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Fungal infections in transplant recipients

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ABSTRACT: Fungi are ubiquitous and the respiratory tract is exposed to aerosolized spores of both fungi that are "pathogenic" even in the normal host, such as *Cryptococcus neoformans*, and those that are "opportunistic", such as *Candida* and *Aspergillus* species, among others. Although these latter species may occasionally form fungal balls or induce allergic phenomena in the normal host, they produce more invasive diseases in immunosuppressed patients. Among these diseases, pseudomembranous aspergillosis has recently been described.

The diagnostic approach to these entities, and, in particular, the thin dividing line between colonization and infection are addressed, along with the diagnostic value of the various procedures.

New prophylactic regimens are reviewed such as the possibility of using amphotericin aerosols in combination with systemic azole administration.

The authors would emphasize the importance of restoring lung defences by not only decreasing immunosuppressive regimens but also considering the use of newly available recombinant cytokines such as growth factors, to reduce neutropenia, for instance, in addition to antifungal drugs when infection is diagnosed. However, immunomodulation procedures are far from being well established.

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Fungi are ubiquitous in nature and their constant aerosol exposure to respiratory tissue is unavoidable. Some, such as *Cryptococcus neoformans* and, in North America, *Blastomyces*, *Coccidioides immitis* or *Histoplasma capsulatum* are "pathogenic" even in the normal host. A separate group of potential fungal pathogens generally infect only those patients with abnormal host defences against infection. Among these so-called "opportunistic" fungi, *Candida* and *Aspergillus* species are those most commonly found in transplant patients [1] but others such as *Torulopsis glabrata* or *Phycomyces* (*Mucor* or *Rhizopus*) can also occur.

The respiratory tract is an obvious portal of entry, especially soon after transplantation, not only because the immunosuppressive regimen is at its highest but also because the anatomical barrier of the mucosa is altered. Nevertheless, the gastrointestinal tract and intravascular catheters may occasionally be the source of fungal infection.

Incidence of fungal infection

Fungal infections have been reported to occur in 15–25% of patients after lung transplantation. In 1993,

a 14% infection rate was reported in Pittsburgh, PA, USA, of which 56% was due to *Candida* and 33% to *Aspergillus*. The mortality rate reached 61% in these patients, with the first death occurring as early as 6 days after transplantation and a median occurrence at 44 days [2]. Although, in some series of heart/lung and lung transplant recipients, *Candida* infections were more frequent than those due to *Aspergillus* [3], in others *Aspergillus* infections predominated [4, 5].

In the present authors' experience of lung transplantation, 84% of patients yielded positive respiratory specimens for fungi. *Candida albicans* were the most frequent isolates and were found in 20 of 31 patients [6]. The second most frequently occurring fungus was *Aspergillus fumigatus*, isolated from 16 patients. Preoperative sputum examinations revealed *A. fumigatus* in three patients, two of whom immediately yielded a positive postoperative respiratory tract specimen, and one of whom developed invasive *Aspergillus* bronchitis. It is thus likely that most infections were hospital-acquired and caused by a contaminated water source, ventilation systems or construction projects. Among the *Aspergillus* species, *A. fumigatus* is the most common human pathogen. *A. niger*, *A. terreus* and *A. nidulans*

are, however, also found. *C. albicans* was found in seven patients, one of whom yielded repeated respiratory tract and pleural fluid specimens positive for *C. albicans* immediately postoperatively. Other species, in particular *C. norwegensis*, *C. lusitaniae* and *C. glabrata* (or *T. glabrata*) were also isolated [6].

After liver transplantation, opportunistic fungi produce up to 37% of pneumonias, usually within 2–3 months of transplantation [7]. Invasive mycoses are also more frequent after prolonged corticosteroid or antibiotic administration, prolonged surgery, retransplantation and systemic cytomegalovirus infections [7, 8]. After kidney transplantations, 2–14% of infectious complications are caused by opportunistic fungi [9]. Opportunistic pulmonary mycoses are more common after allogeneic than autologous bone marrow transplantation. This lower incidence is due to the shorter period of neutropenia and the quicker recovery of efficient immunity [10]. Although *Candida* species are the most common isolates, *Aspergillus* is the most common cause of fungal pneumonia in patients following allogeneic bone marrow transplantation, and develops in up to 20% of them [11, 12], with the worst prognosis [13].

Pathologies related to most common fungi

Aspergillus species

The principal types of aspergillosis that affect immunocompromised patients are angioinvasive, chronic necrotizing and tracheobronchial. Other forms such as aspergilloma, allergic bronchopulmonary aspergillosis or bronchocentric granuloma are less commonly described following transplantation [14].

Invasive pulmonary aspergillosis. Classically, the major risk factors for invasive pulmonary aspergillosis include severe or prolonged neutropenia (absolute neutrophil count $< 500 \times 10^6 \cdot L^{-1}$) and prolonged high-dose corticosteroid therapy [15]. In the absence of an effective host immune response, the spores mature into hyphae that can invade the pulmonary structures, particularly blood vessels. This results in pulmonary arterial thrombosis, haemorrhage, lung necrosis and systemic dissemination.

Macrophages and granulocytes are the major immunoregulatory cells involved in host defences against fungal infections. It has been demonstrated that corticosteroids suppress macrophage and granulocyte function, whereas little effect of the suppression of T-lymphocyte function by cyclosporin has been noted [16].

