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ARTICLE

Aspergillus infection in lung transplant patients: incidence and prognosis

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Abstract Lung transplant recipients experience a particularly high incidence of *Aspergillus* infection in comparison with other solid-organ transplantations. This study was conducted to determine the incidence of *Aspergillus* colonisation and invasive aspergillosis, and the impact on long-term survival associated with *Aspergillus* infection. A retrospective study of 362 consecutive lung transplant patients from a single national centre who were transplanted 1992–2003 were studied. Twenty-seven patients were excluded due to incomplete or missing files. A total of 105/335 (31%) patients had evidence of *Aspergillus* infection (colonisation or invasion), including 83 (25%) patients with colonisation and 22 (6%) patients with radiographic or histological evidence of invasive disease. Most of the infections occurred within the first 3 months after transplantation. Cystic fibrosis (CF) patients had higher incidences of colonisation and invasive disease [15 (42%) and 4 (11%) of 36 patients] than non-CF patients [68 (23%) and 18 (6%) of 299 patients] (P=0.01). Invasive aspergillosis was associated with 58% mortality after 2 years, whereas colonisation was not associated with early increased mortality but was associated with increased mortality after 5 years compared to non-infected patients (P<0.05). An analysis of demographic factors showed that donor age [OR 1.40 per decade (95% CI 1.10-1.80)], ischaemia time [OR 1.17 per hour increase (95% CI 1.01–1.39)], and use of daclizumab versus polyclonal induction [OR 2.05 (95% CI 1.14–3.75)] were independent risk factors for *Aspergillus* infection. Invasive aspergillosis was associated with early and high mortality in lung transplant patients. Colonisation with *Aspergillus* was also associated with a significant increase in mortality after 5 years. CF patients have a higher incidence of *Aspergillus* infection than non-CF patients.

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Introduction

In comparison with recipients of other solid-organ transplantation, lung transplant recipients experience a particularly high incidence of *Aspergillus* infection, and only haematopoietic stem-cell transplantation is associated with a comparable incidence of *Aspergillus* infection [1]. *Aspergillus* causes colonisation of airways, tracheobronchial infection in anastamoses, or true invasive disease with clinical, radiological and histological evidence of tissue invasion [2].

Several patient series [3–5] demonstrate colonisation frequencies of up to 30–40% of all lung transplant recipients. Tracheobronchial infection occurs in 5–10%, and invasive aspergillosis occurs in 3–5% of patients. Colonisation is usually transient but increases the risk of invasive disease. Invasive aspergillosis still carries a high mortality of up to 80% [6–8] and is considered a major problem in lung transplantation; approximately 50% of centres world-wide use some form of post-transplant antifungal prophylaxis [9].

Several factors have been identified as risk factors for infection with *Aspergillus*, although not consistently: colonisation wih *Aspergillus* in patients with cystic fibrosis (CF) [10] and other lung diseases [11], cytomegalovirus (CMV) infection [12], environmental exposure [13] and severe immunosuppressive treatment [14].

This study represents the experience from a large single centre with a close bronchoscopic surveillance programme. All transbronchial and bronchial mucosal biopsies and bronchoalveolar lavage (BAL) fluid cultures were studied for *Aspergillus*, giving a good estimate of the incidence and prognosis of *Aspergillus* infection.

Patients and methods

The study is a retrospective evaluation of all single (SLTX), double (DLTX), and combination heart-lung (HLTX) transplantation cases at our centre from 1992–2003. The Danish national lung transplant programme was established in 1992 and is located at the Copenhagen University Hospital, Rigshospitalet. A detailed report on the programme with methods and results has been published previously [15].

