PCR-Mediated Early Diagnosis of Fungal infections in Patients with Living-Donor Liver Transplants

Akiko Nakamura[1,2], Hideo Wada[*,2], Hiroyuki Sakurai[3], Masanobu Usui[3], Yoshiko Matsushima[1], Junji Nishioka[1], Shuji Isaji[3] and Tsutomu Nobori[2]

[1]*Central Clinical Laboratories, Mie University Hospital,* [2]*Department of Molecular and Laboratory Medicine,* [3]*First Department of Surgery, Mie University Graduate School of Medicine, Tsu, Mie, Japan*

Abstract: Fungal infections are frequently observed in living-donor liver transplantation (LT) and are correlated with the clinical outcome. Early diagnosis of fungus infection is difficult because of the low positive rate of blood culture and the poor specificity of the (1-3)-β-D-glucan assay for fungi. A polymerase chain reaction (PCR)-based method was developed for the early diagnosis of fungal infection. The PCR-based method consists of a two step PCR amplification from peripheral blood samples; the first PCR which detects all species of fungi excluding *Mucor*, *Fusarium* and *Sporothrix* (fungi-specific PCR). Subsequently, nested PCRs is used to determine the species of fungi (species-specific PCR), *Aspergillus*/*Penicillium* (ASP/PEN), *Candida albicans*/*parapsilosis*/*tropicals*/ *guliermondi* (CAN), *Candida glabrata* (C.glab.), and broad spectrum of fungi (Broad spectrum). This method was applied to peripheral blood samples from 31 LT patients, 11 samples from febrile patients in hematological ward (HW), and 15 patients with fever of unknown origin in the general ward (GW). Fourteen of the LT patients were tested positive with the fungi-specific PCR, 22 with the species-specific PCR. The positive rates of species-specific PCR were 12.9% for ASP/PEN, 29.0% for CAN, 9.7% for C.galb. and 45.2% for the broad spectrum, respectively. Five of the HW patients tested positive with the fungus specific-PCR, and 6 with the species-specific PCR. Two of the GW patients were positive with the fungi-specific-PCR. The fungus-specific PCR has higher specificity than the (1-3)-bete-D-glucan measurement, and the clinical outcome of LT patients correlated significantly with the results of this method. In conclusion, that the newly developed PCR-based method might be useful for early detection and diagnosis of fungus infection in LT patients.

Keywords: Living-donor liver transplantation, Fungal infection, PCR, *Aspergillus*, *Candida.*

INTRODUCTION

Fungal infections are opportunistic infections which occur in patients who have serious underlying diseases and who are also in an immunological deficient state during advanced medical treatment. Liver transplantation has been recently performed at increasingly more centers throughout the world for patients with hepatitis-related liver failure and liver cancer [1, 2]. The quality of life and the rates of survival after liver transplantation have greatly improved as a result of advances in surgical techniques, immunosuppressive therapy and medical management of patients.

Liver transplantation surgery is extremely invasive, and serious infections including fungal infections often occur partly because of the need for immunosuppressive therapy after transplantation. Complications such as infections remain the major cause of morbidity and death in organ transplantation. In particular, fungal infections following solid organ transplantation remain a major cause of death [3]. The incidence of infection after transplantation varies according to the type of organ transplanted, the degree of immunosuppression, the need for additional anti-rejection therapy, and the occurrence of technical surgical complications [4]. The high mortality rate associated with fungal infection after organ transplantation is due to the difficulty of early diagnosis. This is because performing a blood culture of fungi is time consuming and β-D-glucan [5] also has a low specificity for fungal infections. Since the clinical management of fungal infection depends on the rapid and unambiguous identification of pathogens, the early detection of fungal pathogens using the polymerase chain reaction (PCR) has recently been investigated for *Candida albicans* [6], other types of *Candida* [7-9] and *Aspergillus* species [10].

A sensitive and semi-diagnostic PCR system including four nested PCR assays was developed for the detection of broad spectrum fungi, *Candida glabrata*, and *Candida* species other than *C. glabrata*, and *Aspergillus*/*Penicillium* species in patients undergoing liver transplantation.

