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【 CASE REPORT 】

A Radical Approach to Acute Lymphoblastic Leukemia Treatment: A Case Study of a Veterinarian Specializing in Livestock who Developed Disseminated Mucormycosis during Induction Therapy

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Abstract:
Mucormycosis has emerged as the third-most common fungal mycosis and is one of the most fatal molds. We herein report a case study of a 30-year-old woman who was a veterinarian, specializing in livestock, who developed disseminated mucormycosis during induction therapy for acute lymphoblastic leukemia. We successfully used a radical approach for treatment, including a surgical procedure and allogeneic transplantation, with continuous administration of antifungal agents. Reports of successful treatments are extremely rare, and our case has had the longest documented remission from disseminated disease. We speculate that our case's occupational environment may represent a risk factor for development of mucormycosis.

Key words: mucormycosis, hematopoietic stem cell transplantation, disseminated disease, dairy workers

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Introduction

Invasive fungal infections (IFIs) are life-threatening complications in patients with hematological malignancies (1). The incidence of mucormycosis has been increasing over the past decade, and this condition is fatal in most patients (2). In clinical situation, hematopoietic stem cell transplantation (HSCT) recipients frequently develop mucormycosis (3). Since rapidly progressive disseminated disease occurs in about 60% of mucormycosis cases, the mortality rate exceeds 58-100% (2, 4). The early diagnosis, prompt administration of appropriate antifungal agents and surgical intervention are known to be critical for the successful treatment of mucormycosis (5). There have been few reports of patients with hematological malignancies in whom HSCT has been conducted after the development of disseminated mucormycosis.

We herein report a case study of a 30-year-old woman who was a veterinarian, specializing in livestock, who developed complicated disseminated mucormycosis during induction therapy for acute lymphoblastic leukemia (ALL). The patient's occupational environment may represent a risk factor for the development of mucormycosis. We demonstrated the efficacy of a radical treatment approach including allogeneic transplantation for disseminated mucormycosis.

Case Report

A 30-year-old woman who was a veterinarian, specializing in livestock, presented to our hospital because of anemia symptoms and abnormalities on a blood examination in February 2017. She had been caring for dairy cows in a barn until the day before her first visit. The patient's white blood cell count was 1.6×10^{9}/L with 21% abnormal cells, and her hemoglobin was 7.6 g/dL. On a bone marrow examination, the abnormal cells were judged to be L2 blastoid cells according to French-American-British (FAB) classification. These cells were positive for CD10, CD19, CD34, and HLA-DR and negative for CD20, indicating a normal karyotype. The patient was therefore diagnosed with B-cell ALL.

The patient started induction therapy according to the CALGB8811 protocol (6) with preventive administration of levofloxacin at 500 mg/day, fluconazole at 200 mg/day and

