Invasive Aspergillosis: New Insights into Disease, Diagnostic and Treatment

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Abstract: Aspergillus infections are a threat to in patients with hematological malignancies. Known risk factors are profound and long lasting neutropenia, uncontrolled graft versus host disease, continuous administration of steroids and environmental factors such as hospital construction. Numerous efforts have been undertaken for prophylaxis of invasive aspergillosis in high-risk populations. Most of them failed to demonstrate survival advantages. Prophylaxis makes sense, since diagnosis and treatment of invasive aspergillosis remain difficult. The introduction of non-culture based tools for the diagnosis of invasive aspergillosis is an important step forward for early and sensitive diagnosis of invasive aspergillosis. Early treatment is the cornerstone of a successful management of invasive aspergillosis. Substantial improvement came with the introduction of lipid formulations of amphotericin B in the late 1990s. Voriconazole was the firstazole that improved the overall survival for patients with invasive aspergillosis. Newer azoles and the echinocandins were introduced for the treatment of invasive aspergillosis in the late 1990s. Voriconazole and liposomal amphotericin B allow a safer and more effective treatment of invasive aspergillosis when compared with amphotericin B-desoxycholate. Combination of antifungal agents has been introduced in clinical trials. Up to now no significant benefit has been obtained with antifungal combination compared to voriconazole alone. Because mortality of invasive aspergillosis remains up to more than 50%, prophylaxis, early diagnosis and early initiation of antifungal therapy are of utmost importance for the reduction of invasive aspergillosis related mortality. Despite all advances in the management of invasive aspergillosis important questions remain unresolved. This article reviews the current state and new insights in the management of invasive aspergillosis and points out clinicians unmet needs.

Keywords: Invasive aspergillosis, diagnostic treatment.

INTRODUCTION

Fungal Infections

Human mycoses are caused by a variety of species. Fungal infections can appear as superficial and invasive mycosis. They are caused by a variety of different fungi. Of particular importance are invasive aspergillosis, candidiasis, mucormycosis, blastomycosis, cryptococcosis and coccidiomycosis among other rare mycoses. Fungal infections are an important cause of morbidity for patients with immunosuppression in particular hematological malignancies. The Prospective Antifungal Therapy Alliance (PATH) performed prospective surveillance of invasive fungal infections (IFI) among patients hospitalized at 25 medical centers in North America between 2004 and 2008, collecting information on the epidemiology of IFIs. In total, 7526 IFIs were identified in 6845 patients. Candida spp. (73.4%) were the most common pathogens, followed by Aspergillus spp. (13.3%), and other yeasts (6.2%) [1]. European surveys indicate that Candida albicans is responsible for more than half of the cases of invasive candidiasis; however, occurrence of non-albicans-related invasive fungal infections appears to be increasing. IFI-related mortality depends on the pathogen, geographical location and underlying patient characteristics. A German autopsy study reported a shift of IFI within recent years. The rate of candida infections decreased towards more mould infections [2]. A major threat in cancer patients are infections due to invasive Aspergillus species with mortality rates ranging from 38 to 80% [3]. In the United States in 1996, there were an estimated 10,190 aspergillosis-related hospitalizations. These resulted in 1873-4286

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Patients with acute myeloid leukemia undergoing intensive chemotherapy were reported to have a 7% to 11% risk of developing proven or probable invasive aspergillosis (IA) [5, 6]. The rate of possible IA with typical pulmonary infiltrates but without microbiological confirmation is estimated in up to more than 20 to 30% in AML patients [7, 8]. After allogeneic stem cell transplantation (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. An early phase around 16 days after aSCT is associated with severe and long lasting neutropenia. A later peak is observed around 96 days after aSCT. Several risk factors in the aSCT setting are well known: During the early post transplantation phase (aSCT), the rate of IA is reported more often (15% to 25%). IA is observed in two timely peaks. A
a surveillance of IA across different populations, studies and institutions remains an unmet need. A North American surveillance study (TRANSNET) from March 2001 through September 2005 included a total of 16,220 patients who underwent at least one SCT procedure at one of their surveillance sites. IFI occurred in 639 patients in the incidence cohort. Invasive aspergillosis was the most common IFI in the surveillance cohort too (425 cases; 43%), followed by invasive candidiasis (276 cases; 28%) and zygomycosis (77 cases; 8%) [13]. The TRANSNET registry (Table 3) reported in an update isolates with Aspergillus fumigatus 44%, followed by A. niger 9%, ahead of A. flavus 7%, and A. terreus 5%. The lung was most frequently affected. The twelve-week-overall survival was 64.4% [14]. A French registry reported similar twelve-week-overall survival of 44.8%. Independent factors for 12-week mortality were older age, positivity for both culture and galactomannan and central nervous system or pleural involvement, while any strategy containing voriconazole was protective [15]. The Italian SEIFEM-2008 registry reported 27% attributable mortality of invasive aspergillosis [6]. Although IA-associated mortality remains high, recently a significant decrease in fungus-related deaths was observed despite a significant increase of IFI over time [16, 17]. The introduction of new diagnostic and therapeutic tools has improved the management of invasive aspergillosis. This review highlights advances in the management of invasive aspergillosis and points out unmet needs.

Epidemiology and Pathogenesis of infections due to Aspergillus spp

The genus Aspergillus includes over 185 species. Out of these around 20 have been reported causative of opportunistic infections in man. Species distribution may differ which means that local epidemiology should be kept in mind (e.g. A. terreus with a lack of susceptibility against amphotericin B) [18]. Infections due to Aspergillus species are caused in most cases by Aspergillus fumigatus, far ahead of Aspergillus flavus, Aspergillus niger, Aspergillus terreus and other Aspergillus species.

Table 1. Host Risk Factors Associated with All-Cause Mortality for Invasive Aspergillosis in allogeneic SCT

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Mortality Rate</th>
</tr>
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<tbody>
<tr>
<td>&gt;40 years of age</td>
<td>+</td>
</tr>
<tr>
<td>Impairment in pulmonary function before SCT</td>
<td>+</td>
</tr>
<tr>
<td>Baseline disease (non remission)</td>
<td>+</td>
</tr>
<tr>
<td>Transplant characteristics (HLA mismatch, cord blood transplantation)</td>
<td>+</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>+</td>
</tr>
<tr>
<td>Elevated creatinine, bilirubine</td>
<td>+</td>
</tr>
<tr>
<td>Disseminated IA &gt;40d after SCT</td>
<td>+</td>
</tr>
<tr>
<td>Respiratory virus infection</td>
<td>+</td>
</tr>
<tr>
<td>CMV disease</td>
<td>+</td>
</tr>
<tr>
<td>Uncontrolled Graft versus host disease (GvHD)</td>
<td>+</td>
</tr>
<tr>
<td>Prolonged administration of steroids (&gt;2 mg/kg)</td>
<td>+</td>
</tr>
<tr>
<td>Blood transfusions</td>
<td>+</td>
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</tbody>
</table>

Table 2. Treatment Associated Risk Factors for Invasive Fungal Infections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mucosa destruction</th>
<th>Neutropenia</th>
<th>Lymphopenia</th>
<th>Immune Suppression HLA Mismatch</th>
<th>Steroid use</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML induction with 3+7</td>
<td>*</td>
<td>*</td>
<td>+</td>
<td>+ / -</td>
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</tr>
<tr>
<td>Hidac/dauno</td>
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<td>+ / -</td>
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<td>**</td>
<td>**</td>
<td>++</td>
<td>+ / -</td>
<td></td>
</tr>
<tr>
<td>ALL Induction</td>
<td>*</td>
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<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Matched sibling transplantation</td>
<td>* - ***</td>
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<td>**</td>
<td>* - **</td>
<td>0 - ***</td>
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<tr>
<td>Mismatch transplantation</td>
<td>* - ***</td>
<td>**</td>
<td>** - ***</td>
<td>**</td>
<td>0 - ***</td>
</tr>
<tr>
<td>Cord transplantation</td>
<td>* - ***</td>
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</tbody>
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The manifestation and severity of the aspergillosis disease depends upon the immune status of the patient. Invasive Aspergillus infections most commonly affect the lung (see Fig. 1) and sinuses. Other forms of the disease are central nervous aspergillosis, osteomyelitis, endophthalmitis, endocarditis and disseminated form of aspergillosis, which are attributed with morbidity and a high risk of infection related death. Invasive Aspergillus infections are rarely observed in healthy hosts [19-21]. Therefore a thorough knowledge of risk factors and potential causative organisms are required for optimal management. Seasonal peaks for invasive aspergillosis have been reported during spring, summer and fall. These data are somewhat inconsistent. This might be due to regional differences of the load of Aspergillus spores in the air [22, 23]. Since Aspergillus is mostly airborne, environmental modification can decrease IA incidence. This emphasizes the use of an environmental strategy, including high-efficiency air filtration (HEPA), in the prevention of IA. Risk factors for Aspergillus infections are outlined in (Table 1) [24, 25].

Aspergillus spores are ubiquitous in the air around the world. The development of invasive aspergillosis requires exposure of a susceptible host. Aspergillus spores are inhaled. The incubation period between exposure to Aspergillus spp and development of disease is unknown. Aspergillus infections are observed more often under new building construction or reconstruction [26], roadwork, contaminated air conditioning systems, and contaminated soils. Potted plants should be removed from patient’s rooms. Patients should avoid contact with contaminated food with aspergillus spores (e.g. tea, nuts, tobacco). The patients should be advised not to stay close to compost heaps when they are at risk for IA. IA due to contaminated water has been reported. Aspergillus species were found in the hospital water system with significantly higher concentrations of Aspergillus in bathrooms, where water use was highest [27]. Water-born infection of IA however is rare. Because Aspergillus spores are in the air, filtering of the air to remove Aspergillus spores makes sense. Suitable measures in the patient’s environment to protect the patient from exposure to spores can reduce the rate of Aspergillus infections. Efficacy of environmental air control with high efficiency filter rather than laminar air flow is recommended to reduce pulmonary Aspergillus infections in the hospital for patients receiving aSCT. No validated recommendations have been presented for solid organ transplant or autologous SCT patients. Patients receiving aSCT are advised to stay within high-efficiency respiratory-protection areas. Masks are suggested when they leave their rooms and when dust-generating construction activities are ongoing in the hospital [28].

A reduction of Aspergillus infections with these measures has been demonstrated during hospital construction periods. Situations with an outbreak of Aspergillus infections can be managed with portable HEPA filters. A Finnish 12-week study reported on the incidence of IA on a SCT ward with HEPA filtration during close-by construction work. Patient room air samples were negative for Aspergillus spores in 32 of 33 cases. All samples of the outside air were positive. During a median follow-up of 214 days, no invasive Aspergillus infections were diagnosed in the 55 patients treated during the construction period [29]. Similar observations of a reduction of invasive aspergillosis are reported by several other

| Table 3. Characteristics of HSCT (Autologous, Syngeneic, and Allogeneic) Recipients (TRANSNET data [13]) |
| --- | --- | --- |
| Variable | Surveillance Cohort | Incidence Cohort |
| HSCT recipients | 875 | 639 |
| No. of HSCT recipients | 445 (51) | 325 (51) |
| 3-Month Mortality (%) | 606 (70) | 431 (68) |
| Pretransplantation Conditioning | 261 (30) | 202 (32) |
| Myeloablative | 3 (0.3) | 6 (1) |
| Non-myeloablative | 187 (44) | 134 (45) |
| Aspergillus fumigatus | 22 (5) | 17 (6) |
| Aspergillus terreus | 36 (9) | 26 (9) |
| Aspergillus niger | 31 (7) | 25 (8) |
| Aspergillus flavus | 27 (6) | 17 (6) |
| Multiple Aspergillus species | 13 (3) | 12 (4) |
| Other Aspergillus species | 109 (26) | 70 (23) |
| Unspecified Aspergillus species | 109 (26) | 70 (23) |
authors with HEPA-filtration [30-34]. Portable air filtration units have been used in older buildings. However, the difference in Aspergillus spores between rooms with and without portable air filtration was found non-significant [35]. In addition, cleaning of HEPA-filtrated rooms in a hematology unit may have an influence on the amount of Aspergillus spores in the air since it may disperse dust. This particular question has been investigated. Data from the MD Anderson Cancer Center looked for a relationship between cleaning status and occurrence of Aspergillus species. Of 627 bioaerosol samples, 7.3% were positive for Aspergillus species. Multiple logistic analyses revealed independently significant associations with detection of Aspergillus species. Cleaned rooms positive for Aspergillus species had a higher density of colonies than that of rooms sampled before cleaning (18.9 vs air colony-forming units [cfu] per cubic meter; P = 0.0047). Rooms with positive airflow had a detection rate for bioaerosol samples equivalent to that of rooms with negative airflow (7.3% vs 7.8%; P = 0.8) [36].

Although high efficient filters techniques can reduce the amount of inhaled Aspergillus spores in patient rooms, the risk for inhalation after departure from these rooms remains. Minimizing the length of time outside the room with HEPA filtration is recommended. But not all patients at risk for IA can be treated in air-filtered facilities. Even in those hospitals providing HEPA-filtrations patients have to leave air filtered areas for some diagnostic or therapeutic procedures. A ten-year air sample analysis of Aspergillus prevalence found it impossible to provide an environment completely devoid of Aspergillus spp. Airborne fungal samples were collected on a monthly basis for 10 years, from 1995 to 2005, at a tertiary university hospital. Recovery of Aspergillus spp at 37 °C in the high-efficiency particulate air (HEPA)-filtered locations was positive for Aspergillus spp. approximately one-third of the time. The rest of the patient care areas were positive half of the time and the outdoor samples were positive 95% of the time. The samples excluded that routine air sampling is not an effective means of predicting hospital-acquired infections. A transient spike, or burst, may be useful in identifying an in-house source of contamination and may be used to consider additional interventional treatments for high-risk patients [37].

