A Risk Profile for Invasive Aspergillosis in Liver Transplant Recipients

M. Rosenhagen, R. Feldhues, J. Schmidt, T. Hoppe-Tichy, H.K. Geiss

Abstract

Background: Given the high incidence (1.5%–10%) of invasive aspergillosis (IA) after liver transplantation and the associated mortality, prophylaxis according to the patients' circumstances is a reasonable approach. The purpose of this investigation was to determine the effect and significance of risk factors for IA in a specialized transplantation center.

Methods: We collected data from patients who underwent liver transplantation at the Transplantation Center of the University Hospital Heidelberg (Germany) between December 2001 and December 2004 in a specifically designed database for retrospective analysis. Invasive aspergillosis was defined according to the European Organization for Research and Treatment of Cancer classifications. Univariate analysis and logistic regression were performed to assess the influence of each assumed risk factor.

Results: A total of 195 liver transplantations were performed in 170 patients, with two patients (1.2%) developing a proven IA, seven (4.1%) developing a probable IA, and five developing a possible IA (2.9%). All patients received oral itraconazole prophylaxis. Of these 14 patients with proven, probable or possible IA, 13 died within 4 weeks after the initial diagnosis; this represents 33.3% of all patients with a fatal outcome. Univariate significant factors were retransplantation ($p = 0.004$), cytomegalovirus (CMV) infection ($p = 0.024$), dialysis ($p < 0.001$), renal insufficiency ($p = 0.05$), thrombocytopenia ($p = 0.001$), and leukocytopenia ($p = 0.002$). Multivariate analysis showed an independent influence of CMV infection (OR 6.032, 95% CI 1.446–25.163) and dialysis (OR 14.985, 95% CI 2.936–76.486).

Conclusion: The rate of IA found in this investigation is within the range reported in published studies. Based on our data, extended antifungal prophylaxis should be given to liver transplant patients with specific risk factors, such as renal insufficiency, requirement for dialysis, CMV infection, or thrombocytopenia. Additional focus should be on the prevention of CMV infections.

Abbreviations: CMV: Cytomegalovirus; CT: Computer tomography; EORTC: European Organization for Research and Treatment of Cancer; IA: Invasive aspergillosis; OLT: Orthotopic liver transplantation SAPS: Simplified acute physiology score; SOT: Solid organ transplantation; UNOS: United Network for Organ Sharing

Infection 2009; 37: 313–319
DOI 10.1007/s15010-008-8124-x

Introduction

Organ transplantation is one of the main therapeutic options in certain acute and chronic diseases. The steady increase in the number of transplantations performed means greater numbers of immunocompromised patients and higher rates of infection-related morbidity and mortality. Given the high incidence of invasive fungal infections after solid organ transplantation (SOT), prophylaxis against fungal infections is a reasonable approach. Although most fungal infections are caused by *Candida* spp., the worst outcomes are due to *Aspergillus* spp. [1, 2]. Invasive aspergillosis (IA) has been reported to occur in 1.5%–10% of liver transplant recipients [3, 4]. Given the potential of antifungal agents [5–8], the threat of emerging drug resistance and the associated costs, strategies for antifungal prophylaxis should be construed for patients with increased risk of invasive fungal infections [9].

However, there is a substantial debate among clinicians on the optimal diagnostic criteria for these infections [10]. Early diagnosis of IA is difficult. The identification of risk factors for IA in transplant recipients could allow the use of antifungal agents for prophylaxis, thus avoiding the development of an invasive mycosis with high morbidity and mortality.

