Note

Invasive pulmonary aspergillosis due to a mixed infection caused by *Aspergillus flavus* and *Aspergillus fumigatus*

Melissa Orzechowski Xavier[1], Alessandro Comarú Pasqualotto[1,2], Maria Da Penha Uchoa Sales[3], Cecília Bittencourt Severo[1,4], José J. Peixoto Camargo[4], Luiz Carlos Severo[4,5]

[1]Programa de Pós-graduação em Ciências Pneumológicas, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil; [2]School of Medicine, The University of Manchester, UK; [3]Faculdade Integrada do Ceará, Brazil; [4]Santa Casa-Complexo Hospitalar, Porto Alegre, Brazil; [5]Pesquisador 1B do CNPq, Faculdade de Medicina da UFRGS, Brazil

Summary Invasive pulmonary aspergillosis is typically caused by a single *Aspergillus* species, most frequently *Aspergillus fumigatus*. Here we report that a lung transplant recipient developed invasive aspergillosis due to a mixed infection caused by *Aspergillus flavus* and *A. fumigatus*. The implications for this unusual finding are discussed.

Key words Aspergillosis, *Aspergillus fumigatus*, *Aspergillus flavus*, Lung transplantation

Aspergilosis pulmonar invasora causada por infección mixta de *Aspergillus flavus* y *Aspergillus fumigatus*

Resumen La aspergilosis pulmonar invasora es producida típicamente por una única especie de *Aspergillus*, siendo habitualmente *Aspergillus fumigatus*. Se presenta el caso clínico de un receptor de trasplante de pulmón que desarrolló aspergilosis invasora por una infección mixta causada por *Aspergillus flavus* y *A. fumigatus*. Se discuten las implicaciones de la rareza de este caso.

Palabras clave Aspergilosis, *Aspergillus fumigatus*, *Aspergillus flavus*, Trasplante de pulmón

A 33 year-old man with primary pulmonary hypertension underwent a single right lung transplantation procedure in 1990. His immunosuppressive regimen consisted of azathioprine, cyclosporine and prednisone. Four months after transplantation, the patient was diagnosed with cytomegalovirus (CMV) pneumonia based on the histopathological findings obtained by transbronchial biopsy, and treated with intravenous gancyclovir. Six weeks later he presented with productive cough and severe dyspnea, reporting very limited tolerance to exercises. At admission to the hospital, chest radiograph showed bilateral pulmonary infiltration with areas of pneumonic-type consolidation. Bronchoscopy and transbronchial biopsy were performed.

Address for correspondence:
Dr. Alessandro C. Pasqualotto
Programa de Pós-graduação em Ciências Pneumológicas
Universidade Federal do Rio Grande do Sul (UFRGS)
Brazil
Tel.: +44 161 291 5818
Fax: +44 161 291 5806
E-mail: acpasqualotto@hotmail.com

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Hyaline septate branched hyphae were seen in the bronchoalveolar lavage fluid after preparation with potassium hydroxide (KOH 10%). Histopathological studies (haematoxylin and eosin, and Gomori-Grocott methanamine-silver nitrate stain) revealed acute suppurative bronchial inflammation. In addition, there was evidence of tissue invasion by hyphae consistent with *Aspergillus* species. Amphotericin B deoxycholate treatment (1.5 mg/kg daily) was commenced, and *Aspergillus flavus* was recovered in culture (Sabouraud dextrose agar with chloramphenicol, and Mycosel agar at 25 ºC and 35 ºC).

The patient died 15 days later despite antifungal therapy in association with broad spectrum antimicrobial drugs. Two courses of steroids were also attempted, without success. At autopsy, the right lung revealed visceral pleura congestion with extensive adhesion. There were multiple yellowish nodules irregularly distributed throughout the lungs, 0.1-1.0 cm in diameter. Some of these nodules had coalesced to form a large reddish mass. Yellowish nodules were observed also in the heart and right kidney (Figure 1) with histopathological evidence of tissue invasion by *Aspergillus* hyphae. In the mycology laboratory, the lung fragment was processed in the laminar flow hood and cut using sterile technique. Three pieces were removed from a deep pulmonary nodule, and culture showed growth of both *A. flavus* and *Aspergillus fumigatus* in triplicate (Figure 2).

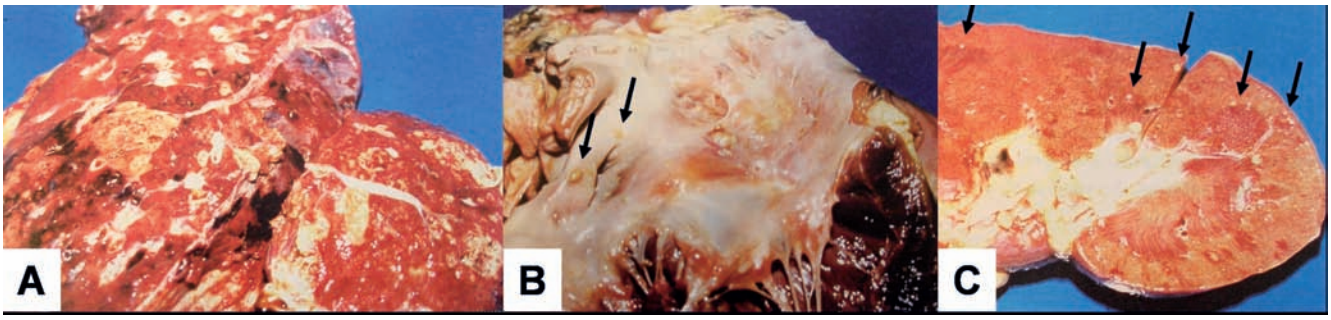

Figure 1. Lesions found at autopsy. Nodular lesions were present in the lungs (A), heart (B), and right kidney (C).