Environmental hospital control measures have been analyzed. The question of a threshold of Aspergillus spores for patients at risk is of clinical relevance. A systematic review summarized mortality rates of infected patients, distribution of Aspergillus spp. in clinical specimens, concentrations of Aspergillus spores in volumetric air samples, and outbreak sources. Fifty-three studies with a total of 458 patients were included. In 356 patients, the lower respiratory tract was the primary site of Aspergillus infection. Species identified most often were Aspergillus fumigatus (154 patients) and Aspergillus flavus (101 patients). Hematological malignancies were the predominant underlying diseases (299 individuals). Construction or demolition work was often (49.1%) considered to be the probable or possible source of the outbreak. Even concentrations of Aspergillus spp. below 1 colony-forming unit/mm³ were found to be sufficient to cause infection in high-risk patients. Virtually all outbreaks of nosocomial aspergillosis are attributed to airborne sources, usually construction. Even small concentrations of spores were associated with outbreaks, mainly due to A. fumigatus or A. flavus [38].

A reduction of Aspergillus spores in the air outside of a HEPA filtration area can be achieved with well-fitting masks and is recommended in general. These masks are used in health care settings to protect from inhaling particles of 0.3-0.5 μm size. The efficacy and tolerability of well-fitting masks in high-risk patients was evaluated in a prospective, randomized, multi-center study compared with standard hospital hygiene procedures. Adult patients undergoing chemotherapy for acute leukemia or aSCT were included. Forty-one patients were randomly assigned to wearing masks and 39 to the control group. In all, 76% of patients were treated in laminar airflow or HEPA-filtered rooms. Antifungal prophylaxis was with oral polyenes in 84%, and three aSCT recipients were given fluconazole. Invasive fungal infections were diagnosed in eight patients in either study arm. One patient in each arm died from proven invasive aspergillosis. There was no difference in the use of systemic antifungals. The trial failed to show a reduction of invasive fungal infections by using well-fitting masks [39]. Although we recommend well-fitting-maks for patients who are exposed to hospital areas with construction or remodeling.

Despite all this, all patients at risk should not be exposed to Aspergillus spores. Therefore, emphasis is necessary on maintaining Aspergillus spores filtration of the outside air from the patients respiratory tract as long as there are high-risk host factors to prevent invasive pulmonary aspergillosis.

Definition of Infections Due to Aspergillus spp

Diagnosis of IA remains difficult. Clinical signs and symptoms are often not specific. Definite diagnosis by culture from sterile body compartments may take weeks but confirms the diagnosis. On the other hand, cultures that yield Aspergillus species do not always reflect invasive disease, because colonization can occur in immunocompromised patients, and false-positive results that result from environmental contamination are occasionally a problem. Because culture has a poor sensitivity in the diagnosis of invasive aspergillosis, reliance on culture alone results in substantial underdiagnosis. Autopsy studies report confirmed IFI up to more than 30% of autopsied patients with hematological malignancies. The prevalence of IFIs at autopsy was reported up to 30% over the last 20 years [2, 40, 41] but these are selected patient series. Autopsy findings over a 30-year period from a single institution revealed IFI in 21.4% of hematological patients. But autopsies are performed less frequently in recent years.

Due to a substantial controversy concerning optimal diagnostic criteria for invasive fungal infections members of the EORTC and the MSG formed a Consensus Committee to develop standard definitions for invasive fungal infections [42]. These guidelines were recently updated [25]. A set of research-oriented definitions for the IFIs in immunocompromised patients with cancer was proposed. Three levels of probability are proposed: “proven,” “probable,” and “possible.” The definitions were developed to facilitate the identification of homogeneous groups of patients for clinical and epidemiologic research. The EORTC definitions are helpful for the design of clinical trials and to evaluate new management strategies. The EORTC criteria allow a better enrollment of similar cases into clinical trials, and therefore better comparison. Diagnosis of probable or possible IA requires host and clinical factors. Probable diagnosis of IA requires a mycological criterion. Accepted mycological criteria for probable IA are direct tests (cytology, direct microscopy, or culture) from sputum, bronchoalveolar lavage, bronchial brush, or sinus aspirate sample indicated by one of the following presence of Aspergillus spp or recovery of Aspergillus spp by culture. Indirect tests that are applicable for IA are Galactomannan (GM) antigen detected in plasma, serum, bronchoalveolar lavage fluid or cerebrospinal fluid. Beta-D-glucan detected in serum is accepted as indirect test and primarily applicable to aspergillosis as well as candidosis. The test has been approved by the FDA. Different thresholds of GM have been reported in clinical studies. For the EORTC/MSG categories there is reliance entirely on the thresholds recommended by the manufacturer. Host factors are not synonymous with risk factors but are characteristics by which individuals predisposed to acquire IA can be recognized. Consequently, the presence of fever was removed as a host factor in the revised 2008 EORTC/MSG criteria because it represents a clinical feature, not a host factor, and is nonspecific for IA. The host factors include history of neutropenia (<0.5/mm³ for >10d) temporally related to the onset of invasive aspergillosis, receipt of a solid-organ transplant, hereditary immunodeficiencies, connective tissue disorders, and
IFI requires cultural and/or histological evidence, i.e. positive cul-
definitions of invasive fungal disease, a definite diagnosis of proven
gillosis. Early preemptive treatment with a safe antifungal agent
ticular institutions. Local environmental situations are only one
differs considerably between clinical trials, drugs used, and in par-
count. The numbers needed to prevent an invasive asperg illosis
aspergillosis prophylaxis of invasive fungal infections would be an
respectively. Given the high mortality associated with invasive
strates refractory to broad spectrum antibiotics.
proven diagnosis at a stage of fever of unknown origin or lung infil-
and appropriate treatment. Most patients have to be treated prior to
stated that the proportion of Aspergillus infections increased from about 10% before 1980 to
more than 30% in the 1990s and fell to 21% by 2005 [2]. Even with
the introduction of newer diagnostic tools most invasive aspergillosis
remain unproven intra vitam. Therefore, early diagnosis of inva-
sive aspergillosis remains important. Outcome depends on early
and appropriate treatment. Most patients have to be treated prior to
proven diagnosis at a stage of fever of unknown origin or lung infil-
brates refractory to broad spectrum antibiotics.

The attributable early mortality of invasive aspergillosis within
the first 6 weeks of treatment has been reported up to 89% in ran-
monized trials [46]. The detection of serum galactomannan (GM)
antigen allows 5-8 days earlier diagnosis of invasive aspergillosis
when compared to clinical signs, imaging or even cultures of As-
pergillus species alone [47]. The Platelia GM Aspergillus EIA is a
commercially available test kit that detects an exoantigen of Asper-
gillus with a high sensitivity and specificity of > 80% and 90%
respectively. Given the high mortality associated with invasive aspergillosis prophylaxis of invasive fungal infections would be an
ideal antifungal strategy [48]. But antifungal treatment may be
associated with adverse events, increasing fungal resistance and has a
substantial economic impact. These aspects must be taken into ac-
count. The numbers needed to prevent an invasive aspergillosis
differs considerably between clinical trials, drugs used, and in par-
ticular institutions. Local environmental situations are only one
reason, including patients and their risk factors for invasive asper-
gillosis. Early preemptive treatment with a safe antifungal agent
would be an alternative concept, if early diagnosis and effective
treatment could reliably be established.

According to the 2008 revised EORTC/MSG consensus based
definitions of invasive fungal disease, a definite diagnosis of proven
IFI requires cultural and/or histological evidence, i.e. positive cul-
tures from normally sterile body fluids and/or positive findings
from tissue biopsies or resection samples. However, these criteria
may not be applicable in hematological patients at risk for invasive
aspergillosis (IA) at an early stage of infection. These patients are
usually in clinical conditions that rarely permit invasive diagnostic
procedures as tissue sampling. The decision to start antifungal ther-
apy in this group of severely immunocompromised patients remains
mostly based empirically. Antibiotic-refractory fever or pulmonary
symptoms with detection of suggestive pulmonary infiltrates by
chest computed tomography (CT) and/or positive biomarker find-
ings are early findings. Since, up to now, no single test is suitable
each to diagnose or to exclude IA definitely in the early course
of invasive aspergillosis, the diagnosis of a suspected IA is based on
a puzzle of findings. This puzzle includes the assessment of
clinical signs and symptoms, cultural as well as non-cultural tech-
niques (e.g. antigen detection, molecular methods) and imaging
procedures. In general, clinical signs and symptoms in IA are non-
specific. Unexplained fever, cough or short-time pleural pain are a
hint of an early fungal infection and should lead to further diagnos-
tic procedures.

Culture Based Techniques

Culture based diagnostic tools in blood samples are disappoint-
ing and show low sensitivity rates. Blood culture samples are very
rarely positive in IA. If positive they were found mostly in patients
near death [49]. This might be due to a circulating pathogen burden
mostly below a threshold of the diagnostic tools. Under the clinical
condition of an early empirically based or pre-emptive antifungal
treatment in suspected IA the value of diagnostic tools may be re-
duced. Recovery of Aspergillus species from respiratory samples of
high-risk patients with clinical signs suggestive for IA should be
regarded as a possible hint of a fungal infection, but it is not con-
clusive [50].

The culture based detection of Aspergillus spp in respiratory
samples is not accepted as a proof of an infection in the revised
EORTC/MSG consensus definitions of 2008 [25]. In fact there is a
wide-spread tracheobronchial colonization with the opportunistic
pathogen, in addition, is a chance of contaminated samples. The
detection of Aspergillus species in deeper compartments of respira-
tory samples as BAL is regarded as evidence for a pulmonary infec-
tion in patients at high risk, if clinical signs and symptoms and ra-
diological findings (lung infiltrates in a chest computed tomography
scan) support the suspected diagnosis. An antifungal treatment is
recommended to be started regularly, if the patient is not already
on Aspergillus treatment.

Despite the problem of differentiating between contamination,
colonization and infection based on culture results, bronchoalveolar
lavage might be the optimal specimen tool for diagnosis of IA.
BAL obviously reflects the samples which most adequately repre-
sent the location of the infection, thus yielding a higher amount of
pathogens, at least detectable in molecular and/or serological as-
says. Sometimes they can be detected by microscopy.

Microscopy

Clinical specimens obtained from relevant clinical sites and in
context with a suspected fungal infection should be investigated by
microscopy in addition to culture. Fungal elements may be missed
or misinterpreted as artifacts in routine Gram or hematoxylin–eosin
stained slide preparations. Fungi are best visualized in general by
periodic acid–Schiff or Grocott’s methamine silver stains and
optical brighteners (e.g. calcofluor white), including BAL and other
liquid clinical samples (CSF, pleural effusion) [51].

Microscopy may provide important information on filamentous
fungi as Aspergillus species such as the presence of septa, ramifica-
tion patterns and other specific morphological aspects that may
yield additional valuable diagnostic hints. Histomorphological in-
vestigations in tissue specimens should be performed by an experi-
enced and skilled pathologist. The microscopic differentiation between Aspergillus spp. and other filamentous fungi may be very difficult. Particularly angioinvasive growth and ramification patterns of the pathogen and/or thrombosed vessels in biopsy specimens may hint at Aspergillus species as the causative pathogen. These findings above are not specific for IA. The cultural investigation of tissue samples is mandatory beyond positive culture results, the histopathological detection of the pathogen is considered as “diagnostic gold standard” for IA. The exact clinical place value and the predictive power investigating tissue samples by histopathological investigation is difficult to assess. Up to now, standards regarding the numbers of specimen, the technique and time schedule for work-up of samples or the interpretation of results in patients with suspected IA are not assessed [52].

Imaging Tools

Attempts to improve the early diagnosis of IA rely on the application of radiological diagnostic tools. Conventional chest X-ray examinations are often not conspicuous of IA. They should therefore not be performed routinely. Radiological findings based on chest computed tomography scan results allow detecting lung infiltrates earlier. Moreover, a normal CT-scan of the lung is a helpful tool to rule out invasive aspergillosis of the lung in most patients. Chest imaging plays a crucial role in a suspected IA. They relate both to the start and the monitoring of the effects of antifungal treatment. Serial CT-scan data from Japan showed that the first sign of invasive pulmonary aspergillosis is an airway invasive pattern, that occurs prior or may be followed by angio invasive signs (halo sign, air crescent signs) [53]. Although the specificity of radiomorphological findings alone is not definite for diagnosis of IA. High resolution computed tomography (HRCT) or thin-section chest CT scans detect more typical lung infiltration patterns of IA at an early stage (Fig. 1). This may translate into an improved outcome. CT scans performed within the first days of a suspected infection (febrile neutropenia of unknown origin unresponsive to antibacterial antibiotics for more than 1-2 days) are strongly recommended [54-56].

Fig. (1). Invasive pulmonary aspergillosis radiological halo signs.

Infiltrates caused by pulmonary aspergillosis consist mostly of small nodules (size 1cm to 3cm). They may be surrounded by a halo (ground-glass opacification). This halo represents perifocal hemorrhage due to an angioinvasive pathogen growth and consecutive infarction. Albeit not specific, halo signs may be considered suggestive of an early invasive pulmonary aspergillosis. Differential diagnoses include bleeding, embolism, leukemic infiltrates and other causes including other pulmonary infections must be kept in mind [57-59].

Other radiological signs (nodules without halo sign) or cavitation (in particular air crescent sign), alveolar consolidation with or without halo sign or cavitation, centriflobular micronodules and “tree-in-bud” opacities, ground glass opacities, septal thickening, and pleural effusion may be suggestive of invasive pulmonary aspergillosis. According to newer data, the pattern of lung infiltrates in patients with invasive pulmonary aspergillosis depends on the underlying hematologic malignancy and the extent of immunosuppression [60].