M. Rosenhagen *(corresponding author)*, **T. Hoppe-Tichy**
Pharmacy Dept., University Hospital of Heidelberg, INF 670, 69120 Heidelberg, Germany; Phone: (+49/6221) 56-38348, Fax: -5413,
e-mail: maike.rosenhagen@med.uni-heidelberg.de
R. Feldhues
Institute of Hygiene, University Hospital of Heidelberg, Heidelberg, Germany
J. Schmidt
Dept. of General, Visceral and Transplantation Surgery, University Hospital of Heidelberg, Heidelberg, Germany
H.K. Geiss
Rhön-Klinikum AG, Schlossplatz, Bad Neustadt, Germany

Received: March 23, 2008 · Revision accepted: November 18, 2008
Published online: July 23, 2009

Patients and Methods

Patients

Definitions

All liver transplantations at the Liver Transplantation Unit of the University Hospital of Heidelberg, Germany from December 2001 to December 2004 were included retrospectively, with the exception of three cases (two patients underwent a prior liver transplantation at another hospital; the third was a child who had undergone a prior liver–kidney transplantation at another unit).

A case of IA was defined as any liver transplant recipient with a proven, probable, or possible diagnosis of IA according to the European Organization for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycosis Study Group (EORTC/MSG) classification [10].

"Proven IA'' refers to histopathological evidence of tissue invasion (needle aspiration or biopsy and/or autopsy specimen) that discloses septated, acutely branching hyaline hyphae compatible with *Aspergillus* or positive results of cultures for *Aspergillus* spp. from a sample of a normally sterile but clinically infected body site (excluding bronchoalveolar lavage fluid and sinus aspirate) obtained by a sterile procedure. ''Probable IA'' implies an appropriate host setting (neutropenia, persistent fever for > 96 h refractory to appropriate broad-spectrum antibacterial treatment in high-risk patients, body temperature either > 38 °C or < 36 °C, or prolonged (> 3 weeks) use of corticosteroids during the previous 60 days) and the presence of positive culture results or cytological evidence for *Aspergillus* spp. from a lower respiratory tract specimen, in conjunction with one major (halo sign or ''air crescent'' sign on a computed tomography [CT] scan) or at least two minor clinical findings (signs of lower respiratory tract infection, pleural rub, and presence of any new infiltrate in a patient who did not fulfill the major criterion but for whom no alternative diagnosis was available). If there was one major clinical criterion (or two minor clinical criteria) in the appropriate host setting without any other clear diagnosis of the symptoms, patients were considered to have ''possible IA''.

Cytomegalovirus Surveillance

The cytomegalovirus serostatus of donors and recipients was determined preoperatively by testing CMV IgG. Following orthotopic liver transplantation (OLT), all patients were screened weekly for active CMV infection by means of a quantitative assessment of CMV pp65 antigenemia and the detection of CMV by PCR. Cytomegalovirus infection was defined as pp65 antigenemia or at least 1 positive cell/10,000 leukocytes.

In the case of clinically relevant elevations of liver enzymes, a percutaneous liver biopsy was performed to verify CMV hepatitis.

Leukocytopenia was defined as ≤ 1 neutrophil/nl, and thrombocytopenia as ≤ 50 thrombocytes/nl.

Data Collection

Electronic and handwritten medical individual patient charts were reviewed and included in the investigation. Clinical and surgical variables were transferred into a specifically designed data base (Microsoft Office Access). Parameters analyzed during the preoperative period were age, sex, underlying disease, concomitant diseases, virological status, Child–Pugh classification, United Network for Organ Sharing (UNOS) status, transplantation urgency, retransplantation, and Karnofsky index. Parameters collected for the intraoperative period were duration of graft cold ischemia, number of packed RBCs, and donor criteria (age, cause of death, liver quality). In the postoperative period, the parameters analyzed included requirement for renal and hepatic dialysis, length of intubation, re-intervention, immunosuppression, liver biopsy, blood count, duration and kind of antibiotic therapy, and other co-morbidity factors.

In addition to these variables, blood samples were used to determine *Aspergillus* antigen using a sandwich-ELISA technique (Platelia; Bio-Rad, Hercules, CA). For valid interpretation of the antigen testing, we took at least two serum samples at different time points. An optical density index of *Aspergillus* antigen detection with a value of 1 ng/μl or higher was considered to be positive.

Simplified Acute Physiology Score

Each patient was scored according to the simplified acute physiology score (SAPS II) to provide a method of predicting mortality [11].

