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Case Report

Liver abscess due to *Sterigmatomyces halophilus* in a boy with acute lymphoblastic leukemia☆

Toshinobu Imashioya [a], Yuichi Kodama [a, *], Tadasuke Ooka [b], Shunsuke Nakagawa [a], Takuro Nishikawa [a], Takayuki Tanabe [a], Yasuhiro Okamoto [a], Naoko Imuta [b], Mari Kirishima [c], Akihide Tanimoto [c], Toyoyasu Koriyama [d], Junichiro Nishi [b], Yoshifumi Kawano [a]

[a] Department of Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[b] Department of Microbiology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[c] Department of Pathology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[d] Department of Laboratory Medicine, Kagoshima University Hospital, Kagoshima, Japan

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ABSTRACT

We report the first case of liver abscess due to *Sterigmatomyces halophilus*. Because this pathogen grows poorly in culture medium without added salts, it was identified by sequencing analysis targeting the rRNA gene internal transcribed spacer (ITS) region. This method could be useful for pathogens that cannot be cultured using standard methods.

1. Introduction

Deep fungal infection during chemotherapy may cause serious complications in patients with acute leukemia. However, the diagnosis of fungal infection in children with leukemia is challenging owing to difficulties with specimen sampling. Furthermore, standard culture may not always be effective for detecting the pathogen due to unsuitable culture conditions and poor pathogen survival. *Sterigmatomyces halophilus* is a marine-derived fungus belonging to the phylum Basidiomycota and was discovered by Fell et al., in 1966 [1]. Here, we report a case of liver abscess due to *Sterigmatomyces halophilus S. halophilus*. To the best of our knowledge, no cases of infectious disease due to *S. halophilus* have been previously reported.

2. Case report

A 6-year-old boy without a personal or family history of cancer received a diagnosis of B cell precursor acute lymphoblastic leukemia with hypodiploidy. Therefore, allogeneic bone marrow transplantation (allo-BMT) was scheduled; the patient achieved complete remission following induction therapy. He had a fever 4 weeks post early intensification therapy, the number of neutrophils was 53/μL, and there was no abnormality upon physical examination; thus, his illness was diagnosed as febrile neutropenia and he received cefozopran. As his fever continued, we changed cefozopran to doripenem on day 3 of onset, started granulocyte-colony stimulating factor on day 4, and added micafungin (MCFG) at dose of 6 mg/kg/day on day 6. However, the fever persisted. The abdominal computed tomography scan on day 28 showed low density areas (ring diameter 3–10 mm) accompanied by ring enhancement scattered throughout the liver, which suggested a liver abscess (Fig. 1a). It was suspected that the abscess was caused by a fungal infection. We changed MCFG to liposomal amphotericin B (L-AMB) at a dose of 2.4 mg/kg/day. Nevertheless, the fever persisted and we increased the dose of L-AMB to 4.7 mg/kg/day. The

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* Corresponding author. Department of Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan.
E-mail address: yuichik@m.kufm.kagoshima-u.ac.jp (Y. Kodama).

