
ASPERGILLOSIS OF THE NOSE AND PARANASAL SINUSES: A REVIEW OF 54 CASES

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ABSTRACT

Aspergillosis of the nose and paranasal sinuses: a review of 54 cases

Aspergillus species are considered opportunistic fungi of increasing clinical importance. Information regarding extrapulmonary involvement is scarce. The aim of this study was to isolate the different species of *Aspergillus* from patients with rhinosinusitis. A retrospective study was conducted in a university hospital in Porto Alegre, Brazil (1986–2014). For mycological diagnoses, paranasal tissue obtained at surgery was subjected to histopathology examination and sent for fungal cultures. Of the 54 samples analyzed, 32 were diagnosed positive by culture. The underlying causes of immunodeficiency were: six with transplantation (three bone marrow, two lung, one kidney) and two with hematological disease (one bone marrow neoplasia and two leukemia). In the present study, the clinical manifestations of rhinosinusitis aspergillosis were: 20 allergic reactions, 20 fungus balls, and 14 acute invasive cases. The species isolated from the 54 samples were: *Aspergillus fumigatus* (n=14); *A. flavus* (n=6); *A. niger* (n=2); *A. terreus* (n=1); *A. fisheri* (n=1); and *Aspergillus* sp., (n=3). Two concomitant species of *Aspergillus* were observed in two patients: *A. fumigatus* and *A. flavus*; and *A. fumigatus* and *A. niger*. In four patients, *Aspergillus* was associated with other

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fungi. These were: *A. flavus* and *Fusarium*, *A. fumigatus* and *Rhizopus*, *A. flavus* and *Mucorales*, and *Aspergillus sp.* and *Mucorales*. The most common species of *Aspergillus* that were responsible for paranasal sinus infections were *A. fumigatus*, *A. flavus*, and *A. niger*.

KEY WORDS: Paranasal sinuses; *Aspergillus*; Mycology; Aspergillosis.

RESUMO

Aspergilose nasal e dos seios paranasais: revisão de 54 casos

Espécies de *Aspergillus* são considerados fungos oportunistas de crescente importância clínica. Informações sobre o envolvimento extrapulmonar é escassa. O objetivo deste estudo foi isolar as diferentes espécies de *Aspergillus* em pacientes com rinosinusite. Um estudo retrospectivo foi realizado em um hospital universitário em Porto Alegre, Brasil (1986-2014). Para diagnóstico micológico, tecido paranasais obtido no momento da cirurgia foi submetido a exame histopatológico e encaminhados para cultivos de fungos. Das 54 amostras analisadas, 32 foram diagnosticados pelo cultivo positivo. As causas subjacentes da imunodeficiência foram: seis com transplante (medula óssea, três, pulmão, dois; rim, um) e dois com doenças hematológicas (neoplasia osso estreito, um; leucemia, duas). No presente estudo, as manifestações clínicas de rinosinusite aspergilar foram: alérgica, 20; bolas fúngica, 20; e aguda invasiva, 14. As espécies fúngicas isoladas foram: *Aspergillus fumigatus*, 14; *A. flavus*, seis; *A. niger*, dois; *A. terreus*, um; *A. fischeri*, um; e *Aspergillus sp.*, três. Duas espécies de *Aspergillus* concomitantes foram observadas em dois pacientes: *A. fumigatus* e *A. flavus*; e *A. fumigatus* e *A. niger*. Em quatro pacientes, *Aspergillus* foi associado com outros fungos: *A. flavus* e *Fusarium*, um; *A. fumigatus* e *Rhizopus*, um; *A. flavus* e *Mucorales*, um; e *Aspergillus sp.* e *Mucorales*, um. Os isolados mais comuns de *Aspergillus* que são responsáveis por infecções dos seios paranasais são *A. fumigatus*, *A. flavus* e *A. niger*.

DESCRITORES: Seios paranasais; *Aspergillus*; micologia; aspergilose.

INTRODUCTION

In aspergillosis, the most frequent site of human infection is the lung, although many other sites may be involved, including the paranasal sinuses.

There are four categories of paranasal sinus aspergillosis, classified based on the host's immune response to the fungus: chronic indolent sinusitis (invasive), fulminant sinusitis (invasive), fungal balls (noninvasive), and allergic sinusitis (noninvasive) (12).

