Pulmonary mucormycosis diagnosed by brushing cytology. A case report

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Abstract
Pulmonary mucormycosis, an uncommon fungal infection occurs among immunocompromised individuals. It is clinically rapidly progressive and often fatal, early diagnosis is critical to patient survival. Bronchoscopy with bronchial brushing is extremely useful for diagnosing pulmonary mucormycosis in immunocompromised hosts as these organisms are morphologically distinct and are infrequently isolated from clinical samples.

We report a case of pulmonary mucormycosis diagnosed on bronchial brushing cytology in a 47 year old male patient presented with pneumonia, acute renal failure and type II diabetes. The Papanicolaou stained filter preparation from the specimen showed broad, ribbon like asceptate fungal hyphae with right angled branching. The characteristic cytologic features permitted a diagnosis of mucor infection. This case demonstrates that brushing cytology is a useful technique in the rapid diagnosis of pulmonary mucormycosis. Familiarity with the cytologic appearances of these fungi assist in the correct diagnosis of this disease. This case is of interest also as it showed associated needle shaped crystals in rosette formations and bilateral renal mucormycosis.

Key words: mucormycosis, zygomycosis, phycomycosis, Rhizopus, brushing cytology


Introduction
Pulmonary mucormycosis is an aggressive fungal infection, usually associated with vascular invasion and infarction and can be fatal if not treated quickly (1). Early diagnosis is essential to successful treatment. We report a case that demonstrates the usefulness of bronchial brushing cytology in the diagnosis of this infection.

Case report
A 49 year old male presented with the clinical features of a severe left upper lobe (LUL) community acquired pneumonia, acute renal failure, diabetic ketoacidosis with a pH on admission of 6.99 and type II diabetes. He was treated with intravenous fluids, intravenous antibiotics, cefuroxime and erythromycin. He improved on antibiotics and became afebrile, and was discharged after seven days.

Five days following discharge, he presented with abdominal pain, left sided pleuritic chest pain, fever, shortness of breath and cough. His sputum was mucopurulent and blood stained with a heavy growth of coagulase negative Staphylococcus, no acid fast bacilli were seen. A blood culture gave no growth after 48 hours incubation. His urine showed only mixed bowel flora on culture. Intravenous cefuroxime and metronidazole were commenced with fever. Pain, left sided pleuritic chest pain, fever, shortness of breath and malnutrition (1-8). The patient presented in this case clearly had predisposing clinical conditions, uncontrolled diabetes with severe diabetic ketoacidosis and acute renal failure.

Seventeen days after admission fungal growth was noted on the urinary and sputum specimens and fluconazole commenced. Bronchoscopy was performed that day and revealed a left upper lobe completely replaced by necrotic tissue. Bronchial washing, brushing and biopsy of left lower lobe were performed via the bronchoscope. Bronchial washing and brushing cytology revealed fungal hyphae consistent with mucormycosis. The biopsy also showed fungal hyphae.

A diagnosis was made of pulmonary and bilateral renal mucormycosis. The patient was treated with amphotericin B intravenously. After full lung function testing, the patient was considered not fit for curative cardiothoracic surgery due to the unacceptably high perioperative risk. Medication was discontinued and the patient was discharged for palliative care in home environment. He died twelve days later.

Material and methods
Bronchoscopy derived bronchial washing and brushing specimens were collected in cytology containers with 30% ethyl alcohol in physiologic saline. The filter preparations were made from both specimens on size 5 μm Sartorius membrane filter (Ag-cellulose acetate, Germany) using the cytosieve method and stained by Papanicolaou method.

Results
Cytologic findings
Papanicolaou stained filter preparations showed numerous acute inflammatory cells, reactive bronchial cells and broad, ribbonlike asceptate fungal hyphae with right angled branching at irregular intervals consistent with Rhizopus/mucor infection (Figures 1A and 1B). In addition, a few rosettes of needle shaped crystals were identified (Figure 2).

Histologic findings
Histologic examination of the bronchial biopsy taken during bronchoscopy confirmed the diagnosis of mucor infection. H & E stained sections of the biopsy showed an aggregates of broad, asceptate hyphae with right angled, irregular branching (Figure 3) and a few rosettes of needle shaped crystals (Figure 4).

