Endobronchitis by *Scedosporium apiospermum* in a child with cystic fibrosis

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Summary

A case of endobronchitis by *Scedosporium apiospermum* in a child with cystic fibrosis is presented. The bronchial aspirate´s cytology showed the presence of a large amount of septated-dichotomized hyphae. The bronchial aspirate´s culture showed the presence of *Scedosporium apiospermum* in a pure culture of three consecutive samples. The scanning electron microscopy study of the mucosal surface revealed scarce mycelia with the presence of abundant conidiae. The transmission electron microscopy of the mucosa revealed inflammatory infiltrates constituted by macrophages, polymorphonuclear leukocytes, a lot of dichotomized mycelia and macrophages with hyphae and conidiae within the phagosomes. The patient was treated with amphotericin B and itraconazole.

Key words *Scedosporium apiospermum,* Cystic fibrosis, Endobronchitis

Endobronquitis por *Scedosporium apiospermum* en una niña con fibrosis quística

Resumen

Presentamos un caso de endobronquitis por *Scedosporium apiospermum* en una niña con fibrosis quística. El diagnóstico se confirmó mediante laboratorio. La citología del aspirado bronquial mostró la presencia de grandes cantidades de micelio dicotomizado septado. El cultivo del aspirado bronquial en tres muestras consecutivas, mostró la presencia de *Scedosporium apiospermum* en cultivo puro. El estudio de la superficie de la mucosa, mediante microscopia electrónica de barrido, reveló la presencia de micelio escaso, contrastando con la presencia de una gran cantidad de conidias. La microscopia electrónica de transmisión realizada en los cortes de la mucosa bronquial, reveló la presencia de infiltrado inflamatorio constituído por macrófagos, leucocitos polimorfonucleares y una gran cantidad de micelio dicotomizado y macrófagos con micelio y conidias en el interior de fagosomas. La paciente fue tratada con anfotericina B e itraconazol.

Palabras clave Fibrosis quística, Endobronquitis, *Scedosporium apiospermum*

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Cystic fibrosis is the most common autosomal recessive disorder in white people, with a frequency of about 1 in 2500 live births [1]. The disease is characterized by the dysfunction of the exocrine glands. This dysfunction causes the production of abnormally viscous bronchial secretions [2].

Oftenly, the lungs of patients with cystic fibrosis are colonized by bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* [3].

In more advanced stages of the disease, the respiratory tract can also be colonized by filamentous fungi due to the entrapment of spores in the bronchial pulmonary mucus and damaged bronchopulmonary epithelial tissue induced by bacterial proteases and leukocytic enastase. The two most frequently isolated fungi are *Aspergillus fumigatus* and *Scedosporium apiospermum* [4].

The *Scedosporium* genus harbors two medically important species: *S. apiospermum* (*Pseudallescheria boydii* teleomorphic phase) and *S. prolificans*.

S. apiospermum has been reported as causing local or disseminated infection in normal hosts and immunocompromised patients [5, 6]. There is only one report on the ability of *S. apiospermum* to invade the bronchial mucosa in an adult immunocompetent patient. These authors found fungal hyphae invading the bronchial mucosa [7]. However, the pathogenic role of *S. apiospermum* in patients with cystic fibrosis is still controversial [2].

Here in, we report a case of endobronchitis by *S. apiospermum* in a patient with cystic fibrosis, where the fungi was shown invading the bronchial mucosa in addition to the presence of conidiogenesis on the mucosal surface.

Case Report

A seven-year old female patient with a history of cystic fibrosis by mutation D-F 508 detected when she was 1 year 9 months-old. From that point on, the patient was admitted to the hospital on several occasions with repetitive bronchial and lung disease and diarrheal episodes.

Six weeks before her last admission, the patient relapsed with a lung infection, secondary to bronchopneumonia. Bronchial sputum was cultured reporting over 100,000 colony forming units of *Pseudomonas fluorputida* and *Staphylococcus aureus* resistant to amikacin and ceftazidime. Instead, she was treated with ciprofloxacin. She responded favorably, and was discharged two weeks later.