The isolation of *Aspergillus* from bronchoalveolar lavage fluid and/or sputum has been shown to correlate with the histopathological changes of invasive pulmonary aspergillosis in bone marrow recipients [17] in whom invasive forms cause the highest mortality [13]. *Aspergillus* isolation from culture of respiratory secretions, pleural fluid or ascitic fluid has also been correlated with invasive *Aspergillus* infection and poor outcome in recipients of both liver and kidney transplants [18, 19]. In cases in which the diagnosis need to be proven, transbronchial biopsy is usually of little

sensitivity, being as low as 20% [20], whereas transthoracic needle biopsy or open lung biopsy provides a higher and more specific diagnostic yield [21].

Mortality rates from infections can be high (50–70%), and patient outcome depends on the early institution of antifungal therapy, the severity of the underlying disease and the speed of granulocyte recovery [15, 22].

Invasive aspergillosis has been described as occurring following up to 18% of heart and lung transplants [23], but mortality can be reduced with pre-emptive therapy and reduced immunosuppression [6, 24].

Invasive pulmonary aspergillosis appears on radiographs as multiple ill-defined 1–2-cm nodules that gradually coalesce into larger masses or areas of consolidation (fig. 1) [25]. An early computed tomography finding, but seen with thin collimation, is the rim of ground-glass opacity surrounding the nodules (computed tomography halo sign) [26]. This sign is, however, nonspecific and has also been described in patients with tuberculosis, mucormycosis and Wegener's granulomatosis [1]. Cavitation is usually a late finding. The intracavitary mass composed of sloughed lung and the surrounding rim of air may be seen as "the air crescent sign". Lobar consolidation is more common and less specific [27]. Pleural effusion is unusual and adenopathy rare.

Ulcerative aspergillus tracheobronchitis. *Aspergillus* tracheobronchitis has been well described by KRAMER *et al.* [4] as occurring at the bronchial anastomosis in the necrotic walls after lung transplantation, where it can cause deep ulceration or dehiscence. In the present authors' series, of the nine patients showing microscopic evidence of fungi on bronchial biopsy, two also showed clinical evidence of bronchial aspergillosis. One patient developed invasive pseudomembranous right-sided bronchial aspergillosis due to *A. fumigatus* ~28 days after transplantation. This occurred on the medial proximal part of the donor bronchi, where a limited area (~2.5 × 3 cm) of necrosis had been observed a few days after transplantation. The infection healed within 19 weeks of itraconazole treatment. In a second case, ~3 weeks after transplantation, a pseudomembranous aspergillosis distal to the bronchial suture of the right main bronchus obstructed ~80% of the luminal area while extending into the intermediate bronchus. Under itraconazole treatment, the bronchial aspirate remained positive for 4.5 months, and the bronchus infection was healed after 6 months.

The present authors had a patient with ulcerative bronchitis due to *Aspergillus* >6 months after lung transplantation on a previously normal mucosa, while receiving high doses of inhaled steroids, and a few days after pulse steroid administration. These ulcerations healed 2 months later under itraconazole treatment (unpublished data). These forms of either invasive tracheobronchial aspergillosis or obstructive bronchopulmonary aspergillosis have been described not only after lung transplantation but also after allogeneic bone marrow transplantation and in patients with acquired immune deficiency syndrome [28, 29]. An example of a pseudomembranous aspergillosis found on autopsy is shown in figure 2.

