

Relationship between mold exposure, specific IgE sensitization, and clinical asthma

A case-control study

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ARTICLE INFO

Article history:

Received for publication February 19, 2018.

Received in revised form June 13, 2018.

Accepted for publication June 18, 2018.

ABSTRACT

Background: Most of the findings related to the noxious effect of mold sensitization on asthma come from investigations based on *Alternaria alternata*, *Cladosporium herbarum*, and *Aspergillus fumigatus*. However, species such as *Penicillium* spp, *Cladosporium sphaerospermum*, *Cladosporium cladosporioides*, or *Aspergillus versicolor* display a more pronounced indoor tropism, and their potential harmful respiratory effects cannot be neglected.

Objective: The goal of this work was to relate mold sensitizations with asthma severity and with the level of indoor mold contamination among mold-sensitized patients with asthma and nonsensitized patients with asthma.

Methods: A case-control study was conducted and several asthma severity markers were compared between patients with asthma with and without mold sensitization. Indoor contamination of patients' dwellings was also investigated.

Results: Our findings confirmed the association between sensitization to *A fumigatus* and severity for patients with asthma in contrast with sensitization to other species. Indoor mold contamination was detected in approximately 90% of dwellings. Overall mold exposure was not associated with asthma severity. However, regardless of the sensitization, exposure to *A fumigatus* and *Penicillium* spp in dust was linked to an increased risk of severe asthma.

Conclusion: The harmful nature of mold sensitization and mold exposure for patients with asthma was not confirmed in this study. However, sensitization to *A fumigatus* was associated with an increased risk for severe asthma. A better investigation of the properties of *Penicillium* spp is recommended because its exposure was found to be associated with a more pronounced impairment of lung function.

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Introduction

Mold sensitizations have been repeatedly reported for their association with exacerbations of asthma symptoms and disease severity. In 1999, Neukirch et al¹ showed that sensitization to *Alternaria alternata* was associated with severe asthma.¹ These findings were later confirmed in the European Community Respiratory Health survey, which showed that the frequency of sensitization to *A alternata* or *C herbarum* was significantly associated with asthma severity.²

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Disclosures: The authors declare that they have no conflicts of interest.

Funding: This study was supported by an Ylief fellowship and by the Belgian Science Policy (BELSPO) contract IAP P7/32 DISCOBEL "Molecular Signaling in Cell Death and Inflammation: an integrative approach from basic mechanisms to disease models."

<https://doi.org/10.1016/j.anaai.2018.06.016>

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Previous studies also suggested a link between sensitization to *A alternata* and severe asthma exacerbation.^{3,4} More recently, Black et al⁵ found that patients admitted to the intensive care unit for a severe asthma attack were more likely to have a positive skin prick test for fungal spores (*A alternata* and *C herbarum*). Likewise, *A fumigatus* is frequently cited for its association with asthma severity,⁶⁻⁹ and the first report of this association dates back to 1978.¹⁰

So far, most studies regarding asthma and molds focused on *A alternata*, *C herbarum* and *Aspergillus fumigatus*.¹¹ Still, the study of mold species more specifically linked to the indoor environment, such as other *Aspergillus* species, *Penicillium* spp, and *Cladosporium* spp, is also of clinical interest.¹²⁻¹⁵ Indeed, in Western countries, people tend to increase their time spent indoors, and for energy-saving purposes, ventilation is often inadequate or limited, which promotes mold growth.¹⁶⁻¹⁸ Over the last years, several hallmark studies and

meta-analyses showed that indoor dampness and mold contamination were associated with respiratory health outcomes. For instance, in their meta-analysis, Fisk et al.¹⁹ showed that “building dampness and molds are associated with increases of 30% to 50% in a variety of health outcomes in a variety of populations.”¹⁹ Another large meta-analysis including 148 studies concluded that a significant association existed between exposure to indoor dampness or molds and cough, wheeze, upper respiratory tract infections, asthma exacerbations, and even asthma development.²⁰

In a previous work, we found that in a cohort of mold-sensitized patients with asthma, 60% of patients exhibited multiple sensitizations.²¹ *Aspergillus fumigatus* (32%) and *A. alternata* (28%) were the most frequent “major sensitizers.”^{21,22} *Penicillium chrysogenum*, *Penicillium brevicompactum*, *Cladosporium cladosporioides*, and *Cladosporium sphaerospermum*, a group of species commonly contaminating indoor environments,^{14,23} were also frequent (approximately 30%) “major sensitizers.” In parallel, we showed in a mouse model that these molds displayed Th1 or Th2 pro-inflammatory effects.²¹ These findings suggested that these mold species could be candidates to asthma expression.

