Disseminated *Rhizomucor pusillus* infection in a patient with acute myeloid leukemia successfully treated with extensive surgical debridement and long-term liposomal amphotericin B

Yusuke Toda¹*, Yuya Nagai¹, Noriyuki Abe², Toshiyuki Hata³, Tatsuo Nakagawa⁴, Gen Honjo⁵, Satoshi Noma⁶, Takashi Misaki⁷, Hitoshi Ohno¹

¹Department of Hematology, Tenri Hospital; ²Department of Clinical Laboratory Medicine, Tenri Hospital; ³Department of Gastrointestinal Surgery, Tenri Hospital; ⁴Department of Thoracic Surgery, Tenri Hospital; ⁵Department of Diagnostic Surgical Pathology, Tenri Hospital; ⁶Department of Radiology, Tenri Hospital; ⁷Isotope center, Tenri Hospital

Received 2018/3/12; accepted 2018/4/18; released online 2018/7/1

A woman in her fifties was diagnosed with hypoplastic acute myeloid leukemia (AML). Soon after induction treatment, she developed pulmonary infiltrates in the lower lobe of the right lung, which shortly evolved into disseminated disease involving the left thorax, spleen, and liver. Although she had this life-threatening complication, complete hematological response was achieved after salvage and consolidation treatments. Positron emission tomography combined with computed tomography demonstrated formation of multiple abscesses, and biopsy of the liver revealed fungal hyphae, the morphology of which suggested mucormycetes; however, culture yielded no growth. As involved lesions remained unchanged or even deteriorated after >4 months of liposomal amphotericin B treatment, and because the underlying AML was in remission with normal hematopoietic recovery, the patient underwent carefully organized two-stage surgery to debride infected organs and tissues. The surgical specimens were composed of necrotic debris, but some areas contained hyphae with similar morphological features as those of the liver biopsy. The causative fungal pathogens were identified as *Rhizomucor pusillus* by a polymerase chain reaction-based molecular method. Currently, she regularly visits the out-patient clinic and is free from leukemia. This report suggests that extensive surgical debridement should be considered for disseminated mucormycosis when clinically possible.

**Keywords:** acute myeloid leukemia, mucormycosis, surgical debridement, liposomal amphotericin B, *Rhizomucor pusillus*

**INTRODUCTION**

Mucormycosis, previously referred to as zygomycosis, has emerged as the third most common invasive fungal infection after aspergillosis and candidiasis that develops in patients with hematological malignancies and/or recipients of hematopoietic stem cell transplantation (HSCT).¹⁻³ The disease incidence has increased primarily because of increasing numbers of patients who receive...
aggressive chemotherapy and/or undergo HSCT. However, it is also possible that prophylactic use of antifungal agents against Aspergillus and Candida has caused the emergence of rare fungi. Indeed, voriconazole prophylaxis has been reported to be an independent risk factor for mucormycosis.

Mucormycosis most commonly presents with pulmonary infection and often progresses to disseminated disease, resulting in a life-threatening condition. Although the mainstay of diagnosis is isolation of mucormycetes in culture and the treatment of choice is amphotericin B (AMPHB), biopsy is often difficult due to thrombocytopenia and cultures may fail to grow a fungal pathogen, delaying the initiation of appropriate antifungal therapy. Concurrently, clinicians must treat the underlying hematological condition and consider timely surgical intervention, requiring a multidisciplinary approach.

We report here a case of disseminated mucormycosis that developed in a patient with acute myeloid leukemia (AML), and the fungal pathogen was identified by a molecular technique. The patient finally underwent extensive surgical debridement of the affected organs and tissues during AML remission in combination with long-term administration of liposomal AMPHB (L-AMB). The course, histopathological findings of the biopsy and surgical specimens, and molecular identification of the causative pathogen are described.

