.i.d. followed by 4mg/kg i.v. b.i.d. until the patient stabilises, then a dosage of 200mg b.i.d. orally is recommended[12]. However, there is substantial intra- and inter-individual variability in voriconazole blood levels. The extensive use of voriconazole in immunosuppressed patients has been associated with a drop in invasive aspergillosis, however, as a counterpoint the incidence of serious invasive zygomycosis, which shows resistance to voriconazole, has increased in these patients [21].

Posaconazole **(Noxafil®)** is a new triazole analogue of itraconazole with broad spectrum antifungal activity against *Candida, Cryptococcus, Aspergillus,* and other emerging moulds including the zygomycetes and *Fusarium.* Posaconazole differs from other azoles by its hepatic metabolic pathways: glucuronation plays a major role, while enzymes of the P450 system are of secondary importance, which may decrease the risk of clinically significant dug-drug interactions. The drug is available in oral form with variable bioactivity, which can be improved to up to 90% if it´s administered with a high-fat meal [45]. Treatment-related adverse events were reported in 38% of an overall study population of 428 patients [55]. The most common adverse events were nausea (8%), vomiting (6%) and other gastrointestinal symptoms (eg diarrhea and abdominal pain). Treatment related serious adverse events occurred in 8% of patients, and included increased hepatic enzymes (2%), corrected QT-interval and/or QT interval prolongation (1%) and rash (2%) [55]. *Posaconazole* is approved as prophylaxis for IA and Candida infections. In two recent randomised studies posaconazole prevented IFIs in neutropenic patients and

in patients with graft versus host disease more effectively than other azoles (e.g. fluconazole) and improved overall survival [42, 49]. Posaconazole has been recently approved for salvage therapy of refractory IA and coccidioidomycosis and for the treatment of infections due to Zygomycetes [41,50]. Dosage for treatment of IA is 400 mg po bid; dosage for prophylaxis 200mg three times daily [16, 42].

5.4.1.3 Echinocandins

Echinocandins are a new class of antifungal agents that are poorly bioavailable and produced in intravenous formulation only. They are natural cyclic hexapeptide antifungal compounds that noncompetively inhibit 1-3-β-D-glucan synthase, an enzyme complex which forms glucan polymers in the fungal cell wall [16]. These agents are fungicidal against *Candida* species and *Pneumocystis*. Specific modifications to the N-acyl aliphatic side chains expand the antifungal spectrum to include *Aspergillus*. Notably, these agents are neither classically fungicidal nor fungistatic for *Aspergillus*, but exert their effect on the growing hyphal tips where the glucan synthase target is located [16]. No cross-resistance with azoles has been reported. Innate resistance to the echinocandins is seen in fungi lacking 1-3-β-D-glucan in the cell wall, which limits its spectrum of activity mainly to *Candida* spp., *Aspergillus* spp. and *Pneumocystis.* Emergent resistance is rarely seen in clinical use [12].

Caspofungin **(Cancidas®)** is the first echinocandin licensed for the treatment of invasive mycoses. Caspofungin is slowly degraded by hydrolysis and N-acetylation by the liver. Therefore, dose reduction to 35mg is recommended in patients with moderate hepatic dysfunction, but there is no need for dose adjustments in patients with renal insufficiency [10]. Based on an open label trial that demonstrated therapeutic efficiacy and safety in 37 of 83 (45%) patients studied, caspofungin is approved for treating patients refractory to or intolerant of standard therapies for IA [22]. In a large multicenter trial including 239 patients (of whom 24 were neutropenic) with invasive candidiasis, caspofungin was more efficious (success rates: 73% vs 62%) and less toxic than amphotericin B deoxycholate (discontinuation for adverse events: 3% vs. 23%). The success rates against *C. glabrata* and *C. krusei* were comparable to those obtained in azole-susceptible *Candida* species [43]. Safety profile of caspofungin is excellent, only about 3% of patients discontinued therapy in clinical trials [22,43]. The few reported adverse events included abnormal liver

function tests, phlebitis and infusion-related reactions, analogous to the “red person´s syndrome” observed with vancomycin infusions, due to histamine release during infusions [45]. Drug-drug interactions (eg with cyclosporine, rifampicin, anticonvulsants, tacrolimus, protease inhibitors) have been observed.

Micafungin was approved in 2005 for prophylaxis of *Candida* infections. **Anidulafungin (Ecalta®)** was approved in 2006 for candidemia and in 2007 for invasive candidiasis. Both agents also have activity against *Aspergillus* spp [16].

5.4.2 Treatment of Invasive fungal infections in general

Invasive fungal infections in patients with hematologic malignancies like acute myelogenous leukemia remain a serious challenge; (a) the epidemiology of fungal infections is changing as the more drug resistant saprophytic fungi (e.g. zygomycosis, scedosporosis) are increasingly being associated with human disease; (b) despite advances in diagnostic fungal essays, early and correct diagnosis of fungal infections is difficult and antifungal drug susceptiibility data are seldom available during clinical decision making; and (c) monotherapy with an antifungal agent is often unsuccessful which leads to additional approaches, including combination therapy and immunenhancement strategies. [14]

For decades the standard therapy for invasive fungal infections has been amphotericin B deoxycholate. However, this drug is often poorly tolerated and has severe side effects with nephrotoxicity and infusion-related acute reactions. In the late 1970s the emergence of azoles provided an alternative therapeutic strategy. In recent years several new antifungal agents eg. lipid formulations of amphotericin B, new azoles (voriconazole, and posaconzole) and echiocandins (caspofungin, and micafungin) have emerged offering additional therapeutic options. [10,14]

Gold standard treatment for IFIs has been the use either of amphotericin B deoxycholate or lipid formulations of amphotericin B. However one big metaanalysis indicated a low response rate to amphotericin B in patients with invasive aspergillosis; the response rate was about 55% in patients with leukemia, with similar results in a combined group of patients with leukemia or lymphoma. Particularly patients with fungal infection of the central nervous system had high case fatality rates [7].

Broad spectrum triazoles, including voriconazole, which became available in 2001 have also been evaluated as treatment option. An often cited report showed that initial therapy with voriconazole in patients with invasive aspergillosis led to better responses (52,8% successfull outcomes at week 12 of treatment vs 31,6%), improved survival (70,8% at week 12 vs 57,9%) and significantly fewer severe side effects than the standard approach of initial therapy with Amphotericin B deoxycholate [13]. However, the intend-to-treat analysis revealed less impressive rates for complete response which were nearly the same in each treatment arm (voriconazole 21%; amphotericin B 17%) [13]. Voriconazole has been used extensively in patients with with cancer or bone marrow transplants which has been associated with a drop in invasive aspergillosis. However, the increased incidence of serious invasive zygomycosis in these immunosuppressed patients has been attributed to voriconazole use, to which these organisms are intrinsically resistant [21]. Prior exposure to voriconazole increased the risk for zygomycosis 20-fold, while patients with fungal sinusitis had a 80-fold higher probability of zygomycosis [21].

Caspofungin, which belongs to the echinocandins has been evaluated as second line therapy in patients with IA. Complete and partial responses to caspofungin occured much more frequently in patients who were intolerant to conventional therapy (75% of patients) rather than in patients who were truly refractory to conventional treatment (39% of patients) [22].

Current treatment approaches also include combination and/or high-dosage therapy and immune-enhancement strategies. In early 2007 a study was published, comparing a higher dose of caspofungin (100mg/day) with standard-dose caspofungin (70mg followed by 50mg daily) in patients with hematologic malignancies and hematopoietic stem cell transplantation (HSCT). Twelve weeks after beginning of treatment, 44% of patients in the higher-dose group (15/34) exhibited a complete or partial response, compared with 29% in the standard dose group (18/63). The better response in the higher-dose group may be, in part, a result of the fact that more patients in this group received immune enhancement with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) and/or recombinant human interferon γ1b [26].

Neutrophils, which are recruited by alveolar macrophages during the immune resonse to an IFI to neutralize microconidia, release proteases that trigger an

adaptive immune response; activated T-helper type 1 lymphocytes migrate from the lymph node back to the site of infection. Several factors, including hematologic malignancies and chemotherapy can inhibit a host´s immune response to an IFI. Immunomodulators restore effector cell numbers, enhance effector cell function, and promote a T-helper type 1 environment and may therefore play a role in this immune recovery process [27]. Two approaches of immune enhancement have shown promise in recent studies; (a) the use of rhGM-CSF and rhIFN-γ and (b) the combination of donor granulocyte transfusion plus rhIFN-γ and/or rhGM-CSF [14].

Given the poor prognosis for *Candida* sepsis and IA in immunocompromised patients, using combinations of antifungal drugs would seem a logical approach to improve outcome. However, until now there have been only relatively few clinical studies supporting this approach. The addition of caspofungin in hematologic patients with invasive Aspergillosis refractory to liposomal amphotericin B was evaluated by Kontoyiannis et al. Nearly equal numbers of patients had documented versus possible fungal infections. While the overall response rate was 42%, the response rate was only 22% in those with documented IFIs compared with 60% in those with possible IFIs [23]. Another study showed an even better overall response rate of 60%, however most patients fell within the “possible” (n=20) rather than the “probable” (n=4) or “proven” (n=6) IFI group, which skewed the data to a more favorable impression of the combination treatment [24]. A third study evaluated the use of either voriconazole plus caspofungin (n=16) or voriconazole alone in patients who developed invasive aspergillosis after receiving stem cell transplants (mostly allogeneic) or cytotoxic chemotherapy, and who failed initial therapy with amphotericin B. Treatment with the combination was associated with a higher survival rate at 90 days compared with voriconazole alone (p=.048) [25]. In a randomised, double blind study in 219 non-neutropenic patients with candidemia, fluconazole (800mg/d iv) was compared to a combination of fluconazole (800mg/d iv) and amphotericin B deoxycholate (0,7mg/kg/d iv). The efficiacy of combination therapy was slightly superior to that of monotherapy (success 69% vs 56%), however, there were statistically significant differences between the two treatment groups, such as disease severity, which was lower in the combination group [43].