Allergic *Aspergillus* sinusitis is analogous to allergic bronchopulmonary aspergillosis. The disease appears in patients with an atopic background, and nasal polyposis may be present (7). The diagnostic criteria include the presence of allergic mucin and fungal hyphae within a sinus. One of the characteristic pathological features of allergic fungal sinusitis is the presence of many Charcot-Leyden crystals. In localized noninvasive aspergillosis, there is an extra mucosal ball of tangled fungal mycelium (fungal balls), frequently (and inappropriately) referred to as mycetoma or aspergilloma, a dense conglomeration of fungal hyphae that form a mass within a sinus cavity without invading the surrounding host tissue. Invasive *Aspergillus* sinusitis is characterized by the spread of fungal mycelium from sinus

air spaces into adjacent structures, with tissue necrosis, chronic inflammation, and fibrosis. Chronic invasive sinusitis is a slowly progressive syndrome that occurs in patients with relatively subtle defects in immunity (e.g., low-dose corticosteroid use and diabetes). Acute invasive sinusitis occurs in profoundly immunocompromised patients and is characterized by a rapidly destructive pansinusitis that spreads to contiguous structures (2, 3, 11, 12).

This paper reports 54 cases of aspergillosis of the nose and paranasal sinuses. We also describe the use of direct fluorescent antibody staining, immunodiffusion, and galactomannan (GM) serology tests for the diagnosis of these infections.

PATIENTS AND METHODS

We reviewed the medical records of patients who had been diagnosed with nasal and paranasal aspergillosis at the Santa Casa Hospital Complex of Porto Alegre, RS, Brazil. The study was carried out with the permission of the Medical Research Ethics Committee of Santa Casa (Protocol number 64705/12).

Over a 28-year period, 54 patients with confirmed aspergillosis of the nose and paranasal sinuses were evaluated. Clinical specimens were processed routinely by light microscopy and stained with hematoxylin and eosin (H&E). Special staining for organisms was also performed with calcofluor white and Gomori's methenamine-silver stain (GMS). Antibiotics were added to clinical specimens, aiming to reduce bacterial contamination, as is done in dermatology (10). The diagnosis was confirmed either histologically and/or by culture. Necrotic tissue was obtained by the Caldwell-Luc procedure or the endoscopic technique.

Patient ages ranged between 15 and 72 years old, with a mean of 46.8 years (32 females, 22 males). At presentation, all patients showed symptoms of chronic sinusitis refractory to medial management (multiple courses of antibiotics).

In addition to basic radiographs, serial computed tomography (CT) was obtained for 27 patients.

Immunodiffusion (Ouchterlony's method) in agar gel double diffusion was used for detection of antibodies against *Aspergillus fumigatus*, *A. flavus* and *A. niger* (Immy - Immuno Mycologics®, USA) and antigen was detected by direct detection of GM (galactomannan) non competitive double sandwich ELSA (Platelia, Bio-Rad, Marnes-la-Coquette, France).

RESULTS

In this series, nine patients were immunocompromised. The underlying causes of immunodeficiency were: six with transplantation (three bone marrow, two lung, one kidney) and three with hematological disease (one case of bone marrow neoplasia and two of leukemia).

In the present study, the clinical manifestations were: 20 allergic reactions, 20 fungal balls (Figure 1) and 14 acute invasive cases (Figures 2 and 3). All patients underwent basic sinus roentgenograms for the confirmation of diagnosis, and because this was a retrospective study over a 28-year period, only 27 of the 54 patients underwent sinus CT scan. The radiological findings demonstrated unilateral lesions in 38 cases; bilateral lesions were found in 16 cases, all of whom showed pansinus disease, and 26 patients demonstrated more than one sinus disease. CT scan findings showed high-attenuation areas coupled with surrounding soft tissue in 25 patients (Figure 1). Also, we found periosteal (bone) sclerosis in 26 patients. The maxillary sinus complex was most frequently involved (92%), followed by the ethmoid (83%), frontal (71%) and sphenoid (67%) sinuses.

