Aspergillus infections in lung transplant recipients: risk factors and outcome

A. Solé[1], P. Morant[2], M. Salavert[3], J. Pemán[4], P. Morales[1] and the Valencia Lung Transplant Group*

[1]Pulmonary Transplant Unit, [2]Rehabilitation Department, [3]Infectious Disease Unit and [4]Microbiology Department, Hospital Universitario La Fe, Valencia, Spain

ABSTRACT

This retrospective study of 251 lung transplant patients aimed to determine the prevalence, clinical presentation and mortality of *Aspergillus* infection in order to define specific risk factors and to compare survival in patients with and without infection. *Aspergillus* was isolated from 86 (33%) cases, which involved colonisation ($n = 50$), tracheobronchial lesions ($n = 17$) or invasive aspergillosis ($n = 19$). Overall, aspergillosis had an impact on survival ($p < 0.05$); in fact the 5-year mortality rate was substantially higher in single lung transplant recipients with bronchial anastomotic infection, and in those with late-onset infections and chronic rejection. A significant association ($p < 0.05$) was found between acute rejection and the time at which fungal infection was diagnosed. *Aspergillus* infection was not related to cytomegalovirus infection or treatment with corticosteroids. The mortality rate for invasive infections was 78% and was related to survival ($p < 0.0001$); invasive aspergillosis was also associated with chronic rejection ($p < 0.05$), but not with high corticosteroid doses ($p\ 0.49$) or use of tacrolimus ($p\ 0.73$). In conclusion, *Aspergillus* infection was associated with a reduction in the 5-year survival rate of lung transplant recipients, and this was particularly true for patients infected with the invasive forms and for patients with single lung transplants, bronchial anastomotic infection and chronic rejection. Isolation of *Aspergillus* spp. from respiratory samples preceded acute rejection, and may be a marker of graft dysfunction and/or airway inflammation. Close monitoring, or even pre-emptive antifungal therapy, is recommended for patients with chronic rejection or bronchial airway mechanical abnormalities and persistent *Aspergillus* colonisation.

Keywords *Aspergillus* infection, bronchiolitis obliterans syndrome, colonisation, fungal infection, lung transplantation, risk factors

INTRODUCTION

Aspergillus is a widely distributed filamentous fungus with branched septate hyphae. Isolation of *Aspergillus* from lung transplant recipients may occur following colonisation, tracheobronchitis, invasive pulmonary aspergillosis or disseminated disease [1–5]. Although the incidence of *Aspergillus* infection in lung transplant patients is lower than that of infections caused by bacteria and viruses, *Aspergillus* causes a similar or even higher mortality rate for several reasons: the difficulty in achieving an early diagnosis; the lack of effective treatment for infections caused by filamentous fungi; the toxicity associated with interactions of antifungal agents with immunosuppressive drugs; the few available data on the effectiveness of antifungal prophylaxis; and, finally, the reduced level of immunosuppression that may lead eventually to graft loss. Fungal infections occur in 15–35% of patients following lung transplantation. Many factors may predispose to fungal

Corresponding author and reprint requests: A. Solé, Unidad de Trasplante Pulmonar, Hospital Universitario La Fe, Av. de Campanar 21, 46009 Valencia, Spain
E-mail: sole_amp@gva.es
*Members of the Valencia Lung Transplant Group also include A. Pastor, C. Lozano, R. Vicente, F. Ramos, E. Blasco, V. Calvo, A. García-Zarza, J. Padilla, J. Pastor and V. Tarazona.

infections in lung transplant patients, including pre-operative chronic lung disease, *Aspergillus* colonisation, lung rejection, enhanced immunosuppression, cytomegalovirus (CMV) infection and the type of antifungal agent used [3,6–8].

Since there are many controversial issues regarding the management of aspergillosis in lung transplant recipients, the present study aimed to determine the prevalence, clinical presentation and mortality of fungal infections caused by *Aspergillus* in lung transplant recipients. The possible association with specific risk factors (such as acute and chronic rejection, corticosteroid dose, CMV infection and type of immunosuppression) was also evaluated. An additional analysis was made in the subgroup of cases with invasive *Aspergillus* infection.

