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ASPERGILLUS FUMIGATUS FUNGUS BALL IN HOSPITALIZED PATIENTS WITH CHRONIC PULMONARY DISEASE. USEFULNESS OF DOUBLE IMMUNODIFFUSION TEST AS A SCREENING PROCEDURE

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Double immunodiffusion (DID) was used as a screening test for the diagnosis of aspergillosis. Three hundred and fifty patients were tested, all of them referred from a specialized chest disease hospital and without a definitive etiological diagnosis.

When DID was positive additional information such as clinical history and radiographic findings were requested and also surgical specimens were obtained whenever possible. Specific precipitin bands for Aspergillus fumigatus *antigen were found in 29 (8.3%) of 350 patients sera. Nineteen (65.5%) of the 29 patients with positive serology were recognized as having a fungus ball by X-rays signs in 17 or by pathological examination in 2 or by both in 8 patients. This two-year prospective study has shown that pulmonary aspergillosis is a considerable problem among patients admitted to a Chest Diseases Hospital, especially in those with pulmonary cavities or bronchiectasis.*

Key words: aspergillosis – fungus ball – double immunodiffusion

Fungus Ball (FB) due to *Aspergillus* species, mainly from the *A. fumigatus* group (aspergilomas; FB) is recognized to occur in patients with sterile pulmonary cavities. However little is known about the magnitude of the health problem represented by this form of pulmonary aspergillosis in Brazil, mainly because of the lack of adequate diagnostic criteria.

A prospective study has been undertaken in order to see if FB is a significant disease problem in a Chest Diseases Hospital in RJ. Such a hospital attends a great number of tuberculosis patients, a lot of them with residual pulmonary cavities and, consequently, belonging to a risk group of FB. For this purpose we applied a double immunodiffusion test (DID) as an aspergillus antibody screening procedure for patients with chronic pulmonary disease.

MATERIALS AND METHODS

Patients: a serological study was done on 350 patients admitted to a Chest Diseases Hospital (Hospital Raphael de Paula Souza). All the patients presented X-ray findings of chronic pulmonary disease and were found to have sputum negative for tubercle bacilli or, if acid fast bacilli were revealed, had responded poorly to specific treatment. Patients were included in the study irrespective of the presence or not of pulmonary cavity.

Antigens: strains of *A. fumigatus* were isolated in our laboratory from 4 patients with FB and were maintained on Sabouraud's agar slants (Difco) at room temperature. The inoculum was prepared from a four-day-old Sabouraud's chloramphenicol (500 mg/l) culture. Each strain was grown for a period of four weeks after which thimerosal 1:10000 (w/v) was added. The culture filtrate was separated from mycelia by passage through a Whatman no. 1 filter (Whatman Ltd. England), centrifuged (1200g-4°C-10 min) and concentrated 20 times by lyophilization. The concentrated culture filtrate was dialyzed against deionized water at 4°C overnight and the four strains were pooled. Protein concentrations were determined according to Lowry (Lowry et al., 1951) using bovine serum albumin as a standard.

Serologic procedure: DID in agarose gel was done on serum from each patient by a modification of Ouchterlony's method (Ouchterlony,

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1962). After diffusion for 48 h at 37°C in a humid chamber, gels were washed with 5% (w/v) sodium citrate for 45 min in order to avoid non specific reactions and then in 10 mM phosphate-buffered saline (PBS), pH 7.2, 1:3 (v/v) in deionized water, with several changes during a 48 h period. The gels were then dried and stained with 0.5% (w/v) Coomassie Brilliant Blue r-250 (Sigma Chemical Co.). A serum was considered positive when a line of identity was detected with a standard human serum precipitin line(s) (Coleman & Kaufman, 1972; Ferreira-da-Cruz et al., 1985).

Mycologic procedure: when possible, pulmonary tissue removed by surgical resection was examined. Part of each specimen was fixed in 10% buffered formalin, processed for paraffin section and stained with Haematoxylin & Eosin (HE) and Gomoris' methenamine silver (Grocott, 1955) for histologic examination. The remaining part of each specimen was planted in Sabouraud (Difco) and Sabouraud plus chloramphenicol agar medium and incubated at 25°C and 37°C. The isolates were identified according to Raper & Fennel (1965).

RESULTS

Specific precipitin bands for *A. fumigatus* antigen were found in 29 (8.3%) of the 350 patients' sera tested. In these 29 patients tubercle bacilli could not be demonstrated in 3 to 5 sputum samples from each even though the great majority (27; 93%) gave a history of past pulmonary tuberculosis. In 17 (58.6%) of the 29 patients with serodiagnosis of aspergillosis the presence of FB was observed by standard post anterior chest X-rays in all except one. In this one tomograph was necessary to demonstrate the FB. In the remaining 12 patients, 2 showed cavities, 4 had residual fibrosis, one of them with an emphysema vesicle, and the 6 others had a wide parenchymal unilateral opacity that masked the possible evidence of cavity. The clinical course of the 29 patients with positive serology was followed. The decision to treat with surgical resection or antifungal therapy was taken by the attending physician. Surgery was recommended for 11 patients and the pathological examination of the specimens demonstrated the presence of FB in 10, including 2 patients in whom no cavitations were apparent on chest roentgenograms; in the remaining one fungus was not demonstrated. The mycological examination confirmed the etiologic agent by isolation of *A. fumigatus* in these 10 patients. The other 18 patients were not surgery candidates because of the underlying conditions of their lungs so pathological and mycological examinations were not done. Therefore, evidence that a serological diagnosis of aspergillosis corresponded to a FB was present in 9 (50%) of these patients by radiographic appearances (Table). The 321 patients with negative serology were found to have pulmonary neoplasia, pneumoconiosis, bacterial pneumonia, tuberculosis, other mycotic diseases and no evidence of aspergillosis was found in any.

