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Molecular biotyping methods for epidemiologic studies of candidemia in patients with acute leukemia

Molecular epidemiology of *Candida tropicalis* fungemia was studied in 8 isolates from patients with acute leukemia using restriction fragment analysis (RFLP) and homogeneous electric field electrophoresis (CHEF). Our data suggest that RFLP is more sensitive than CHEF and that at least two prevalent biotypes are circulating in our hospital.

Sir,

Invasive candidiasis due to *C. albicans* and recently due to other species of Candida is an important cause of morbidity and mortality in patients with acute leukemia.[1,2]

The isolation of yeasts from blood cultures is difficult and subsequently understanding of the epidemiology of Candida infections is unclear, but the potential for nosocomial transmission must be considered.[3,4]

In recent years new methodologies of molecular epidemiology, such as restriction fragment analysis (RFLP) and homogeneous electric field electrophoresis (CHEF), have been used to improve understanding of the epidemiology of these infections and appear to offer important advantages over phenotyping methods.[5,6]

To evaluate the incidence and molecular epidemiology of systemic fungemia in patients with acute leukemia, we used RFLP and CHEF to study isolates of *Candida spp.* obtained from blood cultures from patients admitted to our Department from June 1994 to June 1997.

Febrile episodes were classified according to the EORTC statement.[7]

All isolates were identified by morphology on corn meal agar with the API 20 C gallery system. Identification was independently verified by two laboratories.

For RFLP, *Candida* isolates were grown in YPD (yeast extract 10 mg/mL peptone 20 mg/mL, dextrose 20 mg/mL) for 24 h in a shaker, at 28°C. The DNA was extracted as described elsewhere.[8,9] From each strain 20 γ of DNA were restricted using 2 µL of EcoRI conc. (40 U/µL) for 3 h, at 37°C. The fragments obtained were separated on agarose gel 1.5%, TAE 1X, passing a 75V current for 2 hrs. The gel was then blotted onto nylon membrane (Amersham, Life Science). The filter was hybridized with a DIG ribosomal DNA of *Saccharomyces cerevisiae* λ DNA HINDI-II, and developed with NBT/BCIP chromogene substrate after incubation with an AP conjugate anti DIG antibody (Boehringer, Mannheim, Germany).

For CHEF, cells of *Candida* were grown in YPD medium (glucose 2%, yeast extract 1% and Bactopeptone

Table 1. Clinical characteristics of patients with *C. tropicalis* fungemia.

Pts./Year of isolation	*Age/Sex*	*Disease*	*Status*	*PMN/µL*	*CVC*	*Clinical signs*	*Therapy*	*Outcome*
BM/1994	61/F	AML	R	< 100	NO	Pneumonia	Amphotericin B	Died
GG/1994	62/M	AML	I	< 100	NO	None	Amphotericin B	Improved
CS/1994	69/M	AML	I	< 100	NO	None	Amphotericin B	Died
PL/1995	66/F	AML	I	500-1000	NO	Splenic and cutaneous lesions	Amphotericin B	Improved
RF/1995*	63/F	AML	I	< 100	NO	None	Amphotericin B	Improved
AR/1995*	53/M	AML	R	< 100	YES	None	Amphotericin B	Died
TI/1996	67/F	AML	I	< 100	NO	None	Fluconazole	Improved
GA/1996	16/M	ALL	R	< 100	NO	None	Amphotericin B	Improved
FP/1996	52/M	AML	I	< 100	NO	Pneumonia (ARDS)	Amphotericin B then fluconazole	Improved
MM/1996	54/M	AML	I	< 100	NO	None	Amphotericin B	Improved

*AML= acute myeloid leukemia; ALL= acute lymphoblastic leukemia; I= induction, R= relapse; *strains from these patients were not available for molecular biotyping methods.*

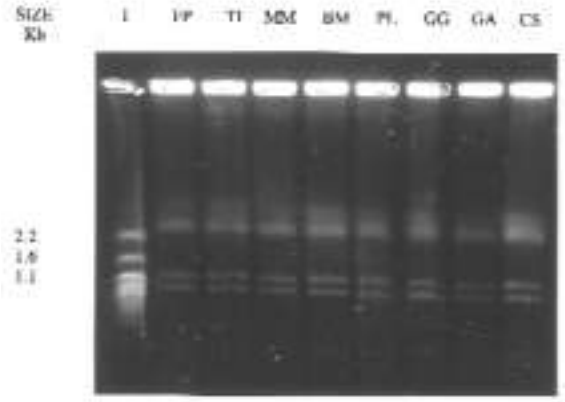

Figure 1. CHEF of *C. tropicalis*. Agarose 1% in 0.5 X TBE, 10°C, angle 120°, 2.4 V/cm. Pulse time 20', run time 82 hrs, 1: marker (*S. cerevisiae*).