PATIENTS AND METHODS

Patients

All patients in the lung and heart–lung transplantation programme of the Hospital Universitario La Fe (Valencia) from January 1991 to January 2004 were included in this study. Information on fungal infection was obtained from the clinical records and computer databases of the Lung Transplant Unit and the Department of Microbiology of the hospital.

Criteria and definitions

For the purposes of this study, the different forms of *Aspergillus* infection were defined as follows [2,3]:

1. *Aspergillus* airway colonisation: patients with *Aspergillus* cultured from the airway specimens in the absence of invasive aspergillosis or tracheobronchitis.
2. Tracheobronchial aspergillosis or anastomotic infections: isolation of *Aspergillus* in culture with histopathological evidence of tissue invasion or necrosis, ulceration or pseudomembranes on bronchoscopy.
3. Invasive pulmonary aspergillosis: invasive fungal infections of the lung caused by *Aspergillus* spp., with clinical, radiological and/or histological findings of pulmonary tissue invasion by *Aspergillus*, together with isolation of the fungus from respiratory samples. Invasive aspergillosis was considered to be disseminated if the infection was documented histopathologically at two or more non-contiguous organ sites.
4. Opportunistic invasive aspergillosis: diagnosed with reference to the standardised criteria and definitions formulated by the Invasive Fungal Infections Cooperative Group (IFICG) of the European Organization for Research and Treatment of Cancer (EORTC) in conjunction with members of the Mycoses Study Group (MSG) of the National Institute of Allergy and Infectious Diseases (NIAID) [9].

Acute rejection, chronic rejection or bronchiolitis obliterans syndrome were defined according to the international classification of the Lung Rejection Study Group [10].

Lung transplant protocols: immunosuppression and prophylactic measures

The immunosuppressive regimen consisted of a combination of prednisone, azathioprine and cyclosporine. Induction therapy was used only in the first 20 cases and in the last three (anti-thymocyte globulin or anti-interleukin-2 receptor antibodies). Rejection episodes were treated with methylprednisolone pulse therapy (1 g/day for 3 days). In cases of recurrent or refractory rejection episodes, cyclosporine was replaced by tacrolimus, and azathioprine by mycophenolate; six cases were treated with OKT3.

Prophylaxis against CMV, fungi and *Pneumocystis carinii* infection was given to all patients as described previously [11]. The antifungal prophylactic protocol comprised fluconazole (200 mg/day), except following the isolation of *Aspergillus*, when the treatment was changed to itraconazole (200–400 mg/day) and nebulised amphotericin B (0.2 mg/kg/8 h) during the first month after transplantation, followed by lower doses of nebulised amphotericin B (< 0.5 mg/kg/day) until months 3–6 after transplant.

Antifungal treatment regimens

Airway colonisation within 6 months of transplantation was treated with amphotericin B aerosols and oral itraconazole until the fungus was eradicated. If *Aspergillus* was detected > 6 months after transplantation, only nebulised amphotericin B (< 0.5 mg/kg/day) was administered. For *Aspergillus* tracheobronchitis, combined treatment with nebulised amphotericin B and itraconazole was used to eradicate the fungus. However, if significant ulcerations were present, intravenous therapy with lipid formulations of amphotericin B (3 mg/kg/day) or caspofungin was started, with a switch to itraconazole or voriconazole after treatment for 4 weeks (both of these drugs have been introduced in the last 2 years). If invasive pulmonary aspergillosis was diagnosed, initial treatment consisted of a lipid formulation of amphotericin by the intravenous route (dose 5 mg/kg/day) or caspofungin, sometimes combined with voriconazole plus high doses of nebulised amphotericin B, until there was a clear clinical improvement in the patient's condition.

