Review

Practical management of deep cutaneous fungal infections

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ABSTRACT

Understanding deep cutaneous fungal infection requires not only reading many case reports and checking the typical clinical images of skin lesions, but also managing the patients properly to prevent misdiagnosis. Herein, I review my recent experiences with eight typical cases of deep cutaneous infections (including protothecosis and nocardiosis) in Japan. It is very important to do the four management processes; namely, KOH direct microscopic examination, skin biopsy, fungal culture, and microscopic examination of the histopathological specimen of PAS and Grocott staining. Also, to aid in memorizing the names of important diseases, I recommend the mnemonic “AC PPPS MD” (Aspergillosis, Cryptococcosis, Phaeohyphomycosis, Protothecosis, Pseudoallescheriosis, Sporotrichosis, Mycetoma, and Dermatophytosis). Isolation of the fungus by culturing from the skin lesion is the best way to carry out quick and correct diagnosis.

Key words : echo image, fungal culture, histopathology, skin biopsy, to homogenize tissue

Introduction

Deep cutaneous fungal infection has become a very important concern for dermatologists because of the increasing number of immunocompromised patients¹⁾. The number of case reports of deep cutaneous fungal infection are increasing²⁾. Understanding these cutaneous disease requires not only reading many case reports and checking the typical clinical images of skin lesions but also managing the patients properly to prevent misdiagnosis. Herein, I review my recent experiences with eight typical cases of deep cutaneous fungal infections in Japan. It is very important to do the four management processes; namely, KOH direct microscopic examination, skin biopsy, fungal culture, and microscopic examination of the histopathological specimen of PAS and Grocott staining. There are many cases for which we primarily suspected deep fungal infection, but herein I also report two cases (Aspergillosis and Dermatophytosis) for which we primarily suspected skin tumor and wherein, surgical impression during operation was different from simple

skin tumor, therefore we performed fungal as well as bacterial culture. The isolation of the fungus from the skin lesion was the most rapid way to the correct diagnosis of deep cutaneous fungal infection. Sometimes fungal infection was suspected from the histopathology, but it was difficult to make the final diagnosis from paraffin-embedded block specimen. This is because formalin-fixed skin lesions do not provide a reliable source for direct isolation of fungal DNA for molecular biological methods.

As a mnemonic to remember the names of important diseases, I recommend use of the letters “AC PPPS MD” (Aspergillosis, Cryptococcosis, Phaeohyphomycosis, Protothecosis, Pseudoallescheriosis, Sporotrichosis, Mycetoma, and Dermatophytosis). To aid in memorizing these names, I created a sentence based on their first letters.

“A Cutaneous lesion of Pustulosis Palmaris et Palantalisis Should be examined by MD.”

During skin tumor operation, if the tumor is found to be an abscess, it is very important for dermatologists to try not only bacterial culture but also fungal culture. Isolation of the fungus by

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Fig. 1. Aspergillosis (67-year-old female).
 (a) Three subcutaneous nodules caused by *Aspergillus calidoustus* that were not cured by surgical treatment.
 (b) Fungal culture slants: Same fungus (*Aspergillus calidoustus*) was isolated from the lesions.

culturing from the skin lesion is the best way to carry out quick and correct diagnosis.

Cases

Case 1: Aspergillosis³⁾

A 67-year-old Japanese female had been treated with oral prednisolone and tacrolimus for adult-onset Still's disease and interstitial pneumonia. She presented with a 2-month-history of three subcutaneous nodules on the left back at nerve block injection sites for post-herpetic neuralgia (Fig. 1a).

At first we suspected skin tumor, such as epidermal cysts. During operation we diagnosed them as abscess, and performed not only bacterial culture, but also fungal culture on Sabouraud agar. We isolated fungus from the skin lesion culture (Fig. 1b). Histopathology of the lesion was consistent with fungal infection. We identified the fungus as *Aspergillus calidoustus* based on microscopic structure of the isolated fungus and fungal DNA sequence analysis. The patient was treated with surgical removal of the lesions and

oral itraconazole at 200 mg/day. She died, however, of infectious interstitial pneumonia due to *Pneumocystis jirovecii* and Cytomegalovirus infection. Percutaneous infection may have been responsible for the incidence of localized infection. There was no evidence of systemic aspergillosis.

