

Allergic sensitization to laboratory animals is more associated with asthma, rhinitis and skin symptoms than sensitization to common allergens.

Running head: Effects of occupational sensitization compared to common sensitization

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Summary

Background Workers exposed to laboratory animals have a high risk of developing laboratory animal allergy (LAA). Atopy seems to be the main risk factor for LAA. We hypothesized that occupational sensitization is a better predictor for the development of asthma, rhinitis and bronchial hyperresponsiveness than common sensitization.

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Objective To investigate the association between occupational sensitization to laboratory animals and clinical outcomes.

Methods This was a cross-sectional study performed at two universities on students and employees dealing with small rodents. The subjects were allocated in groups: non-sensitized, common sensitization or occupational sensitization, according to the results of the skin prick test (SPT). All subjects answered a questionnaire about animal exposures, symptoms, allergic diseases, and underwent spirometry and bronchial challenge test with mannitol. Multivariate analysis was performed by using Poisson regression to estimate the prevalence ratio (PR).

Results Data from 453 volunteers were analyzed. Non-sensitized group comprised 237 subjects; common sensitization group, 142 subjects; and occupational sensitization group, 74 subjects. Occupational sensitization was associated with greater risk for all outcomes studied. When the common sensitization group was reference, skin symptoms had PR of 1.36; 95% confidence interval (CI): 1.01-1.85; wheezing had PR of 1.75; CI 95%: 1.21-2.53; rhinitis had PR of 1.25; 95%: 1.11-1.40; nocturnal dyspnea had PR of 2.40; 95% CI: 1.31-4.40; bronchial hyperresponsiveness had PR of 2.47; 95% CI: 1.50-4.09; and confirmed asthma had PR of 2.65; 95% CI: 1.45-4.85. In addition, the overlap of asthma, rhinitis and skin symptoms in a same subject was significantly more prevalent in the occupational sensitization group, 16.2% versus 4.9% in the common sensitization group.

Conclusion and Clinical Relevance Occupational sensitization is associated with allergic symptoms and respiratory diseases. SPT with occupational allergens along with other parameters may contribute to detection of risk for allergic and respiratory diseases associated with exposure to laboratory animals.

Keywords: asthma, bronchial hyperreactivity, diagnosis, epidemiology, occupational allergies, rhinitis, work.

Introduction

Workers exposed to laboratory animals have a high risk of developing allergy. The development of allergic reactions to laboratory animals is known as laboratory animal allergy (LAA) [1], defined as a syndrome characterized by conjunctivitis, rhinitis, urticaria and asthma [2]. Different definitions of LAA can be found (based on reported symptoms or

laboratory tests), which makes this disease prevalence range from 11 to 44% [3], with symptoms ranging from mild rhinitis to severe asthma [1,4].

Atopy is observed in up to 45% of the population of industrialized countries [5]; however, most atopic workers do not become sensitized to occupational allergens [6]. Occupational sensitization is found in approximately 16% of workers exposed to laboratory animals [7]. After sensitization to occupational allergens, very small exposures to sensitizing agents can induce asthma, which is often associated with rhinoconjunctivitis [8].

Most studies did not discriminate between common sensitization and occupational sensitization. This discrimination may help to assess the risk of occupational sensitization with or without common sensitization. We believe that among workers with occupational sensitization, continuous exposure to occupational allergens is associated with higher risk of allergic reactions as compared to exposed workers with only common sensitization, i.e. occupational sensitization is a major determinant of allergic diseases. Thus, the present study was aimed to investigate the association between occupational sensitization to laboratory animals and clinical outcomes.

Methods

This was a cross-sectional study conducted at two universities, the University of São Paulo at Ribeirão Preto (USP-RP) and the State University of Campinas (UNICAMP), São Paulo state, Brazil. Sample selection and the study protocol have been previously described [7]. Study subjects were workers and students exposed to laboratory animals. Data were collected in loco, i.e., in the workplace and all procedures were performed on Wednesdays, Thursdays, and Fridays. At least 90% of the subjects in each workplace consented to participate; the overall consent and participation rate was 95% [7]. The study was reviewed and approved by the Ethics Committees of both institutions: Medical School of Ribeirão Preto, USP-RP (protocol number 9428/2009), and the School of Medical Sciences, UNICAMP (protocol number 779/2009). Written informed consent was obtained from all subjects after reading and discussing the protocol individually.