TABLE

Chest X-ray findings and histopathology in 29 cases with serological diagnosis of aspergillosis

X-ray findings	Histopathology
Fungus Ball (n = 17)	FB (n = 8) ND (n = 9)
Unilateral opacity (n = 6)	FB (n = 2) ND (n = 4)
Cavity (n = 2)	ND (n = 2)
Fibrosis; emphysema (n = 4)	FB? (n = 1) ND (n = 3)
Total	29

n = number of cases
ND = Not done

DISCUSSION

In a previous report (Ferreira-da-Cruz et al., 1985) from this laboratory the antigen reactivity of the culture filtrate was determined with sera from patients with culturally proven aspergillosis (FB) and the specificity was determined using sera from patients with other lung pathologies, including other mycotic infections, and healthy individuals. The antigenic extract reacted with all reference sera, and precipitins to *A. fumigatus* were not found in the other sera tested. Precipitin lines were sometimes observed which had no identity with precipitins of a standard sera. These results are in agreement with findings of other investigators (British Thoracic and Tuberculosis Association, 1970; British Tuberculosis Association, 1968;

Coleman & Kaufman, 1972) and confirm the usefulness of DID as a diagnostic tool.

In our survey 19 (65.5%) of the 29 patients with positive serology have been recognized as having a FB by X-ray signs (17 patients) or by pathological examination (2 patients). Sputum was not examined by mycological procedures since the clinical relevance of the isolation of *A. fumigatus* from this specimen remains unclear (Cancroft et al., 1984; Pepys, 1969; Pepys et al., 1959; Sandhu & Sandhu, 1973; Severo et al., 1978). The diagnosis was established in the remaining 10 patients only on the basis of seropositivity, in the highly specific DID. On the other hand radiographs by themselves are not proof of the diagnosis of *A. fumigatus* FB since similar X-ray findings may appear in a number of other circumstances (Cancroft et al., 1974; Reeder, 1974). Moreover 2 of our patients had no roentgenographically demonstrable cavitations despite pathological, mycological and serologic evidences of an *A. fumigatus* FB.

It has been suggested that a radiographic appearance of a ball within a cavity plus a positivity in DID indicates that a ball of aspergillus mycelium is present (British Thoracic and Tuberculosis Association, 1970). Indeed we had 8 patients in our study group with these characteristics in whom mycological examination confirmed the pathogen. Actually we have no mycological proof that patients with a positive DID for *A. fumigatus* necessarily have pulmonary aspergillosis. However our present data strongly indicated an infection by *A. fumigatus* in a damaged lung with prolonged antimicrobial therapy in the patients who had precipitins without radiographic signs of a FB or symptoms similar to the mycologically or roentgenographically confirmed cases.

This two-year prospective study has shown that pulmonary colonization with *A. fumigatus* is a considerable problem among patients admitted to a Chest Diseases Hospital in RJ and is apparently more serious than chronic pulmonary paracoccidioidomycosis (Ferreira-da-Cruz et al., 1987) which is the systemic mycosis most frequently diagnosed in Brazil.

We believe that the prevalence of aspergillosis (FB) is considerably underestimated in Brazil since in this survey the studied patients were only those presenting chronic lung disease whilst other possibly eligible patients could be overlooked and individuals with illnesses not severe enough to necessitate hospitalization could be excluded.

Tuberculosis was the most common antecedent, probably owing to its high prevalence in Brazil and also to the study group be referred from a Chest Diseases Hospital with sanatorium characteristics. The incidence and the severity of FB suggests that patients with pulmonary cavities associated with other disorders including sarcoidosis (Israel et al., 1982), lung cancer (Cancroft et al., 1984) and many other pulmonary conditions should be watched very closely.

RESUMO

Aspergilose (bola fúngica) em pacientes hospitalizados com doença pulmonar crônica. Importância da imunodifusão dupla como método de triagem – Trezentos e cinqüenta pacientes sintomáticos respiratórios admitidos no Hospital Raphael de Paula Souza, sem diagnóstico etiológico definitivo, foram triados pela técnica de imunodifusão dupla (IDD) para aspergilose. Quando a IDD foi positiva, informações adicionais como histórico e exames radiológicos foram requisitados e, quando possível, espécimes clínicos foram processados para exames micológicos e histopatológicos. Linhas de precipitação específicas para o antígeno de *A. fumigatus* foram encontradas em 29 (8,3%) dos 350 soros de pacientes testados. Dezenove (65,5%) dos 29 pacientes com sorologia positiva foram reconhecidos como tendo bola fúngica pelos achados radiológicos em 17 ou pelo exame histopatológico em dois ou por ambos em oito pacientes.

Este estudo prospectivo de dois anos mostrou que aspergilose pulmonar é um problema considerável entre pacientes admitidos em um hospital para sintomáticos respiratórios, especialmente aqueles com cavidades pulmonares ou bronquiectasias.

Palavras-chave: aspergilose – bola fúngica – imunodifusão dupla

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