A hematopoietic stem cell transplantation (HSCT) is a curative treatment for hematological malignancies, but it is not generally performed for patients with active invasive fungal infections because of the high treatment-related mortality [1]. However, because persistent invasive fungal infection itself is lethal, HSCT must be performed when disturbed normal hematopoiesis prompts the infection.

Fusarium is a rare fungus transmitted to immunocompromised patients with poor antifungal susceptibility, and the lower neutrophil count is directly linked to the dismal prognosis of the disseminated disease. We report a case of HSCT for refractory acute myeloid leukemia (AML) with disseminated fusariosis that developed during chemotherapy and was refractory to antifungal therapy due to cytopenia.

**Case Report**

A 53-year-old man visited a local physician with complaints of abdominal pain and malaise. He was referred to our hospital because his blood test indicated pancytopenia with blasts in peripheral blood and elevated levels of C-reactive protein. Bone marrow examination revealed that myeloperoxidase-positive myeloblasts accounted for 68.2% of the nucleated cells. The chromosomal test was a normal karyotype; thus, we diagnosed his disease as AML not otherwise specified. Also, computed tomography revealed acute appendicitis with localized peritonitis, and a laparoscopic appendectomy was performed on the day of admission. On postoperative day 11, induction chemotherapy with idarubicin and cytarabine was performed, but there was no recovery of normal hematopoiesis. One month after the commencement of induction chemotherapy, a bone marrow examination revealed persistent pancytopenia.
marrow examination revealed non-remission, with 27.4% myeloblasts. Despite salvage chemotherapy with mitoxantrone, etoposide and cytarabine (MEC therapy), the pancytopenia persisted, and the patient developed a fever exceeding 38°C.

Although blood cultures were negative, we changed the antibiotic from cefmetazole to doripenem for the treatment of febrile neutropenia. However, there was no improvement of the fever, and systemic palpable papules appeared. We suspected that the papules were caused by some sort of infection rather than drug eruption, and introduced vancomycin and changed the antifungal drug from itraconazole (ITCZ) to 5 mg/kg of liposomal amphotericin B (L-AMB). Nevertheless the fever persisted, and myeloblasts reappeared in the peripheral blood. Thus, a bone marrow examination and skin biopsy were performed (Table 1 and Fig. 1).

Because the papules deteriorated even with a high dose of L-AMB and the bone marrow examination again showed non-remission, we assumed that the skin lesions were leukemic infiltrations rather than infectious, and did not perform a culture test. However, the skin specimen had no evidence of malignancy, but a fungal infection was observed instead. The pathological findings revealed the fungi were true hyphae with septa, infiltrating and proliferating into the blood vessels.

