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Efficacy of Galactomannan Antigen in the Platelia *Aspergillus* Enzyme Immunoassay for Diagnosis of Invasive Aspergillosis in Liver Transplant Recipients

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The utility of galactomannan antigen for diagnosing invasive aspergillosis was evaluated in 154 liver transplant recipients. Sample agreement was 98.5%, and patient specificity was 87%. Galactomannan positivity correlated with mortality, even when controlled for the number of tests performed. Whether galactomannan positivity identifies a subgroup at risk for poor outcome warrants further evaluation.

The utility of the Platelia *Aspergillus* enzyme immunoassay (EIA) for the diagnosis of invasive aspergillosis (IA) was prospectively evaluated in liver transplant recipients. The study population comprised 154 patients undergoing liver transplantation between September 2001 and October 2002. Institutional review board approval was obtained, and all patients provided written informed consent. Tacrolimus (with or without mycophenolate mofetil) and low-dose prednisone were employed for immunosuppression. Induction with thymoglobulin followed by tacrolimus was employed in 65 patients (7). Routine antifungal prophylaxis was not used.

IA was diagnosed as per the EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study Group) criteria (1) by investigators who were blinded to test results. Blood samples were collected twice weekly during the posttransplant and subsequent hospitalizations. Galactomannan was detected by one-stage immunoenzymatic sandwich microplate assay (Platelia *Aspergillus* EIA; Bio-Rad Laboratories, Marnes, France) as per the manufacturer's instructions. Samples with an index of 0.5 or greater were retested and considered positive only if the repeat test was also positive. Sensitivity and specificity were calculated in reference to the diagnosis of IA by using the total number of patients in the study.

The clinical characteristics of the patients are outlined in Table 1. IA developed in 1 of 154 (0.6%) of the patients and comprised a patient with probable pulmonary aspergillosis due to *Aspergillus fumigatus* 21 days posttransplantation. A total of 1,594 sera were analyzed, including 31 sera from the patient with probable IA and 1,563 sera from 153 patients without IA. The patient with probable IA had a positive result in three samples on initial, but not on repeat testing. Sample agreement was 98.5% (1,540 of 1,563 samples) in the patients without IA.

Twenty patients without IA had 23 false-positive tests that occurred in a median of 16 days posttransplantation (1 to 174 days). Patients undergoing transplantation for autoimmune liver disease were more likely to have false-positive tests than all others (Table 2). Patients with false-positive tests were more likely to have required dialysis than those with true-negative tests ($P = 0.0015$). Patients with positive tests had longer intensive care unit (ICU) stay; however, the duration of hospitalization and the number of tests performed per patient did not differ significantly for patients with positive tests versus those with negative tests (Table 2). Of 20 patients with false-positive tests, 7 (35%) were receiving piperacillin-tazobactam.

Mortality was 14.9% (23 of 154 patients). Of 23 patients who died, 4 had autopsies performed (none had IA). When controlled for the number of tests performed (positive tests/100 tests performed), the number of positive tests was higher in the patients who died than in those who lived (2.91 versus 1.05; $P = 0.032$).

Dialysis, cytomegalovirus (CMV) disease, intubation, longer length of ICU stay, mold-active antifungal agent use, and false-positive tests were significantly associated with death (Table 3). In a logistic regression model, however, only dialysis (odds ratio [OR], 12.5; 95% confidence interval [CI], 3.70 to 42.52; $P = 0.0001$) and the length of stay (OR, 1.03; 95% CI, 1.004 to 1.05; $P = 0.023$), but not test positivity (OR, 2.41; 95% CI, 0.060 to 9.70; $P = 0.215$) or antifungal agent use (OR, 1.75; 95% CI, 0.46 to 6.58; $P = 0.408$) were significantly associated with mortality. Of 20 patients with false-positive tests, 8 died (none had an autopsy performed). The latter had a longer ICU stay (51.9 versus 11.3 days; $P = 0.0005$) and were more likely to have required dialysis (6 of 8 versus 2 of 12; $P = 0.019$) than those who lived.

In all, 26 of 154 patients received a mold-active antifungal agent (Table 4). In patients who received a mold-active antifungal agent, the false-positive test was documented in 40% (4 of 10) in those who died and in 5.9% (1 of 16) of the patients who lived ($P = 0.055$). The false-positive test preceded death by a median of 3 weeks. Among 128 patients who did not receive a mold-active antifungal agent, galactomannan positivity was observed in 30.8% (4 of 13) of the patients who died compared to 9.6% (11 of 115) of those who lived ($P = 0.047$).

