Clinical Syndromes: *Aspergillus*

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Invasive *Aspergillus* infection (IAI) is the second most common invasive fungal infection among patients with hematological malignancies [1]. Systemic infection with *Aspergillus* spp. is potentially life-threatening for patients suffering from severe diseases.

Persons at risk have severe and prolonged immunosuppression and may suffer from a hematological malignancy (in the first line patients with acute myeloid leukemia and recipients of allogeneic HSCT). Moreover solid organ transplant recipients and patients treated with corticosteroids for exacerbated COPD or with multiple myeloma are increasingly at risk for IAI [2, 3]. The most important risk factor is particularly severe granulocytopenia (<0.5 G/L), but other risk factors also include AIDS in a progressive stage and also intensive care patients with corticosteroid treatment, lung disease, and renal failure [4]. Additionally, patients who have to take immunosuppressive drugs other than corticosteroids or those suffering from inherited immunodeficiency may develop a severe *Aspergillus* infection [5]. IAI may result in a high mortality up to 90% in severely immunocompromised patients with involvement of the central nervous system [6–8]. The variance in epidemiological data may be due to the use of a variety of diagnostic tools. Most studies are based on autopsy results. In these studies *Aspergillus* species identification was rarely done. Although antemortem diagnosis of IAI has improved, some cases of IAI remain undetected. With the reduction in autopsies over time, a reliable estimate of the real prevalence of IAI in high-risk patients will not be available in the near future [9].

The four most common pathogenic species are *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *and Aspergillus terreus*. However, there is a shift in the prevalence of the *Aspergillus* species in transplantation centers in the USA describing a decrease of *A. fumigatus* and an increase of *A. flavus*, *A. niger* and *A. terreus* [6, 10]. Thus, particularly in medical centers caring for patients with hemato-oncological diseases, stem cell, and solid organ transplantation, a specialized mycological laboratory service is pivotal for the modern diagnostic methods and exact identification plus susceptibility testing of pathogenic fungi (see Chap. 1). The knowledge of the locally predominant species is essential for the choice of therapy since, for example, *A. terreus* is resistant against amphotericin B (AMB) [11].

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5.1 Diagnosis

The diagnosis of IAI is challenging. Diagnosis of IAI requires careful evaluation of the patient's history, clinical signs, and laboratory and microbiological work-up.

According to a review of the literature and the guidelines, invasive aspergillosis is defined as proven, probable, and possible [12]. The EORTC criteria classify IAI as proven, probable, and possible for clinical studies. These criteria were mainly defined for performing and comparing clinical studies but are also helpful in the diagnostic work-up and management in the clinical routine. IAI is proven by histopathology and positive culture of a specimen from a sterile site. IAI is defined as probable if there are clinical signs and symptoms of IAI but negative cultures and *Aspergillus* suggestive hyphae in histopathology, or negative cultures but positive galactomannan assay, or beta-D-glucan assay and typical radiologic signs (computed tomography). The sensitivity of chest radiography is low in the earliest stages of pulmonary infection. The radiographic manifestations are variable according to the host and the type of disease. There is a broad range of alterations from unspecific peribronchial infiltrations, nodules, or consolidations with or without cavitation. The mentioned lesions may be single or multiple. The next step in the workflow for diagnosis of IAI is the (high-resolution) computed tomography (CT) of chest and/or any other body site depending on the clinical sites and symptoms. To exclude systemic IAI, a full body CT including the brain is strongly recommended. CT scan is the diagnostic gold standard for imaging IAI. CT reveals alterations in lung tissue at an earlier stage and more precisely. The CT findings largely depend on the host. In patients with neutropenia, initial infiltrates and/or nodules with surrounding ground-glass infiltrates are described. These are called "halo signs," reflecting hemorrhages into the area surrounding the fungal infiltration. In a typical course of the disease, the nodules enlarge; they may cavitate. This cavitation of the former solid nodule is called "air-crescent sign." The appearance of the air-crescent sign is supposed to be linked with reconstitution of the immune response but independent of appropriate therapy. The material inside is infarcted lung tissue containing *Aspergillus*, but it may also be another fungus. This is also called a mycotic lung sequestrum. Any nodules or abscesses at any site in patients at risk for IAI may be caused by *Aspergillus* sp. To prove or to exclude IAI, a needle biopsy with mycological and with histology work-up is recommended [13]. Blood cultures hardly show positive results, even in invasive infection.