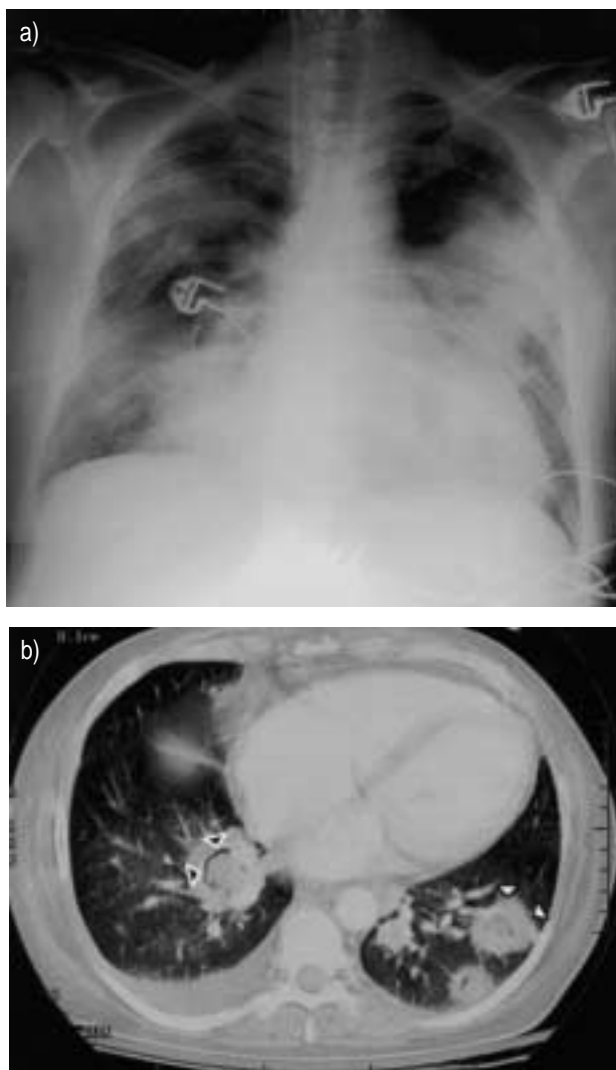


Fig. 1. – A 55-yr-old female with "leukaemia and agranulocytosis". a) Chest radiograph showing multiple bilateral focal areas of consolidation. b) High-resolution chest computed tomography (slice thickness 1 mm) revealing dense focal parenchymal nodules with surrounding air crescents (hollow arrowheads) and ground-glass opacity (solid arrowheads), reflecting progressive necrotizing pneumonia. Invasive pulmonary aspergillosis was confirmed on autopsy.

Other forms of disease related to Aspergillus. Chronic necrotizing or semi-invasive aspergillosis typically occurs in patients with mild immunosuppression such as occurs in chronic obstructive pulmonary disease, sarcoidosis or underlying malignancy. It progresses slowly over a period of weeks or months. Aspergilli invade the tissues adjacent to cavities, increasing their size due to progressive necrosis. In transplantation, these slowly invasive forms are not described as distinct entities compared with the acute invasive forms.

Allergic bronchopulmonary aspergillosis has occasionally been described after transplantation [14]. Milder forms may have local consequences such as bronchocentric granulomatosis that may be underestimated. The physiopathology of allergic bronchopulmonary aspergillosis and bronchocentric granulomatosis may

be better understood in the near future with the recent observation that aspergilli share epitopes similar to the cytoplasmic structures of epithelial cells. These local infections could trigger autoimmune processes [30]. It is possible that such phenomena do occur in the lungs of transplant recipients, leading to bronchial inflammation and stenosis.

Aspergilloma can be present in pre-existing pulmonary cavities before transplantation. With immunosuppression, aspergilli can invade adjacent structures and lead to widespread disease [23]. Preventive surgical removal of such mycetoma remains a matter of debate, especially for mycetoma resulting from previous invasive aspergillosis after bone marrow transplantation [31].

Candida species

Candida species are found in the human gastrointestinal tract, from oropharynx to anus, in the female gynaecological tract and on the skin [32]. Small numbers of yeast colonies are normally present, increasing in number when the normal microbial flora is altered by antibiotics or when there is a defect in immune competence.

Disseminated candidiasis is one of the most common serious opportunistic mycoses in severely immunocompromised patients. Pulmonary candidiasis is uncommon, except in lung transplants, where they colonize and invade necrotic tissues and bronchial anastomoses. The clinical patterns range from mucocutaneous to invasive disease [23]. If isolation of *Candida* from the sputum of many seriously ill patients on antibiotics is very common, pneumonia is considered unusual [33]. Isolation of *Candida* from a patient with pulmonary infiltrates after lung transplantation should, however, not be dismissed as benign colonization, and, clearly, histological confirmation of invasive disease is essential for diagnosis and the institution of aggressive antifungal therapy. The clinical symptoms are usually nonspecific: prolonged fever despite broad-spectrum antibacterial therapy, accompanied or not by cough or haemoptysis. Chest radiographic abnormalities with disseminated candidiasis usually consist of patchy airspace consolidations in a lower lobe distribution [34, 35]. Diffuse reticular or reticulonodular opacities are less common. Pleural effusions are reported in 25% of patients [33]. Pulmonary cavitation and adenopathy are rare and the air crescent sign has also rarely been reported [36].

Mucormycosis

The most frequent genera within the family Mucoraceae to cause human infection are *Mucor*, *Absidia* and *Rhizopus*. *Mucor* is the most frequent, causing mucormycosis. Rhinocerebral mucormycosis in diabetic patients is perhaps the best-described infectious syndrome with *Mucor*, but increasing incidence of this invasive pulmonary infection among immunosuppressed patients has been reported [37]. Other groups at increased risk of pulmonary mucormycosis are

