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Invasive fungal infection by *Peziza ostracoderma* in an immunocompromised patient

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ABSTRACT

We report for the first time a case of disseminated infection caused by *Peziza ostracoderma*, a mold not previously associated with invasive infections in humans. *P. ostracoderma* occurs in natural and sterilized soil and may cause hypersensitivity pneumonitis in greenhouse workers. The immunocompromised patient presented with neutropenic fever that did not respond to broad-spectrum antibiotics and developed multiple skin and lung lesions. A skin biopsy demonstrated an angioinvasive mold, identified as *Peziza ostracoderma* by culture and DNA sequencing. Minimum inhibitory concentration (MIC) for amphotericin B was 0.125 mg/L, for isavuconazole 0.125 mg/L, for voriconazole 0.06 mg/L, and for posaconazole 0.03 mg/L. The skin lesions have resolved completely, and the lung lesions have decreased significantly in size after 14 months of mold-active antifungal therapy, mostly isavuconazole. In conclusion, *Peziza* species can be opportunistic pathogens causing considerable morbidity in immunocompromised hosts. The infection may be successfully treated with mold-active antifungal drugs.

1. Introduction

We report a case of disseminated infection with the mold *Peziza ostracoderma* in the lungs and the skin of an immunocompromised patient with chronic lymphatic leukemia (CLL). *P. ostracoderma* occurs in natural soil and grows particularly well in sterilized soil and peat soil in greenhouses and mushroom farms [1]. *P. ostracoderma* has previously been associated with hypersensitivity pneumonitis in greenhouse workers but has never been reported as an invasive pathogen in humans to the best of our knowledge [1,2]. However, it may be an emerging pathogen in immunocompromised hosts.

After a period of prolonged neutropenia, the patient presented with neutropenic fever that did not respond to broad-spectrum antibiotics. The patient developed lung infiltrates suggestive of a mold infection and painful skin lesions on the extremities. The diagnosis was established through histology, culture, and DNA sequencing of a skin biopsy. *P. ostracoderma* had low minimum inhibitory concentration (MIC) values to voriconazole, posaconazole, isavuconazole, and amphotericin B. The skin lesions have resolved completely, and the lung lesions have decreased significantly in size after 14 months of mold-active antifungal therapy, mostly isavuconazole. This is the first report of invasive infection with pezizales in humans and it may guide clinicians in the treatment of invasive infections with this mold in the future.

2. Case

2.1. Clinical presentation

The 59-year-old subject was diagnosed with atypical CLL and thrombocytopenia requiring treatment. Corticosteroids, romiplostim, rituximab, and intravenous immunoglobulin were administered without effect. A secondary aplastic anemia developed and further therapy with antithymocyte globulin (ATGAM®) and cyclosporine A was given after five weeks of neutropenia. Only two days after receiving ATGAM®, the patient was re-admitted to hospital due to neutropenic fever (day 0). The patient reported fever and chills but had no symptoms of focal infection. At admission, a body temperature of 38.0 °C was noted, but apart from that, routine physical examination was normal. C-reactive protein (CRP) was low (28 mg/L), and white blood cell (WBC) count was $0.2x10^9$ cells/L, with no neutrophils. Piperacillin/tazobactam 4 g 4 times daily was started for neutropenic fever on day 0. On day 3, therapy was changed to Vancomycin 1 g 3 times daily (in combination with ciprofloxacin as

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gram-negative antibacterial prophylaxis), because of growth of *Staphylococcus haemolyticus* in blood cultures both from day 0 and day 2. Echocardiography on day 3 did not show any signs of endocarditis. In nasopharyngeal culture from day 0, *Streptococcus pneumoniae* was found, whereas urine culture was negative. Ultrasound of the liver was performed on day 6, because of elevated levels of alkaline phosphatase (ALP) and alanine transaminase (ALT) in blood, but the examination was normal. Ceftazidime 1 g 4 times daily was added day 9 to day 12 and was later switched to Meropenem 500 mg 4 times daily (day 12 to day 20).