On January 9th, 2004, the patient was re-admitted with respiratory problems, fever, difficulty breathing, cough, dysnea, and vomiting. At this time, the patient had a 10.7 g/dl hemoglobin; 29,900 leukocytes, 81% polymorphonuclear neutrophils, 15% lymphocytes, 3% monocytes, 2% eosinophils, and 700,000 platelets.

A sample of bronchial aspirate was sent to the laboratory for cultured. A serial of three blood cultures and one urine culture were also submited. Two bronchial aspirate cultures were positive for *S. apiospermum*. Colony surface of cultures had a spreading, grey, cottony aerial mycelium with a reverse grey. The microscopic morphology showed septated hyphae with conidiophores bearing oval conidia, either single or in small groups. The blood cultures and the urine culture were both negative.

A serial bronchoscopies and bronchial washing were carried out for three consecutive days, a previous preparations with Dnase was indicated to facilitate mechanical elimination of secretions.

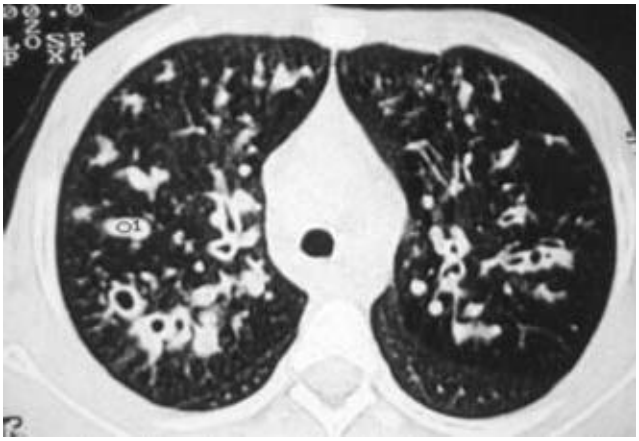

Figure 1. Chest computerized axial tomography showing the presence of multiple bronchiectasias and bronchial thickening.

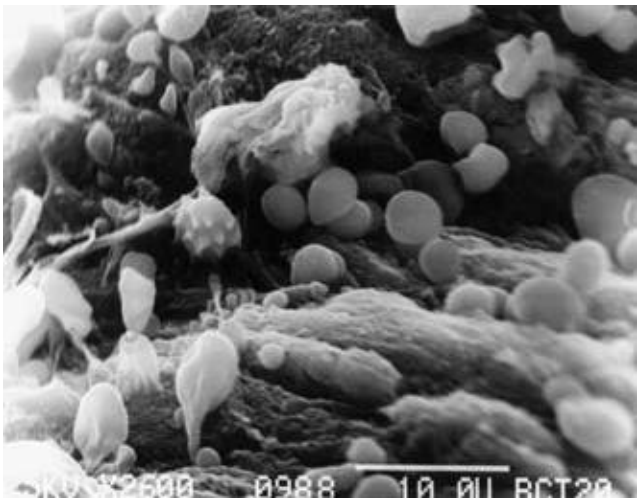

Figure 2. Bronchial mucous biopsy showing scarce mycelia and a large amount of conidia emerging from the mucosa. Scanning electron microscopy x 2600. Bar=10 µm.

The broncoscopy revealed moderate endobronchitis with hyperemia and mucosal edema, as well as scarce mucopurulent secretion. The cytology of the bronchial aspirate showed the presence of a large amount of septated-dichotomized mycelium.

Since no other etiologic agent was isolated in addition to *S. apiospermum*, a biopsy of the mucosa was carried out to determine whether *S. apiospermum* was found only on the mucosal surface, or whether there was an invasive infectious process in the bronchial mucosa that would justify using systemic antimycotics.

The laboratory culture report of the bronchial aspirate obtained by bronchoscopy confirmed the presence of *S. apiospermum* in a pure culture from of three consecutive samples. The patient was treated with amphotericin B and itraconazole at a daily dose of 1 mg/kg/day and 100 mg/day, respectively.

