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REVIEW

Diagnosis and treatment of invasive pulmonary aspergillosis in neutropenic patients

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Diagnosis and treatment of invasive pulmonary aspergillosis in neutropenic patients. F. Reichenberger, J.M. Habicht, A. Gratwohl, M. Tamm. ©ERS Journals Ltd 2002.
ABSTRACT: Invasive pulmonary aspergillosis is a major cause of morbidity and mortality in neutropenic patients.

Microbiological and serological tests are of limited value. The diagnosis should be considered in neutropenic patients with fever not responding to antibiotics, and typical findings on thoracic computed tomography scan. Whenever possible, diagnosis should be confirmed by tissue examination. Newer techniques, such as polymerase chain reaction may change the current diagnostic approach.

Therapeutic strategies consist of prophylaxis in risk groups and the early application of antifungal agents in suspected or probable disease. Amphotericin B as desoxycholate or lipid formulation is the current standard medication in invasive infection, although it has major side effects. Its role is challenged by the new azole derivates, such as itraconazole and voriconazole, and the new echinocandins. Additional therapies with cytokines, such as granulocyte macrophage colony stimulating factor and interferon-γ, and with granulocyte transfusions are under evaluation. In selected cases lung resection is of proven diagnostic and therapeutic value.

This paper analyses the current understanding of the pathogenesis and epidemiology of invasive aspergillosis and reviews the actual diagnostic and therapeutic strategies for invasive pulmonary aspergillosis in neutropenic patients.
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Neutropenic patients are at high risk of infectious complications. Infection with *Aspergillus* spp. is one of the most serious, because it is difficult to diagnose and it is associated with a high mortality despite adequate therapy [1, 2].

Aspergillus is an ubiquitous mould commonly found in humid areas or damp soil. Among 350 species, only seven are facultatively pathogenic. *A. fumigatus* is the most frequent species found in 90% of infections. Other pathogenic species include *A. flavus*, *A. niger*, *A. oryazeae*, *A. vesicolor*, *A. terreus*, and *A. nidulans*. The infectious agent is the conidium of 1–3 μm in diameter, which can be carried by air. After germination, aspergillus grows with 45° dichotomous branching of hyphae of 2–5 μm diameter, which are able to invade tissue [3].

Antifungal defence in humans is based on normal mucosal barriers, macrophage and neutrophil function. The latter can kill conidiae and inhibit the germination of hyphae. Tumour necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-1α are macrophage derived cytokines and crucial in the defence against fungal infections [4]. MIP-1α is a C-C chemokine with chemotactic and leukocyte activating properties. Both TNF-α and MIP-1α are released by alveolar macrophages when exposed to aspergillus conidiae. In neutropenia, TNF-α and MIP-1α are reduced. Antibody mediated blocking of either TNF-α or MIP-1α leads to pulmonary invasion of fungal hyphae in non-neutropenic mice. Intratracheal instillation of biologically active TNF-α prior to aspergillus inoculation was associated with a better survival in neutropenic mice [5]. In contrast, aspergillus spores release factors, that can suppress the synthesis of pro-inflammatory cytokines such as interleukin (IL)-1, and TNF-α in macrophages at the transcriptional levels by inhibition of the transcription factor nuclear factor-κB and activation protein-1 [6].

T-cell function is also important in the development of invasive aspergillosis. Aspergillus antigens are able to induce T-helper (Th)1 and Th2 type reactivity. Th1-reactivity is displayed by an increase of interferon (IFN)-γ and IL-12 and has protective effects on the infection. However, Th2 reactivity is characterized by production of IL-4 and IL-10 and leads to disease progression in a murine model of invasive pulmonary aspergillosis (IPA) [7]. The therapeutic consequences of these findings are discussed later.

Epidemiology and risk factors

Invasive aspergillosis (IA) is a major problem in immunocompromized patients, as in human

immunodeficiency virus (HIV) infection, after solid organ transplantation, under immunosuppressive or steroid therapy, and in chronic granulomatous disease [8–10]. The highest risk is in neutropenia, where the lungs are affected in 90% of cases [11].

Invasive fungal infections are most commonly caused by *Candida* spp. However, while the number of invasive Candida infections declined, there was a ten-fold increase in invasive fungal infections between 1978–1992, mostly caused by *Aspergillus* spp. [12]. Other pathogenic moulds are Fusaria, Mucor, Alternaria, and Scedosporium. The differentiation is important because of the different susceptibility to antifungal therapy [13, 14]. The present review focuses on IPA in neutropenia.

Prolonged neutropenia with a granulocyte count $<500 \cdot \mu L^{-1}$ for >20 days is the strongest risk factor for IA [3]. The risk of developing IA increases with the length of neutropenia and reaches a plateau of 70% after 34 days of granulocytopenia [1].