Briefly, following induction with either polyclonal [antithymocyte globulin (ATG) or antilymphocyte globulin (ALG)], or monoclonal [daclizumab (Zenepax)] preparations, patients received a standard triple-drug maintenance immunosuppression with ciclosporine, azathioprine and prednisolone. All patients had bronchoscopy at 2, 4, 6 and 12 weeks, and 6, 12, 18 and 24 months. Additional bronchoscopy was performed in the event of the development of radiological changes or decline in lung function. BAL was performed in the transplanted lung (SLTX recipients) or preferentially in the right-lung (DLTX and HLTX recipients), usually in the middle lobe or lingula. In case of radiological infiltrates, additional lavage was performed in the affected segment. Aliquots of 50 ml saline were used up to a maximum of 200 ml for each BAL. All BAL fluid was sent for bacterial and fungal culture, and cytological preparations were examined for inflammatory cells, bacteria and fungi. Transbronchial biopsies (TBB) were also performed under fluoroscopic guidance, at all scheduled bronchoscopies and in cases of persistent radiological infiltration. Usually five to six biopsies were obtained to evaluate rejection. Mucosal biopsies were also taken in the event of abnormal macroscopic appearance.

The diagnostic methods (bronchoscopy with BAL and TBB, and culture methods) were unchanged during the study period. At all bronchoscopies, chest x-ray was performed routinely, and in cases with new persistent changes, a computerized tomography (CT) of the thorax was also performed. In case of signs of fungal growth or biopsies with fungal invasion, a subsequent CT scan was usually performed. The standard policy was to repeat bronchoscopy 2–3 months after discontinuation of antifungal treatment in patients with colonisation to ensure that cultures for fungal growth had become negative. Patients with invasive disease were followed with serial bronchoscopies and CT scans.

In this study, infection with *Aspergillus* was defined as colonisation or signs of invasive disease with *Aspergillus.* Colonisation with *Aspergillus* was defined as a positive culture from BAL fluid or expectorate, or the presence of hyphae in BAL fluid without any evidence of tissue invasion at bronchoscopy or in radiological studies. Invasive aspergillosis was defined as the presence of fungal growth with hyphae in mucosal or transbronchial biopsies, or radiological appearances consistent with invasive aspergillosis as evaluated by thoracic CT. Hyphae in BAL fluid without a positive culture were assumed to be *Aspergillus* species, as more than 99% of positive fungal cultures from BAL fluid demonstrate *Aspergillus* species at our institution. The first occurrence of colonisation or invasive disease was recorded for use in the analyses. Patients were treated until signs of invasive disease or colonisation disappeared, however, it was often not possible to establish the exact time for this, and thus, this date was not recorded.

Antifungal treatment regimes

No standard antifungal prophylaxis with oral or intravenous drugs was used in the study period. All patients, however, received oral solution with nystatin (5 ml four times daily) for the first 7 days after transplantation and whenever treated with high dose steroids for acute rejection. Patients with evidence of *Aspergillus* colonisation were treated with oral itraconazole capsules (Sporanox, Janssen-Cilag) at a dose of 200–400 mg depending on weight. With intolerance to or lack of effect of itraconazole, the treatment was supplemented by inhalation of amphotericin B solution for intravenous use (Fungizone, Bristol-Myers Squibb), standard dose 10–15 mg twice daily.

All patients with invasive aspergillosis were initially treated for 3 weeks with liposome-coated amphotericin complex (Ambisome, Pfizer) at a maximum dose of 3–5 mg/kg, followed by oral treatment with itraconazole capsules at a dose of 200–400 mg daily.

Laboratory methods

The methods for processing BAL fluid and TBB were as previously described [16]. Briefly, BAL fluid was cytocentrifuged (2,000 cpm, 5 min) or smeared directly onto glass slides. Routine stainings included silver stains (Grocott-Gomori) for the detection of fungi and *Pneumocystis jiroveci*. This staining method was also applied to one series of sections from the routinely processed paraffin-embedded TBB, for which Periodic Schiff staining (PAS) was also conducted in parallel. The presence of characteristic hyphae or conidiae was reported as consistent with *Aspergillus*.

All BAL, TBB and sputum samples were microscoped using the Gram stain method, and cultured on the following media: 5% Danish horse blood agar, chocolate agar (pre-heated blood), Sabouraud dextrose agar and chromeagar. The plates were incubated for 48 h at 37°C. When moulds were present, all isolates were sent for final identification to the Danish State Serum Institute, which is the central reference laboratory for microbiology in Denmark (http://www.ssi.dk). All isolates were determined to species level.