Methods

Transplantation Procedures and Medication

Donor management and surgery were performed according to internationally accepted standard procedures. Biliary reconstruction occurred by either choledo-choledochostomy or Roux-en-y-choledocho-jejunostomy, whichever was the more appropriate. Postoperative prophylaxis included cefuroxime for 3 days. For selective intestinal decontamination, all patients received subantimicrobial dose doxycycline (SDD) capsules (gentamicin, polymyxin) orally for 3 days. All patients received oral itraconazole (2×200 mg, liquid formulation) and oral amphothericin B (four times a day, 1 ml of liquid formulation) as an antifungal prophylaxis for the duration of 14 days after transplantation.

Fungal infections were treated with liposomal amphotericin B, voriconazole, or caspofungin – mono- or combination therapy – according to microbiological results.

Mupirocin was applied nasally for a period of 14 days after transplantation for methicillin-resistant *Staphylococcus aureus* (MRSA) prophylaxis.

During the first 6 months all patients received cotrimoxazole as *Pneumocystis jiroveci* prophylaxis. Cytomegalovirus prophylaxis was performed with ganciclovir in seronegative recipients or seropositive donors. In all other cases, pre-emptive CMV therapy was initiated when the antigenemia (pp65) result was positive. Ganciclovir doses were adjusted to the renal function according to the manufacturer's guidelines.

The immunosuppressive regime comprised oral cyclosporine A with target trough levels of 200–250 ng/day or tacrolimus with a target trough concentration of 4–8 ng/ml; corticosteroids and azathioprin (1 mg/kg day) provided that the WBC count remained > 60,000/μl). Rejection episodes were initially treated with corticosteroid pulse therapy.

There was no specific ''renal protection protocol''.

Statistical Analysis

Univariate analysis was performed with the assumed risk factors. The χ^2 test and Fisher's exact test were used to compare binary variables. The t-test and box and whisker plots were employed to compare numeric factors. All statistical tests were two-tailed, and the threshold of statistical significance was a p-value ≤ 0.05. Statistically significant variables in the univariate analysis were intro-

Table 1
Underlying diseases of all patients.

Indication	n
Acute hepatic failure (fulminant or subfulminant hepatitis)	10
Benign liver tumors or polycystic disease	3
Cancers (epithelioid hemangioendothelioma, hepatocellular carcinoma)	28
Cholestatic disease (primary biliary cirrhosis, primary sclerosing cholangitis, secondary biliary cirrhosis)	12
Cirrhosis (alcohol cirrhosis, autoimmune cirrhosis, cryptogenic [unknown] cirrhosis)	57
Virus-related cirrhosis	42
Congenital biliary disease (alagille syndrome)	1
Metabolic diseases (alpha-1-antitrypsin deficiency, cystic fibrosis, familial amyloidotic polyneuropathy, hemochromatosis, Wilson disease)	17

duced to a multivariate model by use of logistic regression to identify the independent risk factors for IA. Odds ratios (ORs) and 95% confidence intervals (CIs) illustrated the amount of risk associated with some of the assumed risk factors.

Kaplan-Meier survival curves were constructed from the date of the first transplant as the starting point and death as the endpoint.

Statistical analysis of the data was performed with SPSS 11.0 for Windows (SAS, Chicago, IL) and SAS 9.1 (SAS Institute, Cary, NC)

Results

From December 2001 to December 2004 a total of 198 liver transplantations were performed in 170 patients. Data on 195 transplantations were used in this analysis. Three transplantations were excluded because these patients did not receive their first liver transplantation in the Transplantation Unit of the University of Heidelberg.

The underlying diseases as deciding factors for transplantation are shown in table 1.

Two patients (1.2%) developed proven IA, seven (4.1%) developed probable IA, and five developed a possible IA (2.9%).

Characteristics of the 14 cases are shown in table 2.

Post-mortem examination was performed in only two patients, and in both cases IA was proven by histopathological examination. The other 12 patients had several positive *Aspergillus* cultures in sputum or bronchoalveolar lavage and/or positive antigenemia and therefore fulfilled the criteria of probable or possible IA. Six patients had undergone retransplantation. The median time from transplantation to the onset of IA was 25 days (range 3–282 days). 13 of these patients died within 4 weeks after initial diagnosis of IA, which corresponds to 33.3% of all liver transplant patients with a fatal outcome.