Follow-up

Chest X-rays, serum chemistry (glucose, kidney and liver function tests), a complete blood count and a measurement of blood levels of immunosuppressive drugs were performed daily during the first two post-operative weeks, and every 2–3 days thereafter until discharge. A high-resolution computerised tomography scan and lung function tests were performed after 1 month. In addition, sputum samples were cultured for bacteria and fungi every 5 days during the first month, every 3 months thereafter, and whenever respiratory symptoms were noted. Microbiological tests for CMV in blood (pp65 CMV antigenaemia) and respiratory (shell vial culture) samples were performed every 2 weeks during the first month, monthly up to the third month after transplant, and every 3 months thereafter, or if clinical or radiological signs were evident. Surveillance bronchoscopy, with microbiological studies of bronchoalveolar lavage samples and transbronchial biopsies, was performed 0, 2, 15, 30 and 90 days after transplantation, and if considered necessary for clinical reasons. After the first 3 months post-transplant,

sputum cultures for epidemiological surveillance were obtained every 2 months. *Aspergillus* spp. were identified by morphological characteristics.

Statistical analysis

The Fisher Exact test was used to compare categorical variables such as *Aspergillus* infection (colonisation, airway lesion, invasive forms), rejection (acute or chronic), CMV infection, use of corticosteroids and immunosuppressive drugs. Simple regression and the coefficient of determination were applied to analyse continuous variables as a temporal association between fungal infection and acute rejection or immunosuppressants. Survival rates were calculated by the Kaplan–Meier method and compared with the log-rank test. The alpha level of significance was set at $p < 0.05$.

RESULTS

In total, 251 lung transplants were performed during the 13-year study period. Of these, 186 were bilateral lung, 41 single lung and 19 heart–lung transplants.

Prevalence of aspergillosis and clinical presentation

Eighty-six cases of aspergillosis were diagnosed (a prevalence of 33%), comprising 50 males and 36 females, with a mean age of 40 ± 6 years. The main indications for transplantation in patients with aspergillosis were pulmonary emphysema (29%), pulmonary fibrosis (28%), cystic fibrosis (25%), bronchiectasis (11%), primary pulmonary hypertension (5%) and lymphangioleiomyomatosis (3%).

Colonisation by *Aspergillus* spp. occurred in 50 (20%) patients, involving *Aspergillus fumigatus* (59%), *Aspergillus flavus* (35%), *Aspergillus niger* (4%), and unidentified *Aspergillus* spp. (2%). Tracheobronchitis caused by *Aspergillus* spp. occurred in 17 (6%) cases (Table 1) and invasive infection in 19 (8%) cases (Table 2); most of the latter cases involved invasive pulmonary aspergillosis. Six cases involved disseminated infection; in two of these, the initial symptoms were an epidural abscess and a hip arthritis associated with aortic endocarditis. *A. fumigatus* was identified as the sole pathogen in all invasive cases, with the exception of one pulmonary infection in which *Scedosporium apiospermun* was present simultaneously. In 13 of the 19 cases, cultures of respiratory samples were sporadically or persistently positive for *A. fumigatus* for several months before the onset of invasive infection.

The time of onset was related strongly to the clinical form of aspergillosis. All tracheobronchitis or bronchial anastomotic infections occurred within 3 months of transplantation. In contrast, invasive or disseminated aspergillosis occurred significantly later (33.7 ± 19.6 months post-transplantation). The early invasive pulmonary forms (Table 2) occurred simultaneously with tracheobronchial *Aspergillus* infection.

Table 1. Characteristics of patients with airway lesions associated with *Aspergillus* spp.

No.	Diagnosis	Type	Airway lesion	Site	Treatment	FEV$_1$ (%)	Survival and outcome
1	CF	BL	Bronchial stenosis	Bilateral	Prosthesis	80	8 years
2	CF	BL	Pseudomembrane	Bilateral	–	–	Died
3	LAM	BL	Bronchial stenosis	LMB	Prosthesis	83	4 years
4	Emphysema	SL	Pseudomembrane	LMB	–	–	Died
5	CF	BL	Bronchial stenosis fistula	RMB	Laser	80	6 years
6	CF	BL	Ulcers	LMB	Prosthesis	60	4 years
7	CF	BL	Bronchial stenosis	LMB	Dilatation	60	7 years
8	Emphysema	SL	Bronchial stenosis	RMB	Dilatation	60	Died 3 years
9	CF	BL	Bronchial stenosis	LMB	Dilatation	80	2 years
10	CF	BL	Bronchial stenosis	RMB	–	80	2 years
11	Emphysema	SL	Bronchial stenosis	LMB	Dilatation	50	2 years
12	Bronchiectasis	BL	Bronchial stenosis	LMB	Prosthesis	70	1 years
13	Bronchiectasis	BL	Bronchial stenosis	LMB	Dilatation	80	1 years
14	Emphysema	SL	Bronchial stenosis	RMB	Dilatation	50	Died 1 year
15	Emphysema	SL	Bronchial stenosis	RMB	Dilatation	40	Died 6 months
16	CF	BL	Bronchial stenosis	RMB	–	60	5 months
17	CF	BL	Bronchial stenosis	RMB	Dilatation	70	4 months

