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Invasive Aspergillosis

Epidemiology, Diagnosis and Management in Immunocompromised Patients

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Abstract

Morbidity and mortality caused by invasive *Aspergillus* infections are increasing. This is because of the higher number of patients with malignancies treated with intensive immunosuppressive therapy regimens as well as their improved survival from formerly fatal bacterial infections, and the rising number of patients undergoing allogeneic haematopoietic stem cell or organ transplantation. Early initiation of effective systemic antifungal treatment is essential for a successful clinical outcome in these patients; however, clinical clues for diagnosis are sparse and early microbiological proof of invasive aspergillosis (IA) is rare. Clinical diagnosis is based on pulmonary CT scan findings and non-culture based diagnostic techniques such as galactomannan or DNA detection in blood or bronchoalveolar lavage samples.

Most promising outcomes can be expected in patients at high risk for aspergillosis in whom antifungal treatment has been started pre-emptively, backed up by laboratory and imaging findings. The gold standard of systemic antifungal treatment is voriconazole, which has been proven to be significantly superior to conventional amphotericin B and has led to a profound improvement of survival rates in patients with cerebral aspergillosis. Liposomal amphotericin B at standard dosages appears to be a suitable alternative for primary treatment, while caspofungin, amphotericin B lipid complex or posaconazole have shown partial or complete response in patients who had been refractory to or intolerant of primary antifungal therapy.

Combination therapy with two antifungal compounds may be a promising future strategy for first-line treatment. Lung resection helps to prevent fatal haemorrhage in single patients with pulmonary lesions located in close proximity to larger blood vessels, but is primarily considered for reducing the risk of relapse during subsequent periods of severe immunosuppression. Strict reverse isolation appears to reduce the incidence of aspergillosis in allogeneic stem cell transplant recipients and patients with acute myeloid leukaemia undergoing aggressive

anticancer therapy. Well designed, prospective randomised studies on infection control measures effective to prevent aspergillosis are lacking.

Prophylactic systemic antifungal treatment with posaconazole significantly improves survival and reduces IA in acute myeloid leukaemia patients and reduces aspergillosis incidence rates in patients with intermediate-to-severe graft-versus-host reaction emerging after allogeneic haematopoietic stem cell transplantation. Voriconazole prophylaxis may be suitable for prevention of IA as well; however, the results of large clinical trials are still awaited.

1. Epidemiology of Invasive Aspergillosis

Morbidity and mortality caused by invasive aspergillosis (IA) are increasing. Until most recently, centres treating patients with acute leukaemias or bone marrow or solid organ transplant recipients have reported steadily rising numbers of patients in whom IA has been diagnosed clinically or at autopsy. Data from prospective clinical studies on the use of broad-spectrum triazoles such as itraconazole, voriconazole or posaconazole for antifungal prophylaxis in patients undergoing allogeneic haematopoietic stem cell transplantation (HSCT) or intensive chemotherapy for aggressive haematological malignancies now indicate that among those patients, incidence and fatality rates of IA have declined.[1-4] Among patients with IA, ≈30% each have undergone HSCT and intensive chemotherapy, whereas ≈10% each are solid organ transplant recipients or have an underlying pulmonary disease.[5] Conversely, incidence rates among patients at risk vary according to local epidemiology and presumably many other factors such as strict isolation of patients, use of high-efficiency particulate air (HEPA) filters and the use of strict diagnostic criteria. In acute leukaemia patients, the incidence may be as high as 24%, among allogeneic stem cell transplant recipients up to 10%, among solid organ transplant patients up to 26%, and patients with severe burns may have a risk of 5–10%.[6] Owing to the introduction of new and effective antifungal agents such as voriconazole or caspofungin, mortality rates among patients with IA have declined during the past decade,[2,7] but still >50% of cases are fatal, particularly those with CNS involvement and those affecting patients after allogeneic HSCT.[8]

Among *Aspergillus* isolates from large diagnostic reference centres, *Aspergillus fumigatus* is by far the most common species (50–60%), followed by *A. flavus*, *A. niger* and *A. terreus* (10–15% each), whereas *A. nidulans*, *A. ustus* and other rare *Aspergillus* spp. typically each represent <2% of isolates.[5,9,10]

Preferentially, *Aspergillus* conidia are inhaled by breathing normal air and kept under control by pulmonary macrophages in individuals with normal immune competence. When they germinate into hyphae, invasive tissue infection may occur, if hyphal growth is not prevented by normally functioning neutrophil granulocytes. In individuals with impaired immune defence (specified further in section 1.3), tissue invasion may progress and result in vessel occlusion and ischaemic necrosis of tissue areas affected by this disruption of blood perfusion.

1.1 Clinical Features of Invasive Aspergillosis

IA most frequently manifests as invasive pulmonary disease, accounting for 50–60% of all cases.[5] Apart from that, ≈20% of IA cases are disseminated, while 5% each affect the skin, the paranasal sinuses or the CNS alone.[5] However, a large number of IA cases remain clinically undetermined because clinical signs and symptoms may be nonspecific, as may be the results of imaging diagnostic techniques such as thoracic CT scans. Since autopsy rates in many Western European countries and the US have fallen below 10% of all patients deceased during hospitalisation, the majority of IA cases are only suspected ante mortem.[11,12] Only a

few clinical symptoms may be suggestive of IA,[13] such as pleural pain or pulmonary infiltrates associated with signs of sinusitis, while clinical findings unequivocally indicating IA are lacking. Therefore, the distribution patterns mentioned above are empirical but not clearly proven. More diagnostic details are outlined further in section 2.

1.2 Definitions

The lack of specific clinical findings unequivocally proving IA in combination with the low autopsy rates in many institutions has led to significant confusion with regard to incidences, risk factors, usefulness of diagnostic procedures and efficacy of antifungal agents used for prophylaxis or treatment of IA. A broad multinational consensus facilitating the discrimination between possible, probable and proven IA was therefore welcomed internationally at its first publication in 2002 and has been uniformly used since then.[14] However, it must be emphasised that these definitions have been elaborated for the assessment and evaluation of clinical studies, and that they are restricted to patients with malignancies and HSCT recipients. Thus, they have not been designed to enable clinical assessment or therapeutic decision making (table I).

1.2.1 Proven Invasive Aspergillosis

The diagnosis of proven IA requires histopathological or cytopathological examination showing hyphae from a needle aspirate or biopsy specimen with evidence of associated tissue damage (either microscopically or unequivocally by imaging), or a positive culture result for a sample obtained by sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with infection, excluding urine and mucous membranes.[14]

1.2.2 Probable Invasive Aspergillosis

In cancer patients or HSCT recipients in whom probable IA is diagnosed, a number of different criteria must be fulfilled, defined as at least one host factor criterion from table I and one microbiological criterion and one major (or two minor) clinical criteria from an abnormal site consistent with infection.[14]

1.2.3 Possible Invasive Aspergillosis

The majority of clinically diagnosed IAs in severely immunocompromised patients do not fulfil the criteria required for a proven or probable IA. Invasive procedures required for histopathological or cytological evidence or positive cultures are often avoided because of the poor clinical condition of these patients or severe thrombocytopenia. Apart from that, the results of invasive procedures are often disappointing because histology, cytology and cultures remain negative despite the presence of IA.[12,15-18] Therefore, the decision to start systemic antifungal treatment is based on findings indicative of a possible IA, defined as at least one host factor criterion and one microbiological or one major (or two minor) clinical criteria from an abnormal site consistent with infection. While this clinical practice may be life-saving, because early antifungal therapy is associated with better clinical results than late onset of therapy,[19,20] it leads to a broad use of antifungals in patients in whom no IA has been demonstrated. Apart from costs and drug-related adverse effects, the real efficacy of an antifungal drug cannot be estimated in these cases. Because of these considerations, it is recommended not to use the category of a possible IA for a clinical study on the usefulness of an antifungal for IA therapy.

1.3 Incidence and Fatality Rates of Invasive Aspergillosis

It is not possible to specify precise incidence and fatality rates of IA among the different patients at risk (e.g. those with acute leukaemias or solid organ or stem cell transplant recipients) because clinically used diagnostic procedures often fail to provide positive results despite a present IA.[12,17,18,21] In addition, in many countries, autopsies are performed only in a small minority of patients who die of cancer or after a transplant procedure, and IA proven by autopsy may have been undiagnosed clinically in many cases, so that they have never been treated appropriately ante mortem. Thus, only approximate

Table I. Definition of invasive aspergillosis among cancer patients and haematopoietic stem cell transplant recipients (reproduced from Ascioglu et al.,[14] with permission from the University of Chicago)

PROVEN INVASIVE ASPERGILLOSIS

Deep tissue infection

Histo/cytopathology showing hyphae from a needle aspiration or biopsy with evidence of associated tissue damage (either microscopically or unequivocally by imaging) OR

Positive culture obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with infection

PROBABLE INVASIVE ASPERGILLOSIS

Defined as at least one criterion from host section AND

One microbiological criterion AND

One major (or two minor) clinical criteria from an abnormal site consistent with infection

POSSIBLE[a] INVASIVE ASPERGILLOSIS

Defined as at least one criterion from host section AND

One microbiological OR one major (or two minor) clinical criteria from an abnormal site consistent with infection

CRITERIA FOR PROBABLE AND POSSIBLE INVASIVE ASPERGILLOSIS

Host factors

Neutropenia: polymorphonuclear leucocytes <500/mm^3 for >10 days

Persistent fever for >96h refractory to appropriate broad-spectrum antibacterial treatment

Body temperature either >38°C or <36°C AND any of the following predisposing conditions:

a. Prolonged neutropenia (>10 days) in the previous 60 days
b. Recent or current use of significant immunosuppressive agents in the previous 30 days
c. Invasive fungal infection in a previous episode
d. Coexistence of AIDS

Signs and symptoms indicating graft-versus-host disease

Prolonged use of corticosteroids (>3 weeks)

Microbiological criteria

Positive culture of *Aspergillus* spp. from sputum or BAL

Positive culture or cytology/direct microscopy for *Aspergillus* spp. from sinus aspirate

Positive cytology/direct microscopy for *Aspergillus* spp. from sputum or BAL

Positive *Aspergillus* antigen in BAL, CSF or >2 blood samples

Positive cytology/direct microscopy for *Aspergillus* spp. in sterile body fluids

Pulmonary abnormality and negative bacterial cultures of any possible bacteria from any specimen related to the lower respiratory tract infection including blood, sputum, BAL, etc.

