
Association between sensitization to *Aureobasidium pullulans* (*Pullularia* sp) and severity of asthma

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Background: Recent data indicate that fungi may contribute to increased severity of asthma.

Objectives: To determine the prevalence of allergy to 15 mold allergens among patients hospitalized because of exacerbation of asthma and to evaluate the relationship between the severity of the disease and allergy to particular molds.

Methods: Skin prick tests with standard aeroallergens of airborne allergens, including grass, tree, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, feather, and cat and dog fur, and a panel of mold allergens, including *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Trichothecium*, *Chaetomium globosum*, *Epicoccum*, *Epidermophyton*, *Helminthosporium*, *Aureobasidium pullulans*, *Rhizopus nigricans*, *Fusarium*, *Mucor*, *Merulius lacrymans*, and yeast mix, were performed in 105 asthmatic patients and 30 controls.

Results: Positive skin prick test results were found in 98% of asthmatic patients and 66% of controls. Sensitivity to *A pullulans* was significantly associated with more severe asthma (odds ratio, 1.4; 95% confidence interval, 1.09–1.75; $P = .006$). Sensitization to *Helminthosporium* was associated with an increased number of asthma exacerbations that required hospitalization (17% vs 38%; χ^2 test $P = .03$).

Conclusion: Sensitization to *A pullulans* is a risk factor for severe asthma. Sensitization to *Helminthosporium* may be related to asthma exacerbation that requires hospitalization.

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INTRODUCTION

A study by Stark et al¹³ in a cohort of 405 children from Boston, MA, suggested that specific dust-borne fungi (*Aspergillus*, *Aureobasidium pullulans*) found at home in the first 3 months of life might be associated with an increased risk of developing allergic rhinitis by 5 years of age. Early *Penicillium* exposure may increase the risk of asthma.⁷ Results of the European Community Respiratory Health Survey (ECRHS) (including 1,132 patients in the European Union, United States, and Australia) showed that mold allergy (*Alternaria*, *Cladosporium*) is a strong risk factor for severe asthma in adults.¹⁶ Numerous clinical studies confirmed this observation.^{2,3,7,12} A positive skin prick test result for fungal allergens (*Alternaria*, *Cladosporium*, *Helminthosporium*, *Epicoccum*) is also a risk factor for life-threatening asthma.² Most of the studies analyzed only a few fungal allergens, mainly *Alternaria* and *Cladosporium*. The lack of standardization of some fungal extracts and the allergenic potency of certain species remains an unsolved issue. Although a study by Taylor et al¹⁴ suggested that immunologic reaction to *A pullulans* is associated with immediate reactivity in skin tests

and specific IgE production, other studies have not confirmed allergenic IgE-mediated reaction to this mold. The aim of this study was to determine the association between allergy to 15 mold allergens (*Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Trichothecium roseum*, *Chaetomium globosum*, *Epicoccum*, *Epidermophyton*, *Helminthosporium*, *A pullulans*, *Rhizopus nigricans*, *Fusarium*, *Mucor*, *Merulius lacrymans*, and yeast mix) and severity of asthma and the need for hospitalization because of asthma exacerbation.

MATERIALS AND METHODS

Patients

A total of 105 consecutive asthma patients treated at the Department of Allergology, Medical University of Gdansk, Gdansk, Poland were included in the study. Asthma was diagnosed and graded according to the Global Initiative for Asthma guidelines.⁹ The control group consisted of 30 patients hospitalized in the Department of Internal Diseases, Endocrinology, and Hemostatic Disorders with no symptoms of any allergic disease in the medical history. The exclusion criteria in both groups were pregnancy and severe diseases, such as cancer, tuberculosis, and heart, renal, and liver failure. Clinical and demographic data, including age, sex, personal and family history of asthma, previous hospitalizations, home and work characteristics, environmental exposures, smoking, coexisting diseases, and physical examination findings, were obtained by questionnaire after written informed

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consent was acquired. The study was approved by the Ethical Committee of Medical University of Gdansk.

Skin Prick Tests

Skin prick tests with standard aeroallergens of airborne allergens, including grass, tree, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, feather, and cat and dog fur, and a panel of mold allergens, including *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *T roseum*, *C globosum*, *Epicoecum*, *Epidermophyton*, *Helminthosporium*, *A pullulans*, *R nigricans*, *Fusarium*, *Mucor*, *M lacrymans*, and yeast mix (Stallergenes, Antony, France), were performed by the prick method according to European Academy of Allergology and Clinical Immunology recommendations.⁴ Positive skin prick test results were defined as a mean wheal diameter larger than 3 mm. Glycerol-buffered saline was the negative control, and histamine, 1 mg/mL, was the positive control. SPT lancets from Allergopharma (Reinbeck, Germany) were used.

