Case report

Isolation and identification of Rhizomucor pusillus from pleural zygomycosis in an immunocompetent patient

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Zygomycosis is usually an invasive mycotic disease caused by fungi in the class Zygomycetes. It often occurs in immunocompromised patients, but sporadic cases without apparent immune impairment have been described. This report presents the first case of pleural zygomycosis caused by Rhizomucor pusillus, an uncommon pathogen of human infection. A 19-year-old man was found to have pleuritis several days after a drainage catheter was implanted to cure a pneumothorax caused by a ruptured bulla. Local pneumonectomy to resect the ruptured bulla and vacuuming of the pleural fluid was performed. Rhizomucor pusillus was cultured from the pleural fluid and irregular broad sparsely septate hyphae, consistent with zygomycetes, were histologically detected in the thickened pleura of the resected bulla. The catheter was suspected of having been contaminated with the fungus, but no evidence could be obtained. His fungal pleuritis subsided without any antifungal medical therapy and his immunocompetence seemed to contribute to limiting the infection.

Keywords Human, pleura, Rhizomucor pusillus, immunocompetent

Introduction

Zygomycosis is an infection caused by filamentous fungi within the class Zygomycetes [1] which are ubiquitous being found in soil, plants and decaying material [1]. Common clinical manifestations of zygomycosis are rhinocerebral, pulmonary, gastrointestinal, cutaneous, and disseminated [2]. Most of these occur as a secondary infection in immunocompromised patients, such as those with diabetes mellitus, malignancy or transplantation [2], which are associated with impaired leukocyte immune function [3]. Rarely, primary zygomycosis occurs in immunologically healthy patients as the result of traumatic or surgical wounds that become exogenously contaminated [3]. To our knowledge, the case described here represents the first of pleuritis caused by Rhizomucor pusillus (R. pusillus) in an immunocompetent patient.

Case report

A 19-year-old man, who had three episodes of natural pneumothorax, was referred to a hospital because of chest pain. The pneumothorax episodes which occurred 38 and three months prior to observation were cured by conservative procedures while the one diagnosed 36 months previously was cured by video-assisted thoracoscopic surgery. Chest roentgenography of the present episode was consistent with left side pneumothorax. Therefore, a drainage catheter was implanted in the pleural cavity on Day 1. However, air leak from the left lung continued and the patient was transferred to our hospital for surgical procedures on Day 7. On Day 8, computed tomography...
revealed left pneumothorax and several bullas at the bilateral pulmonary apex. On Day 9, the patient had a fever of 38°C and the peripheral white blood cell count was 11,800/μl. Cough, sore throat and sputum production were not evident. The respiration sound was clear. Pleuritis due to a contaminated catheter tube was suspected. Antibiotics were started and video-assisted thoracoscopic surgery was performed on Day 10. The left lung apex portion with bulla was excised. The left pleural fluid was cloudy and the pleural fluid was presented for culture. The left pleural cavity was cleansed with saline and a new catheter tube was implanted. The fever subsided on Day 12. The peripheral white blood cell count on Day 14 was 7,200/μl. Antibiotics were therefore stopped. The catheter tube was removed and he was discharged on Day 16. He has remained well after 15 months of follow up.

Pathological findings
Grossly, the excised left lung apex showed thickened pleura. The pleural surface was smooth and the ruptured portion could not be detected. On the cut surface of the lung, there was a cleft-shaped bulla under the thickened pleura in connection with paraseptal emphysema. Microscopically, focal purulent inflammation was found in the fibrous-thickened pleura and subpleural space (Fig. 1a). Purulent pneumonia was limited to the focal area adjacent to the bulla. Sparlessly septate, broad hyphae with right-angle branching, consistent with members of the zygomycetes, were noticed in the thickened pleura and purulent exudates of bulla space (Fig. 1b,c). There was no hyphal invasion into the pulmonary parenchyma and no apparent fungal angioinvasion was detected.

