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Selection of *Candida glabrata* Strains with Reduced Susceptibility to Azoles in Four Liver Transplant Patients with Invasive Candidiasis

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The cases of four liver transplant recipients who developed invasive candidiasis (2 cholangitis, 1 perihepatic abscess, 1 candidemia) due to azole-resistant *Candida glabrata* are reported. Three patients were receiving azolic compounds (2 itraconazole, 1 fluconazole) when the infection was diagnosed.. All four patients received fluconazole as intestinal decontamination during the first three weeks post transplantation. The infections occurred two months after transplantation in all patients, and in one patient *Candida* infection was the direct cause of death. Infection of the biliary tree was the origin of candidiasis in three patients; the fourth patient developed neutropenic-related candidemia. Fluconazole MICs exceeded 16 μg/ml in all cases; itraconazole MICs were 16, 2, 1, and 2 μg/ml, respectively. The potential role of *Candida* species other than *albicans* in these patients after administration of azole agents is discussed.

Studies of neutropenic patients have repeatedly shown an overall decrease in the frequency of fungal infections in patients treated prophylactically with azole compounds. This decrease has been documented primarily with infections caused by *Candida albicans* and *Candida tropicalis*; an increase has been reported, however, in infections due to *Candida krusei*, *Candida glabrata*, and *Candida lambica*, which are less susceptible to azoles (1–8).

Prophylaxis with azoles has been used in solid organ transplant recipients, although its benefits in these patients are less clear than in bone marrow transplant recipients or in neutropenic patients. Usually, prophylaxis with azoles is administered to patients undergoing hepatic or pancreatic transplantation, in whom infections due to *Candida* spp. account for 30% of severe infections (9).

Initial studies employing ketoconazole as antifungal prophylaxis in neutropenic patients have documented the emergence of resistant *Candida glabrata* strains following administration of the drug (10). Fluconazole is now part of many antifungal prophylaxis protocols in liver transplant recipients. To date, experience with itraconazole is still sparse, and the potential of this drug to select resistant *Candida* spp. strains is still unknown. Although the efficacy of itraconazole can be affected by fluconazole cross-resistance, the MICs of itraconazole are clearly lower than those of fluconazole; itraconazole can even be used successfully for treatment of some, but not all, patients infect-

Table 1: Clinical parameters and evolution of *Candida glabrata* infection in four liver transplant recipients.

	Patient 1	Patient 2	Patient 3	Patient 4
Age/sex	63/M	55/M	57/M	59/M
Liver disease	hepatitis C	hepatitis C	hepatitis C	hepatitis C
Acute rejection	no	yes	no	yes
Retransplantation	yes	no	no	no
Duration of fluconazole therapy	7 days	20 days	18 days	18 + 12 days
Infectious complications	pulmonary aspergillosis	PCP	none	peribiliary fluid
Antifungal treatment	liposomal AmB, 1600 mg			fluconzale 12 days
Itraconazole dosage	400 mg/day	200 mg/day	none	none
Duration of itraconazole therapy	40 days	15 days		
Days after transplantation that *C. glabrata* was isolated	60	88	74	63
Clinical characteristics of *C. glabrata* infection	cholangitis, choledochal fistula, candidemia	candidemia	cholangitis, choledochal fistula	peribiliary abscess, choledochal fistula
Source of *C. glabrata*	bile, blood	blood, urine	bile	peribiliary abscess
Treatment for *C. glabrata* infection	AmB, biliodigestive anastomosis	AmB	AmB, biliodigestive anastomosis	AmB, surgical drainage
Outcome	invasive cholangitis, liver abscesses, death	cure	cure	cure

* AmB, amphotericin B; PCP, *Pneumocystis carinii* pneumonia.

ed with fluconazole-resistant *Candida* strains, particularly AIDS patients infected with *Candida albicans* (11, 12). The experience with itraconazole in *Candida glabrata* infections is limited, although some authors have reported promising results in patients with persistent vulvo-vaginal candidiasis (13).

Four cases of invasive azole-resistant *Candida glabrata* infection in 101 successive liver transplant patients are reported here. In all four cases, antifungal prophylaxis with fluconazole was administered in the post-transplantation period. In three cases, azole agents (itraconazole in 2 patients, fluconazole in 1 patient) were being administered when the infections were diagnosed. In one patient, death was directly attributed to fungal infection.

