Disseminated *Fusarium* Infection Identified by the Immunohistochemical Staining in a Patient with a Refractory Leukemia

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Department of Pediatrics, *Department of Pediatric Hematology and Oncology, Department of Dermatology, Tohoku University School of Medicine, Sendai 980-8574, and The Second Department of Pathology, Shinshu University School of Medicine, Matsumoto 390-8621

Saito, T., Imaizumi, M., Kudo, K., Hotchi, M., Chikaoka, S., Yoshinari, M., Suwabe, N., Sato, A., Suzuki, H. and Iinuma, K. *Disseminated Fusarium Infection Identified by the Immunohistochemical staining in a Patient with a Refractory Leukemia*. Tohoku J. Exp. Med., 1999, **187** (1), 71-77 —— The difficulty and uncertainty encountered in diagnosing a systemic mycosis often lead to a delay in starting antifungal therapy. We reported a disseminated infection of multiple fungal isolates including *Fusarium species* during donor leukocyte transfusion (DLT) after allogeneic bone marrow transplantation in a 20-year-old woman with a refractory leukemia. Skin lesions are the feature of *Fusarium* and occur in the early period of the infection. In this case, during immunosuppression state after DLT, she presented with the whole body ache and erythematous lesions which appeared rapidly on her trunk and extremities. While administration of amphotericin B was started, her condition was further deteriorated and she died. Autopsy materials revealed that she had multiple fungal infection with different isolates, including *Aspergillus* and *Candida* in the brain, lung and liver, but not in the skin. With the immunohistochemical staining with specific antibody, *Fusarium* or *Aspergillus* infection was identified from the biopsy skin or autopsy brain, respectively. This rapid and specific immunohistochemical method may be useful for the diagnosis and treatment of invasive fungal infection without delay.

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Therapy with intensive multi-drug combined chemotherapy or bone marrow transplantation (BMT) has improved the survival rate of patients with leukemia, while the morbidity caused by opportunistic infections has been increasing in patients with a prolonged immunosuppression (Michel et al. 1982; Ricardo et al. 1993; Daniel et al. 1994; Paul et al. 1994; Pietro et al. 1994; Vicki et al. 1994; Susan et al. 1995). Tollemar et al. (1989) reported that the deep fungal infections were observed in 27 of 209 patients (13%) undergoing allogeneic BMT. Candida and Aspergillus are two common fungal pathogens isolated from patients in immunosuppressive state. Then, Fusarium species (sp.), a plant pathogen which can cause focal infections in human, is the third common fungal pathogen after BMT in the United States (Vicki et al. 1994). In Japan, however, a disseminated infection of Fusarium has been scarcely reported.

We report a patient with leukemia who showed a disseminated infection caused by multiple fungal pathogens including Fusarium sp. during the therapy with DLT after allogeneic BMT. In this patient, Fusarium infection was identified by the immunohistochemical staining method, which may be useful for the rapid diagnosis of a progressive systemic mycosis in the immunocompromised patients.

**Case Report**

A 20-year-old woman with acute lymphoblastic leukemia (ALL) was admitted to our hospital in December 1995 for the purpose of treatment to the second bone marrow (BM) relapse after allogeneic BMT performed from an A-locus mismatched sibling donor in November 1994. After a short period of complete remission achieved with an intensive reinduction chemotherapy, she relapsed again in BM in April 1995. Donor leukocyte transfusion (DLT) from the BMT donor was performed two times on 8 and 25 May following chemotherapy, and then her leukemic cells were decreased in her peripheral blood. Because of a chronic neutropenia during the therapy, she was administrated prophylactically with polimyxin B sulfate (3 × 10⁵ unit/day), sulfamethoxazole-trimethoprim (3 g/day) and fluconazole (100 mg/day).