Table 1  Laboratory data on admission and at the time of occurrence of papules

<table>
<thead>
<tr>
<th>Bone marrow aspiration</th>
<th>On admission</th>
<th>At the time of emerging papules</th>
<th>Peripheral blood &amp; coagulation</th>
<th>On admission</th>
<th>At the time of emerging papules</th>
<th>Biochemistry</th>
<th>On admission</th>
<th>At the time of emerging papules</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCC</td>
<td>16.9</td>
<td>10.6 × 10⁴/μL</td>
<td>White blood cells 1,050</td>
<td>180/μL</td>
<td>C-reactive protein 14.45</td>
<td>12.09 mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megakaryocyte</td>
<td>13</td>
<td>56. μL</td>
<td>Myeloblast 14</td>
<td>1%</td>
<td>Albumin 4.3</td>
<td>3.3 mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloblast</td>
<td>68.2</td>
<td>48.8 %</td>
<td>Neutrophils 5.5</td>
<td>0%</td>
<td>AST 25</td>
<td>20 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>1.8</td>
<td>0%</td>
<td>Lymphocytes 78</td>
<td>94%</td>
<td>ALT 51</td>
<td>19 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myelocyte</td>
<td>0.2</td>
<td>0.4 %</td>
<td>Monocytes 2</td>
<td>1%</td>
<td>LDH 253</td>
<td>161 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metamycelocytes</td>
<td>0.0</td>
<td>0%</td>
<td>Eosinophils 0</td>
<td>0%</td>
<td>ALP 147</td>
<td>288 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.2</td>
<td>0.6%</td>
<td>Basophils 0</td>
<td>0%</td>
<td>T-Bil 2.2</td>
<td>1 mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.0</td>
<td>0%</td>
<td>At-Lymph 0.5</td>
<td>4%</td>
<td>CK 40</td>
<td>43 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophils</td>
<td>0.0</td>
<td>0%</td>
<td>EBL 5</td>
<td>0%</td>
<td>BUN 11.8</td>
<td>9.3 mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.2</td>
<td>0.4%</td>
<td>Red blood cells 379</td>
<td>245 × 10⁴/μL</td>
<td>Cre 0.74</td>
<td>0.89 mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.2</td>
<td>5.8%</td>
<td>Hemoglobin 12</td>
<td>7.5 g/dL</td>
<td>UA 4.1</td>
<td>1.7 mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0</td>
<td>4.2%</td>
<td>Hematocrit 36.2</td>
<td>21.7%</td>
<td>Na 139</td>
<td>143 mEq/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBL</td>
<td>28.2</td>
<td>31.8%</td>
<td>Platelets 4</td>
<td>4.2 × 10⁹/μL</td>
<td>K 3.4</td>
<td>3.5 mEq/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPO stain of myeloblast</td>
<td>82</td>
<td>55%</td>
<td></td>
<td></td>
<td>β-D-Glucan 10.8</td>
<td>10.3 pg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karyotype</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
<td></td>
<td>GM 0.2</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WT1 5.1 × 10³</td>
<td>1.7 × 10² copy/μg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NCC, nucleate cell count; EBL, erythroblast; MPO, myeloperoxidase; Aty-Lymph, atypical lymphocytes; AST, aspartate aminotransaminase; ALT, alanine aminotransaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; T-Bil, total bilirubin; CK, creatine kinase; BUN, blood urea nitrogen; Cre, creatinine; UA, uric acid; Na, sodium; K, potassium; Ca, calcium; GM, galactomannan antigen; WT1, gene of Wilms’ tumor-1 (tumor marker of acute myeloid leukemia).

Fig. 1 A, B, papules are shown (black arrow); C, Grocott stain of the skin biopsy section showing infiltration of the fungi.
Based on the morphology and an increase of β-D glucan to 10.3 pg/mL in 2 weeks, the pathogen turned out to be a filamentous fungus, but Aspergillus was unlikely because the galactomannan antigen remained below the cutoff on the same day of the biopsy. Given the clinical course with fever and skin eruptions, fusariosis was the most suspected condition. Thus, we re-examined the blood and papule culture tests; however, both were negative. Despite adding voriconazole (VRCZ) with a targeted concentration of trough to 2-4 μg/ml, the fever and papules did not improve, and β-D glucan increased to 23.7 pg/ml 6 weeks after initiation of VRCZ. Considering that a superinfection other than Fusarium may be present, we added caspofungin (CPFG). Combination therapy with L-AMB and VRCZ and CPFG stopped the enlargement of the papules and slightly reduced β-D glucan, but it did not make β-D glucan undetectable, and it did not resolve the fever hovering around 38°C or the remaining papules. Although the causative pathogen was not yet detected, we reasoned that the persisting pancytopenia associated with the leukemia hindered the healing of the infection regardless of the pathogen, and we again conducted a salvage chemotherapy to control the underlying disease. However, the case ended in non-remission again and there was no recovery of normal hematopoiesis. We decided to perform allogeneic HSCT to treat leukemia and to restore normal hematopoiesis to overcome the suspected fusariosis. We obtained informed consent from the patient.

**HSCT details.** The donor was the patient’s elder brother, who was 6 of 8 alleles matched in the host-versus-graft direction (HVG) and 8 of 8 alleles matched in the graft-versus-host direction. No donor-specific antibody was detected. The donor source was peripheral blood with $7.3 \times 10^9$/kg of CD34-positive cells to achieve quick engraftment. Blood types were major/minor incompatible. As the underlying disease was non-remission, the conditioning regimen was myeloablative and consisted of fludarabine 21 mg/m² from day −7 to day −2, intravenous busulfan 3.2 mg/kg from day −5 to day −2, and melphalan 40 mg/m² from day −7 to day −6. Graft-versus-host disease (GVHD) prophylaxis consisted of continuous intravenous tacrolimus targeting a concentration of 10-12 ng/ml and short term methotrexate with a dose of 10, 7, 7, and 7 mg/m² on days 1, 3, 6, and 11, respectively. We substituted tacrolimus for cyclosporine, which also served to prevent rejection associated with mismatches in HVG. Among the antifungal drugs, CPFG was ceased during the administration of busulfan due to a high risk of sinusoidal obstruction syndrome (SOS), and was resumed after busulfan administration.