The galactomannan test demonstrated a patient specific-

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TABLE 1. Demographic and clinical characteristics of the patients in this study

Characteristic	Result
No. of patients	154
Mean age in yr (range)	52.4(24–72)
% Male	65.1
% Female	34.9
% with underlying liver disease:	
Hepatitis C virus	32.5(50/154)[a]
Alcohol	16.9(26/154)
Hepatitis C virus and alcohol	8.4(13/154)
Autoimmune liver disease	7.1(11/154)
Primary sclerosing cholangitis	7.1(11/154)
Hepatitis B virus	5.8(9/154)
Hepatitis B and C viruses	1.3(2/154)
Cryptogenic cirrhosis	3.2(5/154)
Primary biliary cirrhosis	3.2(5/154)
Polycystic disease	1.3(2/154)
Hepatocellular carcinoma	1.3(2/154)
Other	11.7(18/154)
% with human immunodeficiency virus coinfection	3.2(5/154)
% with retransplantation	5.2(8/154)
% with immunosuppression[b]	
Tacrolimus	75.3(116/154)
Cyclosporin A	5.2(8/154)
Mycophenolate mofetil	5.2(8/154)
Sirolimus	11.4(17/154)
Thymoglobulin	42.0(65/154)
% on dialysis	14.3(22/154)
% with rejection	18.8(29/154)
Length of ICU stay (median no. of days)	7

[a] No. with result/total no. in the study.
[b] Some patients received more than one agent.

ity of 87%. However, a low incidence of IA precluded meaningful assessment of the sensitivity of the test. Several observations concerning the galactomannan test in liver transplant recipients are nevertheless important. Patients with autoimmune liver disease were more likely to have false-positive tests. These patients comprise a unique group of transplant recipients who are deemed to be at higher risk for immune-mediated complications: e.g., chronic rejection (4). A false-positive test, due likely to autoreactive antibodies or paraproteins, has been reported in other conditions associated with autoimmune phenomenon: e.g., chronic graft-versus-host disease (3).

Patients requiring dialysis were more likely to have a false-positive test than all others. Rapid renal excretion and uptake by the macrophage mannose receptors are the major pathways by which the galactomannan is cleared from the bloodstream (2). In an animal model, excretion into the urine accounted for 35% of the dose by 24 h (2). Whether renal failure or dialysis affects the clearance of galactomannan is not known.

The patients who died had a higher number of positive tests, even when controlled for the number of tests performed. A false-positive test, however, was not an independently significant predictor of poor outcome. Nevertheless, the possibility that the test was in fact truly positive in these patients cannot be totally discounted. Liver transplant recipients with risk factors that confer a higher risk for aspergillosis (e.g., dialysis) (5, 6) were the very patients who had false-positive tests. Thus, IA may have been present but never documented due to the receipt of a mold-active antifungal agent as empirical therapy or treatment in many of these patients.

In summary, although the sensitivity of the Platelia *Aspergillus* EIA for the detection of IA could not be meaningfully assessed due to the fact that there was only a single case of IA, the test exhibited an excellent sample agreement of 98.5%. Correlation of galactomannan positivity with mortality in this patient population warrants validation in further studies.

TABLE 2. Clinical variables in patients with positive and negative galactomannan test

Variable	% of patients with test[a]		*P* value
	Positive (n = 20)	Negative (n = 134)	
Underlying liver disease			
Hepatitis C virus (any)	40 (8/20)	43.3 (58/134)	NS[b]
Alcohol (any)	15 (3/20)	27.6 (37/134)	NS
Autoimmune	20 (4/20)	5.2 (7/134)	0.030
Immunosuppressive agent			
Tacrolimus	80 (16/20)	74.6 (100/134)	NS
Cyclosporin A	5 (1/20)	5.2 (7/134)	NS
Mycophenolate mofetil	0 (0/20)	6.0 (8/134)	NS
Sirolimus	15 (3/20)	10.4 (14/134)	NS
Thymoglobulin	35 (7/20)	43.3 (58/134)	NS
CMV infection	50 (10/20)	42.5 (57/134)	NS
Intubation	25 (5/20)	10.4 (13/134)	NS
Rejection	10 (2/20)	20.1 (27/134)	NS
Dialysis	40 (8/20)	9.8 (14/133)	0.002
Antifungal agent use	25 (5/20)	15.7 (21/134)	NS

[a] The median length of ICU stay for patients with a positive test is 16 days, and that for patients with a negative test is 6 days (P = 0.002). The median length of the hospital stay is 26 days for patients with a positive test and 18 days for those with a negative test (not significant).
[b] NS, not significant ($P > 0.05$).