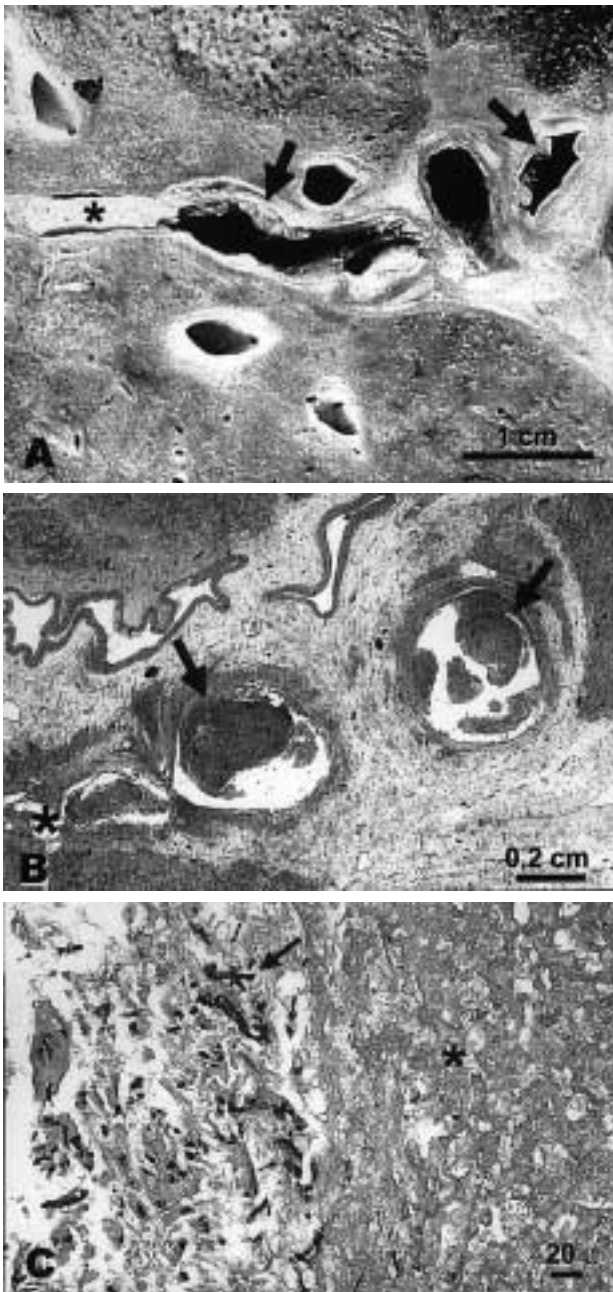


Fig. 2. – Manifestations of pseudomembranous fungal bronchitis in a lung transplantation recipient on autopsy. A) Fibrinous or pseudomembranous bronchitis (arrow) with subocclusion of the airways (asterisk indicates subocclusion of the airways by pseudomembranes); B) fibrinonecrotic material (arrow) from the airway shown in A, with subocclusion of the bronchial lumen (asterisk); and C) rare *Aspergillus* hyphae (arrow) in the lumen without invasion of the necrotic bronchial wall (asterisk). (Internal scale bar a=1 cm; b=0.2 cm; c=20 μ m.)

patients with diabetes mellitus, renal failure and burns [37].

Clinically, after inhalation or aspiration of organisms, pulmonary infection with *Mucor* can be chronic, such as in mycetoma, or acute. Like *Aspergillus*, these fungi have a propensity for invasion of pulmonary arteries, causing pulmonary artery thrombosis, pulmonary infarction and haemorrhage, with possible sys-

temic dissemination. Cavitation is common, but the air crescent sign is seen less frequently in these patients than in aspergillosis.

Diagnosis relies on the demonstration of the typical broad nonseptate hyphae in lung tissues obtained by biopsy, aspiration, or autopsy. Treatment with amphotericin B is recommended, but survival from acute pneumonia in the compromised host is rare.

Rising opportunistic fungal infections

T. glabrata is a yeast-like organism, normally present commensally in the human vagina. *T. glabrata* pneumonias have been reported in myelosuppressed patients with neoplastic disease. *T. glabrata* was isolated from the bronchoalveolar lavage fluid of three of 26 of the present authors' lung transplant patients. Pneumonia can occur and progress despite amphotericin B treatment, but apparent lung infection has also been seen to regress without specific treatment, with bone marrow recovery after bone marrow transplantation [38].

Invasive fungal infections caused by unfamiliar species are increasingly being reported in immunocompromised patients [39]. These emerging opportunistic fungi include *Fusarium*, a common plant pathogen; *Penicillium marneffeii*; *Trichosporon beigeli*; *Blastoschizomyces capitatus* and *Malassezia furfur*. Thus *Fusarium* can cause disseminated infection similar to aspergillosis in profoundly neutropenic patients [40].

Cryptococcosis

C. neoformans is a fungus that exists as a yeast in both nature and tissue. The yeast cell is 4–6 μ m in diameter, with a capsule, which, depending on the growth conditions, can make the entire organism range from 5–20 μ m. It is abundant in soil and pigeon excrement.

Pulmonary cryptococcosis causes symptoms ranging from none to those of overt pneumonia. The resultant radiographic lesions vary from plate-like atelectases to diffuse interstitial infiltrates, from ill-defined tiny nodules to huge space-occupying masses. The progression of the disease varies with the state of immunosuppression [41]. In immunocompromised patients, dissemination to extrathoracic sites such as the central nervous system, bone and skin is common [42]. Immunocompromised patients often show few or no pulmonary symptoms, even in the presence of radiographically evident pulmonary abnormalities [42].

Positive sputum culture does not establish the diagnosis; it is therefore prudent to rely instead on demonstration of the fungus in tissue, either microscopically or on culture. Serum cryptococcal antigen titres are not usually elevated, except in patients with disseminated disease [42].

