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Two cases of *Emergomyces pasteurianus* infection in immunocompromised patients in the Netherlands

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ABSTRACT

We report two cases of *Emergomyces pasteurianus* infection in the Netherlands. Both patients were immunocompromised and had pulmonary symptoms. The first patient died due to a pulmonary infection with *Es. pasteurianus*, concomitant listeriosis, *Pseudomonas aeruginosa* sepsis and invasive pulmonary aspergillosis. The second patient had pulmonary and subcutaneous lesions, and recovered completely after treatment with posaconazole for 14 months. In both cases, diagnosis of *Es. pasteurianus* was made with internal transcribed spacer rRNA PCR and culture.

1. Introduction

Emergomyces is a recently proposed new genus within the family *Ajellomycetaceae* (*Onygenales*), to accommodate the recently described and globally emerging *Emmonsia*-like fungi which cause disease in immunocompromised hosts [1,2]. Patients can be infected with *Emergomyces* species, which are saprophytic dimorphic fungi, presumably by inhaling dust-borne conidia. In the genus *Emergomyces* five species are described: *Es. africanus*, *Es. pasteurianus, Es. europeaus, Es. orientalis* and *Es. canadensis*. All species lack adiaspores, classically associated with the genus *Emmonsia* [2]. *Emergomyces* species have been found in immunocompromised hosts and mostly present as a disseminated infection, with over 80 reported cases of *Es. africanus* in HIV-patients in South Africa [3–5].

To date, only a handful of cases of *Es. pasteurianus* infection have been reported, distributed over Africa, Asia and Europe [6–11]. We report, to the best of our knowledge, the first two cases with an *Es. pasteurianus* infection in the Netherlands.

2. Case

2.1. Case A

In November 2017 an Iraqi male, in the eight decade of life, was admitted to a tertiary academic hospital in the Netherlands with a decreased level of consciousness and fever (day 0). The patient, with a medical history of B-cell chronic lymphocytic leukemia and chronic kidney failure, had developed chronic neutropenia due to his hematological condition and therapy (cyclophosphamide and prednisone since eleven months). The patient had returned two months earlier (day −60) from visiting relatives in Iraq.

Upon admission he was evaluated for meningitis; cerebrospinal fluid (CSF) analysis (day 0) showed pleocytosis (WBC 613×10^6/L), normal glucose (0.43 g/L) and an elevated protein concentration (3.6 g/L). Gram staining of CSF (day 0) showed Gram positive rods and treatment with high dose amoxicillin was started; cultures of CSF and blood showed *Listeria monocytogenes* (day +1). The following days there was some moderate neurological improvement and the fever subsided. On day + 11 fever and tachypnea developed, no skin abnormalities were seen. Broad spectrum antibiotics (cefuroxime/gentamicin) were added to amoxicillin treatment. A thoracic high resolution computed tomography (HRCT) scan (day +11) (Fig. 1) showed

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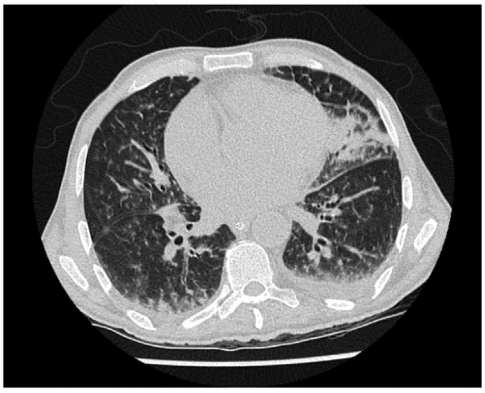

Fig. 1. Thoracic HRCT scan, showing extensive bilateral infiltrative abnormalities, pathognomonic signs for a fungal infection were absent.

extensive bilateral infiltrative abnormalities, consistent with a broad differential diagnosis, including (fungal) infection, but pathognomonic signs for a fungal infection were absent. A bronchoalveolar lavage (BAL) was performed on day + 12 and liposomal amphotericin B 3 mg/kg was started as empirical therapy. The patient was transferred to the Intensive Care Unit (day +12) for respiratory insufficiency, but died within 36 h after onset of fever and tachypnea on day +13, because of respiratory insufficiency, cardiac failure and sepsis caused by *Pseudomonas aeruginosa*.

