ORIGINAL ARTICLE

Sputum Inflammatory Patterns Are Associated With Distinct Clinical Characteristics in Patients with Occupational Asthma Independently of the Causal Agent

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Abstract

Background: Clinical heterogeneity in sensitizer-induced occupational asthma (OA) and its relationship to airway inflammatory profiles remain poorly elucidated.
Objectives: To further characterize interactions between induced sputum inflammatory patterns, asthma-related outcomes, and the high- or low-molecular-weight category of causal agents in a large cohort of patients with OA.
Methods: We conducted a multicenter, retrospective, cross-sectional study of 296 patients with OA confirmed by a positive specific inhalation challenge who completed induced sputum assessment before and 24 hours after challenge exposure.
Results: Multivariate logistic regression analysis revealed that sputum eosinophilia ≥3% was significantly associated with a high dose of inhaled corticosteroid (OR [95%CI], 1.31 [1.11-1.55] for each 250-µg increment in daily dose), short-acting $ß_2$-agonist use less than once a day (3.54 [1.82-7.00]), and the level of baseline nonspecific bronchial hyperresponsiveness (mild, 2.48 [1.21-5.08]; moderate/severe, 3.40 [1.44-8.29]). Sputum neutrophilia ≥76% was associated with age (1.06 [1.01-1.11]), male sex (3.34 [1.29-9.99]), absence of corticosteroid use (5.47 [2.09-15.16]), use of short-acting $ß_2$-agonists once or more a day (4.09 [1.71-10.01]), ≥2 severe exacerbations during the previous 12 months at work (4.22 [1.14-14.99]), and isolated early reactions during the specific inhalation challenge (4.45 [1.85-11.59]).
Conclusion: The findings indicate that sputum inflammatory patterns in patients with OA are associated with distinct phenotypic characteristics and further highlight the differential effects of neutrophils and eosinophils on asthma-related outcomes. These associations between inflammatory patterns and clinical characteristics share broad similarities with findings reported in nonoccupational asthma and are not related to the type of causal agent.

Key words: Eosinophils. Induced sputum. Neutrophils. Occupational asthma. Phenotype.

Resumen

Antecedentes: La heterogeneidad clínica en el asma ocupacional (AO) inducida por agentes sensibilizantes y su relación con los perfiles inflamatorios de las vías respiratorias siguen siendo muy poco conocidas.
Objetivos: Profundizar en la caracterización de las interrelaciones entre los patrones inflamatorios en esputo inducido, diversas variables relacionadas con el asma y la categoría de agentes causales de alto o bajo peso molecular, en una gran cohorte de sujetos con AO.

Métodos: Este estudio multicéntrico, retrospectivo y transversal se llevó a cabo en 296 sujetos con OA confirmada mediante una provocación bronquial específica (SIC) positiva, en los que se obtuvieron muestras de esputo inducido antes y 24 horas después de la SIC.
Resultados: El análisis de regresión logística multivariable reveló que la presencia de eosinofilia en esputo ≥3 % se asoció significativamente con una dosis alta de corticosteroides inhalados (*odds ratio* [intervalo de confianza del 95 %], 1,31 [1,11-1,55] por cada incremento de 250 µg en la dosis diaria), el uso de agonistas ß$_2$ de acción corta menos de una vez al día (3,54 [1,82-7,00]), y un nivel de hiperreactividad bronquial inespecífica inicial (leve: 2,48 [1,21-5,08]); moderado/grave: 3,40 [1,44-8,29]). La neutrofilia en esputo ≥76%, se asoció con la edad (1,06 [1,01-1,11]), el sexo masculino (3,34 [1,29-9,99]), la ausencia de uso de corticosteroides (5,47 [2,09-15,16]), el uso de agonistas ß$_2$ de acción corta una vez o más al día (4,09 [1,71-10,01]), la presencia de ≥ 2 exacerbaciones graves en los últimos 12 meses en el trabajo (4,22 [1,14-14,99]) y reacciones inmediatas aisladas durante la SIC (4,45 [1,85-11,59]).
Conclusión: Los resultados del estudio indican que los patrones inflamatorios del esputo en sujetos con OA están asociados con características fenotípicas distintas y resaltan aún más los efectos diferenciales de la inflamación bronquial neutrofílica o eosinofílica en las distintas variables relacionadas con el asma. Estas asociaciones entre patrones inflamatorios y características clínicas comparten amplias similitudes con lo que se ha descrito en el asma de origen no ocupacional y no están relacionadas con el tipo de agente causal.

Palabras clave: Eosinófilos. Esputo inducido. Neutrófilos. Asma ocupacional. Fenotipo.

Summary box

- **What do we know about this topic?**
  Information on the relationships between sputum inflammatory patterns and clinical characteristics in sensitizer-induced occupational asthma is scarce and often discordant.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  This large cohort study indicates that eosinophilic and neutrophilic sputum inflammatory patterns are associated with distinct clinical phenotypes of occupational asthma that are similar to those described in nonoccupational asthma. In addition, the data provide evidence that a sensitizing occupational agent may induce occupational asthma through various inflammatory pathways, independently of its high- or low-molecular-weight category.

Introduction

Sensitizer-induced occupational asthma (OA), a distinguishable subset of adult asthma, is characterized by the de novo onset of asthma or the recurrence of previously quiescent asthma induced by immune-mediated sensitization to specific agents in the workplace [1,2]. Workplace sensitizing agents are conventionally categorized into high-molecular-weight (HMW) (glycol) proteins of animal, vegetable, or microbiological origin and low-molecular-weight (LMW) agents that include reactive chemicals, metals, and wood dusts [1,2]. OA caused by HMW agents is associated with demonstrable specific IgE antibodies, while LMW agents act as haptens, binding to endogenous proteins to initiate a specific immune response through mechanisms that remain largely unknown [3].

