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Short Communication

Evaluation of mold exposure in cystic fibrosis patients' dwellings and allergic bronchopulmonary risk

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Abstract

Very few studies have been conducted on cystic fibrosis (CF) patients' exposure to the indoor environment and, to our knowledge, there are no studies dealing with the link between specific fungal environmental exposure at home and fungal colonization resulting in allergic bronchopulmonary aspergillosis (ABPA). Fungal exposure of CF adult patients with ABPA (n = 4) with fungal sensitization (n = 7) and with no ABPA (n = 5) was assessed in 16 homes by dust sampling with electrostatic dust fall collectors (EDCs). *Aspergillus fumigatus* was specifically quantified by real-time quantitative polymerase chain reactions (qPCRs), and *A. fumigatus* DNA concentrations were significantly higher in homes of ABPA patients ($p < 0.001$). Results indicate that indoor fungal contamination could be a factor favoring ABPA and suggest that environmental surveys could help in preventing fungal risk in CF patients.

Keywords: Cystic fibrosis (CF); Allergic bronchopulmonary aspergillosis (ABPA); *Aspergillus fumigatus*; Real-time quantitative polymerase chain reactions (qPCRs); Dwellings

1. Introduction

The respiratory tract of Cystic Fibrosis (CF) patients may be colonized by filamentous fungi such as *Aspergillus* spp., *Scedosporium apiospermum* and *Exophiala dermatitidis* [1]. Up to 57% of CF patients are colonized by *Aspergillus fumigatus* [2], which can lead to allergic bronchopulmonary aspergillosis (ABPA) [3].

The consensus conference of the CF foundation defined minimum criteria required for ABPA diagnosis in CF [4]:

1. Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, change in pulmonary function, or increased sputum production) not attributable to another etiology.
2. Total serum IgE concentration of >500 IU/mL if patient is not treated by steroid.
3. Immediate cutaneous reactivity to *Aspergillus* or in vitro demonstration of IgE antibody to *A. fumigatus*.
4. One of the following: (a) precipitins to *A. fumigatus* or in vitro demonstration of IgG antibody to *A. fumigatus*; or (b) new or recent abnormalities on chest radiography (infiltrates

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or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy.

Recent publications have investigated links between allergic respiratory diseases such as asthma, and fungal exposure in indoor environments [5–7]. There is a growing recognition of the interest of such studies in chronically immunocompromised patients' dwellings, at risk of invasive fungal infection [8,9]. In contrast, very few studies have been conducted in the dwellings of CF patients with ABPA risk.

Electrostatic dust fall collectors (EDCs) are new methods that provide an alternative to air samplings and studies have shown that qPCR quantification of targeted species is an easy and reliable tool for characterizing the homes of allergic patients [10]. Thus, we aimed to investigate both the types and levels of fungi in CF patient's dwellings, using EDC, and to compare them with ABPA diagnosis.

2. Materials and methods

Since June 2011, during pneumology consultations, study was presented to patients. Those who had given their written consent received EDC. They put them in their bedrooms for a 10-week period and then sent them back to us by post.

Data written by patient on the EDC cover (opening date, closing date, room of deposit, height of EDC) were collected to check for the correct placement. A housing characteristics questionnaire (60 questions on location and type of dwelling, heating, ventilation, wall and floor covering, pets, number of people living in the dwelling) was also completed by each patient.

Washing solutions (0.1% Tween 80 solution (Merck®, Darmstadt, Germany)) of EDC were inoculated on culture media (Malt agar 2% (Oxoid®, Unipath®, Basingstoke, England) and Dichloran-Glycerol 18 (Oxoid®) added with 0.5% chloramphenicol (Merck®, Darmstadt, Germany) (DG18)). Molds that can colonize CF patients, *A. fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus terreus*, *E. dermatitidis*, *S. apiospermum*, and *Scedosporium prolificans*, were particularly sought and counted.