It should be remembered, that, if CT scans of the lung are performed for follow-up, the volume of pulmonary lesions even may increase during the first week despite effective antifungal treatment. The diminishment of halos or the appearance of air-crescent signs, mostly seen after recovery from granulocytopenia, may indicate response and not a failure to the therapy [59].

Concerning lung infiltrates, the diagnostic standard is computed tomography. Data obtained with magnetic resonance imaging (MRI) are not convincing, up to now. Moreover availability, fast track diagnostic and patient comfort of chest CT-scan procedures versus MRI should be taken into account.

In contrast to the lung, in those cases with disseminated invasive aspergillosis, MRI based diagnostic investigations play a significant role in diagnosing and monitoring fungal infiltrates in liver, spleen, the gastrointestinal tract and the brain [61].

Serological Assays: Galactomannan (GM) and 1→3,β-D glucan (BG)

Early diagnosis and early appropriate antifungal treatment is the cornerstone to reduce mortality and morbidity of IA. Research at present is therefore focused on the development of reliable novel diagnostic modalities. These tools should have high sensitivity and specificity rates. But most important they should detect Aspergillus pathogens earlier, in particular in the group of patients at high risk for IA due to intensive immunosuppressive treatment for hematological malignancies.

The use of non-invasive biological surrogate markers to identify the causative pathogen from blood samples is attractive. The problems of diagnosing IA by histology and/or culture methods have been outlined before. In the late 1970s, antigen assays detecting surrogate markers such as circulating fungal cell wall elements or metabolites were developed. These assays were not influenced by the immune status of the patient and showed higher negative predictive value (NPV) and positive predictive value (PPV) than antibody assays that were formally used. By detecting galactomannan (GM), a carbohydrate constituent of the Aspergillus cell wall, diagnostic sensitivity and specificity rates of 80% and 94% could be reached, respectively, with bronchoalveolar lavage (BAL) samples. However, testing blood samples, the results showed considerably lower detection rates, although a number of techniques, such as enzyme immunoassays (EIA), radioimmunoassay (RIA) and latex particle agglutinations tests have been evaluated. Their routine use has been hampered by low sensitivity rates, detecting the infection only at advanced stages [62].

A sandwich EIA technique that incorporates the B 1–3 galactofuranose specific EBA2 monoclonal antibody as detector for GM improved the sensitivity rates considerably, due to a significantly lower GM detection limit in vitro [63]. Using this technique to evaluate the test clinically, Maertens et al. prospectively screened serial blood samples from patients with hematological malignancies. They observed, based on histologically and culturally proven invasive infections, both high sensitivity and high specificity rates of about 90%. The rate of false positive reactions was up to 14% [64, 65].

Based on these results, the authors recommended appropriate imaging techniques in addition to the serological assay, thus improving the detection rate of IA. Serial screening of blood samples
for GM from a high-risk population with the EIA (Platelia Aspergillus, Bio-Rad Laboratories) seemed to be a sensitive and non-invasive tool for diagnosing and confirming IA in symptomatic hematological patients. However, these data could not be confirmed in other trials. Testing patients in other clinical conditions and using different thresholds for positivity reported distinctly lower sensitivity rates. The comparison of the results between these different studies is difficult, due to other variables. Especially the cut-off values (optical density index (OD)) affect both sensitivity and specificity rates of the test [66].

The influence of different cut-off levels, performed in the range between 0.5 and 1.5, was shown by several authors. In the United States, the test has been approved by the Food and Drug Administration (FDA) with a cut-off value as low as 0.5. In Europe, the manufacturer first recommended a cut-off value of 1.5, before adjusting the OD to 0.5, likewise. According to another study from Maertens et al., a single positive result with an OD index higher than 0.8 (“static threshold”) in blood samples warranted the initiation of specific antifungal therapy, whereas a further lowering of the static threshold reduced the PPV. The demonstration of at least two sequential positive blood samples with an OD >0.5 (“dynamic threshold”) increased the specificity and the PPV to 98.6% and the efficiency to 98%. However, the clinical benefit of lower cut-offs (particularly for earlier diagnosis) depends upon the more or less unknown kinetics of antigenemia and the intensity of serum sampling [67].

Further drawbacks of the GM test are false-positive results. These were observed and occurred in patients treated with antibiotics produced by fungal organisms or due to other conditions. False-positive GM test results have been reported in patient serum or bronchoalveolar lavage (BAL) fluid in association with antibiotics, infant feeding, some enteric bacteria, Plasma-Lyte infusion (contains high levels of GM-antigen) [68], and (1->3)-β-D-glucan blood products. Histoplasmosis and other endemic mycoses may also cause false-positivity of GM-tests. On the other hand, mould-active antifungal drugs may cause false-negativity or reduces sensitivity rates, at least, so that the clinical correlation is imperative [69]. In conclusion, a negative GM result in blood samples does not exclude the diagnosis, and a positive result does not reliably establish the diagnosis of invasive aspergillosis. As with all non-culture tools to diagnose IA, the detection of GM, must be considered in the context of the risk constellation of the patient and clinical, radiological and other laboratory findings [70, 71].

A few years ago several groups started to investigate BAL samples to overcome the blood testing drawbacks. Despite the problem to differentiate between contamination, colonization and infection, bronchoalveolar lavage seems to be a more appropriate specimen than blood. BAL obviously reflects the sample which most adequately represents the primary location of the infection, thus yielding a higher amount of pathogens. One of the major clinical drawbacks investigating BAL samples is the limited availability. BAL has the need of an endoscopic procedure that cannot be repeated arbitrarily. In particular this is a fact in patients with neutropenia, thrombopenia and reduced clinical conditions. Nevertheless, given the fact that cytology or culture of BAL samples are positive in distinctly less than one half of cases, the sensitivity of GM testing may be improved by testing BAL samples [72, 73]. First, Becker et al. reported higher positive results in BAL in 100% compared to serum in 47% of cases of invasive aspergillosis in hematological patients [74]. Further study results supported the practice of galactomannan testing on BAL fluid in suspected IA with acceptable sensitivity rates. A meta-analysis of BAL studies reported estimates of the galactomannan assay with sensitivity and specificity rates of 90% and 94%, respectively, for proven/probable IPA. These studies demonstrated the superior performance of BAL GM over serum GM [75, 76]. This finding was confirmed recently by two other studies performed in well defined groups of hematologic high risk patients [77, 78].

A retrospective analysis of the GM assay performed on BAL samples from high risk hematology patients. Maertens et al. observed that GM detection in BAL may further improve the diagnostic accuracy of the test. BAL GM demonstrated an improved sensitivity profile with an optical density (OD) index cut-off of 1.0, in contrast to the cut-off OD of 0.5 testing from blood samples. A higher detection threshold improved specificity, but impairs the sensitivity rates. In addition, a trend toward decreased sensitivity in patients receiving mold-active prophylaxis was noted [79].

The FDA approved the GM EIA for BAL samples in January 2011 with an GM OD cutoff of 0.5, just the same as for blood samples. Another retrospective study of the Maertens group investigated a mixed population with suspected IA. The aim was to define the clinical significance and the analytical and clinical validity of the detection of GM in BAL samples. Only 20% of patients suffered from hematological malignancies. Data on antifungal prophylaxis remained unreported. There was a low number of proven cases. The authors calculated a GM index of >0.8 as optimal OD index cut-off by a receiver operating characteristic curve (ROC) analysis. At this cut-off, PPV and NPV were 81% and 93.6%, respectively. However, an OD index value of >3.0 corresponded to 100% specificity, thus ruling in the disease. Conversely, an OD cutoff of <0.5 corresponded to a high sensitivity, virtually always ruling the disease out. For all values in between, the (posttest) probability of IPA depends on the prevalence of disease in the particular at-risk population. Therefore the BAL cut-off for GM cannot be clearly defined for mixed population. The test had good diagnostic accuracy. Although, provided results have to be interpreted in parallel with clinical and radiological findings and in relation to pretest probabilities [80].

The role of other factors affecting GM assay performance in BAL samples was investigated by Racil et al. [81]. The authors evaluated the performance of GM assay (BAL) compared to serum samples for the diagnosis of invasive pulmonary aspergillosis in patients with hematological diseases and found the highest (but moderate) sensitivity rate (78.6%) and a good specificity rate (98.6%) with a ‘triple detection’ of GM in bronchial, bronchoalveolar and serum samples. Neutropenia, antifungal therapy, and BAL standardization affected the GM assay performance in this study. This highlighted the open questions in regard of bronchoscopy and BAL in in immunocompromised patients in general. Standards of BAL (sample volume, BAL technique and time schedule) are crucial. Consistent adherence to a standardized BAL protocol may improve laboratory findings, thus potentially facilitating research into the diagnosis and management of lung infiltrates [82].

Combined testing of clinical samples with GM EIA with molecular diagnostic tools seems to be more promising than investigating one single diagnostic biomarker. Trials on this topic have been published some years ago. First, Musher et al. evaluated the performance of the GM EIA and a quantitative PCR assay for the detection of Aspergillus species from BAL samples of patients with hematologic malignancies. The authors found GM indices and DNA quantities corresponding to BAL fungal burdens. They concluded that GM EIA combined with a quantitative Aspergillus PCR assay added to the sensitivity of BAL for diagnosing IPA in high-risk patients, with excellent specificity. The adjunctive use of both tests may reduce dependence on invasive diagnostic procedures [83].

The first prospective, multicenter evaluation of both BAL GM and PCR assays for diagnosing invasive pulmonary aspergillosis used a validated nested Aspergillus PCR assay and a GM EIA. The trial was performed in a strictly defined group of hematological patients. Reinwald et al. prospectively examined BAL samples from hematological patients at high risk of IA. They elucidated the
clinical significance of testing BAL samples both with polymerase chain reaction (PCR) and Aspergillus GM EIA. Via receiver operating characteristic (ROC) analysis of GM optical density (OD) cut-off levels, the authors confirmed a BAL OD of 0.5 to be optimal. Whereas sensitivity and specificity rates for BAL GM were 79% and 96%, respectively, sensitivity and specificity rates for BAL PCR were 59% and 87%, respectively. Combining BAL GM and PCR for diagnosis showed a sensitivity and specificity rate of 55% and 100%, respectively. According to their data, the authors conclude, that positivity for both GM (at a sensitive OD of 0.5) and Aspergillus PCR in BAL makes a pulmonary aspergillosis with high specificity rates highly likely (diagnostic odds ratio > 200), without the need to increase the GM OD threshold at the cost of sensitivity [84].

In conclusion, GM determination in BAL samples is a sensitive and specific test for the diagnosis of proven and probable IA. Despite some drawbacks and open questions, GM from BAL seems to be superior to GM blood sample testing in patients with suspected IA. Further studies focused on diagnostic adjunct for antifungal treatment agents, the threshold value and the clinical significance of the combination of diagnostic tools, in particular molecular assays, are needed.

1→3-β-D Glucan (BG)

Circulating 1→3-β-D glucan (BG) is another diagnostic biomarker. BG derived from fungal cell walls is detected by glucan assays on the basis of its recognition by the innate immune system of horseshoe crabs, specifically Tachypleus tridentatus and Limulus polyphemus. The so-called “G-Test”, established in the 1990s by a Japanese working group, seemed to lack sensitivity and/or specificity and remained unsuitable in the clinical routine, at least in Europe [85]. The novel Limulus amebocyte lysate (LAL) assay and the BG-specific variant Glucatell (FungitellTM, Associates of Cape Cod, East Falmouth, MA, USA) are described in detail in Odabasi et al. [86].

The optimal cut-off value for this test and its efficacy with a multiple-sampling strategy in an immunocompromised population has been established. Ostrosky-Zeichner et al. evaluated the performance of the BG assay as diagnostic adjunct for invasive fungal infection in a single-sample, multicenter validation study [87]. At a cut-off of 60 pg/ml, the sensitivity and specificity rates of the assay were 70% and 87%, respectively, with a PPV of 84% and a NPV of 75%. Reproducible assay results with high specificity rates and a high PPV demonstrated that an assay to detect serum BG derived from fungal cell walls is a useful diagnostic adjunct for IFI. It should be kept in mind, that the test is not species-specific, so that positivity represents the detection of a group of pathogens (Aspergillus, Candida, Fusarium species and Pneumocystis jirovecii). It is unclear, as if (and how) the test is less affected by antifungal therapy. The test shows false positive results. Investigating BG in clinical samples others than blood, particularly in BAL samples, seems to give non-indicative findings. This is due to detection of predominantly colonizing Candida spp in the tracheobronchial system, for example. The (1→3)-β-D-Glucan (BG) assay has been FDA approved for diagnosing invasive fungal disease (IFD), not primarily for IA. Given the lack of pathogen specificity, use of the BG test requires an integrated clinical, radiological and laboratory approach, in any case.

In a recent meta-analysis, including 15 studies, sensitivity and specificity rates were 76% and 85% respectively. Subgroup analyses showed that the BG assay had higher specificities for patients with hematological disorders and a positive BG result with two consecutive (serum) samples. The combination of GM and BG increased the specificity rate to 98% for diagnosing invasive aspergillosis [88]. Data on BG investigating BAL samples for suspected IA are scarce and crude, up to now.

In conclusion, serum BG determination is clinically useful for diagnosing IFD in at-risk patients, especially for hematology patients. The combination of galactomannan and BG was helpful for diagnosing invasive aspergillosis. Since the BG assay is not absolutely sensitive and specific for IFD, the BG results should be interpreted in parallel with clinical findings, particularly when IA is suspected.

The definition and detection of other antigens as diagnostic markers for IA are ongoing, suggesting that some recombinant antigens may have a potential in the serodiagnosis of aspergillosis [89].

In summary, for diagnosis of IA, serological testing (GM and/or BG) should be performed and assessed in combination with imaging tools. These include in particular chest computed tomography scans, and if available, well validated molecular diagnostic tools (Fig. 2).

