

## Disseminated Subcutaneous Phaeohyphomycosis caused by *Exophiala oligosperma* in a Patient with Wegener's Granulomatosis

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Phaeohyphomycosis (PHM) is a rare infectious disease caused by dematiaceous fungi in cutaneous, subcutaneous or visceral organs. Most cases have been reported in immunocompromised patients (1, 2), such as those with autoimmune diseases and post-transplant patients. Various kinds of fungi, including *Alternaria*, *Phialophora* and *Exophiala* species, are known aetiological agents (3). However, few cases of cutaneous or subcutaneous PHM caused by newly recognized *E. oligosperma* have been reported (3, 4) and no treatment for this infection has been established. We report here a case of subcutaneous PHM caused by *E. oligosperma* in a patient with Wegener's granulomatosis that was treated successfully with voriconazole (VRCZ).

### CASE REPORT

A 71-year-old woman with Wegener's granulomatosis presented with an 8-month history of multiple subcutaneous nodules, accompanied by cutaneous erythema and discharge of pus, on her left forearm (Fig. 1A). A small nodule had appeared on the dorsal part of her left hand, and similar lesions had gradually expanded on her left forearm. She had been treated with prednisolone and methotrexate (MTX) for Wegener's granulomatosis,

and fluconazole (FLCZ) for oral candidiasis. A dendritic form of hyphae was observed under potassium hydroxide direct microscopy of the pus discharge. Skin biopsy revealed dense suppurative granulomatous infiltration composed of neutrophils and multinucleated giant cells, mainly in the subcutaneous region (Fig. 2A, B). Haematoxylin and eosin staining (H&E) showed brown hyphae proliferating in and around granulomas. The hyphae were densely stained by Periodic acid-Schiff staining (PAS) and Grocott staining (Fig. 2C). Incubation on Sabouraud's glucose agar medium yielded grey-black and yeast-like colonies in which the hyphae were morphologically expanding with the formation of annelloconidia (Fig. 2E, F), indicating that the isolate was an *Exophiala* species. The sequence of the internal transcribed sequence (ITS) region of the ribosomal RNA of the isolate was determined (DDBJ/EMBL/GenBank accession number AB713430) and showed a 99.8% similarity with *E. oligosperma* CBS 725.88 ex type (DDBJ/EMBL/GenBank accession number AY163551). *In vitro* susceptibility assay proposed for filamentous fungi by the National Committee for Clinical Laboratory Standards (CLSI M38-A2) (5) showed that the minimum inhibitory concentrations (MIC) of micafungin sodium (MCFG), amphotericin B (AMPH-B), flucytosine (5-FC), FLCZ, itraconazole (ITCZ), miconazole (MCZ) and VRCZ against the causative agent were 0.25, 0.25, <0.125, 8, 0.125, 1 and 0.125 µg/ml, respectively.

Oral administration of VRCZ was started at a loading dose of 300 mg twice daily, followed by a maintenance dose of 200 mg twice daily. Since liver damage appeared one month later, the dose of VRCZ was decreased to 150 mg twice daily. An improvement was observed 2 weeks after commencement of treatment, leading to gradual, but dramatic, resolution of the lesions (Fig. 1B). Persisting lesions were surgically excised after 9 weeks, followed by the same maintenance dose of VRCZ. Because of an amnesic episode, which occurred 12 weeks after the operation, possibly in relation to VRCZ, VRCZ was switched to another 7-month administration of oral ITCZ, 200 mg once a day. No recurrence has been observed for 9 months after the completion of ITCZ. Tissue samples excised in the operation revealed the presence of fungi in granulomas in some specimens, but these changed into round sclerotic cells encapsulated by thick walls (Fig. 2D). Based on the diagnosis of this fungal infection, MTX was discontinued for one month, but resumed with intravenous pulse steroid therapy because of newly emerging lung lesions of Wegener's granulomatosis.

### DISCUSSION

*E. oligosperma* was newly identified by de Hoog et al. (6) in 2003 on the basis of sequencing of the ribosomal DNA ITS. Although *E. oligosperma* causes olecranon bursitis (7), fungaemia (8) and chronic sinusitis (9), a review of the literature revealed only 2 reports of isolation of *E. oligosperma* from skin lesions. Gonzalez-

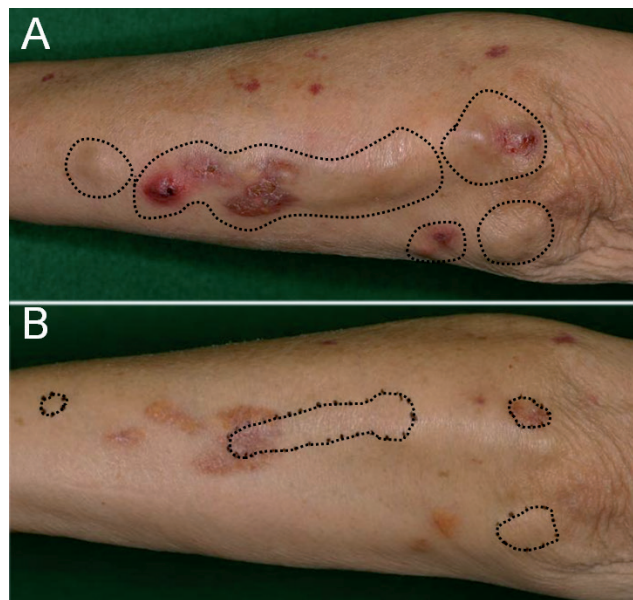


Fig. 1. (A) Multiple subcutaneous nodules with pus discharge on the left forearm. (B) Nine weeks after initiation of voriconazole treatment, the nodules have decreased significantly. (Broken lines indicate the extent of the subcutaneous lesions).