MATERIAL AND METHODS

Patients

This study examined 31 post-liver transplantation patients, 11 patients in the hematology ward and 15 patients in the general ward, between June 1, 2003 and December 31, 2004, at the Mie University School of Medicine. Of the 31 patients who had undergone liver transplantation, 17 had liver cirrhosis, 12 had hepatic cell carcinoma (HCC), 1 had congenital bile duct atresia and 1 had fulminant hepatitis

*Address correspondence to this author at the Department of Laboratory Medicine, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan; Tel: 81-59-232-1111; Fax: 81-59-231-5204;
E-mail: wadahide@clin.medic.mie-u.ac.jp

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(mean age, 54; range, 25-70; sex, F:M=11:20). The 11 patients with hematological diseases suspected of having fungal infections following chemotherapy included 3 with myelodysplastic syndrome (MDS), 2 with multiple myeloma (MM), 1 with acute myeloblastic leukemia (AML), 1 with acute lymphocytic leukemia, 1 with chronic myelocytic leukemia with blastic crisis, 1 with hypoplastic leukemia, 1 with acquired immunodeficiency disease (AIDS) and 1 with renal cancer (mean age 57, range 50-76; sex, F:M=5:6). The 15 infectious patients without opportunistic infection in the general ward included 4 with diabetes mellitus, 3 with pneumonia, 3 with solid cancer, 1 with sepsis, 1 with suffered burns, 1 with hemophagocytic syndrome, 1 with chronic hepatitis and 1 with no underlying disease (mean age, 73, range 28-87; sex, F:M=5:10). The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine.

Amplification of Fungal Cell DNA by PCR

Reference strains of twenty-six fungus reference strains (Japan Collection of Microorganisms RIKEN, Saitama, Japan) were used to test the primers specificity. Fungal cell colonies were peeled off from the agar surface using a sterile micro loop, and the cells were washed with 1 ml of sterilized water. After centrifugation, the cells were resuspended with 100 µl of sodium saline and collected by centrifugation at 12,500 G for 10 min. Cellular DNA was extracted using SepaGene (Sanko Junyaku, Tokyo, Japan) after heat-treatment at 90°C for 20 min in 50 µl of lysis reagent (Roche Diagnostics, Tokyo). Blood samples were obtained from each subject and DNA was extracted from whole blood cells from 100 µl of peripheral blood by means of the same method as that used for the fungal cells.

PCR was performed in a 25-µl reaction mixture containing 500pg DNA, 15 mM Tris (pH 8.0), 50 mM KCl, 1.5 mM MgCl2, 100 µM dATP, dCTP, dGTP and dTTP, and 0.25 U of Taq Gold DNA polymerase (Applied Biosystems, Foster City, CA) using the primer sets listed in Table **1**. The fungi-specific PCR profile was as follows: 15 min at 95°C, 40 cycles of 30 sec at 94°C, 30 sec at 55°C, 60 sec at 72°C and finally 7 min at 72°C. The species-specific PCR (nested PCR) were performed using these PCR products as a template and species-specific PCR primers. These PCR products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining and fluorescence transillumination.

Types of Nested PCR Systems

The fungi-specific PCR was designed to detect various pathogenic fungi and used the primer pair Fung-F and B4R, which amplifies the 18SrRNA region [11]. Subsequently to fungi-specific PCR, four species-specific PCR (nested PCR) methods were also designed to identify pathogenic fungi and their sensitivity to anti-fungal drugs using the fungi-specific PCR amplification products: 1) ASP/PEN: for detection of fluconazole (FLCZ)-resistant *Aspergillus*/*Penicillium* species, 2) C. glab.: for the detection of FLCZ-moderately resistant *C. glabrata*, 3) CAN: for the detection of FLCZ-sensitive *C. albicans, Candida parapsilosis*, *Candida tropicalis* and *Candida guilliermondii*, and 4) Broad spectrum: for the detection of broad spectrum fungi. This system was similar to the fungi-specific PCR. These systems are illustrated in Fig. (**1**) and were used either individually or in combination for the detection of fungi.