Skin prick test

Skin prick tests (SPTs) were applied according to the recommendations of the European Academy of Allergology and Clinical Immunology [9]. All subjects refrained from taking antihistamine drugs for 15 days prior to SPT. A wheal diameter of at least 3 mm was considered positive in the absence of a reaction to physiological saline solution and in the presence of a positive reaction to histamine. The allergens included common allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, *Felis domesticus*, *Canis familiaris*, *Blattella germanica*, *Periplaneta americana*, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and mixed grass) and extracts from laboratory animals (rat, rabbit, mouse, hamster, and guinea pig). These were called occupational allergens. Individuals were allocated in groups according to the sensitization status.

Questionnaire

A self-administered questionnaire with 97 questions was used to inquire about respiratory, nasal, ocular, and skin symptoms and personal history of allergic diseases, smoking, and pet owning. The questionnaire items also included job characteristics, such as the duration of working with laboratory animals, job titles, job contents, frequency of contact with laboratory animals, time spent handling animals, species, use of protective equipment, and knowledge about animal-induced allergies, asthma, or rhinitis. To assess symptoms, we used questions from the European Community Respiratory Health Survey questionnaire, which has been translated into Portuguese, adapted to the Brazilian lexicon, and validated [10].

Spirometry and bronchial challenge test with mannitol

Spirometry was performed using a Koko spirometer and its software (PDS Instrumentation, Inc., Louisville, Colorado, USA) following international recommendation [11].

Dry powdered mannitol (Aridol) was supplied in kit form (Pharmaxis Ltd., New South Wales, Australia), which contained 1 empty capsule (0 mg), capsules containing 5, 10 and 20 mg, and 15 capsules containing 40 mg (cumulative dose of 635 mg). The challenge began with the empty capsule, followed by inhalation of 5, 10, 20, 40, 80, 160, 160, and 160

mg dry powdered mannitol. Forced expiratory volume in one second (FEV1) was measured 60 seconds after every inhalation. This procedure was repeated for each dose step until a 15% fall in FEV1 was achieved or the cumulative dose reached 635 mg. A 15% decrease in FEV1 was regarded as a positive response indicating bronchial hyperresponsiveness (BHR) [12].

Dust samples and endotoxin analysis

Samples of floor dust were collected in workplaces to evaluate endotoxin levels. For details, refer to Freitas et al. [13]. Briefly, dust was collected with the aid of a vacuum cleaner equipped with a filter in the tip. Measurement of endotoxin concentration was performed by limulus amebocyte lysate assay.

Variables

Groups: Three groups were formed in accordance with the allergic sensitization. Non-sensitized group: composed by individuals with negative SPT. Common sensitization group: composed by subjects sensitized to common allergens. Occupational sensitization group: composed by subjects sensitized to occupational allergens. Individuals who were sensitized to these two types of allergens (common and occupational) were allocated to the latter.

Confirmed asthma: An individual was considered an asthmatic subject if he/she had BHR and had experienced symptoms of wheezing, tightness of the chest during the night or dyspnea during the day or at night in the previous 12 months.

Wheezing: Current wheezing was defined by a positive answer to the question: *Have you had wheezing or whistling in your chest any time in the last 12 months?*

Rhinitis: An individual was considered to have rhinitis if he/she answered yes for any of the two following questions: 1 - *Do you have rhinitis (nose allergy)?* 2 - *Have you had a problem with a runny or blocked nose when you did not have a cold or the flu in the last 12 months?*

Daily work hours: This variable was defined by the question: *What are your daily work hours?* Results were categorized into two categories: ≥ 8 hours and < 8 hours.

Exposure years: This variable was the duration of exposure calculated from the date of beginning activities at the job. Results were categorized in the following way: Category 1: up to 1.0 year of exposure; Category 2: between 1.1 and 3.0 years of exposure; Category 3: between 3.1 and 5.0 years of exposure; Category 4: 5.1 or more years of exposure.

Pet ownership: This variable was defined by the question: *In the last 12 months, have you had pets at home?*

Smoking: This variable was defined by question: *Have you ever smoked for more than 1 year?*

Skin symptoms: This variable was defined by the

question: *Have you had redness and irritation of the skin that was coming and going in the last 12 months?* **Nocturnal dyspnea:** This variable was defined by the question: *Have you been woken by an attack of shortness of breath during the night in the last 12 months?* **Endotoxin concentration:** Results were categorized in the following way: High endotoxin concentrations included values above the median (20.4 EU/mg) and low endotoxin concentrations included values lower or equal to 20.4 EU/mg.