Patients 2 and 7 were colonised by *Aspergillus* spp. before lung transplantation.
All cases were diagnosed within 2 months of transplantation. All were treated with nebulised amphotericin and oral itraconazole. Cases 1, 2, 4 and 5 also received liposomal amphotericin; cases 16 and 17 received caspofungin.
CF, cystic fibrosis; LAM, lymphangioleiomyomatosis; LMB, left main bronchus; RMB, right main bronchus; SL, single lung; BL, bilateral lung; FEV$_1$, forced expiratory volume in 1 s.

Table 2. Characteristics of patients with invasive aspergillosis

No.	Onset	Type	Months post-transplant	Risk factor	Treatment	Outcome
1	P, A, EB, End	BL	3	PAC, EB	Amphotericin B	Died
2	P	BL	26	CR	Amphotericin B IV	Died
3	P, EA	BL	17	CR	Amphotericin B	Died
4	P, LM	SL	12	CR	Amphotericin B	Died
5	P, EB	SL	2	EB	Amphotericin B	Died
6	P, EB	BL	1	EB	Amphotericin B	Alive
7	P	BL	12	CR	–	Died
8	P	BL	48	CR	Amphotericin B IV	Died
9	P, B	BL	12	CMV, AR	–	Died
10	P	BL	12	–	Amphotericin B	Alive
11	P, B	SL	34	CR	Amphotericin B IV + itraconazole	Died
12	P	SL	36	CR	Amphotericin B IV + caspofungin	Died
13	P, A	SL	30	EB, CR	Amphotericin B IV + itraconazole	Died
14	P	BL	48	EB, CR	Caspofungin + voriconazole	Alive
15	P, PSAb	BL	60	CR	Amphotericin B IV + itraconazole	Died
16	P	SL	6	EB, CR	Amphotericin B IV itraconazole	Died
17	P	HL	34	CR	Amphotericin B IV + itraconazole	Died
18	P	BL	72	PAC, CR	Caspofungin + voriconazole	Alive
19	P	BL	74	CR	Caspofungin + voriconazole	Alive

P, pneumonia; End, endocarditis; A, arthritis; EA, epidural abscess; EB, endobronchial lesion; PAC, previous airway colonisation; B, brain; LM, leg mycetome; CR, chronic rejection; PSAb, psoas abscess; SL, single lung; BL, bilateral lung; HL, heart–lung; IV, intravenous; CMV, cytomegalovirus..

Anastomotic infections were found more frequently in cystic fibrosis patients. Among the patients with airway problems, those with emphysema had the worse prognosis; indeed, in four of the five cases of emphysema, the initial *Aspergillus* infection progressed to a pulmonary invasive form and the patient died (Table 1). All four of these cases involved single lung transplants, as well as persistent *Aspergillus* colonisation and associated chronic rejection.

Aspergillus colonisation was detected within 3 months of transplantation in 56% of patients and >12 months after transplantation in 20% of patients. All of the patients who developed invasive infections yielded positive cultures, except for one case (diagnosed following autopsy). The time from the first isolation of *Aspergillus* spp. to the development of invasive infection varied from 1 to 7 months (mean, 4 months).

