Candidemia in Allogeneic Blood and Marrow Transplant Recipients: Evolution of Risk Factors after the Adoption of Prophylactic Fluconazole

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The prophylactic use of fluconazole is common in blood and marrow transplant (BMT) recipients. To evaluate how fluconazole has influenced the development of azole resistance and candidemia, weekly mouthwashings were done, and fluconazole susceptibility was determined for 1475 colonizing and invasive isolates obtained from patients undergoing BMT. Of 585 patients, 256 (44%) were colonized with Candida species during the course of BMT. Of these, 136 patients (53%) had at least 1 mouthwashing sample that yielded Candida species other than C. albicans on culture. Only 4.6% of patients developed candidemia. Overall, C. albicans was the most common colonizing isolate, but it caused only 7% of cases of candidemia. About 5% of colonizing C. albicans strains and 100% (2 of 2) invasive C. albicans strains were fluconazole-resistant. Colonization, cytomegalovirus disease, and bacteremia are risk factors for the development of candidemia. The use of prophylactic fluconazole is associated with a low incidence of candidemia and attributable mortality, despite colonization with azole-resistant Candida species in BMT recipients.

Before the availability of azole antifungal agents for prophylaxis, invasive infections caused by Candida species were common in allogeneic blood and marrow transplant (BMT) recipients [1, 2]. Studies done in the early 1990s established the utility of fluconazole administered prophylactically to prevent candidal infections after conditioning chemotherapy for BMT. The results of the first randomized, prospective study comparing the administration of fluconazole with that of placebo during the neutropenic period documented that fluconazole administered during neutropenia decreases both colonization and invasive infection with Candida species [3]. A subsequent study at our institution, the Fred Hutchinson Cancer Research Center (FHCRC), used fluconazole (400 mg/kg/day) prophylactically during the first 75 days after BMT, which is the period extending through neutropenia and acute graft-versus-host disease (GVHD) [4]. This study found a decrease in candidiasis as well as an overall mortality benefit among patients who received fluconazole. This 75-day administration of fluconazole has since been standard practice for patients undergoing allogeneic BMT at FHCRC.

C. albicans has historically been responsible for most mucosal and disseminated candidal infections in BMT patients. Although the incidence of serious candidal infections has decreased with the prophylactic use of azole drugs, there appears to have been a change in the Candida species that cause infection. A number of centers have documented an increase in the azole-resistant species C. krusei and C. glabrata among patients with cancer and BMT recipients [5–7]. Whether this observation is a direct result of fluconazole administration or of other factors (e.g., neutropenia, other drugs) is controversial, because other groups have noted that these organisms caused disease before the use of fluconazole [8] and in patients with no prior antifungal exposure [9]. Also, many centers have reported no increased incidence of infections with azole-resistant Candida species, despite the widespread use of fluconazole [4, 10].

To study the epidemiology of colonization and infection with Candida species in BMT patients receiving fluconazole prophylaxis, weekly surveillance cultures were collected from patients undergoing allogeneic BMT at FHCRC from 1994 to 1997. Candida isolates obtained from mouthwashings were stored frozen and subsequently screened for resistance to azole antifungal drugs. Microbiology records, a clinical database, and patients’ charts were reviewed to determine the incidence of and risk factors for colonization by and invasive bloodstream infection with Candida species, and to assess the outcome of candidemia in patients receiving prophylactic fluconazole.
Materials and Methods

Patients and the BMT procedure. Data from patients undergoing allogeneic BMT at FHCRC from January 1994 to June 1997 were obtained retrospectively. Demographic, clinical, and treatment characteristics were obtained from patient and microbiology databases, and a chart review was performed for all patients who developed candidemia.

Pretransplant conditioning was done as described elsewhere [11, 12]. All patients were treated with either oral or intravenous fluconazole (400 mg) for the first 75 days after transplantation. During the study period, patients were followed for cytomegalovirus (CMV) pp65 antigenemia, and patients were given ganciclovir preemptively as indicated for prevention of CMV disease [13]. Additional care included acyclovir at 250 mg/m² intravenously every 12 h for patients seropositive for herpes simplex virus, from day 7 until initiation of ganciclovir therapy, and 1 double-strength trimethoprim-sulfamethoxazole tablet twice weekly after engraftment for prophylaxis against Pneumocystis carinii infection. During the study period, patients routinely received ceftazidime monotherapy with the onset of neutropenia, and aminoglycosides and/or vancomycin were added to treat fever, depending on whether there was a clinical suggestion of infection with a gram-positive organism. Amphotericin B (0.5 mg/kg/day, or an equivalent lipid amphoter- icin B preparation) was added to treat fever persisting for >3 days or when a fungal infection was suspected. Fluconazole prophylaxis was discontinued during amphotericin B administration.