Statistics

Data analysis was performed using the SPSS 12.0 and SAS 9.1 statistical packages. Continuous variables are expressed as mean ± standard deviation, median and interquartile range (IQR). Inter-group comparisons of continuous data were made using the non-parametric Mann-Whitney test. Two-value comparisons of categorical data were made using the chi-square or Fisher's exact test where appropriate. Holm's correction method was employed for multiple pair-wise comparisons. The evaluation of selected recipient, donor and intra-operative demographic parameters as possible risk factors for post-transplant *Aspergillus* infection was performed using logistic regression. Potential risk factors ($P<0.1$ by univariate analysis) were considered in a backward elimination multivariate logistic regression model with stay criteria set at $P<0.05$. Results of logistic regression analysis are presented as odds ratios (OR) and 95% confidence intervals (CI). Survival data were assessed by the Kaplan-Meier method, and significance between groups was evaluated using the log-rank test. For patients that were re-transplanted ($n=7$), only the first transplantation was considered. The level for statistical significance was set at $P<0.05$.

Results

Out of a total of 362 patients transplanted between 1992 and 2003, 27 patients had to be excluded from the study due to missing or incomplete records. Basic demographic data, however, were available and are presented in Table 1. There was no difference with respect to recipient and donor age, gender, pre-transplant pulmonary disease or type of lung transplant between included and excluded patients. However, because most of the excluded patients originated from the early years of the transplant program, a higher proportion received polyclonal rather than monoclonal induction therapy.

Demographic details for the 335 included patients are provided in Table 2. A total of 105 (31%) patients had microbiological evidence of pulmonary *Aspergillus* infection post-transplantation, 83/335 (25%) with colonisation and 22/335 (6%) with invasive disease. Patients with post-transplant pulmonary *Aspergillus* infection were more

Table 1 Comparison of demographic data for all lung-transplanted patients from 1992 to 2003 according to study inclusion and exclusion

	Included ($n=335$)	Excluded ($n=27$)	P-value
Recipient			
Age (years)			0.954[a]
Mean	49.3±11.8	48.6±15.0	
Median (IQR)	52 (44–57)	51 (44–58)	
Gender			0.441
Male	187 / 56%	14 / 52%	
Female	148 / 44%	13 / 48%	
Disease			0.156[b]
Emphysema	240 / 72%	21 / 78%	
CF/Bronchiectasis	37 / 11%	0 / 0%	
Pulmonary hypertension	30 / 9%	1 / 4%	
Fibrosis	28 / 8%	4 / 15%	
Other[b]	0 / 0%	1 / 4%	
Transplantation type			0.354[b]
SLTX	214 / 64%	14 / 52%	
DLTX	103 / 31%	9 / 33%	
HLTX	18 / 5%	3 / 11%	
LLTX[b]	0 / 0%	1 / 4%	
Induction treatment			0.007[b]
ATG[c]	169 / 50%	21 / 78%	
Daclizumab	165 / 49%	6 / 22%	
None[b]	1 / 0%	0 / 0%	
Donor			
Age (years)			0.407[a]
Mean	40.7±12.8	38.7±14.2	
Median (IQR)	43 (33–51)	41 (30–48)	
Gender			0.707
Male	161 / 48%	12 / 44%	
Female	173 / 52%	15 / 56%	

IQR Interquartile range, *SLTX* single-lung transplant, *DLTX* double-lung transplant, *HLTX* heart-lung transplant, *LLTX* living lobar lung transplant, *CF* cystic fibrosis, *ATG* antithymocyte globulin

[a] Mann-Whitney test

[b] Excluding marked group (due to small sample size)

[c] ATG group includes patients receiving antilymphocyte globulin induction treatment

Table 2 Demographic data according to evidence of pulmonary *Aspergillus* infection (colonisation and/or invasion)

