



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.

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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). Electrosynthesis 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

Table 1
ABPA patient classification.

Status	Patients	Age (gender)	<i>A. fumigatus</i> specific IgE level (KUI/l)	Total IgE level (IU/mL)	Radiographic criteria	Metabolic antigens of <i>A. fumigatus</i> (arcs)	Somatic antigens of <i>A. fumigatus</i> (arcs)	Positive <i>A. fumigatus</i> culture from sputum	Antifungal therapy (inclusion period)	Steroid therapy (inclusion period)
No ABPA	P1	19 (M)	<0.1	3	TT opacities ²	0	1	Yes	No	No
	P2	32 (F)	<0.1	21	Mucoid impaction, nodules, TT opacities ²	1	0	No	No	Fluticasone (inhaled)
	P3	27 (M)	2.3	263	Nodules, TT opacities ²	0	0	No	No	Beclometasone (inhaled)
	P4	29 (M)	<0.1	<20	TT opacities ²	1	1	No	No	No
	P5 ¹	20 (M)	0.2	55	Nodules, TT opacities ²	0	0	Yes	No	No
Sensitized	P6	17 (M)	NA ⁴	26	Nodules, TT and FIG opacities ^{2,3}	2	3	Yes	No	No
	P7	23 (M)	<0.1	111	Nodules, TT opacities ²	5	4	Yes	No	Fluticasone (inhaled)
	P8	25 (F)	0.7	381	Mucoid impaction, TT opacities ²	2	3	No	No	No
	P9	25 (F)	<0.1	<2	No signs	5	4	No	No	Budesonide (nasal)
	P10 ¹	43 (M)	0.2	29	Nodules, TT and FIG opacities ^{2,3}	2	3	Yes		
	P11	35 (M)	<0.1	19	Nodules, TT opacities ²	3	3	No	Itraconazole	Budesonide (nasal)
	P12	22 (M)	<0.1	3986	TT opacities ²	5	5	Yes	Itraconazole	No
ABPA (4)	P13	18 (M)	43.1	789	Bronchiectasis, mucoid impaction, TT opacities ²	6	5	No	Voriconazole	No
	P14	49 (F)	17.2	976	TT opacities ²	5	3	No	No	Prednisone (oral)
	P15	38 (F)	39	1871	Mucoid impaction, TT opacities ²	2	2	No	Itraconazole	Prednisone (oral)
	P16	53 (M)	10.6	2023	Infiltrates, Nodules, TT opacities ²	2	2	No	No	Prednisolone (oral) budesonide (inhaled)

Highlighted boxes correspond to the required criteria.

¹Patients with ABPA remission.

²TT opacities = Tram-Track opacities.

³FIG opacities = Finger In Glove opacities.

⁴NA = not available.

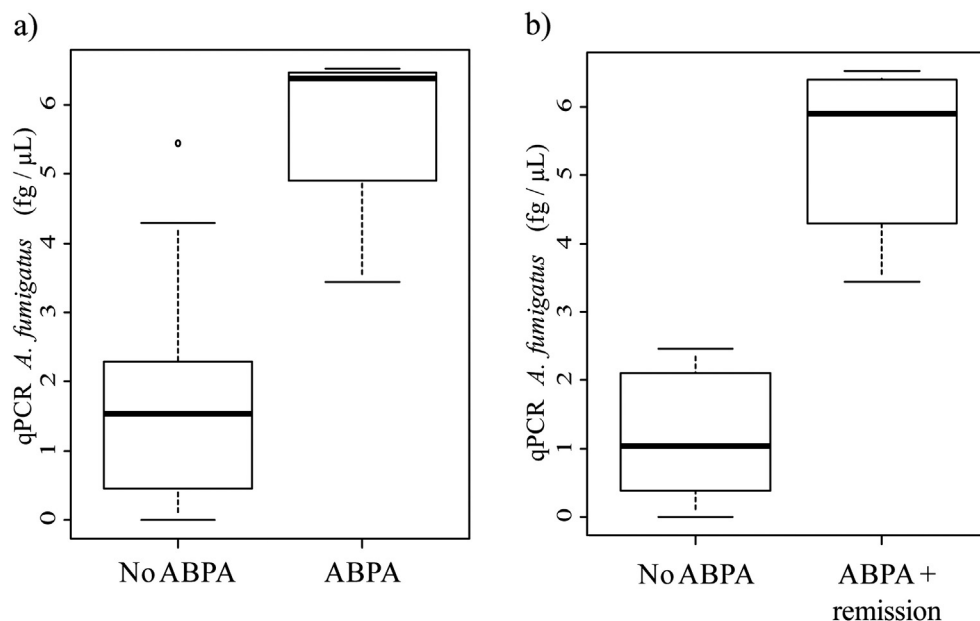
Table 2Fungal analysis of dwellings (culture and *A. fumigatus* qPCR).