Other Tests

C-reactive protein (CRP), β-D-glucan and Aspergillus antigen were measured using the N-assay TIA CRP-S kit (Nitto Boseki, Fukushima, Japan), the β-glucan test (Wako Pure Chemical Industries, Osaka, Japan) and the Platelia Aspergillus kit (Bio-Rad, Paris, France), respectively. Blood culture for fungus was carried out in the BacT/Alert system (Biomērieux, Durham, USA)

Statistical Analysis

A statistical analysis was performed using Fisher's exact probability test. A P value less than 0.05 was considered to indicate the presence of a statistically significant difference. The sensitivities and specificities for poor outcome (death within 24 weeks) were calculated and shown in Table **5**.

RESULTS

Table **2** shows the 26 fungus reference strain stocks analyzed by the species-specific PCR systems. The results indicate that these nested-PCR systems detect pathogenic fungi *Aspergillus* sp., *Penicillium* sp., *Candida* sp. (except *C. krusei*), *C. glabrata*, and most other fungi. This PCR system could detect 1 X 10^2 CFU/ml of fungal infections, but the more commonly used blood culture system could not detect less than 1 X 10^3 CFU/ml of fungal infections, thus indicating that the sensitivity of this PCR system for detecting fungal infections was 10-fold higher than blood culture system.

Table 1. Oligonucleotide Primers Used for PCR Amplification

PCR Name	Forward Primer		Reverse Primer		Product(bp)
Fungi-specific PCR	Fung-F	: 5'-TTCGATGGTAGGATAGTGGCC-3'	B4R	: 5'-TGATCGTCTTCGATCCCCTA-3'	683
Speices-specific PCR					
ASP/PEN	Fung-F	: 5'-TTCGATGGTAGGATAGTGGCC-3'	n-Asp/Pen-R	: 5'-AGCCAGTGAAGGCCATG-3'	410
C.glab.	Fung-F	: 5'-TTCGATGGTAGGATAGTGGCC-3'	n-C.glab.-R	: 5'-CCAAGCCAGAAGGACTTGG-3'	405
CAN	n-Candida-F	: 5'-TTTGATGCGTACTGGACCCA-3'	B4R	: 5'-TGATCGTCTTCGATCCCCTA-3'	337
Broad spectrum	n-Fung-F	: 5'-GAATAAGGGTTCGATTCCGG-3'	n-Fung-R	: 5'-CCCCGACCGTCCCTATTAAT-3'	410

Fourteen of the 31 liver transplant patients were positive by fungi-specific PCR, 22 were positive by species-specific PCR (ASP/PEN, 4; CAN, 14; C. glab., 6; ASP/PEN +CAN, 2; CAN + C. glab., 1; Broad spectrum, 22), 26 were positive by the β-D-glucan assay, and none was positive by culture methods (Tables **3** and **4**). Five of the patients with hematological diseases were positive by fungi-specific PCR, 6 were positive by species-specific PCR (ASP/PEN, 3; Can, 3), and 6 were positive by the β-D-glucan assay (Table **4**). Two patients in the general ward were positive by fungi-specific PCR, 3 by species-specific PCR (CAN, 3) and 2 by the β-D-glucan assay (Table **4**).

One week after liver transplantation, 18 patients were positive by β-D-glucan, in comparison to 12 patients that were positive by species-specific PCR and 7 patients that were positive by fungi-specific PCR (Fig. **2**). However, starting two weeks after surgery, the proportion of patients with fungal infections detected by species-specific PCR was higher than those detected by β-D-glucan. At 12 weeks post surgery, none of the patients was positive by fungi-specific PCR, species-specific PCR, or β-D-glucan (Fig. **2**).