Risk factors

Typically, *Aspergillus* was detected in respiratory samples 1.8 ± 4 months preceding an acute rejection episode. There was a significant association ($p < 0.05$) between the time at which *Aspergillus* spp. were first isolated and the time of diagnosis of acute rejection ($R^2 = 0.92$), administration of tacrolimus ($R^2 = 0.67$) and administration of mycophenolate ($R^2 = 0.44$). There was no significant association with CMV infection or corticosteroid doses. The incidence of acute rejection was similar ($p > 0.05$) between the three different forms of *Aspergillus* infection (61% colonisation, 63% airway infections and 70% invasive infections). There was no correlation of invasive disease with CMV infection (p 0.40), high corticosteroid doses (p 0.49) or the use of tacrolimus (p 0.73); the only significant association was found with chronic rejection ($p < 0.05$). Bronchiolitis obliterans syndrome was diagnosed in 87% of invasive cases, compared to 44% of colonised cases and 57% of those with airway infection.

Survival

The mortality of *Aspergillus* infection was 14% in cases with tracheobronchitis, 28% in colonised cases, and 78% in cases with invasive infections ($p < 0.05$). The mortality caused by invasive fungal infections was related directly to *Aspergillus* spp. Five patients died within 6 months of transplantation, while the remainder died after a

follow-up period of > 1 year. In two cases, the invasive fungal infection was diagnosed at autopsy.

Actuarial survival in patients with any form of *Aspergillus* infection was 81%, 51% and 39% after 1, 3 and 5 years, respectively. The 5-year survival rate was lower ($p < 0.05$) when compared to patients without infection (Fig. 1). Invasive infections were related strongly to survival ($p < 0.0001$) (Fig. 2).

DISCUSSION

Infection with *Aspergillus* poses significant problems for lung transplant patients, with high rates of morbidity and mortality [2,3,7]. Moreover, there is no established and uniform approach to prophylactic regimens and antifungal therapy [8,11–14], and simple colonisation has been associated with progression to endobronchial lesions and invasive infection [3,15,16]. Management decisions are difficult when *Aspergillus* is isolated from respiratory samples, particularly in the absence of respiratory symptoms. Furthermore, the current and potential pathogenicity of these fungi are unknown, although it has been found that patients colonised with *Aspergillus* and other fungi have significantly greater neutrophil counts in bronchoalveolar fluid, a finding that is associated with a poor outcome in lung transplant recipients [8,17].

Given these unanswered questions regarding the management of aspergillosis in lung transplant recipients, the present study aimed to
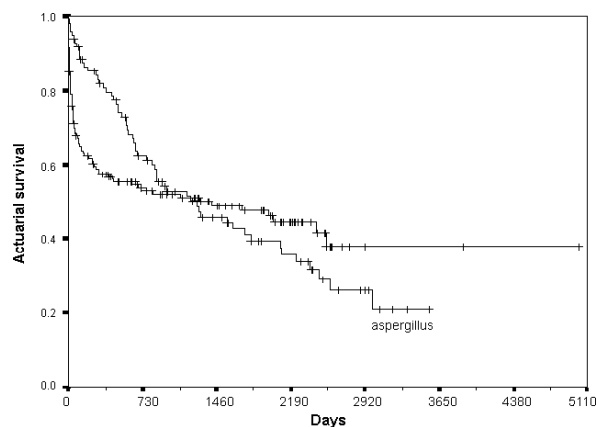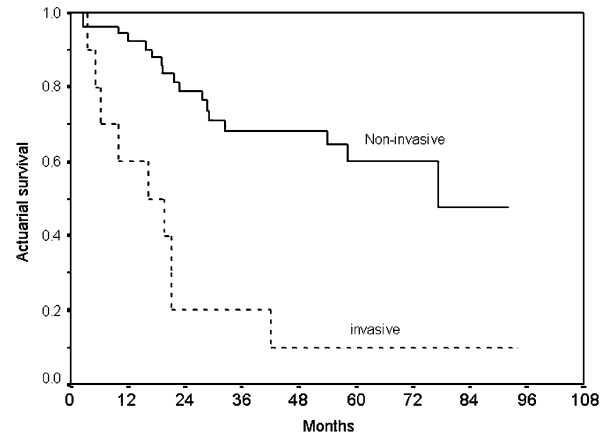

Fig. 2. Actuarial survival in lung transplant recipients with invasive and non-invasive forms of aspergillosis (log-rank test, $p < 0.0001$).