Patients undergoing myeloablative chemotherapy or stem cell transplantation (SCT) for haematological malignancies are especially at risk of IPA. The frequency of IA in allogeneic SCT recipients is between 5–15% [13, 15, 16]. Most cases are found in acute myeloid leukaemia (AML). Surprisingly, the outcome in AML patients seems to be better than that of acute lymphoblastic leukaemia or lymphoma patients, probably due to the greater use of corticosteroids in the latter groups [17]. Steroids inhibit macrophage function and predispose to IA [18]. A dose >0.5 mg prednisone equivalent·kg body weight^{-1} for >30 days is regarded as a substantial risk for IA [16]. IPA develops in ~20% of allogeneic haematopoietic SCT recipients [19].

After SCT, IA occurs in 2 phases, an early phase ~16 days after SCT, and a late phase ~96 days after SCT. Several risk factors of developing IA for both phases have been described (Relative Risk): In the early phase: nonfirst remission of haematological malignancy (8.9), underlying disease (aplastic anaemia, myelodysplasia, nonhaematological malignancy) (5.8), lack of a laminar air-flow room (5.6), SCT in summer (4.4), SCT in autumn (2.2), allogeneic SCT with human leukocyte antigen mismatches (2.1). In the late phase: delayed transplant engraftment with prolonged neutropenia (6.0), age >40 yrs (5.0), underlying disease (aplastic anaemia, myelodysplasia, nonhaematological malignancy) (3.7), first remission of the haematological disease (3.6), chronic graft *versus* host disease (gvhd) requiring treatment with corticosteroids (3.1), nonfirst remission of the haematological disease (3.0), age 20–40 yrs (3.0), acute gvhd >grade 2 (2.6). Further risk factors are a positive cytomegalovirus serostatus at SCT and construction works [13, 14, 16, 20].

The occurrence of aspergillus infection has been clearly related to building hygiene and construction work [21–23]. Building activities have been shown to increase the concentration of aspergillus conidiae in the air with subsequent development of IA [22, 24, 25]. Although this conjunction is still under discussion [21, 26, 27]. The use of high efficiency particulate air (HEPA) filtration units with laminar air flow could markedly reduce the amount of contamination with aspergillus conidiae and the subsequent development of IA [24, 28].

Diagnosis of invasive pulmonary aspergillosis

Clinical signs

Clinical signs are nonspecific, but characteristic. The occurrence of fever despite appropriate antibiotic therapy for >96 h in neutropenic patients is suspicious for IA [29]. Chest pain during breathing and cough are present in ~20% of IPA cases [30].

Haemoptysis is not an initial symptom of IPA. It occurs when granulocytopenia resolves [31]. The leukocyte reconstitution leads to an overwhelming inflammatory response in the infected lung with local necrosis of the pulmonary parenchyma [32]. Life threatening pulmonary bleeding may occur, and therefore haemoptysis is regarded as a poor prognostic sign in IPA [33].

Radiology

Radiological imaging is the cornerstone of the diagnosis of IPA, when pulmonary changes develop in neutropenic patients with antibiotic resistant fever. Infiltrates and macronodules are nonspecific changes representing very early infectious consolidation. The appearance of a haemorrhagic pulmonary nodule, termed "halo sign" is typical for IPA. It consists of a nodule-like centre of $\geqslant 50\%$ (dense fungus ball), surrounded by $\geqslant 180^{\circ}$ ground glass attenuation (coagulation necrosis and haemorrhagic infarction) [34]. The halo is present over a short period of 5–14 days after onset of IPA and has also been found in patients with other pulmonary disorders, such as alveolar haemorrhage, bronchiolitis obliterans organizing pneumonia, viral infections, Kaposi's sarcoma, Wegener's granulomatosis, and angiosarcoma [35, 36, 37]. Based on the few available data its specificity can be calculated at 80% [38]. The air crescent sign, indicating the development of necrosis, has a sensitivity of 48–68% and develops mainly from larger consolidations or masses at the time of bone marrow recovery [39]. A cavitary lesion is the late stage of IPA [40].

Plain chest radiography is too insensitive for the diagnosis of IPA. In the early stages nonspecific infiltrates or nodular lesions may be present and the halo sign is not detectable. In the later course of the disease the air crescent sign and cavitation may become visible on plain films [41, 42].

Thoracic computed tomography (CT) scan is the most sensitive radiological method able to detect early changes of IPA (fig. 1a). CT should be performed early in neutropenic patients with antibiotic resistant fever and further clinical signs for IPA [43]. Some investigators perform weekly CT for early detection of IPA [33]. Notably, the volume of the IPA lesion can increase three- to four-fold within the first 7 days despite adequate antifungal treatment [35]. Ultrafast CT with reduced scanning time has been tested for