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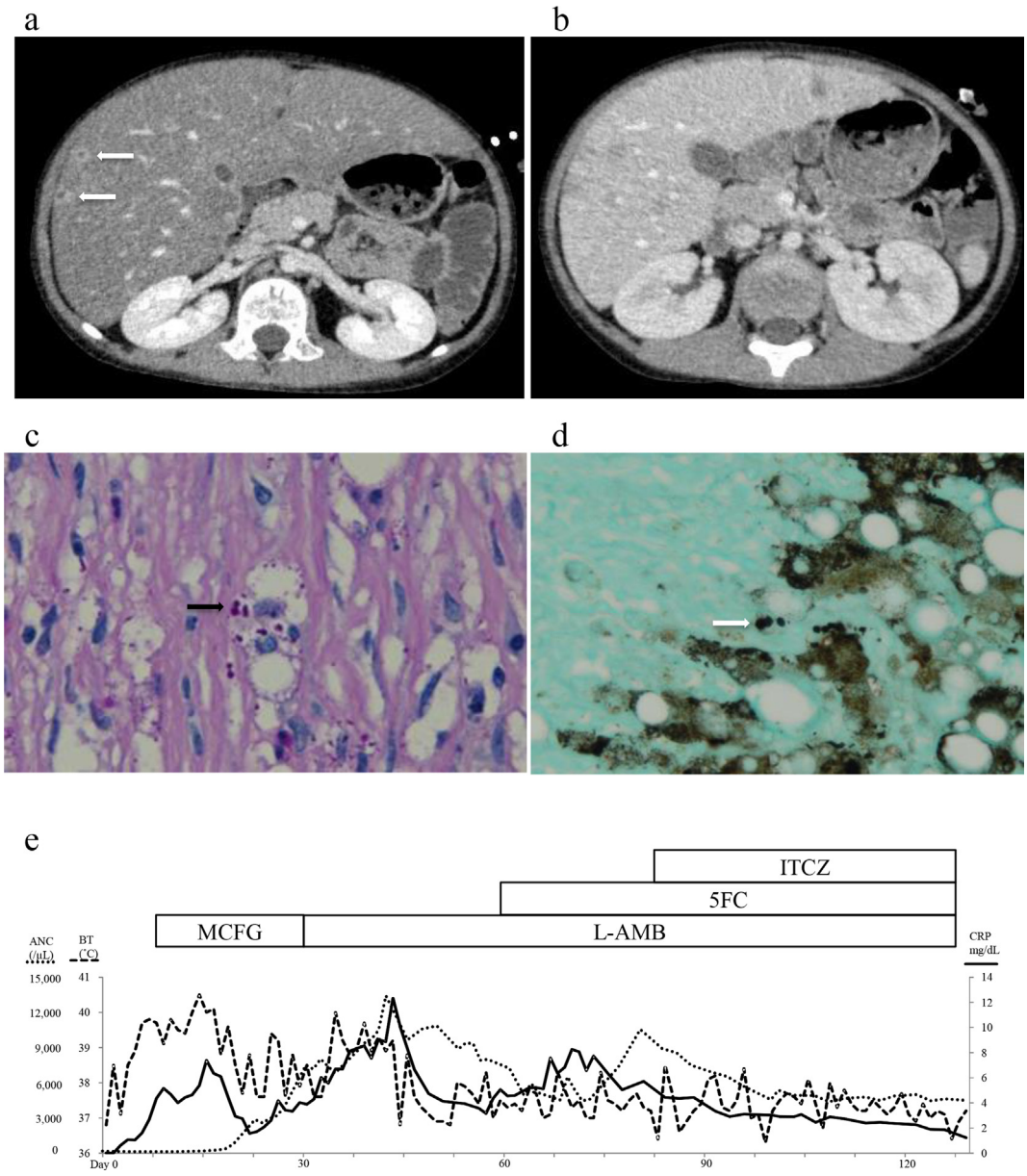

Fig. 1. a and b: Abdominal computed tomography scan on days 28 and 106. The liver abscess is designated with a white arrow (a) and was not observed in (b). **c and d:** Periodic acid-Schiff (PAS) stain and Grocott methanamine silver (GMS) stain. Yeast-like fungi are designated with black arrow (PAS positive) and white arrow (GMS), respectively. **e:** Clinical course of the patient. MCFG: micafungin, L-AMB; liposomal amphotericin B, ITCZ; itraconazole, 5-FC; flucytosine, BT; body temperature, CRP; C-reactive protein, ANC; absolute neutrophil counts.