Clinical Criteria

MAJOR	*MINOR*
Lower respiratory tract infection	
Any of the following new infiltrates on CT imaging: halo sign, air-crescent sign or cavity within an area of consolidation	Symptoms of lower respiratory tract infection (cough, chest, pain, haemoptysis, dyspnoea) Physical finding of pleural rub Any new infiltrate not fulfilling major criterion
Sinonasal infection	
Suggestive radiological evidence of invasive infection in the sinuses (i.e. erosion of sinus walls or extension of infection to neighbouring structures, extensive skull base destruction)	Upper respiratory symptoms (nasal discharge, stuffiness etc.) Nose ulceration or eschar of nasal mucosa or epistaxis Periorbital swelling Maxillary tenderness Black necrotic lesions or perforation of the hard palate

Continued next page

Table I. Contd

CNS infection	
Suggestive radiological evidence of central nervous system infection (i.e. meningitis extending from perinasal, auricular or vertebral processes; intracerebral abscesses or infarcts)	(Provided that CSF is negative for other pathogens by culture, microscopy and malignant cells) Focal neurological symptoms and signs (including focal seizures, hemiparesis and cranial nerve palsies) Mental changes Meningeal irritation findings Abnormalities in CSF biochemistry and cell count

a This category is NOT recommended for use in clinical trials on antifungal agents, but for use in studies on empirical treatment, epidemiological studies and studies on health economics when needed.

BAL = bronchoalveolar lavage; **CSF** = cerebrospinal fluid.

estimates can be given for most frequently affected patient groups (table II).

1.3.1 In Neutropenic Patients

Incidence rates of IA among neutropenic patients vary considerably between centres. In a recent multinational, randomised, controlled study on the efficacy of antifungal prophylaxis in patients with acute leukaemia, an incidence of 6.25% according to the definitions provided in section 1.2 was reported for 240 patients not receiving an antifungal active against *Aspergillus* spp.[3] A report from Israel using less stringent diagnostic criteria showed a striking 50% incidence of aspergillosis among acute leukaemia patients treated during a period of hospital construction and of 43% thereafter.[30] A range of 5–24% had been depicted in a survey in 1998,[6] which appears to be representative. The mortality due to IA in acute leukaemia patients depends on the efficacy of systemic antifungal treatment and the recovery from severe neutropenia. In patients with refractory leukaemia, who do not produce sufficient numbers of granulocytes, a fatal infection, usually an invasive mycosis, typically represents their terminal event. This figure has to therefore be separated from a general assessment of mortality from aspergillosis in acute leukaemia patients. In studies on the efficacy of antifungal treatment regimens for IA, 30–40% of patients including those with acute leukaemia died of their fungal infection.[22,23] However, a separate analysis for acute leukaemia patients is not available.

1.3.2 In Stem Cell Transplant Recipients

Among allogeneic HSCT recipients, the incidence of IA is ≈10% within the first year after transplantation.[1,5,6,24,25] More than half of these infections occur later than 90 days post-transplant, while the majority of patients are treated outside the hospital.[4,31] However, the incidence of IA is significantly increased among patients who develop severe graft-versus-host disease (GVHD) requiring intensified immunosuppression and among patients with concomitant cytomegalovirus (CMV) infection compared with patients without these complications.[1,24,32,33] The fatality rate attributable to aspergillosis in affected patients is ≈60%.[24,33] Among patients undergoing high-dose chemotherapy and autologous HSCT, the risk of IA is generally very low.[25,34-37] This may be different in a patient popu-

Table II. Incidence and fatality rates of invasive aspergillosis among distinct patient populations

Patients	Incidence (%)[a]	Fatality (%)	References
Acute leukaemia	5–24 (–50)	30–40	8,22,23
Allogeneic haematopoietic stem cell transplantation	10	60	1,5,6,24,25
Solid organ transplantation	(1–) 11–14	50–60	25-27
Other causes of immunosuppression (burns, AIDS, alemtuzumab therapy, intensive care unit)	4–7 (–12)	70–85	8,28,29

a Figures in parentheses are from a single report.

lation with a positive history of IA in recent periods of neutropenia;[38] however, it has also been shown that in these patients autologous stem cell transplantation can be performed without an overwhelming risk of aspergillosis relapse.[39]

1.3.3 In Organ Transplant Recipients

The incidence of IA among organ transplant recipients varies according to the type of transplantation performed. Among heart-lung and small bowel transplant recipients, it may be as high as 11–14%,[25,26] whereas after kidney transplantation, it is ≈1%.[25,26] Of note, the anastomotic site of lung transplants is frequently colonised with *Aspergillus* spp.,[26] being a risk for tracheobronchitis and IA. The onset of IA among organ transplant recipients is typically later than 1 month post-transplant.[40,41] The fatality of IA after organ transplantation is 50–60% overall.[27] Among liver transplant recipients, survival in IA patients has been improved from 10% to 40% during the past decade.[42] Mortality in other organ transplant recipients has been reported from 70% (lung transplant) up to 100% (small bowel, pancreas).[26]

1.3.4 In Other Immunosuppressed Patients

Apart from patients with acute leukaemia undergoing intensive chemotherapy and HSCT or solid organ transplant recipients, patients with severe congenital immunodeficiency, severe burns or advanced HIV infection (AIDS) have been identified as risk groups for IA. During the early 1990s, an increasing risk of IA had been described for AIDS patients[43] and an incidence of up to 12% was reported in a review from 1998;[6] however, HIV-infected individuals now appear to have a much lower risk of IA. However, this may be different in regions where effective antiretroviral therapy is not generally available. For patients with severe burns, IA incidences of up to 7% have been reported between 1971 and 1991,[44-46] but more recent publications are sparse.[47,48]

The introduction of new agents for the treatment of T- and B-cell malignancies, particularly the CD52 antibody alemtuzumab, which causes profound and prolonged T-cell suppression, has been associated with considerable rates of infectious complications including a 4–7% incidence of IA among lymphoma patients treated with this antibody for relapsed or refractory disease.[49,50] This relatively high incidence of IA (along with a broad spectrum of other opportunistic infections) appears to be related to a profound humoral immunodeficiency and a severe neutropenia in patients with far advanced and heavily pretreated indolent B-cell malignancies, aggravating the immunosuppression caused by alemtuzumab, because no comparable rates of severe infections have been reported among patients with B-cell lymphocytic leukaemia treated upfront with alemtuzumab. Moreover, it has been observed that among organ transplant recipients treated with alemtuzumab to prevent graft rejection, no unusual incidences or characteristics of infectious complications occurred.[51-53] Fatality rates of IA in AIDS patients have been reported to be higher than among patients with haematological malignancies a decade ago[28] (reported to be ≈85% in a literature review in 2001[8]), but these figures have not been updated. IA may emerge in patients undergoing intensive care treatment. Meersseman et al.[54] reported on 58 patients with underlying diseases, such as chronic obstructive pulmonary disease or liver cirrhosis, who had proven or probable IA out of a total of 1850 intensive care patients evaluated retrospectively. Among 83 intensive care patients with diverse underlying conditions, including corticosteroid therapy, renal replacement therapy and cancer, who developed IA in a Belgian retrospective survey, a mortality rate of 77% was observed.[29] However, it has to be remembered that, in patients dying in an intensive care unit, the diagnosis of IA is frequently established at autopsy only.[55,56]

1.4 Risk Factors

1.4.1 Patient-Related Risk Factors

The most common patient-related clinical risk factor for developing IA is prolonged and profound neutropenia, defined as a neutrophil count <100/μL lasting for >10 days.[57,58] Patients undergoing intensive anticancer chemotherapy for aggressive haematological malignancies and those after allogenic HSCT are most frequently affected.[5,25] Patients

who have gone through sequential periods of profound neutropenia, e.g. for remission induction, consolidation or relapse treatment, have an increasing risk of acquiring IA, and a history of IA represents a high risk of relapse during a subsequent phase of severe immunosuppression.[59-61] In allogeneic HSCT recipients, the majority of IA is related to profound T-cell suppression (e.g. induced by total body irradiation, high-dose corticosteroids or T-cell suppressive drugs used for conditioning pre-transplant or management of severe GVHD), and their IA risk is further increased by concomitant CMV infection.[1,32,62] However, long-term administration of ganciclovir for CMV prophylaxis in these patients has also resulted in an increased risk of IA.[63] Prolonged administration of fluconazole for prevention of *Candida* and *Cryptococcus* infections has also been described as a risk factor for IA.[64,65] In addition, bacteraemia occurring during the early phase of bone marrow aplasia post-transplant has been described as a risk factor for subsequent invasive fungal infections including IA.[66] Among solid organ transplant recipients, hepatic and renal dysfunction could be identified as independent risk factors for IA.[67]

Patient-specific genetic risk factors have been reported recently by several investigators. In allogeneic HSCT recipients, polymorphisms of Toll-like receptors 1 and 6 have been associated with a higher susceptibility to IA,[68] while distinct interleukin-10 promoter polymorphisms may play a protective role[69] or increase the risk of IA.[70] Additionally, differences in the promoter region of tumour necrosis factor-α receptor type 2 gene appear to have an effect on the risk of developing IA in patients with haematological malignancies.[71]

1.4.2 Environmental and Nosocomial Risk Factors

Since *Aspergillus* spores are inhaled with normal room air, the risk of pulmonary aspergillosis outside special facilities with HEPA filtration is ubiquitous.[72] There is no strict correlation between the number of *Aspergillus* colony-forming units per cubic metre of sampled air and the incidence of IA; however, the load of *Aspergillus* spores and the incidence of IA are dramatically increased by building reconstruction performed without specific protective measures.[30,73-77] In general, a lack of HEPA filters has been described as a risk factor for IA in patients at risk;[59,78] however, it must be emphasised that inadequate air filtration systems may themselves represent a high risk of aspergillosis,[79] as has been described for air-conditioning systems.[80,81] Fire-proofing material used in many buildings, including hospitals, may be a source of nosocomial *Aspergillus* spread.[82] Also, carpets were found to be highly contaminated with *Aspergillus* spores.[83] Although systematic data are lacking, elevators appear to be distributing fungal spores via their intense air turbulence (author's own air sampling measurements). Depending on the water supply system of an individual hospital, the hospital water, especially when used for showering, may represent another nosocomial source of *Aspergillus*,[84,85] particularly *A. niger*. In addition, patients undergoing haemodialysis might be at risk for a water-borne fungal infection.[86] In a series of cutaneous nosocomial *A. niger* infections in bone marrow transplant patients, the hospital kitchen was identified by molecular typing as the source.[87]

Food may be a source of aspergillosis as well, because many different types of food, such as tea, pepper, fruits or freeze-dried soups, have been shown to contain high numbers of aspergilli, predominantly *A. flavus* and *A. fumigatus*.[88]

Since soil represents a natural reservoir of filamentous fungi such as aspergilli, potted plants have been identified as being a source of *Aspergillus* spread in hospitals.[72]

Seasonal influences have been described, with higher numbers of aspergilli during autumn and winter, related to rotting leaves;[72] however, other investigators explicitly point out that no seasonal influences have been identified in their epidemiological data analyses.[25,89,90] In clinical practice, patients treated in facilities without HEPA filters and well controlled air-conditioning systems may be at higher risk during seasons with hot outside temperatures by opening their windows and inhaling dust loaded with *Aspergillus*. Geographical factors also appear to have a significant impact on the incidence

of IA,[91] with lower infection rates being reported from countries with temperate climates.

