JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

Pulmonary aspergilloma in immunocompromised patients in a Respiratory Care Unit

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Abstract

Introduction: Pulmonary aspergilloma is commonly associated with comorbidities that cause immunodeficiency such as diabetes mellitus, tuberculosis, human immunodeficiency virus/acquired immunodeficiency syndrome and/or a pre-existing parenchymal lung disease such as chronic obstructive pulmonary disease. Predisposing factors can further increase the risk of acquiring this mycosis. Our objective was to determine the frequency, clinical and microbiological characteristics of pulmonary aspergilloma in immunocompromised patients.

Methodology: Retrospective case series of patients diagnosed with pulmonary aspergilloma in a respiratory care unit in Mexico City from 2000 to 2019 was studied. Bronchoalveolar lavage cultures on Sabouraud-dextrose agar and serum galactomannan determination were performed on each patient.

Results: We identified twenty-four patients with pulmonary aspergilloma (sixteen male and eight female), thirteen had a history of tuberculosis (54%), seven of diabetes mellitus (29%), three of human immunodeficiency virus/acquired immunodeficiency syndrome (13%) and one of chronic obstructive pulmonary disease (4%). The most commonly reported symptoms were hemoptysis in eighteen patients (75%), dyspnea in sixteen patients (67%) and chest pain in thirteen patients (54%). *Aspergillus fumigatus* was identified in all cultures and galactomannan was positive in 21 serum samples (87%).

Conclusions: Coexistence of diseases that could suppress the immune system predispose to pulmonary aspergilloma; clinical presentation is often confused with other systemic diseases. A high degree of clinical suspicion is important for early detection.

Key words: Pulmonary aspergilloma; diabetes mellitus; immunocompromised; HIV.

J Infect Dev Ctries 2022; 16(3):557-563. doi:10.3855/jidc.13120

(Received 23 May 2020 – Accepted 01 November 2020)

Introduction

Aspergillosis is a mycosis caused by a saprophytic and ubiquitous fungus in the air called *Aspergillus*. It has more than 200 species, about twenty of them classified as human pathogens. Featured species include *A. fumigatus* (85%) (The most common, and responsible for lung disease), *A. flavus* (5-10%), *A. niger* (2-3%) and *A. terreus* (2-3%). They usually grow on decomposing plants, animals, soil, water, and organic waste [1,2].

Mycosis notification is not mandatory in Mexico, so aspergillosis incidence is not precisely known, and is probably underestimated. The signs and symptoms are generally nonspecific, leading to a late diagnosis and poor prognosis. Those infected may even remain asymptomatic; nevertheless, when symptoms develop, massive hemoptysis appears as the main clinical manifestation. Radiological features may appear as the only sign leading to disease suspicion. Diagnosis is often delayed, with up to 30% of cases neither diagnosed nor treated and commonly presenting as postmortem findings [3].

Pulmonary mycoses have increased globally due to the increase in chronic diseases and various states of immunosuppression such as diabetes mellitus (DM), human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), transplants, malignancies, prolonged use of corticosteroids and malnutrition. These have been pointed out as well-constituted risk factors; the combination of two factors increases the risk of infection and mortality [4].

Aspergillus generally grows as a solid, round fungal mycelium, showing the "Monod" sign consisting of a mass surrounded by air within a preexisting lung cavity. Systemic and local manifestations include granulomatous disease, allergic bronchopulmonary

disease, or disseminated mycosis. Host and fungus factors that contribute to the development of aspergilloma include strain virulence and patient's immune status [5,6].

The aim of this study is to determine the frequency and clinical characteristics of pulmonary aspergilloma in immunocompromised patients attended at a respiratory care unit of a tertiary care referral center in Mexico City.

Methodology

We included patients who were diagnosed with pulmonary aspergilloma, required hospitalization at our respiratory unit, had symptoms of cough, dyspnea, chest pain, hemoptysis and/or fever, and radiological findings of caverns, halo sign and/or bronchiectasis from 2000 to 2019. Any patient with a report of contaminated culture samples was eliminated from our study.

All patients diagnosed with pulmonary aspergilloma underwent blood cytometry and bronchoalveolar lavage (BAL) by flexible bronchoscopy obtaining a 10 mL sample; seven patients underwent thoracoscopic surgery obtaining biopsies of lung tissue. The biological material was processed in the Mycology Laboratory of the General Hospital of Mexico "Dr. Eduardo Liceaga". Clinical characteristics, laboratory findings, as well as findings in chest computed tomography were documented from clinical records.

Table 1. Characteristics of the participants with diagnosis of pulmonary aspergilloma.

