【 CASE REPORT 】

Successful Treatment of Intestinal Mycosis Caused by a Simultaneous Infection with *Lichtheimia ramosa* and *Aspergillus calidoustus*

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Abstract:
A 53-year-old woman was hospitalized due to septic shock after developing pneumococcal pneumonia after undergoing esophageal cancer surgery. Her transverse colon became perforated after receiving antimicrobial chemotherapy; therefore, emergency subtotal colectomy was performed. Fungi detected in both her colon tissue and a drainage sample indicated intestinal mucormycosis. Early intensive treatment with high-dose liposomal amphotericin B was successful, and she was subsequently discharged from the hospital. The fungal isolates were identified to be *Lichtheimia ramosa* and *Aspergillus calidoustus* via gene sequencing using pan-fungal primers as well as species-specific primers against elongation factor 1 and beta-tubulin for detecting *Lichtheimia* and *Aspergillus*, respectively.

Key words: intestinal mucormycosis, *Lichtheimia ramosa*, *Aspergillus calidoustus*, zygomycosis, fungal infection

(Intern Med 57: 2421-2424, 2018)
(DOI: 10.2169/internalmedicine.0254-17)

Introduction

Mucormycosis, previously called zygomycosis, is a life-threatening infection caused by filamentous fungi that belong to the order Mucorales. Mucormycosis mainly occurs in immunocompromised individuals, and most commonly affects the sinuses or lungs following the inhalation of fungal spores. Gastrointestinal mucormycosis is relatively rare and accounts for only 4-7% of all mucormycosis cases (1, 2). *Lichtheimia ramosa*, formerly known as *Absidia ramosa,* is an uncommon human pathogen belonging to the order *Mucorales* (3). Separately, *Aspergillus calidoustus* was once identified as *Aspergillus ustu*s, another rare pathogen, but it was reclassified as a new species following the discovery of distinct characteristics by Varga et al. in 2008 (4). We herein describe a rare episode of a patient with invasive intestinal mycosis caused by a simultaneous infection with *L. ramosa* and *A. calidoustus*.

Case Report

A 53-year-old woman was admitted to Osaka Police Hospital with septic shock after having contracted pneumococcal pneumonia. She had undergone surgery for esophageal cancer 2.5 years prior to this presentation. Her gastric tube, which had been reconstructed by esophagogastrostomy, had become obstructed and her nutrition status was poor (body mass index: 11.8 kg/m^2). She had a history of smoking (10 cigarettes/day) and alcohol consumption (4 glasses of wine/day). She did not have diabetes mellitus and tested negative for the human immunodeficiency virus antibody. Penicillin-susceptible *Streptococcus pneumoniae* was isolated from hemoculture, and she was treated with meropenem (2 g/

day), immunoglobulin (5 g/day), and hydrocortisone (200 mg/day) for 7 days under ventilation and continuous hemodiafiltration for an invasive pneumococcal infection.