	Aspergillus infection		
	Yes (n=105)	No (n=230)	P-value
Recipient			
Age (years)			0.347[a]
Mean	47.8±13.1	49.9±11.1	
Median (IQR)	51 (40–58)	52 (46–67)	
Gender			0.274
Male	51 / 49%	97 / 42%	
Female	54 / 51%	133 / 58%	
Disease			0.034
Emphysema	72 / 75%	168 / 73%	
CF/Bronchiectasis (36/1)	19 / 18%	18 / 8%	
Pulmonary hypertension	8 / 8%	22 / 10%	
Fibrosis	6 / 6%	22 / 10%	
Transplantation type			0.045
SLTX	68 / 65%	146 / 63%	
DLTX	36 / 34%	67 / 29%	
HLTX	1 / 1%	17 / 7%	
Induction treatment			0.001[b]
ATG[c]	39 / 37%	130 / 57%	
Daclizumab	66 / 63%	99 / 43%	
None[b]	0 / 0%	1 / 0%	
Ischaemic time (min)			0.068[a]
Mean	317±141	282±97	
Median (IQR)	302 (227–370)	282 (223–333)	
Donor			
Age (years)			<0.001[a]
Mean	44.1±12.4	39.1±12.6	
Median (IQR)	47 (29–54)	40 (29–49)	
Gender			0.498
Male	53 / 51%	108 / 47%	
Female	51 / 49%	122 / 53%	
History of smoking			0.967
Yes	39 / 49%	79 / 49%	
No	41 / 51%	84 / 51%	
Use of inotropes			0.926
Yes	85 / 82%	187 / 81%	
No	19 / 18%	43 / 19%	
Ventilation time (h)			0.875[a]
Mean	47.5±44.8	49.0±72.6	
Median (IQR)	30 (24–48)	30 (24–48)	

IQR Interquartile range, *SLTX* single-lung transplant, *DLTX* double-lung transplant, *HLTX* heart-lung transplant, *CF* cystic fibrosis, *ATG* antithymocyte globulin

[a] Mann-Whitney test

[b] Excluding marked group (due to small sample size)

[c] ATG group includes patients receiving antilymphocyte globulin induction treatment

likely to have received allografts from older donors [median 47 years, IQR 29–54 years versus median 40 years, IQR 29–49 years (P<0.001)]. In addition, there were significant differences between *Aspergillus* infected and non-infected groups with respect to pre-transplant recipient diagnosis (P=0.034), lung transplant type (P=0.045) and choice of induction therapy (P=0.001). The difference between pre-transplant diagnosis was mainly caused by CF patients having a significantly higher rate of infection [incidences of colonisation and invasive disease: 15 (42%) and 4 (11%) of 36 patients] versus non-CF patients [68 (23%) and 18 (6%) of 299 patients] (P=0.01). A significantly higher proportion of patients receiving DLTX developed post-transplant *Aspergillus* infection in comparison to SLTX and HLTX recipients (Holm's correction, P<0.05). A higher proportion of patients receiving daclizumab induction developed post-transplant *Aspergillus* infection compared to patients receiving polyclonal induction with either ATG or ALG.

All demographic parameters associated with the development of post-transplant pulmonary *Aspergillus* infection were evaluated by backward elimination logistic regression. This method identified donor age (OR 1.40 per decade, CI 1.10–1.80), ischaemia time (OR 1.17 per hour increase, CI 1.01–1.39), and the use of daclizumab compared to polyclonal induction (OR 2.05, CI 1.14–3.75) as independent risk factors for *Aspergillus* infection.

Figure 1 shows the overall survival function (freedom from *Aspergillus* infection) of the entire cohort of 335 lung-transplanted patients. Most of the infections occurred during the first 12 months, with the infection rate being much lower after 1 year. After 3 years, only a few new pulmonary *Aspergillus* infections occurred. A more detailed analysis of the first 24 months shows that the majority of infections occurred during the first 3 months after transplantation (Fig. 2).