Febrile patients were considered positive for fungal infection when C-reactive protein (CRP) was > 5 mg/dl. The sensitivity (85.7%) of β-D-glucan for fungal infection (CRP >5 mg/dl) was high but the specificity (10.0%) was low (odds ratio [OR]: 0.667, p<0.629). Both the sensitivity and specificity of fungi-specific PCR for detecting infection were high (81.8% and 84.6%, respectively). The sensitivity and specificity of the species-specific PCR for detecting infection were high (90.0% and 50.0%, respectively, OR: 9.0, p=0.050). Only the fungi-specific PCR had a significant association with infection (OR, 24.75, p=0.002). The

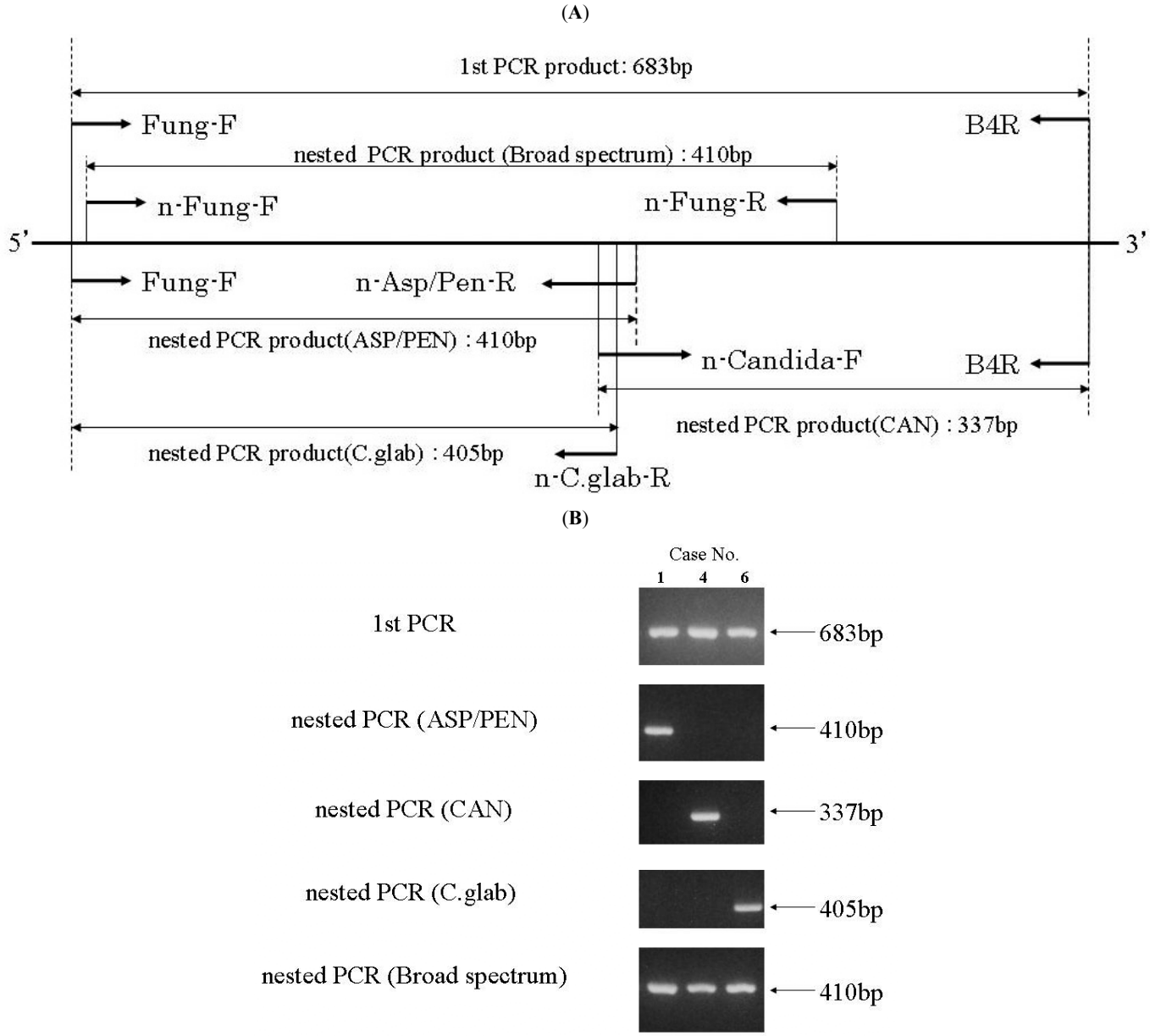

Fig. (1). Primer target areas in fungus 18SrRNA and PCR specificity. (**A**) PCR systems to detect various fungal infections. (**B**) Representative PCR results for reference strains. (a: *Aspergillus fumigatus*, b: *Candida albicans*, c: *Candida glabrata*).