In the current study, mold contamination of patients' dwellings was evaluated and related with mold sensitization and disease expression.

Methods

Study Design

A case-control study was conducted in patients with asthma who were living in the Brussels area and enrolled in CHU Brugmann between May 2012 and September 2015, according to a protocol approved by the Local Ethics Committee (CE2011/71).

Definition and Selection of Cases and Controls

A group of 64 patients with asthma, who were diagnosed according to the Global Initiative for Asthma (GINA) guidelines²⁴ and followed-up in the Clinique d'Immuno-Allergologie in CHU Brugmann, were included. Based on the results of skin prick test (SPT) or cellulose acetate membrane precipitin (CAP) test to molds (*A. fumigatus*, *A. alternata*, *Penicillium chrysogenum*, and *C. herbarum*), subjects were defined either as cases (at least 1 positive SPT or CAP) or as controls (negative for all SPT and CAP). Skin prick tests were carried out using a standardized procedure with reagents from Stallergènes (Antony, France). Positive (histamine and codeine) and negative (allergen diluent) controls were used. Weal size was measured after 20 minutes, and the result was considered positive if the weal was at least 4 mm greater than the negative control. The CAP tests were performed in the Immunology laboratory at CHU Brugmann with the Phadia 1000 FEIA analyzer (Thermo Scientific, Waltham, MA) against *A. fumigatus*, *A. alternata*, *P. chrysogenum*, and *C. herbarum*. According to the manufacturer, the test has a limit of detection of 0.1 kU/L and a positivity threshold of 0.35 kU/L. Thirty-two patients were included in each group, and they all signed a written informed consent. In the following pages, *cases* and *controls* refer to mold-sensitized patients with asthma and patients with asthma without mold sensitizations, respectively.

Dot-Blot Assay for Mold-Specific Immunoglobulin E Detection

The protocol for immunoglobulin E (IgE) detection by dot-blot assay has been described elsewhere.²² Briefly, mold extracts were coated on a nitrocellulose membrane using a 96-well dot-blot system and later blocked with bovine serum albumin. The membrane was then incubated with patients' sera and finally with a rat monoclonal anti-human IgE coupled to horseradish peroxidase. The membrane was developed with the Amersham ECL Prime reagent.

Readings were performed on a Bioanalyzer CCD-Camera and analyzed with the ImageQuant TL software (GE Lifesciences, Chicago, Illinois). Results, given as pixel counts, were analyzed with the ImageQuant TL software (GE Lifesciences, Chicago, Illinois), converted into an index, and considered positive beyond a positivity threshold established for each mold species.²² Because most sera showed multiple reactivities, the strongest response, called “major sensitization,” was defined as the response against a single extract showing the highest difference as compared with the variation of response of the negative control to this extract.

Data Collection

The clinical evaluation was based on symptoms, spirometry measurements (pre- and post- bronchodilator forced expiratory volume in one second [FEV₁], forced vital capacity (FVC), FEV₁/FVC) and the need for treatment. The GINA criteria were used to define the severity of asthma ranging from stages I to V. The GINA classification considers, among others, which kind of medication is needed to control a patient's symptoms (2016 report, Page 43, Box 3-5). In brief, steps I and II are mild asthma controlled with bronchodilators and low doses of inhaled corticosteroids (ICS); step III is a moderate asthma controlled with an association of low-dose ICS and long-acting bronchodilators (LABA); steps IV and V are severe asthma uncontrolled with a high dose of ICS-LABA and required other controllers, including oral CS or anti-IgE/-IL5.²⁴ Total serum IgE and blood eosinophils were measured in each patient.