Molecular Diagnostic Assays (PCR)

As serological assays remained disappointing for a long time, research since the mid-nineties was focused on molecular methods to detect fungal pathogens, especially Aspergillus species, early, specifically, reliably and sensitively. PCR assays are helpful in diagnosing infectious diseases detecting genomic DNA of pathogens difficult to culture in clinical samples. Molecular diagnostics of Aspergillus species attempt to cover a wide range of diagnostic problems mainly to characterize the pathogen earlier in the clinical course of an invasive disease than culture or histopathology. A PCR-mediated detection of the pathogen from blood or other clinical samples of high-risk patients and the identification of the pathogen in tissue specimens is possible. The proof of the pathogen for epidemiological, ecological and typing questions remains a problem to be solved.

PCR-based detection methods for fungi have been developed successfully since parts of the fungal genome, especially multicopy gene targets, were identified and sequenced. Early studies to evaluate PCR-mediated detection of fungi showed significantly improved sensitivity, compared with other diagnostic tools at that time. These fungal PCRs were performed with methodically different assays and different objectives, partly to optimize culture assays, partly for typing in epidemiological studies, often in experimental and non-clinical settings. For epidemiological studies, the identification and typing of the pathogen from culture or environment samples, more and more assays using different PCR techniques or other molecular approaches were published. At present, a “molecular gold standard assay”, addressing these questions, has not been defined. First studies in this regard have been published recently [90-92].

The exact place value of the PCR method detecting fungi, especially Aspergillus species, is still unclear. Many questions are unanswered up to now. This concerns e.g. the course of Aspergillus DNAemia, optimal clinical samples to be tested, time points and standardization of PCR. Further challenges include comparability from in-house-assays or commercially available assays. Nevertheless, the use of non-invasive molecular markers to detect the pathogen from blood and other clinical samples is attractive.

Since the early 1990’s, methods for detecting fungi-specific DNA by means of hybridization and amplification of nucleic acids have been developed to detect fungal DNA in clinical specimen. The primary attention was paid to the detection of moulds. In particular, this was done with Aspergillus spp. in the last decade. Aspergillus spp. are more difficult to isolate from patient samples compared to Candida spp. by conventional methods [93, 94]. Two approaches have been studied in most reports. Looking for genus-specific genomic sequences as well as single copy genes or looking for multiple copy genes which could be detected in almost all fungal species. Primers from either the 18S-rRNA subunit gene, the 28S-rRNA gene or mitochondrial genes have been studied as a so-
called “Panfungus PCR”. The “Panfungus PCR” is followed by hybridization with species-specific probes to gain adequate specificity. A “Panfungus PCR” using a sequence from the 18ssu-rRNA gene followed by species-specific hybridization with probes for Candida species as well as Aspergillus species has been studied most extensively in several clinical studies (blood and BAL) in hematological patients, first by Einsele and his group [95, 96].

The specificity is around 65-75% depending on the number of tests needed to establish the diagnosis with a sensitivity of 100%. The species-specific approach using a nested PCR (two-step PCR) with primers derived from a variable region of the 18S-rRNA gene which is highly conserved among Aspergillus species have been studied in BAL and blood as well [97].

Other assays successfully used the real-time PCR technology, (e.g. LightCycler™, TaqMan™). Both PCR-assays combine a fast in vitro amplification of DNA with immediate detection of the amplicon. Rapid quantification of the DNA amount of the pathogen can be achieved, beside the detection of the pathogen. The sensitive and specific quantification of the fungal burden seems to be of clinical relevance, since the assessment of the individual fungal burden may allow therapeutical monitoring. Data on this topic are scarce, however, up to now [98].

Concerning PCR based investigations of blood samples, Mengoli et al. published a systematic review and meta-analysis on the use of PCR tests for the diagnosis of IA in 2009. Numerous Aspergillus PCR assays had been developed and evaluated in the early years of the last decade. These Aspergillus PCRs are methodological more or less accurate. The meta-analysis encompassed just 16 studies that had investigated blood samples and evaluated both screening and diagnostic studies, despite different methodological attempts. Sensitivity and specificity rates of PCR for two consecutive positive blood samples were found to be 75% and 87% [99]. However, this meta-analysis did not take into account the revolution of antifungal therapy with novel drugs a few years ago. There has been a change in clinical concepts in treating patients earlier with less toxic and/or more effective antifungals such as newer azoles or echinocandins. Furthermore potent antifungal prophylaxis with posaconazole for patients at highest risk for IA reduces also the pathogen load to detect. PCR sensitivity rates investigating blood samples dropped down clearly due the change in clinical concepts. In contrast, the pathogen load in BAL samples, originating from the site of the infection, remained higher in relation to blood samples. The diagnostic accuracy of BAL remained high, even under antifungal therapy, when investigating BAL samples. In the study of Lass-Flörl et al., the sensitivity values of PCR in lung and blood samples for patients with proven infection were 100% and 40%, respectively. The negative predictive value of blood monitoring under conditions of antifungal treatment was 44%. For patients with probable infection the sensitivity values of PCR in lung fluid and blood were 66% and 44%, respectively. The authors recommended, given the disappointing results for blood PCR results under antifungal treatment, to investigate BAL or even tissue samples with PCR technology, in addition to microscopic examination and culture technique for a sensitive detection of fungal infection. Tissue based diagnostic tools confirm a suspected IA diagnosis, but at a later stage of the disease, rather than contributing to an early diagnosis [100].

Similar findings were shown in another study, where samples in a subset of hematologic patients were evaluated. Patients within the AmBiload trial were investigated prospectively by a nested Aspergillus PCR assay. The aim was to re-evaluate the significance of PCR in this setting. A median of four samples per patients were investigated. Seventy percent of samples investigated by PCR were obtained during treatment with antifungal study medication. The overall PCR sensitivity rate was only 43%. Patients with an unfavorable outcome had a significantly higher rate of positive PCR (48% vs 21%). The authors concluded that the sensitivity of Aspergillus PCR is limited during antifungal therapy. The tendency for persistently positive PCR results may indicate a poor prognosis [101].

The investigation of the clinical significance of Aspergillus PCR assays is focussed nowadays on BAL samples. In a systematic

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**Fig. (2).** Diagnostic-driven management of invasive pulmonary aspergillosis.
literature review on the diagnosis of invasive aspergillosis using PCR from bronchoalveolar lavage clinical samples, Tuon showed overall sensitivity and specificity values of PCR-based techniques of 79% and 94%, respectively. Primary contamination of the clinical sample or even in the laboratory, the primer design and the PCR methodology were important variables that could hamper the interpretation of these test assays [102].

The first studies based on the concept of combining diagnostic assays were done to improve the clinical validity of PCR based molecular tools to diagnose IA in high risk patients with hematologic malignancies. Mushan et al. evaluated the performance of the GM EIA and a quantitative PCR assay for the detection of Aspergillus species from BAL samples in a small number of patients with hematologic malignancies. The authors found GM indices and DNA quantities corresponding to BAL fungal burdens. They concluded, that GM combined with a quantitative Aspergillus PCR assay added to the sensitivity of BAL (61% and 67%) for diagnosing IPA in high-risk patients. The adjunctive use of both tests may reduce dependence on invasive diagnostic procedures [103].

A serious drawback of GM and PCR assays is that both are affected by concomitant antifungal prophylaxis and treatment. Although a number of studies for GM have been published, some studies showed a trend toward better performance in patients receiving antifungal therapy and prophylaxis, while others showed the opposite. A recent meta-analysis suggests a reduced sensitivity for GM in patients receiving antifungals prior to BAL sampling [77].

The effect of antifungal therapy and prophylaxis on the results of PCR testing (at least in blood samples) is an ongoing matter of debate [104]. Reinwald et al. recently evaluated the effect of antifungal treatment on the performance of an Aspergillus-specific PCR assay in bronchoalveolar lavage (BAL) samples. They found that the sensitivity of BAL PCR for patients without antifungal treatment prior to BAL sampling was 69% and the specificity was 87%. No significant change in diagnostic performance by the therapy with one antifungal was observed, while patients receiving two or more antifungals prior to BAL sampling showed a significant decrease in the diagnostic performance of BAL PCR testing (P<0.009). These findings highlight the need to perform diagnostic bronchoscopy and BAL investigation as early as possible in the diagnostic schedule and work-up of newly arisen lung infiltrates.

The diagnostic information of Aspergillus PCR from BAL under intensive treatment with antifungals is not promising [105].

PCR data from the investigation of other clinical samples are preliminary. There are promising data in patients with proven invasive mold infection. PCR assays may offer a plausible etiologic primer design and the most reliable method to detect amplicons. Comparative interlaboratory studies of different assays had been performed to harmonize, optimize and standardize assays to provide one or more “gold standard” PCR methods. This was underscored by a recently published meta-analysis. Sun et al. stated PCR assay on BAL is highly accurate for diagnosing IA in immunocompromised patients. However, a standard protocol is needed to enable formal validation of BAL-PCR [108]. This answer and other important questions remain open [94, 109, 110].

Concluding our current knowledge testing BAL samples provide higher sensitivity and specificity rates than testing blood samples. Investigating blood samples may be helpful in screening hematology patients at risk without antifungal therapy. Data on PCR (alone) based starting of antifungal therapy are not convincing. The intention to detect more than one fungal pathogen in one assay (“multifungal/panfungal PCR”) encompasses additional methodical and technical problems and seems to be less suitable to diagnose IA. Efforts to improve diagnostic and to facilitate the establishment of a valid and reliable diagnosis in IA remain an unmet need, to improve the antifungal treatment and, at last, the outcome in IA high-risk patients.

DRUGS FOR TREATMENT OF INVASIVE ASPERGILLOSIS

Amphotericin B (AmB)

Amphotericin B deoxycholate (cAmB) is fungistatic or fungicidal depending on the concentration obtained in body fluids and the susceptibility of the fungus. The drug acts by binding to sterols in the cell membrane of susceptible fungi with a resultant change in membrane permeability allowing leakage of intracellular components. An initial intravenous test infusion is recommended. The dose should be gradually increased to 0.4 and 0.6 mg/kg up to 1 mg/kg daily. AmB circulating in plasma is highly bound (>90%) to plasma proteins and is poorly dialyzable. Approximately two thirds of concurrent plasma concentrations have been detected in fluids from inflamed pleura, peritoneum, and synovium. Concentrations in the cerebrospinal fluid seldom exceed 2.5% of those in the plasma. Little cAmB penetrates into vitreous humor or normal amniotic fluid. Complete details of tissue distribution are not known. Amphotericin B is excreted very slowly (over weeks to months). Amphotericin B shows a high order of in vitro activity against many species of fungi including Aspergillus spp beside Aspergillus terreus.

The administration of cAmB is associated with acute reactions including fever, shaking chills, hypotension, anorexia, nausea, vomiting, headache, and tachypnea which are common 1 to 3 hours after starting an intravenous infusion. These reactions are usually more severe with the first few doses of amphotericin B and usually diminish with subsequent doses. Rapid intravenous infusion has been associated with hypotension, hypokalemia, arrhythmias, and shock and should, therefore, be avoided.

Despite being associated with significant therapy-related toxicity, AmB is an important cornerstone for the treatment of IA, in particular in developing countries. The main disadvantage of cAmB is nephrotoxicity. Increasing number of publications reported long-term nephrotoxicity with cAmB interfering with later nephrotoxic medications especially in SCT patients. These data showed reduced survival rates after SCT [111]. Continuous infusion of cAmB might be better tolerated than administration over 2-6 hours. In a small trial a >2-fold decrease in creatine clearance was observed with continuous cAmB infusion in 5 of 28 patients only. The decrease was dose limiting in only 1 patient. No dialysis was reported [112]. Continuous infusion of cAmB has not been compared head to head with lipid AmB formulations or newer azoles for efficacy in patients with invasive aspergillosis.
Amphotericin B Lipid Formulations

Compared to CAmB, the lipid formulations offered similar efficacy and significantly better tolerability. The optimal dosage of lipid-based amphotericin B is a matter of debate. A phase I-II study evaluated the safety, tolerance, and plasma pharmacokinetics of liposomal amphotericin B (L-AMB; AmBisome) in order to determine its maximally tolerated dosage (MTD). Patients with infections due to Aspergillus spp. and other filamentous fungi were included. Dosage cohorts consisted of 7.5, 10.0, 12.5, and 15.0 mg/kg of body weight/day. A total of 44 patients were enrolled, of which 21 had a proven or probable infection (13 aspergillosis, 5 zygomycosis, 3 fusariosis). The MTD of L-AMB was 15 mg/kg/day [113]. The data provided a scientific backbone for the study of an initial high-dose treatment compared to a lower dose of liposomal amphotericin B. The AmbiBlast trial proved however a better efficacy and safety of 3 mg/kg versus 10 mg/kg of liposomal amphotericin B for induction treatment of patients with IA [45]. The 12-week survival was better for the 3 mg/kg treatment arm. Significantly higher rates of nephrotoxicity and hypokalemia were seen in the high-dose group with 10 mg/kg/d.

AmB lipid formulations have been studied preferably for second-line therapy of IA. Liposomal amphotericin B (L-AmB) and amphotericin B lipid complex (ABLC) are licensed for this clinical scenario. There are large databases for ABLC (CLEAR/Collaborative Exchange of Antifungal Research), which showed a 31% overall and 21% response in patients with GVHD after allogeneic stem cell transplantation. The overall response to first line treatment was 41% [114]. In a randomized study L-AmB resulted in equal antifungal efficacy compared to CAmB [115], but the study was not restricted to aspergillosis as well as the second-line use studies. The efficacy of L-AmB versus ABLC has been compared by an analysis of 8 open label studies with more than 1000 patients resulting in a response of 61% vs. 46% [116]. Studies showed that ABLC has more infusional-related reactions on day 1. After day 1, infusional reactions were less frequent with ABLC, but chills/rivers were still higher (21.0% and 24.3% vs. 50.7%; P<0.001). Less nephrotoxicity was observed with 3 and 5 mg/kg L-AmB compared with ABLC (14.1% and 14.8% vs. 42.3%; P=0.01) [117]. Results of randomized studies comparing AmB lipid formulations to azoles or echinocandins in the treatment of aspergillosis have not yet been published. Therefore liposomal amphotericin B as primary therapy with the conventional dosage of 3 mg/kg is the preferred treatment in the first line of IA when an amphotericin B formulation is used. Lipid based AmB may be also used as second-line treatment of IA. Interactions have been reported rarely when AmB is used for treatment.