Therefore, for the diagnosis of pulmonary IAI, the acquisition of a specimen by bronchoalveolar lavage, CT-assisted transthoracic percutaneous needle aspiration, or even thoracoscopic biopsy is the diagnostic options. These procedures assume that the patient is hemodynamically stable, has sufficient platelet counts, and has normal or only slight-altered coagulation values. For cytological and histopathologic examinations and fungal culture and identification, presentative specimens of bronchial fluid or tissue are required. Hemato-oncological centers have specialized laboratories, where molecular diagnostic methods and antifungal susceptibility testing are available. The identification of the *Aspergillus species* is also recommended in the recently published guidelines of the IDSA [14].

Microscopy and histology are conclusive for IAI, if the specimen is gained from a sterile body site ("relevant specimen"). This diagnostic method allows exclusion of contamination and differentiation between septate and non-septate hyphae and pseudohyphae and is available within a short time. However, microscopy and culture of specimen from BAL which is not considered to be "relevant" display a sensitivity of about 50% in high-risk patients suffering from a hematologic disease. Culture results are available only a few days after the onset and do not assure the differentiation between contamination, colonization, or infection. Advantages of cultures are that they facilitate the identification of *Aspergillus species* and determination of susceptibility testing to antifungal agents for therapy guidance.

Biomarkers like the surface galactomannan (GM) and β-D-glucan (BDG) or molecular (PCR) techniques are considered to be helpful for a reliable diagnosis of IAI in the absence of positive culture results. Information and details for

both, GM and BDG, are given in Chap. 1. Briefly, whether a positive GM result justifies initiation of preemptive therapy for suspected IAI can only be answered in context with the patient's risk factors for IAI and the clinical presentation. But, in case of suspected infection of the central nervous system, this test is also of diagnostic value. Therapeutic monitoring may be facilitated by serial determination of the GM in blood.

Detection of *Aspergillus* nucleic acid in the different specimens is still a challenge. Validated and commercially available assays are a precondition for application in the clinical microbiology lab. The advantage of a PCR in the obtained specimen is the timely delivery of a specific result. However, molecular detection methods, such as PCR, are more sensitive than culture. Again, it is important to consider the particular patient's clinical situation and risk factors for IAI. The presence of fungal nucleic acid in relevant material alone is not the proof of infection but may have the consequence of further diagnostic procedures. For details of molecular fungal diagnostics, see also in Chap. 1.

According to experts' opinion, the *interpretation of the microbiological results* requires several items to be considered, particularly in case of strong suspicion of IAI but negative culture results:

- Was antifungal therapy started before diagnostic samples were taken?
- Is the quality of the specimen relevant, i.e., sterile site of the needle biopsy, bronchoalveolar lavage, superficial swabs, or any respiratory tract secretions?

For example in severely immunocompromised patients with hematological malignancy, the interpretation of the radiological films (preferentially CT-scan) is of utmost importance. An intrapulmonary lesion with halo sign or air-crescent sign is characteristic though not diagnostic for invasive pulmonary aspergillosis. However, similar radiological signs may be described in infections with other fungi like *Zygomycetes*, *Fusarium* spp., or *Scedosporium* spp. but also with bacteria like *Pseudomonas aeruginosa* or *Nocardia* spp. These typical radiological signs are only described for patients with severe neutropenia and hematological malignancies but cannot be extrapolated on other immunocompromised patients [15]. Because of the high risk for invasive *Aspergillus* infection in immunocompromised patients, guidelines for the diagnosis and treatment have been published and are continuously updated [14, 16]. Nevertheless, the diagnostic pathways have to be tailored to patient groups at risk as well as the availability of a microbiological laboratory familiar with mycology and of radiological services optimally equipped with an interventional unit for needle biopsies.

5.1.1 Clinical Presentations of IAI

Aspergillus sp. may enter the body through the lower respiratory tract, the sinuses, or the skin. From there either direct continuation of invasive growth or hematogenous dissemination may follow. Spread of *Aspergillus* infection to the central nervous system (CNS), the thyroid gland, the skin, the cardiovascular tissue, and each possible organ can potentially occur.

5.1.2 Clinical Presentation

On the assumption that the conidia of *Aspergillus* are inhaled into the lungs or sinuses, IAI most frequently presents with respiratory symptoms. Less common ways of inoculation of *Aspergillus* are the ingestion via the gastrointestinal tract or direct inoculation via skin lesions.

Invasive pulmonary *Aspergillus infection* is the most common invasive fungal infection in patients with hematological malignancies, second only to invasive Candida infection. However, in the times of antifungal prophylaxis, invasive fungal infection caused by other fungi may emerge in this distinct patient population. Patients usually present with fever not responsive to broad-spectrum antibacterial therapy, pleuritic chest pain, shortness of breath, cough, and sometimes hemoptysis. Even if antifungal prophylaxis has been administered, the next diagnostic steps will be the CT scan of the lungs, the acquisition of respiratory samples for fungal

culture preferentially by needle biopsy and only second by BAL, and the determination of biomarkers (GM, BDG).

