Established in 1871

Swiss Medical Weekly

Formerly: Schweizerische Medizinische Wochenschrift

An open access, online journal • www.smw.ch

Review article: Current opinion | Published 22 February 2016, doi:10.4414/smw.2016.14281

Cite this as: Swiss Med Wkly. 2016;146:w14281

Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and *Pneumocystis* pneumonia

Yvonne Schmiedel, Stefan Zimmerli

Infectious Diseases, Inselspital, Bern, Switzerland

Summary

Every year, *Candida*, *Aspergillus*, *Cryptococcus* and *Pneumocystis* infect an estimated two million individuals worldwide. Most are immunocompromised or critically ill. *Candida* is the most common fungal pathogen of the critically ill and of recipients of transplanted abdominal organs. In high-risk haemato-oncological patients, in contrast, the introduction of antifungal prophylaxis with fluconazole and later with mould-active posaconazole has led to a remarkable reduction of invasive candidiasis and is likely to have a similar effect on invasive aspergillosis. Invasive aspergillosis remains the dominant invasive fungal disease (IFD) of haemato-oncological patients and solid-organ transplant recipients and is increasingly found in individuals with exacerbated chronic obstructive pulmonary disease on corticosteroids. In the developed world, owing to antiretroviral therapy *Pneumocystis* pneumonia and cryptococcosis have become rare in patients with human immunodeficiency virus (HIV) and are mainly found in solid-organ transplant recipients or immunocompromised patients. In the developing world, cryptococcosis remains a common and highly lethal disease of HIV positive individuals.

With invasive candidiasis and invasive aspergillosis, timely diagnosis is the principal challenge. The clinical presentation is nonspecific and current diagnostic tests lack sensitivity and specificity. The combination of several tests improves sensitivity, but not specificity. Standardised polymerase chain-reaction-based assays may be promising tools for more rapid and specific diagnosis of candidiasis and invasive aspergillosis. Nevertheless, initiation of treatment is often based solely on clinical suspicion. Empirical therapy, however, may lead to over-treatment of patients without IFD or it may miss its target in the case of resistance. Despite the success of antifungal prophylaxis in reducing the incidence of IFDs in haemato-oncological patients, there are a considerable number of breakthrough infections demonstrating not only fungal resistance but also the emergence of rare and often lethal fungal pathogens. Knowledge of the local epidemiology and antifungal resistance is therefore pivotal. Current trial-based guidelines leave major gaps in identifying those most at risk, who may benefit from prophylaxis. Ongoing searches for disease-associated genetic polymorphisms may contribute to the establishment of individual risk profiles and targeted prophylaxis.

Key words: *invasive fungal diseases; invasive candidiasis; aspergillosis; cryptococcosis; Pneumocystis pneumonia*

Introduction

Out of more than 100'000 known fungal species, only about 300 cause disease in humans [1]. Our body temperature may provide a protective thermal barrier against the majority of species that grow best at ambient temperature [2]. The most common pathogens are *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis spp.* – causing more than 90% of reported deaths due to fungal disease [3]. The top ten fungal infections are responsible for at least as many deaths as tuberculosis or malaria [3–5]. Yet, on a global scale, fungal diseases are neglected. This is reflected by the lack of initiatives by the World Health Organization and the paucity of national surveillance programmes [3]. Although current trends show an overall increase of invasive fungal diseases (IFDs), their incidence is likely to be underestimated [6].

IFDs are associated with high morbidity and mortality. Their diagnosis is challenging and their timely treatment often depends on a high level of clinical suspicion. Therefore, this review aims to give an overview of the current epidemiology, clinical presentation, diagnosis, and management of the four most common IFDs: invasive candidiasis, aspergillosis, cryptococcosis, and *Pneumocystis* pneumonia.

Definitions of invasive fungal disease

IFDs are proven by the presence of moulds or yeasts in a deep tissue biopsy or a culture obtained by a sterile procedure [7]. Additional definitions for probable and possible IFDs based on host-specific, clinical, and mycological features, which were originally established for haemato-oncological research purposes, are now commonly applied in the clinical setting [8]. However, these definitions lack pre-

cision and universal applicability to all patients groups – notably the critically ill – and do not include *Pneumocystis* pneumonia.

Invasive candidiasis

Candida is the most common cause of IFDs in the developed world [9]. As a normal commensal of humans, this yeast may be found in the oral cavity, the gastrointestinal tract, the female genital tract or on the skin [10]. An estimated 24–70% of healthy people above one year of age are colonised by *Candida*. Presumably, everyone is temporarily colonised at least once during their lifetime [11, 12]. Invasive candidiasis originates from the patient's own flora: it is introduced into the bloodstream or deep tissue following iatrogenic breaks of the skin or mucosal barrier. Since its introduction in the 1990s, antifungal prophylaxis with fluconazole has led to a remarkable reduction of candidaemia in haemato-oncological patients [13–16]. Invasive candidiasis is predominantly a disease of the critically ill, hospitalised patient.

Epidemiology

The incidence rate of invasive candidiasis shows a large geographical variation. In Europe, invasive candidiasis makes up 2-3% of all nosocomial infections – four times less than in the USA [9, 17–20]. Despite an increased use of antifungals, the incidence of candidaemia is on the rise. Between 2000 and 2010 its incidence rate in Swiss hospitals has doubled from 0.49 to 1.01/10 000 patient days [21]. A similar trend was observed in other European surveillance studies, with an average incidence rate increasing to approximately 0.59/10 000 population and 11/10 000 admissions [22–25]. Incidence rates in intensive care unit (ICU) patients are 5–10 times higher than in patients from medical or surgical wards [20, 22, 26]. The average 30-day mortality rate for candidaemia is 43% [27, 28]. This is substantially higher than for any other blood stream infection [29].

The main risk factor and principal portal of entry for candidaemia is an intravenous (IV) catheter [30]. *Candida* biofilms formed on IV catheters are also an important source of continued infection [31]. Other critical risk factors for candidaemia are the use of broad-spectrum antibiotics, total parenteral nutrition, dialysis, and chemotherapy. Notably, all of these factors are associated with critical illness [32–34]. In the USA TRANSNET study on solid-organ transplant recipients, more than 50% of all fungal infections were due to *Candida* with incidence rates increasing from 1.4% to 2.1% between 2001 and 2006. In particular, recipients of abdominal organs (liver, pancreas, small bowel) were likely to become infected [35]. The principal risk factors for invasive intra-abdominal candidiasis are complicated abdominal surgery and pancreatitis, both often leading to ICU admission. Its mortality rate in recent studies was between 27–38% [36, 37].

Different *Candida* species

Worldwide, *C. albicans* remains the dominant *Candida* species. Between 1991 and 2010, Funginos, the "Fungal infection network of Switzerland", reported a stable distribution of blood-stream isolates of 65% *C. albicans*, 15% *C. glabrata*, 6% *C. tropicalis*, 5% *C. parapsilosis* and 2% *C. krusei* [21]. In contrast to the USA, in Switzerland and Northern Europe no shift to fluconazole-resistant *C. krusei* and *C. glabrata* has been observed [27, 38]. In some areas (Southern Europe, the Americas and Asia) *C. parapsilosis* is the second most common isolate after *C. albicans*. The identification of *Candida* isolates to the species level is important because of species-specific antifungal drug resistance patterns, which have major impact on the choice of best treatment. More than 98% of *C. albicans* isolates are susceptible to fluconazole [39], whereas *C. krusei* is constitutiively resistant to fluconazole, but susceptible to newer-generation azoles. Because of its wide array of resistance mechanisms, *C. glabrata* poses the greatest treatment challenge [23, 27]: azole therapy is usually advised against because of the capacity of *C. glabrata* to develop or to extend resistance under treatment, and some studies also report rapidly occurring and/or increased resistance to echinocandins [40–42]. Previous azole exposure increases the risk for infection with azole-resistant *Candida* isolates [43–46].

Clinical presentation

Signs and symptoms of invasive candidiasis are nonspecific. Candidaemia is the most common manifestation, deep-seated infections with or without concomitant candidaemia are rarer. Intra-abdominal candidiasis may occur after complicated abdominal surgery or with necrotising pancreatitis; simultaneous candidaemia is detected in only 10% of cases [47–49]. Candidaemia may go unnoticed when it produces a single febrile peak among many – typically in the critically ill patient. It may induce sepsis or ultimately septic shock, both indistinguishable from bacterial infection [43]. Signs of intra-abdominal candidiasis include persistent fever and clinical deterioration despite continued antibiotic treatment. Deep-seated organ infections are more common in solid organ transplant recipients, whereas candidaemia occurs more often in haematological or critically ill patients [50]. *Candida* pneumonia is rare [11, 51].