Statistical analysis

Univariate analysis (chi-square test) was performed to compare prevalence between groups (non-sensitized, common sensitization and occupational sensitization). ANOVA was used to compare age among the three groups. Prevalence ratio was estimated by using a modified Poisson regression approach, i.e., Poisson regression with a robust error variance [14]. These analyses were performed to test associations between occupational sensitization and outcomes. All models were adjusted for sex, smoking, exposure years, age, daily work hours and pet ownership. Wheezing model was also adjusted for the endotoxin concentration. Analyses were performed using SAS 9.0 software.

Results

A total of 455 volunteers exposed to laboratory animals were selected. Two subjects were excluded because they did not perform the SPT, thus data from 453 individuals were analyzed. The non-sensitized group comprised 237 volunteers, the common sensitization group comprised 142 volunteers, and the occupational sensitization group comprised 74 volunteers. Table 1 shows characteristics of the subjects. Univariate analysis showed that the occupational sensitization group had higher prevalence of all outcomes compared to the other two groups (Table 2).

The overlap of confirmed asthma, rhinitis and skin symptoms was analyzed. Occupational sensitization was associated with a higher prevalence of workers with overlap of the 3 outcomes when compared to the other two groups ($p < 0.01$) (Figure 1).

Multivariate analysis showed that volunteers with occupational sensitization had higher risk of confirmed asthma as compared to individuals with common sensitization (prevalence ratio (PR): 2.65; 95% confidence interval (CI): 1.45-4.85) and non-sensitized individuals. In addition, workers exposed to animals from 3.1 to 5.0 years had increased risk of confirmed asthma compared to individuals exposed up to 1.0 year (Table 3). Occupational sensitization was associated with higher risk of wheezing in comparison with common sensitization (PR: 1.75; 95% CI: 1.21-2.53) and non-sensitization. High concentration of endotoxin in the workplace was also a risk factor for wheezing (PR: 1.56; 95% CI: 1.09-2.22) (Table 4).

The occupational sensitization group had a higher prevalence of rhinitis than the common sensitization group (PR: 1.25; 95% CI: 1.11-1.40) and the non-sensitized group. Moreover, female sex was a risk factor for rhinitis and increasing age was a protective factor (Table 5). Table 6 presents the risk factors for skin symptoms. Similar to previous outcomes, individuals from the occupational sensitization group had higher risk of skin symptoms than individuals from common sensitization group (PR: 1.36; 95% CI: 1.01-1.85) and non-sensitized group (Table 6). Occupational sensitization was also associated with increased risk of BHR (Table S1) and nocturnal dyspnea (Table S2) compared to common sensitization and non-sensitization.

We compared severity of skin prick test reactions between occupational and common sensitization groups. Among the 11 common allergens tested, the common sensitization group reacted to 2.96 ± 1.29 allergens (mean $\pm$ s.d.) and the occupational sensitization group reacted to 2.89 ± 2.09 ($p = 0.505$); in addition, mean wheal sizes of positive reactions were not different between groups, respectively, 3.92 ± 0.80 mm and 4.02 ± 0.90 mm ($p = 0.373$).

Discussion

In the present study, we investigated the association between occupational allergic sensitization and skin symptoms (redness or irritation), chest symptoms (wheezing and nocturnal dyspnea), rhinitis, BHR and confirmed asthma. All subjects were exposed to laboratory animals. If they had a positive SPT to rat, mouse, rabbit, guinea pig or hamster, they were considered as having occupational sensitization. Occupational sensitization was associated with increased risk for all evaluated outcomes. These associations were found

when the occupational sensitization group was compared to either one of the other two groups, the non-sensitized group or the common sensitization group. that comprised subjects sensitized to common allergens.

In addition, exposure duration between 3 and 5 years was associated with a higher risk of confirmed asthma as compared to exposure for less than one year. Regarding number of allergic manifestations, workers with occupational sensitization had greater overlap of skin symptoms, rhinitis and asthma. Finally, the exposure to a high concentration of endotoxin (> 20.4 EU/mg) was associated with increased prevalence of wheezing.

One could argue that differences and worse outcomes related to occupational sensitization were due to sensitization severity differences. The number of positive allergens in every group was compared as well as the reaction wheal sizes. No difference was found. Therefore, it seems that occupational sensitization leads to more severe clinical outcomes independently of more severe skin prick test findings.

