Infectious Disorders

Breakthrough fungemia caused by fluconazole-resistant *Candida albicans* with decreased susceptibility to voriconazole in patients with hematologic malignancies

A 7-year retrospective analysis of candidemia in patients with hematologic malignancies demonstrated that ten patients, who received itraconazole and fluconazole during neutropenia, developed breakthrough fungemia caused by fluconazole-resistant *Candida albicans* (*C. albicans*) with decreased susceptibility to voriconazole. Eight of these ten patients died of candidemia despite amphotericin B administration. Karyotype analysis of *C. albicans* isolates revealed that all isolates were genetically unrelated. Our findings suggest that blood isolates of *C. albicans* in neutropenic patients receiving azoles could be azole cross-resistant, and that the patients should be treated by other antifungals such as echinocandins.

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Invasive candidiasis is an important cause of morbidity and mortality in patients receiving cytotoxic chemotherapy for hematologic malignancies. A recent multicenter survey demonstrated that the role of *C. albicans* as a major pathogen for fungemia may be increasing in some locations, although blood isolates of *C. albicans* with decreased susceptibility are rare.[1] We describe here ten cases of breakthrough fungemia caused by azole cross-resistant *C. albicans* in patients with hematologic malignancies.

Between January 1996 and December 2002, 18 episodes of breakthrough fungemia caused by *C. albicans* were retrospectively identified in 1024 patients with hematologic malignancies who received antifungal treatment during neutropenia. Fourteen of 18 blood isolates were stored and confirmed to be *C. albicans* and not *C. dubliniensis* using genetic typing at Chiba University.[2] As shown in Table 1 (*see online Appendix*), the *in vitro* susceptibility study according to document M-27 A of the NCCLS demonstrated that ten isolates were resistant to both fluconazole (minimum inhibitory concentration [MIC], ≥64 μg/mL) and to itraconazole (MIC, ≥32 μg/mL), and categorized as having the RR phenotype.[1] These ten isolates showed a decrease in susceptibility to voriconazole (MIC, 2-16 μg/mL). The other four blood isolates were susceptible to fluconazole (MIC, 0.5 μg/mL), itraconazole (MIC, 0.0625 μg/mL), and voriconazole (MIC, 0.0625 μg/mL). Micafungin, a new echinocandin, and amphotericin B were effective against these isolates with MIC of 0.0625 μg/mL and 0.125-1 μg/mL, respectively.

To evaluate the molecular epidemiology of azole-resistant candidemia, the electrophoretic karyotypes of the ten isolates were determined by pulsed-field electrophoresis on agarose gels with a CHEF system.[3] Separation of the chromosomes during karyotyping demonstrated that each of the ten isolates was a distinct variant (Figure 1). Each DNA type represented an individual patient with breakthrough candidemia, indicating different sources of infection. The clinical data of these ten patients are summarized in Table 1. At the time of developing candidemia, all the patients had been neutropenic (neutrophil count, < 500 neutrophils /μL) for 10-39 days and had mucositis due to intensive chemotherapy in combination with steroids. Surveillance cultures demonstrated colonization of *C. albicans* in the digestive tract prior to the development of candidemia. All patients received oral itraconazole (150-200 mg/day) as antifungal prophylaxis and therapy for 14 to 35 days, and were placed on preemptive intravenous fluconazole (400 mg/day) for 3 to 7 days in combination with itraconazole when they became febrile. Fluconazole was discontinued, and intravenous amphotericin B (50 mg/day) was initiated because of persistent high fever. Amphotericin B was administered for 5 to 36 days in combination with itraconazole. Ultimately, eight patients died of candidemia despite receiving amphotericin B and itraconazole without improvement of their clinical signs or symptoms related to the fungal infection and, still showing positive blood cultures. Central venous catheters were not the source of candidemia in these patients. Interestingly, all ten patients with azole-resistant *C.albicans* fungemia had received both itraconazole and fluconazole many times in the previous 6 months, either as antifungal therapy or prophylaxis (Table 1). In contrast, one of the four patients with azole-sensitive *C. albicans* fungemia in this study had received previous azole treatment (*data not shown*). There are reports of fungemia caused by fluconazole-resistant *C. albicans* in immunocompromised patients who received fluconazole for a short period[4,5] or as long-term therapy.[6] Therefore, it is likely in our cases that the previous frequent administrations of both fluconazole and itraconazole were effective against azole-susceptible *C. albicans*, but allowed potentially azole-resistant *C. albicans* to colonize and finally cause breakthrough fungemia during neutropenia. Digestive tract colonization of *C. albicans* and mucositis were observed in all patients before they developed candidemia, suggesting that