Candida chorioretinitis, found in approximately 8–16% of candidaemic patients, may be an easily detectable sign of recent or ongoing candidaemia. It is usually asymptomatic unless progression to vitritis and vision-threatening endophthalmitis produces blurred vision or floating black spots [52, 53]. Progression beyond chorioretinitis is very rare in patients treated for candidaemia but may occur up to several months later if candidaemia was missed

Diagnosis

The diagnosis of candidaemia is often difficult. Because of the lack of disease-specific signs and symptoms, clinical suspicion drives diagnostic testing [56]. Despite their low sensitivity of only 50–70%, blood cultures are still the gold standard to diagnose candidaemia. Intermittent shedding of organisms from deep-seated foci of infection, low pathogen counts per volume of blood or nonviable organisms reduce the sensitivity of modern blood culture methods [56]. Frequent sampling is recommended [57–59]. The diagnosis of deep-seated candidiasis is based on positive cultures of

sterilely collected tissue samples, which are often difficult to obtain [56].

Molecular tests such as the assay for (1–3)-β-D-glucan, a cell wall component of various medically important fungi (except *Cryptococcus* and mucormycetes), may detect candidaemia and intra-abdominal candidiasis. However, the low sensitivity (65–75%) and specificity (80–85%) limit the usefulness of the test, which is not commonly offered by European microbiology laboratories [57, 60, 61].

A commercial, whole-blood, multiplex polymerase chain-reaction (PCR) assay that detects the five clinically most important *Candida* species, *Aspergillus fumigatus* as well as several bacteria had a sensitivity of 94% in candidaemic patients [62]. The T2 magnetic resonance assay, an automated, PCR-based method, appears to be a promising new test for the rapid diagnosis of candidaemia [63]. MALDI-TOF (matrix-assisted laser desorption/ionisation time of flight) has been shown to accelerate correct identification of *Candida* species [64]. To search for endophthalmitis, a dilated fundoscopy is recommended for all candidaemic patients with any visual symptom or those unable to report symptoms (i.e. sedated patients) [52].

The detection of *Candida* in a respiratory or urinary sample is almost always due to colonisation [11, 65]. Symptomatic candiduria in a neutropenic patient, however, may indicate a *Candida* cystitis that merits therapy. Persistence of candiduria after catheter removal may be due to colonised foreign bodies in the urinary tract (i.e. kidney or bladder stones) and requires imaging studies.

Treatment

Indications and agents for antifungal prophylaxis are listed in table 1. Antifungal therapy (table 2) is indicated in all patients with evidence of *Candida* in their blood culture regardless of the presence of clinical symptoms [66]. As a result of the nonspecific clinical presentation as well as the poor sensitivity of diagnostic tests, treatment is mostly initiated on the basis of clinical suspicion. Following the latest European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines all non-neutropenic candidaemia patients – regardless of the underlying disease – should primarily receive an echinocandin or alternatively liposomal amphotericin B [66–68]. For neutropenic patients (mostly after haematopoietic stem cell transplantation [HSCT]) echinocandins and amphotericin B are both first choice [69]. For susceptible organisms, fluconazole may be a reasonable choice for continuation therapy in clinically improved patients. For all non-*albicans Candida spp.* treatment is adapted in accordance with resistance testing. They should initially be treated either with an echinocandin or amphotericin B [66].

Treatment for candidaemia should be continued for 14 days after the first negative blood culture [70]. All patients with candidaemia should have daily blood cultures until clearance of the blood stream infection is documented. In a suspected catheter-associated infection, the invasive device should be removed as soon as possible [66, 69]. The 2016 update of the Infectious Diseases Society of America (IDSA) guideline includes suggested treatment durations for some deep-seated infections [71].Combination therapy is generally not recommended. Chorioretinitis is usually sufficiently treated with the systemic antifungal therapy for candidemia [54, 55]. *Candida* endophthalmitis extending into the vitreous body commonly warrants surgical intervention as well as intraocular antifungals.

Aspergillosis

Aspergillosis is the most common mould infection in humans, accounting for >85% of invasive mould disease [72]. *Aspergillus* is found in soil, decaying vegetation, food, air, and the water supply [73]. Its ubiquitous spores reach the respiratory tract by inhalation. In immunocompromised hosts this results primarily in invasive pulmonary aspergillosis (IPA). Disseminated disease, the end-stage complication of IPA, involves predominantly the brain and kidneys.

Epidemiology

Worldwide there are 200 000 estimated annual cases of invasive aspergillosis (IA) [3]. At present, approximately 50% of all IA are found in patients with haematological malignancy, mostly acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), and recipients of allogeneic HSCT [15, 74]. Prolonged severe neutropenia (>10 days; <500 cell/mm^3) as a result of chemotherapy is still the single most important risk factor for IA. The introduction of mould-active prophylaxis with posaconazole after 2007 [13, 14] is likely to result in a reduction of IA incidence in haemato-oncological patients (AML, HSCT): a French prospective single-centre study described not only

Table 1: Prophylactic regimen with grade A1 and A2 recommendations for invasive candidiasis, aspergillosis, cryptococcal meningitis and Pneumocystis jirovecii pneumonia (PCP).

Risk groups	Aspergillosis	Invasive candidiasis	Cryptococcal meningitis	PCP
Allogeneic HSCT			Currently: no primary prophylaxis recommended	
- Initial neutropenic phase		Fluconazole		TMP-SMX
- GVHD phase (start at 90–100 d)	Posaconazole	Fluconazole / posaconazole		TMP-SMX
Acute myeloid leukaemia (remission-induction chemotherapy)	Posaconazole	Posaconazole		
Acute lymphoblastic leukaemia				TMP-SMX
HIV (CD4 count <200 cells/µl or <14%)		No primary prophylaxis recommended		TMP-SMX
Solid organ transplantation	No definitive recommendations for prophylaxis			TMP-SMX

GVHD = graft-versus-host disease; HIV = human immunodeficiency virus; HSCT = haematopoietic stem cell transplant; SMX = sulfamethoxazole; TMP = trimethoprim
Data from references [66, 69, 149–154].

a reduction in IA incidence in AML patients with posaconazole prophylaxis (7.3%) versus those without prophylaxis (15.5%) but also a decrease in mortality at day 100: 3.6% versus 10.6%, respectively [75]. An Austrian single-centre study found unchanged rates of IFDs but no IA in AML, ALL and HSCT patients after introducing posaconazole prophylaxis. This indicates a remarkable decrease of IA, but also a high rate of breakthrough infections [76]. In keeping with this finding, the continued use of fluconazole for prophylaxis in almost 50% of HSCT recipients in a recent Swiss multicentre study resulted in almost 60% IAs among all proven and probable IFDs [77]. Mortality rates of haematological patients with IA in large clinical treatment trials reached 29% at 3 months [14, 78].

In SOT recipients *Aspergillus* causes one out of five IFDs; it is most commonly found in lung and heart-lung recipients. The 5-year cumulative incidence in all SOT recipients of the TRANSNET study remained stable at 0.7%, with a median time to diagnosis of 184 days [35, 79]. The dominant risk factor in SOT recipients is not a depletion of phagocytic neutrophils but their functional impairment by immunosuppressant drugs.

ICU patients represent the second largest at-risk population for IA. Reported incidence rates are variable but generally high (6.1 to 57/1 000 ICU admissions) [80, 81]. Underlying malignancy in the ICU population is rare. Instead, patients often present with exacerbations of chronic obstructive pulmonary disease, which are treated with high-dose corticosteroids. Severe alcoholic liver cirrhosis is another more recently reported risk factor in ICU patients [82, 83]. Critically ill patients with IA have remarkably high mortality rates ranging from 46 to 80% [84, 85]. Only patients with cerebral involvement, regardless of the underlying disease, reach higher rates of 90% at 4 months [86].

IA has also been associated with concurrent viral respiratory infections due to H1N1 influenza, adenovirus [87], and cytomegalovirus. Particularly in transplant recipients, infection with cytomegalovirus exerts a powerful immunosuppressive effect [88, 89]. In addition, genetic factors such as toll-like receptor-4 (TLR4) haplotypes [90] and pentraxin 3 (PTX3) deficiency [91, 92] have been associated with an increased risk of IA. Ddespite a large number of patients at-risk, only a minority eventually develop IA.