Treatment of fungal infection

Amphotericin B is the traditional drug of choice. The most prominent disadvantage of amphotericin B is its

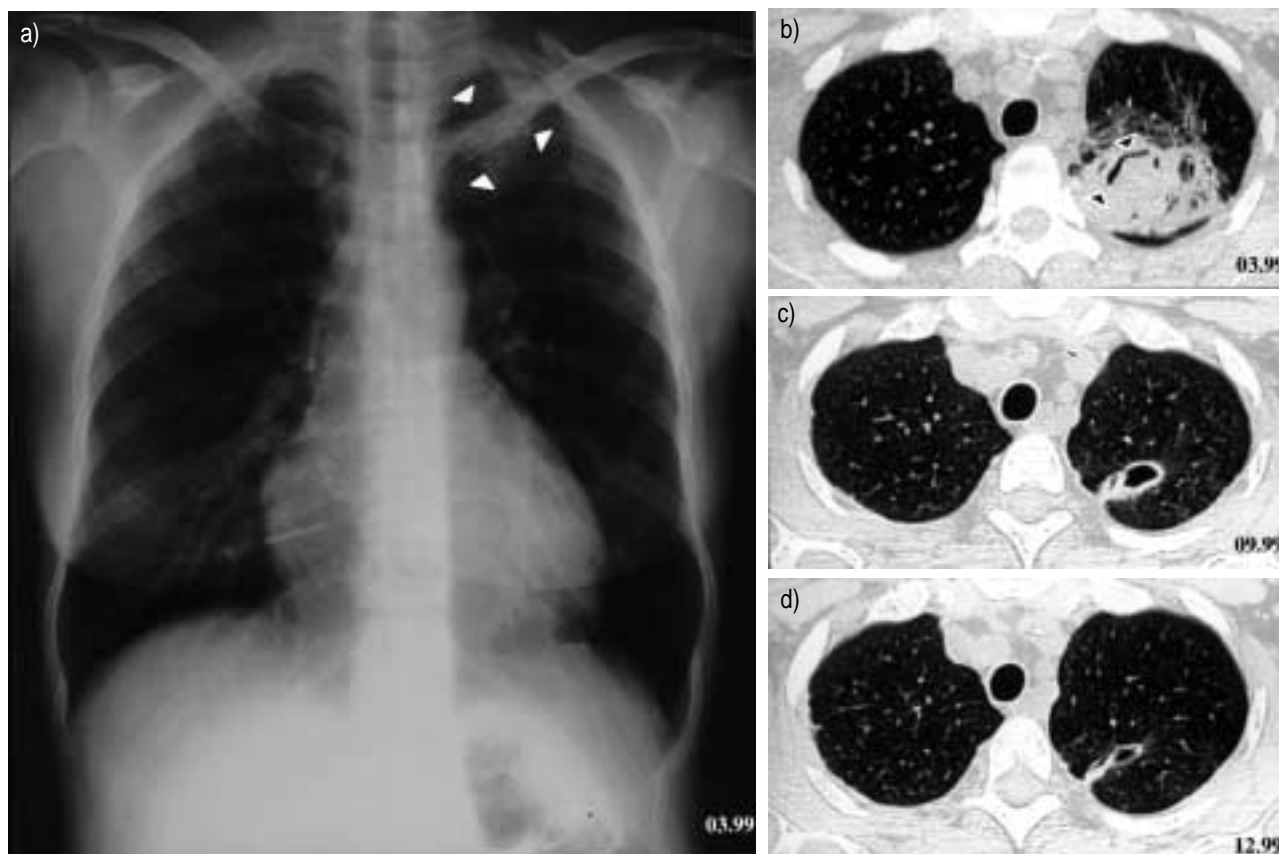


Fig. 3. – A 47-yr-old female who underwent bone marrow transplantation in February 1999 for myelodysplasia. a) The chest radiograph 1 month later shows an ill-defined retroclavicular opacity in the left upper lobe (arrowheads). b) High-resolution chest computed tomography (CT; slide thickness 1 mm) reveals an area of dense focal consolidation with an air crescent (arrowheads) strongly suggestive of invasive aspergillosis. c, d) Follow-up CT examinations after 1 month of AmBisome followed by 9 months of itraconazole, after a further: c) 6 months; and d) 9 months.

nephrotoxic effect. The treatment of aspergillosis with amphotericin B in solid organ transplant recipients results in a higher incidence of nephrotoxicity because of the concomitant use of cyclosporin [43]. Liposomal amphotericin B (AmBisome) has far fewer side-effects and can be much more safely used in patients with solid organ transplants, despite concomitant use of cyclosporin A. In a series of 187 transplant recipients, liposomal amphotericin B was discontinued due to side-effects in 3% of cases. The overall mean increase in serum creatinine levels was 20%. Other side-effects included low serum potassium concentrations (36%) and a rise in alkaline phosphatase levels (26%) [44].

Liposomal amphotericin B markedly decreases the death rate due to aspergillosis in neutropenic patients after bone marrow transplantation from 90% down to 25% even when neutrophil counts are still low [45]. The antifungal efficacy of AmBisome seems to be related to its ability to target fungi rather specifically [46].

Among azoles, fluconazole has been used effectively in localized *Candida* infections, and may also be an option in the treatment of systemic candidiasis [47, 48], but data supporting its use in pneumonia are lacking at the present time. Itraconazole or the new azole voriconazole [49] are effective therapies against aspergillosis, candidiasis or cryptococcosis. They are well tolerated. Enzyme-inducing drugs such as rifampicin

and phenytoin significantly reduce the oral bioavailability of itraconazole, and plasma monitoring of its plasma concentration is recommended when enzyme-inducing agents are coadministered.