Characteristics	n (%)
Total: 24 patients	
Gender	
Male	16 (66.7)
Feme	8 (33.3)
Risk factor	
History of tuberculosis	13 (54)
Diabetes Mellitus	7 (29)
HIV/AIDS	3 (13)
COPD	1 (4)
Symptoms	
Hemoptysis	18 (75)
Dyspnea	16 (66)
Chest pain	13 (54)
Weight loss	11 (46)
Asthenia and adynamia	8 (33)
Fever	7 (29)
Chest computed tomography	
Caverns	12 (50)
Bronchiectasis	8 (33)
Pleural effusion	7 (29)
Halo sign	6 (25)
Solitary pulmonary nodule	1 (4)

BAL and surgical pieces were processed by direct examination with 10% of potassium hydroxide (KOH) and were seeded in Sabouraud dextrose agar medium, incubated for seven days at 28 °C, macroscopically and microscopically analyzed to corroborate the presence of yeasts. Special stains were used such as Schiff periodic acid (PAS), colloidal iron and cotton blue stains. Samples were rapidly processed and centrifuged to avoid overgrowth of bacteria and yeast.

Five milliliters of whole blood were extracted from the patients by means of vacuum venipuncture, serum obtained were tested for galactomannan by the immunoenzymatic technique (Platelia® Aspergillus, Bio-Rad, Marnes la Coquette, France) of the enzyme-linked immunosorbent assay (ELISA) type of double sandwich in microplate, taking a cut-off value of I > 1.5 in two consecutive samples [7]. A confirmed case of aspergilloma was defined as one that presented a positive galactomannan test and a positive culture with the micro-morphology of *Aspergillus* showing dichotomous and septate hyphae.

Results

Twenty-four patients with pulmonary aspergilloma diagnosis were identified, 16 male (67%), 8 female (33%), average age 42 ± 12 years, with history of tuberculosis in 13 cases (54%), carriers of diabetes mellitus 7 (29%), HIV/AIDS 3 (13%), and chronic obstructive pulmonary disease COPD 1 (4%). The main symptoms were hemoptysis in 18 patients (75%), dyspnea in 16 (66%), chest pain in 13 (54%), weight loss in 11 (46%), asthenia and adynamia in 8 (33%) and fever in 7 (29 %). BAL cultures were positive in 92% (22/24) and lung tissue biopsies were positive in the 7 patients undergoing thoracoscopy (Table 1).

Neutropenia was identified in four patients (16%), three of them with moderate neutropenia < 1000 cel/mm3 and one patient with severe neutropenia < 500 cel/mm3. Seven patients had hemoglobin < 10 g/dL; blood cytometry of the rest of the patients were normal.

Chest computed tomography findings were: caverns in 12 cases (50%), bronchiectasis in 8 (33%), pleural effusion in 7 (29%), halo sign in 6 (25%), and solitary pulmonary nodule in 1 (4%) (Figure 1).

Aspergillus fumigatus was identified in 100% of the cultures, serum galactomannan was detected in 21 (87%) patients, and compatible histological study with aspergilloma was identified in 7 patients reporting acute angle branched septate hyphae with PAS staining. Seven patients (29%) died of massive hemoptysis (Figure 2).

Discussion

The lung and paranasal sinuses are the main site of damage by *Aspergillus* spp since their conidia are extremely small (2-3 µm) and they have great sporulative capacity. When inhaled, they can reach the alveoli and are generally expelled by mucociliary clearance and surfactant allowing agglutination and phagocytosis by macrophages and neutrophils. These mechanisms are deficient in immunocompromised patients allowing colonization of the respiratory tract by *Aspergillus*. The prevalence of this disease is unknown; however, it is known that noninvasive forms are common and less lethal than the invasive ones. Of the 24 cases presented in our study, 67% were male, with an average age of 42 years, this group belonged to the country's economically active population, unfortunately pulmonary sequelae caused loss of productive years of life. Several reports worldwide indicate that populations between 33 and 67 years of age become more frequently affected, which coincides with that observed in our study population [8,9,10].

In developing countries, aspergilloma is difficult to treat due to the lack of effective medical treatment and remains as one of the most common forms of lung involvement. The cavitary form consisting of a conglomerate of fungal hyphae, inflammatory cells, mucus, and cellular debris, is the most frequent form of aspergilloma that presents in single or multiple cavities, and is associated with symptoms such as chronic cough, dyspnea and hemoptysis. Mortality associated with aspergilloma ranges between 38% and 80%. Our study population had a mortality of 29%, secondary to intractable hemoptysis plus hypovolemic shock occurring in two patients diagnosed with HIV/AIDS and five patients carrying diabetes mellitus for more than 20 years [11].