The lung computerized tomography did not reveal suggestive data of a pneumonic process or fungal balls, only showed bronchiectasis and bronchial thickening (Figure 1).

The scanning electron microscopy study revealed the scarce presence of mycelium on the mucosal surface. This scarce mycelium on the mucosal surface corresponds the majority of times to terminal hyphae with the presence of anelloconidiae (Figure 2).

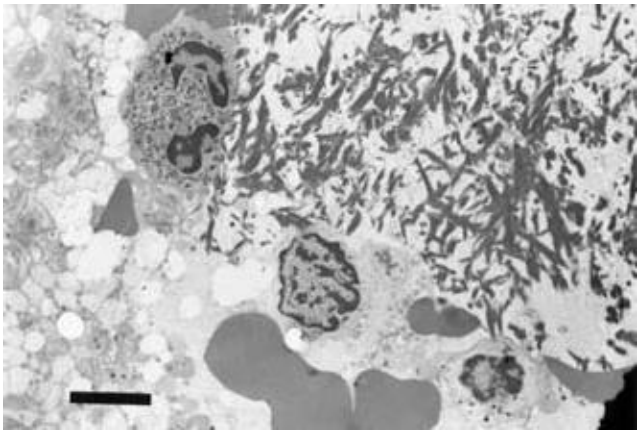

Figure 3. Bronchial mucous biopsy showing the presence of mycelia and inflammatory infiltrate by macrophages and polymorphonuclear cells. Transmission electron microscopy 3200x. Bar=10 µm.

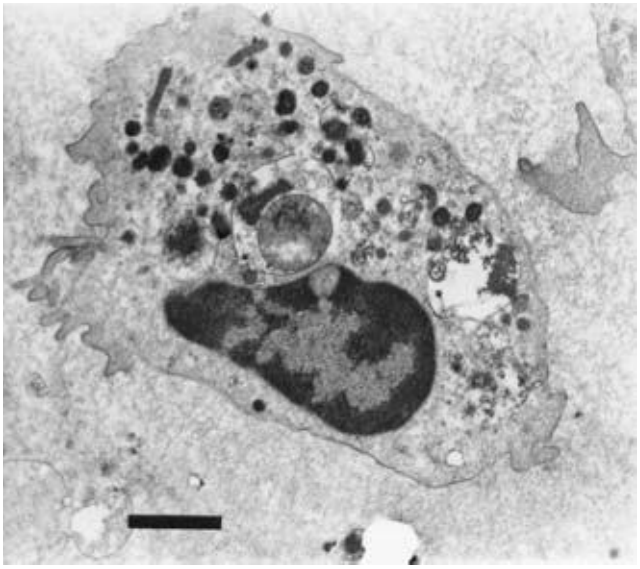

Figure 4. Macrophage and the presence of mycelia remains and a conidia inside a phagosome. Transmission electron microscopy 7000x. Bar= 5 µm.

In 800 nm thick mucosa slices, stained with toluidine blue, we observed inflammatory infiltrates constituted by abundant dichotomized hyphae, macrophages and polymorphonuclear leukocytes found in the process of destruction secondary to macrophagic activity, as well as scarce conidia within the mucosa. Fine 90 nm slices showed under transmission electron microscopy, dichotomized hyphae, leukocytes and macrophages with hyphae and conidiae within the phagosomes (Figure 3 and 4).

With the information obtained through endoscopy, and biopsy, we were able to reach the diagnosis of endobronchitis by *S. apiospermum*. A deeper mycotic infection was discarded and amphotericin B was suspended (total dose of 15 mg/kg). The patient continued with itraconazole. There was a good tolerance with both antifungals, and no secondary or adverse events related to treatment were observed.

The patient evolved satisfactorily with no fever, a progressive decrease of cough and dysnea. During her last lung function test, she responded favorably to bronchodilators.

On February 3, 2004, she was discharged with itraconazole (100 mg/d) as maintenance therapy for four weeks. After-treatment cultures in Sabouraud dextrose agar were negative. Currently, the patient has gained weight and grown, she is in good clinical conditions without the presence of active lung infection.