Itraconazole has been shown to be as effective as amphotericin B in small series of neutropenic patients [50], and in liver or heart transplant recipients [51, 52]. An example of the evolution of invasive aspergillosis initially treated for 1 month with AmBisome followed by 9 months of itraconazole treatment is shown in figure 3. Failure of itraconazole treatment of pulmonary aspergillosis in heart transplant recipients has been reported, but most of these patients had been maintained on high-dose steroids [53].

Modulation of immunosuppression has to be taken into account as a major component of fungal infection treatment. The use of haematological growth factors may prove to be useful in the near future in not only restoring the neutrophil count more quickly, but also increasing the capacity to contain fungal pathogens [54].

Prophylaxis of fungal infections

It is not easy to determine the pathogenic role of fungal isolates from the respiratory tract [55] *i.e.* to

differentiate between infection, colonization and contamination. Several groups are very concerned by the high likelihood of disease when fungi are detected in respiratory tract specimens of either bone marrow or lung transplant recipients, especially with non-*Candida* strains [2, 56]. It is important that potential transplantation candidates are screened for pre-existing fungal infections. Patients with positive cultures for *Aspergillus* and clinical or radiological signs suggesting an infection are usually given a therapy such as fluconazole for *Candida* or itraconazole and aerosolized amphotericin B for *Aspergillus* until signs of infection disappear [57].

Patients colonized with *Aspergillus* either before or after lung transplantation and receiving prophylaxis show a decreased rate of fungal infection. Indeed, aerosolized amphotericin B (0.6 mg·kg body weight⁻¹·day⁻¹) divided into three doses and oral fluconazole (400 mg·day⁻¹) during the postoperative period prevented the early occurrence of any fungal infection in 52 lung transplant recipients [57]. Some groups have started using 15 mg amphotericin B twice daily for 2–3 months, as prophylaxis, with excellent preliminary results and little or no toxicity. In the present authors' lung transplant patients, fungi were found post-transplantation in the lower respiratory tract specimens of 26 of 31 (84%) patients, predominantly *C. albicans* (20 patients) and *A. fumigatus* (16 patients). Mycelia characteristic of *Candida* sp. or *Aspergillus* sp. were found in necrotic tissue at the bronchial suture in nine patients. Fluconazole (200 mg·day⁻¹) or itraconazole (200–400 mg *b.i.d.*) was systematically used according to presumed fungal sensitivity after the discovery of fungi in lower respiratory tract samples. The mean duration of the 38 treatments was 3.6±2 months. After a median follow-up of 16 months, two cases of extended ulcerative and pseudomembranous *A. fumigatus* bronchitis observed early were also resolved under itraconazole treatment [6]. Postoperative pre-emptive azole therapies thus appear well tolerated and effective in lung transplant recipients.

Azoles have been shown to be safe and efficient in the prevention of some invasive fungal infections in bone marrow transplantation [58, 59]. Ketoconazole has been used in recent heart and kidney transplant studies. A cost-sparing effect was obtained by reducing the cyclosporin dosage required and because of its efficacy in preventing fungal infections and achieving a lower rejection rate [60, 61]. However, care should be exercised not only because of drug interactions with azoles, but also because primary or secondary resistance to the antifungal drugs used may occasionally develop [62, 63].

References

1. Connolly JE, McAdams HP, Erasmus JJ, Rosado-de-Christenson ML. Opportunistic fungal pneumonia. *J Thorac Imaging* 1999; 14: 51–62.
2. Paradis II, Williams P. Infection after lung transplantation. *Semin Respir Infect* 1993; 8: 207–215.
3. Maurer JP, Tullis DE, Grossman RF, Vellend H, Winton TL, Patterson GA. Infectious complications following isolated lung transplantation. *Chest* 1992; 101: 1056–1059.
4. Kramer MR, Denning DW, Marshall SE, *et al.* Ulcerative tracheobronchitis after lung transplantation. A new form of invasive aspergillosis. *Am Rev Respir Dis* 1991; 144: 552–556.
5. Laghi F, Yeldandi V, MacCabe M, Garrity ERJ. Common infections complicating lung transplantation. *N J Med* 1993; 90: 313–319.
6. Hamacher J, Spiliopoulos A, Kurt AM, Nicod LP, and the Geneva Lung Transplantation Group. Pre-emptive therapy with azoles in lung transplant patients. *Eur Respir J* 1999; 13: 180–186.
7. George MJ, Snyderman DR, Werner BG, *et al.* The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. *Am J Med* 1997; 103: 106–113.
8. Winston DJ, Emmanouilides C, Busuttill RW. Infections in liver transplant recipients. *Clin Infect Dis* 1995; 21: 1077–1091.
9. Sia IG, Paya CV. Infectious complications following renal transplantation. *Surg Clin North Am* 1998; 78: 95–112.
10. Wilczynski SW, Erasmus JJ, Petros WP, Vrdenburgh JJ, Folz RJ. Delayed pulmonary toxicity syndrome following high-dose chemotherapy and bone marrow transplantation for breast cancer. *Am J Respir Crit Care Med* 1998; 157: 565–573.
11. Walsh TJ, Hiemenz JW, Anaissie E. Recent progress and current problems in treatment of invasive fungal infections in neutropenic patients. *Infect Dis Clin North Am* 1996; 10: 365–401.
12. Worthy SA, Flint JD, Muller NL. Pulmonary complications after bone marrow transplantation: high-resolution CT and pathologic findings. *Radiographics* 1997; 17: 1359–1371.
13. Kaiser L, Huguenin T, Lew PD, Chapuis B, Pittet D. Invasive Aspergillosis clinical features of 35 proven cases at a single institution. *Medicine* 1998; 77: 188–194.
14. Fitzsimmons EJ, Aris R, Patterson R. Recurrence of allergic bronchopulmonary aspergillosis in the post-transplant lungs of a cystic fibrosis patient. *Chest* 1997; 112: 281–282.
15. Denning DW. Diagnosis and management of invasive aspergillosis. *Curr Clin Top Infect Dis* 1996; 16: 277–299.
16. Berenguer J, Allende MC, Lee JW, *et al.* Pathogenesis of pulmonary aspergillosis granulocytopenia versus cyclosporine and methylprednisone-induced immunosuppression. *Am J Respir Crit Care Med* 1995; 152: 1079–1086.
17. Saugier-veber P, Devergie A, Sulahian A, *et al.* Epidemiology and diagnosis of invasive pulmonary aspergillosis in bone marrow transplant patients: results of a 5 year retrospective study. *Bone Marrow Transplant* 1993; 12: 121–124.
18. Braun RS, Lake JR, Katzman BA, *et al.* Incidence and significance of *Aspergillus* cultures following liver and kidney transplantation. *Transplantation* 1996; 61: 666–669.
19. Singh N, Arnou PM, Bonham A, *et al.* Invasive aspergillosis in liver transplant recipients in the 1990s. *Transplantation* 1997; 64: 716–720.
20. Pomerance A, Madden B, Burke MM, Yacoub MH. Transbronchial biopsy in heart and lung transplantation: clinicopathologic correlations. *J Heart Lung Transplant* 1995; 14: 761–773.

