Microbiology and epidemiology of oral yeast colonization in hemopoietic progenitor cell transplant recipients

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Objective. We monitored the epidemiology and microbiology of oral yeast colonization in patients undergoing hemopoietic progenitor cell transplantation (HPCT) to examine associations between yeast colonization and oral mucositis.
Study Design. One hundred twenty-one consecutive HPCT patients were sampled for oral yeasts prior to fluconazole (FLC) prophylaxis, at transplantation, and weekly until discharge. Clinical oral mucositis screenings were performed triweekly.
Results. Yeast colonization was evident at 216 of 510 total visits. *Candida albicans* and *Candida glabrata* were the predominant organisms. Eight patients showed elevated minimal inhibitory concentrations to FLC. One patient developed fungal septicemia. Patients with oral mucositis assessment scale scores <20 had higher colonization rates than those with higher scores.
Conclusions. FLC is effective in controlling a variety of oral yeasts in HPCT recipients. FLC-resistant yeasts do emerge and can be the source of fungal sepsis. A positive association was not shown between yeast colonization and the presence or severity of oral mucositis. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:354-358)

High-dose chemotherapy with hematopoietic progenitor cell transplantation (HPCT) is an established therapy for patients with hematologic malignancies and selected solid tumors.[1] Oral mucositis and fungal infections remain major complications of HPCT.[2,3] Routine antifungal prophylaxis with fluconazole (FLC) in HPCT has greatly decreased the incidence of serious *Candida albicans* infections.[4-6] However, other *Candida* species resistant to FLC, including *Candida glabrata* and *Candida krusei*, have emerged.[3,4] We have previously reported that oral colonization with *C. glabrata*[7] or *C. krusei*[8] can lead to fungal sepsis in hemopoietic progenitor cell recipients. In the era of FLC prophylaxis, there is limited information regarding the oral fungal colonization of transplant recipients before and throughout the transplantation course.

We performed a prospective longitudinal surveillance study of HPCT recipients using oral sampling to evaluate the prevalence of oral yeast microbiology before transplantation and the epidemiology of yeast resistance before and during the HPCT process. The study cohort was also evaluated for levels of oral mucositis because this is a common side effect of HPCT conditioning regimens.[9-11] Severe mucositis can cause painful dysphagia, limiting the patient's ability to maintain a normal diet or take oral medications. This debilitating complication can increase the need for opioid analgesics, prolong inpatient stays, and increase costs.[12,13] Ulcerative mucositis compromises oral mucosal integrity and can increase the risk of bacteremia or fungemia resulting from systemic invasion of endogenous flora.[10,12] Our purpose was to determine the change in oral yeast colonization in patients undergoing HPCT who received FLC prophylaxis. Additionally, we sought to determine whether there was any association between oral *Candida* colonization and the presence of oral mucositis.

MATERIAL AND METHODS

Patient population

We conducted a longitudinal, prospective study of 121 consecutive HPCT recipients with hematologic malig-

Statement of Clinical Relevance

Fluconazole prophylaxis is effective in controlling oral yeasts in hemopoietic progenitor cell transplant recipients. Fluconazole-resistant yeasts rarely emerge but can be the source of fungal sepsis. An association between yeast colonization and presence/severity of oral mucositis was not seen in this study.

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Received for publication Aug 9, 2012; returned for revision Oct 9, 2012; accepted for publication Oct 19, 2012.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2012.10.012

Table I. Diagnosis and conditioning regimens

Diagnosis (number of patients)	*Conditioning regimen*
Multiple myeloma (74)	Melphalan
Non-Hodgkin's lymphoma (27)	CBV or BEAM
Hodgkin's lymphoma (7)	CBV or BEAM
Acute myelogenous leukemia (7)	BuCy or BuF
Testicular germ cell (3)	CEC
Chronic lymphocytic leukemia (2)	BuF or MelF
Myelodysplactic syndrome (1)	BuCy

BEAM, carmustine, cytarabine, etoposide, melphalan; *BuCy*, busulfan, cyclophosphamide; *BuF*, busulfan, fludarabine; *CBV*, cyclophosphamide, etoposide, carmustine; *CEC*, cyclophosphamide, etoposide, carboplatin; *MelF*, melphalan, fludarabine.