In 2006 guidelines for treatment of invasive fungal infections in adults were published by experts of 5 Swiss university hospitals; they recommended for empirical therapy in

patients with neutropenia and persistent fever initial therapy with Amphotericin B deoxycholate with close monitoring of adverse events [10].

5.4.3 Treatment of Invasive Aspergillosis

Antifungal agents licensed for the treatment of invasive aspergillosis (IA) are amphotericin B deoxycholate, the lipid formulations of amphotericin B, voriconazole, itraconazole, posaconazole and caspofungin. Since the mortality of IA ranges between 60 and 90%, an aggressive diagnostic approach, followed by prompt institution of an appropiate first-line antifungal therapy is critical. For decades, amphotericin B deoxycholate has been the drug of choice, but responses rates has been disappointing, especially in patients with profound and persistent neutropenia or with graft-versus-host disease.

The local epidemiology of IA and the patients risk factors, underlying disease and other medication are decisive for the choice of antifungal agent.

The Swiss Guidelines of 2006 stratified the treatment approaches in IA into primary therapy, salvage therapy and combination therapy in critically ill patients. Voriconazole is recommended in invasive Aspergillosis (IA) for primary therapy. Caspofungin, voriconazole (if not used for primary therapy) or liposomal amphotericin B are recommended for salvage therapy of refractory IA, while combination therapy with caspofungin plus voriconazole or liposomal amphotericin B should be considered in critically ill patients [10, 70].

Recently the emergence of multiple-triazole-resistant *A. fumigatus* is challenging the clinicians. In the Netherlands between 2002 and 2007 thirteen isolates of nine patients grew multiple-triazole resistant *A. fumigatus.* Although the emergence coincides with the approval of voriconazole, the factors that may explain this phenomenon remain unclear [56].

In a recent multicenter externally controlled trial posaconazole was studied as salvage therapy of IA in 107 patients refractory to or intolerant of first-line therapy. The overall success rate was 42% for posaconazole recipients and 26% for control subjects, receiving the best available standard care for salvage theapy (odds ratio, 4.06; 95% confidence intervall, 1,50-11,04; P=.006). Posaconazole appeared to confer a survival benefit. The cumulative rate of survival at 30 days was 74%; for

control subjects the rate was 49%. Overall the 12 month survival was 45%. Response rates in high risk subgroups were 24% in neutropenic patients, 31% in allogeneic HSCT recipients and 37% in patients with an underlying hematologic malignancy [41]. Treatment-related adverse event occurred in 44% of the posaconazole population. These included gastrointestinal adverse events (nausea 12%, vomiting 5%, anorexia 3%, and abdominal pain 2%), hepatic adverse events (increased hepatic enzyme level 3%, increased alanine aminotransferase level 2%), and rash (4%) [41]. In another recent study comparing posaconazole with high-dose lipid formulations of amphotericin B alone or in combination with caspofungin as salvage therapy for IA in patients with hematologic malignancies, posaconazole demonstrated significantly greater efficiacy (response rate 40% vs 8% in the amphotericin B group vs 11% for combination therapy) and safety compared to the other regimen [51].

5.4.4 Treatment of Invasive Candidiasis

Rapid initiation of appropiate antifungal therapy is essential for the control of systemic *Candida* infections and has been shown to reduce mortality [30]. Management guidelines have recommended that all patients with the detection of *Candida* in at least one blood culture should be treated with antifungal agents [33].

The Swiss guidelines of 2006 suggest fluconazole as the drug of choice for empirical therapy of Candida bloodstream infection in non-neutropenic patients. If this patients had previous azole exposure amphotericin B deoxychoate or caspofungin would be the treatment option. In patients with severe sepsis or septic shock, caspofungin is the drug of first choice in Switzerland. In neutropenic patients, empirical therapy with amphotericin B deoxycholate is considered first choice. In microbiologically-documented infection due to *C. albicans, C. tropicalis* or *C. parapsilosis* fluconazole is the drug choice; when infections are caused by *C. glabrata* or *C. krusei* caspofungin or amphotericin B deoxychoate are first line therapies [10].

Table 3

General pattern of susceptibility of Candida species (adapted from [33])

Candida spp.	Fluconazole	Itraconazole	Voriconazole	AmphotericinB	Caspofungin
C. albicans	S	S	S	S	S
C. tropicalis	S	S	S	S	S
C. parapsilosis	S	S	S	S	S
C. glabrata	S-DD to R	S-DD to R	S to I	S to I	S
C. krusei	R	S-DD to R	S	S to I	S
C. lusitania	S	S	S	S to R	S

Note: Interpretation based on the use of the NCCLS (CLSI) M27-A methodology

S: susceptible; S-DD: susceptible- dose dependent; I: intermediate; R: resistant

5.4.5 Prophylactic use of antifungal therapy

Prophylactic use of antifungal compounds has become standard clinical practice for patients who are treated for haematological malignancies. Antifungal prophylaxis may be indicated in hematopoietic stem cell transplant recipients with graft-versus-host disease, in persons with hematologic malignancies and prolonged neutropenia due to chemotherapy, and in persons who are at high risk of developing fungal infections [50]. Secondary prophylaxis may be indicated in patients with proven or probable invasive aspergillosis or candidiasis, that run a high risk of reactivation when they undergo further cycles of chemotherapy.

Fluconazole was the first drug used for antifungal prophylaxis of invasive candidiasis. Metaanalysis showed that it decreases overall mortality in allogeneic stem cell transplant recipients and in certain subsets of patients who are treated for acute leukemia, particularly in cases of prolonged neutropenia and mucositis [52]. Itraconazole and voriconazole were adequate prophylaxis for patients treated for graft-versus-host disease and cytomegalovirus disease where treatment puts patients at risk for IA [52]. However, in a recent large, randomized clinical trial comparing fluconazole with itraconazole as prophylaxis in high-risk patients, the efficiacy results for the oral solutions of the two drugs did not differ significantly [53].

However, benefit of antifungal prophylaxis with fluconazole has been proven in allogeneic transplant recipients [69].

It is easy to understand why mould infections are much more difficult to prevent than are infections caused by yeasts. Hematogenous candidiasis is supposed to originate from colonization of the gastrointestinal tract. Orally administered fluconazole both reduces the *Candida* burden in the gut and eliminates the organisms after they have gained access to the bloodstream. Mould infection is pricipally airbourne, and oral itraconazole will not eradicate *Aspergillus* spores and conidia from the airways; therefore itraconazole fully depends on its systemic activity.

Posaconazole was recently approved for prophylaxis of *candida* and *aspergillus* infection. A recent double-blind multicenter study in 600 patients with graft-versus-host-disease who were receiving immunosuppressive therapy compared posaconazole (200 mg t.i.d.) and fluconazole (400mg) for prophylaxis of invasive mycoses. The incidence of IA during the 16 week study period was significantly lower in the posaconazole group (2,3% vs 7%; P=0.006), as was the mortality due to invasive mycoses (1% vs. 4%; P=0.046) and the incidence of breakthrough invasive fungal infections (2,4% vs 7,6%, P=0.004). In preventing all fungal infections posaconazole was found to be as effective as fluconazole (incidence 5,3% and 9,0%; P=0.07). Treatment-related adverse events were similar in the two groups (36% in the posaconazole group and 38% in the fluconazole group), the rates of serious adverse events were 13% and 10%, respectively [42]. A randomised multicenter study compared 304 neutropenic patients receiving posaconazole 600mg, and 298 neutropenic patients receiving fluconazole 400mg (n=240) or itraconazole 400mg (n=58) as antifungal prophylaxis. All the patients were undergoing chemotherapy for AML or MDS. Proven or probable fungal infections were reported in 2% of patients in the posaconazole group and in 8% (8% vs 10%) in the fluconazole or itraconazole group, fulfilling statistical criteria for superiority. Significantly fewer patients had IA (1% vs 7%) and survival was significantly longer among recipients of posaconazole (p=0.04). Serious adverse events possibly or probably related to treatment were reported by 6% of patients in the posaconazole group and 2% of patients in the fluconazole or itraconazole group [49].

6 Materials and Methods

117 patients were identified who received systemic antifungal therapy during a total of 7 months from May to December 2007 at the Department of Internal Medicine, Division of Hematology, Leopold-Auenbrugger Medical University Graz.

In patients receiving systemic antifungal treatment data were collected prospectively of agent, dosage, duration and reason for receiving antifungal therapy, and underlying disease. Patients were retrospectively classified with possible, probable or proven invasive fungal infection according to the EORTC criteria 2007 (Table 4).

The study was done under supervision of ao.Univ. Prof. Dr. Robert Krause and Dr. Ines Zollner-Schwetz of the Department of Internal Medicine, Section of Infectious Diseases, Medical University of Graz.

The study started in the beginning of May 2007 and ended in December 2007. The study was interrupted for one month in August 2007, due to an clinical elective at the University of Toronto. All patients included into this study were admitted to the Department of Internal Medicine, Division of Hematology, Medical University Graz.

The first aim of this study was to identify the numbers of proven, probable and possible invasive fungal infections and to compare the number of cases of fungal infection with the total number of patients receiving antifungal therapy to analyse the basis of desicionmaking for antifungal therapy.

6.1 Study objectives

To determine the incidence of invasive fungal infections among patients at the Department of Internal Medicine, Division of Hematology, Medical University of Graz.

To determine the incidence of Invasive Aspergillosis at the Department of Internal Medicine, Division of Hematology, Medical University of Graz.

To compare the number of patients receiving systemic antifungal therapy with the number of patients with evidence of possible, probable or proven invasive fungal infection.

To describe the basis of desicionmaking for antifungal therapy at the Department of Internal Medicine, Division of Hematology, Medical University of Graz.

To describe the therapeutic regimens used and their efficiacy.

To describe the underlying diseases and the patients risk factors for systemic fungal infection.