Post-mortem, BAL fluid and serum tested strongly positive in the galactomannan assay (Platelia Aspergillus, Bio-Rad). No *Aspergillus* could be cultured, but polymerase chain reaction (PCR) on BAL fluid was positive for *Aspergillus fumigatus* (AsperGenius, PathoNostics, performed by ErasmusMC, Rotterdam, the Netherlands). Culture of BAL fluid showed some small colonies suspected for a fungus after three days of incubation. After three weeks of incubation, a dimorphic fungus was diagnosed without production of adiaspores at 25 °C (Fig. 2). Molecular identification by in-house internal transcribed spacer rRNA (ITS) sequencing revealed *Emergomyces pasteurianus* (ITS1: GenBank accession number KP260922, length 239 nucleotides, similarity 99.2%; ITS2: GenBank accession number KP260922, length 332 nucleotides, similarity 100%), which was confirmed by additional analysis (ITS and large subunit rRNA (LSU) sequencing) at the CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands. The strain of *Es. pasteurianus* was negative in the *Aspergillus species* PCR (AsperGenius, PathoNostics, performed by Leiden University Medical Center).

2.2. Case B

In December 2016 a 62-year old woman of Moroccan descent was

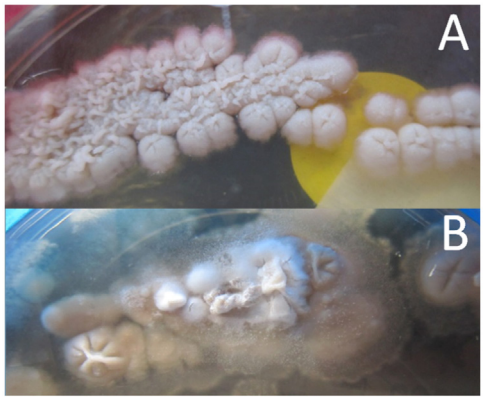

Fig. 2. Yeast (A, 35 °C) and mold (B, 28 °C) phase of *Es. pasteurianus* on Sabouraud agar, after 3 weeks of inoculation.

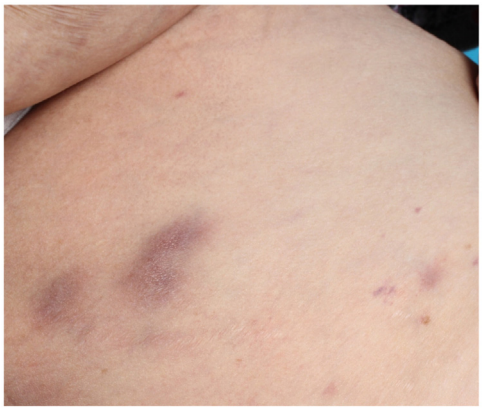

Fig. 3. Two subcutaneous lesions, approximately 1–2 cm in diameter on the left leg of Case B.

admitted to a secondary care hospital in the Netherlands with shortness of breath (day 0). Physical examination showed multiple pink to purple coloured, firm, raised, non-tender, 0.5–2 cm in diameter, subcutaneous lesions on trunk, arms and legs (Fig. 3). She had a medical history of large B-cell non-Hodgkin's lymphoma (2013, in complete remission since 2014), biliary cirrhosis, chronic kidney failure due to diabetes mellitus type 2 and auto-immune haemolytic anaemia treated with 50 mg/day of prednisolone. The patient frequently visited relatives in Morocco and had returned four months earlier from such visit (day −120).

On hospital admission, chest X-ray showed a density in the right upper lobe. Further analysis with a positron emission tomography–computed tomography (PET-CT) scan was performed on day +6, showing two PET positive lesions in the right upper lobe and multiple PET positive subcutaneous lesions, suggestive of malignancy (Fig. 4a).

Biopsy of two subcutaneous lesions showed no malignant cancer cells, but infiltration of predominantly histiocytes and within the histiocytes infiltration of structures, 2 µm in diameter, resembling yeast cells (Fig. 5). Both the size of the structures and its localization within histiocytes could be compatible with histoplasmosis. Additional molecular fungal identification directly on paraffin-embedded tissue biopsy material with ITS PCR revealed *Emergomyces pasteurianus* (GenBank accession number NR_137149, length 261 nucleotides, similarity 100%). On day +26, after 12 days of incubation, cultures of these subcutaneous lesions showed growth of a dimorphic fungus and molecular identification (ITS and LSU sequencing) at the Center of Expertise in Mycology Radboudumc/CWZ, Nijmegen, the Netherlands, confirmed *Emergomyces pasteurianus*. Additional antifungal susceptibility testing was performed by broth microdilution according to CLSI standards [6]. The following MICs were determined (mg/L): amphotericin B 0.031, anidulafungin 0.016, micafungin < 0.008, itraconazole 0.063, voriconazole 0.25, posaconazole 0.063, isavuconazole 1 and fluconazole 64. Patient tested negative for HIV infection and bone marrow biopsy showed no hematological malignancy.

She was treated with posaconazole and the daily prednisolone dose was gradually lowered to 5 mg. A PET-CT scan conducted after 6 months of posaconazole treatment (day +209) showed a decline in number and PET intensity of all lesions (Fig. 4b) and after 14 months of antifungal treatment (day +424) all subcutaneous and lung lesions disappeared (Fig. 4c). Posaconazole treatment was stopped on day +434.