The high fever persisted, and CRP raised to about 200 mg/L despite treatment with broad-spectrum antibiotics. Therefore, to investigate signs of a mold infection, a computed tomography (CT) scan of the chest and sinuses was performed on day 9, even though the patient had no respiratory symptoms. The CT scan revealed rounded infiltrates measuring 2–3 cm in diameter in the left upper and lower lobe and a spiculated infiltrate measuring 1.5 cm in the right upper lobe that raised the suspicion of a mold infection (Fig. 1A). The CT scan of the sinuses was normal. Due to the suspicion of a mold infection in the lung, a bronchoscopy with bronchoalveolar lavage (BAL) was performed on day 10 for microbiological diagnostics. Petechiae were seen in the bronchial tree.

On day 6, a small skin lesion with the appearance of a pimple appeared at the patient's lower leg. In three days, it grew to a 2 cm large red tender lesion. On day 13, the skin lesion was about 10 cm wide, and a central necrosis had developed (Fig. 2A). At this time, multiple painful lesions of similar appearance had emerged at one arm (Fig. 2B). A skin biopsy was performed on day 13 and the histopathology examination reported inflammation and the presence of intravascular fungal hyphae (Fig. 3A and B).

In blood cultures drawn on day 9, *Candida glabrata* was found on day 11. However, there was no growth of *Candida* in repeated blood cultures taken daily for a week thereafter. The patient had received a central catheter on day 2, but there was no signs of a catheter-related bloodstream infection. An echocardiography on day 12 did not show any signs of endocarditis. Abdominal CT and magnetic resonance imaging (MRI) showed a two cm large low attenuation lesion in the spleen that could be due to infection whereas there were no lesions in the liver. However, a single lesion in the spleen is not sufficient for the diagnosis of hepatosplenic candidiasis. No signs of ocular fungal infection were seen at ophthalmoscopy on repeated examinations. In summary, the significance of the candidemia is unclear.

2.2. Microbiology

Microscopy, culture of fungi, and aspergillus PCR in BAL and protected specimen brush (PSB) from day 10 were all negative. Galactomannan test in BAL and serum on day 10 were also negative. Beta-D-Glucan assay (Fungitell®) on day 10 was slightly elevated (156 pg/mL).

Fluorescence microscopy of the skin biopsy taken on day 13 showed fungal hyphae. After 4 days culture (on day 17), a filamentous fungus appeared on Sabouraud agar. The fungus grew at 30 °C and 37 °C, but not at 42 °C. No images of the fungus were taken originally, but the cryopreserved fungus was re-cultured and the appearance of the dark colonies of *P. ostracoderma* on Sabouraud agar and of the ascospores in methyl blue stain in light microscope is shown in Figs. 4 and 5.

The sample was sent to a reference laboratory (Karolinska University Hospital) and was identified as *Peziza ostracoderma* by DNA sequencing of the fungal Internal Transcribed Spacer (ITS) region and pairwise alignment of the obtained sequence against public reference databases of Westerdijk Institute (https://wi.knaw.nl/page/Pairwise_alignment). The isolate showed 100% similarity to the reference sequence *Peziza ostracoderma* CBS 383.61 ex38548 ITS.

The MIC values were determined by the EUCAST method for susceptibility testing of molds after 48 hours incubation at 30 °C. MIC for amphotericin B was 0.125 mg/L, for isavuconazole 0.125 mg/L, for voriconazole 0.06 mg/L, and for posaconazole 0.03 mg/L.

