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ARE HIGH- AND LOW-MOLECULAR-WEIGHT SENSITIZING AGENTS ASSOCIATED WITH DIFFERENT CLINICAL PHENOTYPES OF OCCUPATIONAL ASTHMA?

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Running head:

Occupational asthma due to high- and low-molecular-weight agents

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There is no conflict to declare in relation to this article. The sponsors had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.

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All E-PHOCAS investigators contributed equally to the conception and design of the study and collection of data. OV, JG, NM, LH, and JdB contributed to data collection, analysis, and interpretation, as well as writing of the manuscript. All investigators critically reviewed and approved the final manuscript. OV is the guarantor of the final content of the manuscript.

Keywords: Asthma; bronchial provocation tests; occupational diseases; phenotype

List of abbreviations:

FeNO:	Fractional exhaled nitric oxide
FEV_1 :	Forced expiratory volume in one second
FVC:	Forced vital capacity
GINA:	Global initiative for asthma
ICS:	Inhaled corticosteroids
IQR:	Interquartile range
BHR:	Nonspecific bronchial hyperresponsiveness
OA:	Occupational asthma
$PC/PD_{15\text{-}20\%}$:	Provocative concentration/dose of pharmacological agent causing a 15% or 20% fall in FEV_1
Ppb:	Parts per billion
SABA:	Short-acting $beta_2$-agonist
SIC:	Specific inhalation challenge

ABSTRACT

Background: High-molecular-weight (HMW) proteins and low-molecular-weight (LMW) chemicals can cause occupational asthma (OA) although few studies have thoroughly compared the clinical, physiological, and inflammatory patterns associated with these different types of agents. The aim of this study was to determine whether OA induced by HMW and LMW agents show distinct phenotypic profiles.

Methods: Clinical and functional characteristics, and markers of airway inflammation were analyzed in an international, multicenter, retrospective cohort of subjects with OA ascertained by a positive inhalation challenge response to HMW (n=544) and LMW (n=635) agents.

Results: Multivariate logistic regression analysis showed significant associations between OA caused by HMW agents and work-related rhinitis (OR [95% CI]: 4.79 [3.28-7.12]), conjunctivitis (2.13 [1.52-2.98]), atopy (1.49 [1.09-2.05]), and early asthmatic reactions (2.86 [1.98-4.16]). By contrast, OA due to LMW agents was associated with chest tightness at work (2.22 [1.59-3.03]), daily sputum (1.69 [1.19-2.38]), and late asthmatic reactions (1.52 [1.09-2.08]). Furthermore, OA caused by HMW agents showed a higher risk of airflow limitation (1.76 [1.07-2.91]) whereas OA due to LMW agents exhibited a higher risk of severe exacerbations (1.32 [1.01-1.69]). There were no differences between the two types of agents in the baseline sputum inflammatory profiles, but OA caused by HMW agents showed higher baseline blood eosinophilia and a greater post-challenge increase in fractional nitric oxide.

Conclusion: This large cohort study describes distinct phenotypic profiles in OA caused by HMW and LMW agents, There is a need to further explore differences in underlying pathophysiological pathways and outcome after environmental interventions.

INTRODUCTION

A wide variety of workplace agents can cause the development of sensitizer-induced occupational asthma (OA) which is characterized by an asymptomatic period of exposure before the inception of work-related asthma symptoms (i.e. the "latency period" necessary to acquire immunologic sensitization) and the clinical features of a specific hypersensitivity response to a workplace agent (1-4). These agents are usually categorized into high-molecular-weight (HMW) (glyco)proteins from vegetal and animal origins and low-molecular-weight (LMW) chemicals (<1 kDa) (5). HMW proteins

and a few LMW compounds (e.g., platinum salts, reactive dyes, acid anhydrides, sulfonechloramide, and some wood species) act through a documented IgE-mediated mechanism while for most LMW, the immunological mechanisms leading to airway sensitization remain poorly elucidated (5).

There is evidence form earlier studies that there are differences between OA caused by HMW and LMW agents in terms of clinical characteristics (6-9). A few studies investigated OA due to these two types of agents in terms of the baseline sputum inflammatory profile (10-12) or the pattern of bronchial response (13, 14) and the changes in nonspecific bronchial hyperresponsiveness (BHR) (12, 13) and markers of airway inflammation (12, 15) induced by challenge exposures. However, the clinical and inflammatory patterns associated with these two categories of agents causing OA have not yet been comprehensively described.