3. Discussion

To our knowledge, we report the first two cases of *Emergomyces pasteurianus* infection in the Netherlands. Both our patients were

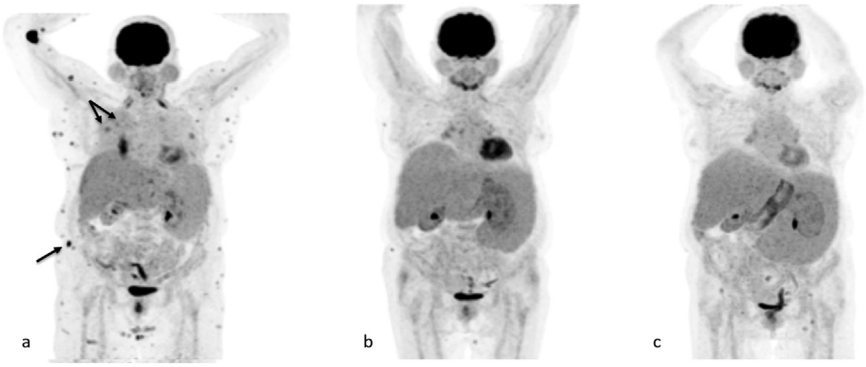

Fig. 4. Position emission tomography–computed tomography images at initial presentation (a), after 6 months (b) and after 14 months (c) of posaconazole treatment. The arrows indicate the two lung lesions and the largest subcutaneous lesion.

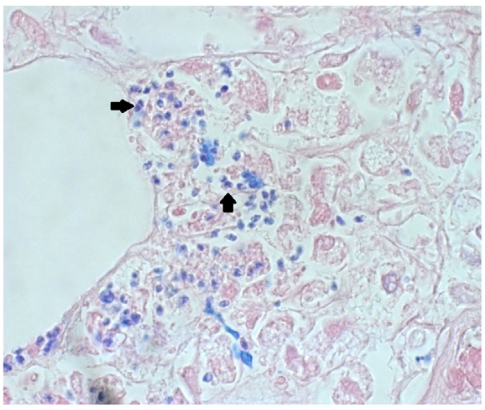

Fig. 5. Histopathologic section (Giemsa stain, x400) of a subcutaneous lesion, showing yeast-like structures (arrows, approximately 2 μm in diameter).

immunocompromised: due to a hematological condition (Case A) or therapy (Cases A and B). Pulmonary symptoms were present in both cases, cutaneous lesions as described in previously reported *Es. pasteurianus* cases [7–12] and in most cases of disseminated *Es. africanus* [3,13], were only present in Case B. *Es. pasteurianus* infection was diagnosed by culture and molecular diagnostic techniques of tissue biopsy material. Case A died on day +13 due to respiratory failure, whereas Case B was successfully treated with long-term posaconazole treatment.

Like *Blastomyces dermatidis* and *Histoplasma capsulatum*, *Es. pasteurianus* is a dimorphic fungus. Emergomycosis seems to be a disease of the immunocompromised host, with few case reports of immunocompetent patients [13,14]. The transmission route of *Emergomyces* species is still unknown. Probably the natural reservoir is soil and infection is presumed to occur via inhalation of conidia [15,16]. In histoplasmosis it is known that reactivation is possible when patients become immunocompromised. The first reported case of *Es. pasteurianus* (previously known as *Emmonsia pasteuriana*) was an Italian women with AIDS with no history of travel abroad [7]. Our patients may have acquired colonization or infection outside Europe, during a visit to their country of origin. It is not known whether *Emergomyces* remains present in the patient after initial infection or colonization and if it can reactivate if the patient becomes immunocompromised. So a more prolonged course of an imported infection in our patients cannot be excluded.

Currently, there are no treatment guidelines for patients with emergomycosis. Guidelines for blastomycosis and histoplasmosis recommend liposomal amphotericin B as initial therapy, followed by itraconazole and therapy duration should be at least 12 months or lifelong when immunosuppression cannot be reversed [17,18]. In Case A the patient was treated with liposomal amphotericin B and in Case B, the patient was successfully treated with 14 months of posaconazole and tapering daily prednisolone dose. A recent in vitro study of 11 strains of *Emergomyces* species showed that posaconazole had the lowest geometric mean MIC [19], supporting that posaconazole may be an alternative for liposomal amphotericin B or itraconazole.

The reported cases emphasize that clinicians should be aware of the presence of mixed, invasive fungal infections with fungi other than *Aspergillus* and *Zygomycetes* in immunocompromised patients. They should also be aware that invasive fungal infections may mimic malignancy. Molecular diagnostic techniques like ITS PCR could accurately and rapidly diagnose these fungi from clinical specimens.

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Conflict of interest

The authors declare no conflicts of interest.

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