Molecular typing studies have shown that in haematological patients with invasive pulmonary aspergillosis, a broad diversity of different strains of *Aspergillus fumigatus* could be identified, so these cases have not been caused by one specific nosocomial *Aspergillus* strain and many different environmental aspergilli may be involved.[92] Reports from an Austrian as well as from a German group indicated that in a proportion of critically ill patients[93] and of patients admitted to a bone marrow transplant centre,[60] *Aspergillus* spp. could be detected by molecular methods in respiratory samples at the time of hospitalisation. Therefore, some patients with severe underlying diseases who have undergone intensive immunosuppressive therapy may already harbour *Aspergillus* spp.; however, this observation is in contrast to reports of virtually zero incidence of aspergillosis in patients with haematological malignancies and allogeneic bone marrow transplant recipients treated under strict isolation.[30,94]

2. Diagnosis of Invasive Aspergillosis

Aspergillosis can only be proven either by histology or by culture from a physiologically sterile source. In theory, this could be a blood culture but in clinical practice *Aspergillus* fungal growth from the blood is extraordinarily infrequent. Because of thrombocytopenia, the two diagnostic pathways can rarely be followed in the populations with the highest incidences of IA, namely leukaemia and allogeneic transplant patients. Thus, diagnosis must rely on noninvasive and thus indirect methods. The recent development of such tools, specifically fungal antigen detection and the amplification of fungal nucleic acid sequences, may revolutionise the field of diagnosing and treating IA. In contrast, cultures from respiratory secretions, usually sputum or bronchoalveolar lavage fluid, in conjunction with a lung infiltrate, may suggest a plausible explanation of a lung infiltrate and thus frequently prompt antifungal treatment. However, since *Aspergillus* is ubiquitous, contamination during sampling or handling of the material in the diagnostic laboratory cannot be ruled out. Thus, *Aspergillus* growing from respiratory samples cannot prove infection.

2.1 Imaging Techniques

A limited number of articles have focused on the optimal use of imaging techniques in IA. Two landmark trials drive our understanding of the dynamics of lung infiltrates and subsequently of how and when to perform the respective imaging studies.

The use of high-resolution CT (HRCT) for early detection of pneumonia in patients with febrile neutropenia has been evaluated. In 112 patients with previously unremarkable chest radiographs, who were persistently febrile for >48 hours despite adequate antibacterial treatment, 60% showed pneumonia and 40% had a normal HRCT. Sensitivity of HRCT was calculated to be 87%, specificity was 57% and the negative predictive value was 88%. For the subgroup of HSCT patients it was even higher. Apart from the systematic approach defining the rate of lung infiltrates in a neutropenic population for the first time, infiltrates were documented 5 days earlier through HRCT than through chest radiography. It became clear that a normal chest radiograph does not rule out pneumonia, so that these patients should undergo HRCT in a timely fashion.[95]

While understanding the value of early CT scanning, how IA infiltrates transform through the course of successful treatment remained imprecise. Once again the setting of febrile neutropenia served as a model to characterise the exact timing of CT imaging. In patients with surgically proven invasive pulmonary aspergillosis, early CT scans displayed a typical halo sign in 96%. The halo sign vanished during the course of IA over the following 2 weeks, so it was present in 68% on day 3 and in only 22% on day 7. The rate of CT scans positive for air-crescent signs reciprocally increased from 8% to 63% of patients. Further analysis proved that the volumes of infiltrates increased, despite adequate antifungal treatment, 4-fold until day 7 and then remained stable during the following week.[96]

The radiographic dynamics have been addressed in a retrospective analysis of 40 consecutive pa-

tients. Regardless of antifungal treatment, the majority of patients had increasing lesion sizes until day 9 after diagnosis, followed by a stable phase of a median 3.5 days. After a median 80 days, 43% of patients presented complete radiographic remission. Formation of a cavitary lesion was associated with a 2.5-fold increase in time to radiographic remission.[97]

Recently an analysis of the imaging studies from a large clinical trial was published.[23] A significantly higher proportion of patients with a halo sign in the baseline CT scan responded to subsequent treatment as compared with patients with other CT imaging findings (52% vs 29%). The proportions of patients surviving until 12 weeks were 71% and 53%, respectively. While 61% of these patients with proven or probable IA exhibited a halo sign on the baseline CT scans, the other signs observed in this population included consolidations (30%), infarct-shaped nodules (27%), cavitary lesions (20%) and less frequently air-crescent signs (10%).[98] Other investigators support the association of halo signs with a diagnosis of invasive fungal infection.[99]

The role of magnetic resonance imaging in IA is ill defined. Today's standard method is CT scanning because of its availability and speed. It is a matter of debate whether HRCT is superior to more recent multidetector techniques in diagnosing halo signs. To date, no randomised comparison has been carried out and both methods seem appropriate. While a contrast agent is not routinely applied in these imaging studies, angiography multidetector CT more readily reveals vessel involvement or angiotropism even if infiltrates are minuscule.[100]

These studies emphasise the importance of a highly standardised approach in daily clinical practice and they define the window of opportunity for diagnosing a halo as decisively important diagnostic evidence. To follow up with the specific kinetics of radiographic signs of invasive pulmonary aspergillosis repeated CT scans are suitable. A first follow-up CT scan on day 7 may define the maximum expanse of pulmonary disease; no major reduction is expected until day 14. Thereafter, the optimum frequency of CT scans is unknown and thus requires an individualised approach.

2.2 Antigen Detection

Aspergillus antigen detection was a synonym for the galactomannan sandwich ELISA until modern 1,3-β-D-glucan tests were developed during recent years.[101,102] Other antigens have been evaluated less successfully for appropriateness in diagnostic tests.[103] The two molecules mentioned above are integral cell wall constituents and both are released from *Aspergillus* cells during the logarithmic growth phase *in vitro*, while DNA is only released after mycelial breakdown.[104] Among the medically important fungi, galactomannan is found predominantly in *Aspergillus* spp., while 1,3-β-D-glucan is vital in many other fungi as well, among them *Candida* spp.[105]

2.2.1 Galactomannan

In a series of 362 consecutive treatment episodes, galactomannan antigen detection had a sensitivity of 90% and a specificity of 98%.[106] These excellent values were corroborated by a second study in a similar patient population. Galactomannan levels in sequential serum samples were evaluated in a population of allogeneic stem cell recipients to determine the temporal onset of antigenaemia. A sensitivity of 94% and a specificity of 99% were calculated; positive and negative predictive values were 94.4% and 98.8%, thus yielding the best statistical profile of any noninvasive test in the field. In the majority of patients, antigenaemia preceded diagnosis based on radiography by 8 days,[107] a result that was in contrast to findings in a further recent study.[108] Another group of investigators found similar results in allogeneic stem cell transplantation. Seventy-four patients were included with a total of 832 serum samples analysed;[109] 89% of the patients did not fulfil any criteria of IA according to the 2002 consensus criteria.[14] Sensitivity and specificity of the test were 75% and 100%, and the positive and negative predictive values were 100% and 97%. However, in this study, detection of antigenaemia did not precede diagnosis.[109] The same finding was reported in another study screening sera for

galactomannan twice weekly.[110] Testing less than three times weekly may thus not be frequent enough to yield a positive result preceding clinical or CT-based diagnosis of IA.

The successful reports on galactomannan detection in serum samples from allogeneic transplant recipients fostered interest in other clinical specimens for which the test has not yet been validated. These include bronchoalveolar lavage fluid, urine and cerebrospinal fluid. Galactomannan has been detected in these materials but, except for bronchoalveolar lavage fluid, the clinical significance is still unclear.[14,111] Lavage fluid is rather easy to obtain and has the potential to support diagnosis. In a series of seven patients with radiological signs of invasive pulmonary aspergillosis, five had galactomannan-positive lavage samples. However, in all five individuals serum samples were positive and usually preceded lavage results.[112] It is of interest that galactomannan can be detected in culture-negative lavage samples.[113]

Different groups explored the performance of the test in various patient populations and reported a high variability in test results.[101] The causes of this variability are not fully understood and seem to be multifactorial. Among other factors test performance may be affected by the release of the antigen itself from the site of infection and its binding to third molecules in the blood. The patient population chosen may be a decisive factor as well as the frequency of serum sampling, when the test is part of a monitoring procedure.[101]

During IA, different patterns of serum galactomannan levels over time have been described.[101] These include a paradoxical increase shortly after initiation of treatment,[114] which may be associated with an increased antigen release from fungal cells. More frequently the galactomannan index will decrease after treatment is begun, as opposed to a persistently positive index which may been seen with treatment failure.[101,115]

The debate on the optimal cut-off index and the potential for false-positive test results discussed in the next sections reflects the complexity of the individual patient situation. A recent meta-analysis of 27 studies found a sensitivity of 71% and a specificity of 89% in patients with proven IA. Of note, the yield of the galactomannan test differed between patient populations, performing better in the setting of haematological malignancy and HSCT.[116] Large clinical series of galactomannan testing in solid organ transplantation are lacking, so currently no recommendation can be given.