Clinical presentation

Like patients with invasive candidiasis, none of the at-risk groups show specific clinical symptoms. In neutropenic patients fever unresponsive to broad-spectrum antibiotics is often an early sign that should prompt further examination. Rarely, a cough or chest pain with or without haemoptysis – both signs of pulmonary infarction due to mould-induced vascular obstruction – are reported [93]. On the other hand, the critically ill ICU patient with IA is mostly mechanically ventilated and may present with deteriorating lung function and refractory fever [86]. Progression to disseminated disease is often an underdiagnosed complication, particularly in patients with severe or advanced underlying disease [94]. Seizures or other focal neurological signs may be a late manifestation of cerebral dissemination [93]. Primary extrapulmonary organ manifestation is rare [11].

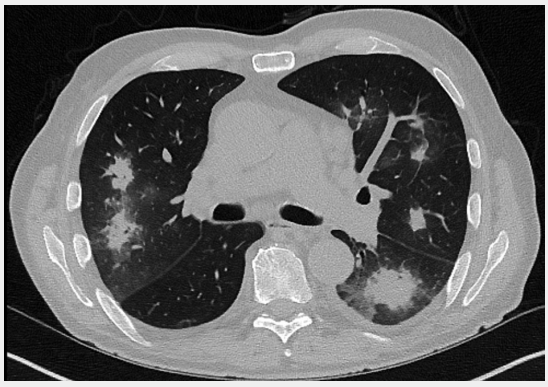
Table 2: Therapeutic regiments with grade A1 recommendation for invasive candidiasis, aspergillosis, cryptococcal meningitis and *Pneumocystis jirovecii* pneumonia (PCP).

Risk groups	Aspergillosis	Invasive candidiasis	Cryptococcal meningitis	PCP
Allogeneic HSCT	**All patients:** Voriconazole (6 mg/kg i.v. BD for 1 d, followed by 4 mg/kg i.v. BD; oral dosage is 300 mg BD). **Alternatively:** Liposomal amphotericin B (3–5 mg/kg/d i.v.).	**Neutropenic patients:** Caspofungin 70/50 mg/d or anidulafungin 200/100 mg or micafungin 100 mg or liposomal amphotericin B 3 mg/kg/d. Fluconazole may be used orally as step-down therapy. Treatment continued for 14 days after first negative blood culture.	**Non-HIV / non-SOT: Induction** – Amphotericin B deoxycholate (0.7–1.0 mg/kg/d i.v.) or liposomal amphotericin B (3–4 mg/kg/d i.v.) plus flucytosine (100 mg/kg/d p.o. in 4 divided doses) for at least 4 weeks. **Consolidation:** Fluconazole (400–800 mg/od p.o.) for 8 weeks. **Maintenance:** Fluconazole (200 mg OD p.o.) for 6 to 12 months.	**HIV negative:** TMP-SMX: 15 mg TMP component/kg/day p.o. or i.v. in 3 or 4 divided doses for 14 days.
Patients without malignancies		**Non-neutropenic patients:** Caspofungin 70/50 mg; anidulafungin 200/100 mg; micafungin 100 mg; fluconazole may be used orally as step-down therapy. Continued for 14 days after first negative blood culture.		
Solid-organ transplantation			**SOT: Induction** – Amphotericin B deoxycholate (0.7–1.0 mg/kg/d i.v.) or liposomal amphotericin B (3–4 mg/kg/d i.v.) plus flucytosine (100 mg/kg/d p.o. in 4 divided doses) for at least 2 weeks. **Consolidation:** Fluconazole (400–800 mg/od p.o.) for 8 weeks. **Maintenance:** Fluconazole (200400 mg/d p.o.) for 6 to 12 months.	
HIV (CD4 count <200 cells/µl or <14%)			**HIV positive: Induction** – Amphotericin B deoxycholate (0.7–1.0 mg/kg/d i.v.) or liposomal amphotericin B (3–4 mg/kg/d i.v.) plus flucytosine (100 mg/kg/d in 4 divided doses p.o.) for at least 2 weeks. **Consolidation:** Fluconazole (400 mg/d p.o.) for a minimum of 8 weeks. **Maintenance:** Fluconazole (200 mg/d) for secondary prophylaxis (min. 1 year) – discontinuation not before CD4 count >100 cell/µl.	**HIV positive:** TMP-SMX: 15 mg TMP component/kg/day p.o. or i.v. in 3 or 4 divided doses for 21 days.

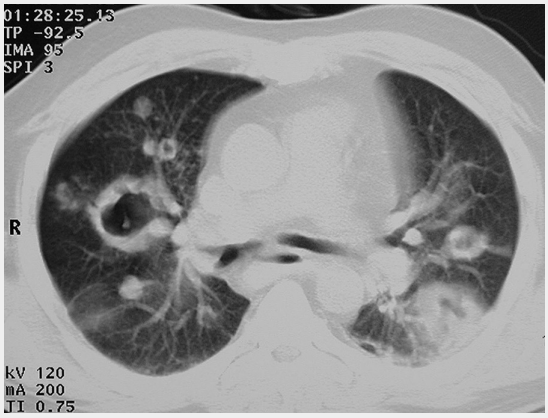
BD = twice daily; i.v. = intravenously; HIV = human immunodeficiency virus; p.o. = orally; SMX = sulfamethoxazole; SOT = solid organ transplantation
Data from references [66, 69, 149, 151, 155–158]

Diagnosis

The diagnosis of IA is challenging and requires a high level of suspicion. The gold standard for proving invasive fungal infection is histopathology or culture of a tissue sample, which is rarely available on time because of the risks involved in performing biopsies during pancytopenia [8]. Detection of galactomannan antigen (GM), a constituent of the hyphal cell wall, in blood is currently the most widely available albeit only moderately accurate noninvasive test for invasive aspergillosis. It has a sensitivity of approximately 71% and a specificity of approximately 89% in high-risk haematological patients [95]. In corticosteroid-treated nonhaematology patients, GM detection in bronchoalveolar lavage (BAL) fluid has higher sensitivity for IPA than in blood [96]. Caution is warranted in patients on mould-active prophylaxis as the sensitivity of the GM assay is reduced [97], making it difficult to identify breakthrough infection in haematological patients [98, 99]. Repeat serological tests of (1–3)-β-D-glucan may be used as adjunct in the diagnosis of aspergillosis, although as a cell wall constituent shared between *Aspergillus spp.*, *Candida spp.*, and *Pneumocystis jirovecii* (but absent from *Cryptococcus* and mucormycetes), it is nonspecific and a high rate of false-positive results may render its interpretation difficult [100, 101]. Blood cultures are virtually always negative in IA [3] and otherwise represent mostly contamination [8].



a



b

Figure 1

Computed tomography images of invasive pulmonary aspergillosis.
a) Neutropenic patient, early stages: sharply demarcated nodes and dense lesions surrounded by a ground-glass halo.
b) Rheumatology patient on corticosteroid treatment, early stages: centrally cavitating dense lesions.

PCR may be a useful tool to diagnose aspergillosis. A recent review reports mean sensitivity and specificity of a single PCR test (plasma and serum) of 80.5% and 78.5%, respectively, and of two consecutive PCRs of 58% and 96.2%, respectively [102]. The European Aspergillus PCR initiative recently introduced a standardised PCR with better sensitivity (95%) and specificity (83%) in plasma than in serum [103]. The majority of IA cases (>90%) are caused by *A. fumigatus*. *A. flavus, A. terreus* and *A. niger*. Of therapeutic relevance is the identification of *A. terreus* (see "Treatment" below).

Imaging studies often provide the first evidence of IA. In neutropenic patients with fever not responding to antibiotic treatment, a high-resolution computed tomography (CT) scan of the thorax may reveal sharply demarcated nodes or dense lesions surrounded by a ground-glass halo compatible with, but not proof of, a fungal aetiology (fig. 1a). In clinical practice, it may be difficult to dismiss the possibility of a fungal aetiology based on the radiomorphology of a lesion [104, 105]. Whereas in neutropenic patients centrally cavitating nodules (crescent sign) are a late sign of invasive fungal infection appearing with the recovery from neutropenia, cavitating pulmonary lesions may be an early sign in, for example, rheumatology patients treated with corticosteroids (fig. 1b). In contrast, in patients on steroid-treatment due to exacerbated chronic lung disease, the CT may only show non-specific pulmonary infiltrates [106].