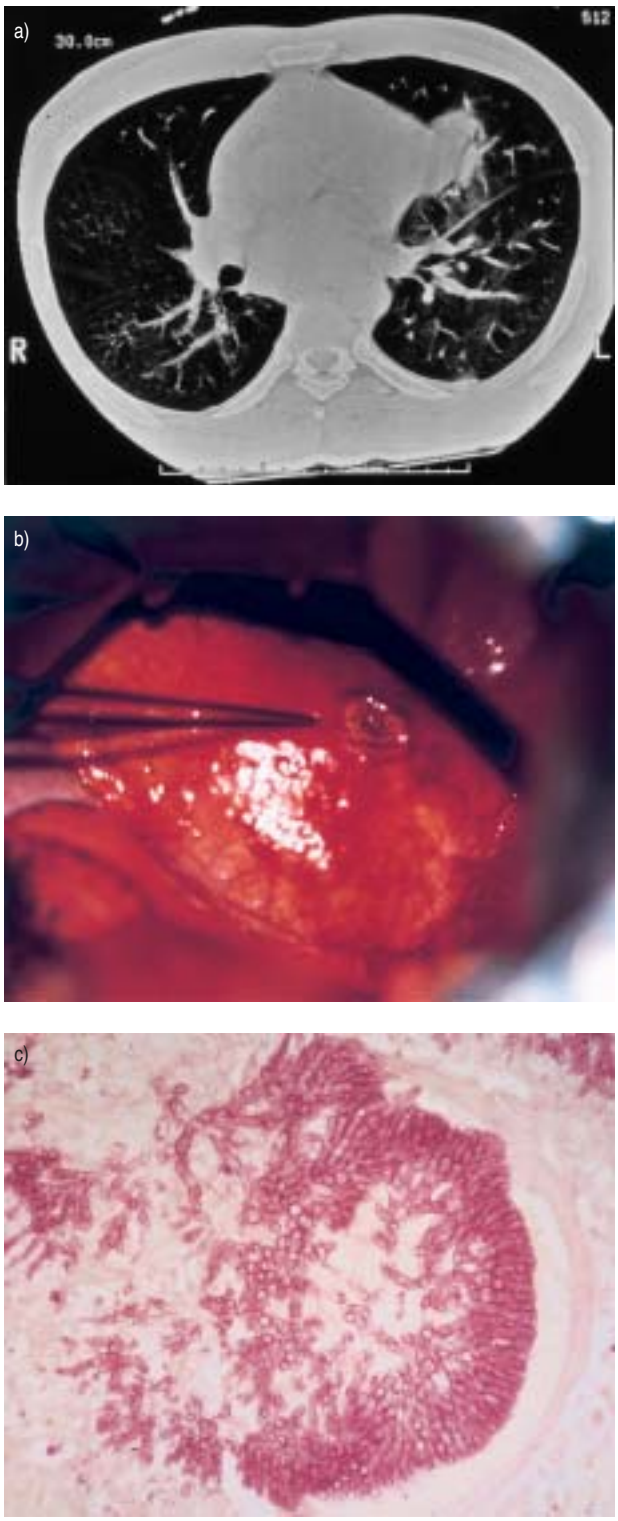

Fig. 1. – a) Thoracic computed tomography scan of a patient with antibiotic resistant neutropenic fever showing a nodular infiltrate in the lingula. When initial antimycotic therapy failed, the patient was scheduled for lung resection. b) The intraoperative appearence during lung resection in the same patient: the invasive fungal infection infiltrates the pericardium. c) Histology of the resected lingula confirmed invasive aspergillosis with fungal hyphae infiltrating the surrounding tissue.

monitoring of IPA in animal studies [44]. Combining the halo sign and the air crescent sign the sensitivity is >80% for the diagnosis of invasive mould infection [35, 36, 39–41, 43, 45, 46]. However, only a few studies have analysed the specificity of these findings reaching 60–98% [36, 45]. Based on their own experience, the present authors believe that the new appearance of pulmonary consolidation or infiltrate on a thoracic CT scan in a neutropenic patient with antibiotic resistant fever is already suspicious of IPA [30, 47]. Thoracic CT scanning is the most important diagnostic tool in IPA and has a prognostic impact on the outcome by detection of early changes (table 1).

Magnetic resonance imaging (MRI) has not been extensively studied in IPA so far. The typical pattern of an isointense nodular lesion on a T1-weighted image and a hyperintense centre on T2-weighted image (target sign) with Gadolinium-Diethylenetriamine pentaacetic acid (Gd-DTPA) enhanced rim margin (perilesional haemorrhagic infarction) is present in the later course of the infection. Actually, early diagnosis of IPA can not be achieved by MRI [41].

^{18}F-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) is a sensitive method to image inflammatory processes such as hypermetabolic foci. FDG-PET has been sporadically used for monitoring of IA [8]. The usefulness of FDG-PET for the management of IPA requires further evaluation.

Laboratory studies

Serological testing for IPA is based on detection of antigens. Antibody testing is not useful in neutropenic patients because of the impaired antigen presentation and lymphocyte function [15].

Antigen testing is based on the Galactomannan (GM) antigen, a polysaccharide of the fungal wall, utilized in several test systems. Latex agglutination with monoclonal antibodies (mAb), which recognizes the (1->5)-β-D-galactofuranoside side chain of the GM, is the commonly used test (Pastorex®) with a sensitivity of 13–95% and specificity of 86–100% [43, 45, 48–50]. A cross reactivity of GM-mAb is known against *Penicillium* spp., and cytotoxic drugs [50].