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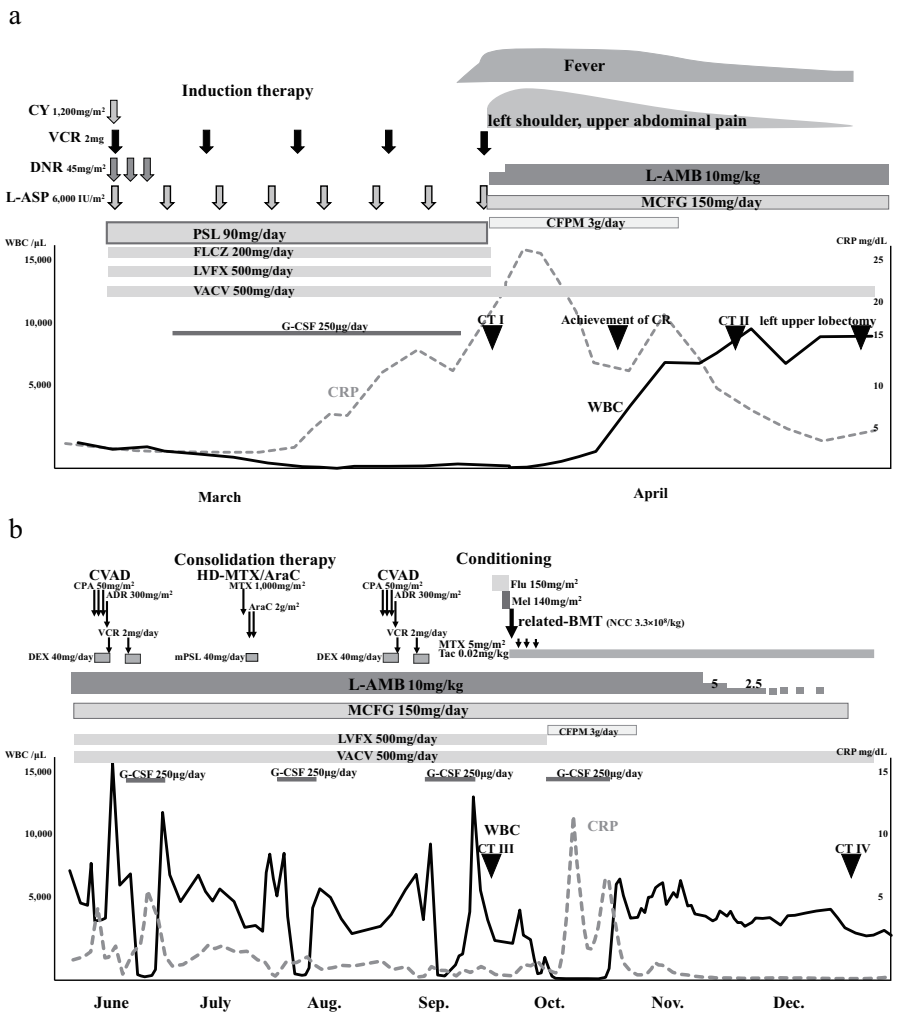

Figure 1. **(a) Clinical course before and after the onset of mucormycosis. CT I: Fig. 2a and 2b, CT II: Fig. 2c. (b) Clinical course before and after bone marrow transplantation. CT III: Fig. 2d and 2e, CT IV: Fig. 2f and 2g. CY: cyclophosphamide, VCR: vincristine, L-ASP: L-asparaginase, PSL: prednisolone, FLCZ: fluconazole, LVFX: levofloxacin, VACV: valacyclovir, L-AMB: liposomal amphotericin B, MCFG: micafungin, CFPM: cefepime, G-CSF: granulocyte-colony-stimulating factor, CRP: C-reactive protein, CPA: Cyclophosphamide, ADR: Adriamycin, VCR: vincristine, DEX: dexamethasone, AraC: cytarabine, mPSL: methylprednisolone, Flu: fludarabine, Mel: melphalan, related-BMT: related-bone marrow transplantation, NCC: nucleated cell count, Tac: tacrolimus, MTX: methotrexate**

valacyclovir at 500 mg/day (Fig. 1a). She developed pain in her left shoulder and upper abdomen on day 21 of therapy. At that time, her body temperature was 38°C, oxygen saturation was 99% on room air, and respiratory sounds were clear. Computed tomography (CT) revealed a reversed halo sign around the left pulmonary apex region (Fig. 2a) and abscessed lesions in other organs, including the liver, kidney and spleen (Fig. 2b). Serum β-D-glucan, galactomannan tests and blood culture examinations were negative, so we suspected disseminated mucormycosis.

We immediately discontinued chemotherapy on this day because of serious infection development and administered a broad-spectrum antibiotic under the diagnosis of febrile neutropenia: liposomal amphotericin B (L-AMB) at 5 mg/kg in combination with micafungin (MCFG). We then increased the dose of L-AMB to 10 mg/kg while monitoring the patient's tolerance.