Microbiology. Surveillance mouthwash samples for culture were collected weekly from each patient by means of a 10-mL “swish” of sterile saline during the period from consolidation chemotherapy to day 75 after BMT (about days 10 to 75). Cultures were done on media selective for fungi (Sabouraud dextrose agar containing chloramphenicol; Diño, Detroit, MI), yeasts were spe- ciated by use of the API 20C AUX system (Analytab Products, Plainview, NY), and isolates were stored frozen at −70°C in Sabouraud dextrose broth containing 10% glycerol. All yeasts isolated from cultures of blood were also stored frozen at −70°C.

To screen Candida isolates for fluconazole resistance, a modification of a technique described elsewhere was used [14]. In brief, the frozen cultures were inoculated into 100 μL of Sabouraud dextrose broth in microtiter plates and incubated for 4 h at 30°C. By means of a multiwell applicator, 10 μL of each isolate was plated on chromogenic media (CHROMagar, CHROMagar Technology, Paris) containing fluconazole (8 μg/mL) and the same media lacking fluconazole. Plates were incubated for 48 h at 30°C, and resistant isolates were identified as those that grew to confluen on both media. Fluconazole MICs were then determined by use of standard- ized microtiter dilution methods, for all C. albicans isolates that were identified as azole-resistant by the initial screening [15]. C. albicans with MICs >16 μg/mL were considered to be fluconazole resistant.

Definitions and statistical analysis. Patients having at least 1 isolate obtained from a mouthwashing were considered colonized, and patients having at least 1 isolate obtained from culture of blood were considered to have candidemia. The day of diagnosis was the day on which a Candida isolate was identified from the sample. Patients were considered to have developed colonization with a particular organism if initial cultures of mouthwashings revealed no growth or growth of a different species of Candida.

CMV disease was considered present if biopsy of an organ (liver, gut, lung) revealed histologic evidence of the virus or if the virus was detected by culture of the tissue. Also, disease was defined by detection of the virus in bronchoalveolar lavage fluid in the presence of pulmonary infiltrates [16, 17].

Risk factors for the posttransplant development of colonization with Candida species were examined in both univariate and multivariate Cox regression models. Only patients known to have initial negative surveillance cultures were considered at risk for the new development of posttransplant colonization. Patients were censored 75 days after BMT or at the time of last surveillance culture, whichever occurred first. Separate analyses looking at risk factors for the development of colonization with azole-resistant species (C. glabrata, C. krusei) and colonization with any Candida species were done. Patient sex, age, type of transplant (matched related vs. mismatched related or unrelated), underlying disease, days until engraftment (absolute neutrophil count >750/μL for 2 consecutive days), receipt of total body irradiation, type of GVHD prophylaxis and treatment (cyclosporin A, methotrexate, FK506, corticoste- roids), acute GVHD grade II-IV, receipt of antibiotics, receipt of intravenous amphotericin B formulations, and receipt of antivirals (acyclovir, ganciclovir, ribavirin) were examined. CMV was ex- amined because of previous reports noting an increased incidence of invasive fungal infection in solid organ transplant recipients with CMV disease [18]. Because retrospective studies have identified receipt of quinolone antibiotics as potentially leading to increased colonization [5] and infection [19] with C. krusei in cancer patients, receipt of quinolone antibiotics was included as an additional variable. Covariates for acute GVHD, CMV disease, and medications initiated after transplant were considered time dependent. Statistical significance was considered to be a 2-sided P<.05.

Univariate and multivariate Cox regression models were also used to identify risk factors for the development of candidemia in patients receiving fluconazole prophylaxis. The risk factor analysis was restricted to the period from day 0 to day 120, which corre- sponds to the period during which patients are followed for micro- biology events in the FHCRC system. Patient age, sex, type of transplant, and the time-dependent covariates for absolute neutro- phil count, recent bacteremia (within 2 weeks of candidemia), colon- ization with Candida species, acute GVHD, CMV disease, recei- pt of antibiotics (specifically quinolone antibiotics), and receipt of corticosteroids were examined. Because of the limited number of candidemia cases, it was not possible to include all covariates in 1 multivariate model simultaneously. The covariates included in this multivariate model were based on clinical interest and selected at the outset to avoid potential bias.

A chart review was done for all 30 patients with candidemia, to determine whether clinical evidence of organ tissue invasion was present and to determine attributable mortality. Death was con- sidered to be associated with candidiasis if there were consistent pathologic findings on autopsy reports and if the death summary listed candidemia as a cause of death.