Enzyme-linked immunosorbent assay (ELISA) of GM antigen using the same antibody for capturing and detection has a 10–15-fold higher sensitivity than latex agglutination [48, 49]. In some studies serum was serially tested for circulating antigen 1–3 times weekly [45, 48–52). An initial study of 19 patients with IPA showed a sensitivity of 95% and a specificity of 99–100% for ELISA using either monoclonal or polyclonal antibodies [50]. Studies using the commercially available GM-ELISA with mAb (Platelia®) reached a sensitivity between 60–95% and specificity of 81–100% in localized and disseminated IPA [48, 49, 51, 53]. A prospective study of serial testing of GM-ELISA in 362 cases of disseminated IA, among them 72 autopsy controlled cases, confirmed a sensitivity of 90–93%, a specificity of 95–98%, a positive predictive value (ppv) of 87–93%, a negative predictive value (npv) of 95–98%, and a false positive rate of 8–14% [52, 54]. In addition, an inhibition ELISA against (1–3)-β-D-glucan (BDG), a carbohydrate antigen of the aspergillus conidiae of all subspecies, has been

Table 1. – Value of different diagnostic methods in invasive pulmonary aspergillosis

Diagnostic methods	Sensitivity	Specificity	Comments
Thoracic CT scan			
Halo/air crescent	>80	60–98	Specificity only assessed in a few studies, findings depend on the disease stage
Serum			
Galactomannan Antigen			
Latex agglutination (Pastorex®)	13–95	86–100	Cross reactivity against penicillium
ELISA (Platelia®)	90–93	95–98	ppv 87–93, npv 95–98 False positive rate 8–14 To measure 1–3 times weekly
BDG-ELISA	16–90	76–100	ppv 59, npv 97
PCR	100	65–92	ppv 15–44, npv 100 Useful to exclude aspergillus
Bronchoscopy with BAL			
Culture	43	100	Use of Sabouraud medium preferred
Galactomannan antigen	0–80	65–95	Highly variable results
PCR	67–100	55–95	ppv 20–46, npv 93–100 Useful to exclude aspergillus

Data are presented as % unless otherwise stated. CT: computed tomography; ELISA: enzyme-linked immunosorbent assay; BDG: (1-3)-β-D-glucan; PCR: polymerase chain reaction; BAL: bronchoalveolar lavage; ppv: positive predictive value; npv: negative predictive value.

developed with a sensitivity of 16–90%, a specificity of 76–100%, ppv of 59% and npv of 97% for IA [45, 55]. Recently, the 18 kDa protein mitogillin has been isolated from *A. fumigatus*, and a role for this antigen in the serodiagnosis of IPA has been suggested [56]. Sensitivity of antigen testing is dependent on the spread of the disease. In localized disease, as in IPA, sensitivity of circulating antigen is significantly lower than in disseminated IA [45, 57].

The sensitivity of antigen testing depends on the severity of disease. In localized disease sensitivity is lower than in disseminated disease. Antigen testing should be performed at least once per week in neutropenia for complementary diagnostic screening. Its use for evaluation of a pre-emptive antifungal therapy should be considered.

Polymerase chain reaction (PCR) in serum for *Aspergillus* spp. was introduced in 1996, using a nested PCR method [58]. Target genes are the 135-bp fragment in the mitochondrial DNA [59], the multicopy 18S ribosomal ribonucleic acid (rRNA) in *Aspergillus* spp. [58–63], and the 401-bp fragment in the ribosomal deoxyribonucleic acid (rDNA) complex of *A. fumigatus* [64]. Several methods including real time PCR, nested PCR, and two step PCR have been described [58–66]. A differentiation of the subspecies of *Aspergillus* spp. at genomic level is feasible using the internal transcribed spacer regions between 18S and the 28S rRNA [67]. PCR testing is of promise but under evaluation. So far the results are highly variable. The largest published study shows a sensitivity of 100%, a specificity of 65–92%, a ppv between 15–44%, and a npv of 100% [60]. These results were achieved by serial testing [59–62]. A study comparing the diagnostic sensitivity of PCR *versus* GM-ELISA revealed a higher sensitivity of ELISA of 40% *versus* PCR of 10% [59]. Further data from prospective histology controlled studies are needed to evaluate this method (table 1).

Bronchoscopy

Bronchoscopy with bronchoalveolar lavage (BAL) is an established tool for the diagnosis of infectious complications in neutropenic patients [68] as well as after bone marrow transplantation [69]. Its value in the diagnosis of IPA, however, requires critical analysis [70]. Aspergillus can be found in the BAL or in bronchial washings by culture, by microscopy with detection of mould hyphae, by detection of the aspergillus antigen, or by PCR.

For identification of aspergillus, BAL is cultured in Sabouraud medium, a fungal culture medium that is superior to the routine bacterial culture medium [71]. Microscopy of BAL for mould hyphae is usually performed after haematoxylin-eosin or Grocott's staining.

Recently the present authors reviewed the value of aspergillus culture and microscopy in BAL and found a sensitivity of 43% and a specificity of 100% in histologically proven cases of IPA. BAL was more often positive in multilobular IPA than in localized disease, irrespective of the duration of pretreatment with Amphotericin B (AmB) [72]. The sensitivity for detecting IPA increases with multiple cultures [71]. It has been reported that a positive fungal culture from secretion indicates a poor prognosis [33].