The aim of this study was to compare a broad range of clinical, functional, and inflammatory characteristics in a large cohort of subjects with a diagnosis of OA induced by HMW and LMW agents ascertained by a specific inhalation challenge (SIC) in order to determine whether these two categories of sensitizing agents are associated with distinct phenotypic profiles.

METHODS

Study design

This study was conducted among an international, multicenter retrospective cohort of subjects with OA assembled by the *European network on Phenotyping of Occupational Asthma* (E-PHOCAS). This cohort included all consecutive subjects who showed a positive SIC result between January 2006 and December 2015 in 20 specialized centers from 11 European countries.

Ethics

Each participating center obtained approval from its local Institutional Review Board for this analysis of retrospective anonymous data. The central database was held at the Strasbourg University and approved by the "*Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé*" (Advisory Committee on Information Processing for Research in the Field of Health) and the "*Commission Nationale de l'Informatique et des Libertés*" (National Commission for Data Protection and Liberties).

Collected data

Demographic and clinical characteristics

The participating investigators were asked to enter in the database the information collected at the time of the diagnostic investigation of subjects with a positive SIC. Data pertaining to respiratory symptoms were not collected through a standardized questionnaire. The requested information included: 1) causal agent and job; 2) demographic characteristics; 3) clinical features (smoking habits, atopic status [defined by at least one positive skin-prick test to a battery of common aeroallergens]); 4) nature and timing of work-related respiratory symptoms and exposure; 5) associated disorders (physician-based diagnosis of work-related rhinitis, conjunctivitis; contact urticaria, and/or dermatitis, and sinusitis); and 6) detailed asthma medications used during the last month of exposure at work.

The severity of asthma during the last month of work exposure was graded *a posteriori* according to the Global Initiative for Asthma (GINA) 2015 update treatment steps (*www.ginasthma.com*; last accessed December 28, 2017). Based on these treatment steps, the level of asthma severity was

categorized as “untreated” (step 0); “mild” (step 1-2); “moderate” (step 3); and “severe” (step 4-5). The level of asthma control could only be characterized by the frequency of short-acting beta$_2$-agonist (SABA) use since most centers failed to use validated instruments for the assessment of asthma control (e.g. Asthma Control Test, Asthma Control Questionnaire, or GINA symptom control tool). For the purpose of this study “poor symptom control” was defined by the need for a SABA at least once a day as a surrogate marker of asthma control. The number of severe exacerbations requiring oral corticosteroids for at least 3 consecutive days or emergency room visit or hospitalization over the last 12 months at work was also recorded (16, 17). In addition, we used a definition of severe asthma adapted from the European Respiratory Society/American Thoracic Society recommendations (18) that require a high intensity treatment (GINA treatment steps 4-5) and any one of the following four criteria indicating uncontrolled asthma: 1) poor symptom control; 2) two or more severe exacerbations in the previous year; 3) serious exacerbations, defined as at least one hospitalization, intensive care unit stay or mechanical ventilation in the previous year; or 4) airflow limitation (as defined below). For the purpose of this study, “poor symptom control” was defined by the use of a SABA once or more a day as recommended the in the American Thoracic Society recommendations issued in 2000 (19).

Lung function parameters

Baseline spirometric values included forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV_1). Baseline airflow obstruction was defined by a FEV_1 <80% predicted value and a FEV_1/FVC ratio <70% since the lower limit of normal values FEV_1/FVC ratio were not available in most centers. The levels of BHR at baseline and 24 h after challenge exposure were recorded and expressed as the concentration or dose of the pharmacological agent inducing a 15% or 20 % fall in FEV_1 ($PC/PD_{15\text{-}20\%}$) according to the bronchoprovocation method used in each center (see Online Supplementary material). Detailed Information on the methodology and interpretation of SICs is available in the Online Supplementary Material.