Renstrom et al. [15] performed a follow-up (median of 18 months) study on LAA subjects (defined as specific IgE and/or symptom related with laboratory animal work). Nine subjects were included in the LAA group and had significantly more responsive airways than non-LAA subjects (n=29). All LAA subjects had lower provocative dose causing 20% FEV1 fall (PD20) values and a steeper slope at follow-up than at baseline. Sensitization to laboratory animals was associated with significant increase in bronchial responsiveness in most exposed workers. The magnitude of the PD20 increase and the final level of bronchial responsiveness were associated with sensitization. However, sensitization to common allergens was not separately considered. Probably, their small sample size would not allow comparisons similar to the comparisons we have done.

Palmberg et al. [16] in a follow-up study showed that in subjects who became sensitized to laboratory animals (n=13), eyes and nose symptoms increased during 12 months of exposure, whereas symptoms did not change over time in the group non-sensitized to laboratory animals (n=57), indicating a causal relationship between occupational sensitization and the development of clinical symptoms of allergic rhinoconjunctivitis. Although the authors showed that bronchial responsiveness to methacholine increased significantly during the study in previously atopic subjects, they failed to detect an association between sensitization to laboratory animals and BHR.

Among workers with less than 4 years of exposure and sensitized to animals to which they were exposed, there were excess declines in forced vital capacity (-148 ml/year), FEV₁ (-83 ml/year) and maximum mid-expiratory flow (-7 ml/s/year) compared to exposed workers with no occupational sensitization [17]. Workers sensitized to mice were nearly 4 times more likely to report chest symptoms when compared to non-sensitized workers [18]; furthermore, work-related chest symptoms (defined as those occurring at work and/or improving when away from work) were observed in 42% of workers sensitized to rat and reported by only 3% of non-sensitized workers [19].

Our data showed that workers with occupational sensitization had greater overlap of allergic diseases. When prevalence studies are performed in groups of persons exposed to laboratory animals, some patterns of overlap are found. These patterns result from the sequence of development, from the risk of allergic manifestation and from the selection of workers that endure working conditions despite their illness [2]. Previous reports on allergic symptoms overlap showed prevalence of 11.6 to 18.3% in laboratory animal workers [1,2]. Elliot et al. [20] observed that workers presenting LAA (defined as self-reported symptoms from working with laboratory animals or their cages) had overlap prevalence of 7.4% at baseline. Overlap was the presence of nasal/eye symptoms, skin symptoms and asthma. In the follow-up analysis, overlap prevalence increased to 23.1%. The authors concluded that in workers who continue to be exposed to animals, allergy symptoms are a major risk to develop asthma.

Interestingly, in our study workers exposed for more than five years showed no increased risk of asthma. This leads us to believe that we may be facing an event called "healthy worker effect", which refers to the bias caused by the phenomenon that sicker or more sensitive individuals may choose workplaces in which exposures are low. They may be excluded from being hired; or once hired, they may seek transfer to less exposed jobs or they may leave work [21]. Similar scenario was reported by Portengen et al. [17] in apprentices who presented more symptoms than "experienced" workers. Moreover, in these "experienced" workers there was no association between exposure to laboratory animals and lung function decline, which was explained by healthy worker effect.

The recommendation for workers with occupational asthma is the cessation of exposure to the sensitizing agent [22,23], whereas the reduction in the exposure seems to be less beneficial than the complete cessation [24]. The use of respiratory protective equipment leads to a significant reduction in respiratory symptoms, but fails to promote complete

protection to workers [24]. In addition, levels of both airborne endotoxin and allergens can be reduced about 50-fold in rooms with filters tops on cages when compared with rooms with open cages [25]. Finally, even though reduction of exposure to occupational allergens is an important issue, identifying a safe exposure threshold is difficult, since several authors have failed to find a cutoff value associated with increased risk of sensitization to laboratory animals or any symptoms [26–28]. For all these features, we believe that the recommendation for the complete cessation of exposure should be taken into consideration for the group of workers with occupational sensitization.

Given the risks presented in this study, alternatives to reduce the incidence of occupational sensitization among atopic workers are very important. Krop et al. [29], based on a multivariate algorithm, suggested that pre-employment counseling among atopic candidates with total IgE higher than 100 IU/ml could reduce occupational sensitization by up to 50%.