On day 12, abdominal radiography and computed tomography revealed free air in the peritoneal cavity (Fig. 1), and emergency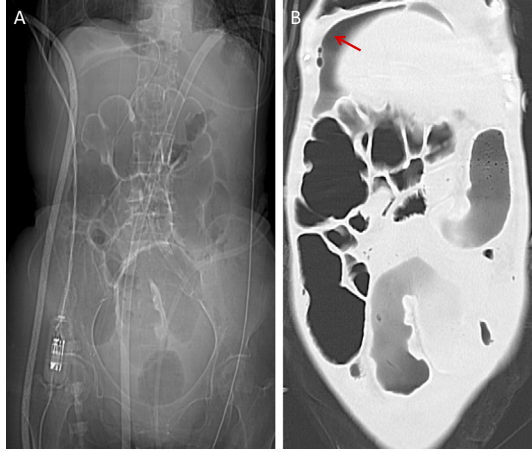 surgery was performed for a transverse colon perforation. Multiple ulcers were observed throughout the colon along with a single perforation associated with thinning of the intestinal wall. Hence, we performed subtotal colectomy from the cecum to the upper rectum and created a stoma. The formation of colonic ulcers and fistula was confirmed by histopathological examinations (Fig. 2A and B). A 38℃ fever and high degree of inflammation were sustained after surgery, although no lung field lesions were observed. Meropenem, teicoplanin, metronidazole, and caspofungin were administered to treat the perforation peritonitis. Fever and rectal bleeding persisted on day 16, and multiple ulcers and bleeding in the residual rectum were confirmed by colonoscopy. *Aspergillus* spp. and other filamentous fungi were isolated from drainage from Douglas' pouch. An *Aspergillus* galactomannan antigen test was positive (0.5 pg/mL). On day 20, filamentous fungi were detected in almost all ulcers in the colon tissue collected during surgery (Fig. 2C-E). Gastrointestinal type mucormycosis and aspergillosis were suspected, and liposomal amphotericin B (L-AMB) administration was started at 8 mg/kg. Subsequently, the fever subsided, filamentous fungus was no longer detected in the drainage fluid from Douglas' pouch, and the inflammatory response both diminished. The multiple ulcers markedly improved along with the patient's general condition, and she was discharged from the hospital
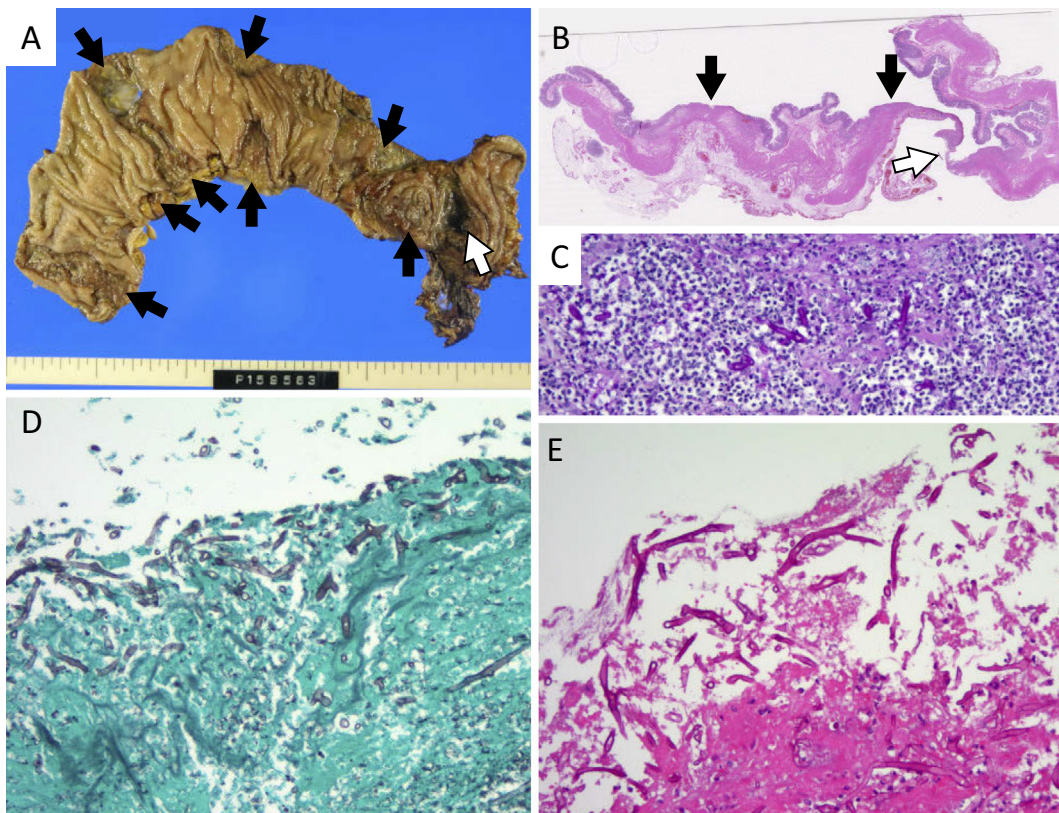

Figure 1. Abdominal radiography and computed tomography findings. Radiograph (A) and computed tomography scan (B) showing the entire colon filled with gas and free air in the suprahepatic area (red arrow).