AZOLES

Itraconazole

Itraconazole is a synthetic triazole antifungal agent. Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (two enantiomeric pairs), each possessing three chiral centers. Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (two enantiomers), each possessing three chiral centers. Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (two enantiomers), each possessing three chiral centers. Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (two enantiomers), each possessing three chiral centers.
of fluconazole-resistant serious invasive Candida infections (including C. krusei), and of serious fungal infections caused by Seesdorrium spp. and Fusarium spp. Voriconazole is p.o. available in two tablet strengths, 50 mg and 200 mg, and liquid powder for solution (40mg/ml). Voriconazole is also presented as a lyophilised sterile powder for solution for infusion. The intravenous formulation of voriconazole contains a novel excipient, sulphobutylether betacyclodextrin sodium (SBECD), used to solubilise voriconazole.

Absorption of voriconazole from the gastrointestinal tract is likely to be high in all species since the apparent oral bioavailability is >75% in all species. Voriconazole penetrates the CNS and CSF well. Voriconazole is eliminated predominantly by metabolism. Metabolites were found not to show any pharmacological activity. Voriconazole is metabolized by CYP2C9, CYP2C19 and CYP3A4. The potential of voriconazole to interact with concomitant medications has led to the instigation of an extensive clinical pharmacology drug interaction program. After single oral dosing of voriconazole, AUC and Cmax increased not dose proportionally over the studied dose range of 100 to 400 mg. Saturation of metabolism is the main responsible factor. Voriconazole was shown to competitively inhibit CYP2C9, CYP2C19 and CYP3A4. The major primary route of metabolism of voriconazole involved fluoropyrimidine N-oxidation to form UK-121, 265 (N-oxide-voriconazole). The CYP2C19 isoenzyme exhibits genetic polymorphism, which in essence divides the population into poor and extensive metabolizer. The extensive metabolizers are further divided into homozygous and heterozygous populations. It has been shown in vitro using specific inhibitors and liver microsomes from poor metabolizer that in the absence of CYP2C9 there is an increased role for CYP3A4 in the metabolism of voriconazole. In poor metabolizers it appeared that Cmax is about 3 fold higher and the AUC about 2 – 5 fold higher compared to extensive metabolizers. After IV and oral administration of radioactive labelled voriconazole 80% and 84% of the dose could be recovered in urine, respectively, and in faeces, 24% and 22%, respectively. The pharmacokinetics of voriconazole was not affected by mild to severe impaired renal function. A moderate hepatic insufficiency resulted in an increase in the systemic exposure necessitating a 50% reduction of the maintenance dose of voriconazole. Patients with severe hepatic insufficiency have not been studied.

Abnormal vision, and headache in the voriconazole treated were reported common adverse events (AE). Abnormal vision was the most frequently reported treated related AE for voriconazole with a rate of 21% to 33%. Vision abnormality is transient and fully reversible [184].

The interaction potential of voriconazole is extensively studied. No significant effect of voriconazole on the pharmacokinetics of indinavir, prednisolone, and digoxin was observed, whereas the AUC of phenytoin, omeprazole, cyclosporin, warfarin and tacrolimus increased due to concomitant intake with voriconazole. The pharmacokinetics of voriconazole is not clinically affected by cimetidine, ranitidine, indinavir, erythromycin, azithromycin and prednisolone, whereas the AUC of voriconazole was decreased due to the concomitant intake with rifampicin, rifabutin, and phenytoin. There was no dose response relationship between voriconazole plasma concentrations (or derived parameter) and efficacy in terms of successful biological response or treatment outcome as determined from available PK-PD data. Indeed successful outcomes were observed for concentrations ranging between 1.0 mg/L and 5 mg/L at the end of treatment.

Trough levels of voriconazole are ongoing a matter of debate. Increased toxicity has been reported in patients with voriconazole levels > 5.5 mg/L (neurological toxicity, skin toxicity), while a lower efficacy was observed with voriconazole plasma levels of <1 mg/L [119-121]. For the most frequent treatment related adverse events such as abnormal vision, fever, rash, and nausea, severe effects occurred in < 1% of the patients. The impact of trough levels of voriconazole for treatment of IA may be overestimated with respect to toxicity, as recently published. The physician can manage important factors that influence trough levels of voriconazole (s. Table 5) [122].

**Posaconazole**

Posaconazole is a broad-spectrum antifungal agent of the triazole class and is available as an oral suspension containing 40 mg/ml posaconazole to be administered at a dose of 400 mg twice a day with a meal or with 240 ml of a nutritional supplement. In patients who cannot tolerate a meal or a nutritional supplement, posaconazole can be administered at a dose of 200 mg four times a day. The approved indication is for use in the treatment of invasive aspergillosis in patients with disease that is refractory to amphotericin B or itraconazole or in patients who are intolerant of these medication products. Furthermore posaconazole is approved for Fusariosis in patients refractory to amphotericin B or in patients who are intolerant of amphotericin B, chromoblastomycosis and mycetoma in patients with disease that is refractory to itraconazole or in patients who are intolerant of itraconazole and coccidioidomycosis in patients with disease that is refractory to amphotericin B, itraconazole or fluconazole or in patients who are intolerant of these medication. Posaconazole received approval for prophylaxis of invasive Aspergillus and Candida infections in patients 13 years and older who are at high risk of developing these infections due to being severely immunocompromised.

Posaconazole is slowly absorbed and slowly eliminated. Bioavailability of posaconazole following oral administration exhibited a very high food effect. Exposure to posaconazole increased with the dose but in a less than proportional manner. A fatty meal provides an ideal oily solvent to solubilize posaconazole into an absorbable form. High fat meals also prolong gastric residence times, increasing the amount of the drug mixing with the contents of the stomach and thus the time to absorb the drug.

In vitro human microsomal enzyme studies showed that posaconazole is an inhibitor of CYP3A4. However, there is no induction of any CYP450 enzymes and no inhibition of CYP1A2, CYP2A6, CYP2C9, CYP2C19, or CYP2D6. Contraindications are the coadministration of sirolimus, CYP3A4 substrates (pimozide, quinidine), simvastatin and ergot alkaloids. Posaconazole increases the concentrations of cyclosporin and tacrolimus. Monitoring of tacrolimus and cyclosporin concentrations are recommended frequently. While adverse reactions to posaconazole are low, there is no result of a first-line trial of posaconazole against invasive aspergillosis.

**Isavuconazole**

Isavuconazole is a new triazole currently undergoing phase III clinical trials. The drug has been investigated as oral and intravenous formulation. Based on AUC values after oral and intravenous administration, an excellent oral bioavailability can be predicted for isavuconazole. Maximum plasma concentrations of isavuconazole were observed 1.5 to 3 h after p.o. drug intake or at the end of the 1-h infusion [123, 124]. Isavuconazole has shown in vitro activity against a large number of clinically important fungal pathogens including Aspergillus spp., Fusarium spp., Scedosporium spp., Candida spp., Zygomyces and Cryptococcus spp. The MICs of isavuconazole was determined against isolates of Aspergillus species and Zygomyces according to the methodology of the EUCAST. The in vitro activity of isavuconazole was found to be comparable to that of voriconazole aside from Mucorales [125, 126]. Similar to voriconazole, reduced in vitro activity is seen against Histoplasma capsulatum. Phase III trials in invasive aspergillosis and Candida infections are recruiting patients. First results are expected late 2013.

**Echinocandins**

Echinocandins are a new class of antifungals. They are significant new tools for our antifungal management. To date, three echi-
nocandins (anidulafungin, caspofungin, and micafungin) have been approved for the treatment of fungal infections. But only caspofungin is approved for 2nd-line treatment of IA. Micafungin is approved for 1st and 2nd-line treatment of IA in Japan only. Echinocandins target the fungal cell wall and have a similar chemical structure. Clinical studies provided sufficient evidence for efficacy against Candida species in the first-line setting against amphotericin B derivatives or fluconazole [127-130]. The echinocandins showed significantly less toxicity when compared with amphotericin B desoxycholate as well as liposomal amphotericin B [131]. At present the maximum tolerated dose of echinocandins is undefined. While toxicity of echinocandins is favorable for all approved echinocandins, there are preclinical data of micafungin that showed development of benign liver tumors in rats treated with extremely high doses of the drug. Micafungin doses in neutropenic patients have been investigated in a trial with escalation dosages from 2 mg/kg up to 8 mg/kg/d, with no grade 3 and 4 toxicity. SCT patients received up to 560 mg micafungin per day [132]. No cases of liver tumors have been reported after use of micafungin in humans. Safety data suggest that micafungin can be administered safely to patients with liver or kidney dysfunction without adjusting the dose [133, 134]. Little is known about the optimal prophylactic dosage and the disposition of micafungin in liver transplant recipients. Small series (n=6) observed a therapeutic drug level by administration of micafungin at a dosage of 40-50 mg/d in liver transplant patients [135]. At present however, current data are insufficient to support the use of micafungin in patients with severe hepatic impairment [EMEA – EPAR Product Information]. The pharmacokinetics of micafungin was studied in 13 critically ill patients receiving continuous hemofiltration (CHDF). There was no progressive accumulation or exclusion of micafungin in patients receiving CHDF. Therefore it is not necessary to adjust the micafungin dose in patients receiving CHDF [136].

Anidulafungin does not require dosage adjustment in patients with renal and/or hepatic failure. This differs from treatment recommendations for caspofungin. At present there is no approval for treatment of IA for anidulafungin.

In adult patients with mild and moderate hepatic impairment, the AUC of Caspofungin is increased about 20% and 75 %, respectively. For caspofungin administered in adult patients with mild hepatic impairment (Child-Pugh score 5 to 6), no dosage adjustment is needed. For adult patients with moderate hepatic impairment (Child-Pugh score 7 to 9), caspofungin 35 mg daily is recommended based upon pharmacokinetic data. Recently, acute renal failure has been reported in recipients of liver transplant while on caspofungin [137].

The value of caspofungin, micafungin and anidulafungin remains to be investigated in randomized clinical trials in comparison with voriconazole or liposomal amphotericin B. The efficacy of echinocandins for the first-line treatment seems questionable at present.

Prophylaxis of Invasive Aspergillosis

The complexity and high cost of therapy and most of all the high case fatality rate of invasive aspergillosis are reasons for the prophylactic approach. A number of comparative studies on the prophylactic use of various antifungal agents in hematology and oncology have been published in recent years. In contrast to patients with solid tumors the incidence of invasive fungal infection is substantially higher in association with hematological malignancies. The wide range in reported incidences (5%-24%) is partly due to a lack of uniform definitions from trials before the year 2000 [44].

Fluconazole is the most extensively studied azole. Daily doses ranging from 50-400mg p.o. have been used in comparative studies. Currently, there is clear evidence that fluconazole prophylaxis is of proven benefit for primary prophylaxis of invasive fungal infections at a daily dose of 400mg in allogeneic bone marrow or hema-topoietic stem cell transplant recipients. But fluconazole (400mg/day) has no activity against Aspergillus spp.

Itraconazole has been studied mainly in AML and SCT patients. Oral itraconazole suspension was studied in a double-blind placebo controlled trial. The dosage regimen was 2.5 mg/kg bid. All patients additionally received nystatin 500 000 IU qid. The itraconazole arm was superior to the placebo arm in terms of reducing the rate of fatal candidemia (1.96% versus 0%). Effective prophylaxis against moulds was not documented [138]. An open-label analysis of high-risk patients suggested that itraconazole oral suspension 100mg bid was superior to polyenes [139]. A randomized trial included allogeneic bone marrow transplant recipients to receive either 400mg itraconazole (intravenous and oral) or 400mg fluconazole (intravenous and oral). Proven invasive fungal infections occurred in 6 of 71 itraconazole recipients (9%) and in 17 of 67 fluconazole recipients (25%). Results suggest itraconazole prophylaxis confers an advantage in terms of incidence of documented invasive fungal infections caused by either yeasts or molds, but was to small to draw a final conclusion for efficacy against IA [140].

A meta-analysis concludes that itraconazole prophylaxis effectively reduces the incidence of invasive Aspergillus infections. But this was only reduced in trials using the itraconazole cycloextrin solution and not itraconazole capsules. The overall mortality was not changed [141]. Itraconazole should be used for the prophylaxis of invasive fungal infections only if plasma level monitoring is conducted at least twice a week for control purposes and only if levels >500mg/mL of itraconazole are reached within a few days [142]. Through levels of the active hydroxy metabolite can be performed by HPLC and provides complementary data in addition to itraconazole levels. The bioassay measurement overestimates itraconazole concentrations (3-5 times compared to HPLC) because of the presence of the hydroxyitraconazole metabolite. Excellent correlation can be achieved between bioassay and HPLC if hydroxyitraconazole is used as the standard in the bioassy [143].

In addition, numerous interactions of several drugs with itraconazole due to a P450-3A4 metabolism are well known. The most common inducers of itraconazole metabolism are antiepileptics (phenytoin, carbamazepin, and phenobarbital) and tuberculosstatics (isoniazid, rifampin, rifabutin).