21. Ellis ME, Spence D, Bouchama A, *et al.*, and the Fungal Study Group. Open lung biopsy provides a higher and more specific diagnostic yield compared to broncho-alveolar lavage in immunocompromised patients. *Scand J Infect Dis* 1995; 27: 157–162.
22. Caillot D, Casasnovas O, Bernard A, *et al.* Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* 1997; 15: 139–147.
23. Kanj SS, Welty-Wolf K, Madden J, *et al.* Fungal infections in lung and heart-lung transplant recipients. Report of 9 cases and review of the literature. *Medicine* 1996; 75: 142–156.
24. Nunley DR, Ohori P, Grgurich WF, *et al.* Pulmonary aspergillosis in cystic fibrosis lung transplant recipients. *Chest* 1998; 114: 1321–1329.
25. Klein DL, Gamsu G. Thoracic manifestations of aspergillosis. *AJR Am J Roentgenol* 1980; 134: 543–552.
26. Kuhlman JE, Fishman EK, Siegelman SS. Invasive pulmonary aspergillosis and acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. *Radiology* 1985; 157: 611–614.
27. Diederich S, Scadeng M, Dennis CH, Stewart S, Flower CH. *Aspergillus* infection of the respiratory tract after lung transplantation: chest radiographic and CT findings. *Eur Radiol* 1998; 8: 306–312.
28. Logan PM, Primack SL, Miller RR, Muller NL. Invasive aspergillosis of the airways: radiographic, CT, and pathologic findings. *Radiology* 1994; 193: 383–388.
29. Kemper CA, Hostetler JS, Follansbee SE, *et al.* Ulcerative and plaque-like tracheobronchitis due to infection with *Aspergillus* in patients with AIDS. *Clin Infect Dis* 1993; 17: 344–352.
30. Mayer C, Appenzeller U, Seelbach H, *et al.* Humoral and cell-mediated autoimmune reactions to human acidic ribosomal P2 protein in individuals sensitized to *Aspergillus fumigatus* P2 protein. *J Exp Med* 1999; 189: 1507–1512.
31. Castagnola E, Bucci B, Montinaro E, Viscoli C. Fungal infections in patients undergoing bone marrow transplantation: an approach to a rational management protocol. *Bone Marrow Transplant* 1996; 18: 97–106.
32. Chu FE, Armstrong D. *Candida* Species Pneumonia. In: Sarosi GA, Davies SF, eds. *Fungal Diseases of the Lung*. 2nd Edn. New York, Raven Press, 1993; 10, 125–131.
33. Pennington JE. Opportunistic Fungal Pneumonias: *Aspergillus*, *Mucor*, *Candida*, *Torulopsis*. In: Pennington JE, ed. *Respiratory Infections: Diagnosis and Management*. 3rd Edn. New York, Raven Press, 1994; 25, 533–549.
34. Buff SJ, McLelland R, Gallis HA. *Candida albicans* pneumonia: radiographic appearance. *Am J Roentgenol* 1982; 138: 645–650.
35. Kassner EG, Kauffman SL, Yoon JJ. Pulmonary candidiasis in infants: clinical, radiologic, and pathologic features. *Am J Roentgenol* 1981; 137: 707–716.
36. Watanakunakorn C. Acute pulmonary mycetoma due to *Candida albicans* with complete resolution. *J Infect Dis* 1983; 148: 1131.
37. Leher RI, Howard DH, Syphred PS, *et al.* Mucormycosis. *Ann Intern Med* 1980; 93: 93–108.
38. Aisner J, Sickles EA, Schimpff SC, Young VM, Greene WH, Wiernik PH. *Torulopsis glabrata* pneumonitis in patients with cancer: report of three cases. *JAMA* 1974; 230: 584–585.
39. Perfect JR, Schell WA. The new fungal opportunists are coming. *Clin Infect Dis* 1996; 22: S112–S118.
40. Martino P, Gastaldi R, Raccach R, Girmenia C. Clinical patterns of *Fusarium* infections in immunocompromised patients. *J Infect* 1994; 28: 7–15.
41. Khoury MB, Godwin JD, Ravin CE, Gallis HA, Halvorsen RA, Putman CE. Thoracic cryptococcosis: immunologic competence and radiographic appearance. *Am J Roentgenol* 1984; 141: 893–896.
42. Patz EF, Goodman PC. Pulmonary cryptococcosis. *J Thorac Imaging* 1992; 4: 51–55.