Figure 2. Macroscopic and microscopic examination of the removed colon. A and B: Multiple ulcers (black arrows) and a fistula (white arrow) were recognized macroscopically (A) or under loupe magnification (B). C-E: A microscopic examination revealed filamentous fungi in the ulcerative region (Magnification: ×400). The tissue specimens were stained with Hematoxylin and Eosin staining (B and E), periodic acid Schiff (C), or Grocott methenamine silver (D).
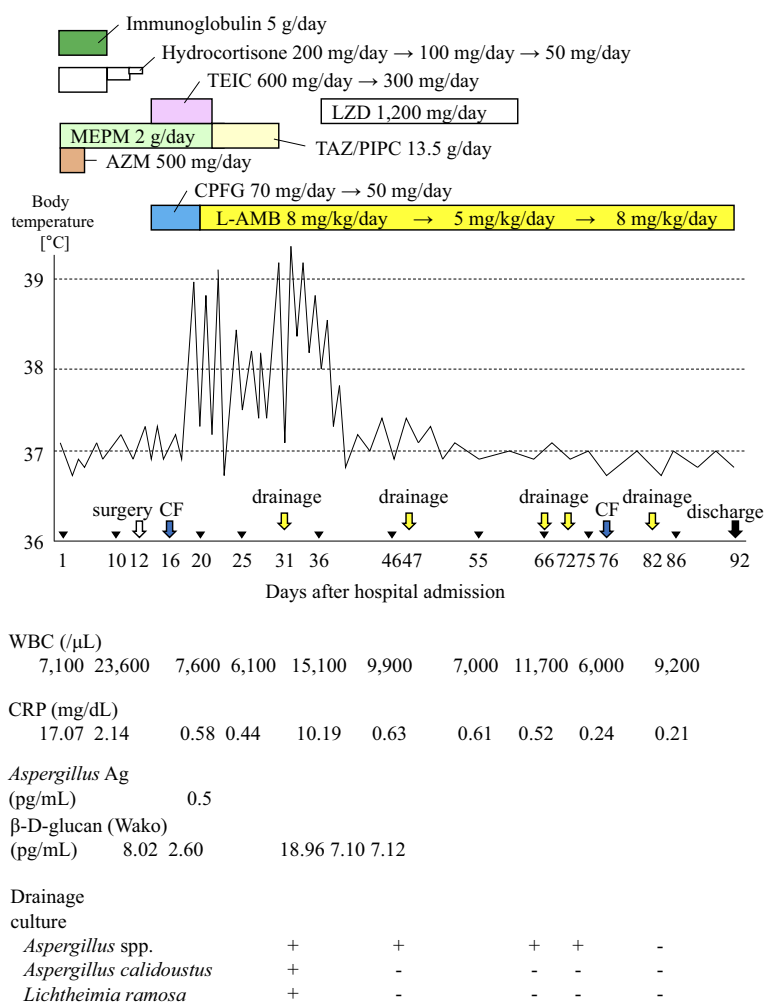
Immunoglobulin 5 g/day
Hydrocortisone 200 mg/day → 100 mg/day → 50 mg/day
TEIC 600 mg/day → 300 mg/day
LZD 1,200 mg/day
MEPM 2 g/day
TAZ/PIPC 13.5 g/day
AZM 500 mg/day
CPFG 70 mg/day → 50 mg/day
L-AMB 8 mg/kg/day → 5 mg/kg/day → 8 mg/kg/day

Body temperature [°C]

39
38
37
36

surgery CF drainage drainage drainage CF drainage discharge

Days after hospital admission
1 10 12 16 20 25 31 36 46 47 55 66 72 75 76 82 86 92

WBC (/μL)
7,100 23,600 7,600 6,100 15,100 9,900 7,000 11,700 6,000 9,200

CRP (mg/dL)
17.07 2.14 0.58 0.44 10.19 0.63 0.61 0.52 0.24 0.21

Aspergillus Ag (pg/mL)
0.5

β-D-glucan (Wako) (pg/mL)
8.02 2.60 18.96 7.10 7.12

Drainage culture

Aspergillus spp.	+	+	+	+	-
Aspergillus calidoustus	+	-	-	-	-
Lichtheimia ramosa	+	-	-	-	-

Figure 3. The clinical course of the patient. Fever subsided after intensive liposomal amphotericin B (L-AMB) treatment. Filled inverted triangles (▼) indicate the date when the WBC and CRP data were obtained. AZM: azithromycin, CF: colonofiberscopy, CPFG: caspofungin, LZD: linezolid, MEPM: meropenem, TAZ/PIPC: tazobactam/piperacillin, TEIC: teicoplanin

(Fig. 3).