Voriconazole for Prophylaxis

A prospective, randomized, open-label, multicentre study compared the efficacy and safety of voriconazole (234 patients) versus itraconazole (255 patients) in alloSCT recipients. The primary composite endpoint, success of prophylaxis, incorporated ability to tolerate study drug for > 100 d (with ≤ 14 d interruption) with survival to day 180 without proven/probable IFI. Success of prophylaxis was significantly higher with voriconazole than itraconazole (48.7% vs. 33.2%, P < 0.01); more voriconazole patients tolerated prophylaxis for 100 d (53.6% vs. 39.0%, P < 0.01; median total duration 96 vs. 68 d). More voriconazole patients received other systemic antifungals (41.9% vs. 29.9%, P < 0.01). There was no difference in incidence of proven/probable IFI (1.3% vs. 2.1%) or survival to day 180 (81.9% vs. 80.9%) for voriconazole and itraconazole respectively. Voriconazole was superior to itraconazole as antifungal prophylaxis, based on differences in the primary composite endpoint. Voriconazole could be given for significantly longer durations, with less need for other systemic antifungals [144].

Voriconazole was evaluated in a randomized, double blind trial that compared fluconazole (n = 295) versus voriconazole (n = 305) for the prevention of IFI. Patients undergoing myeloablative allogeneic SCT were randomized before SCT to receive study drugs for 100 days, or for 180 days in higher-risk patients. There were trends to fewer IFIs (7.3% vs. 11.2%; P = 0.12), in particular Aspergillus infections (9 vs 17; P = 0.09) with voriconazole. Fungal free survival (FFS) rates at 180 days and 6-month overall survival did not differ [145].
A small German trial compared double blind voriconazole prophylaxis to placebo in AML patients. The trial included 25 patients and was prematurely closed when the posaconazole data came out. Adverse events were not different between voriconazole and placebo [8].

**Posaconazole** has been compared to fluconazole 400 mg/d or itraconazole 400 mg/d in a randomized, open-label clinical trial in patients undergoing induction chemotherapy for acute myelogenous leukemia or myelodysplastic syndrome. At a dose of 600 mg/d posaconazole resulted in a significant reduction in proven and probable invasive fungal infections. Significantly fewer patients in the posaconazole group had invasive aspergillosis (2 [1%] vs 20 [7%], \( P<0.001 \)) [5]. In another trial, allogeneic hematopoietic stem cell recipients with severe graft versus host disease (GVHD) were randomly allocated to receive posaconazole 600 mg/d or fluconazole 400 mg/d in a double-blinded fashion. In the posaconazole group, as compared with the fluconazole group, there were fewer breakthrough invasive fungal infections (2.4% vs 7.6%, \( P=0.004 \)), particularly invasive aspergillosis (1.0% vs 5.9%, \( P=0.001 \)). Posaconazole was found to be as safe and tolerated as fluconazole. The incidence of treatment-related adverse events was similar in the two groups (36% in the posaconazole group and 38% in the fluconazole group), and the rates of treatment-related serious adverse events were 13% and 10%, respectively [146]. Posaconazole prophylaxis is therefore recommended for high-risk patients with AML and MDS as well as for patients with GVHD after allo SCT. At present, posaconazole is only available as an oral formulation, with varying absorption from the gastrointestinal tract. Breakthrough infections have been associated with lower plasma concentrations [147, 148].

**Amphotericin B (i.v.) for prophylaxis.** Amphotericin B-desoxycholate, and lipid formulations of amphotericin B. Smaller trials suggested feasibility of low-dose cAmB for prophylaxis of IFI [149]. A prospective randomized study compared the efficacy of fluconazole versus low-dose cAmB in preventing fungal infections during the first 100 days after SCT. Patients undergoing allogeneic or autologous SCT and were randomized to receive fluconazole 200 mg/day p.o. or amphotericin B 0.2 mg/kg/day i.v. starting 1 day prior to commencement of conditioning regimen and continuing until engraftment, drug-associated toxicity was suspected, or systemic fungal infection was suspected or proven. A total of 186 patients were enrolled into the trial, with 100 receiving fluconazole and 86 receiving cAmB. Eighty (43%) patients were removed from prophylaxis for persistent fever despite broad-spectrum antibacterial therapy or suspected fungal infections (Fluc 46 vs AmB 34, \( P >0.05 \)). The incidence of proven fungal infections (Fluc 12% vs AmB 12.8%), suspected fungal infections (Fluc 4% vs AmB 2.3%), superficial fungal infections (Fluc 1% vs AmB 4.6%) did not show any significant difference. The survival at 100 days post transplant was similar between the two groups (Fluc 78% vs AmB 70%, \( P =0.254 \)). Death attributable to fungal infections was similar in both groups (6% vs 7%, \( P >0.05 \)) [150]. The authors concluded that fluconazole was as effective as amphotericin B but less toxic.

**Liposomal Amphotericin B.** Preclinical data suggested that intermittent application of low-dose liposomal Amphotericin B (L-AmB) might be effective for antifungal prophylaxis. A randomized, open-label trial evaluated the efficacy of low-dose liposomal amphotericin B for reduction of invasive fungal infections in patients with hematological malignancies and prolonged neutropenia (>10 days) following intensive chemotherapy. A total of 219 neutropenic episodes of 132 patients randomization was performed. Patients received either 50 mg L-AmB every other day (arm A) or no systemic antifungal prophylaxis (arm B). The incidence of proven or probable IFI, which was the primary end point, was five of 75 patients (6.7%) in arm A and 20 of 57 patients (35%) in arm B (\( P=0.001 \)). Invasive aspergillosis occurred less frequently in patients receiving L-AmB-prophylaxis (\( P=0.0057 \)). Adverse events, possibly related to L-AmB, were observed in five neutropenic episodes (4.6%) and L-AmB was discontinued in three episodes (2.8%). No grade 3 or 4 toxicities were observed. Thus anti-Aspergillus prophylaxis with low-dose L-AmB proved to be feasible and effective [151].

**Aerosolized Amphotericin B.** As IA infections are usually acquired via inhalation of Aspergillus conidia, prevention of invasive pulmonary aspergillosis consists mainly of the reduction of environmental exposure to Aspergillus conidia. Inhalations with amphotericin B aerosols significantly delayed mortality in animal models of invasive pulmonary aspergillosis. High pulmonary concentrations of amphotericin B could be achieved. Aerosolization of amphotericin B is possible with various nebulizers [152]. Pharmacokinetics showed high concentrations of the AmB drug in the lungs with undetectable amounts in the kidneys, spleen, liver, and brain [153]. Conventional amphotericin B is less efficiently nebulized than liposomal amphotericin B, but similar measured aerosol concentrations reached the lungs, although the lipid remained associated with the drug after nebulization [154]. The physiological side effects of aerosolized amphotericin B have been bronchospasm, especially in asthmatic patients, nausea and vomiting.

Most studies with inhaled amphotericin B focused on prophylaxis. A randomized multicenter trial observed no benefit to a control in a high-risk patient population with an expected duration of neutropenia >10 days. The incidence of proven, probable, or possible IA infections was 10 of 227 (4%) in patients who received prophylactic aerosolized AmB. This did not differ significantly from the 11 of 155 (7%) incident in patients who received no inhalation prophylaxis (\( P=0.37 \)). Moreover, no differences in the overall mortality (13% vs 10%; \( P=0.37 \)) or in the infection-related mortality (8% vs 7%; \( P=0.79 \)) were found [155]. The feasibility, tolerability, and outcomes of aerosolized liposomal AmB prophylaxis were investigated in several trials, mostly patients having received lung transplantation. These trials showed feasibility of inhalation of nebulized liposomal amphotericin B.

A total of 271 patients were studied in a placebo-controlled trial including patients with expected neutropenia for >10 days randomized to receive liposomal amphotericin B or placebo inhalation twice a week, using an adaptive aerosol delivery system, until neutrophil counts increased to >300/mm³. The primary end point was the occurrence of pulmonary IA. In the intent-to-treat analysis, 18 of 132 patients in the placebo group developed IPA versus 6 of 139 patients in the liposomal amphotericin B group (\( P=0.005 \)). According to the on-treatment analysis, 13 of 97 patients receiving placebo versus 2 of 91 receiving liposomal amphotericin B developed IA (\( P=0.007 \)). Some adverse effects, but none serious, in the liposomal amphotericin B group were reported, most frequently coughing (16 patients vs 1 patient) [156]. Aerosolized lipid-based amphotericin B has been investigated in the organ setting and was well tolerated in patients after lung transplantation [157-159].

**Empirical Treatment**

There is evidence that some patients with prolonged neutropenia and fever not responding to antibacterial agents are at sufficient risk of developing invasive aspergillosis. Early data from the 1980s suggested an advantage of empirical antifungal treatment versus no treatment. These data warranted empirical antifungal therapy, although superiority of an antifungal agent over placebo has not been conclusively demonstrated [160]. Amphotericin B desoxycholate, liposomal amphotericin B, caspofungin and intravenous itraconazole followed by oral itraconazole solution are licensed in for this indication. In addition, fluconazole and voriconazole proved favorable results in clinical trials of patients with low and high risk of fungal infections, respectively [161, 162] (see Table 4).
Invasive Aspergillosis

Treatment of all episodes with persistent febrile neutropenia exposes more patients to adverse and potential toxic events of antifungal treatment. With the introduction of GM antigen a preemptive strategy was studied in a randomized clinical trial including 293 patients. Empirical treatment was defined as treatment of patients with persistent or recurrent fever. Preemptive treatment was defined as treatment of patients who have clinical, imaging, or GM assay evidence suggesting fungal disease. The study used amphotericin B deoxycholate (1 mg/kg/day) or liposomal amphotericin (3 mg/kg/day), depending on daily renal function. Efficacy outcome was defined by the proportion of patients alive at 14 days after recovery from neutropenia. By intention-to-treat analysis, survival was 97.3% with empirical treatment and 95.1% with preemptive treatment. The lower 95% confidence limit for the difference in mortality was -5.9%, which was within the noninferiority margin of -8%. Probable or proven invasive fungal infections were more common among patients who received preemptive treatment than among patients who received empirical treatment (13 of 143 vs. 4 of 150; P < 0.05), and most infections occurred during induction therapy (12 of 73 patients in the preemptive treatment group vs. 3 of 78 patients in the empirical treatment group were infected during induction therapy; P < 0.01). Baseline invasive aspergillosis infections were 2 in the empirical arm versus 6 in the preemptive treatment arm, while breakthrough invasive aspergillosis were 2 in both arms. Preemptive treatment did not decrease nephrotoxicity but decreased costs of antifungal therapy by 35%. The authors concluded that preemptive treatment increased the incidence of invasive fungal infections, without increasing mortality [163].

Empirical antifungal treatment is still standard of care in persisting febrile neutropenia. Adverse events and emerging fungal resistance are a matter of concern that has to be addressed in the near future, beside pharmacoeconomical aspects.

Treatment of Invasive Aspergillosis

Without adequate therapy invasive aspergillosis will almost progress fatally [164]. A dissemination of IA may occur as disease progresses with spread to the CNS or other thoracic structures.

Voriconazole. The efficacy of voriconazole i.v. followed by p.o. treatment has proven significantly better success rates (52.8% vs 31.6%) compared with amphotericin B deoxycholate [165]. The 12-week overall survival of invasive aspergillosis was 70.8% with voriconazole in that randomized trial. Voriconazole is considered golden standard for the treatment of invasive aspergillosis. No new results from randomized trials comparing voriconazole with other azoles or polyenes in the first line treatment have been reported up to now. The voriconazole study was planned in the 1990s but the EORTC-MSG criteria were established. There are some important differences between the original study pre-specified criteria and the 2008 EORTC/MSG criteria [25]. According to the initial definitions a halo or air crescent sign was a probable IA, while this is considered possible according to the revised EORTC-MSG criteria. A positive mycology in BAL was considered definite IA, while it is defined probable in the new classification. The Herbrecht trial was presented with data adjusted according to the 2008 criteria at the ICAAC. Baseline clinical and microbiological data for 397 patients enrolled in the original study were reclassified. Using the original definitions, 277 patients (pts) had probable or proven IA. Using the revised definitions, 105 patients had possible IA, 174 pts probable IA, and 59 pts had proven IA. Original classification was unchanged in 185 cases and changed in 194 cases. However, reasons for change were: downgraded from definite to probable in 47 (24.2%) based on positive BAL only, downgraded from probable to possible in 65 (33.5%) based on nodule with halo and no mycology, upgraded from not aspergillosis or uncertain to possible in 40 (20.6%) based on nodule without halo and no mycology, upgraded from uncertain to probable in 20 (10.3%) based on nodule without halo and positive mycology. In patients with possible, probable or proven IA, response rate at end of randomized therapy was higher for Voriconazole (54.2%) vs 29.8% for cAmB, P<0.0004. Survival at week 12 was higher for Voriconazole in probable or proven IA (69.9% vs. 55.5%; P=0.014) but not for possible IA (79.6% vs. 68.6%; P=0.18). The application of the revised criteria did not change the outcomes originally reported [166] (Figs. 3 and 4). Additionally, the effective first-line treatment with voriconazole has been shown to be cost-effective. The cost-effectiveness of voriconazole in comparison to cAmB was evaluated with a lifetime Markov model, focusing on the long-term survival of patients treated for invasive aspergillosis. Long-term survival was extrapolated from survival after 12 weeks of treatment, with data obtained from that randomized aspergillosis study. With probabilistic analysis the cost-effectiveness of voriconazole compared with amphotericin B was analyzed and expressed in incremental costs per life-weeks gained. The corresponding incremental cost-effectiveness ratio was 62 euros per life-week gained (i.e., 3224 euros per life-year gained). Hospital costs were approximately 90% of the mean total costs [167].