For hemoptysis symptomatic treatment is sufficient for mild hemoptysis, whereas embolization may be required with this severe complication with bleeding from vascular nexus of small vessels of the systemic circulation. With a skilled interventional radiologist, the chance of a successful embolization is high, though up to 50% of the patients can develop recurrent hemoptysis. A successful long-term antifungal therapy can help to minimize the rate of relapses.

Tracheobronchitis occurs most commonly in patients with severe COPD, lung transplantation, hematological malignancies, and bone marrow transplantation and may be obstructive, ulcerative, or pseudomembranous. In lung transplant recipients, *Aspergillus* tracheobronchitis may develop in the bronchial stump due to infection of the suture material. In this concern nylon sutures should be preferred to silk material [17]. Though immunocompromised patients and patients at risk may only report fever but no pulmonary symptoms. Performing high-resolution CT scan is mandatory when invasive aspergillosis is suspected because conventional x-rays have poor sensitivity.

Disseminated invasive Aspergillus infection refers to IAI with metastatic lesions in many organs including the brain, bone, skin, eyes, thyroid gland, liver, kidneys, and any other body sites. The pathogenesis is either by direct invasive growth when the fungus invades the vessels or most probably via dissemination by macrophages which take up the conidia but are incompetent to kill the fungus [18]. The prognosis of generalized disease is very poor.

Invasive Aspergillus infection of the central nervous system involves the brain, the meninges, or the myelon either by dissemination or continuous infection from local extension from the paranasal sinuses or by dissemination [19]. The clinical symptoms of *Aspergillus* infection of the CNS include focal neurological signs or even generalized seizures. Cerebral mycotic aneurysm is a severe complication because it may rupture and cause a hemorrhage [20].

Invasive Aspergillus infection of the eye includes corneal infection by direct inoculation after trauma with presentation of pain in the eye and visual alterations. The outcome may be poor with loss of vision, and sometimes progression of disease may necessitate enucleation of the usually totally destroyed eye ball [18, 21].

Invasive Aspergillus infection of the heart may present as endocarditis, predominantly as prosthetic valve endocarditis or as invasive *Aspergillus* myopericarditis. The time of inoculation infection may be during or shortly after the surgical intervention. However, the presence of ill-kept central vascular catheters or intravenous drug abuse are risk factors for *Aspergillus* endocarditis. The symptoms are usually fever and septic embolism. In case of *Aspergillus* endocarditis, blood cultures may be positive for *Aspergillus* sp. To exclude contamination more than two sets of blood cultures should be taken. However, negative blood cultures do not exclude *Aspergillus* endocarditis if there are vegetations or paravalvular abscesses, and no other pathogen isolated and GM in serum is repeatedly positive. The demonstration of invasive *Aspergillus* hyphae in the tissue and the growth of *Aspergillus* sp. of the culture of excised valves are the proof of infection. Therapy includes antifungal treatment and surgical therapy. However, the prognosis of cardiac involvement is poor.

The skin may be involved directly via inoculation, by trauma, burns, or during operation. Otherwise the skin may be involved in disseminated IAI particularly in patients with hematological malignancies or allogenic stem cell transplantation. Lesions in skin level may be reddish at first but then get blue to dark gray color (see also cutaneous aspergillosis). Later there may develop ulceration in severe cases. Deep skin biopsy for microscopy and microbiology is the diagnostic method of choice.

Gastrointestinal IAI may present as focal neutropenic enterocolitis, appendicitis, colonic ulcers with gastrointestinal hemorrhage, and abdominal pain. Mucositis in patients with neutropenia and in patients receiving high doses of corticosteroids allow direct invasion of ingested *Aspergillus* cells [22].

Chronic pulmonary aspergillosis (CPA) is a rare pulmonary *Aspergillus* infection and is caused by *Aspergillus fumigatus*, although patients have been described with *A. niger* or *A. flavus* infection. Occasionally, *A. fumigatus* isolates may be atypical, growing slowly with poor sporulation, delaying identification. It is still not clear how chronic pulmonary aspergillosis develops. Patients with chronic *Aspergillus* infection do not have these risk factors and may be considered immunocompetent, but they may have either preexisting pulmonary damage or disease. Patients are not immunocompromised by malignoma, HIV, cytotoxic chemotherapy, or any immunosuppressive therapy but may be malnourished and underweight. Previous infections, e.g., tuberculosis or atypical mycobacterial disease, severe COPD, allergic bronchopulmonary aspergillosis, COPD, precedent pneumothorax, or treated lung cancer, are the predominant risk factors for development of CPA.