Discussion
Mucormycosis, also known as zygomycosis or phycomycosis, is an infection caused by one of a variety of fungi belonging to the class phycomycetes, order mucorales. The common genera causing this infection are Rhizopus, Mucor and Absidia (2,3,6,7). Although these fungi are usually saprophytic and ubiquitous in nature, being found in soil and mouldy bread, they can cause serious and rapidly fatal infection in man as opportunistic disease agents (4).

Infection typically occurs in individuals who are immunosuppressed (5) and have underlying systemic disease such as uncontrolled diabetes (especially diabetic ketoacidosis), haematologic malignancies, renal failure and malnutrition (1-8). The patient presented in this case had underlying clinical conditions, uncontrolled diabetes with severe diabetic ketoacidosis and acute renal failure.

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In diabetes mellitus, acidosis plays an important role in the development of fungal infection. With the fall in blood pH, there is increased release of iron from transferrin that enhances hyphal growth (2). In addition, diabetes causes neutrophil dysfunction, a key defense against fungi.

The organism is ubiquitous in the environment and may be acquired several ways. Intravenous drug abusers are at risk most likely due to direct inoculation by needles contaminated with the organisms (6). In organ transplant recipients immunosuppression is a major predisposing factor either by activation of latent infection or by greatly reducing lethal dose of the organism (2). This infection may occur in immunocompetent patients due to traumatic implantation of soil contaminants (7).

Mucormycosis most commonly involves the rhinocerebral, pulmonary, gastrointestinal and subcutaneous tissues (1,3,6,7). A generalized or disseminated infection may also be seen and renal infection is usually seen in this context. Isolated pulmonary mucormycosis without evidence of dissemination is quite uncommon (2).

The symptoms of pulmonary mucormycosis are fever, cough and shortness of breath, and initially signs of consolidation, which mimics bronchitis and pneumonia. As the organism invades blood vessels, necrosis of the parenchyma leads to cavitation, seen on x-ray. Patients are usually treated with broad spectrum antibiotics, which fail to slow the disease progression – demonstrated by this patient. Without systemic antifungal therapy death occurs in two to three weeks (2). The best chance of survival occurs with early diagnosis, and bronchial brushings are the key investigation to allow this.

Bronchial brushing is an established tool for diagnosis of various pulmonary neoplastic and non neoplastic diseases, and is particularly sensitive in diagnosing peripheral lesions. Sputum culture in pulmonary mucormycosis can fail to give a positive result in over 50% (2) and takes considerable time before a result is available. Transthoracic lung biopsy, thoracentesis, percutaneous needle biopsy and bronchoalveolar lavage are less successful than bronchial brushing in obtaining diagnostic material (2) and are invasive diagnostic procedures unsuitable for in severely ill patients with possible fungal vascular invasion and infarction. By using multiple brushings the diagnostic sensitivity is increased and the need for, but also invasive procedures, is decreased. In addition, bronchial brushing cytology gives a rapid diagnosis facilitating early treatment (7).

The cytologic diagnosis of mucormycosis is based on the recognition of the characteristic mucorales fungal hyphae. Regardless of the species, the hyphae fragments are quite similar and treatment is the same. The hyphae are quite variable in size with average 15-20 µm in diameter and aseptate, unlike Aspergillus, branching at irregular intervals and at right angles as opposed to acute angles as in Aspergillus. The tendency of the hyphae to fold and wrinkle gives them a ribbon like appearance which is very helpful in identification. In the case presented here, the diagnosis was made solely by cytomorphology and supported by biopsy findings.

As well as fungal hyphae a few rosettes of needle shaped birefringent (polarizable) crystals were noted. Various types of crystals such a calcium oxalate, monosodium urate and calcium salts of fatty acids can be associated with the fungal infection (4,9). These crystals were not analysed, but morphologically are probably calcium oxalate, known to be produced occasionally as a metabolic byproduct of mucor infections, especially in high oxalate environments such as in renal failure. After reviewing the literature, we believe that this report is possibly the third case of pulmonary mucormycosis diagnosed on brushing cytology in the English literature and first case with associated crystals on brushing cytology.

Conclusion
This case demonstrates the ability to diagnose pulmonary mucormycosis by bronchial brushing cytology which is less invasive than biopsy and fine needle aspiration biopsy and allows for more rapid identification of fungal organisms than histologic examination or and culture.
References


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