6.2 Methods

This is an observational survey of patients with evidence of aspergillosis and of patients receiving systemic antifungal therapy. The prospective observational study was conducted at the Department of Internal Medicine, Division of Hematology, Leopold-Auenbrugger Medical University Graz. 117 patients receiving systemic antifungal treatment were identified during a total of 7 months from May to December 2007. Elegible patients were identified by performing weekly to twice weekly visits at the Division of Hematology and prospective chart review of the inpatients. The Case Report Forms were completed with the use of MEDOCS, the electronic data base of patients at the Medical University Hospital Graz. Study data have been recorded using a CRF with subsequent electronic data entry. Patients were retrospectively classified with possible, probable or proven invasive fungal infection according to the EORTC criteria 2007 (Table 4).

The following data were extracted from their medical records:

• Demographic data (age, gender, health record number)

• underlying malignancy

• reason for antifungal treatment (considering radiologic, clinical and microbiologic findings.)

• if identified: fungal species, organs involved, and method of fungal identification

• Antifungal agent(s), dosage and duration of therapy

For patients meeting the possible, probable or proven Invasive Aspergillosis/Invasive fungal infection criteria the following information was extracted additionally:

• Current status of *aspergillosis*/IFI: proven, probable or possible

• Diagnosis results: Microscopy, Culture, Antigen tests, PCR tests, Clinical investigations

• Exogenous risk factors: Room conditions, i.e. laminar air flow, HEPA filter use, exposition to construction work / dust

• Fungal species, organs involved

• Antifungals and treatment results

• Survival 4 and 12 weeks post treatment cessation or cause of death

6.3 Definitions

One case was defined as one patient at one hospitalization. One case contained up to five episodes of in time, dosage and agent diverse antifungal treatment. A case was completed at discharge of the patient. If a patient was admitted again to the department and new antifungal treatment was prescribed he was counted as another case. Patients receiving long term prophylactic antifungal treatment were counted as one case no matter how often they were admitted to the division, except a new antifungal agent or a different dosage was started.

De novo IA was defined as an infection in the absence of previous antifungal therapy. Breakthrough IA was defined as an infection in a patient receiving antifungals with a known activity against Aspergillus spp. (e.g. itraconazole, LipoAMP) for at least 7 days before the onset of IA. Improvement was defined as the resolution or major improvement of symptoms and signs of IA (including radiologic changes on chest X-rays or computed tomography scans) and as the requirement of no further systemic antifungal treatment as judged by the treating physician. Failure was defined as the deterioration or lack of significant improvement of these same parameters (including death of the patient or drug withdrawal with evidence of infection still present) after 4 weeks and 12 weeks of therapy. Invasive aspergillosis was considered a contributory cause of death if there was histopathologic involvement of a major organ. Patients who died of other causes during therapy were considered to have responded if the signs and symptoms of IA had resolved before death or if there was no evidence of infection at autopsy.

6.3.1 Prophylactic, Pre-emptive, Empirical and Directed antifungal therapy

An individual may be eligible for one or more of these approaches. Prophylactic therapy targets a population of patients at risk for IFI, but who do not yet have evidence of either infection or clinical disease, for example HSCT recipients with graft-versus-host disease or patients with acute leukemia.

Pre-emptive therapy targets a population of patients with evidence of IFI based on an antigen or genomic detection test, significant colonization and suspect imaging but without evidence of clinical disease. Empirical antifungal therapy is a variant of pre-emptive therapy where persistent neutropenic fever despite broad spectrum antibacterial therapy is considered the surrogate marker of IFI.

Directed or targeted therapy applies to patients with evidence of infection and clinical disease [57].

6.4 Study population

6.4.1 Criteria of including patients

- Age above 18 years
- all patients receiving systemic antifungal therapy at the department of hematology

For the aspergillosis/ IFI study:

- The IA cases were defined as proven, probable, or possible according to the guidelines recently published by the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of cancer (EORTC).
- For IA cases: Zygomycetes and Fusarium spp. were included

Table 4: EORTC criteria 2007

Proven invasive fungal diseases	**Deep tissue disease** **Molds**	Histopathologic, cytopathologic, or direct microscopic examination of a needle aspiration or biopsy specimen showing hyphal forms with evidence of associated tissue damage (either microscopically or as an infiltrate or lesion by imaging).
		OR Recovery of a mould by culture from a sample obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL, cranial sinus cavity, and urine.
	Deep tissue disease **Yeasts**	Histopathologic or cytopathologic examination[2] of a needle aspiration or biopsy specimen from a normally sterile site excluding mucous membranes showing yeast cells (*Candida* species may also show pseudohyphae or true hyphae)
		OR Recovery of a yeast by culture from a sample obtained by a sterile procedure (including a freshly (<24h) placed drain) from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process
	Disseminated and / or pulmonary disease	Must be proven by recovery in culture from a specimen obtained from the affected site, in host with a temporally related illness consistent with a fungal infectious disease process
		OR if culture is sterile or not obtained, histopathologic or direct microscopic demonstration of appropriate morphological forms is considered adequate for dimorphic fungi having truly distinctive appearance.
		OR positive blood culture

	Fungemia **Moulds**	Blood culture that yields a mould e.g. *Fusarium* spp. in the context of a compatible infectious disease process
	Fungemia ***Yeasts***	Blood culture that yields yeast (e.g. *Candida* species) or yeast-like fungi (e.g. *Trichosporeon* spp.)
Probable invasive fungal disease	**Defined by at least**	**a) one clinical criterion AND b) one host criterion AND c) one microbiological criterion**
Possible invasive fungal disease **(provided other plausible causes have been excluded)**	**Defined by at least**	**a) one clinical criterion AND b) one host criterion BUT c) no microbiological criterion**

Clinical criteria

Must be consistent with the microbiological findings, if any, temporally related to current episode and other potential causes must have been eliminated.

Lower respiratory tract fungal disease

A) the presence of one of the following "specific" imaging signs on CT:-

- Well defined nodule(s) with or without a halo sign
- Wedge-shaped infiltrate
- Air crescent sign
- Cavity

B) the presence of a new non-specific focal infiltrate
PLUS at least one of the following symptoms (not necessary if there is mycological evidence):

Pleural rub
Pleural pain
Hemoptysis

Tracheobronchitis Tracheobronchial ulceration, nodule, pseudomembrane, plaque or eschar seen on bronchoscopy
Sinonasal infection Imaging showing sinusitis PLUS at least one of the following:- Acute localized Pain (including pain radiating to eye) Nasal ulcer, black eschar extension from the paranasal sinus across bony barriers, including into the orbit
Endophthalmitis as determined by ophthalmologic examination
CNS infection at least one of the following:- Focal lesions on imaging Meningeal enhancement on MRI or CT **Chronic disseminated candidiasis** Small, peripheral, target like abscesses (new nodular filling defects, bull's-eye lesions) in liver and/or spleen

Host factors

Host factors are not synonymous with risk factors and are characteristics by which individuals predisposed to invasive fungal diseases can be recognized. They are intended primarily to apply to patients treated for malignant disease and to recipients of allogeneic hematopoietic stem cell and solid organ transplant. These host factors are also applicable to those receiving corticosteroids and other T-cell suppressants as well as those with primary immune deficiencies.

1. Recent history of neutropenia (< 0.5 x 10^9 / L for >10 days) temporally related to the onset of fungal disease or ongoing neutropenia
2. Receipt of an allogeneic stem cell transplant
3.Prolonged use of corticosteroids (excluding patients with ABPA) at an average minimum dose of 0.3 mg/kg/day prednisone equivalent for > 3 weeks
4. Treatment with other recognized T-cell immune suppressants such as cyclosporin, TNF-α blockers, specific monoclonal antibodies alemtuzumab, nucleoside analogues during the past 90 days
5. Inherited severe immunodeficiency (e.g., chronic granulomatous disease, severe combined immunodeficiency)

Microbiological Criteria

Cytology, direct microscopy or culture
1. Sputum, BAL and bronchial brush samples demonstrating the presence of fungal elements either by recovery by culture of mould (e.g. *Aspergillus* spp*., Fusarium* spp., Zygomycetes, *Scedosporium* spp.) or detection by cytology or direct microscopy of hyphal forms.
2. Sinus aspirate: recovery by culture of moulds from or detection of hyphal forms by cytology or direct microscopy.
3. Skin ulcers, draining soft tissue lesions or fissure for which both microscopy and culture are required
Detection of antigen, cell wall constituents or nucleic acid
4. Galactomannan antigen EIA (Platelia)
a.) single plasma or serum sample positive for galactomannan
b.) single BAL, pleural fluid or CSF sample positive for galactomannan
5. Glucan Assay is primarily applicable for aspergillosis and candidiasis and does not detect Cryptococcus species nor the Zygomycetes *(Rhizopus spp., Mucor spp. Absidia spp.)*
a.) single serum sample positive for beta-D-glucan

Polymerase Chain Reaction to detect nucleic acid

6. Until a PCR system is developed that has been externally validated, a positive PCR result for blood, tissue, or BAL fluid for the specific fungus studied will not be considered microbiological evidence of invasive fungal disease.

6.4.2 Criteria of excluding patients

- Patients below 18 years
- Patients receiving long term prophylactic antifungal treatment, which have been recorded before

In cases of “possible” aspergillosis (fungal pneumonia based on the clinical and radiologic picture without histopathologic or culture confirmation) we excluded:

- infection by other fungi e.g. Candida, Cryptococcus etc.
- patients who had a concomitant pulmonary or systemic documented infection that could confound the evaluation of the response

7 Results

7.1 Demographic data

A total of 117 patients receiving systemic antifungal therapy were enrolled in the study. The total number of patients admitted to the division of hematology, Medical University Graz during the study period was 805. The average duration of hospitalization was 8,77 days.

Data collected included age, sex, underlying conditions and weight. Demographic data are depicted in Table 5, and Figure 1.