Mold Exposure Assessment

House visits were performed by the Cellule Régionale d'Investigation en Pollutions Intérieures (CRIPI—Bruxelles Environnement), an experimental team for investigation of indoor pollutions. Forty-one inquiries were conducted, 23 in dwellings of cases and 18 in dwellings of control subjects. House visits were not conducted in each patient's house, because even though they all had agreed and signed the informed consent for a house visit, some of them finally refused when they were later contacted by the team. Each visit followed a standardized protocol and started with the completion of a questionnaire by the patient in which information about the time spent in the dwelling, the ownership status, and the dwelling itself were collected. Mold contamination was assessed in the main rooms of the house (eg, kitchen, living room, bathroom, bedroom). Their presence was first investigated visually, and when visible spots were present, imprints were taken on adhesive tapes, colored with lactophenol blue, and species identified in the Mycology laboratory (Health and Environment, WIV-ISP—Brussels). The size of the contaminated area was expressed with a 0 to 3 scale (0: absence of visible molds; 1: contaminated surface < 0.3 m²; 2: contaminated surface between 0.3 and 3 m²; 3: contaminated surface > 3 m²). Contamination was also investigated in air and settled dust samples. Air sampling was performed in the main rooms with an RCS Plus air sampler (Biotest, Rapperswil—Switzerland) filled with high-strength agar strips. A volume of 80 liters was collected for mesophilic molds and 160 liters for thermophilic molds. Agar strips were later cultured for 5 days at 25°C for mesophilic species identification and during 2 days at 45°C for thermophilic (*A. fumigatus*) species. Results are expressed as colony-forming unit/m³ (CFU/m³). To rule out counting bias from passive outdoor entry, indoor fungal concentrations were adjusted for concentration of fungi in outdoor air samples for each species of interest. For dust analysis, Replicate Organism Detection and Counting plates, prefilled with malt extract agar, were applied on horizontal surfaces (eg, shelves, cupboards) to collect settled dust (imprints) and then cultured for 5 days at 25°C for mesophilic species identification and during 2 days at 45°C for thermophilic species. Several values per

dwelling were generated from the counts in the main rooms (kitchen, living room, patient's bedroom, and bathroom): one total count and a count for each species of interest for both air and dust samples.

Environmental surveys are routinely performed by CRIPI and the Mycology laboratory. Based on 1,600 surveys, percentile curves of air contamination by molds were established for the 4 predominant indoor species in the Brussels area: *A. fumigatus*, *Aspergillus versicolor*, *Penicillium* spp, and *C. sphaerospermum* together with a percentile curve of dust contamination by *A. fumigatus*.²⁵ We defined the percentile 90 (P90) as the positivity threshold; that is, an airborne spore count of 14 CFU/m³ for *A. fumigatus*, 75 CFU/m³ for *A. versicolor*, 338 CFU/m³ for *Penicillium* spp, and 38 CFU/m³ for *C. sphaerospermum*. The P90 for settled dust contamination by *A. fumigatus* was 9 CFU/m³. For other species, the P90 was determined based on the results of investigations performed in our cohort after exclusion of outliers. The threshold for *A. versicolor* was 3 CFU/m³; for *Penicillium* spp, 76 CFU/m³; and for *C. sphaerospermum*, 4 CFU/m³.

Statistical Analysis

The Mann-Whitney test was used for comparisons of biological and lung function parameters and spore counts between groups. Results are expressed as medians and interquartile ranges (25th to 75th). Fisher's exact test was used for the comparison of proportions and calculation of the odds ratio. Linear regression was performed to study the link between lung function parameters and exposure variables. Multiple linear regressions were also conducted to investigate simultaneously the impact of mold exposure and mold sensitization on lung function parameters, but because none of these analyses brought significant results, they are not presented here. Analyses were performed with GraphPad Prism version 6.01, version 7.0, and Excel 2010 for Windows 7 Enterprise.

Results

Patient Characteristics

The 2 groups were homogenous (32 patients in each group) and did not differ in age, sex ratio, and smoking status (Table 1). The 2 groups were also comparable and did not differ significantly with regard to frequencies of sensitization to common aeroallergens such as house-dust mite, pet's dander, or birch and weed pollen (data not shown). Regarding housing, the proportion of owners was bigger in the cases group than in the controls group, but the difference did not reach statistical significance (data not shown).

Mold Sensitization and Asthma Severity

Patients sensitized to molds (ie, cases) were characterized by a higher total serum IgE concentration and a higher degree of broncho-obstruction (as measured by FEV₁/FVC). Blood eosinophil count was higher and FEV₁ lower among the cases, although not significantly. Severe asthma was more frequent among the cases (25% vs 15.6%), but the difference was not significant (Table 2).

Table 1
Demographics in Cases and Controls

	Cases (n = 32)	Controls (n = 32)	P value
Median age ^a	55.5 [29.0-66.5]	41 [30.0-60.0]	NS
Sex ratio (F/M) ^b	12/20	11/21	NS
Tobacco exposure ^b			
Smoker	5	3	NS
Nonsmoker	18	22	NS
Former smoker	9	7	NS

Groups were compared with ^aMann-Whitney (median; [25th-75th interquartile range]) and ^bFisher's exact tests.