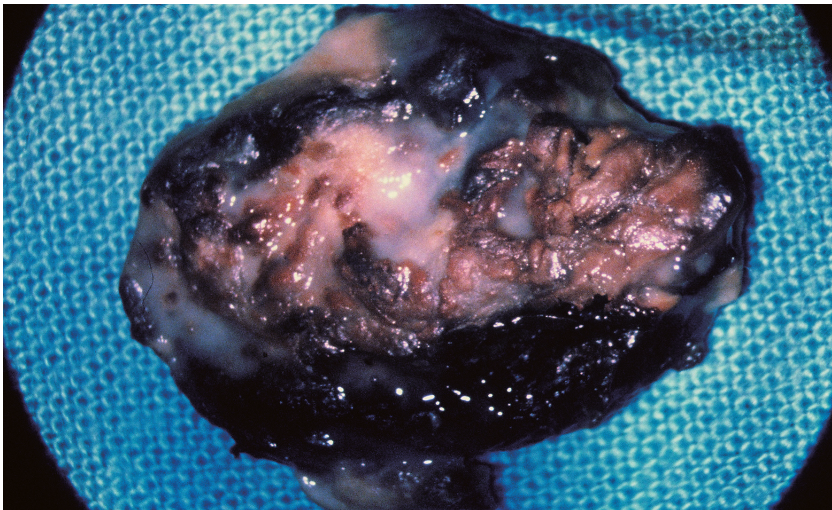


Figure 1. Image of an *A. niger* fungal ball.

In 32 cases, the diagnosis was made by direct examination and culture, and in 22 patients the diagnosis was made exclusively by histology with the visualization of the *Aspergillus* conidiophore. In one patient, the diagnosis was by direct fluorescent antibody staining (*Aspergillus* and *Mucor*, CDC 84 057261), as previously published (15). The species isolated in those 32 patients were: *A. fumigatus* (n=14); *A. flavus* (n=6); *A. niger* (n=2); *A. terreus* (n=1); *A. fischeri* (n=1); and *Aspergillus* sp. (n=3). Two concomitant species of *Aspergillus* were observed in two patients: *A. fumigatus* and *A. flavus*; and *A. fumigatus* and *A. niger*. In four patients, *Aspergillus* was associated with other fungi: *A. flavus* and *Fusarium*; *A. fumigatus* and *Rhizopus*; *A. flavus* and *Mucorales*; and *Aspergillus* sp. and *Mucorales*. Three cases had been previously published: *A. niger* (14), *A. flavus* (16), and *Aspergillus* and *Mucorales* (15).

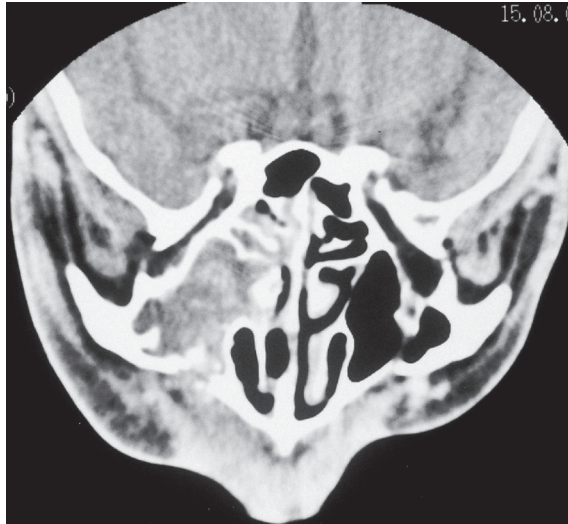


Figure 2. Invasive sinusitis - Axial contrast-enhanced CT images show complete opacification of the right nasal cavity, which is a common presentation for acute invasive fungal disease of the paranasal sinuses. Both left nasal cavity and right maxillary sinus contents have attenuation values higher than those of simple fluid. Also, CT scan demonstrated right nasal and anterior septal soft-tissue thickening and enhancement.