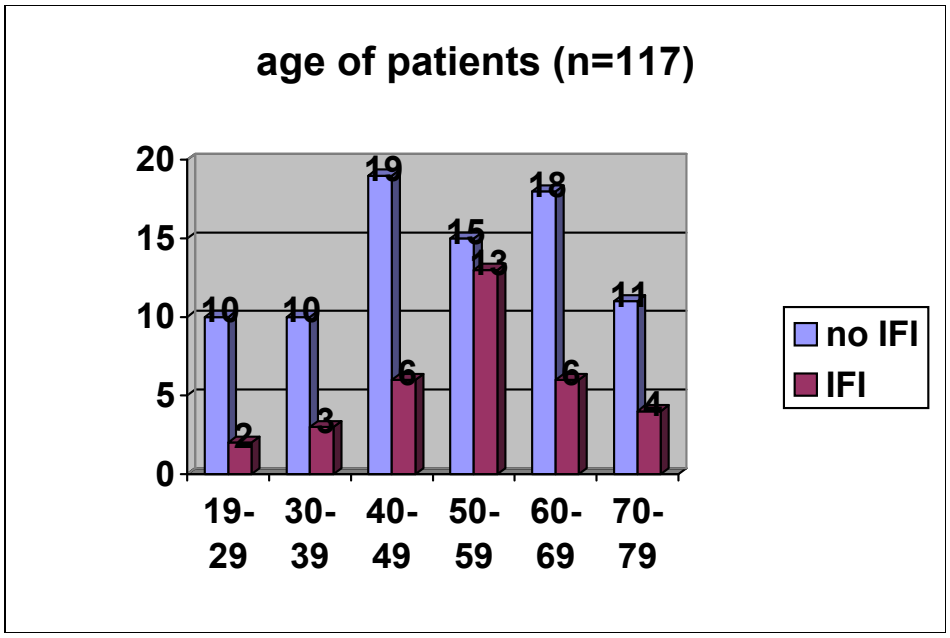
Table 5

Demographic Data of study population

Demographic Data	**All Patients of study group (n=117)**	**Patients with possible/probable or proven IFI (n=34)**
Median age	51,2	55,9
male/female	70/47	18/16

Figure 1

Age of patients



7.2 Underlying diseases

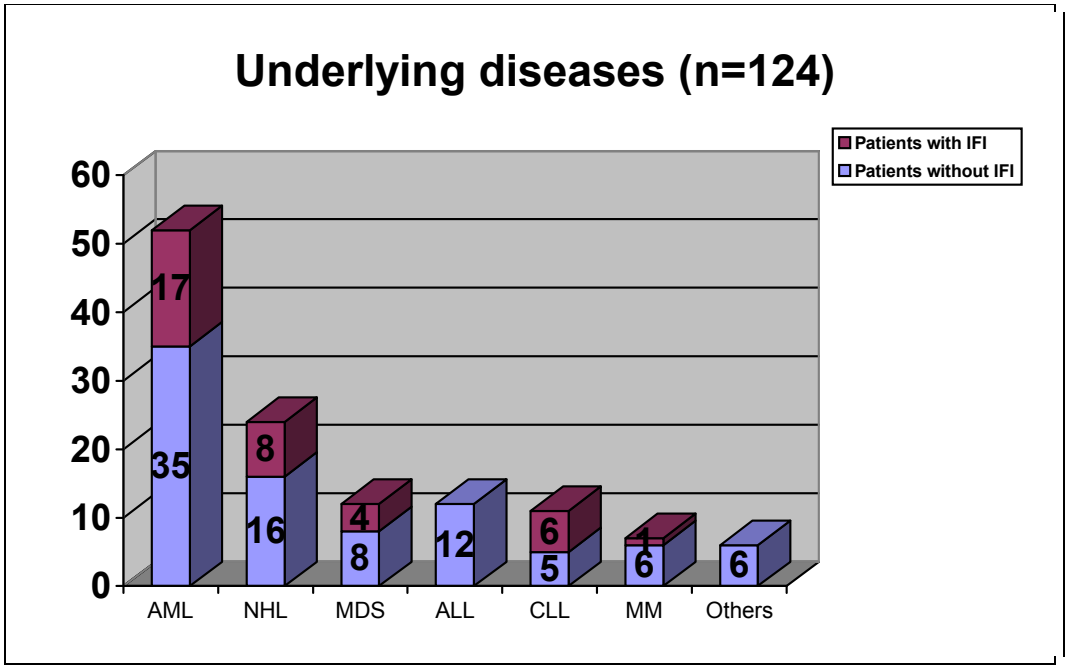
The 117 patients enrolled in the study had 124 underlying diseases. 7/117 (6%) of patients had 2 underlying diseases. The leading primary underlying disease in this patient collective with hematologic malignancies was acute myologenous leukemia (52/117 of patients, 44%), followed NHL (24/117, 21%), MDS and ALL (each 12/117, 10%), CLL (11/117, 9%), MM (7/117, 6%), Mb. Hodgkin (2/117, 2%) and others (4/117 of patients, 3%).

Among patients with possible, probable or proven invasive fungal infection AML was the primary underlying disease (17/34 of patients, 50%), followed by NHL (8/34, 25%), CLL in 6/34 (18%) of patients, MDS in 4/34 (12%) of patients and MM in 1/34 (3%) of patients. 2/34 (6%) of patients had 2 underlying diseases.

The underlying hematologic diseases are listed in Figure 2.

Figure 2

Underlying diseases



7.3 Invasive fungal infections

Of the 117 patients receiving systemic antifungal therapy 24 (21%) had possible, 5 (4,3%) probable and 5 patients (4,3%) proven invasive fungal infection according to the EORTC criteria. 83 patients (71 %) had no invasive fungal infection according to the EORTC. Among the probable and proven infections 7 were due to *Candida spp.*

(4 probable, 3 proven), 2 due to *Aspergillus* (1 probable and proven each) and 1 due to Zygomycetes (proven). Of the infections by *Candida* species 3/7 were due to *Candida albicans* (1 probable, 2 proven), 2/7 were due to *Candida glabrata* (2 probable), and each 1/7 was due to *Candida parapsilosis* (proven) and *Candida krusei* (probable). The proven IA was due to *Aspergillus fumigatus*.

The results are listed in figures 3, 4, 5 and 6.