Results

Patient characteristics. Six hundred fifty-five patients who underwent allogeneic BMT during the study period were en-
rolled in this protocol. The study cohort included 274 females (42%) and 381 males (58%) who underwent BMT for the underlying diseases outlined in table 1. Other demographic and transplant characteristics are outlined in table 1. Of the 655 patients enrolled, 585 had at least 1 mouthwash sample collected. These patients were included in the risk factor analysis.

Colonizing isolates and azole resistance. Of 585 patients who had at least 1 mouthwash sample obtained before BMT or during the first 75 days after BMT, 256 (44%) were colonized with at least 1 Candida species. Most patients (81%) who were colonized had only 1 species isolated. C. albicans was the most frequent species obtained before fluconazole exposure; 132 patients (84%) who were colonized with C. albicans had first positive cultures obtained before conditioning chemotherapy for BMT. A total of 136 patients (53%) were colonized with a Candida species other than C. albicans during the course of BMT. The majority of patients who were colonized with C. glabrata (n = 50; 57%) and C. krusei (n = 25; 76%) became colonized after an initial sample that was negative was obtained or during the period of azole antifungal use. The median day on which colonization with C. krusei or C. glabrata developed was day 36 (range, day 5–75). The day of colonization with a Candida species other than C. albicans was evenly distributed throughout the course of BMT, with no apparent minimum fluconazole exposure necessary (data not shown).

The most common Candida species cultured from mouthwash samples was C. albicans (figure 1). A total of 1475 isolates obtained from mouthwashings were examined for azole resistance by use of the CHROMagar screening method. This screening determined that 394 C. glabrata isolates (99%), all 80 C. krusei isolates, and 62 C. albicans isolates (7%) were resistant to fluconazole. Microtiter dilution testing was done on all “resistant” C. albicans isolates, confirming MICs >16 μg/mL for 49 isolates. Thus, 5.3% of C. albicans obtained from mouthwashings was resistant to fluconazole. The screening method also identified as resistant 9 C. guilliermondii isolates (100%), 6 C. lusitaniae isolates (30%), 3 C. tropicalis isolates (30%), and 2 C. lipolytica isolates (100%).

Risk factors predicting colonization and candidemia. None of the risk factors examined (acute GVHD, CMV disease, receipt of quinolone antibiotics or all antibiotics, receipt of corticosteroids, receipt of amphotericin B, transplant type, patient age, days until engraftment, or receipt of cyclosporin A) had a significant effect on the development of colonization, with all Candida species combined (susceptible and resistant) in either univariate or multivariate analysis (data not shown). The only variable identified as potentially associated with the development of colonization with azole-resistant Candida species (C. krusei, C. glabrata) was CMV disease, with a relative risk (RR) of 4.4 (95% confidence interval [CI], 1.1–18.1) in univariate analysis (data not shown). Multivariate analysis confirmed a trend toward increased colonization with azole-resistant species in the presence of CMV disease (RR, 6.6; P = .07; 95% CI, 0.9–50), although the number of people with CMV disease in the sample was quite small (n = 15).

Four patients were diagnosed with candidemia before BMT. Thirty of the remaining 651 patients developed candidemia after BMT, for a cumulative incidence of 4.7%. The most common species causing posttransplant infection were C. glabrata (n = 14; 47%) and C. parapsilosis (n = 7; 23%). C. krusei caused disseminated infection in 6 patients (20%), and C. albicans and C. guilliermondii caused infection in 1 patient (3%) each. One patient developed candidemia with both C. albicans and C. glabrata. C. albicans isolates obtained from blood of both patients who developed disseminated infection were highly resistant to fluconazole, with MICs >64 μg/mL. The median day on which candidemia developed was day 28 after BMT (figure 2). Figure 3 compares the isolates that caused candidemia during this study with those obtained from blood samples of a cohort of patients who underwent BMT at our institution before the adoption of fluconazole for routine prophylaxis [1].

Risk factors for candidemia are outlined in table 2. Multivariate analysis revealed that colonization with any Candida isolate, bacteremia, and CMV disease contributed to a 3.0-fold, 8.4-fold, and 16.4-fold increased risk for candidemia, respectively. A trend toward an increased risk for candidemia was

<table>
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<th>Characteristic</th>
<th>No. of patients</th>
<th>% of total</th>
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<td>Recipient negative, donor negative</td>
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NOTE. ALL, acute lymphoblastic leukemia; ANL, acute nonlymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; TBI, total body irradiation; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

*Donor HLA matching information was not available for 4 cases.

