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Association of *Torulopsis glabrata* Infections with Fluconazole Prophylaxis in Neutropenic Bone Marrow Transplant Patients

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Because the use of fluconazole prophylaxis had been associated with an increased rate of *Candida krusei* infections at The Johns Hopkins Oncology Center, early empiric amphotericin B plus flucytosine were given to febrile neutropenic patients colonized by *C. krusei*. By this practice, the proportion of fungemias attributable to *C. krusei* was low (12.5%) in patients receiving fluconazole over a 6-month interval. However, *Torulopsis* (*Candida*) *glabrata* assumed a much higher proportion of fungemias (75%) among patients receiving fluconazole. In vitro susceptibility testing combined with this clinical experience suggests that some *T. glabrata* isolates are not susceptible to fluconazole and can cause breakthrough infections in patients receiving fluconazole.

Previous studies have shown fluconazole to be an effective antifungal prophylaxis in chemotherapy-induced neutropenia (2, 8). An association of fluconazole prophylaxis with an increase in infections from *Candida krusei* has also been reported by some centers but not all (4, 7). In vitro susceptibility tests indicated that *C. krusei* isolates recovered from patients receiving and those not receiving fluconazole were resistant (7). There was also an increased rate of colonization with *Torulopsis glabrata* (32% in patients given fluconazole, but only 17% in patients receiving some other or no antifungal prophylaxis; $P = 0.002$) (7). In a multivariate regression analysis, colonization by *T. glabrata* was associated with fluconazole use, and there was also an independent trend toward increased colonization with norfloxacin use (odds ratio = 2.2, $P = 0.07$). Although there was a trend toward an increased rate of infections caused by *T. glabrata* with fluconazole use (2.4 versus 0.9%), this was not significantly different ($P = 0.28$).

With the recognition of a risk for breakthrough infections due to *C. krusei*, amphotericin B at a dose of 1 mg/kg of body weight per day plus flucytosine were given to patients with persistent fevers of unknown origin who were colonized with *C. krusei*. This report examines the effect of this practice over a 6-month interval.

MATERIALS AND METHODS

Patients and cultures. Blood and venous catheter cultures from all leukemia and bone marrow transplant patients from The Johns Hopkins Oncology Center were reviewed between 1 January and 30 June 1991. Seventy-six bone marrow transplant patients and 53 leukemia patients were at risk during this interval. The medical records of patients with *Candida* fungemias were reviewed in order to determine prior use of fluconazole.

All bone marrow transplant patients were given fluconazole; none of the leukemia patients received fluconazole. Amphotericin B and flucytosine were administered to patients empirically on the following basis: a persistent fever despite antibiotics when fever persisted 6 or more days, colonization by *C. krusei*, or a prior or suspected infection by an *aspergillus* species. Forty-seven of the 53 (89%) leukemia patients and 26 of the 76 (34%) bone marrow transplant patients received amphotericin B plus flucytosine.

Susceptibility to antifungal agents. Fungal susceptibility testing for fluconazole and miconazole was performed by a broth macrodilution tube assay (5). Susceptibility to amphotericin B was determined by an agar dilution method (1). *T. glabrata* isolates 1 to 3 were recovered from patients given fluconazole, and *T. glabrata* isolates 4 and 5 were recovered from patients not given fluconazole. Three *C. albicans* isolates were tested for comparison. All isolates were tested in one batch simultaneously. All tests were performed in duplicate.

TABLE 1. *Candida* fungemias in neutropenic cancer patients over a 6-month interval during 1991 at The Johns Hopkins Oncology Center

Candida species	No. (%) of patients receiving:		Total
	Fluconazole	No fluconazole	
T. glabrata	6 (75%)	1 (1%)	7 (41%)
C. krusei	1 (12.5%)	3 (33%)	4 (23.5%)
C. parapsilosis	1 (12.5%)	2 (22%)	3 (17.5%)
C. albicans	0	2 (22%)	2 (12%)
C. tropicalis	0	1 (11%)	1 (6%)
Total	8[a]	9[b]	17

[a] Overall, eight infections occurred in 76 (10.5%) bone marrow transplant patients receiving fluconazole.
[b] Overall, nine infections occurred in 53 (17%) leukemia patients not receiving fluconazole.