Although Granulocyte-colony stimulating factor (G-CSF) administration was started from day +5 after second DLT, neutropenia with absolute neutrophil counts lower than 500/μ was caused by DLT and had never recovered in her peripheral blood. Because graft versus host disease (cholestatic liver dysfunction and erythema on her palms and feet) occurred on day +10, administration of prednisolone (60 mg/m²/day) and cyclosporine (1 mg/kg/day) were started as treatment. Moreover, she presented with a high fever and streptococcal septicemia was revealed by blood culture examination on day +11. Thus, antibiotics were administrated, and then her febrile condition was improved immediately. From day +15 she complained of the whole body ache, and a necrotic lesion was noted two days later around the insertion site of her Broviac
catheter. Then, new indurated erythematous lesions with 2-3 cm in diameter appeared rapidly on her trunk and extremities. The center of these lesions showed necrotic changes with exfoliative epidermis (Fig. 1A). On day +31 she showed right hemiplegia, right facial palsy and dysarthria. Multiple lesions with approximately 1.5 cm diameter of low density areas were detected in the left cerebral hemisphere by computed tomography. Because fungal infection was diagnosed by biopsy examination of the skin lesion, intravenous injection of amphotericin B (AmB) was started, but the patient’s condition did not improve and she died of pneumonia and renal insufficiency on day +34.

**Materials and Methods**

*Tissue preparations*

Skin lesions with necrosis were biopsied and cultured at antemortem phase. Autopsy was performed soon after her death, and tissue materials obtained from the brain, pharynx, lungs, liver, spleen and kidneys were then cultured for the identification of infectious pathogen.

*Pathological staining and immunohistochemical staining*

Tissue sections from the skin and brain were paraffinized for preparation of hematoxylin-eosin (H-E) staining. For immunohistochemical staining, rabbit antisera raised against formalin-killed fungal strains such as *Aspergillus fumigatus*, *Candida albicans*, *Fusarium sp.*, *Trichosporon beigeli*, *Mucor sp.* and *Cryptococcus neoformans* were used as primary antibodies, as described previously (Fukuzawa et al. 1995). Briefly, endogenous peroxidase activity of deparaffinized tissue was inhibited by pretreatment with methanol solution containing 0.3% H₂O₂. Following the reaction with antifungal antisera, tissue sections were then reacted with swine anti-rabbit immunoglobulin conjugated to horseradish peroxidase (DAKO, Hamburg, Germany) after extensive washing. Then the reaction was visualized with solution containing 3,3′-diaminobenzidine and 0.003% H₂O₂.

**Results**

*Pathological and immunohistochemical findings*

Microscopically, fungal infiltration into blood capillary and fungal emboli were observed in the skin lesion (Fig. 1B). Autopsy revealed the presence of multiple hemorrhagic lesions with diameters measuring up to 5 cm in the brain, lungs, liver, spleen, and kidneys (Fig. 1C). *Candida glabrata*, *Aspergillus sp.* and *Trichosporon pullulans* were isolated by tissue culture of these organs (Table 1). The result of immunoperoxidase staining with control sera was negative (data not shown), and *Fusarium sp.* was identified in the skin lesion and *Aspergillus sp.* was in the brain by the immunoperoxidase staining (Fig. 1D and Table 2).
Fig. 1. Multiple lesions and pathological findings.
A: Indurated erythematous lesions with central necrosis. These lesions spread rapidly on her trunk and extremities. B: Microscopic image of the skin lesion (H-E staining). Fungal infiltration into blood capillary and fungal emboli were observed. C: Hemorrhagic lesions in the left cerebral hemisphere of her brain. D: Immunohistochemical staining of the skin lesion. *Fusarium sp.* was dyed by anti-*Fusarium sp.* antibody.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Isolated fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td><em>Aspergillus sp.</em> <em>Trichosporon</em> pullulant</td>
</tr>
<tr>
<td>Pharynx</td>
<td><em>Candida glabrata</em></td>
</tr>
<tr>
<td>Lung</td>
<td><em>Aspergillus sp.</em></td>
</tr>
<tr>
<td>Skin</td>
<td>Yeast like fungus</td>
</tr>
<tr>
<td>Liver</td>
<td><em>Candida glabrata</em></td>
</tr>
<tr>
<td>Spleen</td>
<td>—</td>
</tr>
<tr>
<td>Kidney</td>
<td>—</td>
</tr>
</tbody>
</table>

*Aspergillus sp.* was obtained from pharynx and liver, *Aspergillus sp.* from lungs and brain, and *Trichosporon* pullulant from brain.
—, no fungal isolates.