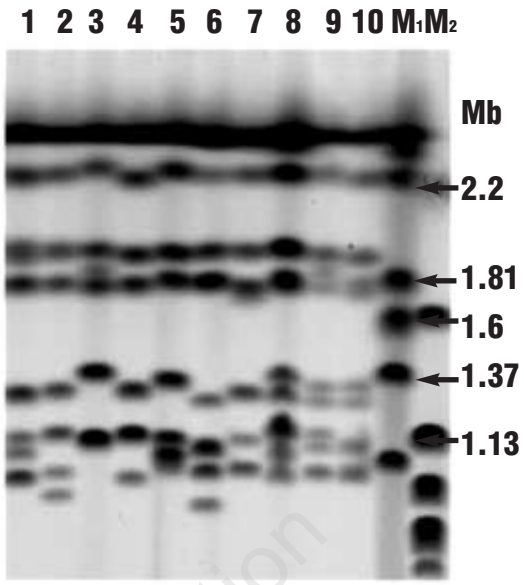

Figure 1. Electrophoretic karyotypes of ten azole-resistant *C. albicans* isolates obtained from patients with breakthrough fungemia are demonstrated by a Typhoon image analyzer. Sizes (in megabase pairs) are denoted on the right. Lane M1, *Hansenula wingei* chromosomes; M2, *Saccharomyces cerevisiae* chromosomes.

endogenous *C. albicans* might have been acquired through the digestive tract rather than from exogenous sources. In addition, karyotyping demonstrated that each case of fungemia was caused by a different strain of *C. albicans*, supporting the assumption of endogenous infection and negating the possibility of nosocomial transmission of a single strain.

Pfaller *et al*. demonstrated that some strains of fluconazole-resistant *C. albicans* displayed cross-resistance to itraconazole (RR phenotype) and that these RR phenotype strains of *C. albicans* are poorly susceptible to new triazoles such as voriconazole (MIC, ≥1 μg/mL). They attributed the possible mechanism of azole cross-resistance to the over-expression of efflux pumps.[1] It is surprising that all of our isolates of *C. albicans* revealed the RR phenotype and that the MIC of voriconazole for these isolates was markedly elevated, indicating azole cross-resistant strains. The mortality associated with breakthrough fungemia due to azole-resistant *C. albicans* in this study (80%) was higher than that in published reports on fungemia due to azole-susceptible *C. albicans* (20-30%),[7] despite amphotericin B administration. These findings strongly imply that the use of echinocandins or a combination of amphotericin B plus echinocandins should be considered the drug of choice for breakthrough fungemia caused by azole-resistant *C. albicans*.[8] In addition, azole prophylaxis should be reserved for high-risk patients with hematologic malignancies to prevent the development of cases of fungemia due to azole-resistant *C. albicans*.

In conclusion, our findings demonstrated the potential for emergence of azole cross-resistant *C. albicans* strains in neutropenic patients with breakthrough fungemia who are receiving azoles and have previously received azoles. Since the infection resulted in a high mortality and the isolates were resistant to azoles including voriconazole, these patients should be intensively treated with alternative compounds, including echinocandins and amphotericin B, until the neutrophils recover.

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References

1. Pfaller MA, Messer SA, Hollis RJ, Jones RN, Diekema DJ. In vitro activities of ravuconazole and voriconazole compared with those of four approved systemic antifungal agents against 6,970 clinical isolates of Candida spp. Antimicrob Agents Chemother 2002;46:1723-7.
2. Tamura M, Watanabe K, Mikami Y, Yazawa K, Nishimura K. Molecular characterization of new clinical isolates of Candida albicans and C. dubliniensis in Japan: analysis reveals a new genotype of *C. albicans* with group I intron. J Clin Microbiol 2001;39:4309-15.
3. Tateishi T, Murayama SY, Otsuka F, Yamaguchi H. Karyotyping by PFGE of clinical isolates of Sporothrix schenckii. FEMS Immunol Med Microbiol 1996;13:147-54.
4. Nolte FS, Parkinson T, Falconer DJ, Dix S, Williams J, Gilmore C, et al. Isolation and characterization of fluconazole- and amphotericin B-resistant Candida albicans from blood of two patients with leukemia. Antimicrob Agents Chemother 1997;41:196-9.
5. Marr KA, White TC, van Burik JAH, Bowden RA. Development of fluconazole resistance in Candida albicans causing disseminated infection in a patient undergoing marrow transplantation. Clin Infect Dis 1997;25:908-10.
6. Munoz P, Fernandez-Turegano CP, Alcala L, Rodriguez-Creixems M, Pelaez T, Bouza E. Frequency and clinical significance of bloodstream infections caused by *C. albicans* strains with reduced susceptibility to fluconazole. Diagn Microbiol Infect Dis 2002;44:163-7.
7. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogenic blood and marrow transplant recipient: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis 2000;181:309-16.
8. Johnson MD, MacDougall C, Ostrosky-Zeichner L, Perfect JR, Rex JH. Combination antifungal therapy. Antimicrob Agents Chemother 2004;48:693-715.