Case Reports. (Table 1) Patient 1 underwent a repeat liver transplant because of acute dysfunction of the initial graft. He developed pulmonary aspergillosis on day 7, which was treated initially with liposomal amphotericin B (cumulative dose 1650 mg) with itraconazole (400 mg/day). He was readmitted to the hospital after 40 days of therapy with itraconazole because of fever and abdominal pain. Exploratory surgery revealed no abscesses, but the choledochus was edematous and a choledochal fistula was discovered and resected. The anastomosis was changed to a Roux-en-Y biliodigestive anastomosis. *Candida glabrata* was recovered from the bile culture and from two blood cultures drawn during surgery. Therapy with amphotericin B and 5-flucytosine was started, but an ascending destruction of the biliary system ensued, with liver infiltration and multiple abscesses. The patient died 12 days after surgery. *Candida glabrata* was recovered from the abscess material.

Patient 2 developed severe pneumonia on day 70 post transplantation. A broncho-alveolar lavage sample showed evidence of *Pneumocystis carinii* and cytomegalovirus. Cotrimoxazole and ganciclovir were started. The patient was mechanically ventilated, requiring high levels of high airway pressure. He became severely neutropenic (540 cell/mm^3), and the risk of fungal infection was deemed high enough to justify therapy with oral

Table 2: Minimum inhibitory concentrations of amphotericin B (AmB), 5-flucytosine (5FC), fluconazole (FLU), itraconazole (ITR), and ketoconazole (KET) for different strains of *Candida glabrata* isolated from four liver transplant patients.

	MIC (μg/ml)				
Patient no.	AmB	5FC	FLU	ITR	KET
1	0.03	0.03	64	16	2
2	0.5	0.06	64	2	2
3	0.5	0.06	32	1	1
4	–	–	16	2	–

itraconazole (200 mg/day). Twelve days later the patient developed fever, and *Candida glabrata* was recovered from blood and urine cultures.

Patient 3 developed a choledochal fistula on day 74 post transplantation. The fistula was surgically resected, and a Roux-en-Y biliodigestive anastomosis was created. The pathological study showed invasion of the choledochus by yeasts, and *Candida glabrata* was recovered from a bile culture.

Patient 4 developed a choledochal fistula and a peribiliary collection of fluid on day 63 post transplantation. The fistula was removed and the fluid drained during surgery to reconstruct the biliodigestive anastomosis; *Streptococcus* spp. and *Candida albicans* were cultured from the peribiliary fluid. Therapy with ampicillin and fluconazole was begun after surgery. Twelve days later the patient developed fever, and a new collection of peribiliary fluid was observed. It was drained under computed-tomographic guided needle aspiration, and *Candida glabrata* was recovered from culture. Although the fever resolved after drainage of the fluid, a course of amphotericin B was administered (Table 1). Patients 3 and 4 both recovered uneventfully.

Microbiological Methods. Susceptibility testing was performed using a microdilution method for amphotericin B, flucytosine, ketoconazole, fluconazole, and itraconazole (Table 2). Tests were based on the method proposed by the National Committee for Clinical Laboratory Standards (NCCLS) (14). RPMI 1640 medium supplemented with extra glucose to a final oncentration of 2% was used (15). Results were read spectrophotometrically at 405 nm after agitation for 24 - and 48 h at 35°C (16). The MIC endpoint was based on spectrophotometric reduction of growth below 50% of control values (17, 18).

Discussion. Although the present study did not include a control group of patients who received no therapy with azole, the recovery of *Candida glabrata* with decreased susceptibility to azoles is a relevant finding. In fact, the infections by *Candida glabrata* occurred during treatment with itraconazole (patients 1 and 2) or fluconazole (patient 4).

In our laboratory, we prefer the microdilution procedure to the macrobroth dilution method (14) because the former is less cumbersome and less expensive. The microdilution procedure correlates well with the NCCLS macrobroth dilution method (19, 20) and has shown close interlaboratory agreement with one of the media employed (RPMI-2% glucose) (15).

There are no defined standards to differentiate resistance from susceptibility in the context of antifungal therapy with azoles, but correlation between the in vitro susceptibility of *Candida* isolates to azoles and clinical response has been demonstrated in patients with AIDS (21). The MICs of fluconazole were 16–32 μg/ml and those of itraconazole > 1–2 μg/ml for the isolates recovered from our patients. These values exceed the MIC90s of these agents for *Candida glabrata*; thus, the isolates we recovered could be considered resistant (22–24).