The patient recovered from myelosuppression at day 28 and achieved complete remission from ALL. A transbronchoscopic lung biopsy and bronchoalveolar lavage were performed on day 30. A histopathological examination of the lung tissue revealed fungal hyphae invading the blood vessels with broad irregular non-septate hyphae with right angles. The hyphae were observed using Grocott's methenamine silver stain (Fig. 3). Based on their morphological features, the mold was classified under the order *Mucorales*. Given these clinical findings, the case was diagnosed as disseminated mucormycosis with the lung as the

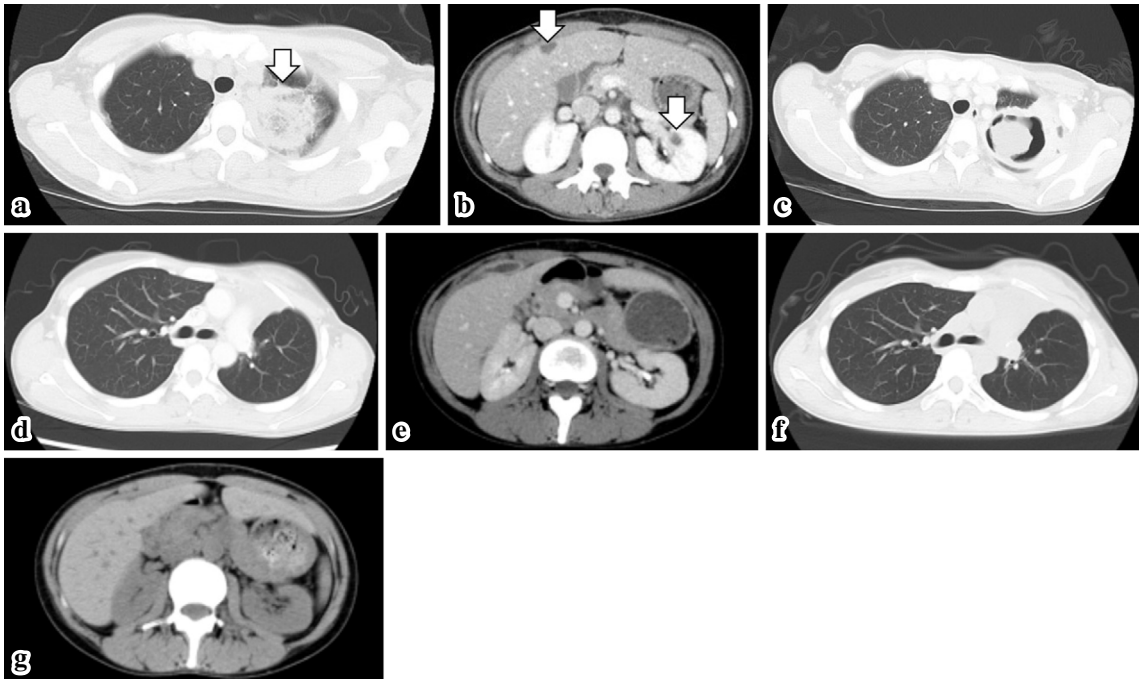

Figure 2. **(a) Chest CT showed a reversed halo sign in the left pulmonary apex region. (b) Abdominal CT revealed abscessed lesions in the liver and kidney. (c) Chest CT before lobectomy revealed a large cavity in the left pulmonary apex. (d) Chest CT before transplantation showed no lesions. (e) Abdominal CT before transplantation showed that the abscessed lesion was smaller and encapsulated but still present. (f) Chest CT after transplantation showed no lesions. (g) Abdominal CT after transplantation showed the residual lesions to have shrunken.**

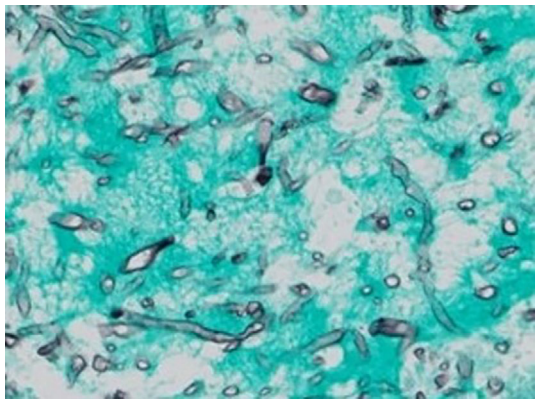

Figure 3. **Grocott's methenamine silver (GMS) staining of lung biopsy tissue showing broad irregular non-septate hyphae with right angles (magnification 400×, GMS stain).**

primary lesion.