CPA starts with nodules containing *Aspergillus*. They appear single or multiple at a size smaller than 3 cm. The lesions contain fungus balls consisting of *Aspergillus* hyphae and of cellular debris and of mucus maintaining chronic inflammation. Most commonly, the lesions remain solid but may progress into cavitary pulmonary aspergillosis as the late manifestation of CPA. The fungus balls and the cystic lesion are morphologically similar to the aspergilloma and consist of fungal hyphae and extracellular matrix. It is formed by collapse of the superficial fungal growth inside its cavity [23, 24].

The clinical signs and symptoms are cough, recurrent pneumonia, and/or exacerbation of asthma or chronic obstructive pulmonary disease. The duration of the symptoms usually persists for at least 3 months. The disease is usually progressing despite of adequate antifungal treatment. Complex genetic factors are supposed to be underlying to CPA [25].

Advanced diagnostic work-up is required to differentiate the nodules from metastases, lung carcinoma, or infiltrations caused by rare fungi or pathogens. Even in rheumatoid arthritis, nodules may occur, and in summary the definite diagnosis on the entity of pulmonary nodules can only be secured by histology. The diagnosis of CPA is done using a combination of procedures: CT scan, a direct evidence of *Aspergillus* infection by culture of relevant (biopsy) material, and immune response to *Aspergillus* spp.

Radiological findings in the CT scan are a sum of the preexisting lung disease and the alterations secondary to CPA with nodular infiltrates, alveolar consolidations, preexisting bronchopulmonary or pleural cavities, and the formation of new cavities, nodules or alveolar consolidations. Positron-emission tomography (PET) shows an isometabolic halo or nodule pattern but does not distinguish between malignancy and CPA.

Aspergillus IgG or a precipitin test will be positive in more than 90% of the patients with CPA. Tissue sections of biopsy material showing hyphae invading lung parenchyma indicate acute or subacute invasive aspergillosis. In summary, the diagnostic criteria for CPA are the detection of *Aspergillus* IgG, IgE, or precipitins in the serum; the detection of galactomannan, *Aspergillus* antigen, or *Aspergillus* DNA in respiratory specimens, a percutaneous or excision biopsy showing fungal hyphae in the tissue section, and/or a growth of *Aspergillus* spp.

Persistent lesions and cavities in the lung are also a breeding place for commensal bacteria present in the respiratory tract. Thus, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and anaerobic bacteria can cause serious bacterial infections on the basis of CPA [26].

Aspergilloma is a generally single fungus ball in a usually preformed single pulmonary cavity and is called a single pulmonary aspergilloma. Pulmonary or systemic symptoms and serological or microbiological proof of *Aspergillus* are usually negative. Progression of the disease is observed for months. Usually, the diagnosis of aspergilloma is incidentally in a chest x-ray or a CT scan done for another purpose. Rarely the patients present with asthma (allergic bronchopulmonary aspergillosis) or even more rarely with hemoptysis. In patients with adequate pulmonary function presenting a single aspergilloma, resection is a promising therapy option. It is very important that the aspergilloma can be

completely resected and that no fungal material can spread into the pleural space. In patients with hemoptysis, embolization of respective arteries is indicated before surgical resection of an aspergilloma. The patient's condition should be carefully observed because a good cardiopulmonary function correlates with the outcome, i.e., fewer complications and lower risk of death.

Allergic bronchopulmonary aspergillosis (ABPA) is a complex hypersensitivity reaction in response to colonization of the airways with *Aspergillus fumigatus*. Tissue sections characteristically show mucoid impaction of the bronchi or eosinophilic pneumonia and bronchial granulomas in addition to histologic features of asthma. In the mucus-filled bronchial lumina, there are septate hyphae, but the fungi do not invade the mucosa.

ABPA occurs in 2% of all asthma patients, particularly in those with frequent exacerbations and corticosteroid treatment and in up to 14% in patients with cystic fibrosis. Chronic ABPA develops after repeated episodes of bronchial obstruction, inflammation, and mucoid impaction which lead to bronchiectasis and respiratory compromise. ABPA may also occur in chronic granulomatous diseases, in hyperimmunoglobulinemia E, and in lung transplant recipients.

Patients with ABPA present with asthma exacerbations, recurrent episodes of bronchial obstruction, malaise, fever, pleuritic chest pain and occasional hemoptysis, or episodic wheezing and expectoration of sputum containing brown plugs. Some patients with ABPA also have allergic *Aspergillus* rhinosinusitis and report nasal obstruction or sinus pressure.

The diagnosis is confirmed by radiological and serological testing. Growth of *Aspergillus* spp. in respiratory samples is only detected in up to 60% of ABPA patients with *Aspergillus*. The skin prick test with *Aspergillus* antigen is positive. Blood tests show a significant elevation of blood eosinophils (>0.5 G/L in patients not receiving corticosteroids) and the serum IgE (>1000 U/L) as well as precipitating IgG antibodies to *Aspergillus*. The immunoassay reveals positive results for IgG antibodies to *Aspergillus* that are diagnostic for ABPA.