Table 2. Detection of Various Fungi by Species-Specific PCR Systems

JCM No.	Fungus	Fungi- Specific PCR	Species-Specific PCR			
			ASP/PEN	CAN	C. Glab.	Broad Spectrum
1542	*Candida albicans*	+	-	+	-	+
2070	*Candida albicans*	+	-	+	-	+
1622	*Candida tropicalis*	+	-	+	-	+
1541	*Candida tropicalis*	+	-	+	-	+
1618	*Candida parapsilosis*	+	-	+	-	+
1784	*Candida parapsilosis*	+	-	+	-	+
3699	*Candida guiliermondii*	+	-	+	-	+
3761	*Candida guiliermondii*	+	-	+	-	+
1539	*Candida glabrata*	+	-	-	+	+
1606	*Candida glabrata*	+	-	-	+	+
1509	*Candida krusei*	+	-	-	-	+
1608	*Candida krusei*	+	-	-	-	+
2061	*Aspergillus flavus*	+	+	-	-	+
10252	*Aspergillus flavus*	+	+	-	-	+
5546	*Aspergillus niger*	+	+	-	-	+
5547	*Aspergillus niger*	+	+	-	-	+
1617	*Aspergillus fumigatus*	+	+	-	-	+
1618	*Aspergillus fumigatus*	+	+	-	-	+
2598	*Aspergillus terreus*	+	+	-	-	+
10257	*Aspergillus terreus*	+	+	-	-	+
3685	*Cryptococcus neoformans*	+	-	-	-	+
3687	*Cryptococcus neoformans*	+	-	-	-	+
1462	*Trichoaporon cutaneum*	+	-	-	-	+
2936	*Trichoaporon cutaneum*	+	-	-	-	+
1809	*Trichoaporon asahii*	+	-	-	-	+
1810	*Trichoaporon asahii*	+	-	-	-	+

mortality rate was lowest in liver transplant patients, followed by febrile patients in the general ward and febrile patients with hematological diseases (Table **4**). The relationship between the clinical outcome and the PCR results for fungus detection (Table **5**) showed that the PCR sensitivity for a poor outcome (death within 24 weeks) was low, but the PCR specificity for a poor outcome was markedly high in liver transplant patients (Odds ratio > 20.0, $p < 0.01$). The PCR specificity for poor outcome was low in patients in the hematological and general wards. The clinical outcome of liver transplant patients correlated significantly with the species-specific PCR results, but the outcome of patients in the general ward did not.

DISCUSSION

A markedly high frequency (71.0%) of fungal infections was detected in liver transplant patients by species-specific PCR. Previous studies using other methods [12-14] reported a much lower frequency ranging from 2 to 50%. These results indicate that this PCR system has high sensitivity and specificity for fungal infections and the assay results are available within a relatively short time period. A high frequency of fungal infections was also observed in patients on the hematological ward. These patients had received high-dose chemotherapy and had severe neutropenia, thus rendering them susceptible to fungal infections. Liver transplant patients and patients with hematological malignancies would benefit from the early detection of fungal infections provided by these PCR assays.

The β-D-glucan results in liver transplant patients had a high rate of positives, especially one week after transplantation. However, Fisher's exact probability test showed no relationship between β-D-glucan and infection. These results suggest that the surgical procedure itself could cause false positives in liver transplant patients. In comparison, the PCR results correlated significantly with fungal infections, thus suggesting that these PCR assays are useful to diagnose and manage fungal infections.