To control the main fungal lesions, L-AMB and MCFG were continued, and the patient underwent left upper lobectomy on day 27 following the onset of mucormycosis. This intervention was necessary because of the risk of hemoptysis due to pulmonary lesions with a large cavity in contact with the subclavian artery, with the goal of reducing the risk of further dissemination from the lung to other organs (Fig. 2c). After surgical intervention, three additional consolidation therapies were administered with concurrent antifungal agents at the same dose (Fig. 1b).

In our assessment of the fungal infection activity, a physical examination revealed that the patient's abdominal findings had disappeared, but her mild fever persisted. A blood examination showed that her C-reactive protein concentration was slightly high, and enhanced CT showed that the abscessed lesion was smaller and encapsulated but still present (Fig. 2d, e). Based on these findings, we deemed the fungal infection activity not completely controlled.

The patient's renal function gradually worsened, although the ALL remained in complete remission. We predicted that it would be difficult to continue long-term consolidation therapy and maintenance therapy while administering antifungal agents. However, since an HLA-matched sibling donor was available, we decided to carry out bone marrow stem cell transplantation (BMT) six months from the day of mucormycosis onset.

The patient was conditioned with fludarabine (150 mg/m^2) and melphalan (140 mg/m^2), and both tacrolimus and methotrexate were administered for graft-versus-host disease (GvHD) prophylaxis. Antifungal agents were continued at the same dose before and after transplant. Engraftment was confirmed on day 20 after transplantation. CT showed that the residual fungal lesions were shrinking over time (Fig. 2f, g) and the C-reactive protein level had dropped to zero. Given these findings, we determined the fungal infection activity to be almost gone.

The patient became complicated with bacterial sepsis during myelosuppression but did not develop any transplant-

related complications, including relapse of fungal infections. However, her creatinine level increased from day 60 after transplantation to a maximum of 5 mg/dL. At that time, the antifungal agents were gradually reduced over a period of about 1 month and discontinued entirely on day 273 after the onset of mucormycosis, while tacrolimus was continuously administered. Thereafter, her renal function improved, and her ALL remained in complete remission without mucormycosis recurrence.

She was discharged on day 101 after transplantation and remains in remission from ALL over 1.5 years after BMT without recurrence of mucormycosis.

Discussion

IFIs are frequent life-threatening complications in patients with hematological malignancies. Over the past decade, several new antifungal drugs suitable for clinical use and novel strategies for treating IFIs have been developed (7). In addition, pre-transplant IFI is no longer necessarily a contraindication for transplantation (8). Among IFIs, mucormycosis remains a particularly life-threatening infection associated with high rates of morbidity and mortality (2, 3). There have been few documented cases in which mucormycosis occurred during the early stages of induction therapy in patients with indications for transplantation.

An important point in the present case report is the timing and risk factors for the development of mucormycosis. We suggest the possibility that the patient's occupational environment (a dairy cattle barn) may have been a risk factor for the fungal infection she acquired. Among veterinarians and dairy farmers, *Mucorales* species are recognized as important pathogens in cows. Most cases of zygomycosis among cows are caused by mucormycetes, and zygomycosis usually produces focal lesions (e.g., gastric mucormycosis, intestinal zygomycosis or lymphadenitis); fatal zygomycosis has also been reported (9-11). These pathogens are ubiquitous within the environments inhabited by cattle and can be isolated from the air (12, 13). In addition, zygomycetes such as *Mucor pusillus* and *Lichtheimia corymbifera* are present in the normal rumen flora (14). Infection can occur from disruption of the normal balance between animals and agents. Such disruptions have been associated with several factors that affect the normal flora in the forestomach, such as ruminal acidosis and broad-spectrum antibiotics. As a result, cattle may develop alimentary mycosis or systemic mycosis (15). We speculate that exposure to factors associated with working in a dairy environment, especially the air, feed and excreta, may be a risk factor for immunocompromised patients. Only one report has focused on the possibility that environmental factors and mucormycosis may be related (16). Our patient was a veterinarian specializing in dairy cows and developed ALL while working in an environment in which she may have inhaled high concentrations of *Mucorales* spores released from hay, fermented hay and composite feed. We suspect that such exposure was responsible for the onset of mucormycosis during the early stages of induction therapy.