Detection of aspergillus antigen in BAL has been studied by only few groups. After early promising results for radio-immunoassay of GM-antigen [73], further studies reported a sensitivity between 0–80% and a specificity of 65–70% using GM antigen latex agglutination in histologically proven IPA [43, 74], and 37–67% sensitivity and 95% specificity for GM-ELISA in probable IPA [75].

PCR in BAL was introduced in 1993 as an indicator of aspergillus infection or colonization. Since then results with several PCR systems and primers have been reported. PCR in BAL has an estimated

sensitivity of 67–100% and a specificity between 55–95%, a ppv ranging between 20–46% and a npv between 93–100%. This technique is useful to exclude aspergillus infection [63, 66, 75–77] (table 1).

Tissue examination

Tissue examination is performed for definite diagnosis or exclusion of IPA. Furthermore, it enables the distinction between colonization and invasive infection to be made, and allows other invasive infections requiring different therapy to be diagnosed.

Transbronchial biopsy (TBB) is an established method in the diagnosis of invasive pulmonary infections in immunocompromized patients. But TBB requires a thrombocyte count of $\geqslant 50{,}000 \cdot \mu L^{-1}$ to avoid a greater risk of bleeding, and this technique can not be routinely performed in pancytopenic patients [78].

CT guided percutaneous lung biopsy has been performed with a diagnostic yield of 80–100%. This procedure requires a thrombocyte count $>60{,}000 \cdot \mu l^{-1}$, but nevertheless bleeding occurs in 46% of cases. Furthermore, it carries a high risk of pneumothorax [37]. This procedure can not be recommended in neutropenic patients.

Open lung biopsy has been advocated for confirmation of the underlying disease. However these patients are high-risk candidates for perioperative complications, and surgery for diagnostic reasons alone is not advisable [79]. The value of lung resection in the therapy of IPA is discussed below.

Classification of the invasive pulmonary aspergillosis diagnosis

Because of the difficulties in diagnosis of IPA, the Mycosis Study Group suggested the following definitions [17, 80, 81]: 1) Definite IPA: septate branching hyphae in tissue histopathology, or positive culture from tissue obtained by an invasive procedure; 2) Probable IPA: appearance of new nodules or cavities on a chest radiograph in neutropenic patients, receipt of a cytotoxic agent for a malignant or immunological disease, steroid use of >10 mg prednisone equivalent or congenital or acquired immunodeficiency. Two sputum cultures or one BAL, bronchial washing/brushing culture positive for *Aspergillus* spp. or cytological examination on BAL showing characteristic hyphae, or two positive PCR for aspergillus in the BAL; 3) Possible IPA: radiological findings typical of invasive aspergillosis such as a halo sign or cavitation in neutropenic or previously neutropenic patients and positive sputum or endotracheal culture for *Aspergillus* spp.

Treatment of invasive pulmonary aspergillosis

Prophylaxis

Patients at high risk of developing IPA should be identified prior to myeloablative therapy. Isolation of these patients and use of a HEPA filtration during neutropenia should be mandatory [21, 24, 28, 82]. Medical prophylaxis of IPA is discussed later.

Amphotericin B

AmB is an ergosterol-binding polyene leading to disintegration of the fungal membrane. Since its development in 1952, different formulations of AmB have been developed. The conventional formulation is AmB-desoxycholate (cAmB). This is the standard therapy for IPA at a dosage of 1–1.5 mg·kg body-weight^{-1}·day^{-1} [83]. The recommended run-in phase with reduced dose can be safely decreased to 24–48 h [29]. Sometimes fever and shivering occur during cAmB infusion. Typical side-effects are electrolyte imbalance and progressive renal failure despite adequate prophylaxis in ~50% of patients, requiring dose adjustment. Electrolyte imbalance can be reduced by appropriate substitution and infusion of electrolytes prior to cAmB application. cAmB itself should be given solely in 5% glucose solution. Continuous infusion of cAmB over 24 h can significantly reduce nephrotoxicity [84]. Approximately 33–54% of patients with IA respond to cAmB therapy [80], however mortality exceeds 64–90% despite adequate treatment [1, 2, 85, 86].

Local administration of cAmB, an effective therapeutic option in aspergilloma, has been performed occasionally in IPA [87]. Percutaneous CT guided application of AmB has been proven to be effective in a small series of IPA [88]. However, thrombocyte counts must be $>50{,}000 \cdot \mu L^{-1}$ to prevent bleeding, and there is a substantial risk of pneumothorax. Endobronchial instillation of AmB has been reported with variable results [89]. A small series of patients successfully treated with a combination therapy of systemic and local AmB has been reported [90].

In the therapy of antibiotic resistant neutropenic fever of unknown aetiology the systemic application of cAmB is recommended [91, 92]. Application of AmB has not been shown to be beneficial for prophylaxis of IPA in neutropenia [93]. Low dose intravenous cAmB, inhalation of cAmB as well as intranasal cAmB application are well tolerated, but failed to be effective in prevention of IPA [21, 82, 94, 95].