The chest x-ray shows consolidations in the upper or middle lobes, central bronchiectasis, parenchymal opacities, and/or mucoid impaction with atelectasis. The CT scan shows bronchiectasis, thickening of the bronchial wall, mucus plugging, or air trapping. The skin prick test with *Aspergillus* antigen is positive. Pulmonary function tests typically reveal reduced forced expiratory volume in 1 second (FEV1) and increased residual volume of the lung.

A negative prick skin test and the absence of precipitins to *Aspergillus* virtually exclude ABPA and should prompt evaluation of other diagnostic possibilities. Differential diagnosis of allergic bronchopulmonary aspergillosis (ABPA) comprises asthma with *Aspergillus* sensitization, bronchocentric granulomatosis, eosinophilic granulomatosis with polyangiitis, and pulmonary eosinophilia due to drugs or parasitic infection and chronic pulmonary aspergillosis [14, 27].

5.2 Superficial Aspergillus Infection

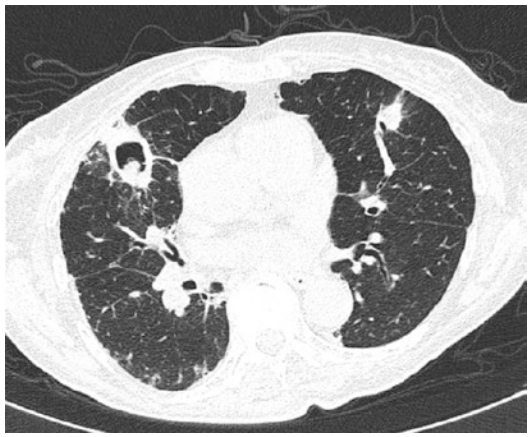
Primary cutaneous aspergillosis can develop at sites of skin injury, near intravenous catheter insertion sites, at areas of traumatic skin lesions and subsequent *Aspergillus* inoculation, and in burn or surgical wounds. Secondary cutaneous aspergillosis may occur during disseminated *Aspergillus* infection through hematogenous spread or through continuous growth. Cutaneous aspergillosis is a rare complication described in AIDS and in non-HIV-infected severely immunocompromised patients or patients with extensive burn wounds. The most common fungus in HIV-associated cutaneous aspergillosis is *Aspergillus fumigatus*, whereas in non-HIV-infected or in burn patients, *A. flavus* and *A. fumigatus* were detected more often.

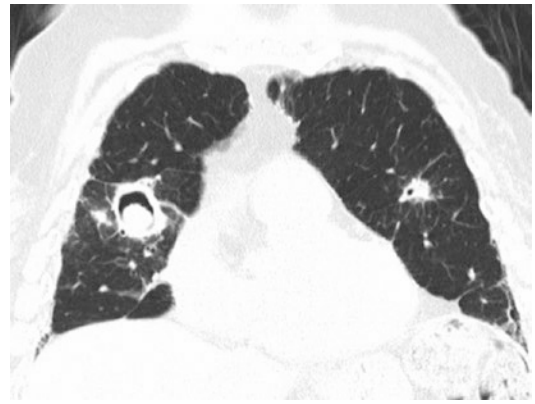
Initial lesions display a variable picture with macules, papules, nodules, plaques, or pustules. A hemorrhagic bulla may develop under an occlusive tape, whereas at the catheter insertion site, erythema and induration are the first manifestations which progress to necrosis. These lesions are preceded by fever, swelling, and

tenderness. Diagnosis is made by skin biopsy of a lesion. The biopsy should reach the subcutaneous fat to rule out invasion of the blood vessels of the dermis and subcutis. A part of the biopsy should be put into saline and sent to the microbiology for microbiologic culture, and the other part should be sent in formalin to perform histopathology.

5.2.1 Case Presentation

An 84-year-old female patient presents with falls and vertigo. In her history she has a fibrosis of the lung, and she has been treated for Waldenström macroglobulinemia since 2001; due to progression of the disease, therapy was intensified with prednisolone 60 mg/day and rituximab. The patient reports fever, cough, dyspnea, and weakness. Because of her clinical presentation and the need of oxygen therapy and the reduced general condition, antibiotic therapy is started immediately. In the x-ray already a node in the lung raises suspicion for a fungal infection. The additional diagnostic investigations result in a positive galactomannan test, sputum microscopy, and culture with proof of *Aspergillus fumigatus*. As the fungus is sensitive to voriconazole, therapy is initiated. The question of a single aspergilloma potentially offering the opportunity for surgical resection arises. Therefore, a CT scan is performed which shows multiple bilateral cavities with central accumulation of soft tissue consistent with a fungus ball.