Some studies have identified asthmatic workers by self-report questionnaires of symptoms [30,31]. An advantage of the present study was the identification of BHR by bronchial challenge test with mannitol, an indirect stimulation agent [32]. The diagnosis of asthma based on positive BHR and symptoms has been seen as a gold standard definition of asthma [33,34]. For instance, we found 42 asthmatic subjects. Among those, 13 (30.9%) reported no asthma, although they had BHR and symptoms.

Another methodological issue is the role of endotoxin. Endotoxin is a constituent of the cell wall of gram-negative bacteria [35], and has been considered in previous studies [13,36]. Using data from the same dataset, our group has previously evaluated the exposure to endotoxin in the workplace and observed that exposure to high concentrations in the dust was associated with wheezing in the last 12 months [13]. Thus, the analysis model for wheezing was adjusted to endotoxin (high and low concentrations).

One limitation of our study is the cross-sectional design instead of the follow-up one. Further, IgE measurement would have provided additional information for better understanding of occupational sensitization and the development of symptoms and diseases. Nevertheless, to the best of our knowledge, our study has the greatest sample among the ones focussing on the same issues, which strengthens our findings.

In conclusion, these data showed that occupational sensitization in workers exposed to laboratory animals is associated with skin symptoms, nocturnal dyspnea, wheezing, rhinitis, BHR and confirmed asthma. Although occupational sensitization raises the risk for symptoms and diseases, we speculate whether it should be a criterion to advise workers to leave the job. We believe that ideally workers should undergo SPT with occupational allergens periodically. If there was occupational sensitization, at least the worker should be warned at an early stage of allergic sensitization to laboratory animals and he/she should receive clear information about the risks and undergo medical follow-up to detect the emergence of any symptom and disease related to exposure to laboratory animals. Ideally, workers should prevent allergic disease with workplace air protection.

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Conflict of interest:

The authors declare that they have no conflict of interests.

Abbreviations:

LAA: laboratory animal allergy; SPT: skin prick test; BHR: bronchial hyperresponsiveness; PR: prevalence ratio; CI: confidence interval.

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Legend for Figure 1: Overlap of asthma, rhinitis and skin symptoms among workers of the non-sensitized group, common sensitization group and occupational sensitization group.

Table 1: Characteristics of subjects.

	Non-sensitized group (n=237)	Common sensitization group (n=142)	Occupational sensitization group (n=74)	p value
Age	33.0±10.6	29.6±8.7	33.0±10.1	<0.01*
Sex				
Female	128 (54.0%)	77 (54.2%)	39 (52.7%)	0.97
Male	109 (46.0%)	65 (45.8%)	35 (47.3%)	
Smoking	40 (16.8%)	23 (16.1%)	17 (22.9%)	0.41
Pet ownership	148 (62.4%)	89 (62.6%)	47 (63.5%)	0.98
Daily work hours				
< 8 hours	45 (19.0%)	34 (24.1%)	8 (10.8%)	0.06
≥ 8 hours	192 (81.0%)	107 (75.9%)	66 (89.2%)	
Exposure years				
≤ 1.0	29 (12.3%)	31 (21.8%)	8 (10.8%)	0.02**
from 1.1 to 3.0	54 (22.9%)	32 (22.5%)	7 (9.5%)	0.03**
from 3.1 to 5.0	49 (20.8%)	16 (11.3%)	12 (16.2%)	0.05**
≥ 5.1	104 (44.0%)	63 (44.4%)	47 (63.5%)	<0.01**
Endotoxin concentration				
Low	83 (37.9%)	54 (43.5%)	32 (47.1%)	0.32
High	136 (62.1%)	70 (56.5%)	36 (52.9%)	

*ANOVA

**Chi-square test

Endotoxin concentration: Low concentration: ≤ 20.4 EU/mg of dust, High concentration: > 20.4 EU/mg of dust.

Missing information for one subject in variable daily working hours in the common sensitization group

Missing information for one subject in variable exposure years in the non-sensitized group

Data for endotoxin concentration were available for 411 subjects.