Among other factors affecting test performance, previous and concomitant antineoplastic and anti-infective treatments have to be considered when interpreting antigenaemia results.[117]

Cut-Off to Positivity

An important variable affecting the performance of any test clearly is the cut-off value chosen to differentiate between positive and negative results. In the early evaluations of the galactomannan antigen test the cut-off chosen was an optical density index of 1.5 (European instructions of the manufacturer) or 1.0.[101]

In a sophisticated approach, test results were evaluated from adult and paediatric patients with haematological or oncological underlying diseases. Results from 3294 serum samples obtained during 797 patient episodes yielded a sensitivity of the galactomannan ELISA of 65% for definite IA (cut-off 1.5). Sensitivity was lower in patients positive for anti-*Aspergillus* antibodies. False-positive results tended to occur more frequently in children and in allogeneic HSCT recipients. The overall specificity was 95% and was lower in the latter two patient groups. When the cut-off was lowered to an index of 0.7, the sensitivity and specificity in the adult non-transplant population were 73% and 94% for definite cases.[118]

Recently, a prospectively evaluated patient cohort with haematological disorders proved the best correlation with the final clinical diagnosis when using a cut-off index of 0.6 in two subsequent samples.[119]

Another analysis based on 986 serum samples from 67 allogeneic HSCT recipients demonstrated that decreasing the index cut-off to 0.5 further increased its sensitivity without losing much specificity. Similar to the first reports in allogeneic transplant

recipients, positive tests preceded diagnosis of IA by 10 days and clinical onset of symptoms by 6 days. An additional finding was a decrease in sensitivity in patients receiving mould-active antifungal treatment.[120,121] Subsequently, the test was approved in the US with the proposed cut-off index of 0.5.

Another approach proposed two independent cut-offs for neutropenic patients. A single test result of ≥0.8 would thus result in *Aspergillus* treatment as well as two consecutive samples with an index of ≥0.5. This strategy increased sensitivity from 62% to 97% and decreased specificity from 100% to 98.6%.[122]

'False-Positive' Galactomannan Detection

'False-positive' does not necessarily refer to galactomannan-free material being tested positive; rather, it addresses finding galactomannan in diseases other than aspergillosis. While cross-reactivity with fungi other than *Aspergillus* has been documented, it may infrequently be of clinical relevance.[123,124] However, several reports have been published on false-positive test results during concomitant use of piperacillin/tazobactam. Subsequently, false-positive galactomannan tests have been described in patients treated with other β-lactam antibacterials, i.e. amoxicillin/clavulanic acid[125,126] and ampicillin/sulbactam.[127] These reports led to altered approaches to febrile neutropenia and clinical trials.[126,128-130] However, β-lactam antibacterials cannot be withheld from patients at risk for IA[131] and galactomannan detection has become a cornerstone in diagnosing IA.[14] Understanding the kinetics of galactomannan should facilitate clinical decision-making. Sequential serum samples were evaluated from patients with false-positive test results when β-lactam treatment was ceased. The average time to negative galactomannan tests at an index <0.5 was 5.5 days, with an elimination half-life of 2.4 days.[132] In a population of patients not at risk for IA, false-positive serum galactomannan levels decreased to negative, i.e. below index 0.7, before the next administration of piperacillin/tazobactam in an 8-hourly schedule.[133]

Recently, a solution (Plasmalyte™)[1] used for bronchoalveolar lavage was found to contain galactomannan and led to a series of false-positive tests. Normal saline solutions should be preferred in this setting, since they have tested negative.[134] It is obvious that knowledge of the aetiology of false-positive results is expanding and a complete listing of all causes has not yet been accomplished.

2.2.2 β-D-Glucan

In view of the shortcomings of existing assays for IA, focus has gone on other target antigens. Effectively detecting 1,3-β-D-glucan could result in early diagnosis of fungal infection. Since the target molecule characterises fungi susceptible to echinocandins, a reliable positive test would even suggest suitability of a specific treatment. Unfortunately, the test cannot differentiate between most fungal infections, e.g. aspergillosis and candidiasis. Two test kits are commercially available, the Fungitell™ (formerly Glucatell™) and the Fungitec-G™.

The plasma 1,3-β-D-glucan level was determined in 202 febrile episodes at the time of routine blood cultures (Fungitec-G™, cut-off 20 pg/mL). Sensitivity was 90% and specificity 100%, the positive predictive value was 59% and the negative predictive value was as high as 97%. Invasive fungal infections leading to positive glucan test results included *Candida*, *Aspergillus*, *Fusarium*, *Cryptococcus* and *Trichosporon*.[135,136]

The Fungitell™ assay was applied to serum and plasma samples from healthy volunteers and patients with positive blood cultures. Sensitivity and specificity of the assay were 93% and 77%; the positive and negative predictive values were 52% and 98%. In the same study, the majority of sera positive for *Histoplasma* antigen or galactomannan were also found to react to the Fungitell™.[137]

Single serum samples from 163 patients with proven or probable invasive fungal infections were tested in the Fungitell™ assay. At a cut-off of 60 pg/mL sensitivity was 70% and specificity 87%. At a cut-off of 80 pg/mL sensitivity decreased to 64% and specificity increased to 92%.[136]

1 The use of trade names is for product identification purposes only and does not imply endorsement.

In a twice-weekly screening procedure, the galactomannan Platelia Aspergillus™ and the Glucatell™ 1,3-β-D-glucan assays were compared in a population of 40 neutropenic adults. The investigators used the galactomannan cut-off index 1.5, and defined results <1.5 but ≥1.0 as indeterminate. All proven and two-thirds of the probable invasive fungal infections were identified by both tests. Sensitivity and specificity were 88% and 90% for both tests, with a negative predictive value of 96%. The test kinetic was similar and both preceded clinical diagnosis and imaging findings, with the glucan assay providing a positive result earlier. The combination of both assays correctly identified all patients with IA.[138]

Recently, the Fungitell™ test was evaluated in a population with acute myelogenous leukaemia or myelodysplastic syndrome.[102] On the basis of results from patients with candidaemia and healthy volunteers, a serum level of ≥60 pg/mL was chosen as the cut-off. In all patients with a proven or probable invasive fungal infection at least one test was positive at a median of 10 days before the clinical diagnosis was made. The fungal infections comprised candidiasis, fusariosis, trichosporonosis and aspergillosis. The negative predictive value was 100%; and the specificity was found to be 90% for a single positive test and 96% if at least two consecutive tests were positive.[102]

The results of these studies are promising and suggest that the 1,3-β-D-glucan assay has at least the potential to exclude invasive fungal infections. Still, other large series evaluating these assays are desirable. In particular, an analysis of intensive care patients at high risk for developing invasive candidiasis would be interesting.

'False-Positive' 1,3-β-D-Glucan Detection

Apart from the variety of fungi testing positive for 1,3-β-D-glucan, 'false-positives' have been reported in patients with bacteraemia. It has been speculated that glucan testing may have the potential to exclude rather than to prove fungal infection in such populations. Furthermore, if the handling of specimens is not kept to a minimum, this will probably increase the number of false-positive results.[137] With the Fungitell™ assay 1,3-β-D-glucan was detected in serum samples of a series of patients without invasive fungal infection while receiving intravenous amoxicillin/clavulanic acid. When the antibacterial was discontinued, glucan test results returned to negative. Subsequently, batches of amoxicillin/clavulanic acid were found to contain 1,3-β-D-glucan. No glucan reactivity was observed in batches of piperacillin/tazobactam.[139] It may be speculated that serum sampling immediately before the administration of the next dose of amoxicillin/clavulanic acid will reduce the magnitude of this problem.

2.3 Polymerase Chain Reaction

Apart from antigen detection from blood and other clinical samples, the detection of specific sequences of the fungal genome is another method promising earlier diagnosis. Since the 1990s, different polymerase chain reaction (PCR) techniques have been used to detect circulating fungal DNA. These either followed a pan-fungal approach or focused on *Aspergillus*. There is still no standardised PCR method for the detection of fungal DNA.

An assay detecting *Candida* and *Aspergillus* 18S ribosomal RNA genes has been tested for clinical use. Blood samples were evaluated from patients with febrile neutropenia, with or without fungal colonisation, and from patients with invasive fungal infection. Sensitivity in samples from patients with invasive fungal infection was 100% if two samples were tested; specificity was 98%. It is of particular interest that PCR positivity preceded radiographic signs by a median of 4 days in patients with chronic disseminated candidiasis or invasive pulmonary aspergillosis. This PCR assay yielded negative results once the invasive fungal infections responded to treatment.[140]

In allogeneic stem cell recipients, a prospective PCR screening was instituted for early diagnosis of IA. The rate of positive blood samples was 14%. PCR was positive by a median of 2 days prior to the first clinical signs in patients with IA. Sensitivity and specificity of the PCR were 100% and 65%, respectively.[141]

In patients with febrile neutropenia or undergoing allogeneic HSCT, *Aspergillus* PCR and galactomannan ELISA were correlated with the final clinical diagnosis. Sensitivity and specificity rates for the PCR assay were both 64%; for the galactomannan ELISA these values were 33% and 99%, respectively.[142]

Adult patients with haematological malignancies were screened for circulating *A. fumigatus* and *A. flavus* DNA twice weekly. For proven and probable IA the sensitivity, specificity and positive and negative predictive values of the PCR assay were 4%, 90%, 64% and 90%, respectively, when positivity was defined as two consecutive positive PCR results. When PCR results were combined with galactomannan results, sensitivity increased to 83% and the negative predictive value improved to 98%, but at the cost of a decrease in specificity to 70%. The false single positive rate was 45%.[143] In another series of haematology patients, the first galactomannan-positive serum samples were screened for *Aspergillus* DNA using real time PCR. There was no clear correlation found between the PCR result and the final diagnosis.[144]

The sometimes disappointing PCR performance from blood samples may possibly be overcome when bronchoalveolar lavage fluid is analysed in patients with lung infiltrates, based on the assumption that the diagnostic yield may be enhanced in the immediate vicinity of the infection process. Consequently, a two-step PCR assay to detect *Aspergillus* DNA was evaluated in bronchoalveolar lavage samples from patients with underlying haematological malignancies. The sensitivity and specificity reached 94%, the positive predictive value 84% and the negative predictive value 98%.[145] The detection of *Aspergillus* DNA by a nested PCR assay was evaluated in cerebrospinal fluid samples from patients with haematological malignancy and cerebral aspergillosis. The cautious conclusion is that *Aspergillus* DNA can be detected from cerebrospinal fluid samples and thus has the potential to improve diagnosis of cerebral aspergillosis.[146]

Clinically, all of the diagnostic tools discussed in this section need to be used as single bricks in the complex strategy finally building the diagnosis of invasive fungal infection. The most frequently used techniques today are CT scans and serum galactomannan detection. Only their incorporation in a well observed standard operating procedure enhances the diagnostic yield.[147,148] The value of the PCR assays is largely dependent on their standardisation and thus availability in the individual hospital. A first inter-laboratory evaluation has been performed, so a process of consensus is on its way.[149] All of these efforts are worthwhile, since the earlier treatment resulting from earlier diagnosis may translate into improved survival.[150]

3. Treatment of Invasive Aspergillosis

The mortality from IA not treated with antifungals is almost 100%. This figure overlaps with the mortality due to the respective underlying disease paving the way for IA, which does not occur in healthy individuals. There may be rare exceptions to this general rule, if IA occurs on the background of a severe, but transient immunosuppression that completely resolves. Although prospective studies have never been conducted on the optimal time to start systemic antifungal therapy after the appearance of the first clinical signs and symptoms, it is generally accepted that early onset of treatment with systemic antifungal agents results in significantly higher survival rates compared with late onset of treatment.[19,20,151-153]

3.1 Principles of Antifungal Drug Treatment in Invasive Aspergillosis

3.1.1 Antifungal Treatment Approaches

Considering the association between early onset of systemic antifungal treatment and improved clinical outcome, it is recommended to start antifungal therapy promptly in a patient at high risk of IA who shows clinical signs and symptoms compatible with IA. In many patients, the diagnosis of IA will never be proven because treatment has led to complete or partial regression of these findings, or because the patient dies and autopsy is refused.