**CASE REPORT**

A woman in her fifties, who had been followed-up for a non-functioning adrenal tumor at the Department of Endocrinology of our hospital, was referred to the Department of Hematology for prolonged neutropenia. Her hemoglobin level on admission was 10.4 g/dL, white cell count was $1.77 \times 10^3 / \mu L$, and platelet count was $197 \times 10^3 / \mu L$. The white cell differential was 82.0% lymphocytes, 1.5% monocytes, 1.0% eosinophils, and 15.5% segmented neutrophils (absolute neutrophil count, 274/µL). Blood chemistry values were normal. The bone marrow (BM) exhibited marked hypocellularity with 42.6% blasts, which were negative for peroxidase cytochemistry, and exhibited the CD13+, CD33+, CD34+, and CD117+ immunophenotype by flow cytometry. No cytogenetic abnormality was found. The mRNA level of the Wilms’ tumor-1 gene was $9.9 \times 10^2$ copies/µg RNA. She was negative for exposure to contaminated materials.

She was diagnosed with hypoplastic AML. Initial induction treatment with daunorubicin and cytarabine were ineffective, but circulating blasts markedly increased. We thus treated her with high-dose cytarabine followed by mitoxantrone and etoposide, successfully eradicating leukemia blasts with hematopoietic recovery. We then performed one cycle of MEC (mitoxantrone, etoposide, and intermediate-dose cytarabine) for consolidation treatment (Figure 1).

The treatment course, however, was complicated by severe prolonged infection, temporarily requiring respiratory and hemodynamic monitoring and hemodialysis in the intensive care unit (Figure 1). On day 30 of admission, she developed pulmonary infiltrates in the lower lobe of the right lung exhibiting radiographic features of fungal infection (Figure 2A), which shortly evolved into disseminated disease involving the left thorax, spleen, and liver, in association with accumulation of bilateral pleural fluid and enlargement of the bilateral kidneys (days 35 and 48, Figure 2B). Positron emission tomography combined with computed tomography (PET/CT) on day 100 revealed hypermetabolic lesions with ring-shaped morphology in the right lung, spleen, and liver, indicating formation of multiple abscesses in these organs (Figure 2C). Repeated blood cultures as well as tests for β-D-glucan and Aspergillus antigen were negative. An ultrasound-guided liver biopsy performed 1 week after the PET/CT scan (day 106) suggested filamentous fungal infection (Figure 3), but the culture yielded no growth. The level of C-reactive protein (CRP) was initially elevated in association with leukemia progression, but it remained high after hematopoietic recovery. The level of serum alkaline phosphatase (ALP) was markedly elevated to the...
peak value of 2,922 U/L on day 45, likely reflecting the liver involvement of AML and fungal infection (Figure 1).

We administered antibiotics targeting both gram-positive and -negative bacteria. Regarding antifungal agents, fluconazole for initial prophylactic use was changed to voriconazole on day 30, and finally to intravenous L-AMB on day 35, according to the CT studies (Figures 1 and 2). After >4 months of L-AMB therapy at 5 mg/kg per day, infected lesions remained unchanged or deteriorated on sequential CT scans on days 147 (Figure 2D) and 164. As her leukemia remained in remission, her clinical state was considered to allow for surgical resection of the involved organs and tissues. The hemoglobin level on day 178 was 9.6 g/dL, the white cell count was $6.02 \times 10^3/\mu L$ with 62.0% neutrophils (absolute neutrophil count, $3.73 \times 10^3/\mu L$), the platelet count was 184 $\times 10^3/\mu L$, ALP was 1,084 U/L, creatinine was 0.9 mg/dL, and CRP was 1.53 mg/dL. Toxicities of long-term L-AMB were not significant, except for mild hypokalemia.