Treatment

Mould-active prophylaxis is recommended for the duration of the neutropenia associated with remission-induction chemotherapy in high-risk patients with AML or myelodysplastic syndrome. Indications for prophylaxis are given in table 1. Starting treatment early is important in order to reduce mortality. First-line treatment for IA is voriconazole (table 2). Compared with amphotericin deoxycholate it showed a survival benefit in haematological patients (71% vs 58%). Furthermore, voriconazole has a more favourable adverse effect profile and, notably, no nephrotoxicity [78]. However, because of extensive drug-drug interactions and large inter- and intra-individual variation in serum levels, therapeutic drug monitoring is advisable when using voriconazole. An equivalent alternative may be liposomal amphotericin B, though no comparative studies have been conducted. Although most *Aspergillus* isolates exhibit good susceptibility to mould-active azoles as well as amphotericin B, studies from the Netherlands, Belgium and Germany have reported an increasing number of azole-resistant clinical *Aspergillus* isolates [107–109]. *A. terreus* is constitutively resistant to amphotericin B [93]. It is recommended to continue treatment for invasive aspergillosis until all clinical signs and symptoms have resolved. In haematological patients combination therapy with anidulafungin and voriconazole showed no significant survival advantage over monotherapy with voriconazole [110]. Nevertheless, concomitant treatment with two antifungal drugs of different classes may occasionally be used to extend the spectrum of activity when the pathogen is unknown. Suspected coinfection with different fungal species may be another reason for combining antifungal agents.

Other measures such as reduction of iatrogenic immunosuppression should be considered [111].

Cryptococcosis

Epidemiology

With more than one million cases and about 650 000 deaths annually, cryptococcosis is the most prevalent fatal fungal disease worldwide [3, 112]. The most common disease manifestation is cryptococcal meningitis presenting as an opportunistic infection principally in the HIV positive with CD4 cell counts below 100 cells/µl or the iatrogenically immunosuppressed [113]. Cryptococcal meningitis is rare in Europe but in sub-Saharan Africa annual incidence rates range from 100/100 000 in high HIV prevalence settings to 4 000/100 000 among the HIV positive [114, 115]. The dominant species in this yeast genus is *Cryptococcus neoformans*, a ubiquitous environmental pathogen. Following inhalation, cryptococcal yeasts are deposited in the lung where they may cause life-long latent infection [116]. In a USA study, 70% of 120 children older than five years were seropositive for *C. neoformans*, indicating oligosymptomatic infection early in life. Only in the immunosuppressed patient, usually after adolescence, infection may reactivate [117].

Globally, HIV remains the leading risk factor for CM, although the widespread use of antiretroviral therapy has led to a steady decline in HIV-associated CM [112, 113]. In the developed world CM is now seen in approximately 2.8% of all SOT recipients, largely kidney transplant recipients. Infection occurs mostly >18 months after transplantation [118, 119]. Rarely, cryptococcosis is seen in apparently immunocompetent hosts. A USA study found that, in non-HIV and non-SOT patients, time to diagnosis was prolonged and outcome was worse compared with HIV positive individuals and SOT recipients [118]. Apparently immunocompetent patients are more likely to have pulmonary disease and less likely to develop CM [120]. Disseminated disease can reach any organ, but the lungs and the skin are predominately affected [11].

Clinical presentation

Symptoms of CM are nonspecific. The clinical picture ranges from minimal to severe symptoms with headache, malaise, fever, visual disturbance, nausea and vomiting. In contrast to bacterial meningitis, meningism is rare [11, 113]. Disease onset is variable, but tends to be more insidious in the immunocompetent than in those with advanced HIV disease. Intracranial pressure is commonly raised and seizures may occur in advanced disease. Pulmonary disease produces nonspecific symptoms such as cough, fever and malaise. Skin lesions, which predominately occur in immunocompromised patients, may present as pustules, papules, ulcers, cellulitis, superficial granulomas, or abscesses [121, 122].

Diagnosis

Diagnosis of CM is established by positive fungal culture or detection of cryptococcal antigen (CrAg) in cerebrospinal fluid. The CrAg test has a sensitivity and specificity of >90% in both cerebrospinal fluid and serum [123]. In developing countries, a point-of-care urine dipstick test has shown promising results [124]. The diagnosis of pulmonary disease is based on a positive sputum culture in conjunction with compatible imaging findings. A positive serum CrAg in pulmonary disease may represent disseminated disease [125]. Extracranial disease manifestation warrants lumbar puncture to rule out possibly asymptomatic meningitis [126, 127].

Treatment

The current treatment guidelines for CM are based on the results of a randomised clinical trial showing superiority of flucytosine over fluconazole in combination with amphotericin B deoxycholate for induction therapy (table 2) [127, 128]. Clinically, control of intracranial pressure is pivotal: if the initial intracranial pressure exceeds 250 mm, it should be decreased by 50% or at least to <200 mm. Repeated daily drainage may be necessary [127]. For pulmonary disease, recommended treatment regimens vary with disease severity: severe disease is treated as CM [129], moderate disease responds to oral fluconazole [11]. In CM patients with HIV, initiation of antiretroviral therapy needs to be delayed for, generally, 5 weeks in order to avoid early excess mortality [130]. In the early phase of antiretroviral therapy a potentially lethal immune-reconstitution inflammatory syndrome occurs in approximately 14–30% of individuals successfully treated for cryptococcosis [131].

Pneumocystis jirovecii pneumonia

Epidemiology

Traditionally considered a protozoan, *Pneumocystis* has been shown to belong to the family of fungi. An important reservoir of this opportunistic organism is children, most of whom are infected early in life and may transmit the organism from person to person via the airborne route [11, 132]. It is currently unclear whether clinical disease results from recent reinfection or from reactivation of latent infection in the naturally colonised host [133]. It is estimated that approximately 400 000 individuals are affected by *Pneumocystis jirovecii* pneumonia (PCP) every year – as many as by *C. albicans* [3]. In the Western world, PCP used to be the most common opportunistic infection in HIV-positive individuals [134]. With the introduction of antiretroviral therapy, the incidence of PCP has significantly decreased. Today, the principal risk group comprises patients who are iatrogenically immunocompromised because of either malignancy, transplantation or rheumatological disease. In a recent European study PCP was the second most common invasive fungal infection in a mixed HIV-positive and -negative population, with an incidence rate of 1.5/100 000 and a fatality rate of 9.5% [135]. Counterintuitively, mortality rates in HIV-negative patients were higher (30–60%) than in HIV-positive individuals (10–20%) [135–137].

Clinical presentation

The clinical course depends on the underlying disease. Generally, in HIV-positive patients the onset of disease is insidious and prolonged, whereas in non-HIV patients dis-

ease progression tends to be more fulminant – possibly explaining the increased mortality in this group. Clinical symptoms are nonspecific including low-grade fever, dry cough and progressive dyspnoea. Lung auscultation is commonly normal [138].

Diagnosis

The diagnosis is confirmed by the detection of the fungal organisms obtained via BAL or induced sputum using fluorescence microscopy in conjunction with a radiological image [139, 140]. Radiological signs include bilateral perihilar interstitial infiltrates [141]. In the early disease stage a chest x-ray may be normal. High-resolution CT is more sensitive, revealing perihilar ground-glass attenuation or cystic lesions [142]. In patients who cannot tolerate bronchoscopy, the serum (1–3)-β-D-glucan is a good adjunct marker with a test sensitivity of 96% and specificity of 87% [143]. The diagnostic yield of any specimen from HIV patients is higher than from HIV-negative patients [144]. Several trials have shown good sensitivities and specificities of real-time PCR [145, 146]. However, so far there is no standardised method and the additional problem of distinguishing between (frequent) colonisation and infection is unresolved.

Treatment

Prophylaxis should be given to all those at risk (table 1). Owing to lack of ergosterol in the plasma membrane, *Pneumocystis jirovecii* is insensitive to polyenes and azoles [11]. Current treatment regimens (table 2) are primarily based on trimethoprim/sulfamethoxazole. In HIV patients with an initial PO_2 <70 mm Hg, the additional administration of corticosteroids has resulted in improved survival [147]. The treatment duration is only well established in HIV-positive patients and lasts for 3 weeks, while non-HIV patients are commonly given a 14-day course. In the case of intolerance to trimethoprim/sulfamethoxazole, clindamycin plus primaquine, atovaquone or trimethoprim/dapsone are alternative regimens [148].

Disclosure statement: No financial support and no other potential conflict of interest relevant to this article was reported.