The noninvasive induced sputum technique enabled the identification of eosinophilic and noneosinophilic inflammatory patterns of asthma that are associated with different clinical phenotypes and are likely related to differences in underlying pathobiological pathways [4-13]. However, clinical heterogeneity in patients with OA and its relationship to sputum inflammatory profiles remain poorly elucidated. Available studies provide sparse and often discordant information on the relationships between eosinophilic inflammation and asthma outcomes [14-21]. In addition, most of these studies failed to specifically investigate the clinical and functional characteristics associated with sputum neutrophilia, although it has been suggested that OA induced by LMW agents may be associated with higher sputum neutrophilia than OA induced by HMW agents [14,16,22].

This study aimed to further characterize the relationships between sputum inflammatory patterns, asthma-related outcomes at the time of the diagnostic evaluation, and the type of causal agent in a large cohort of patients with OA confirmed by a positive specific inhalation challenge (SIC).

Methods

Study Design and Population

This retrospective cross-sectional study was conducted among patients with OA confirmed by a positive SIC completed between 2006 and 2018 in tertiary centers participating in the European network for the PHenotyping of OCcupational Asthma (E-PHOCAS) study [23-27]. Eligible patients were those with complete information on key asthma outcomes (ie, detailed medication and number of severe exacerbations) and induced sputum samples collected both before and 24 hours after the SIC procedure. Cohort recruitment is further detailed in the online Supplementary Materials. This report conformed to the Strengthening of the Reporting of Observational Studies

in Epidemiology statement for cross-sectional studies (www.strobe-statement.org).

Ethics

The retrospective E-PHOCAS study was approved by the local institutional review boards, the Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé, and the Commission Nationale de l'Informatique et des Libertés.

Asthma Outcomes

Detailed information on data collection and interpretation are given in the online Supplementary Materials [23,24]. The analysis was based on information recorded for asthma medication, including the frequency of short-acting $ß_2$-agonist (SABA) use and severe exacerbations while the patients were exposed at work (last 12 months at work for exacerbations). Poor symptom control was defined as the need for an inhaled SABA once or more a day [28]. Severe asthma exacerbations and severe asthma were defined according to the European Respiratory Society/American Thoracic Society consensus guidelines [29].

Lung Function Assessments

We collected prechallenge spirometry values, as well as the level of nonspecific bronchial hyperresponsiveness (NSBH) measured at baseline of the SIC procedure and 24 hours after challenge exposure. NSBH was graded as absent, mild, or moderate-to-severe [23] according to the bronchoprovocation method used in each center (see online Supplementary Materials and Table S1). The methodology and interpretation of SIC are further described in the online Supplementary Materials and conformed with international recommendations [30].

Induced Sputum Assessment

Induced sputum was collected at baseline and 24 hours after the SIC. Detailed information on the methods used to induce and analyze sputum samples in participating centers is included in the online Supplementary Materials. Sputum eosinophilia was defined as a sputum eosinophil count ≥3%; sputum neutrophilia was defined as a sputum neutrophil count ≥76% [11]. Accordingly, the sputum inflammatory patterns were classified as eosinophilic (ie, ≥3% eosinophils and <76% neutrophils), neutrophilic (ie, neutrophils ≥76% and <3% eosinophils), paucigranulocytic (ie, <3% eosinophils and <76% neutrophils), or mixed granulocytic (ie, ≥76% neutrophils and ≥3% eosinophils).

In this study, sputum cell counts obtained 24 hours after the SIC were considered as the primary outcome, since a significant decrease in sputum eosinophil counts has been reported within 2 weeks after removal from exposure [31]. Of note, 173 of 296 (58.4%) patients in this cohort had already been removed from the causal exposure for more than 1 week at the time of the SIC procedure.

Data Analysis

Data are presented as median (IQR) for continuous variables and as percentages for categorical variables. Patients were compared between groups using the Fisher exact or χ^2 test for categorical variables and nonparametric tests for numerical variables. Pairwise comparisons between the 4 sputum inflammatory patterns to identify which groups differed from each other were corrected according to the Benjamini-Hochberg method for multiple comparisons.

Multivariate logistic regression analyses were conducted in order to identify the clinical and physiological characteristics associated with a ≥3% sputum eosinophil count or a ≥76% neutrophil count in samples collected 24 hours after challenge exposure. The multivariate analyses were carried out using a binomial generalized linear model with the most parsimonious models, which were selected using a stepwise procedure based on the Akaike information criterion. The independent variables incorporated into these regression models included both sociodemographic characteristics (age, sex, smoking status, and body mass index ≥30 kg/m^2) and variables with a P value ≤.1 in the univariate comparisons. Missing values were not imputed. Peripheral blood eosinophil counts and fractional exhaled nitric oxide (FeNO) were not included in the multivariate models, because biomarkers and sputum eosinophils are interrelated [32] and reflect type 2 (T2)–high airway inflammation. In addition, these data were missing for a substantial proportion of patients.

Sensitivity analyses were performed by restricting the regressions to the prechallenge (baseline) sputum eosinophil or neutrophil counts in patients who were still exposed to the offending agent within 1 week before the SIC procedure (n=123). The statistical analysis was performed using R version 3.4.1 (www.r-project.org, Vienna, Austria). A P value <.05 was considered significant.

Results

Population

The study included 296 patients with available sputum samples collected both before and 24 hours after the SIC procedure. The clinical and physiological characteristics of the patients grouped according to their postchallenge sputum inflammatory pattern are presented in Tables 1 and 2. Most patients (67.9%) had a postchallenge eosinophilic pattern, whereas 18.2%, 7.8%, and 6.1% had a paucigranulocytic, neutrophilic, or mixed granulocytic pattern, respectively. The clinical and functional characteristics associated with the 4 sputum inflammatory patterns are compared in the online Supplementary Materials. The workplace agents causing OA in this cohort are detailed in Table S2 of the Supplementary Materials.