Status	Patients	Mold culture (CFU/25cm ² of EDC)					qPCR analysis (fg/μL)
		<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. nidulans</i>	Others	Total	<i>A. fumigatus</i>
No ABPA	P1	0	0	0	21	21	0.00
	P2	0	0	0	230	230	1.17
	P3	0	53	0	27	80	0.39
	P4	0	0	0	128	128	0.52
	P5 ¹	0	0	0	75	75	5.44
Sensitized	P6	11	0	5	139	155	1.91
	P7	5	5	0	144	154	0.90
	P8	5	0	0	283	288	2.11
	P9	5	0	0	427	433	2.46
	P10 ¹	0	0	0	187	187	4.29
	P11	0	0	0	27	27	2.10
	P12	0	0	0	107	107	0.07
ABPA (4)	P13	0	5	0	134	139	6.37
	P14	11	0	0	187	198	6.40
	P15	0	0	0	272	272	6.53
	P16	0	11	160	134	304	3.44

¹Patients with ABPA remission.

of precipitating or IgG antibodies against *A. fumigatus*, chest radiographic features consistent with ABPA, an eosinophil count above 500 cells/μL. In our study, P13 and P14 had a total IgE level lower than 1000 IU/mL. So, none of the patients had eosinophil count above 500 cells/μL. Thus, the Stevens classification seemed more suited to our type of patients.

In CF patients, mucociliary clearance is defective and results in the production of sticky and thick bronchial mucus. *A. fumigatus*, ubiquitous in the environment, is the most common species involved in ABPA and patient morbidity. Small size and thermotolerant *A. fumigatus* conidia can deeply penetrate in the lung and easily germinate into hyphae. Therefore,

Fig. 1. Boxplots of *A. fumigatus* qPCR results between dwellings from ABPA and no ABPA patients, according to the Stevens classification at the time of recruitment.

- a). No ABPA versus ABPA (Wilcoxon test; p-value = 0.004) (ABPA remissions are considered no ABPA).
 b). No ABPA versus ABPA + ABPA remission (Wilcoxon test; p-value < 0.001) (ABPA remissions are considered ABPA).

environmental studies are needed to identify the nature of exposure and reservoirs of fungi that can colonize ABPA patients [1,15].

Only a few studies in ABPA patients' environment have ever been conducted. In 1984, a study measured airborne concentrations of *Aspergillus* in the dwellings of two ABPA patients and suggested that diminished exposure to *Aspergillus* could prevent exacerbation of ABPA [16]. Another study, based on an environmental questionnaire, compared the environment of asthmatic patients (n = 202) and that of ABPA patients (n = 101). It showed that only a rural background was significantly associated with ABPA [17].

Indoor environment studies in CF patients with ABPA are even more rare. A study compared airborne fungi of a CF unit with those in CF patients' sputa [18]. Fungal isolates were detected in both patients' sputa and the unit environment, showing that patients with CF were in close contact with viable fungi. In another study, different environmental samples were taken in six homes of CF patients with sputum containing *S. apiospermum*, and the authors demonstrated that indoor plants could be a source of *S. apiospermum* contamination [19].

Despite the lack of a clear link between fungal exposure and ABPA, activities that could result in inhaling large amounts of *Aspergillus* conidia are not recommended for patients [14].

ABPA is caused by hypersensitivity responses to *Aspergillus* antigen exposure. A specific quantification of *A. fumigatus* at home, reflecting the exposure of patients and thus their potential sensitization, could help to detect risk situations. This specific quantification has been performed with qPCR and EDC which represents a standardized and cost-efficient sampling method representative of a patient's chronic exposure. In our study, 75% of patients had *A. fumigatus* sensitization. This could be explained by the great number of dwellings with *A. fumigatus* detected by qPCR at home. The four patients who had neither ABPA (nor ABPA antecedents) nor sensitization have dwellings with the lowest *A. fumigatus* DNA quantities.

A. fumigatus is ubiquitous, but with high variability in conidia concentration from one dwelling to another. Although it is true that the patient sensitivity would make the difference in the development of ABPA, we showed that the massive presence of *A. fumigatus* was associated with the occurrence of ABPA. Consequently, indoor environmental contamination seems to be an inescapable condition of disease development.

However, the additional increase in *A. fumigatus* DNA concentration may also be linked to an increase in indoor spores coming from the extensively colonized patients themselves, who can release spores in their immediate environment. In this study, and as already mentioned in other publications [20,21], sputum culture is not reliably linked to ABPA.

In this study, no correlation was established between environmental exploration by culture and patient status. This could be explained by the difficulty of reviving spores. Detecting DNA from viable and non-viable spores by qPCR may help to overcome this problem and allow us to link *A. fumigatus* DNA concentrations to patient status.

These results must be confirmed in the future. However, this methodology (EDC + *A. fumigatus* qPCR), well accepted by patients, is easy to implement in the context of patient

monitoring. Early in CF monitoring, sampling by EDC would allow us to quantify *A. fumigatus* presence in rooms. We are clearly convinced that long-term environmental surveys (10 weeks), carried out in these fragile patients' dwellings, are important. In fact, the *A. fumigatus* DNA quantification (with a low cost device as presented here) could make it possible to give advice for cleaning, housing refurbishing or renovation and, therefore, to improve the quality of patients' environment.

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