Table 2
Biological and Clinical Parameters in Cases and Controls

	Cases (n = 32)	Controls (n = 32)	P value
Total serum immunoglobulin E (kU/L) ^a	511 [198-934]	170 [44-346]	.0003
Blood eosinophils (cells/ μ L) ^a	269 [180-494]	211 [167-330]	NS
Forced expiratory volume in 1 second (%) ^a	83 [62-103]	96 [77-109]	NS
Forced expiratory volume in 1 second/forced vital capacity (%) ^a	75 [66-82]	79 [75-85]	.03
Severe asthma ^b	25.0%	15.6%	NS

Groups were compared with ^aMann-Whitney (median; [25th-75th interquartile range]) and ^bFisher's exact tests.

Compared with the absence of mold sensitization, "major sensitization" to *A. fumigatus* was associated with higher total serum IgE and lower Tiffeneau index (FEV₁/FVC). Among them, FEV₁ was lower and the blood eosinophil count higher, although the differences did not reach statistical significance (Table 3A). Finally, more patients with "major sensitization" to *A. fumigatus* were suffering severe asthma compared with controls (50% vs 15.6%; odds ratio [OR]: 5.4; 95% confidence interval [CI]: 1.00-29.06; *P* = .059). In contrast, in patients with a "major sensitization" to *A. alternata* or against indoor species (in this paper, *P. chrysogenum*, *Penicillium brevicompactum*, *C. cladosporioides*, and *C. sphaerospermum*), biological and lung function parameters were comparable to those of subjects from the control group (Table 3B, C). When the group of patients sensitized to *A. fumigatus* is excluded, total serum IgE was not significantly higher than in the group of control patients (data not shown).

Indoor Mold Contamination

The presence of 4 mold species commonly isolated in Brussels area,¹⁴ *A. fumigatus*, *A. versicolor*, *C. sphaerospermum*, and *Penicillium*

Table 3
Comparison of Biological and Clinical Parameters between Cases and Controls According to "Major Sensitization"

A.	MS <i>A. fumigatus</i> (n = 8)	Controls (n = 32)	P value
Total serum immunoglobulin E (kU/L) ^a	751 [370-3,638]	170 [44-346]	.0002
Blood eosinophils (cells/ μ L) ^a	363 [195-555]	211 [166-330]	NS
FEV ₁ (%) ^a	75 [57-107]	96 [77-109]	NS
FEV ₁ /FVC (%) ^a	71 [49-77]	79 [75-85]	.016
Severe asthma ^b	50.0%	15.6%	NS
B.	MS <i>A. alternata</i> (n = 7)	Controls (n = 32)	P value
Total serum IgE (kU/L) ^a	317 [25-940]	170 [44-346]	NS
Blood eosinophils (cells/ μ L) ^a	248 [131-347]	211 [166-330]	NS
FEV ₁ (%) ^a	86 [71-106]	96 [77-109]	NS
FEV ₁ /FVC (%) ^a	76 [70-84]	79 [75-85]	NS
Severe asthma ^b	14.2%	15.6%	NS
C.	MS "Indoor species" (n = 7)	Controls (n = 32)	P value
Total serum IgE (kU/L) ^a	373 [143-778]	170 [44-346]	NS
Blood eosinophils (cells/ μ L) ^a	200 [178-360]	211 [166-330]	NS
FEV ₁ (%) ^a	61 [55-103]	96 [77-109]	NS
FEV ₁ /FVC (%) ^a	72 [48-80]	79 [75-85]	NS
Severe asthma ^b	28.6%	15.6%	NS

Groups were compared with ^aMann-Whitney (median; [25th-75th interquartile range]) and ^bFisher's exact tests.

Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

Table 4
Environmental Inquiries

A. Visible mold contamination							
	Cases (n = 23)	Controls (n = 18)	P value				
Presence of visible molds ^a	52.2%	83.3%	.0505				
Size of the visible mark ^a							
<0.3 m ²	26.1%	22.2%	NS				
0.3-3 m ²	26.1%	38.9%	NS				
>3 m ²	0.0%	22.2%	.03				
B. Airborne mold contamination							
Spores count (CFU/m ³)	Cases (n = 22)	Controls (n = 15)	P value	Contamination rates	Cases (n = 22)	Controls (n = 15)	P value
Total spore count ^b	162 [81-245]	158 [88-1,188]	NS	Any contamination ^a	68.2%	60.0%	NS
Spore count <i>A.fumigatus</i> ^b	3.5 [0-14]	0 [0-7]	NS	Contamination by <i>A.fumigatus</i> ^a	31.8%	13.3%	NS
Spore count <i>A.versicolor</i> ^b	13 [13-84]	50 [0-175]	NS	Contamination by <i>A.versicolor</i> [†]	27.3%	40.0%	NS
Spore count <i>Penicillium</i> spp ^b	50 [0-131]	75 [13-675]	NS	Contamination by <i>Penicillium</i> spp ^a	0.0%	26.7%	.020
Spore count <i>C.sphaerospermum</i> ^b	0 [0-19]	0 [0-13]	NS	Contamination by <i>C.sphaerospermum</i> ^a	22.7%	20.0%	NS
C. Settled dust mold contamination							
Spores count (CFU/m ³)	Cases (n = 22)	Controls (n = 15)	P value	Contamination rates	Cases (n = 22)	Controls (n = 15)	P value
Total spore count ^b	63 [38-149]	51 [25-76]	NS	Any settled dust contamination ^a	86.4%	60.0%	NS
Spore count <i>A.fumigatus</i> ^b	9 [2-15]	7 [3-12]	NS	Contamination by <i>A.fumigatus</i> ^a	59.1%	40.0%	NS
Spore count <i>A.versicolor</i> ^b	0 [0-2]	0 [0-1]	NS	Contamination by <i>A.versicolor</i> ^a	22.7%	13.3%	NS
Spore count <i>Penicillium</i> spp ^a	48 [23-121]	35 [18-48]	NS	Contamination by <i>Penicillium</i> spp ^a	36.4%	13.3%	NS
Spore count <i>C.sphaerospermum</i> ^b	0 [0-1]	0 [0-5]	NS	Contamination by <i>C.sphaerospermum</i> ^a	18.2%	20.0%	NS

A. Comparison of visible mold contamination rates and of the size of visible mold marks between dwellings of cases and controls. B and C. Total spore count and spore count per species in the air (B) and in settled dust (C) were compared between dwellings of the 2 groups (left pane). Comparison of contamination rates and rates per species in the air (B) and in settled dust (C) between the 2 groups (right pane). Contamination rates (based on P90 as positivity thresholds, see Material and Methods) were compared with ^aFisher's exact test and spore counts (median; [25th–75th interquartile range]) with ^bMann-Whitney test.

spp, was investigated and quantified in settled dust, in the air, and in visible molds traces if present.

Most of the houses (65.8%) had traces of visible mold contamination in at least 1 of the main living spaces. The average moldy surface ranged between less than 0.3 m² and 3 m². Visible contamination was more frequent in houses of controls than in houses of cases (83.3% vs 52.2%), and the difference almost reached statistical significance ($P = .0505$). Moreover, massive visible contamination, defined by a contaminated area greater than 3 m², was found only in houses from subjects of the control group ($P = .03$) (Table 4A). Another major difference between the 2 groups of patients was the diversity of species isolated in visible marks: only *Cladosporium* and *Aspergillus* were

found in houses of cases, whereas a greater diversity was present in houses of controls. Of note, in both groups, the species most frequently identified in visible spots was *C. sphaerospermum* (Fig 1).

The total and species-specific airborne spore count was not statistically different between houses of cases and controls (Table 4B). In this cohort, almost 65% of housings had the indoor air contaminated by mold spores. The contamination rate was comparable between groups. Of note, *Penicillium* spp was never present in concentrations above the P90 (positivity threshold) in dwellings of mold-sensitized patients with asthma, whereas it was isolated in more than 25% of controls' dwellings ($P = .02$) (Table 4B).

Settled dust spore counts were compared between the 2 groups, and no significant differences were observed, neither for the total count nor for each species (Table 4C). Seventy-five percent of the patients were living in dwellings in which dust was contaminated by molds. Settled dust contamination was comparable between the 2 groups (Table 4C).

More than 90% of dwellings of the cohort had at least 1 type of sample contaminated by molds or affected by visible mold presence. Contamination rates were similar between groups. Findings of all environmental enquiries are summarized in Table 5.