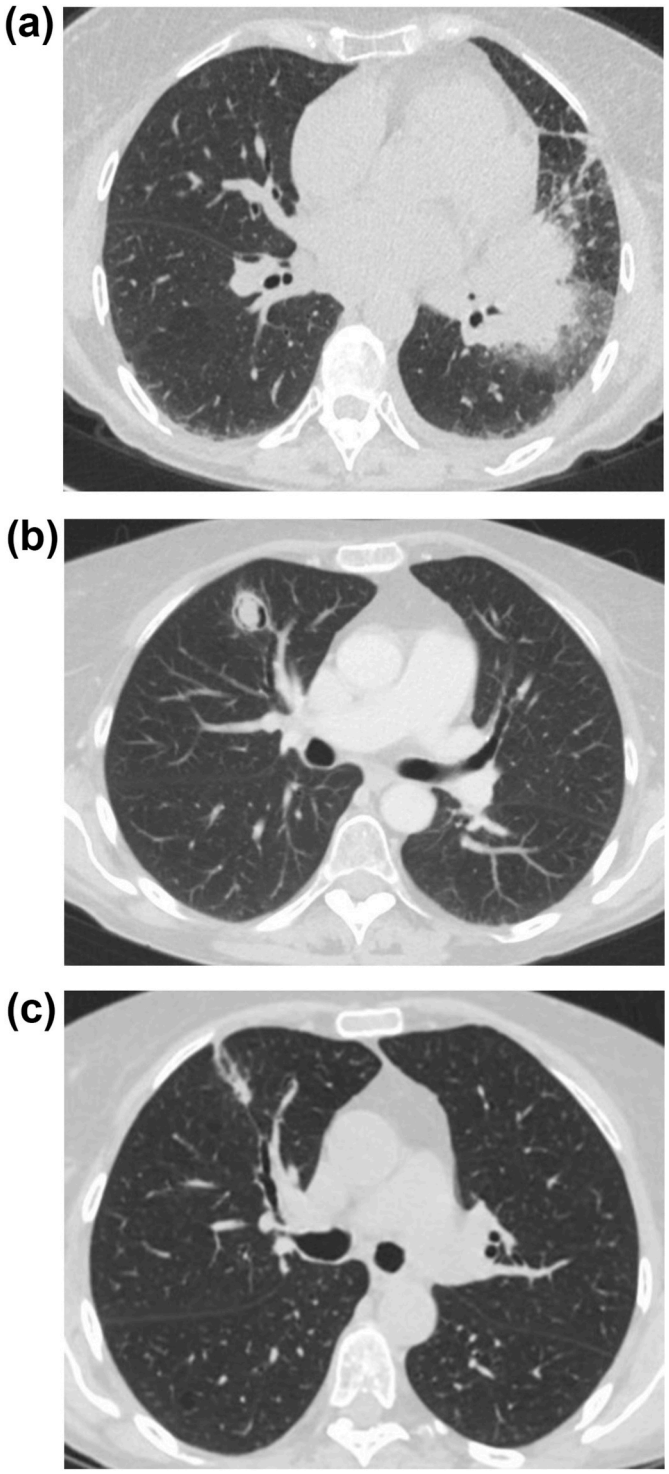

Fig. 1. Chest CT scan on day 9 showed rounded infiltrates measuring 2–3 cm in diameter in the left upper and lower lobe and a spiculated infiltrate measuring 1.5 cm in the right upper lobe, suggestive of a mold infection (1A). On day 81, the lesion in the right upper lung had evolved to a cavern about 2 cm in diameter (1B). After 14 months of antifungal therapy, a nodule measuring 9 × 5 mm remained of the previous cavern in the right upper lobe (1C).

The routine procedure for fungal diagnostics at Uppsala University Hospital is as follows: Biopsies and BAL specimens are stained with Blankophor prior to fluorescence microscopy. The specimens are cultivated on three plates; one with BBL™ CHROMagar™ Candida Medium