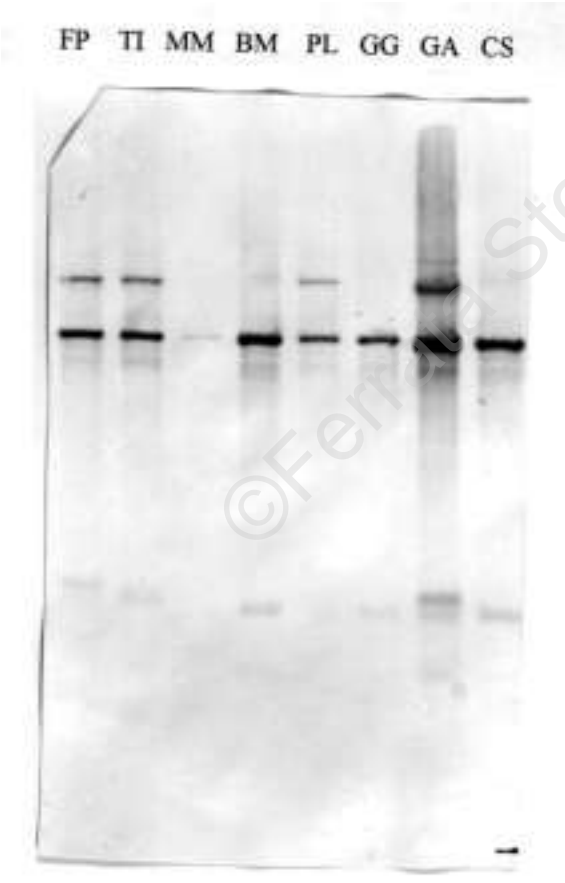

Figure 2. *C. tropicalis* samples. Digestion of 20γ genomic DNA using EcoRI. Separation of fragments of agarose gel 1.5%, TAE 1 X, 75 V 2 hrs. Southern blot and hybridization using as probe ribosomal DNA of *Saccharomyces cerevisiae* λ DNA *HIND*III DIG.

2%; Difco) overnight at 28°C. The DNA samples in agarose inserts were prepared as described elsewhere.[10] CHEF analysis was performed with CHEF-DRII apparatus from Biorad.

Saccharomyces cerevisiae (Biorad) was used as a standard. The parameters for each run were 2.4 V/cm, 10°C, 82 hrs, angle 120°, pulse time 20 min.

During 36 months, 384 febrile episodes were studied in 168 enrolled patients. Of 141 organisms isolated from blood cultures for 123 episodes of bacteremia, fungi were detected in 14 (11%) of all positive cultures; *C. tropicalis* was documented in 10 patients (Table 1) and eight strains were available for DNA detection.

As shown in Figure 1, the karyotype of all 8 isolates was composed of three chromosome bands, in the molecular weight range of about 2.4 to 1 Kbases. The largest band (2.2 Kb) was not well resolved in any of the isolates and could have consisted of more than two chromosome-size components. The numbers and sizes of the bands of all isolates were identical. Using the RFLP method, on the other hand, a degree of heterogeneity between strains was clear: as shown in Figure 2, five isolates had a unique hybridization pattern (BM, CS, PL, GA, TI); the other 3 isolates showed a different pattern, but similar between themselves (GG, FP, MM).

In our experience RFLP appears to be better than CHEF for detecting subtle differences between isolates. These data suggest that some common source of infection could have been present and that at least two prevalent biotypes of *C.tropicalis* were circulating in our hospital.

Further prospective epidemiologic studies are needed to facilitate the development of rational preventive measures.

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Key words

Molecular epidemiology, biotyping methods, Candida infection, candidemia, acute leukemia.