Discussion

Infections by *S. apiospermum* can occur in immunocompetent, as well as immunosuppressed patients. In immunsuppressed, the clinical picture is more severe and usually has a bad prognosis. [8]. The most frequent predisposing factors found in the literature are: immunodepression secondary to treatment with steroids, organ transplants, blood cancer, diabetes mellitus, and AIDS. The infections in these types of patients are usually invasive pneumonia, endophtalmitis, endocarditis, meningitis, sinusitis, brain abscess, skin and subcutaneous tissue infections and disseminated disease. Other clinical scenarios reported as the presence of fungal balls, are related to mycotic colonization in patients not immunologically compromised with pre-existing cavities and the colonization of the airways of patients with pre-existing lung disease such as cystic fibrosis [9].

In Bakerspigel and Schaus´ series, cystic fibrosis and pre-existing lung disease were factors favoring airway colonization by *Pseudallescheria boydii*. All cases presented the fungus in its mycelial form on the mucosal surface and none of the cases had tissue invasion [10].

Yano and cols. reported the first case of bronchial mucosal invasion by *S. apiospermum* in a 74-year old immunocompetent woman [7]. In this report we have presented the first case of endobronchitis by *S. apiospermum* in a child with cystic fibrosis with bronchial mucosal invasion and conidiogenesis on the same surface.

Cimon isolated *S. apiospermum* in 8.6% of a sample that included 128 patients with cystic fibrosis studied for a 5-year period. *S. apiospermum* was found to be the most frequently isolated fungi in the respiratory tract in these types of patients after *Aspergillus* [4]. On the other hand, *S. apiospermum* can trigger an inflammatory response in deeper processes suggesting it may play a pathogenic role in patients with cystic fibrosis [4]. Considering the findings in this study, it is believe that *S. apiospermum* should be consider one of the etiologies expected in patients with cystic fibrosis and with endobronchitis.

Our patient had a great amount of conidiae and scarce hyphae on the bronchial mucosal surface, different from that found inside the mucosa where there was a large amount of hyphae and scarce conidiae. This behavior could possibly correspond to a mechanism of evasion of the immune response on the fungi's part, as has been often seen in cases of invasive candidiasis where macrophages remove and destroy the majority of the blastoconidiae, while those that escape phagocytosis, rapidly transform into mycelial structures that penetrate host tissues, causing damage. The different studies carried out related to the anti-*Candida* activity of macrophages show that they mainly act against blastoconidiae. However, there is evidence that macrophages, as well as other natural effector cells may also affect *Candida* in its mycelial phase [11]. This could explain the presence of conidiogenesis on the bronchial lumen and not in the interior of the mucosa where the cellular response is more intense. Although conidiogenesis is infrequent, it has been reported in biopsies of cases of lung fungal balls and in patients systemically infected with *S. apiospermum* without immunological compromise [8,12].

On the other hand, it should bear in mind that cystic fibrosis is considered as a disease with some functional deficiencies. In this aspect, some authors suggested a problem in cell-mediated immunity that possibly include deficiency in interleukine 10 and the alternative pathway of complement [13].

Amphotericin B, a member of a class of macrolide polyenes, has represented the gold standard as antifungal therapy in cases of severe invasive mycosis. However, *S. apiospermum* is generally resistant to amphotericin B [6,9]. Itraconazole, triazole, and ketoconazole, an imidazole, have shown to have the same efficacy against *S. apiospermum* [6]. In vitro data, animal models and accumulating clinical experience support the use of voriconazole as a primary treatment for pseudallescheriasis [14].

The findings in this study indicate that for an adequate diagnosis, and since *S. apiospermum* is often only a colonizer, a broncoscopy is necessary in search of clinical data on endobronchitis and for obtaining a biopsy sample of the mucosa to confirm or discard tissue invasion. Similarly, we believe that antifungal treatment with itraconazole together with mechanical washings after nebulization with DNAase, was a determining factor in mycological and clinical remission of the respiratory problem.

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