*Conditioning regimen was not available for 124 cases.

*GVHD prophylaxis regimen was not available for 92 cases.

*Pretransplant patient and/or donor CMV serology was not available for 76 cases.
noted among patients who had received quinolone antibiotics (RR, 2.2; \( p = .06 \); 95% CI, 0.96–5.1). Notably, acute GVHD, absolute neutrophil count, receipt of corticosteroids, and receipt of all other antibiotics did not contribute significantly to an increased risk of candidemia in this model.

Of the 30 patients with candidemia, 5 (18%) had tissue invasion, with dissemination into the lungs (2), kidney (2), or spleen (1). Although 7 patients died within 2 weeks of candidemia, only 6 patients were thought to have died secondary to the infection, accounting for a total attributable mortality of 20%. Of the 6 patients who died, 3 were infected with \( \text{C. krusei} \), 1 with \( \text{C. glabrata} \), 1 with \( \text{C. guilliermondii} \), and 1 with an azole-resistant \( \text{C. albicans} \).

We examined the association of \( \text{Candida} \) colonization with the development of candidemia to determine whether colonization with an azole-resistant species predicted subsequent fungemia with the same species. Of 15 patients who had blood cultures positive for \( \text{C. glabrata} \), 9 were previously colonized with the same species. Among patients identified as colonized with \( \text{C. glabrata} \), the cumulative incidence of subsequent candidemia with this organism by day 120 after BMT was 9.8%. Of 6 patients who were candidemic with \( \text{C. krusei} \), 4 were colonized, and, among patients who were colonized with \( \text{C. krusei} \), the cumulative incidence of subsequent invasion with this species was 9.5%. Notably, \( \text{C. parapsilosis} \) differed in that none of the 7 patients infected was previously colonized with this isolate, and none of 30 patients colonized with this isolate developed invasive infection.

### Discussion

By analyzing culture results of prospectively collected mouthwash samples and a large patient database, we have shown that \( \text{C. albicans} \) remains the most common colonizing isolate among BMT patients receiving fluconazole, despite the majority of the
isolates being susceptible to the drug. Furthermore, these isolates are rarely the cause of candidemia. Patients frequently develop colonization with an azole-resistant Candida isolate while receiving fluconazole, and colonization serves as an independent predictor of candidemia. The incidence of candidemia in patients receiving fluconazole has remained consistent with that found in our previous placebo-controlled study, in which fluconazole decreased the incidence of candidiasis from 18% to 7% [4]. Bacteremia and infection caused by CMV are additional independent risk factors for bloodstream invasion with azole-resistant Candida species in patients receiving fluconazole prophylaxis.

The emergence of Candida species other than C. albicans as colonizing and invasive organisms has been well documented among neutropenic cancer and BMT patients. In a chart review of patients with hematogenous candidiasis in a large cancer center, administration of fluconazole was found to be protective from infection with C. albicans and C. tropicalis but predictive of infection with C. krusei and C. glabrata [20]. This protective effect is thought to be due to a reduction in gastrointestinal colonization with C. albicans, thus allowing the less virulent, azole-resistant species to emerge [21]. Although our study was not designed specifically to assess the effect of azole prophylaxis because all patients received the drug, our results support this hypothesis. C. albicans is the most common pretransplant colonizing isolate, whereas resistant species such as C. krusei and C. glabrata are most commonly isolated after transplantation and exposure to fluconazole. Thus, it is likely that fluconazole administration decreases colonization with azole-susceptible organisms, allowing resistant organisms to emerge.

Predictably, many of the isolates obtained from patients after BMT were resistant to fluconazole. The finding that C. krusei and C. glabrata were resistant is not surprising, because C. krusei has been described as inherently resistant and C. glabrata becomes resistant relatively quickly after exposure to the drug [22]. The finding that 5.3% of the C. albicans obtained was resistant to fluconazole is consistent with recent reports by our group [23] and others [24, 25], which described the recent emergence of azole-resistant C. albicans among immunosuppressed patients with hematologic disorders. Both C. albicans bloodstream isolates were highly resistant to fluconazole. These results emphasize that a fungemia that occurs in a patient receiving prophylaxis with high doses of fluconazole (400 mg daily) should be considered resistant to fluconazole, even if germ-tube testing suggests that the isolate is C. albicans. Given the high frequency of cross-resistance with other azole drugs [22], monotherapy with these compounds should be approached with caution.