Correspondence: *Stephan Zimmerli, MD, Inselspital Bern, CH-3010 Bern, stefan.zimmerli[at]insel.ch*

References

1 Chandler F, Watts, J. Mycotic, Actinomycotic, and Algal Infections. In: Mosby CV, ed. Anderson's Pathology. 9th ed. St. Louis; 1996:391–432.

2 Casadevall A. Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection? Fungal Genet Biol. 2005;42(2):98–106.

3 Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012;4(165):165rv13.

4 WHO 2015;Pages. Accessed at WHO at http://www.who.int/mediacentre/factsheets/fs094/en/2015.

5 WHO 2015;Pages. Accessed at WHO at http://www.who.int/mediacentre/factsheets/fs104/en/2015.

6 Das R, Ranganathan, R. An Overview of Changing Trends in Systemic Fungal Infections. WebmedCentral MICROBIOLOGY. 2012;3(5).

7 Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis. 2002;34(1):7–14.

8 De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813–21.

9 Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20(1):133–63.

10 Bennett J, Dolin, R, Blaser M. Mandell, Douglas, and Bennett's Principles and Practice of Infectious diseases. 8th ed. Philadelphia: Elsevier Saunders; 2015.

11 Kauffman CA, Pappas, P.G., Sobel, J., Dismukes, W. Essentials of Clinical Mycology. New York: Springer; 2011.

12 Abu-Elteen K, Hamad, MA Changing Epidemiology of Classical and Emerging Human Fungal Infections: A Review. Jordan Journal of Biological Sciences. 2012;5(4):215–30.

13 Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med. 2007;356(4):335–47.

14 Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med. 2007;356(4):348–59.

15 Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. Haematologica. 2006;91(8):1068–75.

16 Girmenia C, Finolezzi E, Federico V, Santopietro M, Perrone S. Invasive Candida infections in patients with haematological malignancies and hematopoietic stem cell transplant recipients: current epidemiology and therapeutic options. Mediterr J Hematol Infect Dis. 2011;3(1):e2011013.

17 Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. Clin Infect Dis. 2004;38(3):311–20.

18 Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39(3):309–17.

19 Pappas PG. Invasive candidiasis. Infect Dis Clin North Am. 2006;20(3):485–506.

20 Mean M, Marchetti O, Calandra T. Bench-to-bedside review: Candida infections in the intensive care unit. Crit Care. 2008;12(1):204.

21 Orasch CE, Marchetti, O, Durussel, C, Guyaz, C, Ochsner, M, Bille, J. Six-year prospective candidemia survey from the fungal infection network of Switzerland (FUNGINOS) − Candida species distribution and antifungal susceptibility according to recent EUCAST and old vs. new CLSI clinical breakpoints. In: 2013, ed. 23rd ECCMID. Berlin; 2013.

22 Lass-Florl C. The changing face of epidemiology of invasive fungal disease in Europe. Mycoses. 2009;52(3):197–205.

23 Asmundsdottir LR, Erlendsdottir H, Gottfredsson M. Nationwide study of candidemia, antifungal use, and antifungal drug resistance in Iceland, 2000 to 2011. J Clin Microbiol. 2013;51(3):841–8.

24 Presterl E, Daxbock F, Graninger W, Willinger B. Changing pattern of candidaemia 2001-2006 and use of antifungal therapy at the University Hospital of Vienna, Austria. Clin Microbiol Infect. 2007;13(11):1072–6.

25 Almirante B, Rodriguez D, Park BJ, Cuenca-Estrella M, Planes AM, Almela M, et al. Epidemiology and predictors of mortality in cases of Candida bloodstream infection: results from population-based surveillance, barcelona, Spain, from 2002 to 2003. J Clin Microbiol. 2005;43(4):1829–35.

26 Yapar N. Epidemiology and risk factors for invasive candidiasis. Ther Clin Risk Manag. 2014;10:95–105.

27 Bassetti M, Merelli M, Righi E, Diaz-Martin A, Rosello EM, Luzzati R, et al. Epidemiology, species distribution, antifungal susceptibility, and

outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol. 2013;51(12):4167–72.

28 Chen LY, Kuo SC, Wu HS, Yang SP, Chan YJ, Chen LK, et al. Associated clinical characteristics of patients with candidemia among different Candida species. J Microbiol Immunol Infect. 2013;46(6):463–8.

29 Kett DH, Azoulay E, Echeverria PM, Vincent JL, Extended Prevalence of Infection in ICUSGoI. Candida bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med. 2011;39(4):665–70.

30 Denning DW, Kibbler CC, Barnes RA, British Society for Medical M. British Society for Medical Mycology proposed standards of care for patients with invasive fungal infections. Lancet Infect Dis. 2003;3(4):230–40.

31 Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013;62(Pt 1):10–24.

32 Leon C, Ruiz-Santana S, Saavedra P, Galvan B, Blanco A, Castro C, et al. Usefulness of the "Candida score" for discriminating between Candida colonization and invasive candidiasis in non-neutropenic critically ill patients: a prospective multicenter study. Crit Care Med. 2009;37(5):1624–33.

33 Playford EG, Lipman J, Kabir M, McBryde ES, Nimmo GR, Lau A, et al. Assessment of clinical risk predictive rules for invasive candidiasis in a prospective multicentre cohort of ICU patients. Intensive Care Med. 2009;35(12):2141–5.

34 Vardakas KZ, Michalopoulos A, Kiriakidou KG, Siampli EP, Samonis G, Falagas ME. Candidaemia: incidence, risk factors, characteristics and outcomes in immunocompetent critically ill patients. Clin Microbiol Infect. 2009;15(3):289–92.

35 Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–11.

36 Bassetti M, Righi E, Ansaldi F, Merelli M, Scarparo C, Antonelli M, et al. A multicenter multinational study of abdominal candidiasis: epidemiology, outcomes and predictors of mortality. Intensive Care Med. 2015;41(9):1601–10.

37 Montravers P, Mira JP, Gangneux JP, Leroy O, Lortholary O, AmarCand study g. A multicentre study of antifungal strategies and outcome of Candida spp. peritonitis in intensive-care units. Clin Microbiol Infect. 2011;17(7):1061–7.

38 Orasch C, Marchetti O, Garbino J, Schrenzel J, Zimmerli S, Muhlethaler K, et al. Candida species distribution and antifungal susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing and new vs. old Clinical and Laboratory Standards Institute clinical breakpoints: a 6-year prospective candidaemia survey from the fungal infection network of Switzerland. Clin Microbiol Infect. 2014;20(7):698–705.

39 Playford EG, Marriott D, Nguyen Q, Chen S, Ellis D, Slavin M, et al. Candidemia in nonneutropenic critically ill patients: risk factors for non-albicans Candida spp. Crit Care Med. 2008;36(7):2034–9.

40 Lewis JS, 2nd, Wiederhold NP, Wickes BL, Patterson TF, Jorgensen JH. Rapid emergence of echinocandin resistance in Candida glabrata resulting in clinical and microbiologic failure. Antimicrob Agents Chemother. 2013;57(9):4559–61.

41 Alexander BD, Johnson MD, Pfeiffer CD, Jimenez-Ortigosa C, Catania J, Booker R, et al. Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis. 2013;56(12):1724–32.

42 Pfaller MA, Castanheira M, Lockhart SR, Ahlquist AM, Messer SA, Jones RN. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of Candida glabrata. J Clin Microbiol. 2012;50(4):1199–203.

43 Delaloye J, Calandra T. Invasive candidiasis as a cause of sepsis in the critically ill patient. Virulence. 2014;5(1):161–9.

44 Chow JK, Golan Y, Ruthazer R, Karchmer AW, Carmeli Y, Lichtenberg D, et al. Factors associated with candidemia caused by non-albicans Candida species versus Candida albicans in the intensive care unit. Clin Infect Dis. 2008;46(8):1206–13.

45 Antimicrobials for Candida infections – EUCAST clinical MIC breakpoints. In: EUCAST, ed; 2013.

46 Pfaller MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, et al. Epidemiology and outcomes of invasive candidiasis due to non-albicans species of Candida in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004-2008. PLoS One. 2014;9(7):e101510.

47 Bozzette SA, Gordon RL, Yen A, Rinaldi M, Ito MK, Fierer J. Biliary concentrations of fluconazole in a patient with candidal cholecystitis: case report. Clin Infect Dis. 1992;15(4):701–3.

48 Keiser P, Keay S. Candidal pancreatic abscesses: report of two cases and review. Clin Infect Dis. 1992;14(4):884–8.

49 Morris AB, Sands ML, Shiraki M, Brown RB, Ryczak M. Gallbladder and biliary tract candidiasis: nine cases and review. Rev Infect Dis. 1990;12(3):483–9.