CT scan of pulmonary aspergillosis with fungus ball inside the cavity

Due to multiple infiltrations and cavities, surgical resection is not indicated, and the antifungal therapy is continued for 12 weeks (images with kind permission of Prof W. Jaschke, Department of Radiology, Medical University Innsbruck).

5.3 Strategies to Prevent and to Treat IAI

5.3.1 Prevention

Regarding the patients at risk, protective measures have to be provided. After an allogeneic hematopoietic stem cell transplantation (HSCT), exposure to the fungus should be avoided, i.e., especially construction and renovation sites and plants and/or flowers in patients' rooms. Consequently, outpatients are instructed to avoid these places as well as gardening.

5.3.2 Antifungal Prophylaxis

Primary antifungal prophylaxis is the use of antifungal agents before any evidence of fungal colonization or infection starts at the initiation of cytotoxic chemotherapy and immunosuppression in every patient at risk.

Secondary antifungal prophylaxis is the use of antifungal agents after recovery from a proven and documented fungal infection prior to addi-

tional and necessary immunosuppressive therapy or cytotoxic chemotherapy.

The challenge of antifungal prophylaxis is to target the patient population at risk while not overusing antifungal agents that may have toxic side effects, interfere with other medications, foster the emergence of resistant fungi, and, at last, are not needed. Thus prophylaxis strategies are restricted to patients at the highest risk of fungal infections. To avoid overuse of antifungal prophylaxis, the decision to give prophylaxis should be risk-adjusted. Risk adjustment should be based on the incidence and the severity of invasive fungal infections (>10%) [28]. Local epidemiology of invasive fungal infection may be helpful to guide and evaluate the efficacy of antifungal prophylaxis and is therefore recommended in centers caring for patients with hemato-oncological malignancies. Patients at highest risk for IAI are (1) allogenic stem cell recipients (10–20%) with an age >40 years, hematological diseases other than chronic myeloid leukemia, graft failure, long-term steroids, and graft-versus-host disease, and (2) in patients with AML (10%) with an age >40 years, high-dose cytarabine and in an advanced state of the disease without remission [29]. These patient populations should receive antifungal prophylaxis according to current international guidelines, i.e., IDSA and ECIL-6 guidelines [14, 16].To minimize the high risk of IAI, antifungal agents with good efficacy against *Aspergillus* spp. are recommendable. First-line antifungal agents for prophylaxis are posaconazole and voriconazole. Posaconazole (dose, oral suspension 3 × 200 mg po; tablet and intravenous solution, day 1: 2 × 300 mg, following days 1 × 300 mg) is licensed for antifungal prophylaxis in selected hematological high-risk patients, i.e., allogeneic stem cell transplant recipients with graft-versus-host disease and patients with acute myeloid leukemia or myelodysplastic syndrome based on two randomized controlled trials [30, 31]. Voriconazole and micafungin are additional options for prophylaxis in severely immunocompromised patients at high risk [32–34]

Itraconazole is effective for prophylaxis too, but there are problems with absorption and tolerance. It is important to know that itraconazole should not be coadministered with other drugs that might get toxic levels due to metabolic interference with triazoles. Micafungin or caspofungin is recommended as second-line prophylactic agents [14].

5.3.3 Empirical Antifungal Therapy

In patients with prolonged (>10 days) severe neutropenia (<0.5 G/L), hemato-oncological malignancy, and no response to broad-spectrum antibacterial therapy, empirical therapy with antifungal agents may be used. The recommended antifungals are liposomal amphotericin B, caspofungin, micafungin, or voriconazole [14].

5.3.4 Preemptive Antifungal Therapy

Taking an even more refined approach is to initiate treatment only upon positive identification of biomarkers of infection together with appropriate clinical signs and symptoms and/or the presence of lesions in the CT that are suggestive for IAI in patients at risk. Biomarkers that should be continuously determined in patients at high risk for IAI are galactomannan and/or 1,3-beta-D-glucan, both parts of the *Aspergillus* cell wall. This strategy is called preemptive or presumptive therapy. There is excellent evidence that this strategy is effective and safe with the advantage that there are more documented IAI cases [35].