The Departments of Thoracic Surgery and Gastrointestinal Surgery employed a two-stage surgical operation with a 3-week interval. The first-stage on day 183 consisted of open thoracotomy and laparotomy for partial resection of the lower lobe of the left lung, splenectomy, partial resection of the stomach, and debridement of the left subphrenic abscess. The second-stage on day 204 consisted of thoracoscopic partial resection of the lower lobe of the right lung, and open

![Figure 1](image-url). Clinical course during the treatment of AML until day 114 of admission. The counts of white blood cells (WBC), neutrophils, leukemia blasts (blasts), and platelets (PLT) in the blood, and the values of C-reactive protein (CRP) and alkaline phosphatase (ALP) are shown. Chemotherapeutic agents for the treatment of AML, and antibacterial and anti-fungal drugs are shown at the top. Abbreviations: DNR, daunorubicin; Ara-C, cytarabine; HD–Ara-C, high-dose cytarabine; Mit, mitoxantrone; VP-16, etoposide; MEC, mitoxantrone, etoposide, and cytarabine; VCM, vancomycin; LVFX, levofloxacin; CFPM, cefepime; PIPC/TAZ, piperacillin tazobactam; MEPM, meropenem; CPFX, ciprofloxacin; GRNX, garenoxacin; FLCZ, fluconazole; VRCZ, voriconazole; L-AMB, liposomal amphotericin B; and ICU, intensive care unit.
Multidisciplinary treatment of mucormycosis in AML

laparotomy for debridement of the right subphrenic abscess and partial resection of the liver. Intraoperative findings were consistent with multiple intra-thoracic and abdominal abscesses associated with marked adhesion. Her postoperative course was uneventful. She was treated with L-AMB for an additional 3 months, followed by oral itraconazole at 200 mg per day. Currently, she regularly visits the out-patient clinic and is free from leukemia.

HISTOPATHOLOGY OF THE LIVER BIOPSY AND SURGICAL SPECIMENS

The liver biopsy specimen was composed of liver parenchyma and necrotic tissues, the latter of which contained fungal hyphae. The hyphae, which were positive for periodic acid-Schiff and Grocott staining, were broad in width and thin-walled with rare septa, and both acute and right-angle branching, suggesting mucormycetes (Figure 3).

The specimen resected at the first-stage surgery included devitalized lesions extending from the lung base through the diaphragm to the spleen and the serosal surface of the stomach (Figure 4). Microscopic examination of the representative pathological sections revealed marked granulation tissue formation with necrotic debris (Figure 4), whereas areas containing hyphae with similar morphological features as those of the liver biopsy specimen were found within the necrotic debris (Figure 5). The presence of fungal elements in the vessels was confirmed by Elastica van Gieson staining (Figures 5B and D). The second-stage

Figure 2. Imaging studies. (A) Transverse CT images of the chest using a lung window setting on day 30 of admission indicating the initial pulmonary lesion that developed in the S6 segment of the right lung (a to c). The lesion was surrounded by a circular area of ground-glass attenuation, representing the halo sign. Branches of the B6 bronchus were penetrating the infiltrates. (B) Transverse CT images on days 35 and 48 using a soft tissue window setting (a to c) showing deterioration of the lung lesion containing air, accumulation of pleural fluid, involvement of the liver and spleen (arrows in b), and enlargement of the bilateral kidneys (c). (C) FDG-PET/CT images on day 100 demonstrating ring-shaped accumulation of the tracer in the right pulmonary, left subphrenic/splenic, and hepatic lesions. The anterior view of the maximum intensity projection image (a) and representative axial images of the body (b to d) are shown. (D) Transverse or sagittal CT images of the body with contrast media on day 147 highlighting the ring-enhancing lesions (a through f). Low density areas within the liver in e were considered to be liver cysts. The arrow in f indicates the left adrenal tumor with multiple calcification dots.
specimen was composed of devitalized and cavitary lung lesions, but no hyphae were noted under microscopy.

**IDENTIFICATION OF THE CAUSATIVE FUNGUS BY MOLECULAR METHOD**

To molecularly clarify the fungal pathogen that caused the disseminated infection, we employed the comparative sequence-based identification strategy. DNA was extracted from the surgical materials of both first- and second-stage surgeries, and was subjected to polymerase chain reaction (PCR) amplification targeting the ribosomal RNA gene (rDNA), i.e., the internal transcribed spacer (ITS) region, encompassing the ITS1, 5.8S rDNA, and ITS2, and the D1/D2 domain of the 28S rDNA (Figure 6A). As shown in Figure 6B, both ITS and D1/D2 regions were successfully amplified, and the nucleotide

---

**Figure 3.** Histopathology of the liver biopsy. (A and B) Hematoxylin and eosin (H&E) staining. A, low-power magnification image showing necrotic tissue (top) and liver parenchyma (bottom); B, high-power magnification image of the area indicated by the rectangle in A, showing fungal hyphae. (C) Periodic acid-Schiff staining. (D) Grocott staining. Original magnification: A, ×40; B to D, ×400.