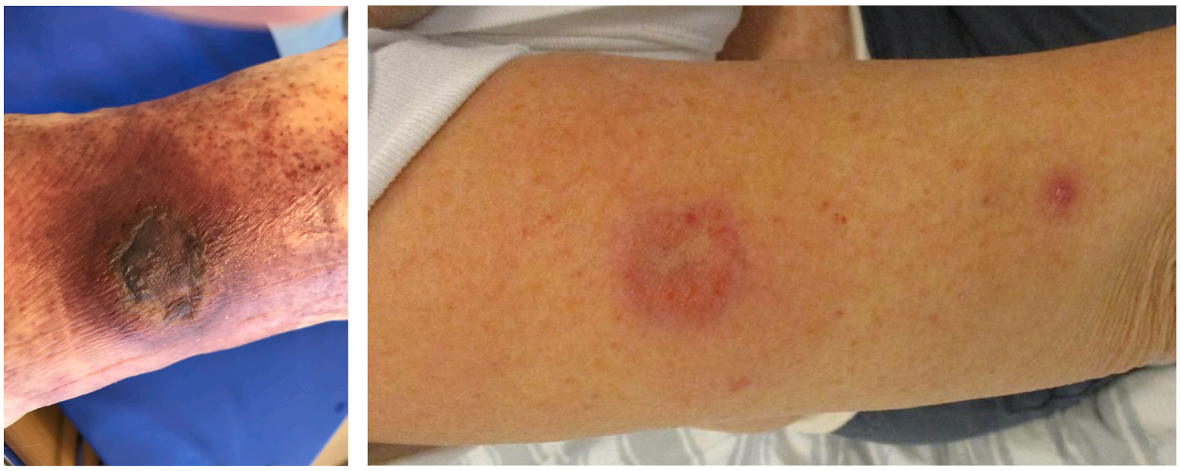

Fig. 2. The patient with neutropenic fever developed multiple painful skin lesions with central necrosis, first on the lower leg (2A), and some days later, on the upper arm (2B).

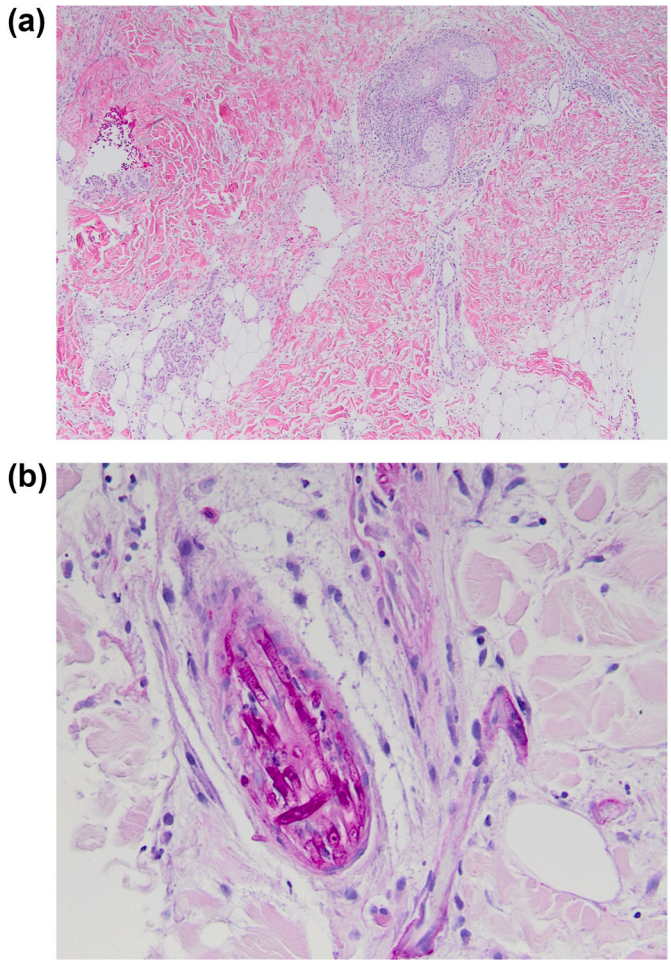

Fig. 3. Histological examination of a biopsy of the skin lesions in hematoxylin and eosin (H&E) staining (3A). In Periodic Acid-Schiff (PAS) stain, fungi are demonstrated in the blood vessels (3B).

(BD) incubated at 37 °C, one with Sabouraud agar (in-house production) incubated at 37 °C, and one with Sabouraud agar incubated at 30 °C. BAL cultures are incubated for a week and examined daily, whereas biopsies are incubated for two weeks. Biopsies are also cultured in Brain Heart Infusion broth with gentamicin added. Microscopy of the cultured fungi is performed after staining with methyl blue.