5.3.5 Antifungal Treatment of IAI

Antifungal treatment of strongly suspected IAI should be initiated as soon as possible. However, samples for fungal cultures should be best taken before the administration of antifungal therapy. Voriconazole is the antifungal drug of choice for treatment of IAI according to current guidelines [14, 16]. Voriconazole achieved a better clinical outcome than amphotericin B deoxycholate in an open-label randomized trial in patients with IAI. For intravenous infusion of voriconazole, a loading dose of 2 × 6 mg/kg on day 1 followed by

2 × 4 mg/kg (maintenance dose) is recommended. Although the enteral absorption of voriconazole depends on the clinical condition, oral administration is justified as a step-down therapy in stable patients. The oral standard dose is 2 × 400 mg on day 1 followed by a maintenance dose of 2 × 200 mg daily. Numerous drug-drug interactions have to be considered, as voriconazole undergoes hepatic metabolism involving CYP2C9, CYP2C19, and CYP3A4 [36]. Enhanced exposition to immunosuppressants can be particularly harmful. Therefore, the doses of cyclosporine A and of tacrolimus have to be reduced and closely monitored during concomitant voriconazole treatment. There are ultrarapid and poor voriconazole metabolizers due to genetic polymorphisms of CYP2C9. Close therapeutic drug monitoring of immunosuppressants is indispensable to avoid excessive immunosuppression and renal damage. Renal impairment at any stage appears to have no relevant influence on voriconazole pharmacokinetics and does not require dose adjustment for the oral voriconazole preparations. However, when the intravenous formulation had been applied, accumulation of the solvent SBECD was reported from patients with renal impairment. Preclinical and available clinical data as well as autopsy studies have shown favorable penetration of voriconazole into the majority of relevant tissues (Table 5.1). However, its complex, nonlinear pharmacokinetics requires therapeutic drug monitoring. The central nervous system, the eye, and the liver are the major targets of voriconazole toxicity with dizziness and hallucinations, visual disturbances, and increase of the liver enzymes [37].

Amphotericin B has been introduced in therapy already in 1958 and has been the standard therapy of invasive aspergillosis for decades. However, the use of its conventional deoxycholate formulation is discouraged now because of poor tolerability. Infusion-related adverse events such as chills, rigors, fever, nausea, hypotension or hypertension, and renal deterioration have been observed in almost 50% of the patients on treatment with this preparation. Lipid formulations of amphotericin B, particularly liposomal amphotericin B, are an alternative to voriconazole for treatment of IAI, particularly, when azole resistance is a concern. The recommended standard dose is 3–5 mg/kg per day. Although the toxicity of liposomal amphotericin B is much lower than that of the conventional formulation, renal safety is a concern, and close monitoring of renal function is strongly advised.

Posaconazole (dose, oral suspension 3 × 200 mg/day; tablet and intravenous solution, day 1: 2 × 300 mg, following days 1 × 300 mg) is licensed for second-line treatment of invasive aspergillosis, because it achieved a response rate of 42% in an open-label, multicenter study on salvage therapy for invasive aspergillosis and other mycoses in comparison to 26% response rate in a retrospective control group [38].

In the current guidelines, posaconazole is mentioned as an option for salvage therapy. If an azole (e.g., voriconazole) had been previously tried, salvage therapy should be performed with an antifungal belonging to a different class [14].

Recently the new broad-spectrum azole isavuconazole with a 5-day half-life has been licensed for treatment of invasive aspergillosis, as it had shown clinical efficacy similar to that of voriconazole in a randomised controlled double-blind trial of 516 patients with invasive aspergillosis and other mold infections [39]. Less severe interactions than voriconazole are expected when isavuconazole is

Table 5.1 Tissue penetration of antifungals for treatment of invasive aspergillosis

	Liver	Spleen	Lung	Kidney	Heart	CNS	Samples
Amphotericin B and its lipid formulations	+++	+++	+++	+/++	+/–	+/–	Autopsy epithelial lining fluid
Voriconazole	++	++	++ +++	++	++	+	Autopsy, biopsy, epithelial lining fluid
Caspofungin	+++	+	+	++	+/–	+/–	Rat, rabbit

+++, favorable penetration; ++, probably sufficient penetration, modest penetration; +/–, poor tissue penetration, probably insufficient for antifungal eradication

administered with drugs being metabolized by CYP. Monitoring of serum drug levels is recommended [14]. Isavuconazole is mentioned as an alternative to voriconazole in the recent guidelines.

Echinocandins are fungistatic to *Aspergillus* spp. Caspofungin is licensed for salvage therapy of IAI based on an open non-comparative trial with failure or toxicity of first-line treatment with lipid-formulated amphotericin B, itraconazole, or voriconazole [40]. However, the clinical response to caspofungin was found to be modest in this condition. As poor outcome was achieved in two studies on first-line treatment of invasive aspergillosis with caspofungin [41–43], it is not licensed for this indication. Caspofungin, however, is a therapeutic option in empirical antifungal treatment of patients with febrile neutropenia [44].