determine the prevalence, outcome and potential risk factors in a large single-centre cohort. *Aspergillus* was isolated from 33% of patients, but simple isolation of *Aspergillus* did not affect overall survival following lung transplantation. The frequency and types of *Aspergillus* infection (airway colonisation, tracheobronchitis and invasive pulmonary aspergillosis), and the time to diagnosis, were similar to those reported previously [4,14]. Interestingly, detection of *Aspergillus* in respiratory samples preceded diagnosis of acute rejection in a substantial proportion of patients, and could be considered an early marker of possible inflammatory events in the airways that might promote infection by *Aspergillus* or predispose to acute rejection, in the same way that fungal infection has been implicated in the development of chronic rejection [17–19]. Pre- and post-transplant colonisation and tracheobronchial aspergillosis [2,6,7,16,20–22] have often been implicated in the development of invasive infections. In the present study, pre-transplant colonisation occurred in only two cases, but six patients with invasive fungal infection had a coexisting airway lesion related to the fungus.

The present study established and confirmed a strong association between invasive infections and chronic rejection. Thus, antifungal prophylaxis and/or pre-emptive therapy with antifungal agents is recommended for patients with chronic rejection and positive respiratory samples for *Aspergillus*, even if no endobronchial involvement or radiological signs are evident. Such treatment should continue for at least 3 months, which is

Fig. 1. Actuarial survival in lung transplant recipients from whom *Aspergillus* was isolated (log-rank test, $p < 0.05$).

the period over which colonisation has been shown to precede disseminated infection [15].

In terms of clinical presentation, previous studies have reported that 5.7% of patients with a positive airway culture progress subsequently to invasive infections [3], and that *Aspergillus* colonisation may precede bronchial complications by 8 weeks [16]. In addition, the present data show a significant increase of bronchial anastomotic infections in cystic fibrosis patients colonised previously with *Aspergillus*. An association between fungal infections and airway anastomotic lesions has also been reported [8,21–23]. In the present series, nine of the 17 patients with airway abnormalities were cystic fibrosis patients, but only two were colonised previously by *Aspergillus*; one died from a disseminated form of aspergillosis, despite full antifungal therapy pre- and post-lung transplant. The time required for development of invasive *Aspergillus* infection was clustered in two periods, i.e., either within the first 6 months post-lung transplant, despite antifungal prophylaxis, or >1 year following transplant. Disseminated infections included uncommon presentations, such as endocarditis, with fewer than ten cases reported previously [24], or a case of epidural abscess, which is a rare complication in transplant recipients [25].

Invasive fungal infections occurred mainly in patients with chronic rejection, suggesting that pre-emptive antifungal therapy should be started for patients with bronchiolitis obliterans syndrome, with clinical, radiological and microbiological monitoring for early diagnosis and subsequent treatment. Since *Aspergillus* infection may be a marker of acute rejection, close monitoring of lung function is recommended for asymptomatic patients from whom *Aspergillus* is detected in respiratory samples. A combination of caspofungin and voriconazole may be optimal for the treatment of invasive aspergillosis, and, in the present series, all of the patients with invasive aspergillosis who received this combination survived (Table 2). New microbiological techniques for detection of galactomannan antigen [26], or molecular methods, such as PCR, may allow early detection of *Aspergillus* in blood or respiratory samples [27]. Early microbiological diagnosis, in conjunction with sequential high-resolution chest imaging techniques, may change the management of invasive pulmonary aspergillosis by facilitating both the pre-emptive and the therapeutic approach.