Mean SAPS II of these patients was 33 compared to 31 in patients without IA, respectively.

The risk factors for the development of IA are shown in table 3. The univariate analysis revealed that IA was associated with retransplantation ($p = 0.004$), CMV infection ($p = 0.024$), dialysis ($p = 0.001$), renal insufficiency ($p = 0.038$), leukocytopenia ($p = 0.001$), and thrombocytopenia ($p = 0.001$).

The multivariate analysis (Table 4) revealed an independent association of IA and CMV infection (OR 6.032, 95% CI 1.446–25.163) and dialysis (OR 14.985, 95% CI 2.936–76.486).

The time relationship between the occurrence of CMV infection and IA is shown in table 2. In only three patients was IA detected before the diagnosis of CMV; in

Table 2
Characteristics of patients with invasive aspergillosis after liver transplantation.

Patient	Age (years)/gender	Retransplantation	*Aspergillus* antigenemia (days post-OLT)	Positive *Aspergillus* tissue sample (days post-OLT)	Diagnosis of CMV (days post-OLT)	Outcome	EORTC diagnosis
1	55/M	No	48	48	23	Death	Probable
2	57/M	No	6	46	48	Alive	Possible
3	60/M	No	282	Neg	Negative	Death	Possible
4	41/M	No	Neg	190	Negative	Death	Possible
5	60/M	No	42	25	Negative	Death	Probable
6	63/M	No	20	20	Negative	Death	Possible
7	44/M	No	146	Negative	29	Death	Probable
8	61/M	No	8	18	32	Death	Proven
9	61/F	Yes	Negative	3[a]	3	Death	Probable
10	61/M	Yes	19[b]	26[b]	Negative	Death	Probable
11	58/F	Yes	13[a]	11[a]	24[a]	Death	Proven
12	41/M	Yes	88[a]	Negative	81[a]	Death	Possible
13	47/M	Yes	30[a]	47[a]	16[a]	Death	Probable
14	57/M	Yes	3[a]	3[a]	Negative	Death	Probable

OLT: Orthotopic liver transplantation; M: male; F: female; EORTC: European Organization for Research and Treatment of Cancer; [a]After retransplantation; [b]After re-retransplantation

Table 3
Univariate analysis of risk factors for aspergillosis development.

Variable	Cases (%)	Controls (%)	Total	p
Male/female	12/14 (85.7)	110/181 (60.8)	122/195 (62.6)	0.085
Age (> median)	9/14 (64.3)	88/181 (48.6)	97/195 (49.7)	0.282
High urgency transplantation	5/14 (35.7)	28/181 (15.5)	33/195 (16.9)	0.065
Child-Pugh score C	9/14 (64.3)	103/181 (56.9)	112/195 (57.4)	0.59
Retransplantation	6/14 (42.9)	19/181 (10.5)	25/195 (12.8)	0.004*
Organ rejection	5/14 (35.7)	62/181 (34.3)	67/195 (34.4)	1.000
CIT (> 8 h)	9/14 (64.3)	96/164[a] (58.5)	105/178[a](59)	0.782
CMV infection	8/14 (57.1)	45/181 (24.9)	53/195 (27.2)	0.024*
Dialysis postoperative	12/14 (85.7)	45/181 (24.9)	57/195 (29.2)	< 0.001*
Renal insufficiency, preoperative	9/14 (64.3)	60/181 (33.1)	69/195 (35.4)	0.038*
Thrombocytopenia (< 50/nl)	14/14 (100)	104/181 (57.5)	118/195 (60.5)	0.001*
Leukocytopenia (< 1/nl)	6/14 (42.9)	15/181 (8.3)	21/195 (10.8)	0.001*
HCV infection	5/14 (35.7)	34/181 (18.8)	39/195 (20)	0.161
Blood requirement > 4 p	4/14 (28.6)	22/159 (13.8)	26/195 (13.3)	0.098
Antibiotic therapy > 7 days	2/14 (85.7)	48/76 (63.2)	60/90 (66.7)	0.129
Metabolic disease	4/14 (28.6)	30/181 (16.6)	34/195 (17.4)	0.273

*Significant at $p \leq 0.05$; CIT: Cold ischemic time; HCV: hepatitis C infection as underlying disease; p: packages; CMV: cytomegalovirus; [a]17 controls missing

Table 4
Multivariate analysis of risk factors for aspergillosis development.

