Clinical Manifestations and Molecular Epidemiology of Late Recurrent Candidemia, and Implications for Management


Abstract The aim of this study was to define the epidemiology and clinical manifestations of late recurrent candidemia. For this purpose, late recurrent candidemia was defined as an episode of candidemia occurring at least 1 month after the apparent complete resolution of an infectious episode caused by the same *Candida* sp. A total of five patients with recurrent candidemia were investigated. For all patients, isolates from the initial and recurrent episodes of candidemia were available for in vitro susceptibility testing and genetic characterization by DNA-based techniques. The results revealed the following salient features: prolonged duration between the initial and recurrent episodes (range, 1–8 months); recurrence of candidemia despite antifungal therapy; importance of retained intravascular catheters, neutropenia, and corticosteroids as factors predisposing to recurrence; high morbidity and mortality; no emergence of antifungal drug resistance between the initial and recurrent episodes; and relapse of infection due to the original infecting strain, rather than reinfection with a new strain. These findings raise several issues about the management and follow-up of patients with candidemia, which require assessment in future studies.

Introduction

Candidemia has emerged as the fourth most common bloodstream infection in the USA, largely due to the increasing number of patients with risk factors such as neutropenia, prior receipt of broad-spectrum antibiotics and corticosteroids, intravascular catheters, renal insufficiency requiring hemodialysis, and prior gastrointestinal surgery. The emergence of *Candida* spp. as significant nosocomial pathogens has been accompanied by shifts in the epidemiology of candidemia. Increased therapeutic and empiric use of antifungal agents has contributed to the development of drug resistance as well as to the increased importance of non-*albicans* Candida spp., which now account for the majority of cases of candidemia at a number of institutions. The significance of candidemia is underscored by mortality rates that frequently exceed 40%.

The clinical manifestations of candidemia range from asymptomatic infection to a septic shock syndrome resembling gram-negative sepsis. Although infections such as *Candida* endocarditis and oropharyngeal candidiasis are well recognized to recur despite antifungal therapy [1–3], recurrent candidemia is not well described. The incidence of recurrent candidemia is not
We report here the clinical manifestations and epidemiology of late recurrent candidemia in five patients. Each patient had an episode of candidemia that occurred at least 1 month after the apparent resolution of an initial episode. *Candida* strains obtained during the initial and recurrent episodes were available for in vitro susceptibility testing and genetic characterization by DNA-based typing methods.

**Patients and Methods**

**Case Definition.** Late recurrent candidemia was defined as a second episode of candidemia caused by the same *Candida* sp., occurring at least 1 month after the last blood culture positive for *Candida* was obtained from the initial episode. Furthermore, patients had to manifest complete resolution of the initial episode of candidemia, as evidenced by clinical and laboratory findings, including sterilization of blood cultures.

**Patients.** A total of five patients who met the case definition were included in the study. Three patients were encountered during the course of a prospective, multicenter study of candidemia (2 patients at the New England Medical Center, Boston, MA, USA, and 1 patient at Duke University Medical Center, Durham, NC, USA) [7]. Two patients were encountered during consultation service at the Shands Teaching Hospital at the University of Florida, Gainesville, FL, USA. Each of these patients had candidemia caused by a single *Candida* sp.

**Antifungal Susceptibility Testing.** Antifungal susceptibility testing was performed on the isolates recovered from the initial and recurrent episodes of candidemia. Each isolate was tested against the antifungal agent administered to the patient from whom it had been obtained. Minimum inhibitory concentrations (MICs) were determined using the standardized macrobroth technique recommended by the National Committee for Clinical Laboratory Standards [8]. For isolates recovered from episodes of candidemia treated with amphotericin B, the minimum lethal concentration (MLC) of amphotericin B was determined using previously described methods [9].