Because of the high morbidity and mortality caused by IAI, antifungal combination therapy has been investigated in numerous preclinical and in several clinical studies. The combination of azoles with amphotericin B yielded variable and contradictory results. Because of a potential mechanism-based antagonism, this combination is discouraged for treatment of invasive aspergillosis. In a small randomised trial, the administration of liposomal amphotericin B combined with caspofungin achieved an improved outcome in comparison with high-dose liposomal amphotericin B monotherapy [45]. The combination of voriconazole with caspofungin was promising as salvage therapy of IAI [46]. In a large randomised clinical trial, however, the benefit of the combination of voriconazole with anidulafungin over voriconazole monotherapy failed its significance [47]. Therefore, the current guidelines advise against routine administration of antifungal combination. It should be considered in proven IAI on a case-by-case analysis as well as for salvage therapy.

The duration of medical treatment of invasive aspergillosis depends on the immunological condition of the patient and on the site of the infection (Table 5.1). Treatment for 6–12 weeks is required in most of the cases. *Aspergillus* endophthalmitis should be treated with a combination of systemic and intravitreal voriconazole. Surgical debridement along with intravenous treatment plays an important role in cardiac manifestation of aspergillosis, as well as in sinusitis, osteomyelitis, and septic arthritis caused by *Aspergillus*. For renal aspergillosis, urological decompression is required. When the renal parenchyma is affected, voriconazole therapy must be administered; otherwise local amphotericin B instillation might be helpful. However, fungus balls within the renal pelvis will have to be surgically removed.

Overall, for those patients with IAI who fail prophylaxis or therapy, it is recommended that antifungal therapy has to be switched to antifungals of another class [14, 48].

5.3.6 Treatment of CPA

Symptomatic patients with pulmonary and/or general symptoms and/or loss of lung funtions should be treated for at least 6 months. Oral voriconazole or itraconazole are the preferred treatment. Itraconazole can be used as well as intravenous therapy with caspofungin, micafungin, or amphotericin B in the intolerability or resistance to triazoles. Hemorrhage has been treated locally in bronchoscopy, arterial embolisation, or even with surgical intervention. Surgical resection may be considered if there is persistent hemoptysis or a resistant *Aspergillus* species. However, the outcome is moderate. Asymptomatic patients may be observed and followed every 3–6 months [26].

5.3.7 Treatment of ABPA

Treatment of ABPA aims to control the exacerbation of asthma episodes and to avoid progressive lung damage. Systemic glucocorticoids and antifungal agents are applied depending on the activity of the disease. Antifungal therapy is able to decrease episodes of exacerbations, whereas inhaled glucocorticoids improve symptoms of asthma. For acute ABPA, prednisone at an initial dose of 0.5 mg/kg/day for 2 weeks is recommended, following a tapering with intermittent dosing over 3 months. Success of therapy can be measured by decrease of IgE. Usually patients

report clinical improvement, in radiography the opacities resolve, and total serum IgE is reduced by about 35%. At the same time, antifungal therapy for acute or recurrent exacerbated ABPA in combination with corticosteroids is recommended. Itraconazole, alternatively voriconazole or isavuconazole, and possibly posaconazole are effective antifungal drugs against *Aspergillus* spp. However, until now are only studies using itraconazole or voriconazole [48, 49]. The dosage of itraconazole in adults is 200 mg thrice a day for 3 days and twice a day from day 4 onward. Concomitant antifungal therapy is considered to help reduce the glucocorticoid dose. The duration of antifungal therapy is at least 16 weeks. For severe cases there are also therapeutic regimens of up to 6 months described. To assure adequate serum levels of the antifungal drug, the monitoring of the drug levels in the blood should be done: voriconazole is recommended with 400 mg twice on day 1, followed by 200 mg twice daily. After 5 days serum concentration should be measured, and throughout the therapy duration liver function tests should be taken. Though as a rare complication under the treatment with itraconazole and systemic glucocorticoids, acute invasive pulmonary aspergillosis may occur, stressing the need of regular controls [14].

5.3.8 Treatment of Cutaneous Aspergillosis

Treatment of cutaneous aspergillosis depends on the general clinical state of the patient [50]. Diagnosis of aspergillosis in the skin should imply the differentiation of primary or secondary fungal infection due to IAI. Secondary cutaneous aspergillosis and complicated and disseminated cases of primary cutaneous aspergillosis are treated as IAI. For primary cutaneous aspergillosis only, itraconazole has been shown to successfully eradicate *Aspergillus* in most cases [51].

In patients with extensive burns, cutaneous aspergillosis occurs as primary disease, which should be treated surgically. Also in AIDS patients, organism directed antifungal medication and surgical therapy are successful. In immunocompromised patients after cytotoxic chemotherapy and with vascular catheter exit sites or tunnel infections due to *Aspergillus* spp., surgical resection followed by antifungal therapy are recommended.

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