blood culture, cytomegalovirus antigenemia, urinary *Legionella* antigen, *Aspergillus* antigen, *Candida* antigen, and C*ryptococcus neoformans* antigen were negative. The level of β-D-glucan was 16.8 pg/mL (the positive cut-off value is 11 pg/mL), which was tested using the dilution and heating turbidimetric method. A laparoscopic liver biopsy was performed on day 37. Pathological examination showed dense infiltration of inflammatory cells composed of an admixture of histiocytes, neutrophils, lymphocytes and plasma cells. Formation of epithelioid cell granulomas with focal necrosis and many denatured yeast-like fungi were seen (Fig. 1c, d); however, no bacterial or fungal growth was detected in CHROMagar *Candida* culture medium (Kanto Kagaku, Tokyo, Japan) during 14 days of incubation. Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region of the fungal ribosomal RNA (rRNA) gene was performed using the ITS1 and ITS4 universal primers [2], which yielded a single DNA band, approximately 600 bp in size. Nucleotide sequence of the PCR product was compared with deposits in GenBank and was 100% identical to that of *S. halophilus* strain CBS4609 (accession No. NR_073302.1). The sequence determined in this study has been deposited in the GenBank/EMBL/DDBJ database with the accession number LC412123. After admission, the patient regularly drank commercial water extracted from the deep sea for at least 4 months, which was delivered by his family. We ceased anti-bacterial therapy and added flucytosine (5-FC) at a dose of 100 mg/kg/day on day 67, and itraconazole (ITCZ) at a dose of 5 mg/kg/day on day 81 to L-AMB. ITCZ dose was raised to 10 mg/kg/day on day 89. Following the commencement of combination therapy, the patient's fever and the level of C-reactive protein appeared to decrease on about day 100 and the liver abscess was not visible on the computed tomography

scan on day 106 (Fig. 1b, e). Allo-BMT from a human leukocyte antigen-identical sibling donor was performed on day 150 from the onset of liver abscess. Eight months post allo-BMT, the patient had acute lymphoblastic leukemia relapses. Therefore, he received chemotherapy and haploidentical hematopoietic stem cell transplantation from his father. He is in remission and there is no recurrence of the fungal liver abscess.

3. Discussion

The patient had a liver abscess due to *S. halophilus*. To date, there have been no reports of patients with infectious disease caused by *S. halophilus*. The fungal pathogen *S. halophilus* was first discovered in sea water [1], and according to initial reports, it grows poorly in culture medium without added salt. However, we did not add salt in the culture media because we were not aware that the microorganism required salt for growth, before it was identified as *S. halophilus*. This is likely one reason why no pathogens were detected in the liver abscess specimen using standard culture methods.

The ITS is a region between the genes encoding the 18S, 5.8S, and 28S rRNA in eukaryotic ribosomal DNA [3] and the sequences are diverse among species. With regards to fungi, several ITS sequence databases such as the UNITE [4], the ISHAM-ITS [5], and the established GenBank RefSeq database are available to assist with species identification. The sequence-based identification method can work in the absence of live cell culture if template DNA for PCR amplification is available in patient specimens [6].

S. halophilus exists in sea water [1]. After he developed a liver abscess, we noticed the fact that he drank commercial water extracted from deep sea and examined this water. No fungi in this water were detected by culture in Sabouraud's broth containing 5% NaCl. In general, *S. halophilus* does not exist in the hospital environment. Therefore, we were unable to determine the root of the infection.

There is no information regarding effective antifungal therapy for *S. halophilus* because this fungus has not been detected in clinical samples. MCFG, L-AMB, ITCZ, and 5-FC were used as antifungal drugs in our patient. Fig. 1c shows the clinical course in this case; ultimately, the patient's fever and the level of C-reactive protein appeared to decrease at the start of ITCZ treatment. However, it was unclear whether ITCZ alone or the combination therapy, including L-AMB, 5-FC, and ITCZ, was effective in treating this infection. As L-AMB denatured the fungus based on pathological examination, we employed combined antifungal therapy in view of the synergistic effect of these three antifungal drugs on *S. halophilus*. Combined antifungal therapy has been reported for *Cryptococcus* meningitis and *Aspergillus* infections [7,8]. Moreover, Candoni et al. [9] have suggested that antifungal combination therapy can be used in hematologic patients suffering from disseminated infection with rare fungi. Future basic and clinical studies are required to identify specific antifungal therapies for *S. halophilus* infection.

In conclusion, we report the first case of a liver abscess due to *S. halophilus* diagnosed using PCR, targeting the ITS, during acute lymphoblastic leukemia treatment. The PCR-based diagnostic method targeting the ITS could be a particularly useful test for this fungus, which is difficult to culture using standard methods.

Conflicts of interest

The authors confirm that there are no conflicts of interests to declare.

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