50 Leroy O, Gangneux JP, Montravers P, Mira JP, Gouin F, Sollet JP, et al. Epidemiology, management, and risk factors for death of invasive Candida infections in critical care: a multicenter, prospective, observational study in France (2005-2006). Crit Care Med. 2009;37(5):1612–8.

51 Schnabel RM, Linssen CF, Guion N, van Mook WN, Bergmans DC. Candida pneumonia in intensive care unit? Open Forum Infect Dis. 2014;1(1):ofu026.

52 Vinikoor MJ, Zoghby J, Cohen KL, Tucker JD. Do all candidemic patients need an ophthalmic examination? Int J Infect Dis. 2013;17(3):e146–8.

53 Khalid A, Clough LA, Symons RC, Mahnken JD, Dong L, Eid AJ. Incidence and clinical predictors of ocular candidiasis in patients with Candida fungemia. Interdiscip Perspect Infect Dis. 2014;2014:650235.

54 Oude Lashof AM, Rothova A, Sobel JD, Ruhnke M, Pappas PG, Viscoli C, et al. Ocular manifestations of candidemia. Clin Infect Dis. 2011;53(3):262–8.

55 Shah CP, McKey J, Spirn MJ, Maguire J. Ocular candidiasis: a review. Br J Ophthalmol. 2008;92(4):466–8.

56 Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013;56(9):1284–92.

57 Cuenca-Estrella M, Verweij PE, Arendrup MC, Arikan-Akdagli S, Bille J, Donnelly JP, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. Clin Microbiol Infect. 2012;18(Suppl 7):9–18.

58 Ostrosky-Zeichner L. Invasive mycoses: diagnostic challenges. Am J Med. 2012;125(1 Suppl):S14–24.

59 Gaspar GG, Menegueti MG, Auxiliadora-Martins M, Basile-Filho A, Martinez R. Evaluation of the predictive indices for candidemia in an adult intensive care unit. Rev Soc Bras Med Trop. 2015;48(1):77–82.

60 Tissot F, Lamoth F, Hauser PM, Orasch C, Fluckiger U, Siegemund M, et al. beta-glucan antigenemia anticipates diagnosis of blood culture-negative intraabdominal candidiasis. Am J Respir Crit Care Med. 2013;188(9):1100–9.

61 Martinez-Jimenez MC, Munoz P, Valerio M, Alonso R, Martos C, Guinea J, et al. Candida biomarkers in patients with candidaemia and bacteraemia. J Antimicrob Chemother. 2015.

62 Lucignano B, Ranno S, Liesenfeld O, Pizzorno B, Putignani L, Bernaschi P, et al. Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. J Clin Microbiol. 2011;49(6):2252–8.

63 Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis. 2015;60(6):892–9.

64 Lacroix C, Gicquel A, Sendid B, Meyer J, Accoceberry I, Francois N, et al. Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of Candida species. Clin Microbiol Infect. 2014;20(2):153–8.

65 Rello J, Esandi ME, Diaz E, Mariscal D, Gallego M, Valles J. The role of Candida sp isolated from bronchoscopic samples in nonneutropenic patients. Chest. 1998;114(1):146–9.

66 Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect. 2012;18(Suppl 7):19–37.

67 Fluckiger U, Marchetti O, Bille J, Eggimann P, Zimmerli S, Imhof A, et al. Treatment options of invasive fungal infections in adults. Swiss Med Wkly. 2006;136(29-30):447–63.

68 Reboli AC, Rotstein C, Pappas PG, Chapman SW, Kett DH, Kumar D, et al. Anidulafungin versus fluconazole for invasive candidiasis. N Engl J Med. 2007;356(24):2472–82.

69 Ullmann AJ, Akova M, Herbrecht R, Viscoli C, Arendrup MC, Arikan-Akdagli S, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). Clin Microbiol Infect. 2012;18(Suppl 7):53–67.

70 Oude Lashof AM, Donnelly JP, Meis JF, van der Meer JW, Kullberg BJ. Duration of antifungal treatment and development of delayed complications in patients with candidaemia. Eur J Clin Microbiol Infect Dis. 2003;22(1):43–8.

71 Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2015.

72 Alastruey-Izquierdo A, Mellado E, Pelaez T, Peman J, Zapico S, Alvarez M, et al. Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). Antimicrob Agents Chemother. 2013;57(7):3380–7.

73 Anaissie EJ, Stratton SL, Dignani MC, Summerbell RC, Rex JH, Monson TP, et al. Pathogenic Aspergillus species recovered from a hospital water system: a 3-year prospective study. Clin Infect Dis. 2002;34(6):780–9.

74 Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. Clin Infect Dis. 2010;50(8):1091–100.

75 Michallet M, Sobh M, Morisset S, Kraghel S, Nicolini FE, Thomas X, et al. Risk factors for invasive aspergillosis in acute myeloid leukemia patients prophylactically treated with posaconazole. Med Mycol. 2011;49(7):681–7.

76 Auberger J, Lass-Florl C, Aigner M, Clausen J, Gastl G, Nachbaur D. Invasive fungal breakthrough infections, fungal colonization and emergence of resistant strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-centre institutional retrospective observational study. J Antimicrob Chemother. 2012;67(9):2268–73.

77 Kuster S, Stampf, S, Baettig, V, Gerull, S, Passweg, J, Mueller, N, Schanz, U, Gerber, B, Berger, C, Chalandon, Y, Van Delden, C, Weisser, M, Khanna, N Epidemiology of invasive fungal infections in patients after allogeneic haematopoietic stem cell transplantation from 2009 until 2013 in a Swiss transplant cohort study. ECCMID Copenhagen: Poster No EP074; 2015.

78 Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med. 2002;347(6):408–15.

79 Singh N, Husain S, Practice ASTIDCo. Aspergillosis in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):228–41.

80 Meersseman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, Van Wijngaerden E. Invasive aspergillosis in critically ill patients without malignancy. Am J Respir Crit Care Med. 2004;170(6):621–5.

81 Tortorano AM, Dho G, Prigitano A, Breda G, Grancini A, Emmi V, et al. Invasive fungal infections in the intensive care unit: a multicentre, prospective, observational study in Italy (2006-2008). Mycoses. 2012;55(1):73–9.

82 Bulpa PA, Dive AM, Garrino MG, Delos MA, Gonzalez MR, Evrard PA, et al. Chronic obstructive pulmonary disease patients with invasive pulmonary aspergillosis: benefits of intensive care? Intensive Care Med. 2001;27(1):59–67.

83 Gustot T, Maillart E, Bocci M, Surin R, Trepo E, Degre D, et al. Invasive aspergillosis in patients with severe alcoholic hepatitis. J Hepatol. 2014;60(2):267–74.

84 Baddley JW, Stephens JM, Ji X, Gao X, Schlamm HT, Tarallo M. Aspergillosis in Intensive Care Unit (ICU) patients: epidemiology and economic outcomes. BMC Infect Dis. 2013;13:29.

85 Meersseman W, Van Wijngaerden E. Invasive aspergillosis in the ICU: an emerging disease. Intensive Care Med. 2007;33(10):1679–81.

86 Taccone FS, Van den Abeele AM, Bulpa P, Misset B, Meersseman W, Cardoso T, et al. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. Crit Care. 2015;19:7.

87 Garcia-Vidal C, Royo-Cebrecos C, Peghin M, Moreno A, Ruiz-Camps I, Cervera C, et al. Environmental variables associated with an increased risk of invasive aspergillosis. Clin Microbiol Infect. 2014;20(11):O939–45.

88 Thursky K, Byrnes G, Grigg A, Szer J, Slavin M. Risk factors for post-engraftment invasive aspergillosis in allogeneic stem cell transplantation. Bone Marrow Transplant. 2004;34(2):115–21.

89 Li L, Wang J, Zhang W, Yang J, Chen L, Lv S. Risk factors for invasive mold infections following allogeneic hematopoietic stem cell transplantation: a single center study of 190 recipients. Scand J Infect Dis. 2012;44(2):100–7.

90 Bochud PY, Chien JW, Marr KA, Leisenring WM, Upton A, Janer M, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. N Engl J Med. 2008;359(17):1766–77.

91 Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. N Engl J Med. 2014;370(5):421–32.

92 Cunha C, Monteiro AA, Oliveira-Coelho A, Kuhne J, Rodrigues F, Sasaki SD, et al. PTX3-Based Genetic Testing for Risk of Aspergillosis After Lung Transplant. Clin Infect Dis. 2015.