Figure 3

IFI in patients with systemic antifungal therapy

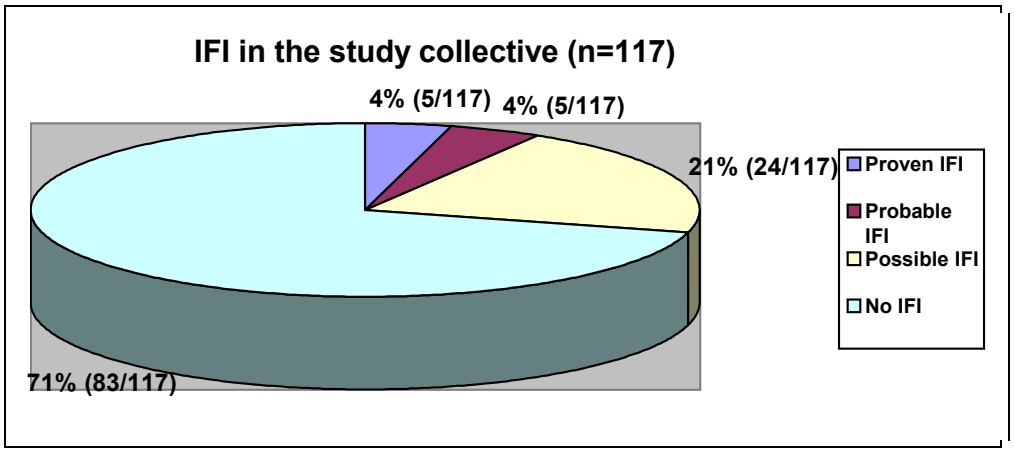


Figure 4

Current status of invasive fungal infection

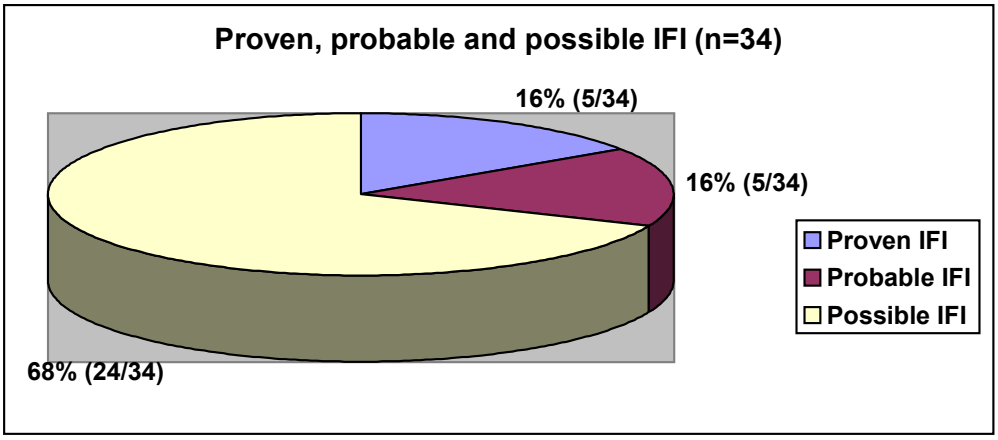


Figure 5

Fungal species of. probable and proven IFI

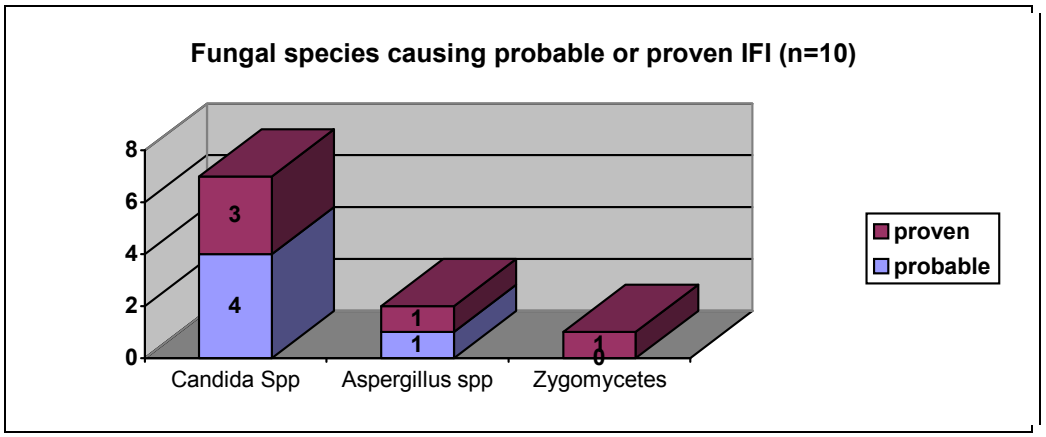
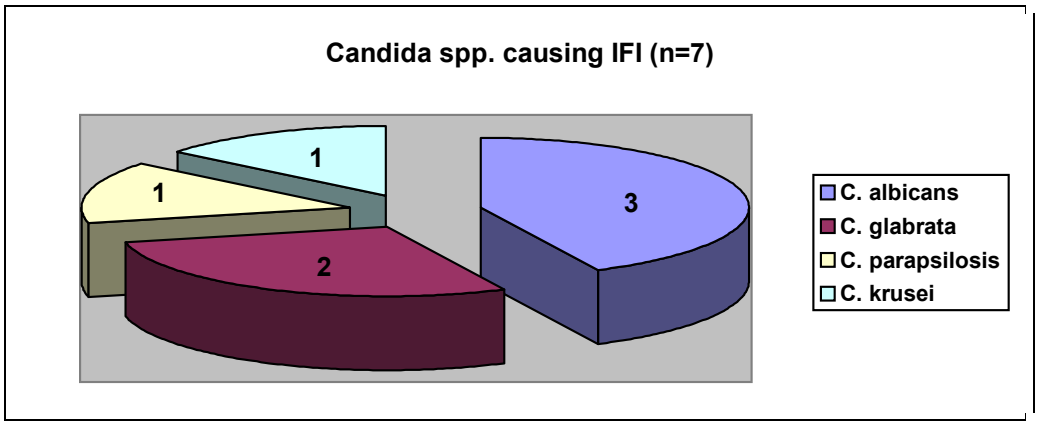


Figure 6

Candida species causing IFI

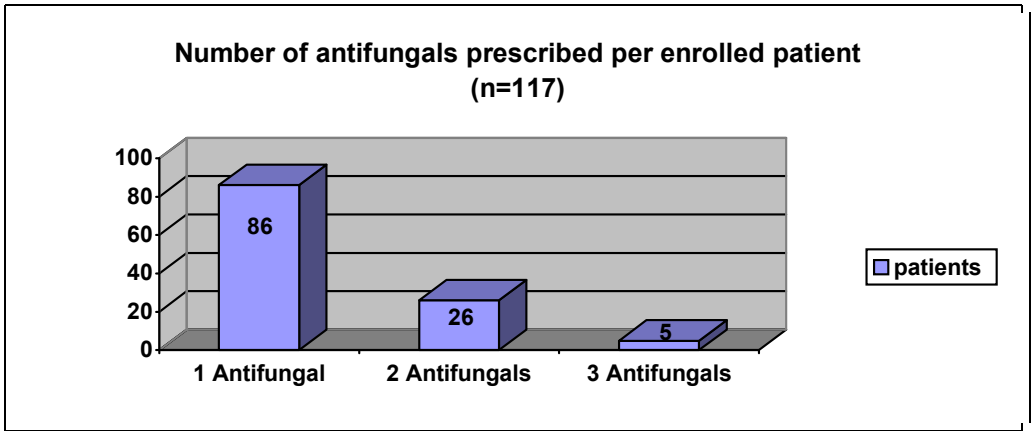


7.4 Antifungal therapy

A total count of 153 antifungal agents were prescribed. 26/117 of patients received two different systemic antifungal agents, 5/117 of patients received three different antifungal agents at one hospitalization while the main population received single agent therapy (86/117 of patients).

Figure 7

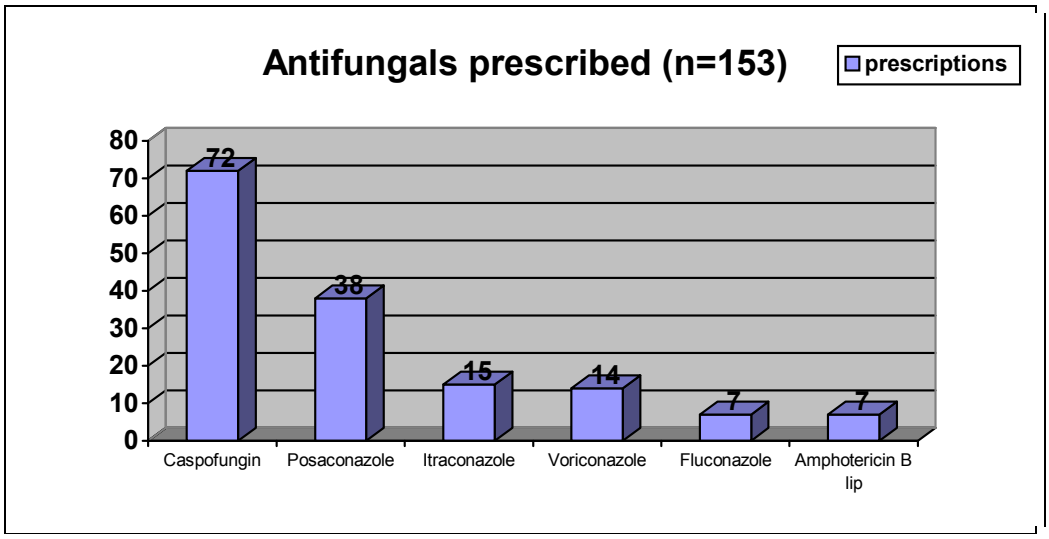
Number of antifungals received by patients



Caspofungin was most prescribed (72/117, 62% of patients), followed by posaconazole (38/117, 32% of patients), itraconazole (15/117, 13% of patients), voriconazole (14/117, 12% of patients), and fluconazole and amphotericinB lip (each 7/117, 6% of patients).

Figure 8

Antifungals prescribed

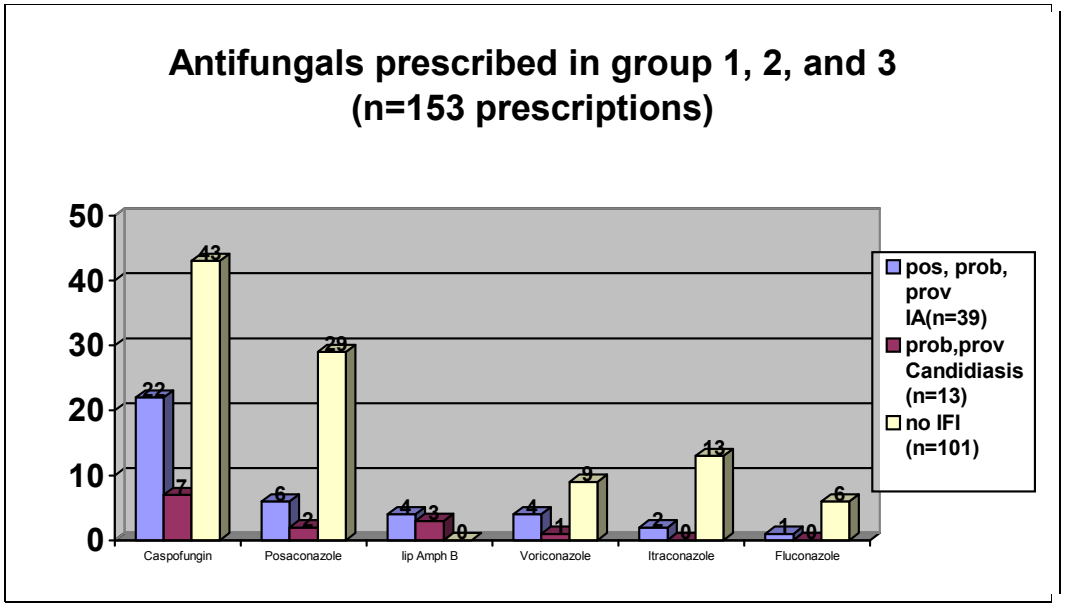


Group 1 was defined as patients with possible, probable or proven IA (n=27). Group 2 was defined as patients with probable or proven invasive candidiasis (n=7). Group 3 was defined as patients with systemic antifungal therapy but no IFI (n=83).

Caspofungin was prescribed in group 1 in 22/27 (81%), in group 2 in 7/7 (100%) and in group 3 in 43/83 (52%) of patients. Posaconazole was prescribed in group 1 in 6/27 (22%), in group 2 in 2/7 (29%) and in group 3 in 29/83 (35%) of patients. Voriconazole in group 1 in 4/27 (15%) in group 2 in 1/7 (14%) and in group 3 in 9/83 (11%) of patients. Itraconazole was prescribed only in group 1 (2/27, 7%) and in group 3 (13/83; 16% of patients). Lip Amphotericin B was prescribed only in group 1 (4/27, 15%) and in group 2 (3/7; 43% of patients), while fluconazole was primarily prescribed in group 3 (6/83; 7% of patients).

Figure 9

Antifungals prescribed in group 1, 2 and 3

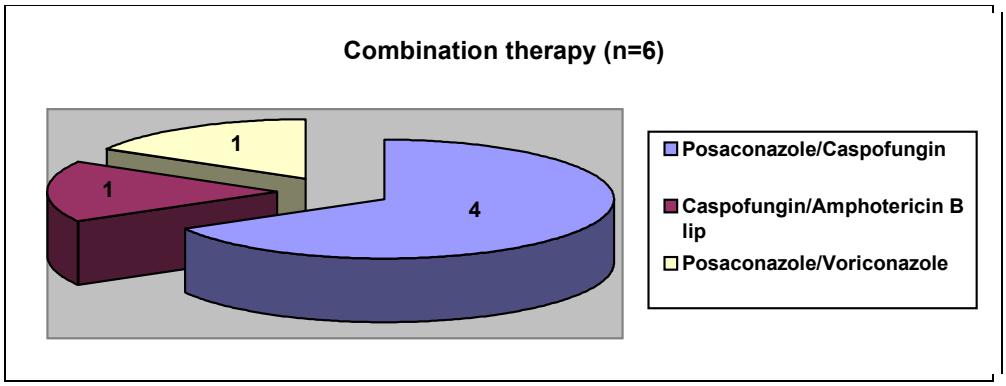


The proven cases of IA and Zygomycoses were treated with lip amphotericin B, posaconazole and voriconazole.