Lipid-bound formulations of AmB exhibit the same microbiological activity and are well tolerated. Because they are less nephrotoxic than cAmB, higher doses of the active antifungal compound can be administered [96]. The overall response rate is around 40–70% [86]. Lipid bound AmB should be used in patients with IPA, who have severe side-effects or fail to respond to cAmB therapy [97].

Liposomal AmB (AmBisome®) achieves response rates of 30–60% in IA. It is less nephrotoxic than cAmB. The recommended dose ranges between 1–3 mg·kg^{-1}·day^{-1}, but can be increased to 6 mg·kg^{-1}. However, reports of beneficial effects of doses >3 mg·kg^{-1} are discrepant [85, 98, 99]. In febrile neutropenia, AmBisome® in a dose of 3 mg·kg^{-1} is as effective as cAmB, but is associated with less side-effects, nephrotoxicity, and breakthrough fungal

infections [83]. The prophylactic administration of AmBisome® three-times weekly could reduce the rate of fungal colonization but not of invasive fungal infections in neutropenic patients [100].

Colloid dispersion of AmB (Amphocil®) consists of an equimolar mixture of AmB and cholesteryl-sulfate. The recommended dose is 3–4 mg·kg body-weight^{-1}·day^{-1}. In patients with IA a response rate of 38–48% has been reported [101]. Amphocil® is also effective in the therapy of neutropenic fever [102]. There is less renal toxicity than with cAmB, although its use is limited by severe side-effects such as fever, chills, and hypoxia despite adequate premedication leading to the early termination of a randomized trial [101–104].

Lipid Complex of AmB (Abelcet®) is supposed to have a response rate of 42–67% but less nephrotoxicity. The recommended dose is 4.8 mg·kg^{-1}·day^{-1}. The use is limited by side-effects such as infusion-related chills, rigor, and fever [105]. Abelcet® is more nephrotoxic and less effective in the therapy of neutropenic fever compared to AmBisome® [106].

Application of cAmB in fatty-acid emulsion is not recommended because antimycotic beneficial effects are lacking and there have been severe renal and pulmonary side-effects, probably due to fat emboli [107].

Resistance of *Aspergillus* spp. to AmB treatment arises from altered ergosterol content of the fungus membrane. *A. fumigatus* and *A. niger* are well susceptible to AmB therapy, but *A. terreus* and *A. flavus* exhibit high minimal inhibition concentrations (MIC) *in vitro*. All of the lipid associated AmB compounds show higher MIC values than cAmB for all *Aspergillus* spp. However the correlation of clinical failure of AmB therapy and resistance is difficult to prove in these severely immunosuppressed neutropenic patients [108]. *In vitro* studies have demonstrated, that previous azole therapy may induce AmB resistance by reducing the amount of ergosterol in the fungus membrane [109] (table 2).

Azoles

Azoles inhibit the P450 dependent lanosterol 14-α-demethylase, a late step in ergosterol synthesis. This leads to disintegration of the fungal cell membrane. The early azoles such as clotrimazole, miconazole, and ketoconazole were not effective enough to be relevant in the therapy of IA. The first triazole, fluconazole showed only *in vitro* activity against IA [110]. The further development of triazoles has led to compounds effective against *Aspergillus* spp. *in vivo*.

Itraconazole has been shown to be as effective as AmB in patients with IA. Oral and intravenous itraconazole is used in the therapy and primary and secondary prophylaxis of IA [111]. The oral formulation has a response rate of between 39–66%. The recommended dose is 400–600 mg·day^{-1} [80, 86, 112]. Side-effects include a nasty taste in the mouth, nausea, vomiting, and diarrhoea leading to a limited compliance in some patients [113]. Furthermore, the gastrointestinal resorption of itraconazole is highly variable depending on the gastric pH, with a consequent danger of breakthrough mould infection, especially in patients with gvhd [114]. The fluid oral solution is able to maintain stable plasma drug

Table 2. – Medical therapy in invasive pulmonary aspergillosis

Medical therapy	Dosage	Response %	Comments
Amphotericine B			
Desoxycholate	1–1.5 mg·kg^{-1}	33–54	Mortality of 64–90% Fewer side effects in continuous 24 h infusion Local instillation possible Not effective in prophylaxis
Colloid dispersion	3–4 mg·kg^{-1}	38–48	Less nephrotoxicity, but severe side-effects as fever, chills, hypoxia
Lipid complex	4.8 mg·kg^{-1}	42–67	Less nephrotoxicity, but chills, rigor, fever
Liposomal	1–3 (-6) mg·kg^{-1}	30–60	Less nephrotoxicity Less breakthrough infections Reduced aspergillus colonization
Azoles			
Itraconazole	Oral 400–600 mg	39–66	Side effects: nausea and vomiting Better resorption as oral solution
	i.v. 200 mg	48	Long term therapy induces resistance
Voriconazole		50–75	Visual, hepatic, and dermal side effects FDA approval pending
Posaconazole		53	FDA approval pending
Racuvonazole			Only animal studies
Echinocandins			
Caspofungin (MK-0991)		41–45	Preliminary data from clinical trials
FK 463			In clinical trials
LY-303366			In clinical trials
Papulacandins			In development
Acidic terpenoids			In development

FDA: Food and Drug Administration.

concentrations [115]. Intravenous application of itraconazole at a dose of 200 mg·day^{-1} was effective in 48% of the patients and it is shortly to be licensed in Europe. Strains of *A. fumigatus* resistant to itraconazole have already been described [116].