Table 3. PCR Results for Liver Transplant Patients

Sex & Age	Disease	Outcome	Results of PCR						Asper-Ag	Culture
			Fungi-Specific PCR	Species-Specific PCR				Presumed Fungal Group		
				ASP/PEN	CAN	C .glab.	Broad Spectrum			
M50	FH	deceased	+	+	-	-	+	A/P	-	-
M54	LC	deceased	+	+	-	-	+	A/P	+	-
F50	LC	alive	+	-	-	+	+	*C. glabrata*	-	-
M48	LC	alive	+	-	+	-	+	*Candida*	-	-
F46	LC	deceased	-	-	-	-	-	Negative	-	-
M54	HCC	alive	-	-	-	+	+	*C. glabrata*	-	-
M52	LC	alive	-	-	-	-	-	Negative	-	-
M58	LC	deceased	-	-	-	-	-	Negative	-	-
M54	HCC	alive	-	-	-	-	-	Negative	-	-
M58	HCC, DM	alive	+	+	+	-	+	*A/P, Candida*	-	-
M44	LC	alive	+	-	+	-	+	*Candida*	-	-
M54	HCC, DM	alive	-	-	-	+	+	*C. glabrata*	-	-
F65	LC	alive	-	-	-	-	-	Negative	-	-
M61	HCC	alive	+	-	+	-	+	*CAN*	-	-
M50	LC	alive	-	-	+	-	+	*CAN*	-	-
F63	LC, DM	deceased	-	-	+	-	+	*CAN*	-	-
F67	HCC	alive	+	+	+	-	+	A/P, *Candida*	-	-
F66	HCC	alive	+	-	-	+	+	*C. glabrata*	-	-
F44	LC	alive	+	-	+	-	+	*Candida*	-	-
M25	CBDA	alive	+	-	+	-	+	*Candida*	-	-
M56	LC, DM	alive	-	-	-	-	-	Negative	-	-
F70	HCC	alive	-	-	+	-	+	*Candida*	-	-
F65	LC	alive	+	-	+	-	+	*Candida*	-	-
M61	LC	alive	-	-	-	-	-	Negative	-	-
F45	LC	alive	+	-	-	+	+	*C. glabrata*	-	-
M38	HCC	alive	-	-	-	-	-	Negative	-	-
M60	LC	alive	+	-	+	-	+	*Candida*	-	-
M58	HCC, DM	deceased	-	-	-	-	-	Negative	-	-
M42	LC, DM	alive	-	-	+	-	+	*Candida*	-	-
F55	HCC	alive	-	-	+	-	+	*Candida*	-	-
M56	HCC	alive	-	-	-	+	+	*C. glabrata*	-	-

FH: fulminant hepatitis, HCC: hepatocellular carcinoma, DM: diabetes mellitus, LC: liver cirrhosis, CBDA: congenital bile duct atresia, A/P: Aspergillus/Penicillium species.

Swoboda-Kopec *et al.* [15] examined liver transplant patients and detected various fungal infections, including *C. albicans* (54.5%), *C. glabrata* (19.1%), *Candida krusei* (5.6%), and other *Candida* species (6.2%). The proportion of patients with ASP/PEN (9.1%) and C. glab. (27.3%) in the current study were slightly higher than in previous reports, thus suggesting a possible increase in fluconazole-resistant fungi. Amphotericin B is the standard treatment for reducing invasive fungal infections in patients with neutropenia and persistent fever [16, 17]. However, amphotericin B is associated with significant dose-limiting nephrotoxicity and infusion-related reactions. Fluconazole has relatively low side effects but has limited use because of a narrow antifungal spectrum restricted to yeasts [18, 19]. Although there are new anti-fungal agents, such as micafungin [20] and voriconazole [21, 22], these treatment options need to be further examined. The prevalence of ASP/PEN appears to be higher in hematological patients than in liver transplant

Table 4. Fungal Infections in Patients Undergoing Liver Transplantations, Hematological Diseases and Other Patients in the General Wards, and the Relationship Between the Clinical Outcome and the Fungus PCR Status