TABLE 3. Factors associated with mortality in the study patients

Factor	Result for patients who:		Significance level
	Died (n = 23)	Lived (n = 131)	
Mean age (yr)	53.5	52.3	NS[b]
% Female	52.2 (12/23)[a]	31.3 (41/131)	NS
% with underlying liver disease:			NS
Hepatitis C virus	39.1 (9/23)	32.1 (42/131)	
Alcohol	17.4 (4/23)	16.8 (22/131)	
Hepatitis C + alcohol	8.7 (2/23)	9.2 (12/131)	
Autoimmune	17.4 (4/23)	5.3 (7/131)	
Hepatitis B virus	0 (0/23)	6.9 (9/131)	
Hepatitis B + C virus	4.3 (1/23)	0.8 (1/131)	
Primary biliary cirrhosis	4.3 (1/23)	3.1 (4/131)	
Primary sclerosing cholangitis	0 (0/23)	8.4 (11/131)	
Cryptogenic	0 (0/23)	3.8 (5/131)	
Other	8.7 (2/23)	13.7 (18/131)	
% with CMV serostatus:			NS
Recipient$^+$/donor$^+$	39.1 (9/23)	40.5 (53/131)	
Recipient$^-$/donor$^+$	30.4 (7/23)	17.3 (23/131)	
Recipient$^+$/donor$^-$	8.7 (2/23)	26.0 (34/131)	
Recipient$^-$/donor$^-$	21.7 (5/23)	16.0 (21/131)	
% with CMV infection	52.2 (12/23)	42 (55/131)	NS
% with CMV disease	13.0 (3/23)	0.8 (1/131)	0.011
% with retransplantation	0 (0/23)	6.1 (8/131)	NS
% with immunosuppression			
Tacrolimus	82.6 (19/23)	74.1 (96/131)	NS
Cyclosporin A	8.7 (2/23)	4.6 (6/131)	NS
Mycophenolate mofetil	0 (0/23)	6.1 (8/131)	NS
Sirolimus	13.0 (3/23)	10.7 (14/131)	NS
Thymoglobulin	52.2 (12/23)	40.5 (53/131)	NS
% with rejection	13.0 (3/23)	19.9 (26/131)	NS
% on dialysis	60.9 (14/23)	6.1 (8/131)	0.0001
Median length of ICU stay (days)	37.5	11.5	0.0001
% human immunodeficiency virus coinfected	0 (0/23)	3.8 (5/131)	NS
% with receipt of an antifungal agent	43.5 (10/23)	12.2 (16/131)	0.0009
% with false-positive test	34.8 (8/23)	9.2 (12/130)	0.003
No. of tests performed	14	7	0.006
No. of positive tests/100 tests	2.91	1.05	0.032

[a] No. with result/no. in the group.
[b] NS, not significant ($P > 0.05$).

TABLE 4. Mold-active antifungal agent use in patients with false-positive and true-negative galactomannan test

Indication	Antifungal agent (no. of patients)[a]
Patients with true-negative tests	
Prophylaxis	Abelcet (7)
Empiric therapy	AmB and then Abelcet (2), Abelcet (1)
Established infections	
Granulomas in the donor liver	AmB (1)
Candida peritonitis	Abelcet (3)
Torulopsis glabrata fungemia	Abelcet (1)
Patients with false-positive tests	
Empiric therapy	Abelcet (2), AmB (1)
Established infection	
Disseminated histoplasmosis	Abelcet (1)
Candida peritonitis	Abelcet (1)

[a] Abelcet, amphotericin B lipid complex; AmB, amphotericin B deoxycholate.

Whether a false-positive galactomannan test is a surrogate for greater severity of illness or an overall debilitated state of the patient or represents true infection that defied clinical detection remains to be determined.

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