Determinants of Sputum Eosinophilia

The clinical and physiological characteristics of patients with and without a postchallenge sputum eosinophil count ≥3% (n=219 and 77, respectively) and the univariate associations with postchallenge sputum eosinophilia are detailed in Table 3. The multivariate logistic regression analysis (Table 4; multivariate model 1) revealed the significant predictors of a postchallenge sputum eosinophilia to be a high dose of inhaled corticosteroids while the patients were exposed at work (OR, 1.31; 95%CI,

Table 1. Demographic and Clinical Characteristics of the Patients According to Their Postchallenge Sputum Inflammatory Pattern.[a,b]

Characteristic	Missing values	Eosinophilic pattern (n=201)	Mixed granulocytic pattern (n=18)	Neutrophilic pattern (n=23)	Paucigranulocytic pattern (n=54)	*P* Value
Age, y[c]	0	42 (34-51)	47 (40-54)	45 (37-54)	46 (34-51)	.566
Sex, male	0	133 (66.2)	14 (77.8)	17 (73.9)	30 (55.6)	.247
Body mass index, kg/m^2[c]	0	27 (24-30)	26 (24-31)	27 (22-30)	28 (25-32)	.413
Smoking status	0					.711
Current smoker	0	46 (22.9)	3 (16.7)	8 (34.8)	14 (25.9)	
Ex-smoker		54 (26.9)	6 (33.3)	7 (30.4)	17 (31.5)	
Never smoker		101 (50.2)	9 (50.0)	8 (34.8)	23 (42.6)	
Atopy[d]	1/0/0/1	95 (47.5)	11 (61.1)	13 (56.5)	25 (47.2)	.616
Chronic rhinosinusitis	1/0/0/1	13 (6.5)	3 (16.7)	3 (13.0)	7 (13.2)	.119
Asthma before the causal exposure	0	12 (6.0)	3 (16.7)	4 (17.4)	6 (11.1)	.061
Duration of exposure before symptom onset, mo[c]	1/0/0/1	120 (60-216)	72 (28-240)	132 (50-228)	84 (25-204)	.362
Duration of asthma symptoms at work, mo[c]	1/1/0/1	36 (18-86)	33 (13-72)	36 (16-48)	24 (15-60)	.246
Interval since last work exposure, mo[c]	1/0/0/0	3.0 (0.03-11.2)	6.5 (0.1-16.8)	5.0 (0.1-14.5)	2.8 (0.1-10.8)	.388
Type of causal agent, HMW	0	139 (69.2)	8 (44.4)	16 (69.6)	25 (46.3)	.005[g]
Work-related rhinitis	0	164 (81.6)	14 (77.8)	14 (60.9)	37 (68.5)	.042[g]
Asthma treatment at work						
ICS use	0	160 (79.6)	10 (55.6)	11 (47.8)	36 (66.7)	.001[g]
Daily dose of ICS, µg[c,d]	0	500 (400-1000)	325 (0-1000)	0 (0-1000)	500 (0-605)	.052
Long-acting ß$_2$-agonist	0	155 (77.1)	10 (55.6)	10 (43.5)	32 (59.3)	.001[g]
Leukotriene receptor antagonist	1/1/1/0	50 (25.0)	3 (17.6)	2 (9.1)	9 (16.7)	.262
Poor asthma control (SABA ≥ once a day) at work	0	45 (22.4)	5 (27.8)	16 (69.6)	13 (24.1)	<.001[g]
≥2 severe exacerbations previous 12 mo at work	0	16 (8.0)	4 (22.2)	2 (8.7)	1 (1.9)	.045[g]
Severe asthma at work[f]	0	35 (17.4)	5 (27.8)	5 (21.7)	8 (14.8)	.549

Abbreviations: HMW, high-molecular-weight; ICS, inhaled corticosteroid; SABA, short-acting ß$_2$-agonist.
[a]The sputum inflammatory patterns were characterized as follows: eosinophilic (ie, ≥3% eosinophils and <76% neutrophils); neutrophilic (ie, ≥76% and <3% eosinophils); paucigranulocytic (ie <3% eosinophils and <76% neutrophils); and mixed granulocytic (ie, ≥76% neutrophils and ≥3% eosinophils).
[b]Data are presented as No. (% of available data) unless otherwise specified.
[c]Median (IQR).
[d]Atopy defined by the presence of at least 1 positive skin prick test result with common allergens.
[e]Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.
[f]Multidimensional definition of severe asthma adapted from the European Respiratory Society/American Thoracic Society guidelines [29].
[g]Statistically significant (*P*<.05).

1.11-1.55 for each 250-µg increment in daily dose of inhaled corticosteroids [ICS]; *P*=.002), SABA use less than once a day at work (OR, 3.54; 95%CI, 1.82-7.00; *P*<0.001), mild NSBH (OR, 2.48; 95%CI, 1.21-5.08; *P*=.012), and moderate-to-severe NSBH (OR, 3.40; 95%CI, 1.44-8.29; *P*=.006).

The multivariate analysis of prechallenge sputum eosinophil count ≥3% restricted to patients still exposed at work within 1 week of the SIC procedure (n=123) (Table 4) also identified the main factors associated with sputum eosinophilia to be a high dose of ICS, SABA use less than once a day, and level of NSBH. The univariate associations with prechallenge sputum eosinophilia are detailed in Table S3.