nancies who underwent high-dose chemotherapy (Table I) followed by transplantation from July 2005 through February 2008. Our patients were referred to the South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio Texas Bone Marrow Transplant Unit, from 28 different referring centers throughout the nation and the Commonwealth of Puerto Rico. Each patient received antifungal prophylaxis with 400 mg oral (p.o.) FLC daily starting with the conditioning regimen and continuing through engraftment. As inpatients, compliance was assured by the daily nursing staff administration and documentation of all medicinal regimens. If the patient was unable to tolerate p.o. medications, intravenous routes were used. Informed consent was obtained from all participants/patients, and all procedures were in accordance with the Institutional Review Board of the University of Texas Health Science Center at San Antonio and the Research and Development Committee of the South Texas Veterans Health Care System.

Oral rinse sample collection and microbiological characterization, mucositis assessment

Sampling consisted of a 20-second oral swish with 10 mL of sterile water. On 3 occasions, an oral swab culture was substituted because oropharyngeal candidiasis (OPC) was evident or the patient was unable to swish and expectorate. Samples were taken before the initiation of the conditioning regimen and FLC prophylaxis (visit 1), the day of transplantation (visit 2), which was 5.5 ± 2.59 days later, and with weekly sampling until discharge, (visits 3 and 4) 12.02 ± 2.77 and 19.04 ± 3.38 days later, respectively.

Samples were plated on CHROMagar *Candida* (DRG International, Mountainside, NJ) medium containing chloramphenicol (0.5 g/L) with FLC (8 and 16 μg/mL) or without FLC for presumptive fungal identification and resistance screening.[14] These chromogenic medium plates were prepared in 100-mm-diameter petri dishes and stored at 4°C for up to 1 week prior to use. CHROMagar *Candida*-specific color patterns were used for presumptive yeast identification. Yeasts were further characterized using germ tube analysis after incubation in human serum at 37°C for 3 hours and by biochemical utilization patterns determined using API 20C (bioMérieux, Marcy-l'Etoile, France). Triweekly oral examinations utilizing both the World Health Organization (WHO)[12,15] and the Oral Mucositis Assessment Scale (OMAS)[16] criteria were used for mucositis assessment and performed by the same 2 health-care providers (SDW and JJT). All patients participated in periodic oral fungal surveillance.

Table II. Yeast distribution with fluconazole minimal inhibitory concentration (MIC) data in 121 hemopoietic progenitor cell transplantation recipients

Yeast	*Colonized visits (216) (%)*	*Total visits (510) (%)*	*MIC range (median)*
C. albicans	96 (44)	96 (19)	0.125-1.0 (0.125)
C. glabrata	76 (35)	76 (15)	2-64 (4)
C. tropicalis	24 (11)	24 (4.7)	0.125-32 (0.25)
C. dubliniensis	23 (11)	23 (4.5)	0.125-32 (0.125)
C. krusei	6 (3)	6 (1)	8-16 (8)
S. cerevisiae	5 (2)	5 (1)	0.5-1 (0.5)
C. parapsilosis	4 (2)	4 (0.8)	0.125-0.25 (0.25)
C. magnoliae	1 (0)	1 (0)	0.5 (0.5)
C. lusitaniae	1 (0)	1 (0)	0.5 (0.5)

Antifungal susceptibility

Minimal inhibitory concentrations (MICs) of these yeasts were determined using the Clinical and Laboratory Standards Institute methodology[17] by the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio. Yeast isolates with FLC MICs of >16 μg/mL were considered resistant in this study.[18]

RESULTS

Our study population was 94% male (112 of 119 patients) with a median age of 58 years (range 19-74, mean 55.4 ± 11.5). Fifty-three percent were white (63 of 119), 25% hispanic (30 of 119), 20% black (24 of 119), and 2% other races (2 of 119). Patients enrolled in this cohort received 106 autologous, 11 allogeneic, and 2 syngeneic transplants. Two patients died prior to transplantation.

A variety of yeasts were cultured from the HPCT patients enrolled in this study. Yeast colonization was evident in 216 of the 510 total visits (42%; Table II). Nine different yeast species were cultured from samples taken at these visits. *C. albicans* and *C. glabrata* were the predominant organisms in the cultures, and most specimens were susceptible to FLC, as shown in Table II.