Mold Exposure and Asthma Severity

Next, the potential link between mold exposure and asthma severity was investigated, first by grouping all patients together then

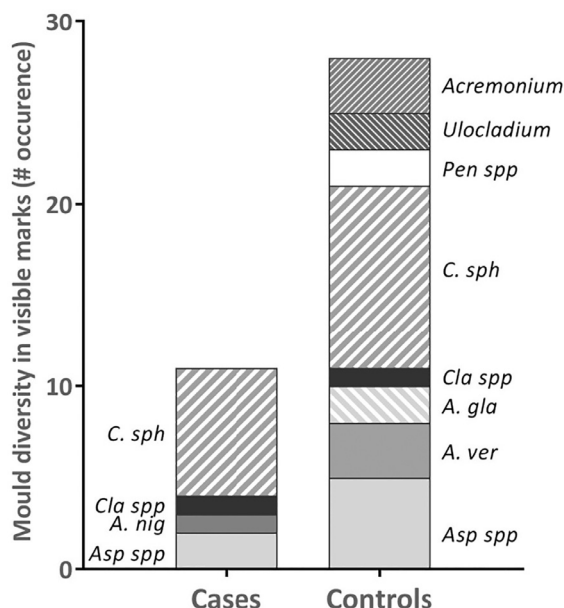


Figure 1. Visible mold contamination. Comparison of the mold diversity in visible spots between the houses of cases (n = 23) and controls (n = 18).

Table 5
Summary of Indoor Mold Investigations

	Cases	Controls	Cases + Controls
Visible mold spots	52.2%	83.3%	65.8%
Indoor air contamination	68.2%	60.0%	64.9%
Dust contamination	86.4%	60.0%	75.7%
Global	91.3%	94.4%	92.7%

Global results refer to the proportion of dwellings in which at least 1 type of sample was contaminated by at least 1 mold species.

Table 6Comparison of Biological and Clinical Parameters among Controls According to *Penicillium* spp Exposure

	<i>Penicillium</i> spp (n = 6)	Noncontaminated (n = 10)	P value
Total serum IgE (kU/L) ^a	217 [37–516]	218 [141–495]	NS
Blood eosinophils (cells/μL) ^a	171 [144–386]	179 [86–376]	NS
Forced expiratory volume in 1 second (%) ^a	77 [64–109]	99 [93–111]	NS
Forced expiratory volume in 1 second/forced vital capacity (%) ^a	71 [57–80]	81 [78–90]	.022
Severe asthma ^b	33.0%	0.0%	NS

Groups were compared with ^aMann-Whitney (median; [25th–75th interquartile range]) and ^bFisher's exact tests. Subjects were considered exposed if at least 1 type of sample was contaminated. N = 16 based on the number of samples (air, dust, and visible molds imprints) for whom a species identification was available.

by stratifying for cases and controls. In this cohort of patients with asthma, overall indoor mold contamination in dust or in air (ie, mold contamination of at least 1 type of sample) was not associated with an increased asthma severity as objectified by several parameters such as FEV₁ measurements, Tiffeneau index, and disease severity classification (data not shown).

The consequence of environmental exposure to particular mold species on asthma severity was also analyzed. Results showed that in this cohort, exposure to *A fumigatus*, *A versicolor*, *Penicillium* spp, or *C sphaerospermum* in the air, in settled dust, or through the presence of visible mold spots was not significantly associated with altered biological markers, neither when cases and controls were analyzed separately nor when they were examined together (data not shown).

Regardless of the sensitization status, however, in this cohort (n = 64), exposure to *A fumigatus* and to *Penicillium* spp in dust was significantly associated with severe asthma (GINA stages IV and V) (OR: 9.3; 95% CI: 1.20–110.6; *P* = .044 and OR: 10.7; 95% CI: 1.15–136.6; *P* = .047, respectively). In addition, in control subjects, overall exposure to *Penicillium* spp was significantly associated with impaired lung function and a trend toward a more severe asthma (Table 6).

Mold Exposure and Sensitization

Only a weak concordance (35.5%) was seen between mold species isolated indoors (in air, dust, or visible mold spot) and “major sensitization.” The best concordance between exposure and sensitization was found for the *Aspergillus* genus and almost reached 40%. It was considerably lower for other mold genera (Table 7).

Although the association was nonsignificant, patients with a “major sensitization” and exposed to *A fumigatus* (n = 5) suffered more frequently from a severe form of asthma than patients whose exposure did not match “major sensitization” (60% vs 25%; OR: 4.5; 95% CI: 0.5–41.2; *P* = .280; n = 17). These patients were characterized by total serum IgE above 350 kU/L. Also, they all received a combination therapy (LABA and ICS), and 1 of them received anti-IgE as add-on therapy as well. Unfortunately, information regarding exacerbations (frequency, severity...) was missing. Finally, these patients had all been living in their homes for more than 20 years. When less stringent criteria of matching were used (eg, concordance between any sensitization and any mold genus isolated indoors), the distribution of the disease severity became very comparable between the 2 groups.