**Figure 4.** Surgical specimens from the first-stage surgery. (A) Gross appearance of the specimens. L, lung; St, stomach; Sp, spleen; Sa, subphrenic abscess. (B to D) Loupe images of representative sections (H&E staining). B, lung (top) and diaphragm (bottom); C, spleen; D, stomach (top) and serosa (bottom). Original magnification: B to D, ×1.
Multidisciplinary treatment of mucormycosis in AML

Figure 5. Histopathology of the necrotic debris of the spleen exhibiting fungal hyphae. (A and B) Loupe image. A, H&E staining; B, Elastica van Gieson staining. (C to F) Low- and high-power magnification images indicated by the rectangles in A and B. C, H&E staining; D, Elastica van Gieson staining; E, H&E staining; F, Grocott staining. (G and H) Another area containing hyphae showing similar morphological features as those of the liver biopsy. G, H&E staining; H, Grocott staining. Original magnification: A and B, ×1; C and D, ×100; E to H, ×200.

sequencing data of the PCR products were submitted to the BLAST tool of NCBI (http://www.ncbi.nlm.nih.gov/blast/), demonstrating 100% or 99% sequence homology with those of *Rhizomucor pusillus* (*R. pusillus*) registered in the database (Figures 6C and D).

**DISCUSSION**

Here, we describe a patient with AML who developed mucormycosis that spread from the primary pulmonary infection and rapidly progressed to dissemination soon after the induction chemotherapy. Although she had this life-threatening complication, complete hematological remission was achieved in response to the salvage and consolidation treatments. After long-term treatment with L-AMB, affected organs and tissues were successfully resected by carefully organized surgery. The causative fungus was identified at the species level as *R. pusillus* by a molecular method. This report suggests that
Figure 6. PCR-based molecular assay to identify the causative fungal pathogen. (A) Schematic diagram of the ITS and D1/D2 regions of fungal rDNA, and the positions of PCR primers. The sequences of the primers, designed to amplify broad-range fungal ITS and D1/D2 regions, are indicated on the bottom. (B) Ethidium bromide-stained gel electrophoresis of the PCR products corresponding to the ITS and D1/D2 regions. DNA was extracted from the subphrenic-infected lesion from the first-stage surgery and the cavitary lung lesion from the second-stage surgery, and was 10× or 100× diluted to eliminate non-specific amplification. No specific PCR products were obtained from sample 2. (C) Alignment of the nucleotide sequences obtained (query) with those registered in the database (sbjct), which was first listed in each NCBI BLAST search. Vertical lines indicate the nucleotide identity. Left, sequences of the D1/D2 region from the first-stage surgery; right, ITS region from the second-stage surgery.

Methods: DNA was extracted from 200 µL of surgical materials by the QIAamp DNA Tissue Kit (QIAGEN, Hilden, Germany). The PCR mixtures in a volume of 20 µL comprised 2 µL of template DNA, 0.2 µL of 50 mmol stock solution of each primer, and 10 µL of 2× GoTaq® master mix (Promega, Fitchburg, WI, USA). PCR was performed in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Inc. Forester City, CA, USA) and run with a temperature profile of 7 min at 95°C, followed by 35 cycles of 20 sec at 94°C, 20 sec at 60°C, and 40 sec at 72°C. The 35 cycles were followed by 7 min at 72°C. After the PCR was completed, a 4-µL aliquot of each PCR product was run on a 1.5% agarose gel with ethidium bromide staining to confirm amplification. The PCR products were excised from the gel and purified using a NucleoSpin® Gel and PCR Clean-up Kit (Takara Bio, Otsu, Shiga, Japan). Direct sequencing of the products was outsourced to Sigma-Aldrich Japan (Tokyo, Japan).
extensive surgical debridement should be considered for disseminated mucormycosis, when clinically possible.