For different antifungal treatment approaches, distinct categories have been defined during the past decade: empirical antifungal therapy, pre-emptive antifungal therapy and (possibly named 'targeted') antifungal therapy of a proven invasive fungal disease. Empirical antifungal therapy is given to a patient at risk for IA, who has persistent fever refractory to broad-spectrum antibacterial treatment, but no other clinical sign or symptom indicating IA. In these patients, administration of antifungals has been shown to be more effective than changing empirical antibacterials.[154-156] This approach has become standard in many cancer centres treating patients with profound and prolonged neutropenia.[131,157] How many of these patients indeed have occult IA remains undetermined. Empirical antifungal therapy is not addressed in this review.

In patients with a high risk of developing IA, e.g. those with acute leukaemia undergoing aggressive chemotherapy or allogeneic HSCT recipients, systemic antifungal therapy is typically initiated on the basis of clinical signs and symptoms indicative of IA. Apart from fever refractory to empirical broad-spectrum antibacterials, characteristic findings on thoracic CT scans, cough, pleural pain or paranasal sinus findings are among the most frequent clinical signs giving reason for suspecting IA.[13] In these patients, systemic antifungals active against *Aspergillus* spp. are given pre-emptively. According to the definition criteria mentioned in section 1.2 (table I), the majority of these cases would be classified as possible IA. In routine clinical practice, criteria for proven IA are rarely fulfilled.[11,12] Therefore, a targeted therapy of documented IA is given only in a minority of patients affected.

It is important to keep these considerations in mind when response to antifungal agents is reported from clinical studies. In patients treated for probable IA, rates of complete or partial response may be >10% higher than in patients with clearly proven IA.[23]

3.1.2 Assessment of Efficacy

For evaluation of the clinical efficacy of an antifungal drug, it must be considered whether the drug has been studied for primary or for salvage treatment of patients with IA. Apart from voriconazole and conventional amphotericin B deoxycholate (D-AmB), which have been used in a properly designed, prospective randomised study on primary antifungal treatment of IA,[23] amphotericin B colloidal dispersion (ABCD)[22] and liposomal amphotericin B (L-AmB)[158] have been tested appropriately for first-line treatment of IA. A successful clinical outcome typically is defined as a partial or complete response, whereas 'stable disease' or 'no change' usually are regarded as clinical failure of the tested drug. Although in many severely neutropenic patients, it should be regarded as a success if progression of IA is halted by an antifungal drug,[159] it must be kept in mind that stable disease has been included in 'overall response' in only a few studies,[22] making these results difficult to compare with study results in which stable disease has been assessed as failure.

Apart from that, it is essential to discriminate between results obtained in an intent-to-treat patient population and those obtained in a patient cohort treated per protocol. Success rates may be substantially worse in an intent-to-treat population if treatment was discontinued or not given in an appropriate dose in a significant proportion of patients, for example, because of intolerance or toxicity.

3.1.3 Pharmacological Considerations

Pharmacological considerations may be important for the clinical choice of an antifungal agent for treatment of IA. *In vitro* susceptibility testing may not be reliable to predict clinical response in patients with IA, because for many antifungal drugs, a standardised susceptibility testing system for *Aspergillus* spp. has not yet been established. Only a limited number of clear *in vitro* and *in vivo* resistances have been demonstrated, particularly for *A. terreus* resistance to amphotericin B[160,161] and for resistance of aspergilli against itraconazole,[162] and more recently multiple triazole-resistant *A. fumigatus* isolates have been described.[163,164] The appropriate dosage of the diverse antifungal agents currently approved for primary or salvage treatment of IA is poorly defined. Basically, azole antifungals such as itraconazole, voriconazole and posaconazole exert their

antifungal activity depending on the time above the minimum inhibitory concentration, whereas polyenes such as D-AmB act in a peak concentration-dependent manner.[165] For the class of echinocandins (caspofungin, micafungin, anidulafungin), it has become clear from animal models that their anti-*Aspergillus* activity is concentration-dependent.[166] However, it has not been demonstrated that dose escalation in echinocandins or amphotericin B translates into higher clinical efficacy.[158,166,167]

Of the pharmacokinetic properties, penetration to the site of IA is essential. While a detailed description of pharmacological data known for the approved antifungal agents is beyond the scope of this article, it is important to note that amphotericin B formulations, itraconazole and echinocandin antifungals do not all achieve sufficient concentrations in brain tissue.[168] Penetration into lung tissue and antifungal killing activity of D-AmB in inflamed lung tissue is suboptimal.[169-172] For posaconazole, precise data on tissue penetration in humans are lacking.

In a large number of critically ill patients with IA, multiple pharmaceutical agents are used concomitantly. Therefore, drug-drug interactions may play an extremely relevant role in these patients, with respect to significant changes in antifungal drug concentrations and to additive or potentiating organ toxicities. Of paramount importance are drug interactions of triazole compounds, particularly itraconazole and voriconazole, with pharmaceutical agents interacting significantly with cytochrome P450 (CYP) isoenzymes; and renal toxicity of D-AmB given in combination with other nephrotoxic drugs such as ciclosporin or aminoglycosides.

In contrast to less life-threatening infections or unexplained fever in neutropenic patients, IA requires prompt therapeutic intervention using the most effective antifungal drug available upfront. Modification or escalation of antifungal therapy in case of non-response to suboptimal first-line treatment is unlikely to influence clinical outcome.[173]

3.2 Prognostic Factors Influencing Treatment Outcome

Patient-related prognostic factors have a marked effect on the likelihood of improvement or cure of IA, with the site of infection being one of the most critical. Isolated pulmonary manifestations may be successfully managed in 40–50% of patients,[5] particularly if pulmonary infiltrates are localised and not disseminated.[8] Aspergillus sinusitis generally has a more favourable outcome with a mortality rate of ≈25%.[8] The most unfavourable treatment results are observed in patients with cerebral aspergillosis (mortality rate of 65–90%)[5,7,8] and in those with disseminated disease, of whom 85–90% have a fatal outcome.[5,8] The underlying disease also has an effect on clinical outcome: most unfavourable results with only 15% survival have been noted in allogeneic HSCT recipients,[5,8] whereas in up to 60% of solid organ transplant patients, IA may be successfully treated.[5] The clinical course of an underlying malignancy or other state of immunosuppression is another critical factor influencing clinical outcome. If acute leukaemia does not respond to intensive chemotherapy and therefore no recovery of neutrophil granulocytes occurs, or if aggressive immunosuppression (e.g. for severe GVHD after allogeneic HSCT) cannot be reduced, the outcome is unfavourable compared with patients going into complete haematological remission or in whom immune recovery can be achieved.[6,59,174]

3.3 Antifungal Drugs

In this section, data on antifungals currently available for clinical systemic antifungal treatment are used. It must be pointed out that not all of these agents are approved in all countries, and that approval may be restricted to second-line treatment of patients with IA refractory to or those intolerant of other licensed antifungal drugs. Apart from that, there may be restrictions on the use of agents in paediatrics. It is recommended to seek updated detailed information on the approval status of an antifungal drug before it is used based on information from this article.

Pharmacological properties of the antifungal agents discussed in this section have been described extensively in specific papers or comprehensive overviews[169] and are not addressed here, apart from aspects important for clinical efficacy, interactions or adverse effects.

3.3.1 Amphotericin B Deoxycholate

Conventional D-AmB was the gold standard for the primary treatment of aspergillosis until 2000.[151] It was approved >40 years ago on the basis of sparse observations of potential activity against pathogenic fungi in humans. However, informative data on the clinical efficacy of D-AmB became available only in the late 1990s. The first prospective, randomised clinical trials comparing the efficacy of D-AmB with that of other antifungals, voriconazole or ABCD, in patients with invasive *Aspergillus* infections were published in 2002.[22,23] In these trials, a response rate (defined as partial or complete response) of <35% to D-AmB was reported. These low response rates are, at least in part, caused by the high proportion of patients not tolerating adequate dosages of D-AmB. The predominant reasons for intolerance are infusion-related adverse effects, such as fever, chills, dyspnoea or skin reactions, and nephrotoxicity. The latter is directly related to the duration of D-AmB therapy[175,176] and has been associated with significantly increased mortality.[175-177]

Infusion-related adverse effects of D-AmB may be ameliorated by co-medication of (non-nephrotoxic) antipyretic drugs and histamine H_1 receptor antagonists, and they may be effectively treated with pethidine, whereas nephrotoxicity can be reduced by hydration and the daily administration of saline (0.9% NaCl) 1.0–1.5L. Glucocorticosteroids, although effective, should be avoided because of their immunosuppressive effects. Prolongation of infusion time to 24 hours, i.e. a continuous intravenous administration, may further improve the tolerability and reduce the toxicity of D-AmB,[178,179] but clinical efficacy requires further confirmation before it can be discussed as a valid option.