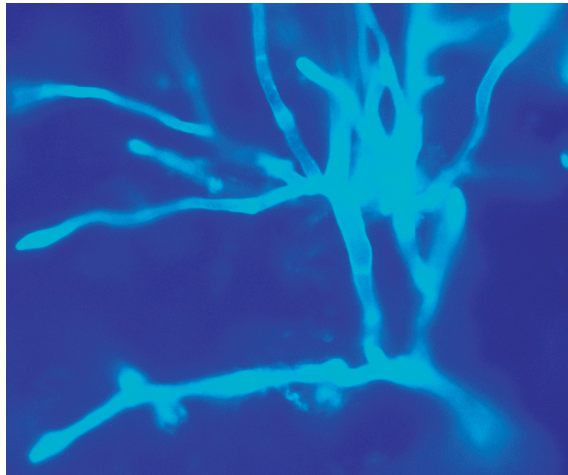


Figure 3. Invasive sinusitis - Microscopic examination of a sinus mucosa biopsy showing septate and dichotomous branching hyphae of *Aspergillus* (calcofluor white).

Immunodiffusion is helpful for the diagnosis of allergic bronchopulmonary aspergillosis and *Aspergillus* fungal balls in the lung. However, in the present cases immunodiffusion for all three species of *Aspergillus* (*A. fumigatus*, *A. flavus*, *A. niger*) was not positive in all five patients tested with paranasal aspergillosis. Conversely, GM, a component of fungal cell walls, is released during tissue invasion by *Aspergillus* hyphae and can be detected in body fluids; in the two cases where GM was performed, patients with invasive rhinosinusitis, serum GM was positive.

DISCUSSION

Fungal infection of the nose and paranasal sinuses is rare, although it has been reported more frequently in recent years.

The finding of sinusitis on sinus roentgenograms of one or more paranasal sinuses is among the diagnostic criteria for allergic *Aspergillus* rhinosinusitis (6). Haziness or clouding of the sinus is the most common feature. Frequently, more than one sinus is opacified, in contrast to the fungal ball manifestation, where most cases show only a single sinus affected. Bilateral involvement has been observed in up to 83% of cases reported (13), contrary to our series, which showed that the vast majority of cases presented unilateral infection of the maxillary sinus (38/54, 70.4%).

In the literature, other manifestations include mucosal thickening and various degrees of bony sclerosis and/or destruction. However, these findings are non-specific because they may also be seen in chronic and/or infective rhinosinusitis or malignancies of the paranasal sinuses.

Currently, CT has emerged as the imaging modality of choice. The characteristic feature of CT is the occurrence of heterogeneous densities, signifying opacification of the sinuses, with serpiginous areas of increased attenuation on non-contrast scans (9). These hyperdense areas are due to the presence of "allergic mucin." The presence of calcium and ferromagnetic elements (iron and manganese) produced by the fungi is said to be responsible for the densities.

In fungal infections of the nose and paranasal sinuses, *Aspergillus* and *Mucorales* are the most commonly implicated fungal organisms (3, 15). The species *A. fumigatus*, the most common isolate, belongs to a complex, which is in fact a section of a group complexes of cryptic species, with the same morphology but different genetic composition. Currently, diagnosis of fungal rhinosinusitis depends on histopathology and recovery of the fungal agent in culture, but, in one of our cases, only formalin-fixed tissue specimens were sent to the laboratory.

GM has played an important role in the non-culture-based diagnosis of invasive aspergillosis. Although GM testing has been widely used for confirmation of the indirect evidence of *Aspergillus* infection (1), experience with GM in patients with rhinosinusitis is limited (4, 5, 8). However, there have been few studies on the use of serum GM assay in patients with nose and paranasal rhinosinusitis. Thus, in the two cases where GM was performed, patients with invasive rhinosinusitis, serum

GM was positive. Chen et al. reported 64% sensitivity of serum GM antigen testing and 60% specificity for the detection of invasive *Aspergillus* rhinosinusitis (4).

CT is capable of precisely defining the extent of the disease process (Figure 2), information that is essential to therapeutic planning. The two cases of *A. flavus* invasive rhinosinusitis presented herein reinforce the importance of performing biopsies (Figure 3) and cultures to identify pathogens. In the present series, all patients were managed with the Caldwell-Luc surgical procedure and, most recently, with conservative endoscopic surgery.

Finally, in cases with negative cultures of paraffin-embedded tissue, the fluorescent antibody technique (15) and the PCR/RLB assay (17) detected mixed infection by *Aspergillus* and Mucorales in a single clinical specimen.

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