Immunohistochemical analysis was kindly performed by Dr Masao Hotchi, Shinshu University, on paraffin sections of the left lung apex lesion following the method of Fukuzawa et al. [4] using rabbit polyclonal anti-

Rhizopus oryzae antibody. This antibody had been confirmed to react with fungal elements of zygomycetes and not with Aspergillus, Candida, Trichosporon, Cryptococcus and Fusarium. All thin-walled broad hyphae in the thickened pleura were positive with the antibody that was consistent with those of a zygomycete (Fig. 1d). They were negative on negative control sections.

Mycology
Within 3 days at 37–45°C, all potato dextrose agar (PDA) cultures inoculated with portions of the pleural fluid produced brownish-gray colonies which covered the agar surface of the petri dishes (Fig. 2a). Microscopic examination of the cultures showed (i) poorly developed rhizoids and distinct stolons, (ii) brown–gray, smooth-walled, unbranched or more commonly branched sporangiophores of up to 16 μm in diameter and 80 μm in length, (iii) brown–gray, globose sporangia 55–80 μm in diameter, obovoid to pyriform columnellae, and (iv) hyaline to light gray, subglobose or ellipsoid sporangiospores of 3–4 μm diameter with smooth walls (Fig. 2b). Zygospores were not observed in the culture. Scanning electron microscopy of the sporangiospores revealed almost smooth surfaces (Fig. 2c–e). Sporangial development was observed at 50°C. On the basis of these morphologic characteristics, the isolate was identified as R. pusillus (Lindt) Schipper [56].

In vitro susceptibility tests
The in vitro susceptibility of the isolate to antifungals was tested according to Clinical and Laboratory Standards Institute guidelines (M38-A) [7]. Minimum inhibitory concentrations (MICs) to antifungals were as follows: amphotericin B 0.25 μg/ml, 5-FC >64 μg/ml, fluconazole >64 μg/ml, miconazole 1 μg/ml, itraconazole 0.25 μg/ml, micafungin 16 μg/ml, and voriconazole 8 μg/ml.

Molecular testing
Fungal DNA was extracted from the isolate according to the rapid method described by Makimura et al. [8]. The 0.5-kbp fragment from the D1/D2 28S rDNA regions was sequenced directly from the PCR products using the 28SF1 and 635 primers as previously reported [9]. The NCBI BLAST database (http://www.ncbi.nlm.nih.gov/blast/) was searched for the DNA sequences of the amplified fragments, which completely (100%) matched only R. pusillus (DDBJ/EMBL/GenBank accession #: AF113474 and AF113475). Based on the results of the DNA sequence analysis, the isolate was confirmed as R. pusillus and was subsequently added to the collection of Teikyo University Institute of Medical Mycology Culture Collection with the identifier TIMM6207.

We also tried to detect 28S rDNA sequences of Rhizomucor pusillus according to the method described by Voigt et al. [10] in paraffin embedded tissue of the pleural lesion. However, we failed to detect identical sequences to those of the fungal isolate.

Discussion
In 1978, the genus Rhizomucor was described as thermophilic Mucor-like fungi with stolons and rudimentary rhizoids [5]. R. pusillus, formerly known as Mucor pusillus, has been reported as an uncommon human pathogen [2]. Our search of the literature found 17 cases with sufficient clinical information to identify this organism as the causal agent of human disease [11–24]. The mean age of the patients was 46.5 years (range, 1.75–78 years), with
12 males and five females. The underlying conditions were leukemia in 13 cases, non-Hodgkin lymphoma in one case, diabetes mellitus in one case, myelofibrosis in one case and aplastic anemia in one case. Cytotoxic agents had been used in 15 cases, which resulted in neutropenia and corticosteroid in 11 cases. The present patient is the first human case with *R. pusillus* infection with no systemic underlying disease. Pre-mortem diagnosis of zygomycosis was accomplished in 13 cases. Methods of diagnosis included biopsy histology in six cases, a combination of biopsy histology and aspiration cytology in two cases, histology of resected material in three cases and a combination of culture and computed tomography scans in two cases. Successful pre-mortem isolation of *R. pusillus* resulted from needle aspiration fluid in two cases, bronchial washing fluid in two cases, bronchoalveolar lavage fluid in one case, nasal swab in one case, biopsied tissue in five cases and resected tissues in three cases. *R. pusillus* was isolated only from autopsied materials in six cases. Lungs were the most commonly affected organ and pulmonary lesions were seen in 13 cases [11–13,17–22,24]. In five cases infection was restricted to the lung, whereas in another eight cases, lung involvement was a part of disseminated infection. In one of these, initial rhino-orbito-sinus infection spread to the brain and lung. Mere sinus involvement was seen in two cases [15,23] and cutaneous infections were only found in two cases [14,16]. The overall mortality was high in that nine patients with visceral organ involvement died, including two with bacterial sepsis. Four recent cases of pulmonary or sinus-orbital infection were treated with lipid formulations of amphotericin B with or without G-CSF and were cured [20–23].