Response rates of IA were obtained with liposomal amphotericin B in the first-line treatment. But the AmBiLoad trial did not compare liposomal amphotericin B to any other antifungal drug. That trial randomized an initial high dose (10 mg/kg) to a lower dose (3 mg/kg) of liposomal amphotericin B within the first 14 days of treatment. Liposomal amphotericin B (3 mg/kg) achieved response rates of 50% in the first-line treatment. Treatment of prob-

Table 4. Empirical Antifungal Treatment in Patients with Febrile Neutropenia (FUO)

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment Arm</th>
<th>Aim</th>
<th>n=</th>
<th>Endpoint</th>
<th>Fav. Outcome</th>
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<tr>
<td>Viscoli</td>
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</tr>
<tr>
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<tr>
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<td>Eff/Safety</td>
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<td>no diff/IAMB</td>
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<tr>
<td>Winston</td>
<td>cAMB vs Fluc</td>
<td>Eff/Safety</td>
<td>317</td>
<td>F/BIF/Sur/T</td>
<td>no diff/Fluc</td>
</tr>
<tr>
<td>Wingard</td>
<td>IAMB vs ABLC</td>
<td>Safety</td>
<td>240</td>
<td>Toxicity</td>
<td>IAMB</td>
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<tr>
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<td>F/BIF/Sur/T</td>
<td>no diff/varies</td>
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<td>Walsh</td>
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<td>Eff/Safety</td>
<td>1133</td>
<td>F/BIF/Sur/T</td>
<td>no diff/varies</td>
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</table>

Eff:efficacy; Fs:fever; BIF:breakthrough IFI; Sur=survival; T=Toxicity; no diff=no outcome difference
able or possible invasive aspergillosis is associated with a better outcome and survival compared to proven invasive aspergillosis [16, 45]. This might be explained to a lower fungal burden in the early course of disease. IA patients with unfavorable results had significantly higher rate of positive PCR results under antifungal treatment [103]. A correlation between serum galactomannan levels and inferior outcome of invasive aspergillosis has been reported elsewhere [168]. All treatment guidelines [169-171] underline early initiation of antifungal therapy as the cornerstone of treatment.

Caspofungin has proven efficacy in the 2nd-line treatment of refractory or intolerant invasive fungal infections [172]. Ninety patients with IA who were refractory to or intolerant of amphotericin B, lipid formulations of amphotericin B, or triazoles were enrolled to receive caspofungin with efficacy data for 83 patients
available. The patients had hematological malignancy (48%), allo-
genic blood and marrow transplantation (25%), and solid-organ transplantation (11%). Seventy-one patients (86%) were refractory to and 12 patients (14%) were intolerant to prior treatment. Re-
ponse was observed in 37 (45%) of 83 patients, including 32 (50%) of 64 with pulmonary aspergillosis and 3 (23%) of 13 with disseminated aspergillosis. Only a minority of patients was neutro-
penic (<500/mm³) at baseline (22.9%). Caspofungin was never compared with voriconazole or amphotericin B in the 1st-line setting of invasive aspergillosis. Efficacy of caspofungin against inva-
sive aspergillosis in the first-line was analyzed in a single arm, multicenter phase II-trial including patients in the SCT setting. The primary efficacy end point was complete or partial response at end of caspofungin treatment. Response at week 12, survival and safety were additional end points. Enrollment was stopped prematurely because of low accrual. Duration of caspofungin treatment was a median of 24 days. At the end of caspofungin therapy, 10 (42%) patients had complete or partial response (95% CI: 22-63%). Survival rate at week 12 was 50% for patients with proven and probable aspergillosis [173]. Caspofungin was also examined in hematological patients in the 1st-line setting. The dosage was 70 mg d1 followed by 50 mg d2. The dosage was the same as in the SCT setting. Adults with proven or probable IA, according to EORTC/MSG criteria, were eligible. The primary endpoint was the complete or partial response at the end of caspo-
fungin therapy. Secondary endpoints were response and survival at day 84 and safety. Most patients (85%) were neutropenic at enrol-
ment. At end of treatment, 1 and 19 patients had complete and par-
tial response, respectively (success rate 33%), while 31 (51%) had disease progression. The 12-week survival rate was 53% (32/60) for patients with proven or probable aspergillosis. Recovery from neutropenia at the end of treatment was significantly associated with survival [174]. There were low rates of adverse events due to caspofungin. Efficacy of caspofungin in these single arm trials against proven or probable IA seems low. The optimal dosage of caspofungin is unknown. Unlike the triazoles, it is not metabolized through the cytochrome P450 enzyme system. This makes caspo-
fungin a candidate for dose escalation. A dose escalation phase II-
study in patients with invasive aspergillosis examined dosages ranging from 70 mg to 200 mg caspofungin per day. The primary endpoints of the study were the safety and tolerability of caspo-
fungin. Endpoints of safety and tolerability were the numbers of toxicity-related study therapy discontinuations and predefined grade ≥3 clinical and laboratory events. Secondary endpoints included pharmacokinetic parameters for each dosage level and efficacy of caspofungin at four escalating dosages. A total of 46 patients with hematological malignancies received caspofungin (in the 70-, 100-, 150-, and 200-mg cohorts) for a median of 24.5 days. The rate of complete or partial responses was 54.3%, and the overall mortality at 12-week follow-up was 28.3%. In first-line treatment of invasive aspergillosis, daily doses of up to 200 mg caspofungin were well tolerated and the maximum tolerated dose was not reached. Re-
ponse rates were similar to those previously reported for voricona-
zole and liposomal amphotericin B. However, the trial was not designed to determine the efficacy of caspofungin in the treatment of IA. All differences across trials must be rated with caution [175].

Combination of Antifungal Treatment

Combination of antifungal treatment seems a reasonable ap-
proach. Azoles and echinocandins target different sites of the fungal cell membrane. The results of in vitro studies and animal models suggest that combinations with azoles and echinocandins have additive activity against Aspergillus species [176-179]. A small retro-
spective single (n=47) center trial suggested improved 3 months overall survival of voriconazole and caspofungin (n=16) over vori-
conazole alone (n=31) in the salvage setting for patients failing initial therapy with amphotericin B in the stem cell transplantation setting. Most patients had received allogeneic SCT (n=43). Histori-
cal data were collected between 1997–2001 where initial therapy was with AmB-desoxycholate (1 mg/kg q.d.) and to change to vori-
conazole (6 mg/kg, followed by 4 mg/kg b.i.d.) for salvage therapy. A change to a salvage regimen was indicated by progressive infec-
tion, intolerance to the initial regimen, or renal dysfunction (creatin-
ine clearance of <30 mL/min or creatinine level of >2.5 μg/mL) after 7 days of amphotericin-based therapy. Patients who had pre-
existing renal dysfunction or a history of AmB-d intolerance could receive lipid based AmB-formulations (amphotericin B lipid complex or liposomal amphotericin B, both at a dosage of 5 mg/kg q.d.) prior to receipt of voriconazole as salvage therapy. Included pa-
tients had proven or probable IA. The institutional practice was altered to caspofungin that was administered (70 mg on d1, fol-
lowed by 50 mg q.d.) in combination with voriconazole (6 mg/kg,
followed by 4 mg/kg b.i.d.) for salvage therapy in February 2001. Patients were well matched with regard to age, sex, underlying disease, type of conditioning therapy, and maximum corticosteroid doses received for GVHD. Most patients in the salvage arms had clinical failure of their treatment against IA. More patients who received combination salvage therapy for extrapulmonary dissemi-
ated and proven aspergillosis; however, differences were not sta-
tistically significant. Overall survival at 3 months after the day of diagnosis of aspergillosis was highest among patients who received the combination regimen (p=0.048) suggesting an advantage of antifungal combination in the study cohort [180]. Several other small cohorts reported antifungal combination with no fully pub-
lished data from prospective comparative studies.

Combination antifungal therapy has been reported in several other series or clinical trials [181-183]. Micafungin was examined in a multinational, non-comparative trial. The inclusion criteria accepted a wide variety of patients with infections due to proven or probable (pulmonary only) aspergillosis. In that open-label study micafungin alone or in combination with another systemic antifun-
gal was allowed. Of those only treated with micafungin in the combination treatment groups favorable response was seen in 5/17 (29.4%) and 60/174 (34.5%) of the primary and salvage treatment groups, respectively [184]. Some studies reported on patients with IA after solid organ transplantation. Solid organ transplant recipients who received voriconazole and caspofungin (n=40) as primary therapy for proven or probable IA between 2003 and 2005 were compared to a control group comprising a cohort of consecutive transplant recipients be-
 tween 1999 and 2002 who had received a lipid formulation of AmB as primary therapy (n=47). Survival at 90 days was 67.5% (27/40) in the cases, and 51% (24/47) in the control group (HR 0.58, 95% CI, 0.30-1.14, P=0.117). In transplant recipients with renal failure (adjusted HR 0.32, 95% CI: 0.12-0.85, P=0.022), and in those with A. fumigatus infection (adjusted HR 0.37, 95% CI: 0.16-0.84, P=0.019), combination therapy was independently associated with an improved 90-day survival in multivariate analysis [185]. The first prospective, randomized, double blind clinical trial investigated the efficacy of the combination of voriconazole and anidulafungin for the treatment of IA. Patients had active hemato-
logical malignancies, including allo-SCT recipients, with a diagno-
sis of proven or probable IA were eligible. Patients were stratified by host and transplant-related characteristics and randomized to receive initial treatment with the combination of voriconazole and anidulafungin or voriconazole monotherapy (with placebo). Study treatment was administered for > 2 weeks, followed by voricona-
zole maintenance to complete 6 weeks. The primary endpoint was all-cause mortality at 6 weeks. 454 patients were enrolled from 93 sites with 277 patients adjudicated proven or probable. Mortality at week 6 was 26/135 (19.3%) in patients treated with the combination of voriconazole and anidulafungin, compared to 39/142 (27.5%) for monotherapy (p=0.09). The MSG 03 trial included patients on the basis of presumed IA, those with possible IA had to be upgraded to probable IA within 7 days. 228 patients received voriconazole in
combination with anidulafungin vs. 226 voriconazole alone. The modified intent-to-treat analysis included only 135 and 142, respectively. There was no advantage for the combination treatment but a high rate of patients were not evaluable due to early death within 6 weeks of treatment and/or no CT-scan for follow up. In a post-hoc analysis of a subgroup of 218/277 (78.7%) patients with probable IA based on detection of galactomannan (GM) in bronchoalveolar lavage or serum, mortality at week 6 was 17/108 (15.7%) for combination and 30/110 (27.3%) for monotherapy p<0.05 (95% CI -22.69, -0.41). Safety parameters did not show significant differences between treatment arms. This first prospective, randomized, clinical trial of combination antifungal failed to show a difference in all-cause mortality, which was the primary endpoint of the trial [186]. Overall survival rates for first-line treatment of invasive aspergillosis are presented in Figure 4.

### Dose Escalation for the Treatment of Invasive Aspergillosis

Further strategies addressed higher dosages of antifungal therapy. The aim of these strategies is to overcome antifungal resistance and improve treatment outcome in invasive aspergillosis while minimizing drug-related toxicities and interactions. Dose escalation of voriconazole is not pursued due to the nonlinear disposition of the compound and a narrow therapeutic window. A dose escalation of oral posaconazole suspension between 50-800 mg/d is associated with an increasing plasma level. Because the saturation of absorption was observed in doses greater than 800 mg/d, a dose escalation does not make sense for posaconazole [187]. Itraconazole 400 – 600 mg/d orally has variable absorption even with the oral suspension. A loading phase is necessary. An intravenous administration of itraconazole circumvents resorption problems. Many patients failed to have or had not acceptable levels of itraconazole when the drug was given orally (itraconazole trough level >500 ng/mL). Substantial drug-drug interactions have to be taken into account, if itraconazole is used. The efficacy of intravenous itraconazole was reported from a small single arm trial with 21 patients. All had AmB refractory IA. Eleven of 21 patients (52%) had a complete or partial response. During intravenous treatment 18 patients experienced adverse events, most related to itraconazole. Taken together

<table>
<thead>
<tr>
<th>Category</th>
<th>Factors potentially influencing voriconazole trough levels (↓↑ = potential effect on voriconazole exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical characteristics</td>
<td>Body weight ↓ (if fixed dose)</td>
</tr>
<tr>
<td>Organ function</td>
<td>Liver: hepatic insufficiency ↑ Vascular endothel: increased permeability ↓ Kidney: no effect on voriconazole (but accumulation of SBECD without known clinical effects)</td>
</tr>
<tr>
<td>Switch to oral therapy</td>
<td>Dose reduction when switching to oral therapy (e.g. from 4 mg/kg b.i.d. to 200 mg b.i.d. if body weight &gt;50 kg) ↓</td>
</tr>
<tr>
<td>After oral administration</td>
<td>With food ↓ With fatty food ↓</td>
</tr>
<tr>
<td>Inadequate dosing</td>
<td>Omission of loading doses ↓ Poor treatment adherence ↓ Dosage errors ↓↑</td>
</tr>
<tr>
<td>Comedications</td>
<td>CYP450 inhibitors ↑ CYP450 inducers ↓ (beware of non-prescription drugs: St. John’s Worth ↓)</td>
</tr>
<tr>
<td>Food</td>
<td>Grapefruit (juice) ↑</td>
</tr>
<tr>
<td>Absorption</td>
<td>Impaired gastrointestinal motility ↓, diarrhea ↓, Intestinal GvHD ↓, malabsorption syndrome ↓</td>
</tr>
<tr>
<td>Genetic factors</td>
<td>CYP2C19 polymorphisms (poor metabolizers ↑; ultrarapid metabolizers ↓)</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
<td>Non-linear pharmacokinetics (enhanced ↓↑), Increased oral dose (200 mg b.i.d. to 300 mg b.i.d.)</td>
</tr>
<tr>
<td>Time of sampling</td>
<td>Prior to steady state (false ↓) Excessive time gap before next dose (= no true trough level) (false ↑)</td>
</tr>
</tbody>
</table>
there is limited data with itraconazole for the treatment of proven or probable invasive aspergillosis [188, 189].