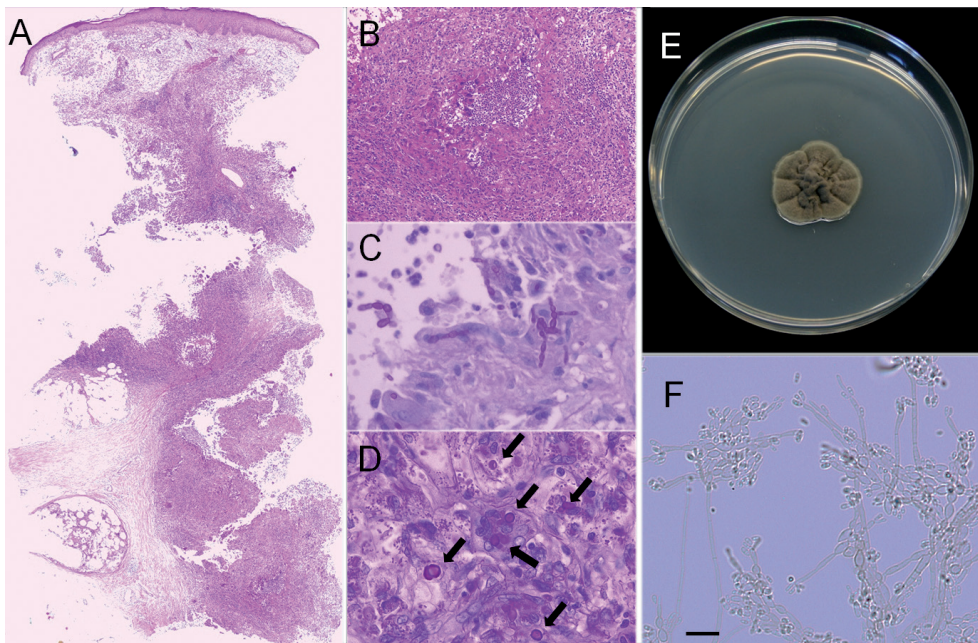


Fig. 2. (A) Subcutaneous regions were involved through suppurative granulomatous inflammation. (haematoxylin and eosin (H&E); original magnification  $\times 20$ ). (B) Granulomas consisted of neutrophils and multinucleated giant cells. (H&E  $\times 100$ ). (C) Biopsy specimen, showing hyphae in and around granulomas. (Periodic acid-Schiff (PAS)  $\times 400$ ). (D) Sclerotic cells (arrows) were observed in the tissue excised during the operation (PAS  $\times 400$ ). (E) A grey-black colony on Sabouraud's glucose agar medium. (F) Hyphae expanding with the formation of annelloconidia. Bar = 10  $\mu\text{m}$ .

Lopez et al. (3) mentioned a case of subcutaneous PHM in a post-renal transplant patient who presented multiple subcutaneous nodules on the right leg, and was treated with 3-month administration of oral ITCZ. Tokuhsa et al. (4) also reported a case of cutaneous PHM of the face in a healthy patient without relevant history, who was treated with topical terbinafine cream. In our case, the patient, taking corticosteroid and immunosuppressant for Wegener's granulomatosis, presented multiple subcutaneous nodules on her forearm, and was treated successfully with oral VRCZ combined with surgical excision. The MIC of our isolate indicated susceptibility to MCFG, AMPH-B, 5-FC, ITCZ, MCZ and VRCZ, but not to FLCZ. Bossler et al. (7) also reported that *E. oligosperma* from their case of olecranon bursitis was very susceptible to 5-FC, ITCZ, VRCZ and AMPH-B.

Dematiaceous fungal infections are classed into PHM and chromoblastomycosis on the basis of the morphology of causative agents in lesions. The form of the "hyphae" and "sclerotic cells" can be characteristic in the lesion of PHM and chromoblastomycosis, respectively (10). In our case, fungi in the biopsy specimen were observed in the form of "hyphae", which changed to "sclerotic cells" after treatment of the fungal infection (Fig. 2C, D). *Exophiala* species in PHM tissues are generally present in the form of hyphae, but may be sclerotic cells in inactive and durable parasitic conditions. Both treatment with VRCZ and the host defence recovery may reduce the activity of fungi and shift its morphology to a strong durable parasitic form of sclerotic cells. However, the tissue with sclerotic cells obtained by operation did not reveal signs of recovered host defence, such as maturation of granulomas or a reduction in abscesses.

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