Case 2: Cryptococcosis⁴⁾

Second case was a 66-year-old female suffering from chronic renal dysfunction and undergoing hemodialysis; she had skin erythematous tumor on the upper arms at manchette compression sites for checking blood pressure (Fig. 2a). We suspected erythema nodosum of the upper arms, cellulitis, and malignant lymphoma, among other diseases. Skin biopsy revealed many Grocott-positive round cells in the dermis and subcutis (Fig. 2b). We isolated *Cryptococcus neoformans* serotype A from the cutaneous lesion. As dermatologists, we recommended chest CT for checking systemic infection of *Cryptococcus* spp.. We identified lung lesion of *Cryptococcus* spp., and final diagnosis was systemic cryptococcosis. This

case shows the usefulness of the skin biopsy in the diagnosis process for systemic fungal infections.

Case 3: Phaeohyphomycosis⁵⁾

Third case was an 80-year-old female who had been treated with oral prednisolone, 15 mg daily, for Takayasu's arteritis and presented with a 6-month history of a hyperkeratotic, brownish, erythematous lesion on the right cheek measuring 6 mm in diameter. Actinic keratosis was suspected, and an excisional skin biopsy was performed. Histopathology showed granuloma formation in superficial dermal layer by multinucleated giant cells that contained black septate hyphae (Fig. 3a). Tissue specimens and skin scrapings were obtained and incubated on Sabouraud dextrose agar, yielding black colonies that were morphologically identified as belonging to *Exophiala* species (Fig. 3b). Sequence analysis of the internal transcribed spacer region of the ribosomal RNA gene showed homology to *Exophiala oligosperma* sequences. We performed chemotherapy and oral KI, but the lesion did not improve. We performed total resection and 2-week pulse administration of oral itraconazole (200 mg/day), but the lesion recurred after 40 days (Fig. 3c). We performed a second total resection, after which the lesion did not recur again. The patient, however, died of aspiration pneumonia from sudden suffocation 1 year and 2 months after the first biopsy. In this case, resection and rebiopsy with culture enabled us to make the final diagnosis. When culturing skin biopsy tissue, it is very important to homogenize the tissue before culture on the agar (Fig. 3d). Aseptic technique in fungal culture to avoid contamination is important for the final diagnosis.

Case 4: Protothecosis

Focus should be made on protothecosis in determining the cause of recent skin ulcers. Fourth case is a 73-year-old immunocompromised female with cellulitis on the forearm that became skin ulcer. *Prototheca* species are algae closely related to chlorella, but they do not have chlorophyll. Basically, *Prototheca* spp. is not a fungus but appears like yeast in culture. Skin biopsy revealed PAS-positive morula-like cells in the dermis (Fig. 4). Since *Prototheca* spp. can be cultured on Sabouraud agar, if fungal infection is suspected, final diagnosis of protothecosis can be

made by culturing on fungal culture media. The International Human and Animal Mycology (ISHAM) – Medical Phycology Working Group was established by flagship members including Japanese scientists⁶⁾. Collaboration of laboratory staff of the hospital is important for proper diagnosis.

Case 5: Pseudoallescheriosis⁷⁾

Pseudoallescheriosis is an emerging and important fungal infection that affects immunocompromised patients. *Pseudoallescheria boydii*/*Scedosporium apiospermum* complex is a ubiquitous filamentous fungus. We report a case of cutaneous *P. boydii* infection of the left knee in a 79-year-old Japanese immunocompromised male patient. Culture of the pus from the skin specimen confirmed the diagnosis of cutaneous *P. boydii* infection. Cutaneous injury may be responsible for the incidence of localized infection. Septate hyphae were found on the histopathological specimen (Fig. 5). It is difficult, however, to differentiate it from other filamentous fungi. Fungal culture morphology and DNA sequence analysis are important for the diagnosis of *P. boydii* infection.

Nomenclature rules have recently been revised under the slogan of 'one fungus, one name,' and the new species epithet for the fungus is still under discussion as a result of the revision⁸⁾.

Case 6: Sporotrichosis⁹⁾

Sporotrichosis is the most common deep cutaneous fungal infection in Japan. This disease is caused by a dimorphic fungus, *Sporothrix schenckii* complex, which comprises three phylogenetic sibling species; namely, *Sporothrix brasiliensis*, *Sporothrix globosa*, and *Sporothrix luriei*. In East Asia including Japan, *Sporothrix globosa* is the main causal species; while in America, Australia, and South Africa, *S. schenckii* sensu stricto is the main species causing sporotrichosis¹⁰⁾.

An 84-year-old farmer had been suffering from skin ulcer on the left back of the hand for the past four months, which did not respond to oral antibiotics treatment for one month (Fig. 6). We suspected squamous cell carcinoma and sporotrichosis. We did skin biopsy and, at the same time, homogenized part of the sample and cultured it on Sabouraud agar without antibiotics (Fig. 6). Sequence analysis of the internal trans-

Fig. 2. Cryptococcosis (66-year-old female).

(a) Erythematous tumor on the right upper arm.

