DNA identification of the pathogen of candidal aspiration pneumonia induced in the course of oral cancer therapy

Tetsuya Yamamoto, Eisaku Ueta, Takaai Kamatani and Tokio Osaki

Department of Oral Surgery, Kochi Medical School, Kohasu, Oko-cho, Nankoku-city, Kochi 783-8505, Japan

Aspiration of oropharyngeal bacteria and fungi is occasionally suspected in patients with pneumonia. A patient with oral carcinoma underwent chemoradioimmunotherapy and, about 4 weeks from the start of the therapy, the patient suffered from severe oral mucositis induced by chemoradiotherapy, and candidal pneumonia was subsequently induced. The candidal pneumonia was insufficiently improved by potent antifungal drugs, taking a lethal course. Randomly amplified polymorphic DNA analysis and DNA sequence examination of strains isolated from the oral cavity 1 week before the onset of pneumonia and autopsied lung revealed the identity of both strains as Candida albicans, and the DNA analysis supported aspiration of oral Candida. These results indicate that the pathogen of the pneumonia, C. albicans, was aspirated from the oral cavity and that oral Candida is easily aspirated and becomes the pathogen of pneumonia.

Methods

Case report – clinical course. In August 2001, an 88-year-old woman with advanced tongue squamous cell carcinoma (T4N2bM0) underwent neck dissection and chemoradioimmunotherapy (Fig. 1). The tongue tumour responded well to the chemoradioimmunotherapy and clinically disappeared after 3 weeks of treatment. However, severe oral mucositis was induced at the end of the third week and the body temperature elevated occasionally to approximately 38.5 °C. Blood examination revealed increases of C-reactive protein and β-D-glucan. Being suspicious of deep candidiasis, the cancer treatment was stopped. A chest X-ray photogram indicated pneumonia in the right-side inferior field. An antifungal drug (fluconazole) was then administered intravenously for about 1 month. The drug administration induced improvement of the infection. About 3 weeks later, β-D-glucan tended to elevate again and white blood cells decreased. Fluconazole and amphotericin B aerosol were concomitantly administered. The β-D-glucan level was not decreased. However, the patient’s general condition was stable, and the patient was discharged at the end of the year.

In July 2002, the metastatic lymph node recurred and the patient was again hospitalized and underwent chemoradioimmunotherapy. About 3 weeks after the secondary treatment, the β-D-glucan level increased and the pulmonary shadow spread on both sides to each upper region. Fluconazole and amphotericin B were administered but no improvement was obtained. The patient finally died of pneumonia on 16 August 2002. The body was immediately autopsied.

Identification of the causative Candida cells

Isolation of Candida. Oral mucosa-swabbed material, which was obtained 1 week before the onset of pneumonia, and lung tissue obtained at the autopsy were inoculated into Sabouraud’s dextrose agar and cultured by transplanting onto yeast extract-peptone-dextrose agar plates. Both isolates were confirmed as Candida by conventional morphological and physiological methods. A standard strain of Candida albicans, TIMM0134 (serological type A), was used as the reference.

PCR for internal transcribed spacer 2 (ITS2) region. DNA was extracted from Candida isolates by using the Dr. GenTLE kit (Takara Bio Inc.). The ITS2 region of the rRNA gene was amplified in a DNA
thermal cycler (model 9700; Applied Biosystems) by PCR using primers ITS3 (5'-TCCGTAGGTGAACTGCGC-3') and ITS4 (5'- TCCTCCGCTATTGATATGC-3') (Chen et al., 2000). Amplification was performed under the following conditions: 95 °C for 6 min followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 20 s with a final extension at 72 °C for 4 min.