Zygomycetes including *R. pusillus* are ubiquitous and humans undoubtedly repeatedly inhale airborne zygomycetes spores. In epidemiological studies, *R. pusillus* was cultured from the hospital rooms and from all parts of an air-conditioning system [15]. Meanwhile, in a separate study, spores were also isolated from air samples taken in a corridor adjacent to a patient-hospitalized room [19]. In-hospital acquisition of the the etiologic agent is
highly suspected to be associated with the infection. Furthermore, direct contamination of skin or wounds by traumatic implantation of spores from soil and other environmental sources may occur, even in the hospital setting. Cellulitis caused by R. pusillus at the needle insertion site of continuous insulin infusion pump has been reported [16]. The route of entry of zygomycetes cannot be confirmed in the present case because cultures were only inoculated with pleural effusion fluid. Nevertheless, the drainage catheter or guiding needle for the catheter may have been contaminated with zygomycetes before insertion or at the unsterile skin site, and its insertion to the pleural cavity might have caused fungal pleuritis. Pleural zygomycosis has been reported only once so far, and it was caused by a biliary drainage catheter which accidentally traversed the right pleural cavity [25]. The causative agent was not cultured in that case. A spread of pulmonary zygomycosis to the pleura is unlikely in the present case because only pleuritis was detected without apparent pulmonary zygomycosis.

The most definitive method to obtain a diagnosis of zygomycosis is to visualize the characteristic hyphae in tissue biopsies, i.e., the presence of irregularly shaped, broad (6–25 μm in diameter), sparsely septate hyphae with right angle branching [26]. They have a high affinity for invading blood vessels causing thrombosis, hemorrhage and tissue infarction [12,13,16–20]. A neutrophilic infiltrate is usually absent in the setting of a compromised immune system [3]. The present patient was immunocompetent without neutropenia and as a result purulent inflammation was obvious with characteristic hyphae in the pleural tissue.

Immunohistochemistry using specific antibodies is helpful for the accurate diagnosis of a number of important mycoses. In the present case, all hyphae of the pleura were immunohistochemically found to be zygomycetous, but no specific genus or species could be confirmed by this technique.

Culture is the only method that allows for species identification in most cases of zygomycosis. Recently, Iwen et al. showed that molecular methods for the diagnosis of zygomycosis were promising for species identification of the fungus in culture and even in tissue extracts [23]. In their study, they targeted the internal transcribed spacer (ITS) regions of the rDNA. The 18S and 28S rDNA can
be also used as target to the identification of species of zygomycetes, as shown in the present case [9,10]. Identification of zygomycetous fungi is a challenge for clinical laboratories. The ability to identify uncommon pathogenic fungi, including *R. pusillus*, to the species level by 28S rDNA sequencing will continue to emerge as a viable alternative for confirmatory diagnosis.

Amphotericin B is the agent of choice for zygomycosis. In addition, surgical debridement or excision of infected tissue is essential in many cases of zygomycosis [3,26]. Partial or complete pneumonectomy can be curative in patients with pulmonary zygomycosis confined to one lung [27]. Partial pneumonectomy without antimycotic medical treatment seemed to be satisfactory in the present case, possibly due to the good immune status and the fortunate lack of fungal angioinvasion.

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**References**