**Clinical course after HSCT.** After stem cell transfusion, the patient’s fever temporarily resolved (Fig. 2). On day 3, however, the patient lapsed into septic shock and experienced refractoriness to platelet transfusion, an elevation of total bilirubin (T-Bil) to 4.2 mg/dL, and right costal pain. We suspected SOS based on the modified Seattle Criteria and added recombinant human soluble thrombomodulin (rTM), but we could not perform a liver biopsy because of severe thrombocytopenia. For this reason, we changed antibiotics and had to stop CPFG. For early engraftment, we increased granulocyte-colony-stimulating factor (G-CSF), which had little effect; the pancytopenia was prolonged and the remaining papules gradually enlarged again. The bone marrow examination on day 14 was a dry tap, which prompted us to attempt granulocyte transfusion; however, we did not have sufficient time to obtain the approval of the ethical board. On the same day, Fusarium was finally detected in a blood culture that had been collected on day 11, and the diagnosis of disseminated fusariosis was confirmed. Because β-D glucan also had jumped up to 49 pg/mL, we resumed CPFG even though T-Bil had already risen to 10.5 mg/dL. However, the fever did not resolve. Thus we replaced the central venous catheter and dwelled blood access for the blood purification on day 15. On day 16, because congestion had progressed, we started ventilator management and continuous hemofiltration dialysis. Granulocytes finally appeared on day 18; however, β-D glucan surged to 82 pg/mL, and Fusarium was repeatedly detected in the blood culture. Although exacerbation of fusariosis was evident, ferritin also increased to 26,039.9 pg/mL, which led us to suspect hemophagocytic syndrome, and we reluctantly administered a small amount of steroids.

However, with the exacerbation of hemophagocytic syndrome, lactate dehydrogenase increased rapidly afterward, and organ function deteriorated. The patient died on day 20. We respected his family’s wishes and did not dissect his body. Gene analysis performed by sequencing the translocation elongation factor 1α of the species complex (SC); antifungal susceptibility for this
species in vitro is poor, except for L-AMB (Table 2).

**Discussion**

Fusarium species are filamentous fungi with septa 3-8 μm in diameter, acute-angle branching, and a wide distribution in soil and water [2]. Fusaria are subdivided into approximately 200 types of species. These species are roughly grouped into 10 SCs. Of these, *Fusarium solani* SC, *Fusarium oxysporum* SC, and *Fusarium fujikuroi* SC are thought to be the most common etiologic agents of human infection [3]. In immunocompetent patients, the infections are sporadic, often converging with keratitis or onychomycosis [4]. In contrast, immunocompromised patients, especially those with long-term neutropenia, can be infected via contact with contaminated water or by inhalation of airborne fungi, developing disseminated disease with a dismal prognosis, in some cases progressing to outbreaks [5,6]. The prevalence of fusariosis is <1% of...
patients with HSCT or AML in Europe and North America [7], whereas it is relatively common in South America, where fusariosis accounts for 5% of deep mycosis in patients undergoing HSCT [8]. The etiological species also vary widely by region. For instance, Fusarium fujikuroi SC is predominant in Europe [3], whereas Fusarium solani SC is common in Brazil [9].

Fusarium fujikuroi SC is the pathogen that causes bakanae disease (blight seedling disease) in rice, which is roughly classified into group G and group F. The former produces gibberellin, which has phytohormonal activity, and the latter produces fumonisins, a mycotoxin [10]. Gibberellin produced by group G is thought to play a role in tissue infiltration [11]. In the aforementioned European study, Fusarium fujikuroi SC infection accounted for not only a high percentage of total fusariosis but also a high frequency of disseminated diseases, as described below. This situation could reflect the strength of tissue invasiveness by gibberellin.

The initial symptom of fusariosis is a fever that is resistant to antibacterial and antifungal therapies. Along with the disease progression, sinusitis, pneumonia, and disseminated diseases can occur. In disseminated fusariosis, as in this case, numerous erythema and painful papules appear within a few days, mainly in the extremities. Also, blood cultures are positive in approximately 40% of the cases because Fusarium can grow and sporulate in vivo [12]. Diagnosis is relatively simple in cases with positive blood cultures. In contrast, it is difficult to make a definitive diagnosis without cultivations, since disease-specific staining material for pathological examination is not widely available. In such cases, clinicians must start the treatment using clinical symptoms. However, not only is Fusarium resistant to many antifungal drugs, but also the minimal inhibitory concentrations of these drugs vary widely among species, making empiric treatment difficult. In real clinical practice, treatments with L-AMB or VRCZ are often adopted [13]. Moreover, combination therapies using multiple antifungal drugs are often adopted to ensure that some of the drugs would be effective, including echinocandins, which are reported to be ineffective in vitro [13,14], because there is a great diversity in antifungal susceptibility for each species as described earlier, and the mortality rate of disseminated fusariosis reaches 60-80% [15].