Determinants of Sputum Neutrophilia

Table 5 presents the characteristics of patients with and without a postchallenge sputum neutrophil count ≥76% (n=41 and 255, respectively) and the univariate associations with

Table 2. Functional Characteristics and Markers of Airway Inflammation According to the Postchallenge Sputum Inflammatory Pattern.[a,b]

Characteristic	Missing values	Eosinophilic pattern (n=201)	Mixed granulocytic pattern (n=18)	Neutrophilic pattern (n=23)	Paucigranulocytic pattern (n=54)	*P* Value
Baseline spirometry						
FVC, % pred[c]	0	102 (91-110)	100 (91-104)	105 (91-112)	102 (91-109)	.928
FEV_1, % pred[c]	0	90 (78-97)	88 (72-92)	85 (73-96)	93 (84-101)	.251
FEV_1/FVC, %[c]	0	74 (66-80)	71 (66-77)	73 (64-78)	78 (72-81)	.024[g]
Baseline level of NSBH[d]	14/2/0/4					
Absent		30 (16.0)	2 (12.5)	4 (17.4)	18 (36.0)	.020[g]
Mild		99 (52.9)	10 (62.5)	12 (52.2)	23 (46.0)	.703
Moderate-to-severe		58 (31.0)	4 (25.0)	7 (30.4)	9 (18.0)	.324
Post-SIC change in NSBH	71/6/2/11					
Pre/post-SIC NSBH ratio[c]		2.33 (1.40-4.64)	2.40 (1.31-4.05)	1.48 (1.00-2.67)	2.58 (1.00-6.70)	.293
Maximum fall in FEV_1, % baseline[c]	0	24 (19-33)	29 (24-37)	22 (18-27)	22 (19-27)	.193
Pattern of bronchial response to SIC	12/4/4/4					.008[g]
Isolated immediate reaction		67 (35.4)	9 (64.3)	13 (68.4)	17 (34.0)	
Late reaction[e]		122 (64.6)	5 (35.7)	6 (31.6)	33 (66.0)	
Blood eosinophils	33/3/4/14					
Cells/μL[c]		300 (200-428)	203 (150-300)	211 (144-305)	200 (100-291)	<.001[g]
>300/μL		93 (55.4)	5 (33.3)	6 (31.6)	9 (22.5)	<.001[g]
Baseline FeNO, ppb[c]	136/6/5/25	26 (14-39)	24 (16-61)	12 (7-26)	18 (9-28)	.123
Post-SIC change in FeNO	139/6/5/31					
ppb[c,f]		20 (6-46)	11 (4-29)	2 (0-12)	2 (0-12)	.001[g]
>17.5 ppb[f]		35 (56.5)	3 (25.0)	3 (16.7)	4 (17.4)	.001[g]
Baseline sputum inflammatory pattern	0					ND
Eosinophilic		95 (47.3)	0	1 (4.3)	7 (13.0)	
Neutrophilic		18 (9.0)	6 (33.3)	14 (60.9)	6 (11.1)	
Mixed granulocytic		10 (5.0)	4 (22.2)	3 (13.0)	1 (1.9)	
Paucigranulocytic		78 (38.8)	8 (44.4)	5 (21.7)	40 (74.1)	

Abbreviations: FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one-second; FVC, forced vital capacity; ND, not done; NSBH, nonspecific bronchial hyperresponsiveness; SIC, specific inhalation challenge.

[a]The sputum inflammatory patterns were characterized as follows: eosinophilic (ie, ≥3% eosinophils and <76% neutrophils); neutrophilic (ie, ≥76% and <3% eosinophils); paucigranulocytic (ie, <3% eosinophils and <76% neutrophils); and mixed granulocytic (ie, ≥76% neutrophils and ≥3% eosinophils).

[b]Data are presented as No. (% of available data) unless otherwise specified.

[c]Median (IQR).

[d]See Table S1 for the grading of NSBH.

[e]Late asthmatic reactions including isolated late and dual reactions.

[f]Difference from baseline value

[g]Statistically significant (*P*<.05).

postchallenge sputum neutrophilia. The multivariate analysis (Table 6) showed that sputum neutrophilia was significantly associated with older age (OR for a 1-year increase, 1.06; 95%CI, 1.01-1.11; *P*=.014), male sex (OR, 3.34; 95%CI, 1.29-9.99; *P*=.019), absence of ICS use while at work (OR, 5.47; 95%CI, 2.09-15.16; *P*<.001), poor asthma control in the workplace (OR, 4.09; 95%CI, 1.71-10.0; *P*=.024), a history of 2 or more severe exacerbations during the previous 12 months while exposed at work (OR, 4.22; 95%CI, 1.14-14.9; *P*=.025), and onset of isolated immediate reactions during the SIC (OR, 4.45; 95%CI, 1.85-11.59; *P*=.001).

Table S3 in the online Supplementary Materials provides the univariate associations for prechallenge sputum neutrophil count ≥76% among the 123 patients still exposed at work. The multivariate analysis of prechallenge sputum neutrophilia retained only absence of ICS use (OR 5.09; 95%CI, 1.82-

Table 3. Univariate Associations With Postchallenge Sputum Eosinophilia.[a]