93 Segal BH. Aspergillosis. N Engl J Med. 2009;360(18):1870–84.

94 Dimopoulos G, Piagnerelli M, Berre J, Eddafali B, Salmon I, Vincent JL. Disseminated aspergillosis in intensive care unit patients: an autopsy study. J Chemother. 2003;15(1):71–5.

95 Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis. 2006;42(10):1417–27.

96 He H, Ding L, Sun B, Li F, Zhan Q. Role of galactomannan determinations in bronchoalveolar lavage fluid samples from critically ill patients with chronic obstructive pulmonary disease for the diagnosis of invasive pulmonary aspergillosis: a prospective study. Crit Care. 2012;16(4):R138.

97 Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. J Infect Dis. 2004;190(3):641–9.

98 Kriengkauykiat J, Ito JI, Dadwal SS. Epidemiology and treatment approaches in management of invasive fungal infections. Clin Epidemiol. 2011;3:175–91.

99 Eigl S, Prattes J, Reinwald M, Thornton CR, Reischies F, Spiess B, et al. Influence of mould-active antifungal treatment on the performance of the Aspergillus-specific bronchoalveolar lavage fluid lateral-flow device test. Int J Antimicrob Agents. 2015.

100 Marchetti O, Lamoth F, Mikulska M, Viscoli C, Verweij P, Bretagne S, et al. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. Bone Marrow Transplant. 2012;47(6):846–54.

101 Theel ES, Doern CD. beta-D-glucan testing is important for diagnosis of invasive fungal infections. J Clin Microbiol. 2013;51(11):3478–83.

102 Cruciani M, Mengoli C, Loeffler J, Donnelly P, Barnes R, Jones BL, et al. Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. Cochrane Database Syst Rev. 2015;9:CD009551.

103 White PL, Barnes RA, Springer J, Klingspor L, Cuenca-Estrella M, Morton CO, et al. Clinical Performance of Aspergillus PCR for Testing Serum and Plasma: a Study by the European Aspergillus PCR Initiative. J Clin Microbiol. 2015;53(9):2832–7.

104 Curtis AM, Smith GJ, Ravin CE. Air crescent sign of invasive aspergillosis. Radiology. 1979;133(1):17–21.

105 Kuhlman J, Fishman, EK, Siegelman, SS. Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis Radiology. 1985;157:611–4.

106 Ader F, Nseir S, Le Berre R, Leroy S, Tillie-Leblond I, Marquette CH, et al. Invasive pulmonary aspergillosis in chronic obstructive pulmonary disease: an emerging fungal pathogen. Clin Microbiol Infect. 2005;11(6):427–9.

107 van der Linden JW, Snelders E, Kampinga GA, Rijnders BJ, Mattsson E, Debets-Ossenkopp YJ, et al. Clinical implications of azole resistance in Aspergillus fumigatus, The Netherlands, 2007-2009. Emerg Infect Dis. 2011;17(10):1846–54.

108 Steinmann J, Hamprecht A, Vehreschild MJ, Cornely OA, Buchheidt D, Spiess B, et al. Emergence of azole-resistant invasive aspergillosis in HSCT recipients in Germany. J Antimicrob Chemother. 2015;70(5):1522–6.

109 Vermeulen E, Maertens J, De Bel A, Nulens E, Boelens J, Surmont I, et al. Nationwide Surveillance of Azole Resistance in Aspergillus Diseases. Antimicrob Agents Chemother. 2015;59(8):4569–76.

110 Marr KA, Schlamm HT, Herbrecht R, Rottinghaus ST, Bow EJ, Cornely OA, et al. Combination antifungal therapy for invasive aspergillosis: a randomized trial. Ann Intern Med. 2015;162(2):81–9.

111 Smith TJ, Khatcheressian J, Lyman GH, Ozer H, Armitage JO, Balducci L, et al. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J Clin Oncol. 2006;24(19):3187–205.

112 Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23(4):525–30.

113 Sloan DJ, Parris V. Cryptococcal meningitis: epidemiology and therapeutic options. Clin Epidemiol. 2014;6:169–82.

114 Mbanya DN, Zebaze R, Minkoulou EM, Binam F, Koulla S, Obounou A. Clinical and epidemiologic trends in HIV/AIDS patients in a hospital setting of Yaounde, Cameroon: a 6-year perspective. Int J Infect Dis. 2002;6(2):134–8.

115 McCarthy KM, Morgan J, Wannemuehler KA, Mirza SA, Gould SM, Mhlongo N, et al. Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. AIDS. 2006;20(17):2199–206.

116 Velagapudi R, Hsueh YP, Geunes-Boyer S, Wright JR, Heitman J. Spores as infectious propagules of Cryptococcus neoformans. Infect Immun. 2009;77(10):4345–55.

117 Goldman DL, Khine H, Abadi J, Lindenberg DJ, Pirofski L, Niang R, et al. Serologic evidence for Cryptococcus neoformans infection in early childhood. Pediatrics. 2001;107(5):E66.

118 Brizendine KD, Baddley JW, Pappas PG. Predictors of mortality and differences in clinical features among patients with Cryptococcosis according to immune status. PLoS One. 2013;8(3):e60431.

119 Neofytos D, Fishman JA, Horn D, Anaissie E, Chang CH, Olyaei A, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. Transpl Infect Dis. 2010;12(3):220–9.

120 Kiertiburanakul S, Wirojtananugoon S, Pracharktam R, Sungkanuparph S. Cryptococcosis in human immunodeficiency virus-negative patients. Int J Infect Dis. 2006;10(1):72–8.

121 Chayakulkeeree M, Perfect JR. Cryptococcosis. Infect Dis Clin North Am. 2006;20(3):507–44, v-vi.

122 Baer S, Baddley JW, Gnann JW, Pappas PG. Cryptococcal disease presenting as necrotizing cellulitis in transplant recipients. Transpl Infect Dis. 2009;11(4):353–8.

123 Kauffman CA, Bergman AG, Severance PJ, McClatchey KD. Detection of cryptococcal antigen. Comparison of two latex agglutination tests. Am J Clin Pathol. 1981;75(1):106–9.

124 Jarvis JN, Percival A, Bauman S, Pelfrey J, Meintjes G, Williams GN, et al. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. Clin Infect Dis. 2011;53(10):1019–23.

125 Smith JA, Kauffman CA. Pulmonary fungal infections. Respirology. 2012;17(6):913–26.

126 Baddley JW, Perfect JR, Oster RA, Larsen RA, Pankey GA, Henderson H, et al. Pulmonary cryptococcosis in patients without HIV infection: factors associated with disseminated disease. Eur J Clin Microbiol Infect Dis. 2008;27(10):937–43.

127 Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. Clin Infect Dis. 2010;50(3):291–322.

128 Day JN, Chau TT, Lalloo DG. Combination antifungal therapy for cryptococcal meningitis. N Engl J Med. 2013;368(26):2522–3.

129 Pappas PG. Cryptococcal infections in non-HIV-infected patients. Trans Am Clin Climatol Assoc. 2013;124:61–79.

130 Boulware DR, Meya DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. N Engl J Med. 2014;370(26):2487–98.

131 Haddow LJ, Colebunders R, Meintjes G, Lawn SD, Elliott JH, Manabe YC, et al. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. Lancet Infect Dis. 2010;10(11):791–802.

132 Ramana K, Kandi, S, Bharatkumar P, Sharada CH V, Rao R, Mani R, Rao SD. Invasive Fungal Infections: A Comprehensive Review. Am J Infect Dis Microbiol. 2013;1(4):64–9.

133 Morris A, Norris KA. Colonization by Pneumocystis jirovecii and its role in disease. Clin Microbiol Rev. 2012;25(2):297–317.

134 Kelley CF, Checkley W, Mannino DM, Franco-Paredes C, Del Rio C, Holguin F. Trends in hospitalizations for AIDS-associated Pneumocystis jirovecii Pneumonia in the United States (1986 to 2005). Chest. 2009;136(1):190–7.

135 Bitar D, Lortholary O, Le Strat Y, Nicolau J, Coignard B, Tattevin P, et al. Population-based analysis of invasive fungal infections, France, 2001–2010. Emerg Infect Dis. 2014;20(7):1149–55.

136 Pareja JG, Garland R, Koziel H. Use of adjunctive corticosteroids in severe adult non-HIV Pneumocystis carinii pneumonia. Chest. 1998;113(5):1215–24.

137 Sepkowitz KA. Opportunistic infections in patients with and patients without Acquired Immunodeficiency Syndrome. Clin Infect Dis. 2002;34(8):1098–107.