Markers of airway inflammation

The data pertaining to markers of airway inflammation included whenever available: 1) blood eosinophils (within one month of the SIC procedure); 2) fractional exhaled nitric oxide concentration (FeNO) at baseline and 24 hours after the SIC challenge; and 3) sputum eosinophils and neutrophils expressed as a percentage of total cell count at baseline and 24 hours post-challenge. Information on induced sputum and FeNO assessments is available in the Online Supplementary Material. The sputum inflammatory pattern was characterized as "eosinophilic" (i.e. ≥3% eosinophils and <76% neutrophils); "neutrophilic" (i.e. ≥76% and <3% eosinophils); "paucigranulocytic" (i.e. <3% eosinophils and <76% neutrophils); or "mixed granulocytic" (i.e. ≥76% neutrophils and ≥3% eosinophils) (23). A post-challenge increase in sputum eosinophil count ≥3% (24, 25) or in FeNO level ≥17.5 ppb (15) as compared to baseline value was considered significant. A ≥17.5 ppb increase in post-challenge FeNO has been shown to provide a specificity of 90% and a sensitivity of 45% for a positive SIC (15).

Statistical analysis

Quantitative data are presented as median and interquartile range (IQR). Comparison between groups of subjects was made using the Chi-squared test for categorical variables and the Wilcoxon rank-sum test for numerical variables. Multivariate logistic regression analysis was carried out using a binomial generalized linear model to identify the clinical and physiological characteristics that were associated with OA caused by HMW agents as compared to LMW agents. The independent variables incorporated into these regressions were selected based on p-value ≤0.1 in univariate comparisons after elimination of variables with >10% missing values (Online Supplementary Material). Additional multivariate logistic regressions were performed in order to identify the variables associated with a baseline blood eosinophil count >300/µl and a post-challenge increase in FeNO >17.5 ppb (Online

Supplementary Material) in subgroups of subjects for whom the data were available (blood eosinophils, n=516; pre- and post-challenge FeNO, n=356). Statistical analysis was performed using the R software version 3.4.1 (*www.r-project.org*). A p-value <0.05 was considered significant.

RESULTS

Population

The cohort included 1,180 patients with OA ascertained by a positive SIC result. The agents that induced a positive SIC response are presented in Table 1. These agents were LMW compounds in 635 (53.8%) and HMW agents in 544 (46.1%) of the SICs, while the causal agent was not precisely identified in 13 subjects.

Clinical characteristics

When compared to subjects with OA caused by LMW agents, those who demonstrated a positive response to a HMW agent were slightly younger, more often never smokers, and more frequently atopic (Table 2). They reported more often work-related rhinitis, conjunctivitis and wheezing at work, but less often chest tightness and sputum production.

The median duration of exposure to the causal agent before the onset of work-related asthma symptoms (i.e. the "latency period") and the duration of asthma symptoms while exposed at work was slightly longer in subject with OA caused by HMW agents compared to those with OA due to LMW agents. The median (IQR) daily dose of inhaled corticosteroid (ICS) was slightly higher in subjects with OA caused by HMW agents (500 µg [0-1000]) than in those with LMW agents-induced

OA (400 µg [0-1000]; p=0.040), which seemed to be mainly related to a higher rate of subjects who were not treated with ICS in the latter group while more subject received a low dose of ICS in the former group. Subjects with OA caused by a LMW agent reported a higher rate (26%) of severe asthma exacerbation while exposed at work as compared to subjects exposed to HMW agents (19%; p=0.008).

Lung function parameters

Baseline spirometry (Table 3) showed a slightly higher proportion of subjects with a FEV_1 <80% of predicted value and airflow obstruction in subjects with OA due to HMW agents (23% and 13%, respectively) as compared to OA related to LMW agents (18% (p=0.020) and 10% (p=0.080), respectively. There was no significant difference between the two groups with regard to the baseline level of BHR and the magnitude of the increase in BHR assessed 24 hours after the challenge exposure. However, BHR was slightly more often absent at baseline assessment in subjects with LMW agent-induced OA (31.7%) than in those with OA due to HMW (25.6%; p=0.027).

Multivariate analysis

The multivariate logistic regression analysis conducted in 877 subjects demonstrated significant and independent associations between OA caused by HMW agents and work-related rhinitis (OR [95% CI]: 4.79 [3.28-7.12], p<0.001), conjunctivitis (OR: 2.13 [1.52-2.98], p<0.001), atopy (OR: 1.49 [1.09-2.05], p=0.012), baseline airflow limitation (OR: 1.76 [1.07-2.91], p=0.026), and the development of an early asthmatic reaction during SIC (OR: 2.86 [1.98-4.16], p<0.001) as compared to OA due to LMW agents (Figure 1). Conversely, OA due to LMW agents was associated with a significantly higher likelihood of experiencing chest tightness at work (OR: 2.22 [1.59-3.03], p<0.001), daily sputum (OR:

1.69 [1.19-2.38], p=0.004), and late asthmatic reactions during SIC (OR: 1.52 [1.09-2.08], p=0.014). In addition, subjects with OA due to LMW tended to experience more often severe asthma exacerbations (OR: 1.32 [1.01-1.69]; p=0.042). The multivariate models revealed no association between the type of causal agent and the daily dose of ICS categorized as low, medium, or high according to the GINA guidelines (data not detailed) nor with the severity of asthma defined according to the ERS/ATS criteria (Online Supplementary Figure S1).