Characteristic	Missing values	Sputum eosinophils ≥3% (n=219)	Sputum eosinophils <3% (n=77)	Univariate analysis	
				OR (95%CI)	*P* Value
Age, y[b]	0/0	43 (34-51)	45 (35-51)	1.00 (0.97-1.02)	.891
Sex, male	0/0	147 (67)	47 (61)	1.30 (0.76-2.22)	.335
Smoking habit	0/0				
Never smoker		110 (50)	31 (40)	-	
Ex-smoker		24 (31)	60 (27)	0.70 (0.38-1.32)	.267
Current smoker		22 (29)	49 (22)	0.63 (0.33-1.20)	.155
Body mass index, ≥30 kg/m^2[b]		63 (29)	25 (32)	0.84 (0.48-1.28)	.541
Atopy[c]	1/1	106 (49)	38 (50)	0.95 (0.56-1.60)	.836
Chronic rhinosinusitis	1/1	16 (7)	10 (13)	0.52 (0.23-1.25)	.129
Childhood asthma	0/0	15 (7)	10 (13)	0.49 (0.21-1.18)	.101
Exposure before symptom onset, mo[b]	1/1	120 (58-216)	108 (36-212)	1.00 (1.00-1.00)	.814
Duration of asthma symptoms at work, mo[b]	1/1	36 (16-84)	36 (15-58)	1.00 (1.00-1.01)	.163
Interval since last work exposure, mo[b]	0/1	3 .0 (0.1-12.0)	3.0 (0.03-12.0)	0.90 (0.72-1.12)	.344
HMW causal agent (vs LMW agent)	0/0	147 (67)	41 (53)	1.79 (1.05-3.05)	.031[g]
Associated work-related rhinitis	0/0	178 (81)	51 (66)	2.21 (1.23-3.95)	.007[g]
Asthma treatment at work					
ICS use	0/0	170 (78)	47 (61)	2.21 (1.26-3.86)	.005[g]
Daily dose of ICS, µg[b,d]	0/0	500 (250-1000)	500 (0-800)	1.20 (1.03-1.40)	.021[g]
SABA ≥ 1/d at work	0/0	50 (23)	29 (38)	0.49 (0.28-0.86)	.012[g]
≥2 severe exacerbations previous 12 mo at work	0/0	20 (9)	3 (4)	2.48 (0.82-10.74)	.152
Baseline spirometry	0/0				
FVC, % pred[b]		102 (91-110)	102 (91-109)	1.01 (0.99-1.03)	.259
FEV_1, % pred[b]		89 (77-97)	92 (80-101)	1.26 (0.71-2.33)	.445
FEV_1/FVC <0.70		85 (39)	20 (26)	0.96 (0.93-0.99)	.021[g]
Baseline level of NSBH[e]	4/16				
Absent		32 (16)	22 (30)	-	
Mild		109 (54)	35 (48)	2.14 (1.10-4.16)	.024[g]
Moderate-to-severe		62 (31)	16 (22)	2.66 (1.24-5.85)	.013[g]
Pre/post-SIC NSBH ratio >2[b]	13/77	94 (66)	35 (55)	1.62 (0.89-2.97)	.115
Maximum fall in FEV_1, % baseline[b]	0/0	24 (19-33)	22 (19-27)	1.27 (0.99-1.65)	.067
Isolated immediate reaction during the SIC[f]	8/16	76 (37)	30 (43)	0.78 (0.45-1.36)	.375
Baseline blood eosinophil count	18/36				
cells/µL[b]		300 (200-410)	200 (100-296)	1.00 (1.00-1.01)	<.001[g]
>300/µL		98 (54)	15 (25)	3.38 (1.79-6.68)	<.001[g]
Baseline FeNO, ppb[b]	30/142	25 (15-40)	17 (8-28)	1.02 (1.00-1.04)	.042[g]
Post-SIC change in FeNO, ppb[b]	36/145	18 (5-46)	2 (0-13)	1.03 (1.01-1.05)	.002[g]

Abbreviations: FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HMW, high-molecular-weight; ICS, inhaled corticosteroid; LMW, low-molecular-weight; NSBH, nonspecific bronchial hyperresponsiveness; OR, odds ratio; SABA, short-acting ß$_2$-agonist; SIC, specific inhalation challenge.
[a]Data are presented as No. (% of available data) unless otherwise specified.
[b]Median value with interquartile range within parentheses.
[c]Atopy defined as the presence of at least 1 positive skin prick test result to common allergens.
[d]Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.
[e]See Table S1 for the grading of NSBH.
[f]The SIC was considered positive based on a significant increase in the postchallenge level of NSBH (ie, pre/post ratio >2), while the changes in FEV_1 remained <15% in 24 patients.
[g]Statistically significant (*P*<.05).

Table 4. Multivariate Models for Sputum Eosinophilia.[a]

Independent variables	Postchallenge sputum eosinophils ≥3% (n=219/296)			Prechallenge sputum eosinophils ≥3%[b] (n=60/123)		
	OR	(95%CI)	*P* Value	OR	(95%CI)	*P* Value
Final model 1						
Causal agent, HMW vs LMW	1.68	(0.87-3.24)	.119	2.43	(0.86-7.47)	.104
Work-related rhinitis	1.69	(0.83-3.41)	.146	0.35	(0.10-1.15)	.095
ICS daily dose, per 250 µg	1.31	(1.11-1.55)	.002[d]	1.36	(1.09-1.76)	.010[d]
SABA ≥1/d	0.28	(0.14-0.55)	<.001[d]	0.28	(0.09-0.84)	.028[d]
Level of baseline NSBH, vs. no NSBH[b]						
Mild	2.48	(1.21-5.08)	.012[d]	3.64	(0.97-18.01)	.075
Moderate/severe	3.40	(1.44-8.29)	.006[d]	7.74	(1.85-41.72)	.008[d]
Final model 2						
HMW causal agent plus rhinitis at work	2.78	(1.55-5.08)	.001[d]	1.07	(0.45-2.53)	.881
ICS daily dose, per 250 µg	1.32	(1.12-1.57)	.001[d]	1.30	(1.05-1.67)	.024[d]
SABA ≥1/d	0.28	(0.14-0.54)	<.001[d]	0.32	(0.10-0.93)	.042[d]
Level of baseline NSBH, vs no NSBH[c]						
Mild	2.57	(1.25-5.29)	.010[d]	3.54	(0.97-17.10)	.075
Moderate/severe	3.54	(1.48-8.73)	.005[d]	8.83	(2.18-46.42)	.004[d]

Abbreviations: HMW, high-molecular-weight; ICS, inhaled corticosteroid; LMW, low-molecular-weight; NSBH, nonspecific bronchial hyperresponsiveness; OR, odds ratio; SABA, inhaled short-acting β_2-agonist.
[a]Multivariate model 1 incorporated HMW causal agents vs LMW agents and work-related rhinitis (yes/no) as independent variables, whereas model 2 included only the association between an HMW agent and work-related rhinitis. The multivariate models included 275 patients for the analysis of postchallenge sputum eosinophilia and 111 patients for prechallenge sputum eosinophilia.
[b]Multivariate analysis conducted among 123 patients still at work at the time of assessment (see Table S3 in the supplementary materials for univariate analyses of prechallenge eosinophilia).
[c]SeeTable S1 for the grading of NSBH.
[d]Statistically significant (*P*<.05).