At the broncoscopy day 10, samples were also taken for culture of bacteria, microscopy, PCR and culture of mycobacteria, PCR for *Pneumocystis jirovecci*, *Legionella*, *SARS-CoV-2*, *Influenza A* and *B*, *Respiratory syncytial virus*, *Human metapneumovirus*, *Adenovirus*, *Parainfluenza virus 1–4*, *Rhino/enterovirus*, *Coronaviruses 229E*, *OC43*, and *NL63*, *Cytomegalovirus*, and *Herpes simplex virus*. All tests were negative apart from non-significant growth of *Actinomyces* and *Veionella* species.

2.3. Treatment and outcome

The patient had not received antifungal prophylaxis and since pulmonary aspergillosis was the work diagnosis after the chest scan, therapy with voriconazole was started after the bronchoscopy, on day 10. Voriconazole was given orally in standard dosage (400 mg two times a day the first day, thereafter 200 mg twice a day) and measurement of through concentration on day 12 showed adequate levels (4.3 mg/L). The patient developed a generalized exanthema and therefore voriconazole was switched to liposomal amphotericin B (7 mg/kg/day) on day 14. After 10 days of antifungal therapy, the patient became afebrile (day 21). After three weeks of antifungal therapy, the skin lesions had improved and a thoracic CT scan on day 31 exhibited partial regression of the infiltrates.

On day 39, the patient was also diagnosed with secondary hemophagocytic lymphohistiocytosis (HLH), based on the presence of fever, persistent pancytopenia, hemophagocytosis in the bone marrow, high ferritin levels (>40 000 μg/L), hypertriglyceridemia (6 mmol/L), and high soluble CD25 (2798 U/mL). Dexamethasone, intravenous immunoglobulin, and anakinra (IL-1 receptor antagonist) were administered for HLH as well as venetoclax for CLL.

When the MIC values were available, the treatment was changed back to voriconazole on day 59 without reappearance of the previous exanthema. However, the patient then developed an approximately 50% increase in creatinine and was switched back to liposomal amphotericin B (5 mg/kg/day) until creatinine was normalized. The neutrophil count was below 0.5×10^9 cells/L until day 68.

A thoracic CT scan on day 81, after ten weeks of antifungal therapy, showed continued regression of the left lung lesions but also the development of a cavern about 2 cm in diameter in the right upper lobe (Fig. 1B). Thereafter, on day 83 the antifungal therapy was changed to isavuconazole, and the patient could be discharged after a three-month hospital stay. Measurement of plasma-isavuconazole showed adequate trough concentration (2.6 mg/L).