138 Thomas CF, Jr., Limper AH. Pneumocystis pneumonia. N Engl J Med. 2004;350(24):2487–98.

139 Choe PG, Kang YM, Kim G, Park WB, Park SW, Kim HB, et al. Diagnostic value of direct fluorescence antibody staining for detecting Pneumocystis jirovecii in expectorated sputum from patients with HIV infection. Med Mycol. 2014;52(3):326–30.

140 Armstrong-James D, Meintjes G, Brown GD. A neglected epidemic: fungal infections in HIV/AIDS. Trends Microbiol. 2014;22(3):120–7.

141 DeLorenzo LJ, Huang CT, Maguire GP, Stone DJ. Roentgenographic patterns of Pneumocystis carinii pneumonia in 104 patients with AIDS. Chest. 1987;91(3):323–7.

142 Gruden JF, Huang L, Turner J, Webb WR, Merrifield C, Stansell JD, et al. High-resolution CT in the evaluation of clinically suspected Pneumocystis carinii pneumonia in AIDS patients with normal, equivocal, or nonspecific radiographic findings. AJR Am J Roentgenol. 1997;169(4):967–75.

143 Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME. Accuracy of beta-D-glucan for the diagnosis of Pneumocystis jirovecii pneumonia: a meta-analysis. Clin Microbiol Infect. 2013;19(1):39–49.

144 Catherinot E, Lanternier F, Bougnoux ME, Lecuit M, Couderc LJ, Lortholary O. Pneumocystis jirovecii Pneumonia. Infect Dis Clin North Am. 2010;24(1):107–38.

145 Huggett JF, Taylor MS, Kocjan G, Evans HE, Morris-Jones S, Gant V, et al. Development and evaluation of a real-time PCR assay for detec-

tion of Pneumocystis jirovecii DNA in bronchoalveolar lavage fluid of HIV-infected patients. Thorax. 2008;63(2):154–9.

146 Orsi CF, Bettua C, Pini P, Venturelli C, La Regina A, Morace G, et al. Detection of Pneumocystis jirovecii and Aspergillus spp. DNa in bronchoalveolar lavage fluids by commercial real-time PCr assays: comparison with conventional diagnostic tests. New Microbiol. 2015;38(1):75–84.

147 Kaplan JE, Benson C, Holmes KK, Brooks JT, Pau A, Masur H, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep. 2009;58(RR-4):1–207; quiz CE1-4.

148 Helweg-Larsen J, Benfield T, Atzori C, Miller RF. Clinical efficacy of first- and second-line treatments for HIV-associated Pneumocystis jirovecii pneumonia: a tri-centre cohort study. J Antimicrob Chemother. 2009;64(6):1282–90.

149 Karthaus M. Guideline based treatment of invasive aspergillosis. Mycoses. 2010;53(Suppl 1):36–43.

150 Neumann S, Krause SW, Maschmeyer G, Schiel X, von Lilienfeld-Toal M, Infectious Diseases Working P, et al. Primary prophylaxis of bacterial infections and Pneumocystis jirovecii pneumonia in patients with hematological malignancies and solid tumors: guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). Ann Hematol. 2013;92(4):433–42.

151 Sax P, Bartlett, John, Mitty, Jennifer 2015; Pages http://www.uptodate.com/contents/treatment-and-prevention-of-pneumocystis-infection-in-hiv-infected-patients.

152 WHO. Rapid Advice Diagnosis, Prevention and Management of Cryptococc al Disease in HIV -infected Ad ults, Ad olescents and Children. Geneva, Switzerland: WHO; 2011.

153 Martin SI, Fishman JA, Practice ASTIDCo. Pneumocystis pneumonia in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):272–9.

154 Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective. Preface. Bone Marrow Transplant. 2009;44(8):453–5.

155 Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis. 2008;46(3):327–60.

156 Cox G, Perfect, J 2015;Pages http://www.uptodate.com/contents/treatment-of-cryptococcus-neoformans-meningoencephalitis-and-disseminated-infection-in-hiv-seronegative-patients.

157 Cox G, Perfect, J 2015;Pages http://www.uptodate.com/contents/treatment-of-cryptococcus-neoformans-meningoencephalitis-in-hiv-infected-patients.

158 Thomas C, Limper, A. 2015;Pages http://www.uptodate.com/contents/treatment-and-prevention-of-pneumocystis-infection-in-non-hiv-infected-patients.

Figures (large format)

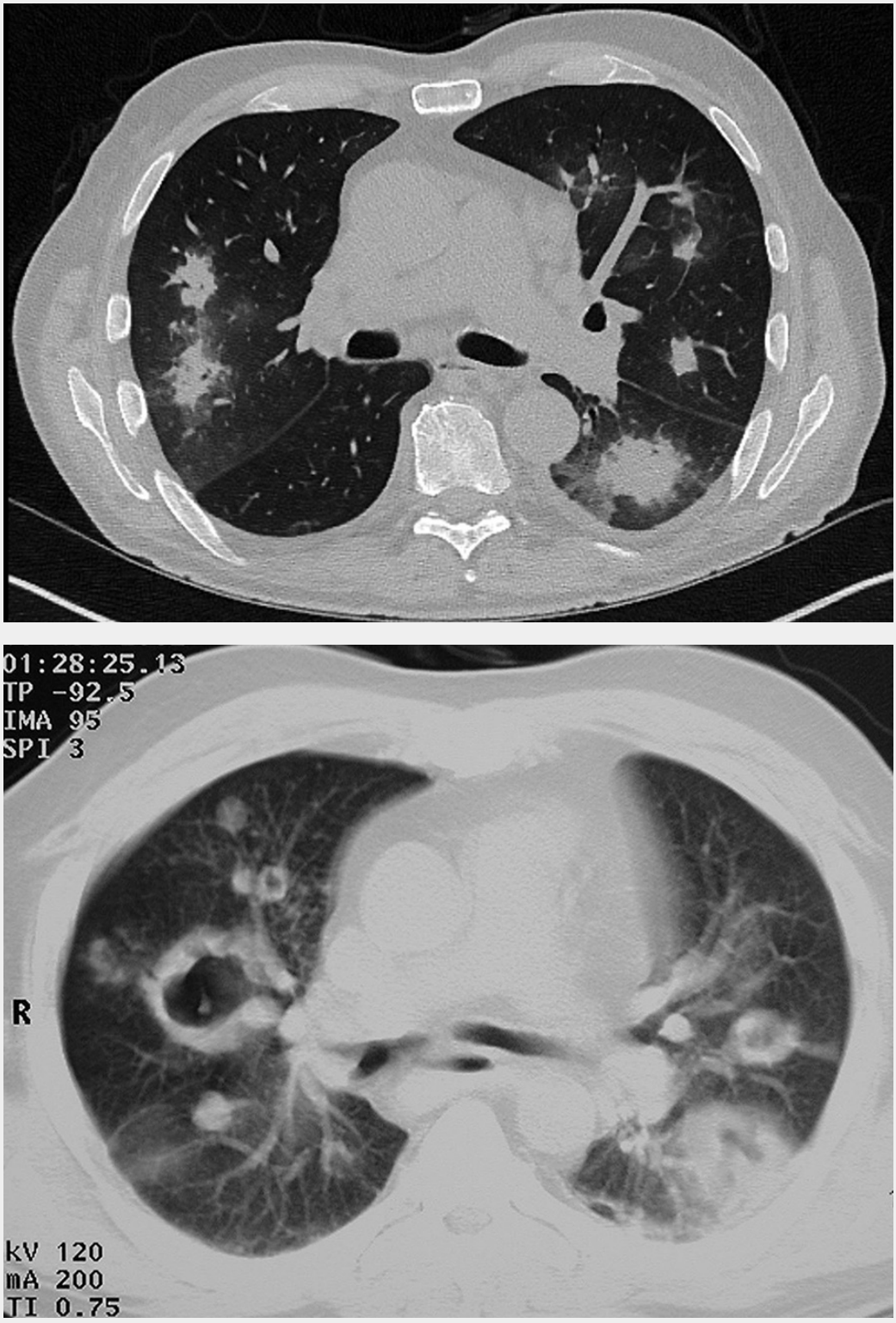


Figure 1

Computed tomography images of invasive pulmonary aspergillosis.
a) Neutropenic patient, early stages: sharply demarcated nodes and dense lesions surrounded by a ground-glass halo.
b) Rheumatology patient on corticosteroid treatment, early stages: centrally cavitating dense lesions.