Mucormycosis developing during HSCT has a high mortality rate (2), but recently, there have been reports of successful treatment (17, 18). L-AMB, starting at 5 mg/kg/day, is now preferred as the first-line therapy, and posaconazole has been mentioned as a viable second-line therapy (5, 19). The appropriate treatment duration for mucormycosis is unclear, and urgent surgical debridement is recommended for localized lesions. The benefits of surgery in the setting of disseminated mucormycosis remain unclear (19).

Our literature search revealed 11 case reports of mucormycosis occurring prior to transplantation and allogeneic transplantation (Table 1) (20-28). Eight cases had disease limited to the lungs, and three cases were disseminated. Acute myeloid leukemia was the most common malignancy, and the onset of mucormycosis frequently occurred during induction therapy. These results are consistent with the fact that cases of induction therapy for acute leukemia are classified as a high-risk group for fungal infections according to the Japanese domestic guidelines for the management of deep-seated mycosis (29). In most cases, the time from the diagnosis to transplantation has been reported to be several months. In the disseminated cases, high doses (e.g., 10 mg/kg) of L-AMB were frequently administered for the treatment of mucormycosis prior to HSCT, and surgical intervention was performed in 3 of 4 patients, including the case described here. We summarized the clinical course and outcomes post-HSCT from these reports (Table 2). There was no clear trend in the patient outcome associated with the status at transplantation, conditioning regimens or donor source. Two of the three disseminated cases died from transplantation-related complications. One case died from CMV, pneumonia and the other died from idiopathic pneumonia syndrome after developing refractory acute GvHD. Pulmonary complications were the cause of death in both cases. Uryu et al. (30) reported that fungal cell components can trigger lung GvHD in mouse models; in this respect, the outcome of these cases is presumed to be the result of fungal infection affecting local immunity in the lung. The optimal administration period of antifungal agents for treating mucormycosis after transplantation remains unclear. One reason for this uncertainty is difficulty in assessing the disease activity of mucormycosis. In the literature describing the duration of treatment with antifungal agents, three cases finished treatment after engraftment. In two cases, including our own patient, treatment was discontinued due to adverse events that occurred within a few months after engraftment. Three cases received maintenance therapy with a small dose of L-AMB or posaconazole over several months, even after discharge. Based on our experience, we consider that it may be possible to withdraw antifungal drugs within a few months of transplantation if there is no apparent fungal infection activity according to the physical findings and other data, including the C-reactive protein concentration, even if immunosuppressants are being administered following trans-

Table 1. Patient Characteristics Pre-HSCT.

Case/ Reference	Age/ Sex	Disease	Clinical Presentation	Diagnosis	Organism	Onset of mucormycosis	Time from diagnosis to HSCT	Treatment of mucormycosis prior to HSCT
1 (20)	31/M	ALL	Pneumonia	Histopathology	*Mucorales*	Induction	21 days	AmB, L-AMB
2 (21)	32/M	AML	Pneumonia	Culture	*Mucorales*	N.A.	60 days	AmB, L-AMB, ope.
3 (22)	28/F	AML	Disseminated disease	Histopathology	*Mucorales*	Consolidation	6 months	AmB, L-AMB 3.5 mg/kg
4 (23)	24/F	AML	Pneumonia	Histopathology	*Mucorales*	Consolidation	35 days	L-AMB, ope.
5 (24)	32/M	AML	Pneumonia	Histopathology	*Mucorales*	Induction	79 days	L-AMB 3 mg/kg, ope.
6 (25)	39/M	AML	Pneumonia	Culture	*Rhizomucor*	Induction	6 months	L-AMB 5 mg/kg, ope.
7 (25)	43/F	ANL	Pneumonia	Culture	*Rizopus*	Induction	9 months	L-AMB 5 mg/kg, Pos, ope.
8 (25)	59/F	AML	Pneumonia	PCR	*Lichtheimia*	Induction	4 months	L-AMB 7.5 mg/kg, ope.
9 (26)	49/M	AML	Disseminated disease	PCR	*Rizomucor*	Induction	3 months	L-AMB 10 mg/kg, ope.
10 (27)	8/M	AML	Disseminated disease	PCR	*Lictheimia*	Induction	79 days	L-AMB 10 mg/kg, ope.
11 (28)	54/F	AML	Pneumonia	Histopathology	*Mucorales*	Consolidation	14 months	L-AMB, ope.
This study	30/F	ALL	Disseminated disease	Histopathology	*Mucorales*	Induction	6 months	L-AMB 10 mg/kg, MCFG, ope.