Multivariate logistic regression analysis showed significant associations between OA caused by HMW agents and work-related rhinitis (OR [95% CI]: 4.79 [3.28-7.12]), conjunctivitis (2.13 [1.52-2.98]), atopy (1.49 [1.09-2.05]), and early asthmatic reactions (2.86 [1.98-4.16]). By contrast, OA due to LMW agents was associated with chest tightness at work (2.22 [1.59-3.03]), daily sputum (1.69 [1.19-2.38]), and late asthmatic reactions (1.52 [1.09-2.08]). Furthermore, OA caused by HMW agents showed a higher risk of airflow limitation (1.76 [1.07-2.91]) whereas OA due to LMW agents exhibited a higher risk of severe exacerbations (1.32 [1.01-1.69]).

Markers of airway inflammation

Peripheral blood eosinophilia >300/µl was significantly more frequent in OA due to HMW agents (49%) than in OA induced by LMW agents (34%; p<0.001) (Table 4). Subjects with OA caused by HMW agents showed a slightly higher baseline FeNO level (25 ppb [14-42]) and a significantly greater increase in post-challenge FeNO (14 ppb [3-42]) as compared to subjects with OA due to LMW agents (22 ppb [12-35]; p=0.009 and 4 ppb [-1-21]; p<0.001) (Table 4). The multivariate logistic regressions performed in the subsets of subjects with available information on the markers of airway inflammation (Online Supplementary Table S3, S4, and S5) confirmed that HMW agents were significantly and independently associated with a baseline blood eosinophilia count >300/µl (OR

[95%CI]: 2.00 [1.37-2.94]; p<0.001) and a post-challenge increase in FeNO >17.5 ppb (OR: 3.12 [2.03-5.48]; p<0.001).

There were no differences between the two groups with regard to the baseline and post-challenge sputum eosinophil and neutrophil counts. However, among subjects for whom a suitable sputum sample was available both at baseline and post-challenge assessments (n=288), a higher proportion of subjects with OA caused by HMW agents showed a switch from the paucigranulocytic to the eosinophilic pattern (52 of 73, 71.2%) as compared to LMW agents (24 of 47; 51.1%; p=0.025).

DISCUSSION

This study is, to our knowledge, the first that thoroughly compared OA caused by HMW and LMW agents with respect to clinical and functional characteristics in a large cohort of subjects with a diagnosis of OA ascertained by SIC. The finding of this study are consistent with previous studies that assessed rhinitis, conjunctivitis, atopy, and the pattern of asthmatic reactions in OA due to HMW and LMW agents (6, 9, 13, 14, 26). In addition, this study demonstrated differences in the pattern of work-related asthma symptoms, since the subjects with OA due to LMW agents showed a higher likelihood of experiencing chest tightness at work and daily sputum production. These findings may be relevant to the development of predictive models and algorithms for diagnosing OA based on simple clinical features and further indicate that these instruments should be developed separately for workers exposed to HMW and LMW agents, as already suggested by a prospective study that assessed the usefulness of questionnaire items for predicting the diagnosis of OA (27). In this study, wheezing, nasal and ocular itching at work were positively associated with the presence of OA in the case of HMW, but not LMW agents.