Table 2: Prevalence of the outcomes studied

	Non-sensitized group (n=237)	Common sensitization group (n=142)	Occupational sensitization group (n=74)	p value
Confirmed asthma	8 (3.3%)	14 (9.8%)	20 (27.0%)	<0.01 [*]
BHR	10 (4.2%)	21 (14.7%)	26 (34.2%)	<0.01 [*]
Wheezing	34 (14.3%)	34 (23.9%)	34 (45.9%)	<0.01 [*]
Rhinitis	133 (56.1%)	107 (75.3%)	67 (90.5%)	<0.01 [*]
Nocturnal dyspnea	21 (8.8%)	15 (10.5%)	18 (24.3%)	<0.01 [*]
Skin symptoms	68 (28.6%)	49 (34.5%)	38 (51.3%)	<0.01 [*]

^{*}Chi-square test

Table 3: Assessment of risk factors for confirmed asthma

Variables	Categories (n)	Crude model				Adjusted model			
		PR	95% CI		p value	PR	95% CI		p value
Group	Occupational (n=73) vs non-sensitized (n=234)	7.98	3.67	17.35	<0.01	7.68	3.52	16.75	<0.01
Group	Occupational (n=73) vs common (n=140)	2.72	1.46	5.06	<0.01	2.65	1.45	4.85	<0.01
Smoking	Yes (n=77) vs no (n=370)	1.30	0.65	2.61	0.45	1.48	0.74	2.96	0.27
Sex	Female (n=240) vs male (n=207)	1.27	0.70	2.28	0.43	1.28	0.72	2.27	0.40
Exposure years	From 1.1 to 3.0 (n=91) vs ≤ 1.0 (n=68)	1.10	0.32	3.76	0.87	1.46	0.42	5.10	0.55
Exposure years	From 3.1 to 5.0 (n=77) vs ≤ 1.0 (n=68)	2.61	0.88	7.71	0.08	3.29	1.06	10.14	0.04
Exposure years	≥ 5.1 (n=210) vs ≤ 1.0 (n=68)	1.60	0.57	4.50	0.38	2.16	0.65	7.19	0.21
Age	Continuous	0.97	0.94	1.00	0.09	0.96	0.92	1.01	0.12
Daily work hours	≥ 8h (n=359) vs < 8h (n=87)	0.89	0.44	1.79	0.75	0.76	0.35	1.65	0.48
Pet ownership	Yes (n=278) vs no (n=169)	0.73	0.41	1.31	0.29	0.74	0.42	1.32	0.31

PR: prevalence ratio; CI: confidence interval; Bronchial hyperresponsiveness data from six subjects were missing

Table 4: Assessment of risk factors for wheezing

Variables		Categories (n)		Crude model			Adjusted model			
			PR	95% CI		p value	PR	95% CI		p value
Group	Occupational (n=71) vs non-sensitized (n=225)	3.50	2.33	5.27	<0.01	3.69	2.44	5.57	<0.01	
Group	Occupational (n=71) vs common (n=127)	1.73	1.19	2.52	<0.01	1.75	1.21	2.53	<0.01	
Smoking	Yes (n=74) vs no (n=349)	0.89	0.55	1.45	0.65	0.91	0.59	1.42	0.69	
Sex	Female (n=228) vs male (n=195)	1.30	0.91	1.86	0.15	1.21	0.86	1.70	0.28	
Exposure years	From 1.1 to 3.0 (n=81) vs ≤ 1.0 (n=61)	1.19	0.64	2.20	0.58	1.33	0.73	2.43	0.35	
Exposure years	From 3.1 to 5.0 (n=73) vs ≤ 1.0 (n=61)	1.32	0.71	2.43	0.38	1.50	0.82	2.73	0.18	
Exposure years	≥ 5.1 (n=207) vs ≤ 1.0 (n=61)	1.02	0.59	1.75	0.95	1.12	0.61	2.08	0.71	
Age	Continuous	0.98	0.97	1.00	0.09	0.99	0.97	1.02	0.45	
Daily work hours	≥ 8h (n=344) vs < 8h (n=78)	0.85	0.56	1.31	0.47	0.94	0.59	1.49	0.79	
Pet ownership	Yes (n=266) vs no (n=157)	1.30	0.89	1.90	0.17	1.29	0.89	1.88	0.18	
Endotoxin concentration	High (n=288) vs low (n=115)	1.47	1.00	2.15	0.04	1.56	1.09	2.22	0.01	

PR: prevalence ratio; CI: confidence interval; Low endotoxin concentration: ≤ 20.4 EU/mg of dust; High endotoxin concentration: > 20.4 EU/mg of dust. Wheezing data from thirty subjects were missing.