3.3.2 Liposomal Amphotericin B

The most widely used alternative to conventional D-AmB is L-AmB. It has the advantage of improved tolerability compared with D-AmB. No clinical study has been conducted in a sufficiently large number of patients with proven IA to compare its efficacy with that of D-AmB or another antifungal active against *Aspergillus* spp. From a study in a small number of patients, similar efficacy of L-AmB and D-AmB and significantly superior tolerability and lower nephrotoxicity of the liposomal formulation were reported.[180] This is in accordance with the results of a large trial comparing L-AmB and D-AmB given for empirical treatment in febrile neutropenic patients.[181] A rate of nephrotoxicity in the order of 10–20% has been observed in patients treated with L-AmB at a daily dose of 3 mg/kg. In patients given L-AmB without nephrotoxic co-medication, however, significant renal impairment is rare.[182,183] The incidence of other adverse effects of L-AmB, in particular infusion-related adverse events, has been reported to be around 30%.[181,184,185] Treatment with L-AmB is discontinued because of adverse events in 5–8% of patients.[184,185]

To better define the appropriate daily dose, one clinical study compared 1 mg/kg with 4 mg/kg in patients with invasive *Aspergillus* infections. Surprisingly, this trial has not been able to demonstrate a superiority of the higher dosage.[167] However, only a small number of patients included in this trial had clearly proven IA, and in these patients, the higher dosage of L-AmB was superior. A theoretical disadvantage of L-AmB is the slow release of the free amphotericin B compound, so that ≈20% of the free drug is bioavailable 72 hours after administration.[186] After preclinical studies had shown that dosages of up to 10 mg/kg/day did correlate with increasingly higher tissue concentrations and were well tolerated at the same time,[187] it was hypothesised that initial loading doses of L-AmB might lead to faster fungicidal activity in patients with IA. In a prospective, double-blind, randomised study, 201 neutropenic or transplant patients with IA were given L-AmB 3 versus 10 mg/kg/day for 14 days, followed by 3 mg/kg/day until antifungal treatment

was completed. Partial or complete response was achieved in 50% of patients treated with 3 mg/kg/day and the outcome was not improved by the higher dosage. A difference in overall survival was not observed, while infusion-related adverse events and nephrotoxicity (31% vs 14%) were more frequent in patients receiving the higher dosage.[158] Comparing response rates and survival from this trial with that reported by Herbrecht et al.[23] for voriconazole, the clinical outcome for both antifungal drugs appears similar, so L-AmB in a daily dose of 3 mg/kg can be considered as alternative to voriconazole for first-line treatment of IA.

An expert group of the Infectious Diseases Society of America (IDSA) recommended a daily dosage of at least 5 mg/kg of a lipid formulation of amphotericin B for the treatment of patients with IA.[151] This recommendation is based on the assumption of a dose-efficacy relationship for amphotericin B. A clinical correlation of such a dose-efficacy relationship has not yet been confirmed.

3.3.3 Amphotericin B Lipid Complex

The lipid complex formulation of amphotericin B (for extensive pharmacological review see Janoff et al.[188]) allows a rapid release of free amphotericin B from the lipid molecules and a high concentration of amphotericin B in lung tissue. The clinical efficacy of amphotericin B lipid complex (ABLC) as first-line treatment for documented IA has not been studied. In a large series of case records requested by the US FDA after the approval of ABLC for salvage therapy in the 1990s, clinical outcome data of patients with IA treated with ABLC have been documented. Of 368 patients evaluable, 44% had partial or complete response, and a further 21% of patients showed stable disease.[189] The data confirmed the report on ABLC treatment results from an extensive compassionate-use programme. Here, 42% of 130 patients with IA had shown partial or complete response to ABLC.[190] The daily dose recommended by the manufacturer is 5 mg/kg. In a Spanish report on patients with invasive fungal infections treated with 3 mg/kg/day, 61% of 49 patients with proven or probable IA had a partial or complete response to ABLC.[191] In the light of study results on L-AmB,[158,167] it appears well possible that a daily dose of 3 mg/kg might be sufficient for the treatment of patients with IA. Infusion-related adverse events and nephrotoxicity were observed in 15% and 7% of patients, respectively, receiving 3 mg/kg/day.[191]

Approximately 10% of patients discontinue ABLC treatment because of drug-related adverse events.[191] However, in comparison with D-AmB, the rate of drug-related adverse events, nephrotoxicity and treatment discontinuation is lower with ABLC.[192] Importantly, renal function impaired by antifungal treatment with D-AmB usually improved significantly after a switch to ABLC.[190,193] Renal impairment observed under ABLC treatment has been associated primarily with the coadministration of other nephrotoxic drugs.[194]

3.3.4 Amphotericin B Colloidal Dispersion

A study comparing the efficacy of ABCD 6 mg/kg/day in a randomised comparison with D-AmB (1.0–1.5 mg/kg/day) demonstrated equally poor response rates to both amphotericin B formulations in patients with aspergillosis.[22] By intent-to-treat, complete or partial response had been observed in 15% of patients treated with ABCD. Nephrotoxicity was noted in 25% of ABCD recipients; however, treatment was discontinued because of nephrotoxicity in <4% of patients. The overall rate of drug-related adverse events is similar in patients treated with ABCD and D-AmB, because the incidence of infusion-related adverse events is >50%.[22] As a result, the rate of treatment discontinuation related to toxicity among patients treated with ABCD for IA is ≈22%.[22]

3.3.5 Voriconazole

Voriconazole, a new broad-spectrum triazole, has been studied in a randomised comparison with D-AmB for the treatment of patients with IA. The principal results of this trial are shown in table III. With respect to these results, voriconazole has become the drug of choice for primary treatment of IA.[23]

In a nonrandomised clinical study, a response of 48% to voriconazole was reported in 116 patients, who in part had been refractory or intolerant to other

Table III. Voriconazole compared with amphotericin B deoxycholate for primary treatment of invasive aspergillosis; response rates after 12 weeks (reproduced from Herbrecht et al.,[23] with permission © 2002 Massachussetts Medical Society)

Response	Voriconazole (n = 144) [no. (%)]	Amphotericin B (n = 133) [no. (%)]
Successful outcome*	76 (52.8)*	42 (31.6)
Complete response	30 (20.8)	22 (16.5)
Partial response	46 (31.9)	20 (15.0)
Unsuccessful outcome	68 (47.2)	91 (68.4)
Stable disease	8 (5.6)	8 (6.0)
Failure of therapy	55 (38.2)	78 (58.6)
Indeterminate	5 (3.5)	5 (3.8)

* $p < 0.05$.

antifungal drugs.[195] Of note, voriconazole has shown significant activity (up to 35% response rate) in patients with CNS aspergillosis, so voriconazole appears to be the drug of choice for these patients.[7]

Voriconazole induces transient and reversible visual disturbances in approximately one-third of patients receiving this drug intravenously or orally. The mechanism of this effect is not yet completely understood. Furthermore, it is important to note the potential of drug-drug interactions with a broad spectrum of substances metabolised by, inducing or inhibiting the CYP isoenzyme system. Voriconazole administration is contraindicated in patients receiving astemizole, long-acting barbiturates, carbamazepine, cisapride, quinidine, rifampicin, sirolimus or terfenadine, whereas dose adjustment and/or careful clinical monitoring is required for patients given voriconazole in combination with rifabutin, warfarin, ciclosporin or tacrolimus (monitoring of blood concentrations), sulphonylureas, pravastatin and other HMG-CoA reductase inhibitors (control of serum creatine kinase required), benzodiazepines, vinca alkaloids, omeprazole and phenytoin.

A substantial advantage of voriconazole is the option of oral administration to continue antifungal treatment after the clinical situation of the patient has improved.

3.3.6 Posaconazole

Data on the efficacy of posaconazole for clinical treatment of patients with IA are sparse. Within an extensive compassionate-use programme, 107 patients with IA refractory to or intolerant of other antifungal agents were treated with posaconazole oral suspension. Their outcome was compared with that of 86 control patients treated in the same participating institutions with other antifungals. Complete response was noted in 7% versus 9%, partial response in 36% versus 16%, stable disease in 9% versus 8% and non-response in 36% versus 60% of patients.[196] In patients with neutrophil counts below 500/μL, the response rate dropped to 24%. Posaconazole is available for clinical use in oral form only. It is an inhibitor of CYP3A4, which is responsible for a considerable spectrum of drug-drug interactions;[197] however, these are less pronounced than observed for voriconazole (section 3.3.5).

3.3.7 Itraconazole

Although itraconazole was licensed for clinical use more than a decade ago, no clear data on its efficacy in primary treatment of IA are available. Most reports of clinical response data represent empirical treatment,[198] retrospective observational studies,[199] e.g. from compassionate use in a variety of patients or from nonrandomised trials.[200,201] In a study conducted by the Mycoses Study Group,[200] a favourable response was reported in 39% of 76 evaluable patients with various underlying diseases including tracheobronchial aspergillosis. A total of 30% of patients discontinued itraconazole, mainly because of tolerance problems. Until recently, no intravenous formulation of itraconazole was available for clinical use, and only a small number of patients with IA were treated with itraconazole capsules or itraconazole cyclodextrin solution for primary therapy of aspergillosis. The only published prospectively randomised study comparing itraconazole capsules (200mg every 12 hours) with intermediate-dose D-AmB (0.6 mg/kg/day) showed a response in six of eight patients given itraconazole and two of five patients receiving D-AmB.[202] The number of patients studied here is much too small to allow any conclusion on the efficacy of itraconazole in patients with aspergillosis. The bioavailability of itraconazole capsules is unpredictable in individual patients. The bioavailability is improved significant-

ly with administration of itraconazole cyclodextrin solution; however, the tolerability of this solution is limited by gastrointestinal discomfort. It might be preferable for oral itraconazole treatment to combine capsules and solution, or to choose the best oral formulation on an individual basis and to adjust the daily dose by serial determination of plasma trough concentrations. This trough concentration should be higher than 500 ng/mL in order to achieve a reliable antifungal efficacy of the drug.[203]

The development of an intravenous formulation of itraconazole significantly facilitates the clinical application of this antifungal and allows the assessment of the efficacy of itraconazole for primary treatment of IA. In a nonrandomised clinical trial, 31 patients, the majority of whom were neutropenic and had haematological malignancies, received intravenous itraconazole for primary treatment of IA.[201] A partial or complete response was noted in 48% and stable disease in a further 19% of patients. Of note, itraconazole had to be discontinued as a result of adverse events in 11 of 31 patients, and in 5 of these patients, these adverse events were obviously itraconazole related.

Another critical aspect of the clinical application of itraconazole is the interaction of this drug with the CYP isoenzyme system. Table IV shows a list of drugs for which combination with itraconazole is contraindicated or requires dose adjustment. Taking into account that interactions with cyclophosphamide potentially resulting in higher hepatic and renal toxicity[204] and that cardiac adverse events in terms of negative inotropic effects have been documented,[205] itraconazole has no role in the primary treatment of IA to date. However, it may be considered for oral maintenance treatment in patients who have responded to intravenous antifungal treatment and who have no contraindications (intolerance, comedication, chronic renal or hepatic insufficiency). Monitoring of adequate drug concentrations may be recommended for those patients.