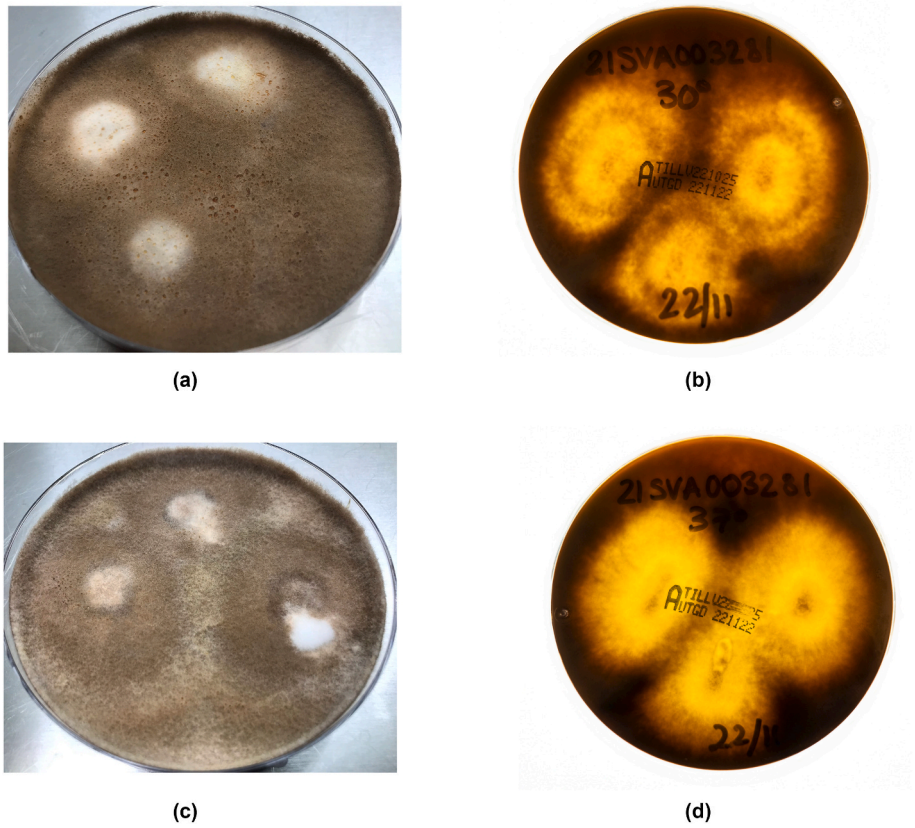

Fig. 4. Images of the culture of *Peziza ostracoderma* on Sabouraud agar incubated for 7 days at 30 °C from observe (4A) and reverse (4B), and at 37 °C from observe (4C) and reverse (4D).

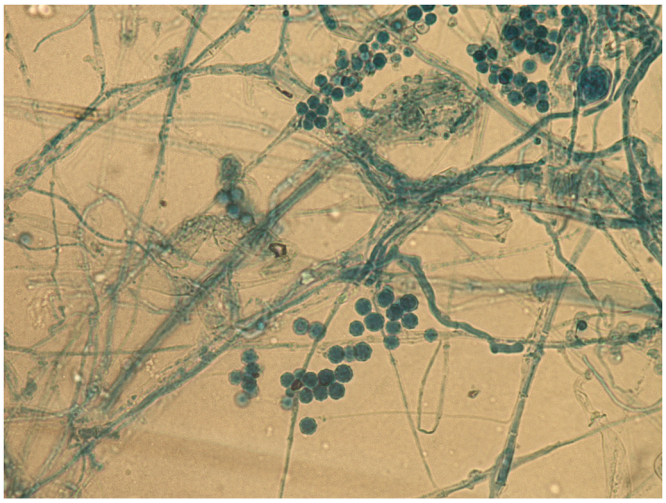

Fig. 5. Ascospores of *Peziza ostracoderma* stained with methyl blue, ×400 magnification in light microscope. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The patient experienced neuropathy and taste disturbance that could be side effects to isavuconazole. After 5.5 months of antifungal therapy, whereof the last three months with isavuconazole, the therapy was changed to posaconazole as secondary prophylaxis on day 178. The corticosteroids were gradually tapered down and five months after the diagnosis of HLH, anakinra was withdrawn.

However, two months after the switch to posaconazole, a chest CT scan, performed because of a viral respiratory infection, showed progression of the lesion in the right upper lobe. Plasma trough concentrations of posaconazole had been adequate (1.4 mg/L). Therapy was changed back to isavuconazole again on day 267.

The latest chest CT scan on day 436 demonstrated a residual nodule measuring 9×5 mm at the place of the former cavern in the right upper lobe (Fig. 1C). No clinical symptoms of infection remained. Venetoclax has been withdrawn due to excellent treatment effect, but immunosuppression with cyclosporine A and low dose corticosteroids is ongoing together with hematopoietic growth factors. Since all lung infiltrates have not resolved and further tapering of the immunosuppressive therapy has not been possible, the patient is still on isavuconazole at the time of writing this report.

3. Discussion

This is the first reported case of invasive infection with *P. ostracoderma* in humans. No prior reports were found in the PubMed database or in other sources on *Peziza* species and invasive infections in humans, nor when broadening the search to the family Pezizaceae, the order Pezizales, or the class Pezizomycetes [3]. *P. ostracoderma* belongs to the phylum Ascomycota and well-recognized human pathogens such as *Aspergillus, Candida, Fusarium,* and *Pneumocystis* belong to the same phylum [4].