Remarkably, this study revealed a higher risk of airflow obstruction in OA caused by HMW agents whereas OA due to LMW agents was associated with a higher rate of severe exacerbations independently from global assessments of asthma severity graded according to either the GINA treatment steps (mild, moderate, and severe) or the ERS/ATS criteria (18). These results seem discordant from a recent monocentric study of 73 subjects with OA which reported a higher risk of moderate/severe persistent asthma (OR: 7.16 [1.13–15.20]; p=0.036) in subjects with OA caused by LMW agents (9). However, in the study by Meca et al. (9), the severity of asthma was graded using a global outcome measure based on GINA guidelines 2010. As highlighted in recent years (16, 17), the amount of medication required to achieve asthma control is a key element for assessing asthma severity (i.e. the intrinsic intensity of the disease process) during treatment while the level of asthma control is best reflected by both the magnitude of asthma-related impairment and the frequency of exacerbations. Our results further outline the importance of assessing separately the diverse components of asthma control since the rates of airflow limitation on spirometry and exacerbations were differently impacted in OA due to HMW and LMW agents, which has not been reported previously. Lemière et al. (28) investigated the rate of severe exacerbation requiring medical resource use in subjects with OA, but found no difference according to the type of causal agent. Population-based epidemiological studies revealed significant associations between severe asthma exacerbations and exposure to any occupational asthmagen, without differences between HMW and LMW agents (29).

There is currently discordant information on the pattern of airway inflammation in OA caused by either HMW or LMW agents. Two studies reported a lower sputum eosinophilia at baseline in OA due to LMW agents as compared to HMW agents (10, 14), although this was not confirmed in the cohort described by Prince et al. (12). In addition, the latter study failed to find differences between HMW and LMW agents in the post-challenge changes in the level of BHR and granulocyte counts as

compared to baseline values (12). In keeping with this study (12), OA due to HMW and LMW agents did not differ in the baseline and post-challenge sputum eosinophil and neutrophil counts in our cohort. Interestingly, a high proportion of the subjects in our cohort (44.1%) demonstrated a paucigranulocytic pattern of sputum cells at baseline assessment. This paucigranulocytic pattern has been considered as a stable non-inflammatory phenotype of asthma associated with a distinct outcome in terms of severe exacerbations and responsiveness to ICS (30, 31). However, we observed that a high proportion of our subjects with paucigranulocytic asthma at baseline (76/120, 63.3%) shifted toward an eosinophilic pattern after challenge exposure to the causal agent. Of note, HMW agents induced more frequently a switch from "paucigranulocytic asthma" to "eosinophilic asthma" than LMW agents. Our finding may provide novel insight into the understanding of paucigranulocytic asthma by indicating that it may be a transient phenotype which can be rapidly affected by environmental exposures, such as occupational sensitizing agents and perhaps non-occupational allergens. In addition, these findings further support the study of Lemière et al. (32) who suggested that the changes in the type of airway inflammation induced by exposure to an occupational agent may be more important than the baseline inflammatory profile to predict the outcome of OA. These investigators found that a noneosinophilic response (i.e. a post-challenge increase in sputum eosinophils <2%) was associated with worse asthma control and a greater decline in FEV_1 at follow-up assessment.

There is considerable interest in the non-invasive assessment of eosinophilic airway inflammation through the measurement of FeNO and blood eosinophils in the diagnosis and management of asthma (33, 34). In our cohort, asthmatic reactions induced by HMW agents were more frequently associated with a post-challenge increase in FeNO compared to LMW agents, corroborating a recent report by Lemière et al. (15). We also found that OA caused by HMW agents exhibited more frequently a baseline peripheral blood eosinophilia as compared to LMW agents, although the two

groups were similar with regard to the baseline sputum eosinophil counts. Currently, only one study has evaluated the usefulness of measuring blood eosinophils in OA (35). These investigators reported that a blood eosinophil count >300/µl was unable to differentiate subjects with positive and negative SICs and that post-challenge changes in blood and sputum eosinophil counts did not correlate, although they failed to provide separate information for HMW and LMW agents. Overall, our findings further highlight an important degree of discordance between sputum eosinophilia and TH2-related biomarkers, indicating that these indices are likely to reflect different phathophysiological pathways (33, 34, 36, 37).

The strength of this study was the international, multicenter design of the study that aimed to minimize potential selection biases due to local clinical practices and recruitment patterns and to enhance the generalizability of the results. However, this study has several limitations that deserve further consideration. First, markers of airway inflammation (peripheral blood eosinophil counts, FeNO and/or sputum cells) were unavailable in a large portion of the subjects and could therefore not be incorporated into the multivariate analysis of the clinical and functional factors that are associated with HMW vs. LMW agents. A major limitation results from the lack of validated instrument for assessing the level of asthma control. Therefore, the diverse components of asthma control could not be fully evaluated (e.g. about nighttime symptoms and limitation of daily activities) and the "poor symptom control" component of the most recent definition of severe asthma (18) had to be defined by the daily use of a SABA as proposed in the ATS recommendations issued in 2000 (19). This may affect the comparison of our findings with those from recent studies on non-occupational asthma. Another limitation arises from the lack of follow-up data that could be correlated with the baseline phenotypic characteristics in order to determine their predictive value. It is now well established that a substantial proportion of patients with OA do not recover from their disease after reduction or even avoidance of exposure to the causal agent (1, 2, 38-40). However,