Spirometry

Spirometry was performed (Lungest 500, MES, Krakow, Poland), and forced expiratory volume in 1 second, peak expiratory flow, and vital capacity were measured. Bronchodilator response was measured 15 minutes after the patient inhaled 400 µg of salbutamol. Spirometry and classification of asthma severity were evaluated according to the Global Initiative for Asthma guidelines.⁹ Additionally, chest x-ray and standard biochemical examinations were performed.

Statistical Analysis

Results expressed as the percentage of the patients and differences in proportions were measured by the χ^2 test. Logistic regression analysis was used to estimate odds ratios for associations between severity of asthma and sensitization. Data for this study were analyzed with Statistica statistical software, version 7.0 PL. (StatSoft, Tulsa, OK).

RESULTS

The study characteristics are presented in Table 1. Severe asthma was diagnosed in 44 patients (42%), moderate asthma in 44 (42%), and mild asthma in 17 patients (16%). Mean forced expiratory volume in 1 second in the study group was 90% (range, 35%–140%). The results of the skin prick test for allergens other than molds were positive in 88 asthmatic patients (83%) (Table 2). Sensitization to molds expressed as the rate of positive skin prick test results in asthmatic patients and controls occurred in 103 (98%) and 20 (66%), respec-

Table 2. Prevalence of Positive Skin Prick Test Results (Wheal >3 mm) to Studied Allergens

Allergen	Asthma patients, No. (%) (n = 105)	Control group, No. (%) (n = 30)	χ^2 P value
<i>Dermatophagoides farinae</i>	52 (50)	3 (10)	.002
<i>Dermatophagoides pteronyssinus</i>	50 (48)	6 (20)	.01
Feather	23 (22)	3 (10)	.23
Grass mix	42 (40)	3 (10)	.008
Cat	40 (38)	4 (13)	.01
Tree Betulaceae	38 (36)	3 (10)	.008
<i>Fusarium</i>	34 (33)	2 (7)	.01
Dog	28 (27)	1 (3)	.01
<i>Aureobasidium pullulans</i>	28 (27)	0 (0)	.003
<i>Mucor</i>	28 (27)	3 (10)	.09
Tree Fagaceae	26 (25)	0 (0)	.005
Yeasts	26 (25)	4 (13)	.28
<i>Trichothecium roseum</i>	25 (24)	2 (7)	.07
<i>Helminthosporium</i>	25 (24)	4 (13)	.21
<i>Alternaria</i>	25 (24)	4 (13)	.32
<i>Merulius lacrymans</i>	25 (24)	3 (10)	.16
<i>Chaetomium globosum</i>	22 (21)	2 (7)	.12
<i>Rhizopus nigricans</i>	21 (20)	3 (10)	.32
<i>Aspergillus</i>	21 (20)	1 (3)	.06
<i>Epicoecum</i>	18 (17)	0 (0)	.03
<i>Cladosporium</i>	15 (14)	2 (7)	.42
<i>Penicillium</i>	14 (13)	3 (10)	.86

tively (Table 3). The incidence of positive skin prick test results was significantly higher in asthmatic patients for *D farinae*, *D pteronyssinus*, grass, cat and dog fur, and trees and among the molds for *Epicoecum*, *A pullulans*, and *Fusarium* ($P < .03$).

Sensitivity to *A pullulans* was associated with severity of asthma (odds ratio, 1.4; 95% CI, 1.09–1.75; $P = .006$) for severe vs mild and moderate asthma. No association was found between the sensitization to other allergens and asthma. The incidence of sensitization to *Helminthosporium* in the asthmatic patients hospitalized for the first time compared with those hospitalized previously for asthma was higher (17% vs 38%; χ^2 test $P = .03$).

DISCUSSION

This study demonstrated that sensitization to *A pullulans* is associated with severity of asthma. *A pullulans*, *Alternaria*, and *Aspergillus* measured at home during the first 3 months of life were associated with the development of allergic rhinitis in the first 5 years of life.¹³ The study by Woodcock et al¹⁵ showed that bedclothes contain a high level of fungi. The most common 3 species isolated from pillows were *Aspergillus fumigatus*, *A pullulans*, and *Rhodotorula mucilaginosa*. Although *A pullulans* is an established factor related to extrinsic allergic alveolitis caused by profuse colonization of air conditioning systems, immunologic reaction to this mold and its role in asthma are still debatable.¹⁵ Recent

Table 1. Age and Sex of Study Population

Group	Male/female	Age, mean (range), y
All asthma patients (n = 105)	33/72	39 (18–77)
Mild asthma (n = 17)	8/9	33 (16–59)
Moderate asthma (n = 44)	12/32	36 (20–59)
Severe asthma (n = 44)	13/31	44 (19–77)
Control group (n = 30)	12/18	41 (18–69)