43. Harari S. Current strategies in the treatment of invasive *Aspergillus* infections in immunocompromised patients. *Drugs* 1999; 58: 621–631.
44. Ringdén O, Andström E, Remberger M, Svahn B-M, Tollemar J. Safety of liposomal amphotericin B (AmBisome) in 187 transplant recipients treated with cyclosporin. *Bone Marrow Transplant* 1994; 14: S10–S14.
45. Mills W, Chopra R, Linch DC, Goldstone AH. Liposomal amphotericin B in the treatment of fungal infections in neutropenic patients: a single-centre experience of 133 episodes in 116 patients. *Br J Haematol* 1994; 86: 754–760.
46. Adler-Moore J. AmBisome targeting to fungal infections. *Bone Marrow Transplant* 1994; 14: Suppl. 5, S3–S7.
47. Anaissie E, Bodey GP, Kantarjian H, *et al.* Fluconazole therapy for chronic disseminated candidiasis in patients with leukemia and prior amphotericin B therapy. *Am J Med* 1991; 91: 142–150.
48. Kauffman CA, Bradley SF, Ross SC, Weber DR. Hepatosplenic candidiasis: successful treatment with fluconazole. *Am J Med* 1991; 91: 137–141.
49. Murphy M, Bernard EM, Ishimaru T, Armstrong D. Activity of voriconazole against clinical isolates of *Aspergillus* species and its effectiveness in an experimental model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 1997; 41: 696–698.
50. Van't Wout JW, Novakova I, Verhagen CAH, Fibbe WE, de Pauw BE, van der Meer JWM. The efficacy of itraconazole against systemic fungal infections in neutropenic patients: a randomised comparative study with amphotericin B. *J Infect* 1992; 22: 45–52.
51. Denning DW, Tucker RM, Hanson LH, Stevens DA. Treatment of invasive aspergillosis with itraconazole. *Am J Med* 1989; 8: 791–800.
52. Viviani MA, Tortorano AM, Pagano A, *et al.* European experience with itraconazole in systemic mycoses. *J Am Acad Dermatol* 1990; 23: 587–593.
53. Nanas JN, Saroglou G, Anastasiou-Nana MI, *et al.* Itraconazole for the treatment of pulmonary aspergillosis in heart transplant recipients. *Clin Transplant* 1998; 12: 30–34.
54. Gaviria JM, van Burik JA, Dale DC, Root RK, Liles WC. Modulation of neutrophil-mediated activity against the pseudohyphal form of *Candida albicans* by granulocyte colony-stimulating factor (G-CSF) administered *in vivo*. *J Infect Dis* 1999; 179: 1301–1304.
55. Paradowski LJ. Saprophytic fungal infections and lung transplantation – revisited. *J Heart Lung Transplant* 1997; 16: 524–531.
56. Trulock EP. Lung transplantation. *Am J Respir Crit Care Med* 1997; 155: 789–818.
57. Calvo V, Borro JM, Morales P, *et al.*, and the Valencia

- Lung Transplant Group. Antifungal prophylaxis during early postoperative period of lung transplantation. *Chest* 1999; 115: 1301–1304.
58. Goodman JL, Winston DJ, Greenfield RA, *et al.* A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Engl J Med* 1992; 326: 845–851.
 59. Slavin MA, Osborne B, Adams R, *et al.* Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation. A prospective, randomized, double blind study. *J Infect Dis* 1995; 171: 1545–1552.
 60. Sobh M, El-Agroudy A, Moustafa F, Harras F, El-Bedewy M, Ghoneim M. Coadministration of ketoconazole to cyclosporin-treated kidney transplant recipients: a prospective randomized study. *Am J Nephrol* 1995; 15: 493–499.
 61. Warnock DW. Fungal complications of transplantation: diagnosis, treatment and prevention. *J Antimicrob Chemother* 1995; 36: Suppl. B, 73–90.
 62. Denning DW, Venkateswarlu K, Oakley KL, *et al.* Itraconazole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 1997; 41: 1364–1368.
 63. Working Party of the British Society for Antimicrobial Chemotherapy. Chemoprophylaxis for candidosis and aspergillosis in neutropenia and transplantation: a review and recommendations. *J Antimicrob Chemother* 1993; 32: 5–21.