Table 5: Assessment of risk factors for rhinitis

Variables	Categories (n)	Crude model				Adjusted model			
		PR	95% CI		p value	PR	95% CI		p value
Group	Occupational (n=73) vs non-sensitized (n=234)	1.61	1.41	1.83	<0.01	1.58	1.38	1.81	<0.01
Group	Occupational (n=73) vs common (n=141)	1.21	1.08	1.36	<0.01	1.25	1.11	1.40	<0.01
Smoking	Yes (n=80) vs no (n=368)	1.07	0.92	1.25	0.38	1.17	1.01	1.35	0.04
Sex	Female (n=242) vs male (n=206)	1.27	1.11	1.44	<0.01	1.23	1.09	1.40	<0.01
Exposure years	From 1.1 to 3.0 (n=92) vs ≤ 1.0 (n=68)	0.93	0.77	1.13	0.48	0.99	0.82	1.20	0.93
Exposure years	From 3.1 to 5.0 (n=75) vs ≤ 1.0 (n=68)	0.88	0.71	1.09	0.23	0.94	0.77	1.15	0.55
Exposure years	≥ 5.1 (n=212) vs ≤ 1.0 (n=68)	0.90	0.76	1.07	0.24	1.04	0.86	1.26	0.69
Age	Continuous	0.99	0.98	0.99	<0.01	0.99	0.98	0.99	<0.01
Daily work hours	≥ 8 h (n=362) vs < 8 h (n=85)	0.95	0.81	1.10	0.47	1.03	0.88	1.21	0.70
Pet ownership	Yes (n=282) vs no (n=166)	1.12	0.98	1.29	0.09	1.12	0.98	1.28	0.09

PR: prevalence ratio; CI: confidence interval; Rhinitis data from five subjects were missing.

Table 6: Assessment of risk factors for skin symptoms

Variables	Categories (n)	Crude model				Adjusted model			
		PR	95% CI		p value	PR	95% CI		p value
Group	Occupational (n=72) vs non-sensitized (n=229)	1.77	1.32	2.38	<0.01	1.64	1.22	2.20	<0.01
Group	Occupational (n=72) vs common (n=131)	1.43	1.04	1.96	0.03	1.36	1.01	1.85	0.04
Smoking	Yes (n=75) vs no (n=357)	1.15	0.84	1.56	0.39	1.14	0.83	1.56	0.42
Sex	Female (n=232) vs male (n=200)	1.31	1.01	1.71	0.04	1.30	0.99	1.69	0.05
Exposure years	From 1.1 to 3.0 (n=88) vs ≤ 1.0 (n=64)	0.89	0.55	1.46	0.66	0.94	0.58	1.54	0.81
Exposure years	From 3.1 to 5.0 (n=75) vs ≤ 1.0 (n=64)	1.09	0.68	1.76	0.72	1.17	0.72	1.90	0.53
Exposure years	≥ 5.1 (n=204) vs ≤ 1.0 (n=64)	1.28	0.86	1.91	0.22	1.44	0.92	2.26	0.11
Age	Continuous	1.00	0.99	1.01	0.81	0.99	0.97	1.00	0.17
Daily work hours	≥ 8h (n=348) vs < 8h (n=83)	1.08	0.77	1.50	0.66	1.01	0.70	1.46	0.97
Pet ownership	Yes (n=269) vs no (n=163)	1.19	0.90	1.55	0.22	1.18	0.90	1.55	0.23

PR: prevalence ratio; CI: confidence interval; Skin symptoms data from twenty-one subjects were missing.