REFERENCES

1. Alexander BD, Tapson VF. Infectious complications of lung transplantation. *Transpl Infect Dis* 2001; **3**: 128–137.
2. Nicod LP, Pache JC, Howarth N. Fungal infections in transplant recipients. *Eur Respir J* 2001; **17**: 133–140.
3. Singh N, Husain S. *Aspergillus* infections after lung transplantation: clinical differences in type of transplant and implications for management. *J Heart Lung Transplant* 2003; **22**: 258–266.
4. Mehrad B, Paciocco G, Martínez FJ, Clark T, Iannettoni MD, Lynch JP. Spectrum of *Aspergillus* infection in lung transplant recipients. Case series and review of the literature. *Chest* 2001; **119**: 169–175.
5. Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. *Chest* 2002; **121**: 1988–1999.
6. Gordon SM, Avery RK. Aspergillosis in lung transplantation: incidence, risk factors, and prophylactic strategies. *Transpl Infect Dis* 2001; **3**: 161–167.
7. Patterson TF, Kirkpatrick WR, White M *et al.* Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. *Medicine (Baltimore)* 2000; **79**: 250–260.
8. Helmi M, Love RB, Welter D, Cornwell RD, Meyer KC. *Aspergillus* infection in lung transplant recipients with cystic fibrosis. *Chest* 2003; **123**: 800–808.
9. Ascioglu S, Rex JH, de Pauw B *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**: 7–14.
10. Yousem SA, Berry GJ, Cagle PT *et al.* Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. *J Heart Lung Transplant* 1998; **15**: 1–15.
11. Calvo V, Borro JM, Morales P *et al.* Antifungal prophylaxis during the early post-operative period of lung transplantation. *Chest* 1999; **115**: 1301–1304.
12. Monforte V, Román A, Gavaldá J *et al.* Nebulized amphotericin B prophylaxis for *Aspergillus* infection in lung transplantation: study of risk factors. *J Heart Lung Transplant* 2001; **20**: 1274–1281.
13. Denning DW. Early diagnosis of invasive aspergillosis. *Lancet* 2000; **355**: 423–424.
14. Paterson BL, Singh N. Invasive aspergillosis in transplant recipients. *Medicine (Baltimore)* 1999; **78**: 123–138.
15. Plit ML, Singleton LG, Scot A, Rainner S, Malouf MA, Glanville AR. Is invasive fungal disease in lung transplant recipients predictable? *J Heart Lung Transplant* 2003; **22**(Suppl 1): A367.
16. Nathan SD, Shorr AF, Schmidt ME, Burton NA. *Aspergillus* and endobronchial abnormalities in lung transplant recipients. *Chest* 2000; **118**: 403–407.
17. Elssner A, Vogelmeier C. The role of neutrophils in the pathogenesis of obliterative bronchiolitis after lung transplantation. *Transplant Infect Dis* 2001; **3**: 168–176.
18. Husain S, Singh N. Bronchiolitis obliterans and lung transplantation: evidence for an infectious etiology. *Semin Respir Infect* 2002; **17**: 310–314.
19. Cainelli F, Vento S. Infections and solid organ transplant rejection: a cause-and-effect relationship? *Lancet Infect Dis* 2002; **2**: 539–549.
20. Cahill BC, Hibbs JR, Savik K *et al. Aspergillus* airway colonization and invasive disease after lung transplantation. *Chest* 1997; **112**: 1160–1164.

21. Nunley DR, Ohori P, Grgurich WF *et al.* Pulmonary aspergillosis in cystic fibrosis lung transplant recipients. *Chest* 1998; **114**: 1321–1329.

22. Nunley DR, Gal AA, Vega JD, Perlino C, Smith P, Lawrence EC. Saprophytic fungal infections and complications involving the bronchial anastomosis following human lung transplantation. *Chest* 2002; **122**: 1185–1191.

23. Hadjiliadis D, Howell DN, Davis RD *et al.* Anastomotic infections in lung transplant recipients. *Ann Transplant* 2000; **5**: 13–19.

24. Gilbey JG, Chalermskulrat W, Aris RM. *Aspergillus* endocarditis in a lung transplant recipient. A case report and review of the transplant literature. *Ann Transplant* 2000; **5**: 48–53.

25. Denning DW. Invasive aspergillosis. *Clin Infect Dis* 1998; **26**: 781–805.

26. Boutboul F, Alberti C, Leblanc T *et al.* Invasive aspergillosis in allogeneic stem cell transplant recipients: increasing antigenemia is associated with progressive disease. *Clin Infect Dis* 2002; **34**: 939–943.

27. Raad I, Hanna H, Huaringa A, Sumoza D, Hachem R, Albitar M. Diagnosis of invasive pulmonary aspergillosis using polymerase chain reaction-based detection of *Aspergillus* in BAL. *Chest* 2002; **121**: 1171–1176.