Variable	Odds ratio/confidence limits	p
CMV infection	6.032 (1.446–25.163)	0.0137*
Dialysis	14.985 (2.936–76.486)	0.0011*
Retransplantation	2.162 (0.560–8.353)	0.1233
Leukocytopenia	3.191 (0.879–11.584)	0.0777

*Significant at $p \leq 0.05$

all other patients IA occurred at the same time (n = 1) or after (7–117 days) the initial diagnosis of CMV infection.

Kaplan-Meier survival curves are shown in figure 1. There is a significant difference in survival between the IA and non-IA-group (p = 0.001).

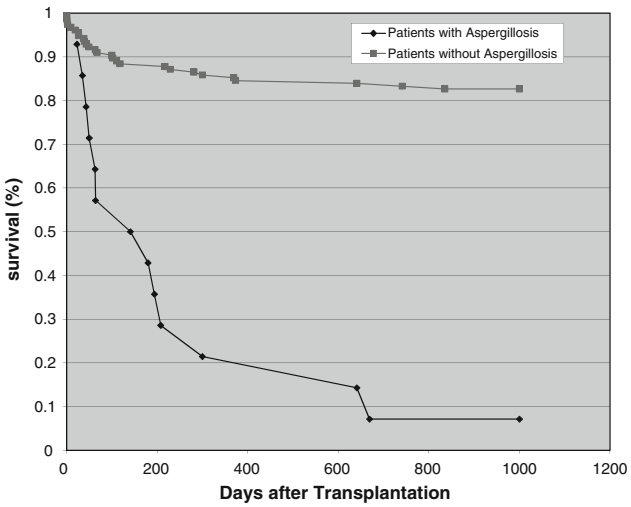

Figure 1. Survival curves.

Discussion

Invasive aspergillosis has been reported to occur in 1.5% to 10% of all liver transplantation patients [12–14], and the mortality rate among liver transplant recipients with IA has been shown to exceed 90% [2, 15]. However, IA remains a diagnostic and therapeutic challenge, with the efficacy of therapy being largely dependent on an early diagnosis.

In our retrospective analysis of 170 patients, we were only able to meet the criteria of proven IA based on post-mortem examination findings in two patients because post-mortem examination was refused by the relatives of the other deceased patients. Therefore, we assume that the number of cases which would have been classified as proven IA would likely have been higher, an assumption underscored by the significantly higher mortality in this group than in patients without signs of IA. Risk factors for the development of IA have been assessed in several reports. *Singh* et al. [15, 16] recently emphasized three main risk factors, which have also been identified in other studies: (1) poor allograft function or primary failure of the allograft; (2) renal dysfunction, particularly the cases requiring dialysis; (3) increased immunosuppression, especially during CMV co-infection. Cytomegalovirus has long been recognized as the most common opportunistic pathogen in transplant recipients. More than 50% of transplant recipients have laboratory evidence of primary or reactivated CMV infection in the first year after transplantation [17]. Over the years, it has been recognized that, aside from direct effects (i.e., morbidity directly attributable to CMV infection), CMV as an immunoregulatory virus is also

responsible for indirect effects, i.e, acute and perhaps chronic allograft rejection and secondary fungal and bacterial infections [18, 19]. In a survival analysis of 146 liver transplant patients, *George* and colleagues [20] showed that about one-third of the patients with CMV disease developed invasive fungal disease within the first year post-transplant compared with 8% of those without CMV disease. Similar data have been reported for other solid-organ transplant patients. Different approaches have been used to mitigate morbidity and mortality associated with CMV infection, but there is still an ongoing debate whether preemptive therapy or prophylactic (long-term) administration of antiviral agents should be applied to all patients [21, 22]. The clinical importance of CMV infection was also confirmed in our study. The multivariate analysis revealed that both CMV and the requirement for dialysis after transplantation were independently associated with IA. In accordance with international recommendations, CMV prophylaxis for the first 3 months after transplantation was given only to seronegative patients receiving a CMV-seropositive organ. All other patients received preemptive antiviral therapy when CMV infection was imminent.