Table IV. Interaction of itraconazole with other drugs

Drug	Type of interaction with itraconazole
Diphenylhydantoin, rifampicin	Accelerated itraconazole elimination
Astemizole, lovastatin and other HMG-CoA reductase inhibitors (statins), midazolam, triazolam, ciclosporin, tacrolimus, vinca alkaloids, digoxin, rifampicin, rifabutin, calcium channel antagonists, oral anticoagulants, busulfan, sulphonylureas, indinavir, saquinavir, nelfinavir	Enhanced activity of these drugs
Antacids, sucralfate, histamine H_2 receptor antagonists, proton pump inhibitors	Reduced itraconazole absorption
Isoniazid	Lower plasma itraconazole concentration

3.3.8 Caspofungin

A significant proportion of patients with IA will not respond to treatment with amphotericin B in conventional or lipid formulation or to voriconazole. In part, they are refractory to this therapy; in part they do not tolerate it. For these patients, echinocandins such as caspofungin, a semisynthetic echinocandin, represents an efficacious treatment alternative. Echinocandins attack the synthesis of 1,3-β-D-glucans, which are essential components of the fungal cell wall. This mode of action is different from that of polyene or azole antifungals, both targeting ergosterol receptors of the fungal cell membrane. Since 1,3-β-D-glucans are not present in mammalian cells, and echinocandins such as caspofungin do not interact with the CYP isoenzyme system, the tolerability of the echinocandins is remarkably good, and their potential for toxic adverse effects and interactions with other drugs essentially low.

No results from prospective, randomised studies on its use for primary treatment of IA are available for caspofungin. Up to 45% of patients with IA who had been refractory to or intolerant of other antifungal agents showed a successful outcome, defined as a partial or complete response, to salvage antifungal treatment with caspofungin.[206] The different mode of action by which echinocandins attack the fungal cell wall opens the possibility of a combination of two classes of antifungals. Preclinical[207,208] as well as clinical[209-211] studies have indicated synergistic effects of an echinocandin in combination with amphotericin B or voriconazole (section

3.3.11). As yet, no data from controlled clinical trials on echinocandin combination therapies are available.

The clinical tolerability of caspofungin is favourable,[212] with no reported serious drug-related clinical or laboratory adverse event reported among 263 patients treated with caspofungin in different dosages for oral and/or oesophageal candidiasis. Among 83 patients with IA treated with caspofungin because of resistance or intolerance to other antifungal agents, no serious clinical adverse event unequivocally related to caspofungin was noted.[206] The concomitant administration of ciclosporin and caspofungin leads to an increase of plasma caspofungin concentrations and appears to be associated with a slight increase of liver transaminases. Clinically relevant adverse effects have not been observed in patients undergoing allogeneic HSCT being treated with caspofungin in combination with ciclosporin.[213] Since blood concentrations of tacrolimus are lowered by up to 20% when it is given in combination with caspofungin, monitoring of tacrolimus blood concentrations is recommended when this drug combination is administered.[212]

3.3.9 Micafungin

As with caspofungin, no results are available from prospective, randomised studies on the use of micafungin for the treatment of IA. In a small number of patients from Japan, partial or complete response was observed in 6 of 10 patients.[214] In a multinational nonrandomised trial, 331 patients (children and adults) with IA were included for primary treatment with micafungin or salvage treatment with micafungin alone or in combination with other antifungals. The daily dose of micafungin was 75mg (or 1.5 mg/kg for children weighing <40kg) but it could be escalated to >200mg. The mean daily dose given was 108mg in adults and 2.1 mg/kg in children. Of 225 patients who fulfilled diagnostic criteria for IA, 80 (35.6%) showed partial or complete response. Of note, none of seven patients with *A. terreus* infections had responded. Combination with other antifungals had no obvious benefit, and higher daily doses were not reported to be associated with more favourable response rates. The number of adverse events associated with micafungin was low and comparable to that for caspofungin, with 3% of patients discontinuing treatment because of drug-related adverse events. Relevant interactions between micafungin and ciclosporin or tacrolimus were not observed.[215]

3.3.10 Anidulafungin

Anidulafungin is the third licensed echinocandin antifungal. It is not yet approved for treatment of IA in any country. Safety analyses have been performed in neutropenic children at risk for invasive fungal infections[216] and in adults with IA treated with anidulafungin in combination with L-AmB,[217] but data on therapeutic efficacy in patients with documented aspergillosis have not been published to date.

3.3.11 Antifungal Drug Combinations

Considering the fact that combination of antimicrobial drugs is routine practice in the treatment of numerous difficult-to-treat infections such as tuberculosis, it appears obvious to combine antifungal agents with different modes of action for the treatment of IA. Echinocandins, which attack fungi by inhibiting the synthesis of β-D-glucans, have been shown to act synergistically in combination with voriconazole or ravuconazole, which inhibit the fungal ergosterol synthesis, in animal models of IA and severe immunosuppression.[208,218] The combination of an echinocandin with amphotericin B, which acts fungicidally by attacking the ergosterol membrane complex, is less well studied in preclinical models of invasive pulmonary aspergillosis. A combination (or sequential administration) of a triazole such as itraconazole or ravuconazole with amphotericin B appears to be potentially antagonistic in preclinical models of aspergillosis.[219,220]

Prospective, randomised clinical studies on the use of combination antifungal therapy of IA using the triazoles mentioned in sections 3.3.8–9 and echinocandins are not available. Case series from different institutions comparing combination treatment with historical control patients[211] or using antifungal combinations for salvage treatment in patients with refractory aspergillosis[209,210,215] have shown promising[209,211,221] or disappointing[210,215]

results. Overall, available clinical data do not provide sufficient evidence that combining an echinocandin with either a triazole (voriconazole, itraconazole or posaconazole) or an amphotericin B formulation results in a superior outcome compared with antifungal monotherapy using voriconazole or L-AmB.

3.4 Adjunctive Drug Treatment

Sufficient numbers of neutrophil granulocytes are mandatory for a successful treatment of IA. For this reason, patients with long-lasting severe neutropenia are at highest risk for a poor outcome in invasive *Aspergillus* infection.[57,222] The option of shortening the period of chemotherapy-induced neutropenia and of activating granulocyte and macrophage function by the administration of recombinant haematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF), macrophage CSF or granulocyte-macrophage CSF has been considered as a promising option. Irrespective of case reports on the addition of those growth factors to antifungal drug treatment in patients with IA, no controlled clinical trial has yet shown a significant benefit from such a combination. Apart from that, cases of severe pulmonary adverse events[15,223] have been observed among patients treated with CSFs in addition to antifungal treatment. A clinical deterioration in patients with invasive pulmonary aspergillosis may occur during (rapid) neutrophil recovery.[224] Therefore, it is not surprising that the transfusion of granulocytes from HLA-matched, G-CSF-primed donors has not yet shown a clear benefit in neutropenic patients with IA, despite promising results from early pilot studies.[225] Prophylactic granulocyte transfusions to prevent relapse of IA, e.g. in patients undergoing allogeneic HSCT, have been reported to be effective in adult and paediatric patients.[226,227]

3.5 Nonpharmacological Treatment of Invasive Aspergillosis

Surgical resection of lung tissue has been reported to be safe and associated with favourable long-term outcome.[228] In patients with incipient fatal pulmonary haemorrhage, thoracic surgery has also been successfully performed in an emergency.[229] Data from controlled clinical trials are not available, and the effect on patient survival[230] must be critically reconsidered in the light of new diagnostic tools for early detection of IA and the broad spectrum of effective new antifungal agents available today.

4. Prophylaxis of Invasive Aspergillosis

A large number of clinical trials have been conducted to evaluate the efficacy and safety of antifungal drugs given prophylactically.[231] Only a minority of these trials were able to prove a substantial benefit, resulting in heterogeneous recommendations.[157,232] A method to overcome the shortcomings in the design of individual trials can be seen in meta-analyses. Several meta-analyses have been performed but again homogeneous conclusions were not achieved.[233-238]

The advantages of antifungals that are active against *Aspergillus* and have been evaluated prospectively are discussed in the following sections.

4.1 Primary Antifungal Prophylaxis

The clinical situations where a prophylactic approach appears desirable are diverse. The most frequent is where a patient who has never had an invasive fungal infection becomes at risk for a first IA. For this primary antifungal prophylaxis setting the following options have been evaluated in clinical trials.

4.1.1 Amphotericin B Deoxycholate

D-AmB oral solution is frequently used as a prophylactic agent, although it is not systemically active and has thus never been shown to be effective.[232] Prevention of its primary target, invasive candidiasis, or of aspergillosis has not been shown. The latter can obviously not be expected in an infection acquired by inhalation. Therefore, D-AmB has been given by aerosol inhalation. Noncomparative studies were promising,[239,240] but in the only large multicentre trial, inhaled D-AmB did not prevent invasive pulmonary aspergillosis. Frequent ad-

verse events were coughing, bad taste and nausea.[241]

Intravenous D-AmB has been evaluated in dosages ranging from 0.1 mg/kg/day to 1.0 mg/kg three times weekly. Low dosages had no benefit over placebo,[242] and a prospective trial of amphotericin B 0.2 mg/kg/day versus fluconazole 400 mg/day orally in allogeneic and autologous HSCT recipients concluded that D-AmB had comparable efficacy but higher toxicity.[243] The higher dosages frequently result in nephrotoxicity and high withdrawal rates.[244] Nephroprotection through saline loading has evolved over time,[245] and historical controls suggested efficacy of intravenous prophylaxis with D-AmB 1 mg/kg every 48 hours in reducing proven and probable invasive fungal infection.[246,247] Since the D-AmB formulation is a rather toxic drug and its administration requires an experienced team, it has never become a standard in antifungal prophylaxis. Prophylactic use is generally discouraged.

4.1.2 Liposomal Amphotericin B

Prophylactic use of L-AmB is attractive because of its lower toxicity compared with D-AmB, and it has shown efficacy in a murine model.[248] L-AmB has been evaluated in a placebo-controlled, but small study population at 1 mg/kg/day and no significant effect could be detected.[249-251] A second placebo-controlled, but as underpowered, trial also failed to show an advantage of L-AmB 2 mg/kg three times weekly.[252] Recently, a prospective, randomised, open-label clinical trial compared L-AmB 50mg every other day with no prophylaxis in 219 neutropenic episodes of 132 patients. The primary endpoint was defined as the incidence of proven and probable invasive fungal infections; these were found in 7% versus 35% of patients and comprised a significant reduction in IA. Possibly related adverse events in the L-AmB group occurred in 4.6% and none were grade 3 or 4 toxicities.

L-AmB at different doses and routes of administration has been used for prevention of fungal infections in solid organ, predominantly liver, transplantation.[253] A large well designed trial is currently lacking.