In vitro testing showed resistance to itraconazole in 1.5–4.2% of isolates with a MIC >8. Long-term therapy with itraconazole can induce resistance to that compound [117]. Cross reactivity to other triazoles has been reported, mainly to posaconazole, but not to voriconazole so far.

Voriconazole is a newly developed triazole with a high activity against *Aspergillus* spp., but also against Scedosporium and Fusarium. It has been tested since 1995 in IA. This compound is more effective than itraconazole or AmB in animal models of IPA [118, 119]. Oral and intravenous application exhibits a 50–75% response rate in patients with IA [96, 109]. Voriconazole is well tolerated, typical side-effects consist of visual disturbances, hepatotoxicity, and dermal rash.

Voriconazole is as effective as AmBisome® in neutropenic fever, but has less nephrotoxicity, hepatotoxicity, breakthrough fungal infections, and acute infusion-related toxic effects. However its use is associated with a higher rate of infusion related visual side-effects [120]. *In vitro* testing of voriconazole showed a MIC >8 in 3.5% of isolates. [108]. The approval by the Food and Drug Administration (FDA) is still pending [109].

Among the new and more powerful triazoles, posaconazole and racuvonazole are two compounds highly active against aspergillus compared to other antimycotics [121, 122]. In a preliminary study in therapy-refractory IA in humans, posaconazole showed a response rate of 53%. Furthermore, this compound possesses lower MIC *in vitro* against all *Aspergillus* spp. compared to the other triazoles [108]. Ravuconazole has only been tested in animal studies so far [109] (table 2).

Echinocandins

Echinocandins are natural inhibitors of the BDG synthetase, an enzyme that forms glucan polymers in the fungus wall [109, 123]. Echinocandins are antifungal lipopeptides firstly isolated from *Aspergillus* spp. in 1974. The distinct term pneumocandin is derived from the originally observed activity against pneumocystis and candida [124]. In the last 20 yrs biologically stable semisynthetic derivatives have been developed, which have proved to be effective in prophylaxis and therapy of IPA in animal models [125].

Caspofungin (MK-0991), a noncompetitive inhibitor of the BDG-synthetase derived from an antifungal substance of Zalerion arboricola, has been recently approved by the FDA. In a preliminary study of 54 patients with IA, a 41% response rate with caspofungin has been achieved, among these were 40 IPA patients with a 45% response rate. In the subset of 44 patients with resistance or intolerance to AmB and azole therapy, a favourable response to caspofungin has been observed in 34% [81].

Other echinocandins such as FK 463 and LY-303366 are being tested in clinical trials. Additional BDG-synthetase-inhibitors, for instance the glycolipid papulacandins and the acidic terpenoids are in development [123].

Other antifungal compounds

Several new classes of antifungals with activity against aspergillus are in development or have been tested in animal studies. Liposomal nystatin showed good *in vitro* activity against *Aspergillus* spp. [126]. Pradimicins destroy the fungal wall by binding to mannosides. Nikkomycins inhibit chitin synthesis and are supposed to act synergistically with triazoles [109]. Further antifungal strategies are reviewed elsewhere [127] (table 2).

Combination therapy

Several compounds have been evaluated for combination therapy in IA and IPA including AmB, itraconazole, flucytosin, and echinocandins.

Application of AmB and itraconazole has been shown to be more effective than either AmB or itraconazole alone [17, 128]. However, case fatality rates are similar with a combination of AmB and itraconazole compared to AmB only [86]. Furthermore, an antagonistic mechanism of these compounds were described *in vitro* [129].

The combination of AmB and 5-Flucytosin is effective in several fungal infections, but the combination was not beneficial compared to AmB monotherapy in neutropenic patients with systemic mycoses [1, 130]. Flucytosin has myelosuppressive side-effects and may multiply the nephrotoxic side-effects of AmB [96].

There are few data on combined application of AmB and echinocandins. In an animal study of IPA, AmB and the echinocandin FK 463 showed synergistic action [109]. Results from clinical trials of combination therapy of AmB or triazoles with echinocandins have not yet been published.

Combination antifungal therapy is not recommended routinely, however it might be effective in individual cases.

Cytokines

Use of cytokines has been suggested to increase antifungal immune response. Colony stimulating factors have been used to shorten the neutropenic phase. In particular granulocyte macrophage stimulating colony (GM-CSF), with its ability to increase the lifespan of neutrophils and to promote monocyte differentiation has been used successfully. GM-CSF has been shown to be effective in invasive fungal infections other than aspergillosis [131]. However, during bone marrow recovery with an increase in

granulocyte count there is a substantial risk of potentially fatal pulmonary haemorrhage, especially when granulocyte counts normalize within one week after aplasia [31, 32, 132, 133]. The use of GM-CSF in invasive aspergillosis is not recommended routinely.