The following limitations of this investigation have to be taken into account. A statistical adjustment for the time dependence of the important risk factors, such as CMV infection, leukocytopenia, and thrombocytopenia, was not performed, and the incidence of fungal infections other than aspergillosis was not evaluated as this topic fell outside the scope of our study. Because of the small number of patients with IA in this retrospective analysis, we did not distinguish between early- and late-onset aspergillosis [13, 23].

As the impaired renal function and the need for hemodialysis are known risk factors for the outcome of transplantations, the protection of the kidney is of outmost importance. Calcineurin inhibitors have a significant adverse impact on renal function [24], and the use of these drugs, therefore, has to be closely monitored. *Sommerer* et al. [25] showed that the individualization of cyclosporin therapy is supposed to be a useful tool for optimizing the long-term immunosuppression with respect to safety. The use of mycophenolatmofetil instead of azathioprine – which is known to cause severe myelosuppression, including leukocytopenia and thrombocytopenia – was introduced as a routine therapy in our transplantation center in 2005 and resulted in a reduction of the target trough blood level of cyclosporin to 150–200 µg/l in the first 3 months. Furthermore, an azathioprine replacement protocol may also prevent severe leukocytopenia.

As most of the IA in our patients has to be considered to be breakthrough infections, the use of an oral formulation of itraconazole must be critically reassessed. The efficacy of itraconazole as antifungal prophylaxis has been examined in several studies [26–28], but all of these studies compared itraconazole to fluconazole, which is not effective against *Aspergillus* spp.

Several studies have found a preventive effect of conventional intravenous amphotericin B with common dosing schemes [29, 30], but other investigations resulted in a failure of this approach [4, 31]. Furthermore, intravenous amphotericin B is also associated with toxic side effects on the often already impaired renal function of transplant recipients. However, the use of low dose amphothericin B deoxycholate (0.1–0.2 mg/kg day) did not successfully prevent aspergillosis in liver transplant patients [4].

The results of an Italian group [32] showed that chemoprophylaxis did not have a significant effect on the prevention of fungal infections and suggested that the administration of antifungal prophylaxis should be limited to patients at risk.

The new triazoles are attractive alternatives for antifungal prophylaxis as all triazoles have enhanced potency and a broad spectrum of activity, and they are available in oral formulation [33]; for example, posaconazole is an extended-spectrum triazole with clinical activity against a wide range of fungal pathogens, including *Candida* spp., *Aspergillus* spp., zygomycetes, and *Fusarium* spp. [34]. Recent studies have shown that posaconazole was as effective as fluconazole in preventing fungal infections and was superior in preventing IA in a high-risk population. The superiority of posaconazole reflects the lack of efficacy of fluconazole against filamentous fungi [35, 36].

The data summarized by *Hamza* et al. [33] indicate that the echinocandins, especially caspofungin, are promising therapeutic options for antifungal prophylaxis as a result of their excellent safety profile, extended half-life, efficacy against most *Aspergillus* species and their reduced drug interactions. Their major drawback, however, is that they are not available as oral formulations.

All these studies show that, in fact, there is no universally valid recommendation for antifungal prophylaxis in liver transplant patients [37]. When the costs and drug-related side effects of antifungal prophylaxis is taken into consideration, such therapy for liver transplant patients can not be recommended and should be restricted to high risk patients.