4.1.3 Amphotericin B Lipid Complex

Patients with acute myeloid leukaemia (AML) or high-risk myelodysplastic syndrome (MDS) undergoing induction chemotherapy received prophylactic intravenous ABLC 2.5 mg/kg three times weekly. Among 131 ABLC-treated patients the breakthrough rate was 5%.[254]

Depending on the nebulizer system used, ABLC is deposited in the lung of lung transplant recipients.[255] Noncomparative case series in allogeneic HSCT recipients have been reported but cannot replace well designed clinical trials.[256,257]

4.1.4 Amphotericin B Colloidal Dispersion

ABCD has been evaluated in a trial on prophylaxis in neutropenic patients; however, this study was too small to provide valid clinical information.[258]

4.1.5 Itraconazole

Several randomised trials evaluated itraconazole in primary antifungal prophylaxis. A double-blind, double-dummy, placebo-controlled trial compared the oral suspension at a dose of 2.5 mg/kg twice daily plus nystatin 500 000IU four times daily with nystatin alone. Invasive mould infection and death due to fungal infection were not prevented.[259] Lower daily doses of itraconazole oral suspension did not effectively reduce the incidence of invasive fungal infections or mortality.[260]

The intravenous formulation followed by oral itraconazole was evaluated in the allogeneic HSCT setting and compared with fluconazole 400 mg/day. Both regimens were given until day 100 post-transplant. The dose administration schedule appears rather complex, but was chosen to address the loading phase necessary and the clinical circumstances, e.g. the ability of the patient to swallow: days 1–2 400mg intravenously, days 3–14 either 200mg intravenously or switch to 400mg oral solution until day 100. In this study, itraconazole reduced proven invasive fungal infections more effectively than fluconazole, but this did not translate to improved attributable or overall mortality.[4] The study has been criticised for being underpowered because it only included 140 patients.[261] Another controlled trial in allogeneic transplant recipients compared

intravenous itraconazole 200 mg/day or oral suspension 7.5 mg/kg/day with intravenous or oral fluconazole 400 mg/day. In the intent-to-treat population no statistically significant reduction of breakthrough invasive fungal infections was accomplished. Itraconazole patients had a lower mould infection rate of 5% versus 12%, but this was accompanied by a higher rate of liver and kidney toxicity leading to a withdrawal rate of 36% compared with 16% in the fluconazole group. Overall mortality and fungal-free survival were not improved by itraconazole.[31] In fact, the itraconazole group had a significantly shorter survival time until the protocol was amended to avoid concomitant itraconazole and cyclophosphamide administration.

A recently published large, randomised, open-label trial compared itraconazole oral solution 2.5 mg/kg twice daily and fluconazole oral solution 400 mg/day. No difference in efficacy was found between the two regimens and IA was diagnosed at a rate of 1% in each group. The withdrawal rates due to adverse events were 36% and 28% for itraconazole and fluconazole, respectively.[262] Another trial on the same drugs yielded basically identical results.[263]

High withdrawal rates from itraconazole oral solution 2.5 mg/kg twice daily and 400 mg/day had been previously reported in the non-transplant setting. Usually these were caused by gastrointestinal adverse events.[259,264] Besides intense patient motivation to overcome the unpleasant taste of the oral suspension, the use of itraconazole demands plasma concentration monitoring on a regular basis, i.e. at least twice weekly, to ensure plasma concentrations >500 ng/mL.[265]

Seventy-one adults undergoing orthotopic liver transplantation were randomly allocated to either itraconazole 5 mg/kg/day orally or placebo. Prophylaxis with itraconazole effectively prevented fungal infections (4% vs 24%).[266]

4.1.6 Voriconazole

Voriconazole 200mg twice daily in tablet form has been used prophylactically in a placebo-controlled trial of patients with AML to prevent lung infiltrates, i.e. the trigger for antifungal treatment in this patient population. Because of ethical concerns referring to the placebo arm, the study was prematurely stopped when results of two posaconazole prophylaxis trials became available. However, the incidences of lung infiltrates were 33% versus none in the placebo group.[267] Currently, two large trials in allogeneic HSCT recipients are comparing voriconazole with fluconazole (ClinicalTrials.gov Identifier NCT00322088) and voriconazole with itraconazole (NCT00289991).

Voriconazole is frequently used in solid organ transplantation. A retrospective series of lung transplant recipients received either voriconazole or, if colonised with *Aspergillus*, itraconazole with or without amphotericin B inhalation. Voriconazole was found to be more effective in preventing IA. Fourteen percent of patients in the voriconazole group compared with 8% in the itraconazole group discontinued because of adverse effects.[268]

4.1.7 Posaconazole

Posaconazole has recently been shown to effectively prevent IA in patients undergoing induction chemotherapy for AML or MDS. The incidence rate of invasive fungal infections (mainly *Aspergillosis*) was 2% versus 8% in the standard azole (fluconazole or itraconazole) comparator arm.[269] In an intention-to-treat analysis, overall and attributable mortality were also significantly improved.[270] In a second large, randomised trial in patients developing high-grade GVHD, the rates of IA with posaconazole and fluconazole were 1% and 6%, respectively. Attributable mortality was improved in this trial also but crude mortality was not significantly decreased.[271]

4.1.8 Caspofungin

Caspofungin 50 mg/day has been compared in a single clinical trial versus intravenous itraconazole 200 mg/day enrolling patients with AML or MDS. Efficacy and safety was similar for both groups. IA occurred in 2% and 1%, respectively.[272]

4.1.9 Micafungin

In a large double-blind trial comparing micafungin 1 mg/kg/day and fluconazole 400 mg/day, invasive candidiasis was effectively prevented by both

regimens, but micafungin was more effective against IA. No significant reduction of the overall and attributable fungal mortality was detected. Unfortunately, the study population comprised autologous and allogeneic HSCT recipients with various underlying malignant diseases.[273] The results are difficult to put into the context of other trials, since for the 46% autologous transplant patients the two treatments are equally experimental.[274]

4.2 Secondary Antifungal Prophylaxis

A particularly difficult question is how to proceed with a survivor of IA who is in need of further profoundly immunosuppressive therapy. Treatment of proven or probable invasive fungal infection is long lasting and requires administration of antifungals well after the end of neutropenia to prevent early relapse of infection.[151,152,157] Continued antifungal treatment is difficult to differentiate from secondary prophylaxis. The prospective definition of risk factors and the evaluation of therapeutic strategies are medical needs still unmet.

However, a variety of risk factors for relapse of infection have been identified in HSCT recipients in mostly retrospective studies. In a population of 48 patients with an overall relapse rate of 33%, the lack of systemically active secondary prophylaxis resulted in a relapse rate of 57%.[275] The largest cohort reported comprised 395 allogeneic HSCT recipients. IA occurred as a second invasive fungal infection or a relapse in 8%. Multivariate analysis revealed moderate to severe GVHD and corticosteroid prophylaxis for GVHD as risk factors.[24]

Among 129 patients with a history of probable or proven IA undergoing allogeneic HSCT, 22% of patients progressed during the 2 years after transplant. A total of seven risk factors for relapse of infection were identified: (i) longer duration of neutropenia after transplant; (ii) advanced status of underlying disease; (iii) <6 weeks of systemic antifungal treatment before allogeneic HSCT; (iv) conventional myeloablative conditioning; (v) CMV disease; (vi) bone marrow or cord blood as source of stem cells; and (vii) grade II–IV acute GVHD.[62] A small group of 11 patients with leukaemia and prior *Aspergillosis* infection received secondary prophylaxis with voriconazole 400 mg/day during allogeneic HSCT. None of the patients had a relapse of the fungal infection.[276]

In a prospective clinical cohort of 166 patients with AML and prior proven or probable pulmonary invasive fungal infection who underwent chemotherapy, we found a 16% rate of breakthrough infections. Risk factors for breakthrough invasive fungal infection were identified by logistic regression and comprised duration of neutropenia, recent high-dose cytarabine and the total number of different antibacterials received. In this population, lack of systemically active prophylaxis was not a risk factor.[277]

4.3 Patient Isolation

Various isolation precautions have been applied over time. These range from a simple reverse isolation with the patient wearing a surgical face mask to elaborate air filtering and climate-control systems, including laminar air flow as used in operating theatres as well as HEPA filters. It is generally believed that reverse isolation is not beneficial and that the technical solutions above are advantageous. They have been proven to almost eliminate the number of colony-forming units in room air. However, well designed clinical trials randomising types of air conditioning usually failed or lacked statistical power.[278] A specific obstacle is the patient being moved from one room to another. Moreover, patients may be exposed to construction work intermittently, e.g. on the way to imaging units during evaluation for febrile episodes. Meta-analyses may not be the appropriate tool to obtain evidence for air-conditioning technology.[279-281]

5. Conclusion

IA affects a broad spectrum of patients at risk, including primarily those with acute leukaemia and recipients of HSCT or solid organ transplant. While increasing morbidity and mortality rates due to IA are reported from the majority of centres involved in the treatment of these patient groups, new diagnostic and therapeutic options have become available during the past decade. Early clinical diagnosis is

based on pulmonary CT scan findings and non-culture-based diagnostic techniques such as galactomannan or DNA detection in blood or bronchoalveolar lavage samples. The most favourable results are achieved in patients in whom early antifungal treatment has been started pre-emptively backed up by these findings. The gold standard of systemic antifungal treatment is voriconazole (6 mg/kg every 12 hours on day 1, 4 mg/kg every 12 hours maintenance dose), which is significantly superior to D-AmB and has led to a large improvement of survival rates in patients with cerebral aspergillosis. L-AmB at a standard dosage of 3 mg/kg once daily appears to be a suitable alternative for primary treatment, while caspofungin (70mg once-daily loading dose on day 1, 50mg once-daily maintenance dose), ABLC (5 mg/kg once daily) or posaconazole (400mg every 12 hours or 200mg every 6 hours) have yielded partial or complete responses in 40–50% of patients refractory to or intolerant of primary antifungal therapy.

Combination therapy using two antifungal compounds may be a promising future strategy for first-line treatment; however, results of properly designed clinical trials are still lacking. Lung resection may help to prevent fatal haemorrhage in individual patients with pulmonary lesions located in close proximity to larger blood vessels, but is primarily considered for reducing the risk of relapse during subsequent periods of severe immunosuppression.

For prophylaxis, strict reverse isolation appears to be effective in allogeneic HSCT recipients and patients with AML undergoing aggressive anticancer therapy; however, prospective randomised studies on infection control measures effective to prevent aspergillosis are lacking. Prophylactic systemic antifungal treatment with oral posaconazole (200mg every 8 hours) significantly improves survival and reduces IA in AML patients and reduces aspergillosis incidence rates in patients with intermediate to severe GVHD arising after allogeneic HSCT.

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