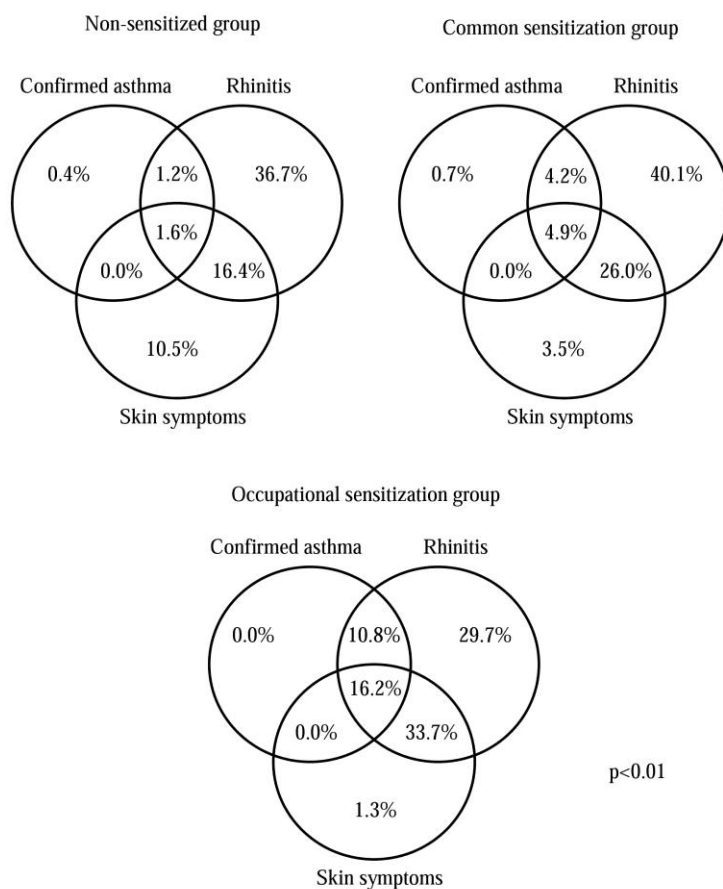


Table S1: Assessment of risk factors for bronchial hyperresponsiveness

Variables		Categories (n)		Crude model			Adjusted model		
		PR	95% CI		p value	PR	95% CI		p value
Group	Occupational (n=73) vs non-sensitized (n=234)	8.30	4.20	16.39	<0.01	8.25	4.10	16.62	<0.01
Group	Occupational (n=73) vs common (n=140)	2.36	1.43	3.89	<0.01	2.47	1.50	4.09	<0.01
Smoking	Yes (n=77) vs no (n=370)	1.02	0.54	1.92	0.96	1.15	0.62	2.15	0.66
Sex	Female (n=240) vs male (n=207)	1.19	0.72	1.94	0.50	1.19	0.74	1.91	0.47
Exposure years	From 1.1 to 3.0 (n=91) vs ≤ 1.0 (n=68)	0.65	0.27	1.61	0.36	0.90	0.38	2.11	0.80
Exposure years	From 3.1 to 5.0 (n=77) vs ≤ 1.0 (n=68)	1.35	0.63	2.93	0.44	1.79	0.85	3.77	0.13
Exposure years	≥ 5.1 (n=210) vs ≤ 1.0 (n=68)	0.92	0.45	1.87	0.82	1.31	0.60	2.89	0.50
Age	Continuous	0.97	0.94	0.99	0.02	0.96	0.93	1.00	0.08
Daily work hours	≥ 8h (n=359) vs < 8h (n=87)	0.75	0.43	1.30	0.30	0.70	0.38	1.29	0.25
Pet ownership	Yes (n=278) vs no (n=169)	0.72	0.45	1.17	0.19	0.72	0.45	1.16	0.17

PR: prevalence ratio; CI: confidence interval; Bronchial hyperresponsiveness data from six subjects were missing.

Table S2: Assessment of risk factors for nocturnal dyspnea

Variables		Categories (n)		Crude model			Adjusted model		
		PR	95% CI		p value	PR	95% CI		p value
Group	Occupational (n=73) vs non-sensitized (n=234)	2.74	1.54	4.85	<0.01	2.60	1.43	4.71	<0.01
Group	Occupational (n=73) vs common (n=141)	2.30	1.23	4.30	<0.01	2.40	1.31	4.40	<0.01
Smoking	Yes (n=79) vs no (n=369)	1.06	0.56	2.01	0.87	1.12	0.55	2.27	0.76
Sex	Female (n=242) vs male (n=206)	2.21	1.26	3.89	<0.01	1.96	1.10	3.48	0.02
Exposure years	From 1.1 to 3.0 (n=92) vs ≤ 1.0 (n=68)	1.14	0.47	2.80	0.77	1.27	0.49	3.26	0.62
Exposure years	From 3.1 to 5.0 (n=76) vs ≤ 1.0 (n=68)	0.88	0.33	2.38	0.80	1.03	0.37	2.85	0.96
Exposure years	≥ 5.1 (n=211) vs ≤ 1.0 (n=68)	1.32	0.60	2.86	0.49	1.95	0.76	5.03	0.17
Age	Continuous	0.98	0.96	1.01	0.26	0.97	0.93	1.00	0.12
Daily work hours	≥ 8h (n=360) vs < 8h (n=87)	0.85	0.47	1.54	0.59	0.86	0.42	1.75	0.68
Pet ownership	Yes (n=282) vs no (n=166)	2.06	1.11	3.79	0.02	2.09	1.16	3.77	0.01

PR: prevalence ratio; CI: confidence interval; Nocturnal dyspnea data from five subjects were missing.