Dose escalation has been performed with liposomal amphotericin B. In a double-blind trial, patients with proven or probable invasive mold infection were randomized to receive liposomal amphotericin B at either 3 or 10 mg/kg per day for 14 days, followed by 3 mg/kg per day (AmBisome-trial). The primary end point was favorable (i.e., complete or partial) response at the end of study drug treatment. Secondary endpoints included survival and safety. The overall response assessment was based on clinical, radiological, and (if available) microbiological findings at the end of the study drug treatment regimen. A total of 339 patients were enrolled. Out of these 105 (32%) did not have a proven or probable diagnosis of invasive fungal infection established within 4 working days after enrolment. These patients were disqualified from the efficacy analysis. Eight patients were enrolled but never received antifungal therapy. The data review board verified 201 infections out of this group 104 received 3 mg/kg while 10mg/kg was administered to the remaining 97 patients in the originally published Ambisome trial. Out of these 66% in the standard-dose group and 50% in the high-dose group completed 14 days of randomized study drug treatment. The primary factor for this difference was discontinuation of treatment because of adverse events (13% in the standard vs. 24% in the high-dose group; P=0.04). The median duration of study drug treatment was 15 days (range, 1–60 days) and 14 days (range, 1–57 days) in the standard vs the high-dose group. There was no significant difference in favorable overall responses between the treatment groups (50% in the standard vs. 46% in the high-dose group; P=0.65). Survival at the end of study drug treatment was 93% versus 88% standard- versus the high-dose group (difference, 4%; 95% CI, -4% to 12%; P=0.05). The 12-week survival rates were 72% and 59% (difference, 13%; 95% confidence interval, -0.2% to 26%; P=0.05). Significantly higher rates of nephrotoxicity and low potassium serum levels were seen in the high-dose group. The data were reanalyzed according to the revised EORTC-MSG criteria 2008. Response at the end of treatment and the 12-week survival was recalculated. Of 201 patients with invasive mould disease, 118 (59%) had a diagnosis based on halo signs (possible cases). Mycological evidence was present in 83 (41%) cases (probable/proven cases). Survival rates at 12 weeks for possible versus probable/proven cases in the 3 mg/kg group were 82% versus 58% (P = 0.006), and 65% versus 50% (P = 0.15) in the 10 mg/kg group. Taken together this initial intensified dosing (10 mg/kg) of liposomal amphotericin B did not prove any better efficacy when compared to standard dosage of 3 mg/kg [190] (Figs. 3 and 4).

Central Nervous Aspergillosis

Extension of invasive aspergillosis to the central nervous system (CNS) is associated with an exceeding high mortality. Early diagnosis requires a high degree of clinical suspicion. Changes in mental status and seizures have been reported as well as hemaneparesis. In addition, nonspecific neurological findings may occur. However, there are no typical clinical symptoms or CSF findings [191]. Recently a high sensitivity has been reported in a retrospective analysis of CSF with an Aspergillus PCR assay [192]. Currently this is not a standard lab diagnostic procedure. When a CNS complication of IA occurs in the immunocompromised host, disease evolution is almost fatal. A major factor for this inferior outcome is a poor penetration into the CNS for most antifungal agents. Amphotericin B-desoxycholate and itraconazole do not penetrate the blood-brain barrier sufficiently. A retrospective cohort of 17 patients with definitive or probable CNS aspergillosis treated with AmB alone (n=15) or in combination with fluocytosine (n=3) or itraconazole (n=2) showed a mortality of 100% with a median survival of 10 days after first evidence of CNS aspergillosis [193]. A more recent retrospective trial included 192 patients with CNS aspergillosis with proven (137) or probable (55) CNS infections. Survival was calculated from the start of voriconazole therapy. In all, 49% of patients died, 71% (67/94) due to fungal infection. The overall median survival was 297 days (range 3 to >2,000). Additional antifungal combination therapy (37 patients) gave a trend towards an improved response rate (p=0.09) and superior survival (p=0.0149) [194].

Abelcet proved to be efficacious in a murine-model with aspergillosis, but not curative, while caspofungin was not effective for the CNS [195]. Posaconazole showed potential for treating CNS aspergillosis in a murine model [196]. At the moment however, there are no further clinical data that support the use of posaconazole in this setting.

Voriconazole has a pharmacological property that allows penetration of the blood-brain barrier [197]. Clinical data of larger cohorts demonstrated efficacy with voriconazole. A retrospective analysis of 48 definitive and 33 probable IA of the CNS has been performed. Complete or partial response rates of 35% were achieved with voriconazole in patients with CNS aspergillosis. There were 31 patients who underwent additional neurosurgical procedures. Multifactorial analysis revealed that neurosurgery was associated with improved survival. Patients who underwent SCT had a poorer survival (22%; 7 out of 32) [198]. Combination therapy of voriconazole with echinocandins or liposomal amphotericin B has been reported in smaller series or retrospective cohorts. Schwartz et al. found a trend towards improved response and an improved overall survival of voriconazole in combination with other licensed antifungal drugs. Patients receiving additional neurosurgical intervention showed superior responses as well as superior overall survival. Therefore voriconazole alone or in combination and/or CNS surgery is recommended for patients with IA of the CNS.

Second-Line Treatment of Invasive Aspergillosis

Data for second-line treatment of IA in clinical trials are available for caspofungin, micafungin, posaconazole, itraconazole and the lipid based amphotericin B formulations [163, 168, 199, 200, 201]. These drugs are licensed for second-line treatment of refractory IA or patients intolerant to first-line treatment.

Invasive Aspergillosis in Children

Pediatric patients undergoing leukemia treatment or SCT often receive antifungal prophylaxis and treatment. There is a clinical need for antifungals in these patients [199]. Only a few drugs have been approved by FDA or EMEA in that population. Furthermore prophylaxis of IA in these patients is a hot topic. At present there are no general accepted guidelines for antifungal prophylaxis in this patient group. Voriconazole was studied in children.

Caspofungin is approved for pediatric patients (3 months and older) for treatment of Candida infections, fever of unknown origin in neutropenic patients, and second-line treatment of IA. Dosing of Caspofungin is based on body surface. There is no approval for prophylaxis and first-line treatment of IA. Anidulafungin is not approved for pediatric patients with IA. Anidulafungin has currently not been studied in a sufficient number of neutropenic patients. Micafungin was studied in an open-labeled neonatal candidiasis study, but this was a post marketing survey. As with many drugs, the pharmacokinetic profiles observed in the pediatric population differ from those seen in adult studies. Although micafungin is active against Aspergillus spp, it has been mainly used in combination therapy for invasive aspergillosis. While there is an approval for prophylaxis of invasive candidiasis in patients undergoing SCT, there is at present no approval for treatment or prophylaxis of IA in the pediatric population for micafungin.

Posaconazole is not yet approved for primary systemic chemotherapy in children under 13 years of age. Voriconazole studies showed that a pediatric dose of 11 mg/kg administered every 12 h are approximately bioequivalent to an adult dosage of 4 mg/kg given every 12 h [202]. Voriconazole has been approved in the...
pediatric population for antifungal treatment as is liposomal amphotericin B (1 months to 16 years) for empirical and targeted antifungal treatment.

Adjunctive Treatment

Confirmation of Aspergillus infection is both a diagnosis and a symptom of a broader cellular immunodeficiency up to more than 50% of patients with IA still failing to antifungal treatment. While neutropenia at time of diagnosis had no impact on prognosis in HSCT patients, a higher response to treatment is found when patients have a recovery of their neutrophil counts [5]. Immune response is the major contributor to host defense against opportunistic fungal infections such as aspergillosis. Aspergillus conidia are effective inducers of host chemokine responses both in vitro and in vivo. Further defined, TNF-α and GM-CSF play a central role in the recruitment of neutrophils into the lung in response to the Aspergillus pathogen [203, 204]. Small studies used granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and interferon-gamma (IFN-gamma) as adjunctive treatment. Cytokine administration to patients together with antifungal agents, as well as transfusion of cytokine-upgraded phagocytes, seems to be a promising immunotherapeutic modality [205]. Since these observations have been obtained in smaller series or case reports, these concepts have to be validated in prospective clinical trials.

Since the late 1990s donated granulocyte transfusions have been used to treat IA in patients with severe neutropenia. Granulocyte transfusions seem to be beneficial in selected cases, as in patients with severe aplastic anemia and prolonged periods of neutropenia [206, 207]. A meta-analysis focused on treatment of infections in patients with neutropenia. Eight randomized-controlled trials were included with 310 total analyzed patient episodes. Each study used different criteria for neutropenia (range < 100 to < 1000 / mm3) for the treatment of IA with cytokines. For mortality, the summary favored transfusion (RR = 0.64; 95% CI 0.33, 1.26), but with evidence of significant statistical heterogeneity. In addition to the observed clinical diversity between all studies, uncertainty about the quantitative and qualitative analyses for these studies is compounded by methodological deficiencies. Although there has been some success with granulocyte transfusions for treatment of IA and improvement in the duration of neutropenia, the conclusive answer of the value of adjunctive granulocyte transfusions for treatment of IA remains open [208, 209].

Surgical procedures. In selected cases lung resection is of proven diagnostic and therapeutic value. Lung resection has both diagnostic and therapeutic impact. The decision to perform lung resection is based on clinical and radiological signs of IPA rather than microbiological findings. Thoracic surgery (lobectomy) is indicated for the prevention of hemoptysis when the mass is in contact with the pulmonary artery or one of its branches. In addition, a persistent residual mass after antifungal treatment may justify a lung resection (lobectomy or wedge) before a new aggressive treatment protocol [211]. The patient should get surgical intervention as soon as clinically possible. A Swiss study reported on 27 consecutive neutropenic patients who underwent lung resection for suspected IPA. Histological findings, outcome concerning recurrence of aspergillosis and survival were evaluated. Seventeen patients with hematological diseases had previously undergone high-dose chemotherapy and 4 of them stem cell transplantation. In only one case a diagnosis of IPA could be made preoperatively. Twenty patients underwent lobectomy and 7 wedge resection. At day of surgery the neutrophil count was below 500/mm3 in 78% of patients, and the platelet count below 50,000/mm3 in 58% of patients. Invasive fungal infection was confirmed histologically in 22 of 27 patients (81.5%), while in the remaining five patients no fungal infection was documented. The median duration of surgery was 120 min. Major surgical complications occurred in two patients (bronchial dehiscence; pleural aspergilllosis). At 30 days postoperative mortality was 11% and 3-months survival was 77%. After lung resection, seven patients underwent stem cell transplantation without recurrence of IPA [212]. Over a 7-year period, ten patients with suspected invasive pulmonary aspergillosis of two university hospitals were retrospectively reviewed. After an antifungal therapy, surgical resection was performed with lobectomy/segmentectomy in 60% and with wedge-resection in 40%. Postoperative courses were uneventful in seven patients, two patients died due to infectious circumstances, and one patient was reoperated because of empyema [213]. There are no randomized trials comparing lung resection and antifungal treatment versus antifungal treatment alone. Reports of a favorable outcome from surgical interventions come from retrospective cohorts [214].

Upcoming Issues

A number of drugs are available for prophylaxis and empirical antifungal treatment of patients with persistent fever and neutropenia. Antifungal therapy exclusively to selected patients with persistent febrile neutropenia may be an over-treatment without impact on mortality. Innovative diagnostics, a better understanding of risk profiles and clinical criteria allow more and more the selection of patients requiring early and focused antifungal therapy. The efficacy of a broad empirical antifungal treatment starting 72 to 120 h after onset of fever was mainly studied in the 1980s and 1990s. At that time the antifungal armamentarium was small and toxic. Empirical antifungal therapy in neutropenic patients after 5-7 days of persistent fever (FUO) has been recommended by the Infectious Diseases Society of America. A broad and uncritical use of antifungals may induce resistance [215]. Selection of high-risk patients for empirical treatment and prophylaxis is of utmost importance to minimize risk of resistance. A more tailored approach for FUO that identified patients not at risk has been reported recently [216].

Several studies reported high breakthrough rates of invasive aspergillosis. A French cohort of 46 patients that received caspofungin for empirical antifungal treatment (median 9 days) reported invasive aspergillosis in 6/46 (12%) during follow up after a median of 8 days. Breakthrough infections occurred under caspofungin while patients receiving AmB (0/16) had no breakthrough aspergillosis [217].

There is growing concern of resistance against antifungals. Rates of azole-resistant Aspergillus fumigatus are currently low, but there are reports of emerging resistance, including multi-azole resistant isolates in parts of Europe [218, 219]. A Dutch trial recently reported a continued increase of azole resistance in Aspergillus fumigatus in the Netherlands. Azole resistance rose from 2% in 2005 to 6.6% in 2010 [220]. Beside the increasing azole use in humans there is a development of resistance due to agriculture antifungal compounds that causes multi drug cross resistance among human fungal pathogens [221]. Currently there are only a few new antifungals on the horizon. These drugs include isavuconazole, oral echinocandins (MK-3118), second-generation echinocandins (ASP97726) and newer formulations of posaconazole (i.v. and pill). Avoidance of emergence of antifungals and development of fungal resistance is an important topic. Implementation of an antifungal stewardship program has been reported to avoid overuse of antifungals. The stewardship program breaks the gap between quality of care and costs. Decentralization of empiric antifungal therapy is one of the most challenging aspects of antifungal stewardship to implement. Refinement of both target populations and clinical pathways incorporating antifungal use is required. Performance indicators including structural, process and outcome measures are integral for demonstrating the value of antifungal stewardship programmes [222, 223].

CONFLICT OF INTEREST

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