Rapid DNA extraction was performed from the washing solution, using mechanical and thermal lysis (runs in MagNA Lyser Instrument (Roche Applied Science®, Mannheim, Germany), boiling water bath and ice) as previously described [9]. Then, quantification of *A. fumigatus* (main mold implicated in ABPA) DNA was performed by real-time quantitative polymerase chain reactions (qPCRs) [11].

Total IgE and specific IgE were assayed by ImmunoCAP™ (Phadia® Thermo Fischer Scientific Inc.® Waltham, USA). Electrosyneresis on cellulose acetate with metabolic and somatic antigens of *A. fumigatus* (Bio-Rad®, Marnes-la-Coquette, France) was also carried out [12]. Patients' sputum was inoculated on DG18 media and CHROMagar™ Candida media (BD, France).

Wilcoxon rank sum and correlation tests were run to investigate possible statistical differences in mold levels related to the patients' status (ABPA or no ABPA) and dwelling characteristics collected by questionnaire. Statistical analyses were performed with R 3.0.2 [13].

3. Results

During the study, 16 consecutive CF patients who gave their written consent were included. ABPA classification was done according to the Stevens classification. Thus, four patients (P13, P14, P15 and P16, Table 1) were diagnosed with ABPA. Seven patients, without ABPA clinical symptoms, but having precipitin antibodies against antigens to *A. fumigatus* (threshold > 1 precipitin arc) were classified in sensitized patients. Five patients had neither ABPA (nor ABPA antecedents) nor sensitization. Two patients, P5 and P10 were previously diagnosed with ABPA (under antifungal therapy until 2012) and were in remission at the time of recruitment. *A. fumigatus* were isolated from sputum samples in six patients (P1, P5, P6, P7, P10 and P12) without ABPA at the time of sputum analyses. Antifungal and steroid treatments are mentioned in Table 1 too.

In the 16 dwellings, only *A. fumigatus*, *A. flavus*, and *A. nidulans* were detected using the culture-based method. *A. fumigatus* was detected in 5/16 dwellings (Table 2): the dwellings of four sensitized patients (P6, P7, P8 and P9) and one ABPA patient (P14). *A. fumigatus* DNA was detected by qPCR in 15/16 dwellings (Table 2).

The six patients with the highest amount of *A. fumigatus* DNA in their dwellings (from 3.4 to 6.5 fg/μL) were the four patients with ABPA and the two patients (P5, P10) previously diagnosed with ABPA, in remission at the time of the study. Among the 6 sensitized patients with no ABPA history, mean *A. fumigatus* DNA quantity was 1.6 fg/μL (min: 0.07; max 2.46). Among the 4 patients with neither ABPA nor ABPA history, mean *A. fumigatus* DNA quantity was 0.5 fg/μL (min: 0.00; max 1.17). Thus, globally, non-sensitized and non-ABPA patients are those who have the lowest *A. fumigatus* DNA quantity in their dwellings.

Wilcoxon sum rank test showed statistically significant differences between *A. fumigatus* DNA concentrations in ABPA patients' dwellings and those found in others patients (p-value = 0.004). A similar result was observed when patients with ABPA remission were grouped with ABPA ones (p-value < 0.001) (Fig. 1). No significant difference between mold contamination and the dwelling variables was found.

4. Discussion

A. fumigatus DNA concentrations were higher in homes of ABPA patients. Conversely, non-ABPA and non-sensitized patients' dwellings present the lowest *A. fumigatus* DNA quantity.

As for asthmatic and immunocompromised patients, environmental contamination may be a factor favoring the occurrence of ABPA.

Diagnosing ABPA in CF patients is difficult. In this study, we used the Stevens classification [4] to categorize our ABPA patients even if we know that these criteria have been controversial and might be revised [3]. ABPA classification complicating asthma has been proposed by the ISHAM working group [14] and is as follows: a) predisposing conditions as bronchial asthma or cystic fibrosis, b) high levels of total IgE (>1000 IU/mL) and specific IgE against *A. fumigatus* (or a positive skin test), c) two of the three following criteria: presence