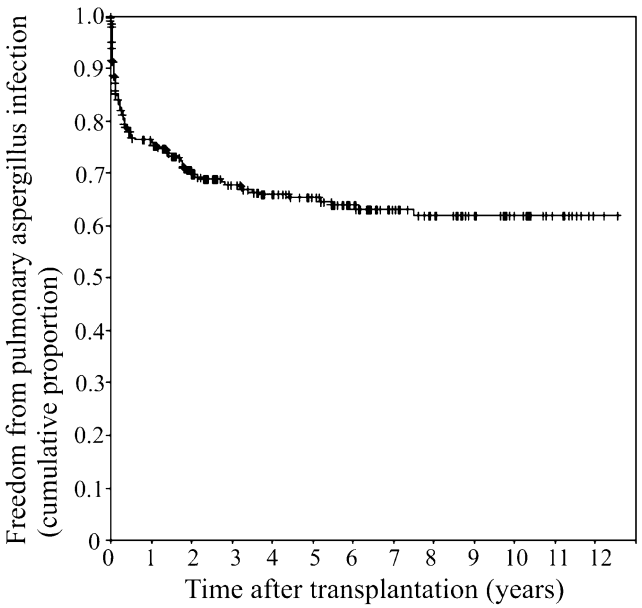

Fig. 1 Kaplan-Meier plot demonstrating freedom from pulmonary *Aspergillus* infection for the entire cohort (n=335) for the complete follow-up period (1992–2004)

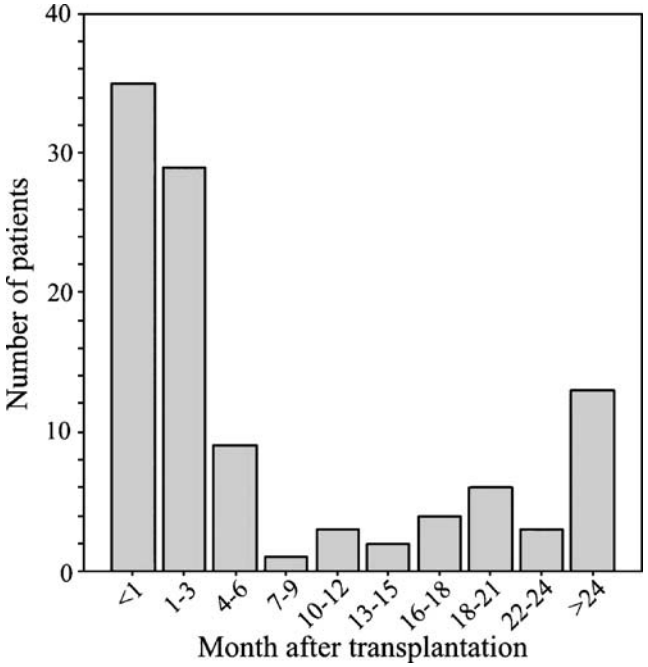

Fig. 2 Histogram demonstrating the time at which lung transplant patients have evidence of pulmonary *Aspergillus* infection

A Kaplan-Meier plot of the survival of infected versus non-infected patients with pulmonary aspergillosis shows no difference for the first 4 years but a significant increase in mortality thereafter in the infected group (log-rank test, $P=0.002$) (Fig. 3). The 1-, 5- and 10-year survival rates were 83, 68 and 48% in non-infected patients and 83, 53 and 6% in patients with pulmonary *Aspergillus* infection.