(b) Histopathology of the Grocott stain: Grocott-positive round yeast cells in the dermis.

Fig. 3. Phaeohyphomycosis (80-year-old female).

(a) Black septate hyphae were seen in the giant cells in the dermis. (Hematoxylin and eosin stain)

(b) Black fungus was isolated three times from the same lesion.

(c) Recurrent lesion on the right cheek after excisional biopsy.

(d) Before culture on the media, the skin tissue should be homogenized.

Fig. 4. Protothecosis (73-year-old female). Histopathology of the PAS stain. Abundant morula-like cells in the dermis and subcutis.

Fig. 5. Pseudoallescheriosis (79-year-old male). Histopathology of the PAS stain. Filamentous fungus was seen in the abscess.

cribed spacer (ITS) region of the ribosomal DNA gene showed homology to *S. schenckii* sequences. This is a case of fixed cutaneous type of sporotrichosis.

In Japan, common sites of infection are arms of adults and faces of children. Since the fungus lives in the soil, wood, and surfaces of plants, farmers are in most danger of infection. Although culturing is necessary, clinical symptoms are also important. The description “sporotrichoid” comes from clinical symptoms of lymphocutaneous sporotrichosis. Cutaneous sporotrichosis is classified into fixed cutaneous, lymphocutaneous, disseminated cutaneous, and hematogenous cutaneous types. Sporotrichosis caused by *S. brasiliensis* is significantly associated with cat transmission in Brazil. In Japan, an epidemic of sporotrichosis has not occurred in decades, but since cats are very popular companion animals, we should pay attention to sporotrichosis as an imported infectious disease¹⁰⁾.

Case 7: Mycetoma (Nocardiosis)¹¹⁾

The clinical symptom complex “mycetoma” encompasses chronic inflammatory swelling with deformity of the affected region and fistulas that discharge granules (grains). The lower limbs are most often affected¹²⁾. Nodules, fistulas, and ulcers were important skin eruptions observed in the case of a 27-year-old man with right knee lesion that did not respond to antibiotics for 3-years (Fig. 7a). He had a history of motorcycle accident in Chiba city and was treated by skin suturing, which was cured as a scar about ten years ago. We needed to perform skin biopsy three times before

Fig. 6. Sporotrichosis (84-year-old farmer). Skin ulcer on the left back of the hand (After skin biopsy).

making the final diagnosis.

Biopsy is essential for the diagnosis of mycetoma. Grains are usually found in the center of the microabscess (Fig. 7b). It is very important to try rebiopsy and re-culture from chronic, unresponsive skin ulcers. Mycetoma is caused not only by *Nocardia* spp. but also fungi. Although *Nocardia* spp. is not a fungus, it can be cultured on SDA. *Nocardia brasiliensis* is the most frequently isolated *Nocardia* sp. from cutaneous nocardiosis in Japan.

Case 8: Dermatophytosis¹³⁾

Dermatophytes are the most common cause of superficial fungal infections in Japan, but they

Fig. 7. Mycetoma (Nocardiosis) (27-year-old male).
 (a) Right knee lesion of patient; Nodules and fistulae with discharge.
 (b) Grain in the center of the abscess (H & E stain).

Fig. 8. Dermatophytosis (58-year-old female).
 (a) Echo image of the hip lesion before operation was consistent with epidermal cyst.
 (b) Grocott stain shows septate hyphae branching at an acute angle in the cystic lesion.

rarely cause deep cutaneous fungal infections. Kerion Celsi is the most common deep fungal infection caused by dermatophytes. We encountered deep cutaneous fungal infection caused by dermatophyte in an immunocompromised host. A 58-year-old female patient with Castleman disease suffered from left hip tumor. Clinical examination and cutaneous echo image (Fig. 8a) were consistent with an epidermal cyst diagnosis; hence was diagnosed as cystic-type deep tinea based on culture during operation and histopathological examination of the tumor (Fig. 8b). Patients with cystic granuloma trichophyticum sometimes re-

quire surgical resection¹⁴.

Conclusions

For the diagnosis of deep cutaneous fungal infections, it is very important to suspect fungal infection and perform skin rebiopsy and fungal culture. Fungal infection must be considered in clinical practice for skin lesions with discharge of pus and that are unresponsive to antibiotics. If yeast is suspected, culture can be performed using the cotton swab method. Dermatologists themselves, however, should culture the pus on

the agar if filamentous fungus is suspected.

Whole clinical symptoms must be considered, and fungi isolated from culture must be differentiated from contamination by filamentous fungi from the environment. Fungal culture and isolation from the cutaneous lesion is essential in the diagnosis of deep cutaneous fungal infections.

Conflict of interest

None

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