Table 3. Association Between Sensitization to Studied Allergens and Severity of Asthma

Allergen	Mild asthma patients, No. (%) (n = 17)	Moderate asthma patients, No. (%) (n = 44)	Severe asthma patients, No. (%) (n = 44)
Grass	7 (42)	19 (43)	16 (37)
Trees Betulaceae	8 (47)	12 (27)	18 (41)
Tree Fagaceae	7 (42)	10 (23)	9 (20)
<i>Dermatophagoides farinae</i>	7 (41)	23 (52)	22 (50)
<i>Dermatophagoides pteronyssinus</i>	8 (47)	23 (52)	20 (45)
Feather	8 (47)	6 (14)	9 (20)
Dog fur	5 (29)	12 (30)	10 (23)
Cat fur	6 (35)	17 (39)	17 (39)
<i>Alternaria</i>	2 (11)	14 (32)	9 (20)
<i>Cladosporium</i>	2 (11)	4 (9)	9 (20)
<i>Aspergillus</i>	3 (18)	7 (16)	11 (25)
<i>Penicillium</i>	0 (0)	7 (16)	7 (16)
<i>Candida</i>	10 (59)	25 (57)	25 (57)
<i>Trichothecium</i>	4 (24)	9 (21)	12 (27)
<i>Epicoccum</i>	2 (12)	7 (16)	9 (21)
<i>Helminthosporium</i>	4 (23)	8 (18)	13 (30)
<i>Aureobasidium*</i>	3 (17)	8 (18)	17 (39)
<i>Rhizopus</i>	2 (12)	8 (18)	11 (25)
<i>Fusarium</i>	4 (24)	14 (32)	16 (36)
<i>Mucor</i>	4 (24)	13 (30)	11 (25)
<i>Merulius</i>	4 (24)	11 (25)	10 (23)
Yeasts	3 (18)	12 (27)	11 (25)
<i>Chaetomium</i>	2 (12)	10 (23)	10 (23)

* Statistically significant at $P = .006$.

studies by Taylor et al¹⁴ suggested that *A pullulans* is associated with immediate reactivity on skin tests, specific IgE production, and allergic disease of the upper and lower airways. Results of the present study seem to support those findings.

The results of the ECRHS¹⁶ showed the relationship between sensitization to *Alternaria* and *Cladosporium* and asthma severity. Results of the present study suggest that asthma severity is also related to *A pullulans* sensitivity. The explanation of this finding requires further studies especially in light of mentioned data that suggest that *A pullulans* is prevalent in the environment,¹⁵ causes IgE-mediated reactions,¹⁴ and is associated with the development of allergic diseases.¹³

The study by Black et al² suggested the relationship between sensitization to *Helminthosporium* and hospitalization caused by asthma exacerbation. Authors compared skin prick tests with *Alternaria*, *Cladosporium*, *Helminthosporium*, and *Epicoccum* in 3 groups of patients: those admitted to an intensive care unit (ICU) with an attack of asthma, those hospitalized with asthma exacerbation but not requiring ICU treatment, and those who never required emergency treatment for that reason. A positive SPT result for studied allergens was a risk factor for admission to an ICU with an acute attack of asthma. In the present study the relationship between

sensitization to some molds and asthma severity and hospitalization was observed but did not reach statistical significance. This might have been caused by the relatively small number of patients included in the study.

The surprising result is a high rate of sensitization to mold allergens in asthmatic patients and in the control group (98% and 66%, respectively). In the study by Katz et al,¹⁰ in Netzer Tzireni mold sensitization was found in 10.6% of asymptomatic and 61.9% of symptomatic subjects. In the ECHRS study, 73% of patients were sensitized to at least one allergen.¹⁶ The present study examined allergens that were not included in ECRHS study: *Aspergillus*, *Penicillium*, *T roseum*, *Chaetomium globosum*, *Epicoccum*, *Epidermophyton*, *Helminthosporium*, *A pullulans*, *R nigricans*, *Fusarium*, *Mucor*, *M lacrymans*, and yeast mix. Sensitization to molds, especially to *Dermatophytes*, may not be associated with allergy but with defense against fungal infection.⁸ Subjects in the control group were hospitalized because of endocrine and hemostatic disorders. The risk of fungal sensitization in these patients is relatively high, comparable to patients with severe asthma hospitalized and treated with corticosteroids and antibiotics. The cross-reactivity between particular fungal allergens may result in positive results of SPT with other noninfectious molds. *Epicoccum*, *A pullulans*, and *Fusarium* induced a statistically significant higher rate of sensitization in the asthmatic group. Therefore, SPT results may not be necessarily related to cross-reactivity with antigens of infectious molds.

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