	Age	Sex F:M	β-D-Glucan	Positive Fungi-Specific PCR	Positive Species-Specific PCR				Death (Mortality)	Fungi-Specific PCR for Death		Species-Specific PCR for Death	
					ASP/PEN	CAN	C. glab.	Broad Spectrum		Sensitivity (Specificity)	Odds Ratio (Fisher's P Value)	Sensitivity (Specificity)	Odds Ratio (Fisher's P Value)
L T	54 (25-70)	11:20	26 (83.9%)	14 (45.2%)	2 (6.5%)	13 (41.9%)	6 (19.4%)	22 (71.0%)	6 (19.4%)	55.6% (95.5%)	26.25 (0.004)	50.0% (95.5%)	20.00 (0.007)
H W	57 (50-76)	5:6	6 (54.5%)	5 (45.5%)	3 (27.3%)	3 (27.3%)	0 (0%)	6 (54.5%)	6 (54.5%)	80.0% (66.7%)	8.0 (0.175)	66.7% (60.0%)	3.0 (0.392)
G W	73 (28-87)	5:10	2 (13.3%)	2 (13.3%)	0 (0%)	3 (20.0%)	0 (0%)	3 (20.0%)	7 (46.7%)	100% (61.5%)	- (0.200)	66.6% (41.7%)	2.8 (0.446)

Fungi-specific PCR: positive 1 week after liver transplantation. Species-specific PCR: positive for more than one PCR assay 2 weeks post-liver transplantation. Outcome: death within 24 weeks of liver transplantation. LT: liver transplantation, HW: hematological ward, GW: general wards.

patients. It should be noted that 4 liver transplant patients with fungal infections could not be identified out of the 22 patients who were species-specific PCR-positive. Therefore, this PCR system cannot diagnose fungal infections with 100% accuracy.

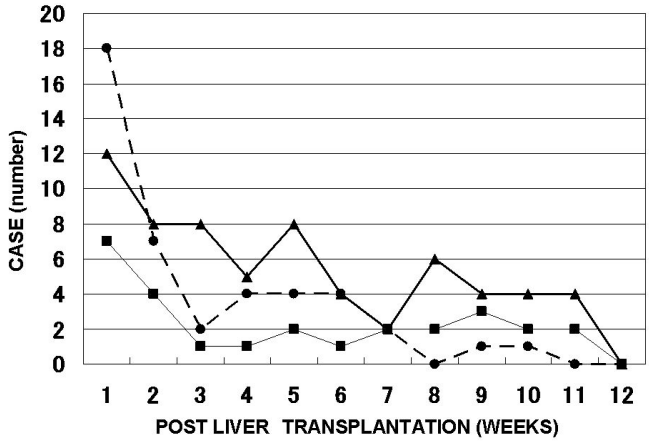

Fig. (2). Number of liver transplant patients who tested positive by the Fungi-specific PCR (squares), Species-specific PCR (triangles) and β-D-glucan (circles) during the 12-week post-operative period.

The current data show that the clinical outcome correlated significantly with the detection of fungal infection by PCR assays in liver transplant patients, but not with patients in the general ward. Fungal infections were frequently observed in liver transplant patients and the detection of fungal infection by species-specific PCR correlated with the outcome. Therefore, the semi-diagnostic PCR system may potentially be useful for detecting fungal infections and to confirm the diagnosis of fungal infections in liver transplantation.

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REFERENCES

[1] Poordad FF. Liver transplant and recurrent disease. Clin Liver Dis 2004; 8: 461-73.

[2] Cahill BA, Braccia D. Current treatment for hepatocellular carcinoma. Clin J Oncol Nurs 2004; 8: 393-9.

[3] Paya CV. Fungal infections in solid-organ transplantation. Clin Infect Dis 1993; 16: 677-88.

[4] Snydman DR. Epidemiology of infections after solid-organ transplantation. Clin Infect Dis 2001; 33: S5-8.

[5] Ohno N, Emori Y, Yadomae T, Saito K, Masuda A, Oikawa S. Reactivity of limulus amoebocyte lysate towards (1→3)β-D-glucans. Carbohydr Res 1990; 207: 311-8.