Nodules, cavitation, consolidated lung, halo and crescent moon signs were observed by chest computed tomography. Yet, these findings are not specific and may be caused by other microorganisms including *Zygomycete*, *Fusarium* sp, *Pseudomonas aeruginosa* and *Nocardia* sp. Radiological findings that predominated in our patients were thick-walled cavities in upper lobes in 50% of cases, bilateral bronchiectasis in 33%, unilateral pleural effusion in 29%, halo sign in 25% and solitary pulmonary nodule in 4%, all commonly described in pulmonary aspergilloma suggesting a chronic course of the disease. It is estimated that aspergilloma is present in 11% of patients with residual cavities or pulmonary or pleural scars observed by imaging studies. No computed tomography technique is 100% sensitive nor specific for the diagnosis of pulmonary mycosis [5,12].

Massive hemoptysis, the main documented symptom, is caused by the release of hemolytic

Figure 1. CT of the thorax. Cavern surrounded by solid tissue, irregular edges of 19.9 × 15.4 mm, solid lesion of 26.0 mm, in the upper and lower lobe, cavitated lesion, hydro-air level and pleural effusion.

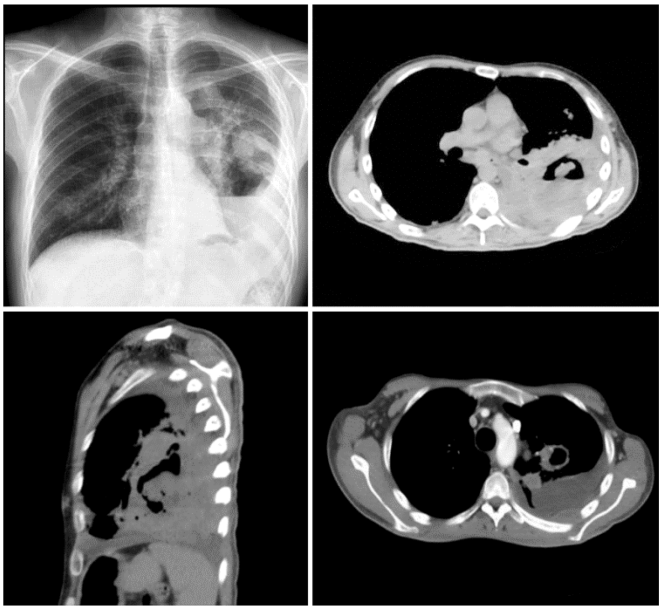
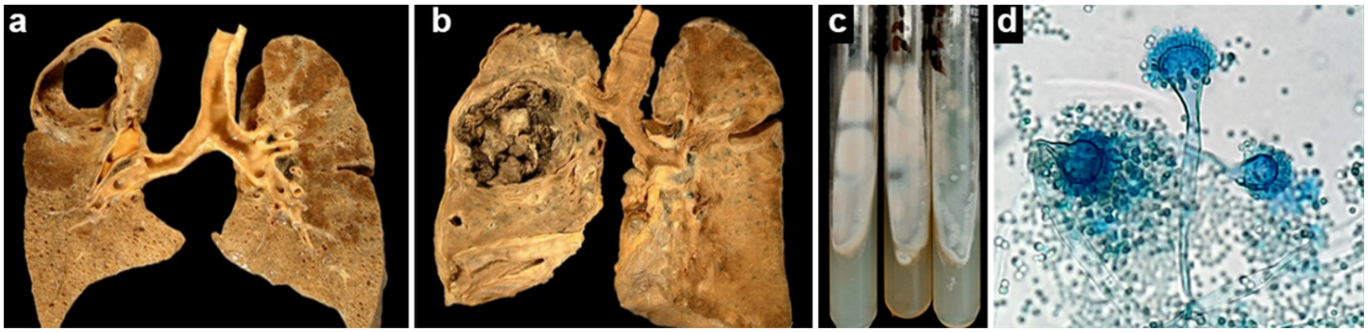


Figure 2. a. Pulmonary tuberculous cavern; **b.** Pulmonary aspergilloma; **c.** Sabouraud dextrose agar culture with *Aspergillus fumigatus* colony, powdery, velvety, limited, bluish green and whitish border; **d.** Aspergillary heads of *Aspergillus fumigatus* in 40X Microscopy with blue cotton lactophenol staining.



endotoxins and injury to the fungal ball with the wall of the cavern. Vascular invasion occurs if components on the fungal surface bind to components on the blood vessel wall, causing necrosis, infarction, and hemorrhage. Hemoptysis was present in 75% of patients in our series accompanied by weight loss, asthenia, adynamia, fever, dyspnea and chest pain, according with what has already been described by other authors; however, these symptoms remain nonspecific and common to a wide variety of infectious respiratory diseases, sometimes showing along with a pre-existing lung disease, making the suspicion of aspergilloma difficult to confirm [10,13].