Our study showed – similar to results reported elsewhere – that CMV infection, severe renal impairment, requirement of hemodialysis, and thrombocytopenia are significant risk factors for IA. Further studies on the efficacy of antifungal prophylaxis in SOT recipients should consider these patients as a primary target group.

Acknowledgments

Grant and financial support: none.

References

1. Paterson DL, Singh N: Invasive aspergillosis in transplant recipients. Medicine (Baltimore) 1999; 78: 123–138.

2. Kusne S, Troke PF, Marino IR: Factors associated with invasive lung aspergillosis and the significance of positive *Aspergillus* culture after liver transplantation. J Infect Dis 1992; 166: 1379–1383.
3. Paya CV, Hermans PE, Washington JA, et al. Incidence, distribution and outcome of episodes of infection in 100 orthotopic liver transplantations. Mayo Clin Proc 1993; 64: 555–564.
4. Singh N, Mieles L, Yu VL, Gayowski T: Invasive aspergillosis in liver transplant recipients: association with candidemia and consumption coagulopathy and failure of prophylaxis with low-dose amphotericin B. Clin Infect Dis 1993; 17: 906–908.
5. Denning DW: Echinocandin antifungal drugs. Lancet 2003; 362: 1142–1151.
6. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E, Haas A, Ruhnke M, Lode H: Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin Infect Dis 2002; 34: 563–571.
7. Fisher NC, Singhal S, Miller SJ, Hastings JGM, Mutimer DJ: Fungal infection and liposomal amphotericin B (AmBisome) therapy in liver transplantation: a 2 year review. J Antimicrob Chemother 1999; 43: 597–600.
8. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, Kern WV, Marr KA, Ribaud P, Lortholary O, Sylvester R, Rubin RH, Wingard JR, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar PH, Hodges MR, Schlamm HT, Troke PF, de Pauw B, The Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 2002; 347: 408–415.
9. Alexander BD: Prophylaxis of invasive mycoses in solid organ transplantation. Curr Opin Infect Dis 2002; 15: 583–589.
10. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, Denning DW, Donnelly JP, Edwards JE, Erjavec Z, Fiere D, Lortholary O, Maertens J, Meis JF, Patterson TF, Ritter J, Selleslag D, Shah PM, Stevens DA, Walsh TJ: 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.
11. Le Gall J-R, Lemeshow S, Saulnier F: A new simplified acute physiology score (SAPS II) based on a European/North American multicenter study. JAMA 1993; 270: 2957–2963.
12. Singh N: The changing face of invasive aspergillosis in liver transplant recipients. Liver Transpl 2002; 8: 1071–1072.
13. Singh N, Avery RK, Munoz P, Pruett TL, Alexander B, Jacobs R, Tollemar JG, Dominguez EA, Yu CM, Paterson DL, Husain S, Kusne S, Linden P: Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. Clin Infect Dis 2003; 36: 46–52.
14. Singh N, Paterson DL: *Aspergillus* infections in transplant recipients. Clin Microbiol Rev 2005; 18: 44–69.
15. Singh N, Arnow PM, Bonham A, Dominguez E, Paterson DL, Pankey GA, Wagener MM, Yu VL: Invasive aspergillosis in liver transplant recipients in the 1990 s. Transplantation 1997; 64: 716–720.
16. Singh N: Antifungal prophylaxis for solid organ transplant recipients: seeking clarity amidst controversy. Clin Infect Dis 2000; 31: 545–553.
17. Snydman DR: Infection in solid organ transplantation. Transpl Infect Dis 1999; 1: 21–28.
18. Rubin RH: The indirect effects of cytomegalovirus infection on the outcome of organ transplantation. JAMA 1989; 261: 3607–3609.
19. Singh N: Interactions between viruses in transplant recipients. Clin Infect Dis 2005; 40: 430–436.
20. George MJ, Snydman DR, Werner BG, Griffith J, Falagas ME, Dougherty NN, Rubin RH: The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland. Am J Med 1997; 103: 106–113.