Two filamentous fungi that were isolated from the Douglas pouch drainage sample were further investigated. Each of their macroscopic and microscopic characteristics was distinct. Gene sequencing using panfungal primers against the internal transcribed spacer and D1/D2 of the 26S nuclear ribosomal RNA gene revealed one strain to be *Lichtheimia* spp. and the other to be *Aspergillus* spp. Further analysis using species-specific primers targeting elongation factor 1α of *Lichtheimia* and β-tubulin of *Aspergillus* identified the species as *L. ramosa* (100% match in a 407 bp region) and *A. calidoustus* (100% match in a 343 bp region), respectively. The specific primers for internal transcribed spacer were: for ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC); for D1/D2: NL1 (GCATATCAATAAGCGGAGGAAAA) and NL4 (GGTCCGTGTTTCAAGACGG); for elongation factor 1α: EF1alpha M1 (GCTGGTATCTCCAAGGATGG) and EF1alpha M2 (CGACGGACTTGACTTCRGTGG); and for β-tubulin: bTub1 (AATTGGTGCCGCTTTCTGG) and bTub2 (AGTTGTCGGGACGGAATAG) (3, 5-7).

Discussion

We successfully treated a patient with intestinal mycosis caused by a coinfection with *L. ramosa* and *A. calidoustus*. These species are often isolated from patients who have undergone transplantations (4, 8) and are currently regarded as emerging fungal pathogens. Although the epidemiological status of mucormycosis in Japan is unclear, autopsy case studies indicate that Mucorales fungi are the third most frequent cause of invasive fungal infection (9, 10). A rising incidence rate of mucormycosis has been reported in European countries, with *Lichtheimia* spp. being one of the most frequently involved species (11). Most patients who develop such infections have serious background diseases or other conditions such as hematological malignancies or trauma/surgical wounds, or else are receiving immunosuppressant therapy (11). The risk factors in our patient included alcohol consumption, malnutrition, immunocompromised status induced by invasive pneumococcal disease, broad-spectrum antibacterial drug administration, and high-dose steroid ther-

apy. Because she initially had no gastrointestinal symptoms such as abdominal pain and melena, the time of gastrointestinal mycosis onset was unknown. A histopathological analysis indicated the presence of filamentous fungi, but did not clearly differentiate between *Aspergillus* and *Mucorales* spp. The endothelial cells exhibited slightly positive immunostaining for cytomegalovirus, but the patient's blood tested negative for C7-HRP at admission.

This episode was rare in that the patient was simultaneously infected with both *L. ramosa* and *A. calidoustus*. Moreover, this is the first reported case of gastrointestinal *A. calidoustus* infection to our knowledge, although one patient with gastrointestinal mucormycosis caused by *L. ramosa* was previously reported (12). An accurate diagnosis of mycosis requires the genetic investigation of fungal isolates, which is difficult to perform at medical facilities lacking specialized laboratories. Therefore, a significant number of patients with *A. calidoustus* infection may have been overlooked in the past, and its incidence rate may be much higher than the currently accepted estimate.

Mucormycosis has a high mortality rate mainly owing to its rapid progression and challenging premortal diagnosis (11, 13). *Mucorales* spp. are generally difficult to cultivate from tissue specimens. Moreover, many patients are ineligible for surgery because of their poor physical condition. Therefore, the proportion of patients with confirmed premortal diagnoses of mucormycosis is quite small; a relatively large number of patients are diagnosed for the first time at autopsy. To achieve a successful outcome, clinicians must often commence antifungal therapy based on a presumptive diagnosis at an early stage of infection. High-dose L-AMB is very effective against most *Mucorales* species with a few exceptions, such as *Cunninghamella bertholletiae* (11). In our patient, emergency surgery was performed immediately, and fungal culturing and histopathological examinations were conducted; intestinal mucormycosis was suspected based on these clinical findings, and high-dose L-AMB therapy was therefore initiated. We therefore would like to emphasize that an early presumptive diagnosis and the immediate initiation of antifungal therapy are critical for achieving a favorable outcome in such patients.

The authors state that they have no Conflict of Interest (COI).

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