The genera in the order Mucorales that are most commonly found in human infections are *Rhizopus*, *Mucor*, and *Rhizomucor*, whereas *Cunninghamella*, *Absidia*, *Saksenaea*, and *Apophysomyces* are less commonly implicated in infection.\(^1,8,9\) *R. pusillus* is an unusual species of the genus *Rhizomucor*,\(^9\) and is ubiquitous in air, soil, water, and organic matter, including a variety of food items.\(^8,9\) *R. pusillus* is not commonly associated with human disease, but typically causes opportunistic infections in susceptible hosts.\(^8,9\) Although it has lower pathogenicity than other mucormycetes in human hosts, *R. pusillus* is still angioinvasive, resulting in thrombosis, hemorrhage, and tissue infarction.\(^8,9\) In a review of reports in English, of 465 cases with culture-confirmed mucormycosis that were reported between 1940 and 2003, *R. pusillus* was isolated in 19 (4\%) cases in both adults and children, and most of the cases were associated with marked neutropenia.\(^8,10\) Another literature review found a total of 22 cases of mucormycosis with sufficient clinical information to identify *R. pusillus* as the definite infecting agent.\(^9\) Of these, 16 (73\%) cases developed in the setting of hematological malignancy and/or HSCT, or renal transplantation. The disease presented with soft tissue, pulmonary, rhino-orbito-cerebral, or intra-abdominal infection, whereas disseminated disease originating from pulmonary or cutaneous infection that progressed to systemic infection occurred in 9 (41\%).\(^8\) The mortality rate was as high as 46\% (10 of 22).\(^9\)

In the major treatment guidelines, early initiation of AMPHB treatment is recommended for any form of mucormycosis that develops in patients with hematologic malignancies,\(^3,11,12\) as delayed AMPHB therapy can lead to a two-fold increase of mortality.\(^13\) In this patient, prophylactic use of fluconazole and switching to voriconazole (Figure 1), both of which are inherently ineffective for *R. pusillus*,\(^9\) delayed the initiation of L-AMB, potentially leading to the development of disseminated mucormycosis. Indeed, breakthrough pulmonary and disseminated *R. pusillus* infection was observed in patients with hematological malignancies and in recipients of solid organ transplants who received fluconazole- or voriconazole-based prophylaxis.\(^14,15\) On the other hand, higher doses of L-AMB, i.e. up to 10 mg/kg every 24 hours, or the combination of L-AMB with posaconazole has been reported to control mucormycetes infection.\(^3,11,12\) However, both >5 mg/kg L-AMB and posaconazole are not approved in our country.

Extensive surgical debridement of mucormycetes-infected organs and/or tissues in combination with effective antifungal agents has been performed for patients with hematological malignancies and/or those who underwent HSCT.\(^3\) The organs for surgical intervention included the lungs,\(^16\) liver,\(^17\) gastrointestinal tract,\(^18\) brain,\(^19\) and skin.\(^20\) Treatment was performed during leukemia remission or neutropenic periods after chemotherapy. However, as the therapeutic approach to mucormycosis is multidisciplinary, consisting of not only antifungal agents and surgical debridement but also correction of the underlying condition predisposing the patient to the fungal infection, control of underlying hematological malignancy is critical.\(^3\) For the present patient, we performed salvage treatment containing high-dose cytarabine even with the severe infectious complication and deterioration of cardiopulmonary/renal function, resulting in the eradication of leukemia cells. Successful surgical debridement was likely possible because of the remission of underlying AML with normal hematopoietic recovery.