**Discussion**

*Fusarium sp.*, a common soil organism and a well-known plant pathogen, has
### Table 2. Immunoperoxidase staining

<table>
<thead>
<tr>
<th>Specificity of antisera</th>
<th>Brain</th>
<th>Skin</th>
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<tbody>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Trichosporon beigelii</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Mucor sp.</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Fusarium sp.* or *Aspergillus sp.* was identified in the skin lesion or brain. 
+ , positive; –, negative.

long been recognized as etiologic agents of focal infections of the skin, nails, and cornea in human (Paul et al. 1994). Occasionally immunocompromised patients with intensive chemotherapy can be affected by a disseminated infection of *Fusarium sp.* (fusariosis). Although *Fusarium sp.* is thought to be distributed widely, patients with fusariosis has not necessarily been reported evenly in the world. Greater than 60% of cases with fusariosis have been reported in the United States and 15% in Italy, while scarcely in other countries including Japan. The background of this peculiar epidemiology has not been clarified (Pietro et al. 1994).

*Fusarium sp.* is living in soil, but often found in the air (Caplin and Unger 1983). Consequently, through respiratory tract are the common portals of entry for disseminated *Fusarium* infection, as well as through the gastrointestinal tract, skin, toenails and central venous catheters (Pietro et al. 1994). In our patient, *Fusarium sp.* was identified at the necrotic lesion around the insertion site of her Broviac catheter, suggesting the catheter as the portal of the infection. Because *Fusarium sp.* has an ability to adhere to the silastic material, central venous catheters may have a risk to become the primary site of this infection when kept in place for a long time (McNeely et al. 1981).

Most of clinical features of disseminated *Fusarium* infection are similar to those of other fungal infections. However, the nature of skin lesions may be useful in suspecting *Fusarium* infection, because they frequently appear in the early period of *Fusarium* infections and spread immediately on the trunk and extremities. These lesions are painful, erythematous, indurated papules and nodules whose center quickly develops necrosis (Pietro et al. 1994; Susan et al. 1995). Interestingly, isolation of *Fusarium sp.* from blood culture can be observed in a high rate, because of a high ability to invade into bloodstream (Anaissie et al. 1993). Moreover, biopsy examination of the skin lesion reveals fungal organisms within and around the dermal blood vessels, causing thrombosis and tissue necrosis.

Michael et al. (1982) reported that 7 of 26 patients (27%) with acute leukemia
exhibiting invasive fungal infections had multiple fungal isolates. In our
patient, the infection with Candida, Aspergillus, Trichosporon and Fusarium was
proved from her organs despite administration of fluconazole. Accordingly, a
whole body survey may be needed to reveal whether multiple organs were
involved in the patients affected with deep fungal infection during a prolonged
immunosuppression.

Immunosuppressive state caused by a prolonged severe neutropenia is likely
to give rise to fungal infection and underlie the deterioration of infection in spite
of the antifungal therapy. Furthermore, the difficulty and uncertainty en-
countered in diagnosing a systemic mycosis often lead to a delay in starting
antifungal therapy. In practice, antifungal agents are administrated conven-
tionally when a high fever continues even with the administration of antibiotics
(Michel et al. 1982). Therefore, a rapid and specific diagnosis is needed for an
effective and specific therapy without a delay in immunocompromised patients
with a suspicion of disseminated fungal infections. Detection of plasma (1–3)-β-d-glucaN, a characteristic fungal cell wall constituent, has a diagnostic value
in screening the presence of an invasive fungal infection, but not the specific to
fungal species. Because of emerging resistance for some antifungal agents, fungal
pathogen should be identified rapidly and specifically (Working Party of the
British Society for Antimicrobial Chemotherapy 1997). Tissue culture not only
takes time to obtain the final result, but also fails occasionally to identify fungal
genus. We could not identified Fusarium by the skin culture, the result of which
was “yeast like fungi.” Identifying Fusarium by culture may be difficult because
the identification of pathogen depends solely on the observation of cultured fungi,
such as the shape of giant colony and hypha. Thus, it may be possible that this
is one of the reasons why Fusarium infection has been rarely reported in Japan.
By contrast, diagnosis of Fusarium and Aspergillus infection was obtained by a
rapid and specific method of immunohistochemistry in our patient. Therefore,
this method may be useful for the rapid diagnosis and effective treatment of
invasive fungal infections in the immunocompromised patients with hematological malignancy.

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