there is considerable uncertainty about the determinants of asthma outcome after interventions for the treatment of OA while patients with OA and their medical advisers need to make important decisions that most often result in substantial socioeconomic consequences (1, 2, 38-40). Two systematic reviews of follow-up studies in subjects with OA provided some evidence that subjects with OA related to HMW agents are more likely to have persistent BHR after complete avoidance of exposure to the causal agent (39, 41).

In conclusion, this study highlighted phenotypic differences between OA caused by HMW and LMW agents in their clinical and functional characteristics, and inflammatory biomarkers profiles. Whether specific pathobiologic pathways are involved in these OA subphenotypes need to be addressed in future studies. From a clinical perspective, further investigation is required to evaluate the impact of the observed phenotypic differences between HMW and LMW agents on the outcome of OA. Ultimately, these studies would help to determine whether the identification of phenotypic characteristics associated with a higher risk for a worse outcome might contribute to a more personalized management approach.

Table 1. Causal agents

High-molecular-weight agents	n (%)*	Low-molecular-weight agents	n (%)*
Flour/grains	369 (31.3)	Isocyanates	206 (17.4)
Latex	36 (3.0)	Persulfate salts	78 (6.6)
Enzymes	26 (2.2)	Metals	42 (3.6)
Storage mites	12 (1.0)	Quaternary ammonium compounds	38 (3.2)
Rodents	11 (0.9)	Acrylate compounds	35 (3.0)
Cow dander	11 (0.9)	Wood dusts	35 (3.0)
Fish/seafood	8 (0.7)	Welding fume	30 (2.5)
Insects and derived products	6 (0.5)	Cleaning products/disinfectant (NOS)	27 (2.3)
Ornamental plants	6 (0.5)	Epoxy resins	18 (1.5)
Moulds	5 (0.4)	Aldehydes	17 (1.4)
Soybean flour	3 (0.2)	Drugs	16 (1.4)
Spices	3 (0.2)	Metal working fluids	15 (1.3)
Vegetable gums	3 (0.2)	Resins/glues/paints (NOS)	15 (1.3)
Various plant-derived products	26 (2.2)	Acid anhydrides	11 (0.9)
Various animals and derived products	18 (1.5)	Amines	10 (0.8)
		Colophony	4 (0.3)
		Styrene	3 (0.2)
		Reactive dyes	2 (0.2)
		Triglycidylisocyanurate	2 (0.2)
		Other low-molecular-weight agents (NOS)	21 (17.8)
Total :	543 (46.5)	Total :	624 (52.9)

Legend: NOS: not otherwise specified

* % of total identified agents (n=1,167); the causal agent was not precisely identified in 13 subjects.

Table 2. Clinical characteristics of subjects with occupational asthma caused by high- and low-molecular-weight agents