Colonization was predictive of bloodstream infection with all Candida species except C. parapsilosis. These findings support the hypothesis that infection with azole-susceptible isolates while undergoing fluconazole prophylaxis is more likely if the infection is acquired through an indwelling catheter, because C. parapsilosis usually is acquired through this exogenous route.
The small increase in *C. parapsilosis* infections noted during the most recent time period (figure 3) likely represents a cluster of infusate-related bloodstream infections that occurred in 1994 (unpublished data, present authors).

Other *Candida* species are often acquired endogenously, through invasion of the gastrointestinal tract [7, 20]. The finding that the majority of patients who developed bloodstream infection with either *C. glabrata* (9/15) or *C. krusei* (4/6) had previous evidence of oral colonization with the same species supports the premise that these *Candida* species usually are acquired through the gastrointestinal tract [7]. Molecular typing, which is currently underway, will help to determine the source of infection in these patients. It is important to note that a possible source of bias in this study is the frequency of mouthwash sampling, since >5 mouthwash samples were obtained from only 71% of patients and since only 1 site was examined. This may have resulted in an underestimation of the risk associated with colonization.

The results of this study suggest an interaction between CMV disease and subsequent breakthrough fungemia with azole-resistant *Candida* species, because CMV was associated with an increased risk for disseminated infection (RR, 16.4; 95% CI, 3.6–75.0). Multivariate analysis confirmed that the increased risk for candidemia was independent of colonization. CMV disease has been previously found to be associated with the development of pulmonary aspergillosis in lung transplant recipients [26] and with deep fungal infection (intra-abdominal infection and fungemia) in liver transplant recipients [18]. It is possible that other opportunistic infections, such as candidemia, are caused by CMV-associated shifts in the ratios of lymphocyte helpers and suppressors, as well as by overall lymphocyte function. CMV disease may serve as a marker for receipt of large amounts of ganciclovir, which can increase the risk of fungal infection through the production of neutropenia [13, 16, 27], although this explanation is less likely because absolute neutrophil count was included in the multivariate model.

Bacteremia was found to be predictive for the development of candidemia, with an RR of 8.4, supporting the results of several previous retrospective analyses [20, 28, 29]. This association is most frequently explained by the possible presence of gastrointestinal mucosal lesions that may provide low-virulence organisms access to the bloodstream and tissues. In this study, the presence of unrecognized gastrointestinal mucosal lesions could explain the associations with both bacteremia and CMV disease, although we cannot rule out the possibility that there exists an underlying, unrecognized immune deficit linking these complications from infection.

Patients who were receiving a quinolone antibiotic had a trend toward an increased risk for the development of candidemia with an azole-resistant *Candida* isolate (P = .06; 95% CI, 0.96–5.1), independent of bacteremia and candidal colonization. One previous retrospective study found that BMT patients who had received norfloxacin had a 2.5-fold increased incidence of colonization with *C. krusei* [5]. Another retrospective study of cancer patients found that prophylaxis with quinolones was associated with an increased risk of breakthrough fungemia [19]. Whether this association involves an effect on host response [30], fungal pathogenicity, or another unrecognized factor is unknown and warrants further study.

In this study, the mortality associated with fungemia, 20%, is consistent with recently published reports from other centers.
[31], although it differs from the historical candidiasis-associated mortality rate of 39%, reported previously from our center [1]. This decreased mortality is likely to be related to the virtual elimination of the more virulent, tissue-invasive species C. albicans and C. tropicalis during the period of fluconazole use. The overall mortality associated with candidemia has decreased dramatically, given the overall decrease in incidence.

Initial cultures for fungi were done on a medium that was not selective for different Candida species, which may have caused us to miss colonization with multiple species. Likewise, since only 1 colony was tested for susceptibility phenotype, intrastrain variability [32] was not detectable. These 2 factors may have caused us to underestimate the prevalence of fluconazole resistance among colonizing Candida species.

Although further studies are necessary to determine how bacteremia, CMV disease, and the administration of quinolone antibiotics cause an increased risk for candidemia, it has become apparent that several risk factors previously noted, such as prolonged neutropenia [1, 20, 31], patient age [1, 31], receipt of total body irradiation [31], presence of acute GVHD [1], and type of transplant [1] are less significant predictors of candidemia in BMT patients receiving fluconazole. In the setting of effective antifungal prophylaxis, drug-resistant organisms with low virulence may proliferate and invade with the help of an additional insult to the host defense, thus explaining the emergence of risks such as concomitant infection and antimicrobial therapy. As the care of the BMT patient continues to evolve with the addition of different cytotoxic chemotherapies, GVHD prophylaxis regimens, and antimicrobial strategies, continued monitoring is necessary to detect important, previously unrecognized interactions between host and pathogens.

Acknowledgment

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References

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