Candida glabrata has a wide range of susceptibility to azole agents. Most strains are initially resistant and are selected in the presence of azoles, but there are susceptible strains that usually respond to azole treatment (6, 22–24). In some cases resistance to azoles developed in strains initially susceptible (25, 26). This finding could indicate an induction phenomenon, possibly occurring by mutation. This possibility has been related to the ability of *Candida glabrata* to develop punctual mutations, given the haploid nature of its genome (27). Hitchcock et al. (28), however, using restriction-fragment-length-polymorphism (RFLP) analyses of genomic DNA, observed that pre- and post-treatment isolates of *Candida glabrata* were clonally unrelated. This proves that the resistant organism was not a mutant derived from the susceptible organism; rather, it was selected from a mixed population of both organisms by imidazole treatment (28). This finding should justify the use of high doses of fluconazole to control *Candida glabrata* infections caused by moderately resistant strains.

We have observed the selection of non-*albicans Candida* strains, particularly *Candida glabrata* and *Candida inconspicua*, with reduced intrinsic susceptibility to azole agents in 23% of AIDS patients with oropharyngeal candidiasis after a low-dose course of fluconazole (21). These findings prompted us to evaluate the need for fungal surveillance cultures in solid organ transplant recipients receiving prophylaxis with azoles, e.g., in bone marrow transplant recipients. Surveillance cultures for *Candida albicans* in bone marrow transplant patients have a good negative predictive value but a poor positive predictive value. For non-*albicans Candida* spp., however, the positive and negative predictive values are better (29).

After considering these findings, we have made two modifications in our liver transplant program: (i) a 10% solution of amphotericin B (5 ml every 6 h) is administered to all patients during the period of fluconazole administration; and (ii)

routine fungal surveillance cultures are taken from all patients (mouth, rectum, and urine) during the first month of treatment.

In summary, the selection of *Candida glabrata* strains with diminished susceptibility to azoles in liver transplant patients is possible. Additional controlled studies in solid organ transplant recipients are warranted to evaluate more thoroughly the role of azoles in the selection of non-*albicans Candida* strains with reduced intrinsic susceptibility.

Acknowledgement

The authors thank Luis de Rafael for his advice and review of the manuscript.

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Determination of the Number of Blood Samples Needed for Optimal Detection of Cytomegalovirus Viremia in Immunocompromised Patients Using a Shell-Vial Assay

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To establish the number of blood samples necessary for the diagnosis of viremic episodes caused by cytomegalovirus (CMV), a prospective analysis was conducted of 238 patients (38 renal transplant recipients and 200 HIV-infected patients) who developed CMV viremia. The usefulness of samples and the volume of blood required to demonstrate the presence of viremia by CMV was also studied. The first blood sample was diagnostic for CMV viremia in 53.3% of the viremic patients; the second sample documented an additional 22.2% of cases of viremia (75.5% of infected patients); and the third sample demonstrated viremia in the remaining 24.5%. Thus, a diagnosis of CMV viremia was established in every patient (100% of episodes of viremia). In this study, the use of three 3 ml blood samples collected at 24 h intervals was sufficient to detect all episodes of CMV viremia in patients clinically suspected to have disseminated disease.

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Symptomatic cytomegalovirus (CMV) infection is one of the principal causes of morbidity and mortality in immunocompromised patients, especially transplant recipients and AIDS patients (1, 2). Because drugs with proven efficacy against CMV are available, i.e. ganciclovir and foscarnet, rapid diagnosis of CMV infection is of great importance. Of the clinical samples that may be used to diagnose disseminated CMV infection, blood (particularly leukocytes) has shown the greatest usefulness and yield (3–5). Although several diagnostic techniques are currently available, the detection of specific antigens to CMV in polymorphonuclear leukocytes (PMNLs) in peripheral blood (pp65 antigenemia) (6, 7) and the isolation of CMV in cell culture (viremia) have shown the greatest utility as markers of active infection that are also predictive of the development of invasive CMV infection (8, 9).

Because the antigenemia assay is easy to perform, it is routinely used to screen for the presence of viral antigen in PMNLs. However, several authors (5, 10) have reported that antigenemia does not always reflect the replicative viral load present in the patient's blood. This conclusion has been reached as a result of frequent discrepancies between the antigenemia values and the number of infectious foci detected in shell-vial culture (10). To avoid this problem, it is advised that the same blood sample be used for culture and for determination of the presence of antigenemia (10, 11).

The biological cycle of CMV in peripheral blood has not yet been clearly established, so it is uncertain whether viremia caused by CMV is a continuous or an intermittent process. Therefore, it is