Characteristic	High-molecular-weight agents (n=544)	Low-molecular-weight agents (n=623)	P-value
Age, yr*	41 (32-49)	43 (34-52)	**0.001**
Sex (male)	338 (62.1)	352 (56.5)	0.060
Body mass index, kg/m² *	26.8 (23.6-30.1)	26.4 (23.8-29.7)	0.530
Smoking habits:			**0.020**
Never smoker	296 (55.3)	301 (49.7)	
Ex-smoker	124 (23.2)	185 (30.5)	
Current smoker	115 (21.5)	120 (19.8)	
Atopy†	316 (58.5)	260 (43.6)	**<0.001**
Pre-existing asthma	59 (10.9)	76 (12.3)	0.520
Nature of respiratory symptoms at work:			
Cough	440 (81.6)	466 (80.1)	0.540
Wheezing	366 (68.2)	357 (61.9)	**0.030**
Dyspnea	482 (89.4)	519 (88.6)	0.700
Chest tightness	176 (33.6)	280 (49.9)	**<0.001**
Sputum	129 (24.6)	173 (31.6)	**0.010**
Associated disorders:			
Work-related rhinitis	473 (87.3)	345 (56.4)	**<0.001**
Onset of rhinitis before asthma	253/447 (56.6)	120/287 (41.8)	**<0.001**
Work-related conjunctivitis	286 (53.3)	158 (27.3)	**<0.001**
Work-related contact dermatitis	86 (15.8)	88 (15.0)	0.740
Work-related urticaria	83 (15.3)	41 (7.0)	**<0.001**
Work-related dysphonia	65 (12.5)	74 (13.1)	0.790
Sinusitis	63 (11.7)	69 (11.8)	1.000
Chronology of symptoms:			
Duration of exposure before asthma onset, mo*	96 (44.5-180)	61 (21-168)	**<0.001**
Duration of symptomatic exposure, mo*	36 (12-72)	24 (12-60)	**<0.001**
Interval since last work exposure, mo*	1 (0.03-8)	1 (0.03-7)	0.950
Short-acting β_2-agonist			**<0.001**
Never	114 (22.0)	192 (33.1)	
Once or less per week	108 (20.8)	101 (17.4)	
2 or more times a week	155 (29.9)	122 (21.0)	
1 or 2 times a day	94 (18.1)	118 (20.3)	
≥3 times a day	47 (9.1)	47 (8.1)	
ICS daily dose¥			
No ICS	157 (30.1)	229 (39.5)	**0.002**
Low (≤500 µg per day)	182 (34.9)	143 (24.6)	
Medium (>500-1,000 µg per day)	139 (26.7)	150 (25.9)	
High (>1000 µg per day)	43 (8.2)	58 (10.0)	
Long-acting β_2-agonist	311 (59.2)	307 (52.8)	**0.030**
Leukotriene receptor antagonist	83 (15.8)	72 (12.3)	0.100
Oral corticosteroid	5 (0.9)	4 (0.7)	0.740
Oral H_1-antihistamine	216 (41.3)	100 (17.3)	**<0.001**
Nasal corticosteroid spray	119 (25.1)	71 (13.1)	**<0.001**
Asthma severity (GINA classification):			**0.002**
Untreated	66 (12.8)	106 (18.4)	
Mild (GINA treatment step 1-2)	107 (20.7)	143 (24.8)	
Moderate (GINA treatment step 3)	202 (39.2)	161 (28.0)	
Severe (GINA treatment step 4-5)	141 (27.3)	166 (28.8)	
"Poor symptom control"§	141 (27.2)	165 (28.4)	0.650
≥1 asthma exacerbation (last 12 month at work)	100 (19)	139 (26)	**0.008**
Severe asthma#	66 (13.9)	66 (15.2)	0.65

Legend: Data are presented as n (% of available data) unless otherwise specified. ICS: inhaled corticosteroid; GINA: Global Initiative for Asthma, 2015 update (*www.ginasthma.org*). Values in boldface are statistically significant

* Median value with interquartile range within parentheses;

† Atopy defined by the presence of a positive skin-prick test to at least one common allergen;

¥ Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent;

§ "Poor symptom control" defined by the use of a short-acting β_2-agonist ≥1 time a day;

Definition of severe asthma adapted from the European Respiratory Society/American Thoracic Society criteria (see Methods) (18).

Table 3. Functional characteristics of subjects with occupational asthma caused by high- and low-molecular-weight agents

Characteristic	High-molecular-weight agents (n=544)	Low-molecular-weight agents (n=623)	P-value
Baseline FEV_1, % pred*	91 (81-100)	92 (83-101.5)	0.060
Baseline FEV_1 <80% pred	124 (22.9)	109 (17.5)	**0.020**
Baseline FEV_1/FVC, %*	76.2 (70.4-81)	77 (71-82)	0.140
Baseline FEV_1/FVC <70%	125 (23.1)	133 (21.4)	0.480
Airflow obstruction[†]	72 (13.3)	62 (10.0)	0.080
Baseline level of BHR[¥]:	(n=492)	(n=586)	0.080
Absent	126 (25.6)	186 (31.7)	
Mild	223 (45.2)	241 (41.1)	
Moderate-to-severe	144 (29.2)	159 (27.1)	
Post-challenge change in BHR[¥]:	(n=295)	(n=370)	
Pre/post-SIC BHR ratio*	2.0 (1.0-4.0)	2.0 (1.0-4.0)	0.190
Pre/post-SIC BHR ratio >2	166 (56.3)	193 (52.2)	0.310
Pattern of asthmatic reaction during the SIC:			
Presence of an early asthmatic component	454 (83.6)	367 (63.7)	**<0.001**
Presence of a late asthmatic component	265 (50.5)	389 (66.4)	**<0.001**
Isolated early reaction	257 (49.2)	182 (31.9)	**<0.001**
Isolated late reaction	68 (13.0)	204 (35.7)	**<0.001**
Dual reaction	197 (37.7)	185 (32.3)	0.140