21. Singh N: Late-onset cytomegalovirus disease as a significant complication in solid organ transplant recipients receiving antiviral prophylaxis: a call to heed the mounting evidence. Clin Infect Dis 2005; 40: 704–708.
22. Snydman DR: Counterpoint: prevention of cytomegalovirus (CMV) infection and CMV disease in recipients of solid organ transplants: the case for prophylaxis. Clin Infect Dis 2005; 40: 709–712.
23. Singh N, Limaye AP, Forrest G, Safdar N, Munoz P, Pursell K, Houston S, Rosso F, Montoya JG, Patton PR, Del BR, Aguado JM, Wagener MM, Husain S: Late-onset invasive aspergillosis in organ transplant recipients in the current era. Med Mycol 2006; 44: 445–449.
24. Flechner SM, Kobashigawa J, Klintmalm G: Calcineurin inhibitor-sparing regimens in solid organ transplantation: focus on improving renal function and nephrotoxicity. Clin Transplant 2008; 22: 1–15.
25. Sommerer C, Giese T, Schmidt J, Meuer S, Zeier M. Ciclosporin: A tapering monitored by NFAT-regulated gene expression: a new concept of individual immunosuppression. Transplantation 2008; 85: 15–21.
26. Potter M: Strategies for managing systemic fungal infection and the place of itraconazole. J Antimicrob Chemother 2005; 56 (Suppl 1): i49–i54.
27. Morgenstern GR, Prentice AG, Prentice HG, Ropner JE, Schey SA, Warnock DW: A randomized controlled trial of itraconazole versus fluconazole for the prevention of fungal infections in patients with haematological malignancies. U.K. Multicentre Antifungal Prophylaxis Study Group. Br J Haematol 1999; 105: 901–911.
28. Winston DJ, Busuttil RW: Randomized controlled trial of oral itraconazole solution versus intravenous/oral fluconazole for prevention of fungal infections in liver transplant recipients. Transplantation 2002; 74: 688–695.
29. Mora NP, Klintmalm G, Solomon H, Goldstein RM, Gonwa TA, Husberg BS: Selective amphotericin B prophylaxis in the reduction of fungal infections after liver transplant. Transplant Proc 1992; 24: 154–155.
30. Fortun J, Martin-Davila P, Moreno S, Barcena R, de Vicente E, Honrubia A, Garcia M, Nuno J, Candela A, Uriarte M, Pintado V: Prevention of invasive fungal infections in liver transplant recipients: the role of prophylaxis with lipid formulations of amphotericin B in high-risk patients. J Antimicrob Chemother 2003; 52: 813–819.
31. Lorf T, Braun F, Ruchel R, Muller A, Sattler B, Ringe B: Systemic mycoses during prophylactical use of liposomal amphotericin B (Ambisome) after liver transplantation. Mycoses 1999; 42: 47–53.
32. Biancofiore G, Bindi ML, Baldassarri R, Romanelli AM, Catalano G, Filipponi F, Vagelli A, Mosca F: Antifungal prophylaxis in liver transplant recipients: a randomized placebo-controlled study. Transpl Int 2002; 15: 341–347.
33. Hamza NS, Ghannoum MA, Lazarus HM: Choices aplenty: antifungal prophylaxis in hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2004; 34: 377–389.

34. Walsh TJ, Raad I, Patterson TF, Chandrasekar P, Donowitz GR, Graybill R, Greene RE, Hachem R, Hadley S, Herbrecht R, Langston A, Louie A, Ribaud P, Segal BH, Stevens DA, van Burik JA, White CS, Corcoran G, Gogate J, Krishna G, Pedicone L, Hardalo C, Perfect JR: Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. Clin Infect Dis 2007; 44: 2–12.
35. Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR, Greinix H, Morais de AW, Reddy V, Boparai N, Pedicone L, Patino H, Durrant S: Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med 2007; 356: 335–347.
36. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, Helfgott D, Holowiecki J, Stockelberg D, Goh YT, Petrini M, Hardalo C, Suresh R, ngulo-Gonzalez D: Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med 2007; 356: 348–359.
37. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Segal BH, Steinbach WJ, Stevens DA, van Burik JA, Wingard JR, Patterson TF: Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2008; 46: 327–360.