In this case, Fusarium sacchari continued to be relatively susceptible to L-AMB in vitro, in line with a previous report (Table 2) [16]. Nevertheless, it was difficult to control the infection with L-AMB alone in this case. This challenge reflects the fact that the recovery of neutrophil counts is the most important prognostic factor in disseminated fusariosis. Previous studies evaluating the therapeutic effects of L-AMB or VRCZ have reported a better prognosis for patients whose neutrophil counts were recovered [17,18]. Thus, the treatment for disseminated fusariosis requires a long period of time until normal hematopoiesis returns and the sign of infection improves. In other words, it is very difficult to cure disseminated fusariosis in persistent pancytopenia. A new azole antifungal agent, posaconazole, has been approved and reported to be effective for treating resistant/intolerable disseminated fusariosis overseas [19]; however, its sensitivity to Fusarium fujikuroi SC is comparable with that of VRCZ [16]. It is unlikely that this agent would have been helpful in our case. On the other hand, VRCZ or posaconazole is thought to be effective for the primary prophylaxis of fusariosis [20]. Therefore, if we had changed the antifungal drug to VRCZ earlier, the disseminated fusariosis may not have occurred.

We performed allogeneic HSCT because the underlying disease was refractory AML, which prevented our using G-CSF. Because this patient had various organ dysfunctions in addition to disseminated fusariosis before transplantation, the hematopoietic cell transplantation-specific comorbidity index score was 4 points and the 2-year non-relapse mortality rate was predicted to be approximately 40% [21]. However, given the high mortality rate of both refractory AML and disseminated fusariosis and the previous reports that hematopoietic stem cell transplantation cured refractory deep mycosis other than that by fusarium due to cytopenia associated with the underlying disease [22,23], we decided to perform HSCT with informed consent from the patient.

The disseminated fusariosis often recurs when neutrophil counts decline again with chemotherapy, even after the disease resolves with antifungal therapy [15]. Only two reports described disseminated fusariosis before allogeneic transplantation [24,25], and both these patients had long-term survival after transplantation. The survival in both cases was attributed to the recovery of normal hematopoiesis before transplantation, which cured the disseminated fusariosis. To our knowledge, our present report was the first to describe...
allogeneic transplantation with active fusariosis, but unfortunately we could not save the patient. This difference may also have been related to the pathogens involved. In the two studies mentioned above, the pathogen was *Fusarium solani* SC, while in the present case the pathogen was *Fusarium fujikuroi* SC, which might be more invasive due to its production of Gibberellin.

One study had reported that granulocyte transfusion was effective [26], although we were unable to perform this procedure because we did not receive a review by the ethical board on time. If we had been able to perform granulocyte transfusion during the transplantation, the fusariosis exacerbation before engraftment might have been prevented. Moreover, a report from the Center for International Blood and Marrow Transplant Research showed that fungal infections increase non-relapse mortality in HSCT for hematologic malignancies, although this effect was smaller than mortality by the underlying disease, and therefore the authors concluded that the presence of fungal infections is not a contraindication for HSCT [27]. Thus, aside from the presence of fusariosis, the lack of control of the underlying disease might affect the outcome.

We chose a conditioning regimen using fludarabine/busulfan/melphalan, which is reported to be effective for relapsed or refractory myeloid malignancies [28]; however, the patient developed SOS after transplantation. This result could have been due to the overlapping use of SOS-risk agents, including long-term use of L-AMB and busulfan used in conditioning [29]. We also considered the use of total body irradiation (TBI) to avoid busulfan, but we abandoned that idea because of a report of disseminated cutaneous candidiasis after TBI [30]. Therefore, differentiation-inducing therapies using novel agents should be considered in the future, instead of cytotoxic agents for patients with active hematological malignancies and fusariosis, as in our case.

In conclusion, if physicians encounter patients with prolonged neutropenia and high fever during the treatment of hematological diseases, disseminated fusariosis should be suspected. In such cases, physicians should not only modify the antifungal drugs but should also make an effort to restore normal hematopoiesis, including G-CSF, granulocyte transfusion and HSCT in some cases.

References