[6] Buchman TG, Rossier M, Merz WG, Charache P. Detection of surgical pathogens by *in vitro* DNA amplification. Part I: rapid

identification of Candida albicans by *in vitro* amplification of a fungus-specific gene. Surgery 1990; 108: 338-46.

[7] Shin JH, Nolte FS, Morrison CJ. Rapid identification of Candida species in blood cultures by a clinically useful PCR method. J Clin Microbiol 1997; 35: 1454-9.

[8] Shin JH, Nolte FS, Holloway BP, Morrison CJ. Rapid identification of up to three Candida species in a single reaction tube by a 5' exonuclease assay using fluorescent DNA probes. Clin Microbiol 1999; 37: 165-70.

[9] Maaroufi Y, Heymans C, De Bruyne JM, *et al.* Rapid detection of Candida albicans in clinical blood samples by using a TaqMan-based PCR assay. J Clin Microbiol 2003; 41: 3293-8.

[10] de Aguirre L, Hurst SF, Choi JS, Shin JH, Hinrikson HP, Morrison CJ. Rapid differentiation of Aspergillus species from other medically important opportunistic molds and yeasts by PCR-enzyme immunoassay. J Clin Microbiol 2004; 42: 3495-504.

[11] Makimura K, Murayama S, Yamaguchi H. Detection of wide range of medically important fungi by the polymerase chain reaction. J Clin Microbiol 1994; 40: 358-64.

[12] Echaniz-Quintana A, Pita-Fernandez S, Otero-Ferreiro A, Suarez-Lopez F, Gomez-Gutierrez M, Guerrero-Espejo A. Risk factors associated with invasive fungal infection in orthotopic liver transplantation. Med Clin (Barc) 2004; 122: 444-8.

[13] Said A, Safdar N, Lucey MR, *et al.* Infected bilomas in liver transplant recipients, incidence, risk factors and implications for prevention. Am J Transplant 2004; 4: 574-82.

[14] Hagerty JA, Ortiz J, Reich D, Manzarbeitia C. Fungal infections in solid organ transplant patients. Surg Infect (Larchmt) 2003; 4: 263-71.

[15] Swoboda-Kopec E, Kawecki D, Wroblewska M, Krawczyk M, Luczak M. Epidemiology and susceptibility to antifungal agents of fungi isolated from clinical specimens from patients hospitalized in the Department of General and Liver Surgery of the Medical University of Warsaw. Transplant Proc 2003; 35: 2298-303.

[16] Hughes WT, Armstrong D, Bodey GP, *et al.* 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. Clin Infect Dis 1997; 25: 551-73.

[17] Karp JE, Merz WG, Charache P. Response to empiric amphotericin B during antileukemic therapy-induced granulocytopenia. Rev Infect Dis 1991; 13: 592-9.

[18] Viscoli C, Castagnola E, Van Lint MT, *et al.* Fluconazole *vs* amphotericin B as empirical antifungal therapy of unexplained fever in granulocytopenic cancer patients: a pragmatic, multicentre, prospective and randomised clinical trial. Eur J Cancer 1996; 32: 814-20.

[19] Winston DJ, Hathorn JW, Schuster MG, Schiller GJ, Territo MC. A multicenter, randomized trial of fluconazole *vs* amphotericin B for empiric antifungal therapy of febrile neutropenic patients with cancer. Am J Med 2000; 108: 282-9.

[20] Tawara S, Ikeda F, Maki K, *et al. In vitro* activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi. Antimicrob Agents Chemother 2000; 44: 57-62.

[21] Ostrosky-Zeichner L, Oude Lashof AM, Kullberg BJ, Rex JH. Voriconazole salvage treatment of invasive candidiasis. Eur J Clin Microbiol Infect Dis 2003; 22: 651-5.

[22] Walsh TJ, Pappas P, Winston DJ, *et al.* National Institute of Allergy and Infectious Diseases Mycoses Study Group. National Institute of Allergy and Infectious Diseases Mycoses Study Group: voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. N Engl J Med 2002; 346: 225-34.

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