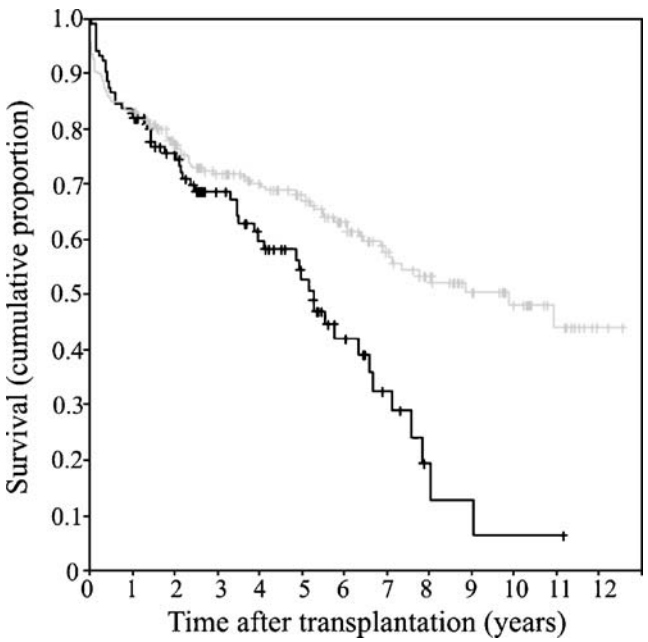

Fig. 3 Kaplan-Meier plot showing survival of infected (*black line*) versus non-infected (*grey line*) patients with pulmonary *Aspergillus* infection (log-rank test, $P=0.002$)

Among the 105 patients with pulmonary *Aspergillus* infection, 83 (79%) had evidence of lower respiratory tract colonisation with *Aspergillus*, and 22 (21%) had histological and/or radiological evidence of invasive pulmonary aspergillosis (Table 3). At the time of the study, the cumulated mortality was 51% in patients with colonisation, 55% in patients with invasive aspergillosis, and 39% in patients without pulmonary *Aspergillus* infection. The median follow-up at time of study was 3.6 years (IQR 1.5–6.4 years).

The group with invasive aspergillosis had a poor prognosis with a high mortality of 58% in the first 2 years, with additional mortality in the following years, Fig. 4. Graft survival with respect to pulmonary *Aspergillus* non-infection, colonisation and invasion was significantly different between groups (log-rank, $P=0.004$), with a demonstrable trend of reduced survival with *Aspergillus* colonisation and progression to invasion (trend test, $P<0.001$).

Discussion

Infection with *Aspergillus* is probably the most serious infectious complication in lung transplantation, and invasive aspergillosis carries a high mortality and morbidity. However, the role of colonisation with *Aspergillus* without evidence of invasion remains undetermined.

The purpose of this single-centre retrospective study was to determine the incidence and time-course of colonisation and invasive disease, and to evaluate its impact on the prognosis of lung-transplanted patients. Due to incomplete files, we could only obtain full information on 92.5% of 362 patients. However, the study included a large number of patients and is the largest published series from a single centre. The excluded patients had survival, age and sex comparable to the included patients; and since the majority of excluded patients originated from the first years of the program, the difference in proportions with respect to monoclonal versus polyclonal induction therapy was not surprising. This centre switched from ATG to daclizumab induction in 1999 [17]. In all other respects, the post-transplant management of lung allograft

Table 3 Cumulated incidence of *Aspergillus* infection and mortality

	Cumulated incidence		Cumulated mortality	
	n	(%)	*n*	(%)[a]
No *Aspergillus* infection	230	69	89	39
Colonisation with *Aspergillus*	83	25	42	51
Invasive aspergillosis	22	7	12	55

[a] Mortality in % is estimated from the populations with no evidence of *Aspergillus* infection, *Aspergillus* colonisation, and invasive aspergillosis

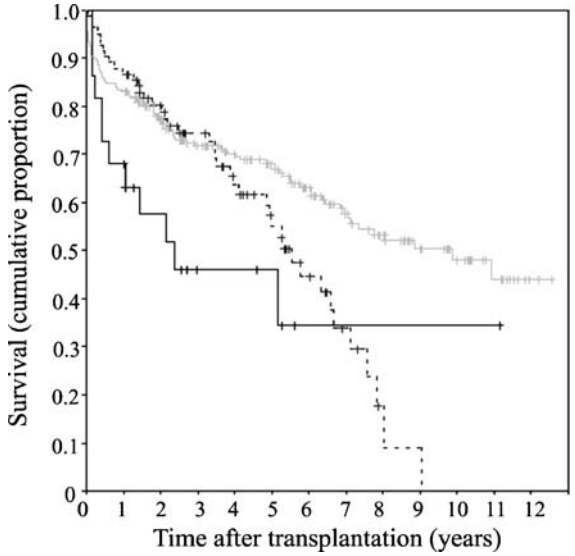

Fig. 4 Kaplan-Meier plot showing survival for patients without pulmonary *Aspergillus* infection (*grey line*), patients with pulmonary *Aspergillus* colonisation (*dashed line*), and patients with invasive pulmonary aspergillosis (*black line*) (log-rank test and trend test, $P=0.004$ and $P<0.001$ respectively)