15.22; *P*=.002) as a significant factor associated with sputum neutrophilia (Table 5).

Associations Between Causal Agents and Sputum Inflammatory Patterns

Although Table 3 indicates that HMW agents were more frequently involved in patients with an eosinophilic pattern, exposure to an HMW agent was not retained as a significant determinant for sputum eosinophilia in the multivariate analysis (Table 4; multivariate model 1). Interestingly, however, the presence of work-related rhinitis in patients exposed to an HMW agent (Table 4; model 2) was significantly associated with a postchallenge sputum eosinophil count ≥3% (OR, 2.78; 95%CI, 1.55-5.0; *P*=.001), while the dose of ICS, SABA use less than once a day, and NSBH remained significant. In contrast, the multivariate analysis conducted among patients still exposed at work at the time of the SIC procedure (n=123) failed to show an association between prechallenge sputum eosinophilia and exposure to HMW agents, even when associated with work-related rhinitis (Table 4; models 1 and 2).

Table S4 in the online Supplementary Materials provides detailed information on the HMW and LMW agents that elicited asthmatic reactions associated with a neutrophilic pattern (n=23) or a mixed granulocytic pattern (n=18). Notably, HMW agents were involved in 24 of 41 (58.5%) patients with postchallenge sputum neutrophilia ≥76%. Flour was the predominant cause, accounting for 19 of 24 cases related to HMW agents. Interestingly, IgE-mediated sensitization to flour was documented by skin-prick testing and/or determination of specific IgE antibodies in 20 of the 24 patients with HMW-induced sputum neutrophilia.

Discussion

This large cohort study is, to our knowledge, the first to comprehensively characterize the clinical and functional characteristics associated with neutrophilic and eosinophilic airway inflammation in OA.

The multivariate analysis revealed that postchallenge sputum neutrophilia ≥76% was significantly and independently associated with older age, poorer asthma control, more frequent severe asthma exacerbations, and low ICS use during exposure at work. These findings are consistent with studies conducted in general adult asthma populations that documented associations between neutrophilic airway inflammation and age [6-8], more severe disease, and poor asthma control [4,5,12], although sputum neutrophilia was not associated with poor lung function in our cohort of patients with OA [6,8].

While data for this cohort indicated that neutrophilic OA was phenotypically similar to nonoccupational asthma, slight

Table 5. Univariate Associations With Postchallenge Sputum Neutrophilia.[a]

Characteristic	Missing values	Sputum neutrophils ≥76% (n=41)	Sputum neutrophils <76% (n=255)	Univariate analysis	
				OR (95%CI)	*P* Value
Age, y[b]	0/0	46 (37-55)	43 (34-51)	1.02 (0.99-1.06)	.154
Sex, male	0/0	31 (76)	163 (64)	1.75 (0.85-3.91)	.148
Smoking habit	0/0				
Never smoker		17 (41)	124 (49)	-	
Ex-smoker		13 (32)	71 (28)	1.34 (0.60-2.90)	.467
Current smoker		11 (27)	60 (24)	1.34 (0.58-3.00)	.487
Body mass index, ≥30 kg/m^2[b]	0/0	12 (29)	76 (30)	0.97 (0.46-1.97)	.944
Atopy[c]	0/2	24 (59)	120 (47)	1.56 (0.81-3.10)	.189
Chronic rhinosinusitis		6 (15)	20 (8)	2.00 (0.69-5.06)	.166
Childhood asthma	0/0	7 (17)	18 (7)	2.71 (0.99-6.74)	.038[g]
Exposure before symptom onset, mo[b]	0/2	120 (48-240)	120 (48-204)	1.00 (1.00-1.00)	.560
Duration of asthma symptoms at work, mo[b]	1/2	36 (12-52)	36 (16-84)	1.00 (0.99-1.00)	.738
Interval since last work exposure, mo[b]	0/1	5 (0-15)	3 (0-11)	1.19 (0.90-1.59)	.225
HMW causal agent (vs LMW agent)	0/0	24 (59)	164 (64)	0.78 (0.40-1.55)	.476
Associated work-related rhinitis	0/0	28 (68)	201 (79)	0.58 (0.29-1.22)	.138
Asthma treatment at work	0/0				
ICS use		21 (51)	196 (77)	0.32 (0.16-0.62)	.001[g]
Daily dose of ICS, µg[b,d]		250 (0-1000)	500 (250-1000)	0.90 (0.74-1.07)	.249
SABA ≥ 1/d at work		21 (51)	58 (23)	3.57 (1.81-7.08)	<.001[g]
≥2 severe exacerbations previous 12 mo at work	0/0	6 (15)	17 (7)	2.40 (0.82-6.22)	.085
Baseline spirometry:	0/0				
FVC, % pred[b]		103 (91-110)	102 (91-110)	1.00 (0.98-1.02)	.906
FEV_1, % pred[b]		88 (73-96)	90 (79-98)	1.59 (0.78-3.14)	.192
FEV_1/FVC <0.70		16 (39)	89 (35)	0.97 (0.94-1.0)	.163
Baseline level of NSBH[e]	2/18				
Absent		6 (15)	48 (20)	-	
Mild		22 (56)	122 (51)	1.44 (0.58-4.11)	.456
Moderate-to-severe				1.31 (0.47-4.04)	.615
Pre/post-SIC NSBH ratio >2	8/82	15 (45)	114 (66)	0.43 (0.20-0.92)	.029[g]
Maximum fall in FEV_1, % baseline[b]				0.94 (0.68-1.26)	.693
Isolated immediate reaction during the SIC[f]	8/16	22 (67)	84 (35)	3.69 (1.74-8.25)	.001[g]
Baseline blood eosinophil count	7/47				
cells/µL[b]		207 (145-300)	292 (200-400)	1.00 (1.00-1.00)	.065
>300/µL		11 (32)	102 (49)	0.50 (0.22-1.05)	.074
Baseline FeNO, ppb[b]	11/161	16 (9-38)	23 (10-37)	1.00 (0.98-1.01)	.717
Post-SIC change in FeNO, ppb[b]	11/170	4 (1-15)	13 (2-38)	0.99 (0.97-1.00)	.154