Combination Therapy was prescribed in 6/117 (5%) of patients. The combination posaconazole and caspofungin was prescribed in 4 patients (one of them had a possible IA and improved), caspofungin plus amphotericinB and posaconazole plus voriconazole were prescribed in one patient each.

Figure 10

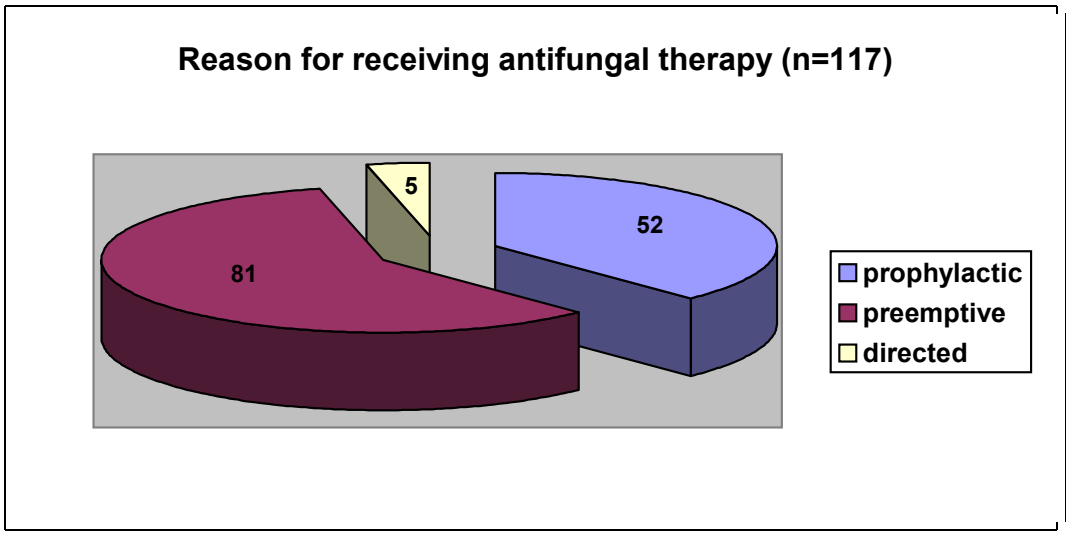
Combination therapy



Out of the 117 patients 52 (44%) received prophylactic antifungal treatment mainly because of undergoing chemotherapy or HSCT and having graft versus host disease or as fungal prophylaxis for AML. 81/117 (69%) of patients received empirical/ preemptive antifungal therapy mainly because of neutropenic fever or suspect imaging or clinical findings. 5/117 (4%) of patients received directed antifungal treatment. 31/81 (38%) of patients receiving empirical/preemptive antifungal therapy had only persistent neutropenic fever and no suspect imaging. Results are shown in figure 11.

Figure 11

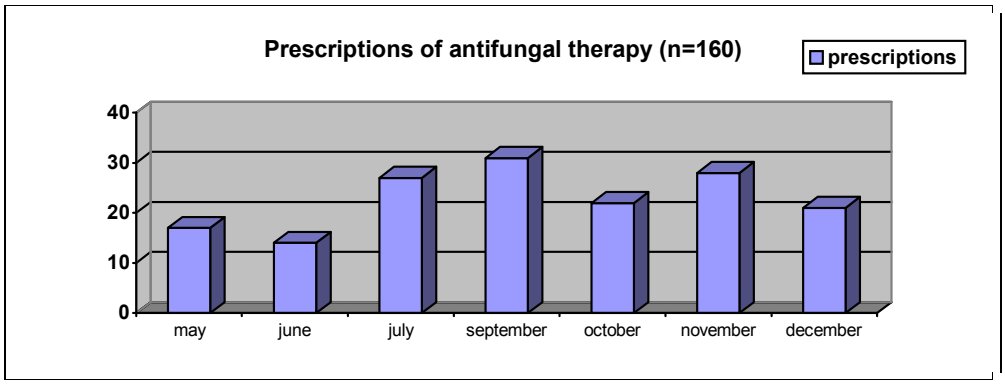
Reason for antifungal therapy



The study was conducted from may to december 2007 with a 1 month break in august/september 07. The total number of antifungal prescriptions was 160 and differed from the total number of antifungals prescribed (n=153), because in some cases the same antifungal was prescribed twice. The amount of antifungal therapies prescribed per month are shown in figure 12.

Figure 12

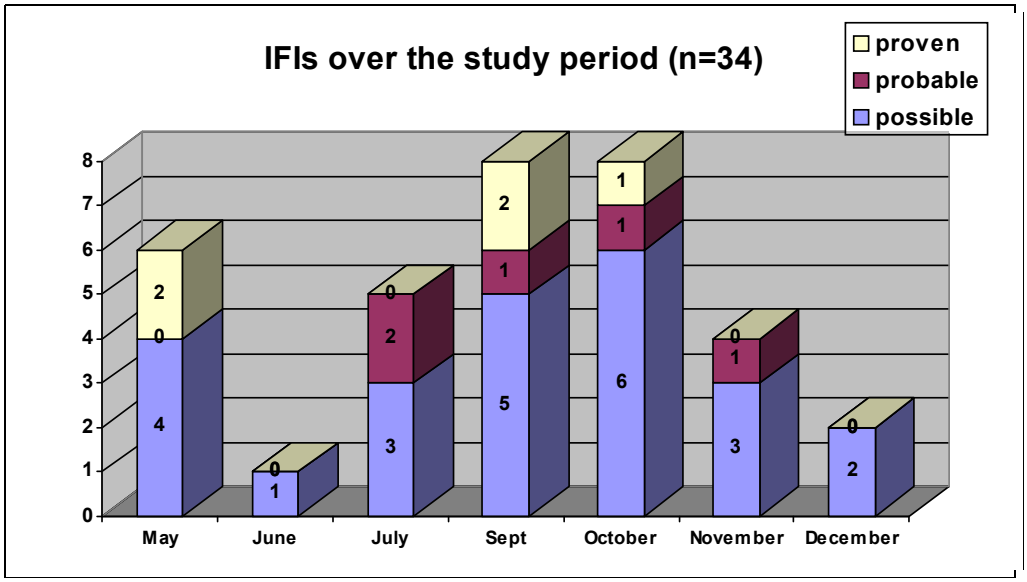
Prescriptions of antifungal therapy



The amount of IFIs per month are shown in figure 13. Most Invasive fungal infections occurred in september and october.

Figure 13

Invasive fungal infections over the study period

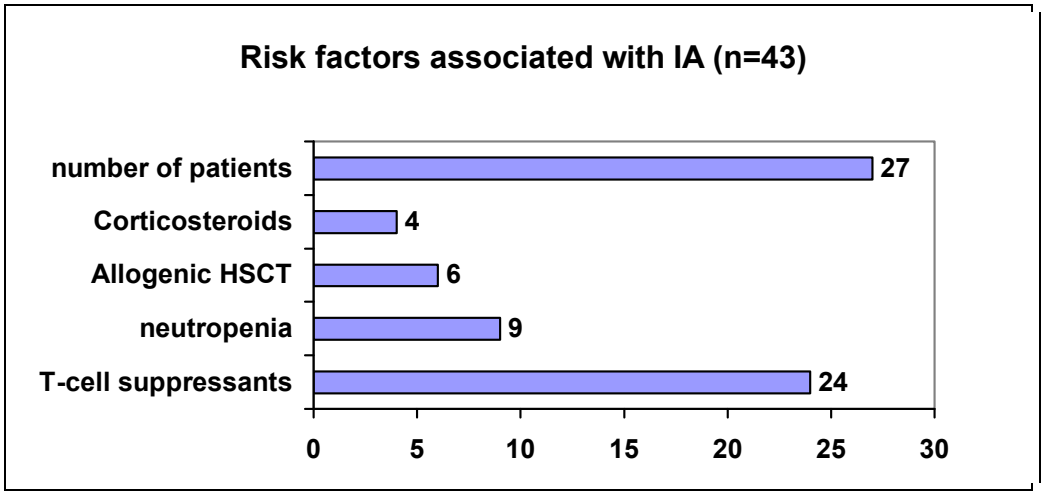


7.5 Risk factors and outcome

The main risk factors associated with possible and probable IA were T-cell suppressants (24/27, 89% of patients), followed by neutropenia (9/27, 33% of patients), allogenic stem cell transplantation (6/27, 22%) and use of corticosteroids (4/27, 15% of patients). No patient had an inherited severe immunodeficiency.

Figure 14

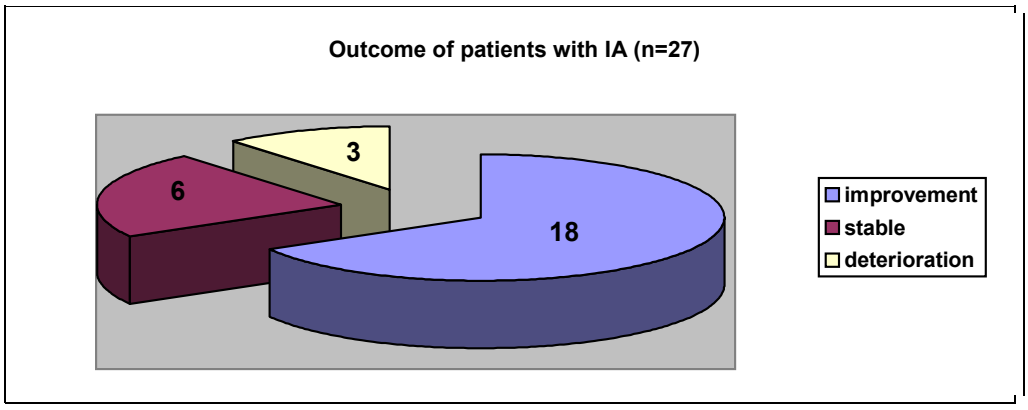
Risk factors associated with IA



Of the 27 patients with possible, probable or proven IA or Zygomycosis 18/27 (66%) showed improvement within 2 weeks of antifungal therapy, 6/27 (22%) presented stable and 3/27 (11%) deteriorated.