**DNA-Based Typing of Candida Strains.** The initial and recurrent strains of Candida isolated from all five patients were available for analysis by DNA-based typing methods. Strains were identified by morphological and biochemical criteria using the API 10 C kit (bioMérieux, France), and stored at −70°C until tested. Yeast genomic DNA was extracted as described previously [10] and suspended in 100 μl of TE buffer (10 mM Tris hydrochloride, 1 mM Na2EDTA, pH 8.0). The discriminatory power of both typing methods was demonstrated by the simultaneous evaluation of 45 Candida albicans, 24 Candida glabrata, and 5 Candida krusei bloodstream isolates obtained from patients enrolled in the multicenter study from 1991 to 1994 [7]. *Candida albicans* isolates ATCC 90028 and 90029 were incorporated into each set of experiments as quality controls.

Restriction enzyme analysis of genomic DNA (REA G) was performed using 10 μl of each extracted sample, which was digested to completion with EcoRI (Boehringer Mannheim, Germany). Digests were separated through 0.8% agarose gel in 1 x Tris-borate-EDTA (TBE) buffer overnight at 30 V. HindIII-digested phage lambda (Bio-Rad, USA) was used as a size marker. Inter-repeat polymerase chain reaction (IR-PCR) was performed using the primer TEL01 (5′-TGGGTGTGGTGTTG GGGTGTGGTGTCG-3′) (Genenco, Italy) as previously described [11]. Reaction mixtures (50 μl) consisted of primer (50 pmol), MgCl2 (2.5 mM), KCl (50 mM), Tris-HCl (10 mM, pH 9.0 at 25°C). Triton X-100 (0.1%), dNTPs (0.2 mM each), and Taq polymerase (2 U; Stratagene, Italy). DNA (50 ng) was added in a 1 μl volume. PCR was performed in a Thermolyne Temp-Tronic thermocycler (Barnsted/Thermolyne Corporation, USA) as follows: 40 cycles of 1 min at 94°C (DNA denaturation), 2 min at 52°C (primer annealation), and 3 min at 74°C (DNA extension). Amplified DNA was separated through 2% agarose gel in 0.5 x TBE for 5.5 h at 60 V. Lambda ladder (50-2000 bp; Bio-Rad, Italy) was used as DNA size marker. For both REAG and IR-PCR, ethidium bromide-stained gels were visually inspected after being photographed under UV illumination. Strains were determined to be different if any detectable electrophoretic band for one strain did not match with a band for its partner strain [12].

**Results**

**Patient 1.** A 46-year-old woman had undergone bone marrow transplantation for breast cancer and was receiving cyclophosphamide, cis-platinum, and nitrogen mustard via a Hickman catheter. She had an absolute neutrophil count (ANC) of 24 cells/mm3 when she developed fever to 39.1°C, hypotension (blood pressure of 100/70) and tachycardia (heart rate of 124 beats/ min). She was treated initially with broad-spectrum antibiotics. Eight blood cultures yielded no growth. Amphotericin B (0.6 mg/kg/day) was added empirically several days later due to persistence of symptoms. A single blood culture drawn 4 days after the addition of amphotericin B yielded Candida albicans. After 6 days of therapy with amphotericin B, she remained febrile, and the Hickman catheter was removed. She deferredly promptly thereafter and completed a 24-day course of amphotericin B for a total dose of 975 mg. Two follow-up blood cultures were negative in the ensuing 3 weeks. One month following resolution of the initial episode of candidemia, the patient was still neutropenic (ANC < 100 cells/mm3), and she developed fever to 38.4°C. She was treated again with broad-spectrum antibiotics; antifungal therapy was not started. Two days later, she developed septic shock requiring pressor support and mechanical ventilation. She died 3 days after the onset of fever. Three antemortem blood cultures yielded Candida albicans. Amphotericin B MICs exhibited by both the initial and recurrent isolates were 0.5 μg/ml. MLCs did not differ from MICs.

**Patient 2.** A 70-year-old woman was treated for B cell lymphoma via an indwelling central venous catheter with cyclophosphamide, doxorubicin, vincristine, and prednisone. She had an ANC of 100 cells/mm3 when...