Table 7

Concordance Between Exposure and “Major Sensitization”

<i>A fumigatus</i>	26.3%
<i>Aspergillus</i> spp	36.8%
<i>Penicillium</i> spp	10.5%
<i>Cladosporium</i> spp	10.5%

Discussion

In a previous work, we conducted investigations in a mouse model of asthma, and the analysis of inflammatory and allergenic properties of a panel of molds showed that different species displayed different immune properties.²¹ Indeed, we showed that *C sphaerospermum* and *A alternata* triggered inflammatory responses rather than typical Th2 allergic responses. In contrast, *P chrysogenum* and *A fumigatus* seemed more pro-allergic (Th2), whereas *A versicolor* and *C cladosporioides* displayed a mixed allergenic/inflammatory profile.

Based on these findings, we investigated, in this case-control study, indoor mold contamination and how mold exposure possibly affects inhabitants' mold sensitizations and the expression of the disease in patients with asthma.

We observed that, in patients with asthma, mold sensitization was associated with an increased impairment in lung function. In this cohort, when sensitizations were considered species by species, the harmful effect of sensitization appeared to be attributable to only *A fumigatus*. Indeed, a reduced Tiffeneau index (FEV₁/FVC) and an increased risk for severe asthma were only detected in this subgroup of patients with asthma, as described previously.²⁶ In contrast to previous studies,^{2,4,5,27} in our cohort, “major sensitization” to *A alternata* or to species with an indoor tropism (*P chrysogenum*, *P brevicompactum*, *C cladosporioides*, and *C sphaerospermum*) were not associated with an increased disease severity. Based on results of the dot-blot assay, the concept of “major sensitization” was developed and validated to address the issue of cross-reactions and of potential polysensitizations.²² However, most studies showing an association between *A alternata* sensitization and disease severity relied on SPTs, well known for their relative lack of specificity, for the detection of sensitizations.^{6,28} Therefore, one cannot exclude that patients in whom sensitization to *A alternata* was detected based on SPT results could actually be sensitized to other relevant species.^{2,4,5,27}

Because of the small sample size and possible recruitment bias, results from environmental investigations performed in this study cannot be extrapolated to all housing in the Brussels area. However, with an overall contamination rate above 90%, the issue of unhealthy dwellings should be further investigated. In addition, even if other studies generally reported much lower contamination rates in dwellings or workplaces, ranging from 5% to 30% according to climates,^{29,30} the visible mold contamination rate was nevertheless similar to what Chasseur et al³¹ reported from their surveys in the Brussels area between 2000 and 2013. A recent study reported a visible mold contamination rate of 100% in 18.2% of dwellings, but the size of the contaminated areas was very limited.³² Differences in contamination rates between studies could be explained, at least partly, by differences in study design. Indeed, in our cohort, investigation of mold contamination was quite exhaustive and relied on the analysis of air and dust samples but also of visible marks, whereas other studies were generally based on the analysis of 1 or 2 types of samples. A second explanation for these differences can also lie in the fact that here, investigations were performed by trained professionals, whereas some studies rely on self-report of visible mold presence. Also, in our study, results were quantitatively expressed. In addition, the choice of positivity thresholds differed between our study and others with quantitative reports of mold contamination.^{18,22,33–35} Finally, studies reporting on indoor mold contamination were conducted in a wide range of countries with different climates, socioeconomic backgrounds, and cultural habits. This heterogeneity complicates the comparison of findings.

In this study, visible mold presence was less frequent in dwellings of patients with asthma than in those of controls (*P* = .0505). Possibly exposure to visible mold spots is not associated with disease severity. Another hypothesis could be that patients with asthma who are sensitized to molds implemented sanitation measures to remove visible mold marks from their dwellings, which could have caused a

reporting bias that could explain the difference of contamination rate. But this seems unlikely, because our results showed that dwellings of cases and controls were homogenous regarding indoor airborne and settled dust contamination. Total spore counts and counts per species and per sample type were globally similar between cases and controls. These data point to a lack of association between indoor mold contamination or exposure and the course of asthma of the inhabitants. Indeed, Nambu et al³⁵ made the same observation when comparing dust contamination rates between allergic children with or without mold sensitization. In contrast, Reboux et al²³ reported a stronger indoor air contamination in the houses of allergic as compared with nonallergic subjects.