**Randomly amplified polymorphic DNA (RAPD) analysis.** Two oligonucleotide primers (RAPD1, 5'-GGCGATCCCCA-3'; RAPD2, 5'-TCGCAGCCA-3') were used for RAPD analysis (Sullivan et al., 1995). Briefly, approximately 10 ng Candida DNA was added to a 0.5 ml microfuge tube containing 20 pmol oligonucleotide primer, 250 μM each of dATP, dTTP, dCTP and dGTP, 3 mM MgCl2, 2.5 U La-Taq DNA polymerase and GC buffer II (Takara Bio Inc.) in a final volume of 50 μl. Amplification reactions were performed under the following conditions: four cycles of 94 °C for 5 min, 36 °C for 5 min and 72 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min. This was followed by a final incubation at 72 °C for 10 min. The products were separated in 2 % (w/v) agarose gels containing 0.5 μg ethidium bromide ml⁻¹ and viewed on a UV transilluminator.

**rRNA gene nucleotide sequence analysis.** A PCR product of approximately 600 bp was amplified from yeast isolates using primers rRNA1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and rRNA2 (5'-GGTCCGTGTTTTCAAGACG-3') (Fell, 1993) specific for the V3 variable region of the large subunit rRNA gene. Amplification was performed under the following conditions: 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 66 °C for 90 s and 72 °C for 15 s with a final extension at 72 °C for 4 min. The amplified DNA was then sequenced in both directions using the PRISM Ready DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems). Completed sequencing reactions were electrophoresed on an ABI 310 Genetic Analyzer (Applied Biosystems), an automated fluorescence capillary electrophoresis system.

### Results

**PCR for ITS region and RAPD analysis**

The PCR products amplified from the ITS2 region appeared as a single band (339 bp). Two clinical isolates gave a PCR product identical in size to that of the standard strain, indicating that both isolates were *C. albicans* (Fig. 2a). The overall RAPD profiles of the clinical isolates were very similar to each other, but very distinct from the profiles of the standard strain (Fig. 2b). These results suggest that the isolates from the oral cavity and lung were derived from the same strain but were different from the standard strain.

**DNA sequence**

The nucleotide sequences of the amplified DNA from the V3 variable region of the large (26S) ribosomal subunit gene were identical in *C. albicans* isolated from the oral cavity and lung.
lungs, but the sequence in the standard strain was different from that in the clinical isolates at position 342, with a T in the standard strain replaced by C in the clinical strains (Fig. 3).

**Discussion**

Infections such as pneumonia and urinary tract infection are a serious problem in cancer treatment (Gucalp, 1991; Homsi et al., 2000). Of these infections, pneumonia is the most frequent and is highly lethal in cancer patients (Yamamoto et al., 2001). Bacterial and fungal invasions through the bloodstream appear to be rare and almost all bacterial and fungal pneumonias are probably caused by aspiration of the causative agents (Kikuchi et al., 1994). Treatment of head and neck carcinomas induces oropharyngeal and tracheal mucositis, and necrotic debris, which is rich in bacteria and fungi, is formed. Therefore, the risk of aspiration pneumonia is very high.

However, the causative micro-organisms of pneumonia have not yet been identified. Diagnosis of deep-organ candidiasis is generally difficult because of the lack of candidiasis-specific screening tests, although an extremely high serum level of the cell wall antigen β-D-glucan indicates candidiasis (Kondori et al., 2003). *C. albicans* is the most frequent causative species not only in deep organs and the genital tract but also in oral candidiasis (Stenderup, 1990; Chen et al., 2001). In the present case, *C. albicans* obtained from the autopsied lungs was identified as the same strain as that obtained from the oral cavity by direct DNA sequence analysis. The oral *Candida* was isolated just after the onset of pneumonia. The DNA examination results therefore suggest that *Candida* was aspirated into the lungs before the onset of pneumonia and multiplied in the lungs. The present DNA analysis is the first identification of the pathogen of candidal pneumonia. Not limited to *Candida*, multiple species of oral microorganisms have the potential to be aspirated into the lungs, possibly inducing pneumonia in compromised hosts. The present case highlights the importance of oropharyngeal hygiene for the prophylaxis of aspiration pneumonia.

**References**


---

**Fig. 2.** (a) PCR amplification of the ITS2 region. (b) RAPD analysis using primers RAPD1 and RAPD2.

**Fig. 3.** Nucleotide sequence of the V3 variable region of the large (26S) subunit rRNA genes.