recipients has changed very little, and in particular, the surveillance programme, with eight scheduled bronchoscopies with BAL and TBB during the first 2 years and when clinically indicated thereafter, remained unchanged during the study period.

The cumulated incidence and time-pattern of infection is comparable to previous studies. Cahill et al. 1997 [3] studied 151 lung transplant patients retrospectively, and 46% developed positive cultures for *Aspergillus* and 3% developed invasive aspergillosis. Patterson et al. in 2000 [4] studied intervention against *Aspergillus* infection in 95 patients in which 42% developed infection (colonisation plus invasive disease). Singh et al. in 2003 [7] estimated from a literature search that the overall mortality rate was 52% in patients with invasive (bronchial or pulmonary) *Aspergillus* infection.

Sole et al. in 2005 [8] studied 251 patients retrospectively with isolation of *Aspergillus* in 86 (33%), where 50 (20%) had colonisation, 17 (7%) tracheobronchial lesions and 19 (8%) had invasive aspergillosis; the mortality rate for invasive disease in this series was 78%. *Aspergillus* colonisation was detected within 3 months of transplantation in 56% of patients and within 12 months of transplantation in 80% of patients. All tracheobronchial infections developed within 3 months, whereas the invasive disease developed at a mean of 34 months after transplantation. *Aspergillus* infection (colonisation plus invasion) resulted in increased early and late mortality. Sole et al. performed four bronchoscopies in the first 3 months (as in our study) but only on clinical indication thereafter. Morgan et al. in 2005 [1] studied prospectively the incidence of *Aspergillus* infection in stem-cell and solid-organ transplantation, with 290 cases of lung transplantation. The cumulated incidence of invasive aspergillosis was 2.4% after 12 months, and 70% of the cases were diagnosed in the first 6 months.

The colonisation rate of 25% and the rate of invasive aspergillosis of 7% in our own series are very similar to Patterson [4] and Sole [8]. However, in contrast with Sole and Singh et al. (where 59 and 63% of the cultures were positive for *Aspergillus fumigatus* respectively), *Aspergillus fumigatus* was seen in 99% of the cultures from this centre. This is probably due to differences in environmental exposure, Sole being a Spanish study and Singh from the United States, in contrast to our centre at a more northern latitude. The temporal pattern with most of the cases occurring in the first 3–6 months after transplantation was observed in all studies.

The latest study from Morgan et al. employed the international consensus definition [14], which was developed for patients with cancer and stem-cell transplantation. The definition of invasive aspergillosis in the series presented here and in the study by Sole et al. would qualify as proven infection, whereas colonisation without endoscopic (with biopsy) and radiological evidence of invasive disease would not qualify as probable or possible invasive disease. The classification, however, was constructed for stem-cell transplantation and does not take the problem of colonisation in lung transplantation into account. The recent recommendation from the American Society of Transplantation [18] on reporting of fungal infections in immunosuppressive trials follows the same principle. This means that positive cultures from BAL fluid without any evidence of bronchial or lung tissue invasion would not be required to be reported. In light of the apparent association between fungal colonisation and late mortality in the present study, however, we suggest that future immunosuppressive trials in lung transplantation should also report transient colonisation of lower airways without evidence of invasion.