The clinical diagnosis of mucormycosis is challenging, as cultures often fail to grow a fungal pathogen, no serum test is currently available, and histopathological identification of an organism with a structure typical of Mucorales may provide the only evidence of infection.\(^3\) PCR-amplification of the ITS and/or D1/D2 region of rDNA, and sequence comparison with available database libraries provide a reliable tool for identification of mucormycetes at the genus and species levels.\(^6,7\) In two comparison studies between culture-based and PCR-
based molecular assays of 6 and 13 biopsy samples with histopathologically-confirmed mucormycetes, only 2 (33%) and 8 (62%) samples yielded positive cultures, whereas PCR identified the genus/species in all 6 and 13 samples, respectively.\(^1\)\(^\text{2}\)\(^1\)\(^\text{2}\) Thus, as observed in the present case, the PCR-based technique is promising for establishing the diagnosis of mucormycosis when the culture is negative. In a pediatric HSCT recipient, \textit{R. pusillus} DNA was serially detectable in the serum by quantitative PCR despite negative blood culture for fungi.\(^2\)^\(^3\)

**ACKNOWLEDGEMENTS**

The authors wish to thank the nursing staff of the relevant wards of Tenri Hospital for their sincere contribution to the treatment.

**REFERENCES**

急性骨髄性白血病患者に発症し、広範囲外科的デブリドマンとリポソーマルアムホテリシン B の長期投与が奏効した播種性 Rhizomucor pusillus 感染症の一例

戸田有亮 1, 永井雄也 1, 阿部教行 2, 畑 俊行 3, 中川達雄 4, 本庄 原 5, 野間惠之 6, 御前 隆 7, 大野仁嗣 1

1 天理よろづ相談所病院 血液内科
2 天理よろづ相談所病院 臨床検査部
3 天理よろづ相談所病院 消化器外科
4 天理よろづ相談所病院 呼吸器外科
5 天理よろづ相談所病院 病理診断科
6 天理よろづ相談所病院 放射線科
7 天理よろづ相談所病院 アイソトープセンター

症例：55歳女性、好中球減少症のため紹介受診。骨髄検査で CD13+, CD33−, CD34+, CD117+, POX− の芽球を 42.6% 認めた AML-M0 と診断した。ダウノルビシン+シタラビンによる寛解導入療法に不応であったが、高用量シタラビンを含むサルベージ療法によって血液学的寛解に至り、地固め療法を 1 サイクル追加した。

ムーコル症の経過：寛解導入療法後に右肺下葉に病変が出現し、急速に播種した。画像検査で、右肺、肝臓・右横隔膜下、脾臓・左横隔膜周囲に膿瘍を認めた。肝生検病理標本の一部に真菌を認め、形態からムーコル属が疑われたが、培養は不成功であった。β-D-glucan, アスペルギルス抗原陰性。リポソーマルアムホテリシン B (L-AMB) を 4か月以上にわたって継続投与したが画像上の改善は見られなかった。内科的治療による根治は困難と判断し、胸部外科・消化器外科で、左開胸開腹下に腫瘍摘出術を一部骨部切除・横隔膜下腫瘍摘出術と、胸腔鏡下右肺下葉部分切除・開胸右横隔膜下腫瘍デブリドメント・肝部分切除を二期的に実施した。術後は L-AMB を続いてイトラコナゾールを経口投与した。現在外来で経過を観察しているが、AML の再燃は認めない。

ムーコル症の診断：摘出腫瘍検体から DNA を抽出し、異種間で相違が大きく、同種間で相違の少ないリボソーム DNA の internal transcribed spacer (ITS) 領域と D1/D2 領域を PCR 増幅し塩基配列を調べたところ、Rhizomucor pusillus の配列に一致した（ITS は 100%, D1/D2 は 99% 一致）。菌を培養することはできなかった。

考察：本症例は、AML の寛解導入療法中に発症した播種性ムーコル症である。播種性病変に対する外科的治療は容易ではないが、AML が寛解状態を維持し、全身状態が手術に耐えうる状況であれば、検討するべきであると考えられた。

キーワード：急性骨髄性白血病、ムーコル症、外科的デブリドマン、リポソーマルアムホテリシン B, Rhizomucor pusillus