P. ostracoderma is called a peat mold, because it typically grows on peat [1]. Besides peat, *P. ostracoderma* is generally found in sterilized soil, on paper pots and growing media in greenhouses, on mushroom beds, and in garden and forest soil [1,5]. High concentrations of *P. ostracoderma* in the air have been found in greenhouses and mushroom farms [1,6]. Exposure to airborne *P. ostracoderma* may cause hypersensitivity pneumonitis and precipitins against *P. ostracoderma* have been demonstrated in greenhouse workers with allergy symptoms [1,2]. *Aspergillus fumigatus* and *A. niger* are more well-known causes of

hypersensitivity pneumonitis [1].

Ascomycota is one of three fungal lineages that have the ability to infect humans; the other two being Mucoromycota and Basidiomycota [4]. In order to cause an invasive infection in humans, a fungus must possess certain characteristics, namely growth at human body temperature, penetration of surface barriers, lysis and absorption of tissue, and resistance to immune defenses [7]. This case report shows that *P. ostracoderma* possesses these features.

It is probable that our patient contracted the infection by inhaling fungal spores either from houseplants or nature soil during a period of prolonged neutropenia, even though no anamnestic data of digging in the soil in the garden or visiting greenhouses was found. The infection spread to the lungs and the skin. The diagnosis was made through microscopy and culture of a skin biopsy followed by DNA sequencing, which highlights the benefits of genomic methods in the diagnostics of invasive fungal infections of unknown etiology.

We assess that there was a hematogenous spread of the fungus in the body given that new skin lesions appeared at different parts of the body for a week during hospital stay. In addition, there was evidence of angioinvasive infection with hyphae detected inside the vessels in a skin biopsy. This spreading pattern resembles what is seen in disseminated fusariosis [8].

We cannot prove that *P. ostracoderma* caused the lung lesions since the fungus did not grow in BAL or PSB cultures and the lung lesions were never biopsied. Nevertheless, since the appearance on thoracic CT scan was similar with lesions seen in other invasive mold infections such as aspergillosis and all diagnostics for other fungi were negative from BAL, we firmly believe that the same fungus caused the skin and the lung lesions. Moreover, *Candida glabrata*, which was found in blood, does not cause this kind of lung infiltrates. *Actinomyces*, which grew in BAL, although in non-significant amounts, could possibly cause similar lung lesions. However, the patient did not receive long-term treatment for actinomycosis and would not have recovered from this diagnosis with the relatively short course of antibiotics given. Furthermore, it cannot be verified whether the lesion in the spleen were due to *P. ostracoderma*, *Candida glabrata*, or other causes. *P. ostracoderma* has beta-D-glucan in its cell wall, as do all ascomycetes. However, it cannot be determined whether the elevated beta-D-glucan level was due to the infection with *P. ostracoderma* or the concurrent candidemia.

P. ostracoderma can be treated with mold-active antifungal drugs such as voriconazole, posaconazole, isavuconazole, and amphotericin B. This patient mostly received isavuconazole due to side effects of voriconazole and progression of lung lesions during treatment with posaconazole. The skin lesions have resolved totally, and the lung lesions have decreased significantly in size. Antifungal therapy is still ongoing at the time of writing this report, adding up to a total treatment time of 17 months so far. Optimal treatment duration is not known and is dependent of ongoing immunosuppressive therapy. The heavy immunosuppression given, first for aplastic anemia and thereafter for HLH, can at least in part explain the long recovery time.

In conclusion, we report for the first time that *Peziza* species can be opportunistic pathogens causing considerable morbidity in immunocompromised hosts. It may be successfully treated with mold-active antifungal drugs.

Declaration of competing interest

Mia Furebring has received honoraria from UniMedic Pharma and Gilead. The other authors report no conflict of interest.

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