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TABLE 2. In vitro susceptibility results for blood isolates of *T. glabrata*

Species and isolate no.	Result (μg/ml) for:								
	Fluconazole				Miconazole				Amphotericin B MIC (24 h)
	MIC		MLC[a]		MIC		MLC		
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	
T. glabrata[b]									
1	5	10	>80		≤0.6	≤0.6	5	20	0.5
2	5	10	>80		≤0.6	≤0.6	5	10	1.0
3	>80		>80		≤0.6	≤0.6	5	>20	1.0
4	5	>80	>80		≤0.6	≤0.6	5	>20	1.0
5	10	10	>80		≤0.6	≤0.6	2.5	5	1.0
C. albicans									
1	≤1.25	≤1.25	>80		≤0.6	≤0.6	20	>20	0.5
2	≤1.25	≤1.25	80	>80	≤0.6	≤0.6	2.5	10	0.5
3	≤1.25	≤1.25	>80		≤0.6	≤0.6	10	>20	0.5

[a] MLC, minimal lethal concentration.
[b] Isolates 1 to 3 were recovered from patients who had received fluconazole; isolates 4 and 5 were recovered from patients not given fluconazole.

RESULTS

Between 1 January and 1 July 1991, there were 17 fungemias in The Johns Hopkins Oncology Center which occurred in 76 bone marrow transplant patients and 53 leukemia patients. Seven were from *T. glabrata*, four were from *C. krusei*, three were from *C. parapsilosis*, two were from *C. albicans*, and one was due to *C. tropicalis* (Table 1). Six of the seven *T. glabrata* fungemias were in bone marrow transplant patients who were all receiving fluconazole. The only other fungemias in bone marrow transplant patients were one due to *C. krusei* and one due to *C. parapsilosis*. The remainder of the fungemias were in non-bone marrow transplant leukemia patients (who were not receiving fluconazole). Of interest is the finding that even among patients not given fluconazole, *Candida* species other than *C. albicans* and *C. tropicalis* accounted for the majority of fungemias.

The overall rates of fungal infection were 10.5% (8 of 76) in bone marrow transplant patients receiving fluconazole and 17.0% (9 of 53) in leukemia patients ($P = 0.29$). The rates of *T. glabrata* infection were 7.9% (6 of 76) and 1.9% (1 of 53), respectively ($P = 0.14$).

Five *T. glabrata* isolates were tested for susceptibilities to fluconazole, miconazole, and amphotericin B (Table 2). The high fluconazole MICs at 24 and 48 h are noteworthy. All isolates were susceptible to miconazole. The 24-h amphotericin B MICs ranged between 0.5 and 1.0 μg/ml.

DISCUSSION

C. krusei was a less frequent pathogen during this interval when the combination of amphotericin B plus flucytosine was used empirically in febrile patients colonized with *C. krusei*. *C. krusei* no longer represented the predominant cause of fungemias in patients given fluconazole. *T. glabrata* was the most common cause of fungemias in patients given fluconazole but was an uncommon pathogen in patients not receiving fluconazole.

T. glabrata isolates from both patients receiving and those not receiving fluconazole were resistant. Earlier reports suggest that exposure to fluconazole may select for azole resistance in initially susceptible *T. glabrata* pathogens (3, 6); this appears to contrast with *C. krusei* isolates, which appear to be natively resistant, since isolates recovered from patients not given fluconazole are as resistant as isolates from patients receiving fluconazole. In this report, the finding that two resistant *T. glabrata* isolates were recovered from patients not receiving fluconazole suggests that some *T. glabrata* strains may be natively resistant to fluconazole.

The lack of susceptibility of isolates of *T. glabrata* to fluconazole suggests a good correlation between in vitro susceptibility assays and clinical observations of breakthrough infections during fluconazole use. For both *T. glabrata* and *C. krusei* isolates (7), in vitro susceptibility assays suggest that resistance to one azole compound (fluconazole) does not necessarily confer resistance to another (miconazole). The *T. glabrata* isolates were less susceptible to amphotericin B than most *T. glabrata* isolates for which MICs are ≤0.5 μg/ml (3a), possibly because of an alteration in sterol composition, concentration, or stereochemistry in the cell membrane accompanying the resistance to fluconazole.

Undoubtedly, there are a variety of factors which contribute to the emergence of relatively uncommon organisms such as *T. glabrata* and *C. krusei* as opportunistic pathogens. Such factors include prolonged neutropenia, broad-spectrum antibiotics, chemotherapy-induced damage to the gastrointestinal mucosa, the use of central venous catheters, exogenous exposure from hospital staff, the environment, and food sources. We cannot exclude the possibility that a common source or some unique feature of the care or treatment of the bone marrow transplant patients versus that of the leukemia patients may have accounted for differences in the frequency or types of the fungal infections. However, since bone marrow transplant patients were dispersed on three different units with separate nursing and physician staffs and both the transplant and leukemia patients were served by common kitchen and environmental services, we believe that a point source is less likely to fully explain the outbreak of *T. glabrata* infections in the transplant patients. We believe that selection of less susceptible organisms by the pressure of antifungal agents is an important contributor. The universal use of fluconazole in the transplant patients along with the less frequent use of amphotericin B appears to be contributory in this cohort of patients. Clinicians should be alert to the possibility of breakthrough infections from *T. glabrata* in patients who are receiving fluconazole prophylaxis.

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