Two interesting observations emerged in our cohort, however. First, the well-described allergenic *A fumigatus* was the most frequent species isolated in the air of dwellings from cases, whereas it was the less frequent in controls, although the difference did not reach significance. Second, a concentration of *Penicillium* spp in the air exceeding the positivity threshold was only found in the dwellings of control subjects. A similar observation was made by Nambu et al, but for dust contamination by *Penicillium* spp.³⁵

In our cohort, the overall concordance between objectified indoor exposure and “major sensitization” was low, only reaching 35%. This is somehow not surprising if we keep in mind that dwellings do not represent the exclusive source of mold exposure and therefore of sensitization. In addition, information was often lacking regarding the length of exposure, and sensitization was possibly anterior to exposure, which highlights again the limits of cross-sectional studies. It was worth notice, however, that a higher concordance between exposure and sensitization was observed with *Aspergillus* spp, the genus consistently associated with asthma severity.

In accordance with previous findings,^{36–39} our results did not show an association between quantitative reports of indoor mold contamination and increased disease severity, even if contradictory results also exist.^{19,20,29} Nevertheless, in more powerful, longitudinal studies, the strength of positive association tended to decrease.^{30,40,41} In addition, the viable fungal count was not an appropriate tool to investigate adverse respiratory effects of mold exposure, and better concordance was reached between qualitative reports of mold contamination and adverse respiratory outcomes.^{20,41–45} We did not make such an observation, and in our cohort, patients living in dwellings with visible mold marks were not more severely affected than others. Some authors highlighted the absence of standardized protocols for indoor inquiries as a source of discrepancy between results,²⁰ whereas others suggested that expression of results for all species together may annihilate positive associations observed with particular species.^{39,43} In our study, genus or species identification was performed and differential spore counts as well. Even when considering spore counts species by species, we did not find any correlation between spore counts and the severity of the disease. Among the whole study population, dust contamination (above the P90) by *A fumigatus* and *Penicillium* spp was associated with increased asthma severity. Of note, in our cohort, overall exposure to *Penicillium* spp in control subjects was associated with an increased impairment of lung function that could potentially be explained by nonspecific inflammatory mechanisms.⁴⁶ However, the sample size was small, and further investigation regarding the impact of exposure to *Penicillium* spp in patients with asthma without mold sensitizations would be of interest. The added value of the determination of “major sensitization” with the dot-blot assay was again illustrated by the positive trend between overall exposure and “major sensitization” to *A fumigatus* and asthma severity. This observation also indicated that even if indoor exposure may not directly influence sensitization or the severity of asthma, in *A fumigatus*–sensitized patients with asthma, exposure to *A fumigatus* might worsen disease severity. A similar

observation was made by Gent et al³⁴ in a prospective study but for exposure and sensitization to *Penicillium*.

The current study has several limitations. First, 64 patients were included, and among them only 41 agreed to having home inquiries. This relatively small sample size limits the robustness of statistical analysis, and we may have missed the significance of some associations. Second, as discussed earlier, only a prospective study is appropriate to investigate the potential causal role of mold exposure in relation to allergy and asthma but is considerably more costly and difficult to organize. Third, for logistic constraints, mold exposure was assessed in dwellings only, but schools, workplaces, or even cars represent other potential sources of mold exposure that also should be investigated.

In a previous work, we observed that in more than 300 serum sample from “normal subjects without asthma,” although mold sensitization was common (32.8%), no “major sensitization” was observed against this mold. On the other side, in our group of patients with asthma with mold sensitization, this “major sensitization” was the most frequent. This suggests that sensitization against *A fumigatus* might be associated with clinical symptoms.²¹

Taken altogether, these findings highlight the interest in investigating asthma symptoms and severity in a cohort of patients with asthma sensitized and exposed or not to *A fumigatus* but also more globally in patients with asthma in whom indoor exposure matches their major sensitization. In such a study, the severity of symptoms with regard to the indoor mold contamination could be addressed in detail. In addition, the harmful potential of *Penicillium* spp exposure for patients with asthma also should be further investigated. Indeed, our findings showed that such an exposure was associated with increased lung function impairment and signs of asthma severity even in the absence of specific sensitization.

In conclusion, this work indicates an absence of the overall harmful nature of heightened mold exposure and mold sensitization for patients with asthma. However, our data showed that a sensitization to *A fumigatus* was associated with an increased risk for severe asthma and that exposure to *A fumigatus* and *Penicillium* spp were associated with increased impairment of lung function, regardless of the sensitization status of the patients. In contrast to other species considered in this study, *A fumigatus* plays an unusual role as an invasive pathogen as well as an airway colonizer, which might explain the link between sensitization to *A fumigatus* and asthma severity. This would further support our result that mold exposure is not, sui generis, a risk factor for more severe asthma.

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