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Disseminated toxoplasmosis after CD34+-selected autologous peripheral blood stem cell transplantation

A patient with adult T-cell leukemia/lymphoma was successfully treated by autologous CD34+ peripheral blood stem cell transplantation (PBSCT) after fractionated total body irradiation (TBI) and high-dose cytarabine and cyclophosphamide. However, the combination of CD34+ cell-selected PBSCT and TBI regimen resulted in a delayed immune-cell reconstitution and rendered the patient susceptible to disseminated toxoplasmosis.

We report a case of disseminated toxoplasmosis which occurred 4 months after a CD34+-selected autologous peripheral blood stem cell transplantation (PBSCT) for acute-type adult T-cell leukemia/lymphoma (ATLL). While the patient achieved a sustained molecular remission after transplantation, immunosuppression as a consequence of T-cell depletion and resultant *Toxoplasma gondii* reactivation ruined the promising outcome. A prudent clinical attitude toward immunologic fragility, and awareness of the occurrence of toxoplasmosis in transplantation of selected CD34+ cells, are needed.

A 49-year old female was found to have an acute-type of ATLL and treated to hematologic remission with conventional chemotherapy. Peripheral blood progenitor cells were harvested and subjected to CD34+ cell selection by an immunomagnetic bead method (Isolex system; Nexell Therapeutics Inc., Irvine, CA, USA). Assessment of the purging efficacy of CD34+ positive selection by inverse polymerase chain reaction revealed an apparent reduction of ATLL cells.[1] After pre-transplant conditioning consisting of 1,200 cGy of total body irradiation (TBI), cytarabine 8 g/m^2, and cyclophosphamide 120 mg/m^2, a total of 6.0×10^7 CD34+ cells (1.2×10^6/kg) were infused. Engraftment was uneventful and the patient was discharged in sustained hematologic and molecular remission.[1] However, four months after transplantation, she suffered a persisting fever and became dyspneic. A chest X-ray demonstrated interstitial ground glass shadowing (Figure 1A); she underwent a bronchoscopy with alveolar lavage, which failed to reveal any specific etiology. Over 24 hours she developed acute respiratory failure, deteriorated rapidly (Figure 1B), in spite of intensive supportive therapies, and eventually died without any definite diagnosis having been made. At autopsy, cysts of *Toxoplasma gondii* were detected in several organs (Figure 1 C, D, E), but there was no evidence of recurrence of ATLL or other opportunistic infections. The serologic test retrospectively performed on sera from the storage sample obtained before transplantation suggested reactivation of the parasite (Table 1).

Toxoplasma gondii is a widespread opportunistic parasite of humans, but rarely seen in transplanted patients.[2,3] In North American reviews,[4] toxoplasmosis occurred in 0.31 cases per 100 allogeneic transplantations, but no case of toxoplasmosis after CD34+-purified autologous PBSCT has been previously reported. The acute infection is asymptomatic but establishes itself within many organs, persisting

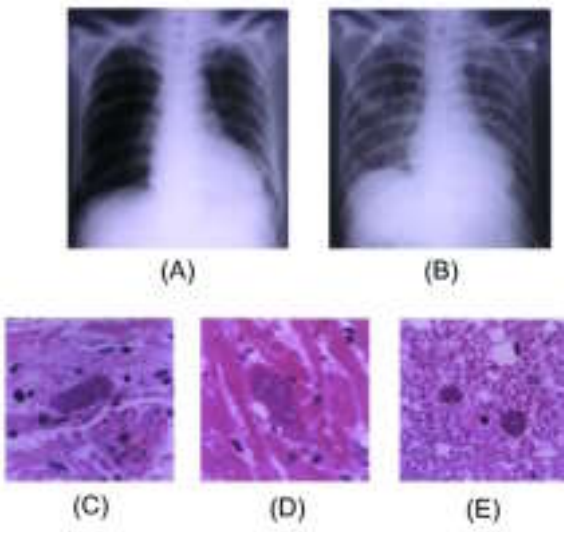

Figure 1. (A) Plain chest X-ray at the onset of respiratory symptoms demonstrated diffuse micronodular and reticular opacities. (B) Chest X-ray, taken 24h later, showed severe bilateral pulmonary infiltration. Multiple cysts of *Toxoplasma gondii* were detected in several organs including the lungs (C), heart (D), and spinal cord (E) at *post-mortem* examination, suggesting disseminated toxoplasmosis.