HSCT: hematopoietic stem cell transplantation, M: male, F: female, AML: acute myeloid leukemia, ALL: acute lymphoid leukemia, PCR: polymerase chain reaction, AmB: amphotericin B, L-AMB: liposomal amphotericin B, MCFG: micofungin, ope: operation

Table 2. Clinical Course and Outcomes Post-HSCT.

Case	Disease status at SCT	Conditioning	Donor source	Antifungal therapy during SCT	Antifungal therapy after discharge	Outcome and follow-up time
1	No CR	ETP/TBI	R-PB	L-AMB 3-5 mg/kg 3 times/wk	No	Alive, in CR for 1 y
2	CR	Bu/Cy	N.A.	N.A.	N.A.	Alive, in CR for 9 mos
3	CR	Cy/TBI	UR-BM	L-AMB 3-5 mg/kg for 5 wks	No	Died from CMV infection at 8 wks
4	CR	Flu/ATG	R-PB	N.A.	N.A.	Alive, in CR for 4 y
5	CR	Bu/Cy	R-HSC	L-AMB until engraftment	No	N.A.
6	CR	Cy/TBI	R-HSC	L-AMB 1 mg/kg	L-AMB 1 mg/ kg for 7 mos	Alive, in CR for 80 mos
7	No CR	Cy/TBI	UR-HSC	L-AMB 3 mg/kg, Pos.	Pos. for 2 y	Alive, in CR for 41 mos
8	CR	Flu/Bu	UR-HSC	L-AMB 3 mg/kg	L-AMB 1 mg/ kg weekly, Pos	Alive, in CR for 22 mos
9	No CR	Flu/Bu	R-PB	L-AMB 7.5-10 mg/kg	No	Alive, in CR for 100 days
10	No CR	Flu/Mel/TBI	CB	L-AMB 3-10 mg/kg	No	Died from IPS at 5 mos
11	No CR	Flu/Mel/AraC/TBI	Haplo-PB	L-AMB, discontinued due to A.E.	No	Alive, in CR for 15 mos
This study	CR	Flu/Mel	R-BM	L-AMB 10 mg/kg, MCFG	No	Alive, in CR for 19 mos

HSCT: hematopoietic stem cell transplantation, SCT: stem cell transplantation, CR: complete response, ETP: etoposide, TBI: total body irradiation, Bu: busulfan, Cy: cyclophosphamide, Flu: fludarabine, ATG: antithymocyte globulin, Mel: melphalan, AraC: cytarabine, R: related, PB: peripheral blood, UR: unrelated, BM: bone marrow, HSC: hemopoietic stem cell, CB: cord blood, Haplo: haploidentical, L-AMB: liposomal amphotericin B, wk: week, Pos: posaconazole, A.E.: adverse event, MCFG: micafungin, mo: month, y: year, CMV: cytomegalovirus, IPS: idiopathic pneumonia syndrome

plantation.

The clinical course of the present case can be a valuable guide for similar cases. Immunodeficient patients in certain workplaces, especially dairy farms, may be at an increased risk for developing mucormycosis. To confirm this speculation, further epidemiological surveys and statistical analyses will be necessary. In this case report, the urgent and continuous administration of antifungal agents, surgical intervention and allogeneic transplantation made it possible to maintain durable complete remission from both ALL and mucormycosis. Although the appropriate treatment for disseminated cases remains unclear, we hope that this case report will help determine the clinical direction for similar cases in the future.

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

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