Several other cytokines have been discussed in antifungal strategies [9, 10]: 1) IFN-γ has been shown to increase the antifungal activity of macrophages and neutrophils. It is able to prevent the injurious effects of steroids on neutrophil activity against aspergillus; 2) IL-12, normally derived from macrophages, is able to stimulate Th1 lymphocytes, which produce IFN. Furthermore IL-12 stimulates natural killer cells which also have antifungal activity and can produce IFN; 3) Neutralization of IL-4 and IL-10 attenuates the Th2 response and is associated with increased antifungal activity [9].

None of these cytokines are established in the therapy of IPA, and results from randomized, controlled trials are not available. The use of cytokines cannot currently be recommended.

Granulocyte transfusion

Other options include granulocyte transfusion, which has been performed with variable success [8, 134, 135]. It seems to be beneficial in selected cases, as in patients with severe aplastic anaemia and prolonged periods of neutropenia. Furthermore, patients with proven IPA, who are planning to undergo allogeneic SCT should benefit from granulocyte transfusion from the stem cell donor. Results from randomized trials are not yet available.

Surgery

Lung resection has been performed as an emergency procedure in IPA when haemoptysis has occurred [1]. The radical removal of the IPA lesion seemed to influence the outcome of IPA and enabled subsequent immunoablative therapy [30, 47]. Therefore, surgery has been advocated as a therapeutic option in localized IPA.

Lung resection has both diagnostic and therapeutic impact. It is able to obtain a specimen for precise histological diagnosis of the pulmonary changes. Furthermore the infectious focus is removed, and complications arising from IPA such as disseminated infection or haemoptysis are prevented.

Early studies on highly-selected patients have proved the feasibility of this procedure [136]. However perioperative complications including infections, fungal relapse, and bleeding need to be considered [33, 137]. Nevertheless, surgery has been advocated in the early stages of IPA [43]. The decision to perform lung resection is based on clinical and radiological signs of IPA rather than microbiological findings. Surgery is also feasible in profound neutropenia and thrombocytopenia [30, 31, 138, 139]. Also in multilocular IPA multiple wedge resections can be performed [138]. Besides lobectomy, lung parenchyma saving procedures such as open wedge resection and video-assisted thoracoscopic surgical interventions are feasible [30].

In recent published studies 165 patients were reported, who underwent lung resection for IPA. Twelve patients had nonfatal postoperative complications that needed intervention (7%), and 24 died postoperatively (14%). In 27 patients uncontrolled or recurrent mould infection was reported (16%). After lung resection, 35 patients underwent SCT, whereas in three patients fungal relapse occurred (8%) [30, 33, 43, 47, 136, 137, 140].

In a retrospective analysis of the outcome of patients with IPA comparing medical and surgical therapy, lung resection was associated with better survival and reduced IPA related mortality [141]. Therefore lung resection should be considered in patients with clinical and radiological signs of localized or multilocular IPA: early in the course of IPA, who failed to respond to antifungal therapy, prior to further immunosuppressive therapy, *e.g.* ablative chemotherapy or SCT, when haemoptysis occurs.

The preoperative microbiological detection of a mould infection is not essential. Neutropenia is not regarded as contraindication, but disseminated IPA is not regarded as indication for surgery (table 3, fig. 1a–c).

Prognosis

The strongest prognostic factor for IPA is successful therapy of the underlying disease. In patients with acute leukaemia and IPA, the complete remission of the haematological disease was the main prognostic factor associated with a significantly better outcome [142]. After surgery for IPA, the survival of patients was limited due to relapse or uncontrolled malignancy, but not by complications of the surgical procedure [30].

Summary

Invasive pulmonary aspergillosis in neutropenia, carrying an increasing morbidity and still a high mortality, is a diagnostic and therapeutic challenge.

Table 3. – Surgery in invasive pulmonary aspergillosis (IPA): indication and risks

Indication for Surgery	Localized or multilocular IPA Early stage IPA Failure of antifungal therapy Planned immunosuppression, *e.g.* HDC or SCT Haemoptysis
Perioperative mortality	14% (24 of 165 patients)
Postoperative complications (nonfatal)	7% (12 of 165 patients)
Recurrent mould infection	16% (27 of 165 patients)
Fungal relapse post SCT	8% (3 of 35 patients)

HDC: high dose chemotherapy; SCT: stem cell transplantation.

It requires a network approach of diagnostic, prophylaxis and therapeutic strategies. Identification of high-risk patients, appropriate prophylaxis, diagnostic surveillance, and early diagnosis are important for early initiation of adequate therapy. In addition to thoracic computed tomography scanning, newer diagnostic strategies should incorporate polymerase chain reaction techniques in serum and bronchoalveolar lavage. Therapeutic options include new antifungal compounds (triazoles and echinocandins) and cytokine therapy. Parenchyma saving surgical procedures should be considered early in the course of invasive pulmonary aspergillosis in patients with localized disease.

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