Discussion

Mixed invasive fungal infections are reported infrequently in the literature [1,6]. Previous reports have usually been associated with states of marked immunosuppression, or with the presence of infected medical devices, such as central venous catheters. However, the isolation of two different fungal species belonging to the same genus from clinical specimens is particularly rare. Fungaemias probably represent an exception to this general rule [17]. For instance, 2-9% of cases of candidemia have been shown to be mixed [2,8], caused by more than one *Candida* species. Interestingly, both the clinical presentation and the severity of mixed fungaemias seem to be similar to that seen with monomicrobial fungaemias [8]. In cases of mixed infections, the existance of a drug-resistant isolate might complicate patient management.

Cimerman et al. seem to be the first to report the occurrence of aspergillosis caused by two *Aspergillus* species [5] in a report of osteomyelitis due to *A. flavus* and *A. fumigatus*. *Chalara ellisii* was also recovered in culture. This case was that of a healthy young man who suffered a closed transverse fracture of the femur as a result of a traffic accident. In a review of the literature up to the year 2000, Singh and Husain [15] found that 5% (two of 40) of aspergillosis cases following lung transplantation were associated to mixed infections due to two *Aspergillus* species. However, no details were given about these cases.

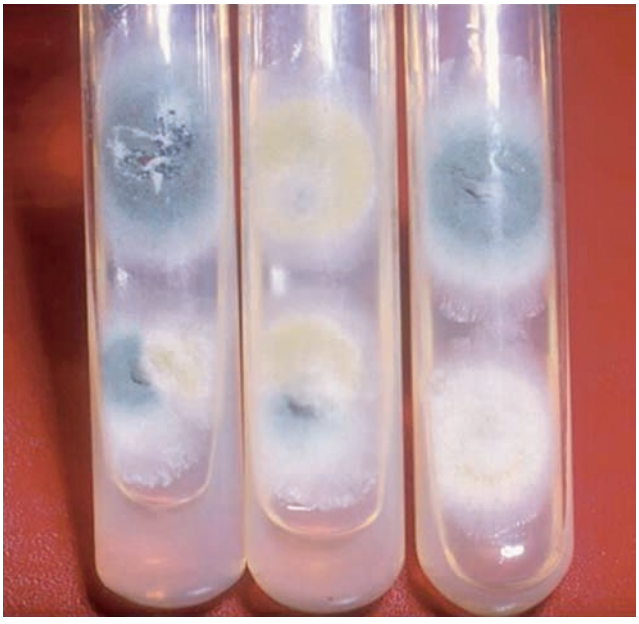

Figure 2. Cultures in triplicate of lung fragments removed after autopsy. The mixed pattern is evident by the repeated growth of both *A. fumigatus* (green to fairly blue colonies) and *A. flavus* (yellow-greenish colonies).

In contrast to the invasive forms of aspergillosis, cases of chronic pulmonary aspergillosis are commonly associated with infection by multiple genotypes, particularly in the presence of fungal balls [3,4]. Mycological studies in patients with chronic cavitary aspergillosis and fungal balls usually show the presence of various morphologies and considerable antigenic variability among isolates [4,9-11], which is probably part of a dynamic process consequent to the continual growth and death of fungal elements [14]. Neuvéglise et al. also showed by molecular typing techniques that cystic fibrosis patients can harbour several strains of *Aspergillus fumigatus* over time [12]. The recovery of two isolates belonging to different *Aspergillus* species is, however, rare.

The precise mechanisms leading to a mixed infection as seen for our patient are unknown. Also unknown is the reason why mixed *Aspergillus* infections are so rare. Our patient had been infected by CMV, an immunomodulatory virus that impairs cellular immunity, therefore increasing susceptibility to opportunistic fungal infections [13,16]. He was also potentially exposed to a high infectious load of *Aspergillus* species, since renovations were being undertaken in the hospital area. However, this assumption is only speculative since we do not routinely measure spores in the hospital air, and this case was not part of an outbreak.

Although invasive pulmonary aspergillosis is usually acquired by inhalation of *Aspergillus* conidia, the diameter and surface characteristics of the conidia seem to be important factors in the pathogenesis of aspergillosis. Accordingly, the bigger size of *A. flavus* conidia favours their deposition in the upper respiratory tract, and this species is the main aetiology of *Aspergillus* sinusitis [7]. The rarity of mixed *Aspergillus* infections in cases of invasive pulmonary aspergillosis probably results from the difficulty imposed for the conidia (mainly to *A. flavus*) to reach the pulmonary alveoli during this acute condition. However, the importance of this and other factors remain to be elucidated.

In conclusion, we report in this study a rare case of mixed *Aspergillus* infection causing invasive pulmonary aspergillosis. The incidence of this condition is unknown and might be underestimated, considering the low yield of microbiological methods in the diagnosis of invasive aspergillosis. Theoretically, the association of different species in mixed fungal infection could be a cause of treatment failure as, for example, one of the isolates showing resistance to any particular antifungal drug. The importance of multiple sampling to promote greater overall sensitivity to the microbiological methods also deserves further investigation.

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