Abbreviations: FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HMW, high-molecular-weight; ICS, inhaled corticosteroid; LMW, low-molecular-weight; NSBH, nonspecific bronchial hyperresponsiveness; OR, odds ratio; SABA, short-acting ß$_2$-agonist; SIC, specific inhalation challenge.
[a]Data are presented as No. (% of available data) unless otherwise specified.
[b]Median (IQR).
[c]Atopy defined as the presence of at least 1 positive skin prick test result to common allergens.
[d]Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.
[e]See Table S1 for the grading of NSBH.
[f]The SIC was considered positive based on a significant increase in the postchallenge level of NSBH (ie, pre/post ratio >2)
[g]Statistically significant (*P*<.05).

Table 6. Multivariate Models for Sputum Neutrophilia.[a]

Independent variables	Postchallenge sputum eosinophils ≥3% (n=219/296)			Prechallenge sputum eosinophils ≥3%[b] (n=60/123)		
	OR	(95%CI)	*P* Value	OR	(95%CI)	*P* Value
Final model						
Age, y	1.06	(1.01-1.11)	.014[c]	1.05	(1.00-1.11)	.073
Sex, male	3.34	(1.29-9.99)	.019c	2.75	(0.92-9.77)	.089
Body mass index ≥30 kg/m^2	-			0.32	(0.08-1.00)	.064
No ICS use	5.47	2.09-15.16	<.001[c]	5.09	(1.82-15.22)	.002[c]
SABA ≥1/d	4.09	(1.71-10.01)	.024[c]	-		
Severe exacerbation, ≥2 previous 12 mo at work	4.22	(1.14-14.99)	.025[c]	-		
Isolated early reaction vs late reaction	4.45	(1.85-11.59)	.001[c]	-		

Abbreviations: ICS, inhaled corticosteroid; OR, odds ratio; SABA, short-acting ß$_2$-agonist.
[a]The multivariate models included 271 patients for the analysis of postchallenge sputum neutrophilia and 123 patients for prechallenge sputum neutrophilia among patients still exposed at work.
[b]Multivariate analysis conducted among patients still at work at the time of assessment (see Table S3 in the online supplementary materials for univariate analyses of prechallenge neutrophilia).
[c]Statistically significant (*P*<.05).

differences were also detected compared with the findings of studies conducted in general adult asthma populations. Male sex was associated with an increased likelihood of sputum neutrophilia, while female preponderance has been reported in asthma in the general population [7]. Another intriguing observation was the association between postchallenge sputum neutrophilia and isolated immediate asthmatic reactions. To our knowledge, such an association has not been described after inhalation challenges with common inhalant allergens in individuals with allergic asthma [33].

The mechanisms underlying neutrophilic airway inflammation and its role in asthma remain an area of intense research [34,35]. It is currently acknowledged that neutrophilic inflammation may reflect innate immune responses to environmental triggers, such as ozone, diesel exhaust particulate, and bacterial endotoxin (lipopolysaccharide), which have been reported to induce an increase in sputum neutrophils. Remarkably, a substantial proportion (58.5%) of persons with sputum neutrophilia in our cohort were challenged with HMW protein agents, mainly flour, and IgE-mediated sensitization to these HMW agents was documented in the vast majority (83.3%) (Table S4). This observation is consistent with the findings of an earlier study, which demonstrated neutrophilic airway inflammation in individuals with OA who developed a predominantly immediate asthmatic reaction after challenge with cereal grain dust extracts [36]. Sputum neutrophilia, whether isolated or associated with sputum eosinophilia, was documented in 11% of persons with allergic asthma after an inhalation challenge with nonoccupational inhalant allergens [37]. Endotoxins that likely "contaminate" allergen extracts and HMW workplace agents may promote neutrophilic inflammation via Toll-like receptor-4 signaling [38]. Collectively, these data further indicate that an HMW agent is capable of initiating either T2, non-T2, or mixed immune responses. Further prospective investigations should be performed to determine whether the elicitation of T2 vs non-T2 immune responses is affected by host-related factors (eg, airway microbiome) and environmental factors (eg, endotoxin) that interact with HMW occupational agents.

Most patients with OA (67.9%) demonstrated a postchallenge sputum eosinophilic pattern, which is consistent with what has been described in individuals exposed to common inhalant allergens [37]. However, there is limited information about the determinants of eosinophilic airway inflammation and its relationship with clinical and functional phenotypes in patients with OA. Previous studies in OA-related sputum eosinophilia report more severe disease at the time of the diagnostic work-up. This was associated with worse quality of life [16] and more frequent use of ICS [16] than in noneosinophilic OA in individuals sensitized to LMW agents. In this OA cohort, the eosinophilic pattern was associated with higher doses of ICS and mild disease activity in terms of symptoms, exacerbations, and airway obstruction. Therefore, eosinophilic OA shared common features with the "eosinophilic inflammation–predominant" cluster described by Haldar et al [9] in a secondary care cohort of adult asthma patients. These findings further highlight the possible dissociation between eosinophilic inflammation and asthma symptoms [9].