Legend: Data are presented as n (% of available data) unless otherwise specified. FEV_1: forced expiratory volume in one-second; FVC: forced vital capacity; BHR: nonspecific bronchial hyperresponsiveness; SIC: specific inhalation challenge. Values in boldface are statistically significant

* Median value with interquartile range (IQR) within parentheses;

[†] Airflow obstruction defined by a FEV_1 <80% predicted value and a FEV_1/FVC ratio <70%;

[¥] See Online Supplementary Material for threshold values used for grading the level of BHR.

Table 4. Airway inflammation markers in subjects with occupational asthma caused by high- and low-molecular-weight agents

Characteristic	High-molecular-weight agents (n=544)	Low-molecular-weight agents (n=623)	P-value
Blood eosinophils:	(n=356)	(n=292)	
Cells/µl*	291 (190-401)	204 (110-331)	**<0.001**
>300/µl	175 (49)	98 (33.6)	**<0.001**
Baseline FeNO:	(n=265)	(n=363)	
ppb*	25 (14-42)	22 (12-35)	**0.009**
Post-challenge FeNO:	(n=199)	(n=284)	
ppb*	47 (26-88)	29 (15-58)	**<0.001**
Post-SIC change in FeNO, ppb*†	14 (3-42)	4 (-1-21)	**<0.001**
Pre/post-SIC change in FeNO >17.5 ppb†	90 (46.6)	79 (28.3)	**<0.001**
Baseline sputum eosinophils:	(n=191)	(n=131)	
%*	2 (1-5)	2 (0.8-6)	0.450
≥3%	81 (42.4)	58 (44.3)	0.820
Post-challenge sputum eosinophils:	(n=168)	(n=120)	
%*	8.5 (3-19)	7 (2-19.8)	0.250
Post-SIC change, %*†	4 (1-10.8)	3.3 (0.4-9.9)	0.370
Baseline sputum neutrophils:	(n=197)	(n=136)	
%*	52 (34-70)	47.6 (30-66.2)	0.140
≥76%	41 (20.8)	23 (16.9)	0.400
Post-challenge sputum neutrophils:	(n=170)	(n=123)	
%*	47 (30.2-66.8)	48 (24.5-63.9-	0.410
Post-SIC change, %*†	-3 (-14.2-8)	-0.9 (-12-12)	0.180

Baseline sputum inflammatory pattern:	(n=191)	(n=131)	0.160
Eosinophilic	69 (36.1)	56 (42.7)	
Neutrophilic	24 (12.6)	17 (13.0)	
Paucigranulocytic	86 (45.0)	56 (42.7)	
Mixed granulocytic	12 (6.3)	2 (1.5)	
Post-challenge sputum inflammatory pattern:	(n=168)	(n=120)	0.189
Eosinophilic	124 (73.9)	81 (67.5)	
Neutrophilic	11 (6.5)	6 (5.0)	
Paucigranulocytic	23 (13.7)	28 (23.3)	
Mixed granulocytic	10 (6.0)	5 (4.2)	

Legend: Data are presented as n (% of available data) unless otherwise specified. Values in boldface are statistically significant. FeNO: fractional exhaled nitric oxide concentration; SIC: specific inhalation challenge.

* Median value with interquartile range (IQR) within parentheses;

† Compared to baseline value.

LEGEND TO FIGURES

Figure 1

The figure illustrates the main results of the multivariate logistic regression analysis for the clinical and functional characteristics that are associated with occupational asthma caused by high-molecular-weight agents as the outcome (odds ratio >1) and low-molecular-weight agents as the reference (odds ratio <1). Work-related rhinitis and conjunctivitis were physician-based diagnoses. Airway obstruction was defined by a FEV_1 <80% predicted value and a FEV_1/FVC ratio <70%. The presence of atopy was documented by at least one positive skin-prick test response to a battery of locally relevant inhalant allergens. Asthma exacerbations were defined by the need for oral corticosteroids for at least 3 consecutive days or emergency room visit or hospitalization during the last 12 months at work (16).

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