Pulmonary tuberculosis is a known risk factor for pulmonary aspergilloma. In our series, it was found that 54% had this antecedent, presenting multiple pulmonary sequelae. In addition to the fact that eight of these patients presented with chronic malnutrition, it should be mentioned that one of them had a recent diagnosis of active pulmonary tuberculosis under pharmacological treatment. Pulmonary tuberculosis remains a public health problem in developing countries, and is considered by the World Health Organization (WHO) as a global emergency with 10 million new cases and 1.57 million deaths per year worldwide. Mexico's incidence of pulmonary tuberculosis is of 22 cases per 100,000 inhabitants, and 23,000 new cases were reported in 2018. The prevalence of aspergillosis secondary to tuberculosis is estimated at 1.2 million cases, one-third of them been aspergillomas [14].

DM is another comorbid condition that predisposes to aspergilloma. In Mexico it is ranked as the 11th leading cause of morbidity and the second leading cause of mortality in adults. From 2000 to 2017, 7.32 million new DM cases were reported, with a prevalence of 9.4%. In our hospital, DM is responsible for up to 26% of annual appointments. Its presence carries a three times greater risk of developing aspergillomas. In our study population, this antecedent was found in 29% of cases.

DM causes a poor response of T cells, neutrophils, and humoral immunity that predisposes to opportunistic infections. Persistent hyperglycemia increases the virulence of opportunistic microorganisms and precipitates apoptosis of nuclear polymorphic leukocytes. In our study population, 90% had serum glucose levels ≥ 250 mg/dL and a mean HbA1c of 15%, 40% of them presented diabetic macro and micro angiopathy [15].

Diagnosis of aspergilloma requires biopsy of the lesion and visualization of the septate hyphae. Cultures establish a certainty of diagnosis, and identify gender and species. We found that cultures from lung biopsies obtained by thoracoscopy were positive in all cases and those obtained by BAL were positive in 22 cases; negative BAL specimens do not rule out aspergillosis, including invasive forms. *Aspergillus fumigatus* was found in all 24 cases, consistent with the reported literature as the main etiologic agent causing aspergilloma. Unfortunately, cultures of secretions from the respiratory tract lack high sensitivity for *Aspergillus*, and it was isolated from sputum in only 35% and from BAL in 63% of patients with active infection [16].

In aspergillosis, antibodies are raised against *Aspergillius*, tests detect IgG, however, aspergillosis is caused by different species, causing false negative results. It has been documented that serum galactomannan can be detected several days before the presence of clinical symptoms, abnormalities on chest X-ray or positive cultures; unfortunately, it can turn negative if patients receive corticosteroids. Serum galactomannan is useful for diagnosis in patients without immunosuppression, being positive in more than 90% of cases, with an estimated sensitivity ranging between 29% and 89%. In our study, galactomannan was positive in 87% of serum samples, showing false negative results in patients with neutropenia and could last more than five weeks on average; two with history of HIV/AIDS, and one patient with COPD and DM respectively. Neutrophils are crucial in protecting the host against invasive aspergillosis, these patients presented a high degree of immunosuppression [17,18,19].

Aspergillosis was a poorly recognized fungal infection in the early years of the HIV/AIDS epidemic; however, a marked increase was recently observed, mainly in advanced stages of the disease due to its association with neutropenia and corticosteroid use. When its prognosis is presented, it is unfavorable, so early diagnosis and aggressive antifungal therapy are required. In our series, it was documented that the three patients showed average CD4 counts of < 100 cel/mm3, two of them were previously diagnosed with tuberculosis and had bilateral caverns and pulmonary bronchiectasis [20].

COPD is considered a risk factor for the development of pulmonary aspergilloma. The common use of steroids and antibiotics lead to decreased macrophage function and neutrophil and mucociliary activity. In this series, a patient with advanced COPD (stage IV of GOLD), had multiple bilateral bronchiectasis and a negative result of serum

galactomannan determination. Detection of serum galactomannan is less useful in COPD patients than in neutropenic patients, and has better results if it is determined in BAL with a sensitivity of 90% and a specificity of 100% [21,22].

Due to the angioinvasive capacity of *Aspergillus*, surgery such as cavernostomy and thoracoplasty should be considered in lesions that are close to large vessels or vital organs, a single focus, and massive hemoptysis, with low mortality and complication rates secondary to the surgical procedure. Continuous monitoring is recommended in patients with asymptomatic aspergilloma and without lesion progression. Recommended treatment includes amphotericin B, triazoles and deoxycholate [23]. This study provides data on aspergilloma characteristics, presentation, and outcomes. Some limitations include its retrospective design and its sample size; however, since it is considered a rare disease, it is worth reporting even if statistical test cannot be applied.

Conclusions

Patients with a decreased immunity have a high degree of predisposition to pulmonary aspergilloma, and hemoptysis is the most frequent clinical manifestation. Health personnel must have a high index of clinical suspicion to establish an accurate and early diagnosis, in addition to that an effective treatment is essential to fight this disease.

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Conflict of interests: No conflict of interests is declared.