Figure 15

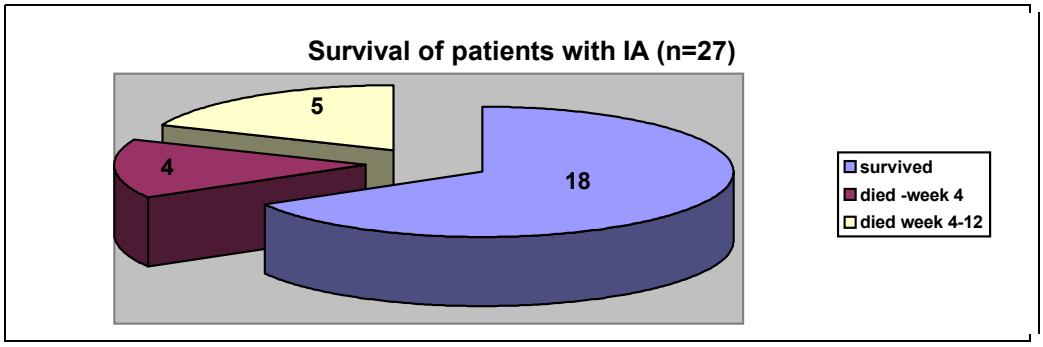
Outcome of patients with IA



Of the 27 patients with possible, probable or proven IA or Zygomycosis 4 (15%) died within 4 weeks. 5/ 27 (19%) of IFI´s ended fatal between week 4 and 12. 18/27 (67%) of patients survived.

Figure 16

Survival of patients with IA



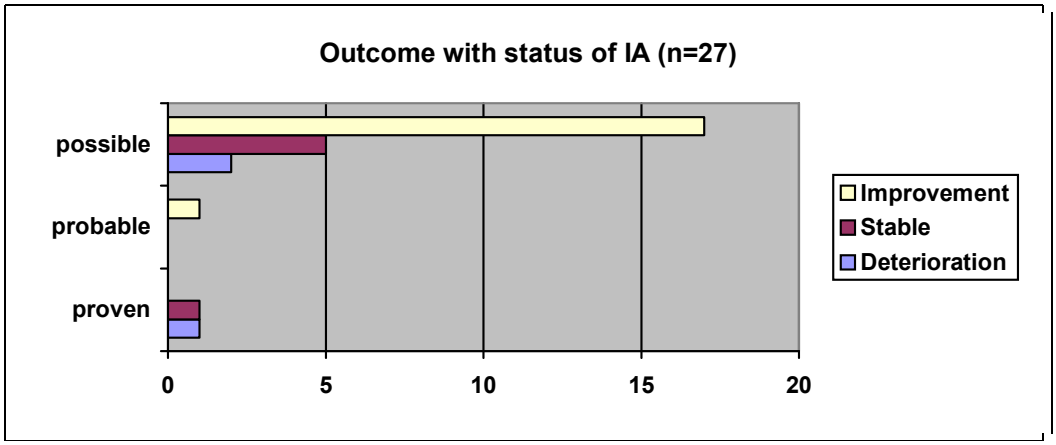
The definition of breakthrough infection was met by 2 cases with caspofungin preemptive treatment and by 2 cases with posaconazole prophylaxis. However, only one of the four breakthrough infection cases was a probable case according to the EORTC criteria with findings of hyphae in cytology of BAL. This patient received preemptive therapy with caspofungin 0,8 mg/kg for 18 days before detection of the hyphae. The other three cases were possible IFIS without any microbiological evidence of infection.

17/24 (71%) of patients with possible IA improved after 2 weeks of systemic antifungal therapy; 5/24 (21%) remained stable, 2/24 (8%) deteriorated. The case of probable IA showed improvement, the case of proven IA remained stable, while the patient with proven Zygomycosis deteriorated. All of the 7 *Candida* cases showed improvement.

Formatiert

Figure 17

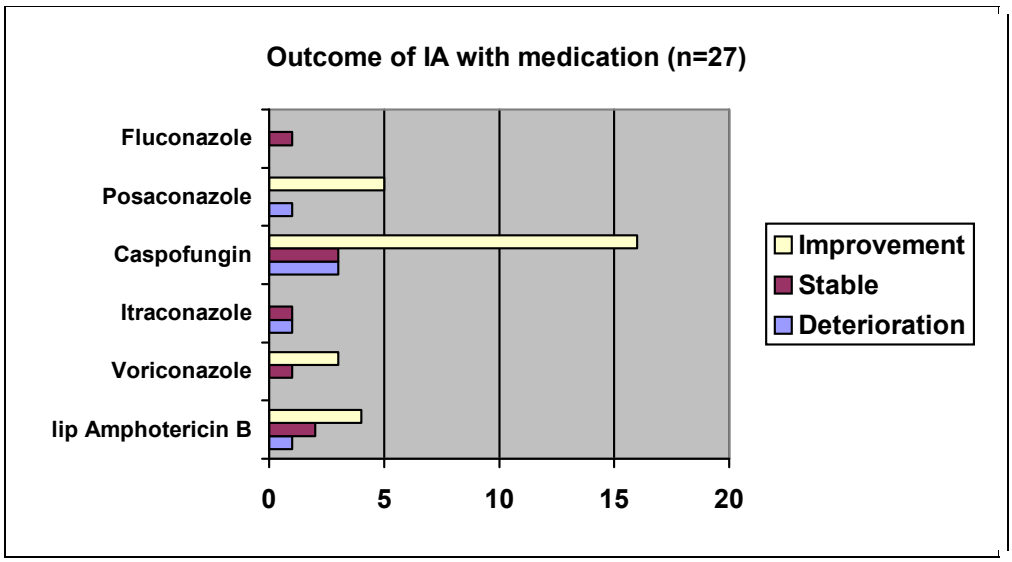
Outcome with status of IA



Outcome with medication is shown in figure 18.

Figure 18

Outcome of IA with medication



7.6 Room with filter use versus standard room

8/27 (30%) of patients with possible, probable and proven IA were hospitalized in a room with filter use, 19/27 (70%) of patients were hospitalized in a standard room.

8 Discussion

A retrospective cohort study of 11802 patients admitted between 1999 and 2003 to 18 hematology wards in Italy reported an probable or proven IFI rate of 4,6%. 69% of the IFIs occurred in patients with AML, the incidence in AML patients was the highest of all underlying diseases with 12%. 64% of all IFIs were caused by molds, in most cases (90%) by *aspergillus spp.* 36% were caused by yeasts, nearly all of them were candidemia. The mortality rate attributable to IFI was 39%, with the highest mortality rates associated with zygomycosis (64%) followed by fusariosis (53%), aspergillosis (42%) and candidemia (33%) [58].

In our study 10/117 (9%) of hematologic patients with systemic antifungal treatment developed probable or proven IFI. The rate of probable and proven IFI among all patients admitted to the hematologic department was very low with 1,24% (10/805) of patients. The rate of probable/proven mold infections was lower compared to the study in italy with 2,6% among patients receiving systemic antifungal therapy and 0,37% among all patients admitted to the department of hematology during study period.

The reasons for this low rate of probable and proven invasive mold infections could be the broader prophylactic use of posaconazole (35% of patients with no IFI), which is highly active against molds, compared to fluconazole and itraconazole which have been shown before to be less effective in preventing invasive mold infections [42, 49].

Another reason however may be the few invasive diagnostic methods performed at the Department of Hematology, Medical University Graz, and that in fact there is no galactomannan or ß-D-Glucan testing available, meaning that many in fact probable IA cases may have remained possible because they did not meet the microbiological criteria. If the possible IFI´s were added, the incidence of IFI would be 4,22% among all patients admitted during study period.

Bischoff et al presented a 6 year review of North American candidemia data in 2003, which disclosed an annual incidence of candidemia of 4,0-5,4 per 10.000 hospital admissions. Species differentiation of 1596 *Candida* isolates showed 52,8% *Candida albicans,* 19,0% *Candida glabrata,* 11,8% *Candida parapsilosis,* 11,3% *C. tropicalis,* and 2,1% *C.krusei* [60]. Results of our study found similar rates among patients with

hematologic malignancies. The rate of probable or proven invasive candidiasis was also very low with 0,87% among patients admitted to the department of hematology, meaning a rate of 9,9 per 10000 hospital days among this patients at special risk.

AML was the primary underlying disease (50% of patients) in our study. The incidence of fungal infections in AML patients however was not higher than in patients with other underlying diseases. 2/3 of patients with probable or proven invasive mold infection died within 12 weeks. All patients with yeast infection survived.

Caspofungin was the drug mostly subscribed in our study as well in the group of patients with IFI as in the group without IFI. Liposomal Amphotericin B was only subscribed in patients with possible, probable or proven IFI, mainly because of its severe adverse events.

117/805 (14,53%) of patients admitted to the department of hematology received prescription of systemic antifungal therapy. In this study we did not differ between empirical and preemptive treatment, because of the lack of microbiological antifungal diagnostic tests available at the Medical University Graz.