The review by Singh [7] suggested that SLTX was a risk factor for *Aspergillus* infection. This could not be confirmed by this study. Indeed, univariate analysis of demographic parameters as potential risk factors for the development of pulmonary *Aspergillus* colonisation and/or invasion demonstrated that DLTX recipients were at higher risk compared to SLTX or HLTX recipients. In addition, there were significant differences between infected and non-infected patients with respect to pre-transplant pulmonary disease which might explain the association. However, both parameters were insignificant once donor age and ischaemia time were taken into account. Ischaemia times are generally longer in patients undergoing bilateral sequential DLTX, thus it is not easy to tease apart the effects of the individual parameters. In addition, since all

CF patients receive DLTX, this may contribute to some of the differences in *Aspergillus* incidence with respect to pre-transplant disease. Furthermore, differences between centres with respect to the selection of recipients and transplant type may also explain these apparent contradictions. The findings of increasing donor age as an associated risk factor, however, and the high cumulative incidence of pulmonary *Aspergillus* infection soon after transplantation seen in this and other studies are mutually compatible.

The risk associated with daclizumab versus polyclonal induction (predominantly ATG) was an unexpected finding. Traditionally, polyclonal induction therapy, such as ATG, has been reportedly associated with increased risk of infection due to more wide-ranging immunomodulatory effects. At this centre, daclizumab was introduced in 1999 after promising reports of a reduction in the incidence of acute cellular rejection versus placebo in renal and cardiac transplantation. However, ATG was reinstated in 2004 as the primary induction therapy following an analysis demonstrating more frequent and severe acute rejection episodes requiring high-dose corticosteroid treatment in the daclizumab induction patient cohort [17]. Thus, the apparent association between post-transplant pulmonary *Aspergillus* infection and daclizumab induction could be a consequence of the much higher doses of corticosteroids used in the daclizumab induction group. Indeed, high-dose corticosteroid treatment is a known risk factor for fungal infection. Alternatively, since daclizumab induction covers a specific later time interval, the attributable risk in this analysis may comprise other management changes such as the acceptance of more marginal donors (i.e. marginal donor criteria other than donor age and other donor variables assessed here), or evolving virulence and pathogenecity of *Aspergillus*, although there exists no evidence to support this. With the exception of induction therapy, our lung transplant programme has been essentially unchanged during the study period.

The very high mortality in invasive aspergillosis known from previous studies was confirmed in this study. The role of colonisation has only been studied in a few series of patients. Colonisation has been described as a risk factor for invasive disease although the rate of progression to invasive disease is low. The role of colonisation without progression to invasive disease, which means clearance of the infection spontaneously or through treatment, remains controversial. Sole et al. reported no effect on survival from colonisation but from overall infection (colonisation and invasive disease). In this study, infection also resulted in lower overall survival with a significant difference between non-infected, colonised and invasive forms of the disease.

Whereas invasive disease had immediate high mortality, colonised patients had increased mortality only after 5 years. As yet there is no biological explanation for this finding, which has to be confirmed in other studies. Furthermore, the actual cause of the increase in mortality in colonised patients will have to be investigated in an extension of this study, although we have already determined that the long-term prognosis was primarily determined by the development of severe chonic rejection (BOS) in our population of lung transplantation patients [19]. We hypothesize that the strong immunological reactions elicited in even colonised patients might be of importance in lung transplant patients. *Aspergillus* conidia and hyphae promote strong immunological reactions with cytokine production when conidiae are inhaled and have the possibility to grow in immunologically impaired individuals [20]. Conidia and hyphae are taken up by dendritic cells which migrate to regional lymph nodes amplifying T-cell-driven immunological reactions [21]. Fungal antigens are recognised by the innate immune system on toll-like receptor-4 (TLR4) and TLR2. Surface receptor CD14 promotes signalling through TLR4, and recently it has been demonstrated that polymorphisms in CD14 in lung transplant patients can influence the number of acute rejections [22] and BOS-free survival [23]. Strong immunological stimulation by fungal antigens due to colonisation of airways might thus influence long-term outcome in lung-transplanted patients.

Conclusion

Aspergillus infection continues to represent a serious challenge in lung transplantation. Invasive disease is a recognised cause of mortality. This study suggests that transient colonisation may also be associated with increased late mortality.

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