Previous studies conducted in limited series of patients with OA predominantly exposed to LMW agents, such as isocyanates or Western red cedar, found that sputum eosinophil counts correlated positively with the degree of baseline airflow obstruction [15,16] and a higher level of NSBH [16], although sputum eosinophilia failed to correlate with NSBH in some of these studies [14,15]. Our large cohort study provides definitive evidence that sputum eosinophilia is strongly associated with a higher level of baseline NSBH, consistent with findings for general asthma populations [6,11,39]. In contrast, we failed to document a relationship between sputum eosinophilia and the baseline FEV_1/FVC ratio or FEV_1 [15,16]. Such relationships

might have been blunted in this OA cohort, because spirometry was measured at the baseline SIC, when 58.4% of the patients were no longer exposed to the offending workplace. Nevertheless, analyzing prechallenge sputum eosinophils among individuals still exposed at work within 1 week of the SIC procedure further confirmed that sputum eosinophilia was not associated with the level of airflow obstruction (Table 4 and Table S3).

Remarkably, this cohort study demonstrates that both sputum eosinophilia and neutrophilia develop independently of the molecular weight category of the causal agents, which is in line with the study by Prince et al [17], who compared the changes in sputum cells during SICs with HMW and LMW agents. Nevertheless, in our cohort, HMW agents were significantly associated with postchallenge sputum eosinophilia only when there was coexisting work-related rhinitis. This finding is consistent with the observation that nasal exposure to inhalant allergens enhances eosinophil recruitment into the lower airways in persons with allergic rhinitis [40,41] and may account for the discordant information on the patterns of airway inflammation induced by HMW and LMW agents reported by previous studies that did not take into account the co-occurrence of occupational rhinitis [14,16,19]. Of note, the effect of HMW agents on sputum eosinophilia was not confirmed when prechallenge sputum cells were analyzed in persons still at work at the time of the SIC procedure, even when exposure to HMW agents was associated with work-related rhinitis, suggesting that the synergic effect of rhinitis on lower airway eosinophilia is predominantly apparent during acute exposures to occupational agents, as demonstrated for common allergens [40,41].

The findings of this study challenge the traditional concept of categorizing the agents causing OA into HMW and LMW, presuming implicitly that they act through different underlying pathophysiological mechanisms. Our data provide convincing evidence that the molecular weight category of the causal agent does not determine the pattern of airway inflammation, although HMW and LMW agents are associated with distinct clinical characteristics, especially those pertaining to IgE-related clinical features. Indeed, a previous analysis conducted in a larger sample of the E-PHOCAS cohort that did not take into account sputum inflammatory data demonstrated that OA caused by HMW agents was characterized by a higher rate of work-related rhinitis, atopy, isolated early asthmatic reactions, and a greater postchallenge increase in FeNO compared with OA induced by LMW agents [23]. In addition, recent data from this E-PHOCAS cohort revealed pathophysiological heterogeneity among LMW agents. Compared with other LMW agents, acrylate-induced OA [25] was characterized by factors that are similar to those of OA caused by HMW agents (ie, concomitant work-related rhinitis and a greater postchallenge increase in FeNO), while OA caused by quaternary ammonium compounds was associated with a more marked eosinophilic response than other LMW agents [27]. However, the number of patients with OA caused by most of the LMW agents was too limited in this cohort to allow further comparison of the clinical and inflammatory characteristics between the various types of LMW agents.

The major strengths of this study were the homogeneous diagnostic criteria used to identify OA and the multicenter design, which allowed for the recruitment of a large cohort of patients evaluated by SIC and induced sputum. Nevertheless, several limitations deserve further consideration. The major potential limitation of the study resulted from the use of sputum cell counts obtained 24 hours after challenge exposure to the causal agents as a surrogate for the airway inflammatory profile of patients while exposed at work. However, the multivariate analysis of baseline sputum eosinophilia restricted to those who were still exposed at work at the time of the diagnosis yielded results similar to those of the analysis of postchallenge sputum eosinophilia among the whole cohort (Table 4). In contrast, low ICS use was the only determinant of baseline neutrophilia among patients still at work, while postchallenge neutrophilia was significantly associated with demographic and clinical characteristics (Table 6), suggesting that neutrophil counts after acute exposure to occupational agents may be more clinically relevant than those recorded during long-term exposure at work. Although the multicenter design of this study enabled the recruitment of a large cohort of patients who had undergone assessment of induced sputum, the number of patients with sputum neutrophilia was still low and might not have enabled us to fully capture the influence of potential environmental and host factors and distinguish accurately between neutrophilia alone (n=23) and neutrophilia in combination with sputum eosinophilia (ie, mixed granulocytic pattern, n=18) (see Table 1 and online Supplementary Materials).

The retrospective cross-sectional design did not allow us to determine whether the persistence of sputum eosinophilia despite ICS treatment might result from suboptimal doses of ICS, poor treatment adherence, or mishandling of inhaler devices. Nevertheless, functional stability was established by monitoring FEV_1 on the control day before challenge exposure to the causal agents. Likewise, we were not able to evaluate whether the higher rates of poor asthma control and severe exacerbations in patients with sputum neutrophilia were related to the neutrophilic inflammation per se or to the less frequent use of ICS.

Another limitation of this retrospective multicenter study resulted from the use of slightly different methods to induce and process sputum samples and the lack of quality control. There is conflicting information as to whether variations in the methods may have impacted the differential sputum cell counts [42], although using different nebulizers and saline concentrations does not affect sputum cell counts [43,44]. There were also between-center differences in the bronchoprovocation methods used to assess the level of NSBH; nevertheless, the interpretation of results was standardized for the whole cohort (Table S1).

Conclusion

This large cohort study indicates that sputum inflammatory patterns are associated with distinct clinical phenotypes of OA. However, the associations between sputum inflammatory patterns and phenotypic characteristics in OA share broad similarities with data reported for nonoccupational asthma. These findings may improve our understanding of the pathophysiological mechanisms involved in OA and enhance precision medicine. Nevertheless, the question of

whether inflammatory patterns have an impact on the long-term outcome of the disease should be further addressed in prospective studies. In addition, our data provide definitive evidence that a sensitizing occupational agent can induce OA through different inflammatory pathways, independently of its HMW or LMW category.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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