In most cases antifungal therapy is prescribed empirically. Basically three considerations justificate empirical therapy for presumed IFI in persistently neutropenic patients: first, the undisputed success of empirical antibacterial therapy for febrile neutropenia; second, that delayed treatment of IFI increases mortality and, finally, the fact that early diagnosis of IFI is difficult. Most of what we know about empirical antifungal therapy results from three prospective, randomised, controlled studies by Walsh et al [61, 62, 63]. These studies have been performed under the same leadershipand in the same institution, and have applied identical criteria for patient inclusion and evaluation, making comparision between them possible [64]. By reviewing the studies by Walsh et al 2002, and Walsh et al 2004 [62, 63] the hypothesis was made that microbiologically proven fungal infections detected after initiation of an empirical regimen (so called breakthrough infections), actually represented failures of the empirical strategy. Breakthrough infections occurred in 45/961 (4,6%) of patients treated empirically with lip AmpB and 23/45 (51%) of breakthrough IFIs caused by *Aspergillus spp;* in 8/415 (1,9%) of patients treated emprically with Voriconazole, with 4/8 (50%) caused by *Aspergillus spp*; and in 29/556 (5,2%) of patients treated emprically with Caspofungin, with *Aspergillus spp*

causing 10/29 (34%) of breakthrough IFIs. The rates for breakthrough IA were 2,4% with lipAmpB, 1% with Voriconazole, and 1,8% with Caspofungin.

The significance of these rates must be put into perspective with the incidence of IFI in patients with persistent neutropenic fever who might have been candidates for empirical therapy without receiving any. The incidence of IFI in 2 recent studies by Maertens et al and Corey et al was 45% [65] and 21% [59]. This would mean that empirical therapy eradicates up to 90% of IFIs in this group of patients, because a failure rate of 4,3% was observed in average for the empirical approach as reported by Walsh and colleagues.

Reviewing the studies by Walsh et al Caspofungin and Voriconazole both appeared suitable, and, considering that they are both better tolerated, they may be preferable alternatives to liposomal Amphotericin B as empirical antifungal therapy in patients with persistent fever and neutropenia [64]. In our patient collective Caspofungin was the most used antifungal agent for empirical therapy in patients with persistent neutropenic fever.

However, overtreatment resulting in increased toxicity and treatment-related costs is a major shortcoming of empirical antifungal therapy. The risk for occult IFI is present only in 25% of patients with persistent febrile neutropenia, meaning with the empirical approach 3 out of 4 patients are treated with potentially toxic and expensive drugs although they are not at risk for IFI [66]. This is the reason why another strategy, the “preemptive approach” has been proposed to fill the gap between over- and undertreatment. However, the success of the preemptive strategy depends on the timely and repeated use of reliable and readily applicable diagnostic tools, like imaging and antigen testing, such as ELISA for galactomannan and the detection of 1-3-β-D-glucan in serum [68].

A study by Maertens et al in acute leukemia patients receiving fluconazole prophylaxis examined an algorithm-based-preemptive approach. Only seropositive patients and patients with a positive galactomannan test result plus supportive radiological findings on computed tomography scanning received liposomal amphotericin B. A total of 41/117 (35%) neutropenic episodes had persistent neutropenic fever; however only 9 patients satisfied the pre-defined criteria for antifungal therapy- a 78% relative risk reduction in antifungal therapy use. One case (due to Zygomycoses) was missed, on the other hand early therapy could be initiated

in 10 clinically not-suspect cases. The overall mortality rate of 18%, although high, was in the range for a population with probable invasive fungal disease [59]. However, despite these promising observations larger randomized studies are needed to prevent the cautious, caretaking clinician to innitiate antimold therapy independent of molecular markers, in high risk patients with clinical or radiological markers of IA. This really presents a dilemma for the clinician, on the one hand there is the risk of delay of therapy which may be life threatening in 25% of patients with persistent febrile neutropenia, on the other hand there is the risk of overtreatment in 75% of febrile neutropenic patients.

In our patients collective 69% (81/117) received empirical/preemptive antifungal therapy, based on clinical and radiological findings. In fact there is no galactomannan testing available at the Division of Hematology, Medical University Graz, meaning that to distinguish clearly between empirical and preemptive antifungal therapy in our patients collective was not possible. 31/81 (38%) of patients receiving empirical/preemptive antifungal therapy had only persistent neutropenic fever and no suspect imaging; still even in these patients the risk for occult IFI is 25%.

4% received directed antifungal therapy and 10/117 (9%) of patients had probable or proven IFI.

To conclude most systemic antifungal therapy at the department of hematology, Medical Univesity Graz was subscribed empirically/preemptively during the study period. 29% of patients receiving systemic antifungal treatment met the EORTC criteria of mostly possible IFI. Proven invasive fungal infections are rare in patients with hematologic malignancies at the Division of Hematology, Medical University Graz.

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10 Tables and Figures

11 Curriculum vitae

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Date of birth: 25th February 1981 in Graz

Nationality: Austrian

Civil Status: Single

Present Status: Student

EDUCATIONAL HISTORY

October 2002 – present	**Leopold – Auenbrugger Medical University Graz**
June 2001 – May 2002	**Civil service at the Psycho-Social Centre Graz-West,** **an ambulatory of the State Hospital of Psychiatry Sigmund Freud**
October 1999- May 2001	**Medicine at the Karl Franzens University Graz**
22nd June 1999	**Bundesrealgymnasium Pestalozzi, Graz (Austria)** Matura (A-Levels Equivalent) with good success

WORK EXPERIENCE/ CLERKSHIPS

August/September 2007	**St.Michaels Hospital, University of Toronto, Toronto, Canada** Internship in the **Department of Infectious Diseases** Dr. I. W. Fong
August 2006	**Cho-Ray Hospital, Ho Chi Minh City, Vietnam** Internship in the **Department of Infectious Diseases and Tropical Medicine** Prof. Dr. Binh

September 2005	**Charité University Hospital, Berlin, Germany** Internship in **Internal Medicine**, **Infectious Diseases** Univ. Prof. Dr. Schneider
September 2004	**University Clinic Graz, Austria** Internship in **Psychiatry** Univ. Prof. Dr. Kapfhammer

SCIENCE WORK

May 2008	Participant at the **31. Austrian congress for Microbiology and Hygiene** in Bad Ischl, presenting a Case Report (Catheter related bacteremia caused by Gordonia Terrae).
May 2008	Participant of the **26. Annual Meeting of the European Society for Pediatric Infectious Diseases** in Graz.
March 2008	Participant at the **Austrian Congress for Infectious Dieseases in Leogang,** presenting a Case Report (Rare cause of a masseteric abcess: Mycoplasma salivarium), and a Study (Epidemiology of invasive fungal infections and antifungal therapy in patients with hematologic malignancies). (Report and study will be published in summer 2008)
May 2007 – present	**Dissertation '**The incidence of invasive aspergillosis and treatment with antifungals in immunosuppressed patients at a university hospital**'** **Ao, Univ. Prof. Dr. Robert Krause** Division of Infectious Diseases, Department of Pulmonary Medicine, University Clinic of Internal Medicine, Medical University Graz

April 2007 – present	Member and participant of the Austrian Aspergillus work group

PUBLISHED PAPERS

Pulmonary Histoplasmosis in Three Austrian Travelers after a Journey to Mexico – Hoenigl M, Schwetz I, Wurm R, Scheidl S, Olschewski H, Krause R. – 2007. Infection. 2008 Jun;36(3):282-4. Epub 2007 Sep 28.

Isolation of Gordonia terrae from a patient with catheter-related bacteraemia. Grisold AJ, Roll P, Hoenigl M, Feierl G, Vicenzi-Moser R, Marth E.

J Med Microbiol. 2007 Dec;56(Pt 12):1687-8.

SPECIAL FUNCTIONS, ABILITIES

April 2008 – present	member of the **Austrian Society for Infectious Diseases**
January 2003 – present	active member of the **Grazer ARGE Alpine-medicine**, December 05-December 07 also **member of the board**
November 2004 – present	member of the Austrian Society for Alpine- and High Altitude Medicine
October 2000 - May 2001	University course for Basic Psychotherapeutic Interventions

October 1998 – April 1999	Matura - Fachbereichsarbeit (the Austrian equivalent of a dissertation for high school students) "Sexual Abuse"
May 1995 – May 1996	organisational assistance at the medical Symposium for Children and Youth - Psychiatry in Pöllau, Austria

EXAMS / ADVANCED EDUCATION

2002/03	Physics, chemistry, biology, physiology, anatomy, histology
2003/04	Pathology, pharmacology, functional pathology, biochemistry, psychology, dermatology
2004/05	Surgery, hygiene, social medicine, internal medicine, dentistry, radiology, statistics, endokrinology
2005/06	Ophthalmology, neurology, casualty surgery, orthopaedy, pediatrics, parasitology, gynecology, psychiatry
2006/07	Urology, nephrology, anesthesia, emergency medical aid, quality management in health care, pediatric infectious diseases, dermatology, cardiology, angiology, ent
2007/08	Practical year. 10 weeks transplant surgery, 10 weeks neurology ICU, 5 weeks general medicine.

LANGUAGES

- German (native)
- English (fluent in spoken and written)
- Spanish (advanced)

COMPUTER SKILLS

- Proficient in MS Excel, Word, PowerPoint

OTHER INTERESTS

- Learning to know new people, cultures and languages
- Travelling
- Fitness
- Skiing
- Wind-Surfing
- Tennis
- Climbing

STAYS ABROAD

1996 - 1999	Boston, MA, Harrisburg, PA, USA and Vancouver, BC, Canada altogether 4 months.
2001	Trekking in Nepal / Annapurna area and Tibet with rounding of the Mount Kailash over the Dalmalah-Pass (5726m)

2002	Trekking in the Moroccan Atlas and climbing of the Djebel Toubkal (4090m)
2003	Trekking in Darjeeling, India
2004	Israel, Cuba, Sri Lanka
2005	Argentine
2006	Vietnam
2007	Canada

REFERENCES

Dr. Ignatius William Fong	Head of the Department for Infectious Diseases, St.Michaels Hospital, University of Toronto, Toronto, Canada
Dr. Robert Krause	Head of the Department for Infectious Diseases, Medical University Hospital Graz, Austria
Dr. Gerd Lyon	Head of the Psycho-Social Centre Graz-West,an ambulatory of the State Hospital of Psychiatry Sigmund Freud