Original Article

Characterization of Occupational Eosinophilic Bronchitis in a Multicenter Cohort of Subjects with Work-Related Asthma Symptoms

Marta Wiszniewska, MD[a], Perrine Dellis, MD[b], Vera van Kampen, PhD[c], Hille Suojalehto, MD, PhD[d], Xavier Munoz, MD[e], Jolanta Walusiak-Skorupa, MD[a], Irmeli Lindström, MD, PhD[d], Rolph Merget, MD[c], Christian Romero-Mesones, MD[e], Joaquin Sastre, MD[f], Santiago Quirce, MD[g], Paola Mason, MD, PhD[h], Catherine Rifflart, MSc[b], Julien Godet, PharmD, PhD[i], Frédéric de Blay, MD[j], and Olivier Vandenplas, MD, PhD[b], on behalf of the European network for the PHenotyping of OCcupational ASthma (E-PHOCAS)* *Lodz, Poland; Yvoir, Belgium; Bochum, Germany; Helsinki, Finland; Barcelona and Madrid, Spain; Padova, Italy; and Strasbourg, France*

What is already known about this topic? Although several cases of occupational eosinophilic bronchitis have been described, the condition has never been investigated in a large cohort of subjects with work-related asthma symptoms.

What does this article add to our knowledge? The findings indicate that a substantial fraction of subjects without any functional evidence of asthma may develop an isolated sputum eosinophilic response consistent with a diagnosis of occupational eosinophilic bronchitis on exposure to workplace-sensitizing agents.

How does this study impact current management guidelines? This study highlights the relevance of induced sputum analysis in the investigation of work-related asthma symptoms, whereas measurements of fractional exhaled nitric oxide offer a low sensitivity in identifying occupational eosinophilic bronchitis.

BACKGROUND: Occupational eosinophilic bronchitis (OEB) has been described only as anecdotal case reports.
OBJECTIVE: We investigated the clinical and inflammatory characteristics of subjects with OEB identified in a cohort of subjects who completed a specific inhalation challenge (SIC) with occupational agents.
METHODS: In this retrospective multicenter study, OEB was defined by (1) a fall in FEV_1 less than 15% during the SIC and the absence of nonspecific bronchial hyperresponsiveness both before and after the SIC and (2) a postchallenge increase of 3% or more in sputum eosinophils. The subjects who fulfilled these criteria were compared with 226 subjects with a negative SIC and 30 subjects with a positive SIC who failed to show baseline nonspecific bronchial hyperresponsiveness.
RESULTS: An isolated increase in postchallenge sputum eosinophils was documented in 33 of 259 subjects (13%) with a negative SIC. These subjects reported significantly more often an isolated cough at work compared with the negative and positive SIC controls. When compared with positive SIC controls, the subjects with OEB experienced less frequently work-related wheezing and reported a shorter duration of symptoms at work. The sensitivity of the post-SIC increase in fractional exhaled nitric oxide in identifying OEB among subjects with a negative SIC was low, ranging from 43% to 24% using cutoff values of 8 ppb to 17.5 ppb, whereas the specificity was high (90%-97%).
CONCLUSIONS: This study highlights the relevance of induced sputum analysis in the investigation of work-related asthma

[a]Department of Occupational Diseases and Environmental Health, Nofer Institute of Occupational Medicine, Lodz, Poland
[b]Department of Chest Medicine, Centre Hospitalier Universitaire UCL Namur, Université Catholique de Louvain, Yvoir, Belgium
[c]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Ruhr University, Bochum, Germany
[d]Occcupational Health, Finnish Institute of Occupational Health, Helsinki, Finland
[e]Servei Pneumologia, Hospital Vall d'Hebron, Universitat Autonoma de Barcelona and CIBER de Enfermedades Respiratorias (CIBERES), Barcelona, Spain
[f]Department of Allergy, Fundacion Jimenez Dıaz, Universidad Autonoma de Madrid and CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain
[g]Department of Allergy, La Paz University Hospital, IdiPAZ and CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain
[h]Department of Cardiac-Thoracic-Vascular Sciences and Public Health, University of Padova, Padova, Italy
[i]Groupe Méthode Recherche Clinique, Pôle de Santé Publique, Strasbourg University, Strasbourg, France
[j]Division of Asthma and Allergy, Department of Chest Diseases, University Hospital of Strasbourg and Fédération de Médecine translationnelle, Strasbourg University, Strasbourg, France

* The E-PHOCAS investigators are listed in Appendix E1 of this article's Online Repository at www.jaci-inpractice.org.

P.D., C.R., and O.V. were supported by a grant from the *Fondation Mont-Godinne*.
Conflicts of interests: The authors declare that they have no relevant conflicts of interest.
Received for publication June 5, 2020; revised July 31, 2020; accepted for publication August 15, 2020.
Available online September 10, 2020.

Corresponding author: Olivier Vandenplas, MD, PhD, Department of Chest Medicine, CHU UCL Namur, 1 Avenue G. Therasse, B-5530 Yvoir, Belgium. E-mail: olivier.vandenplas@uclouvain.be.

2213-2198

https://doi.org/10.1016/j.jaip.2020.08.056

Abbreviations used

FENO- Fractional exhaled nitric oxide
FVC- Forced vital capacity
HMW- High molecular weight
LMW- Low molecular weight
NAEB- Nonasthmatic eosinophilic bronchitis
NSBH- Nonspecific bronchial hyperresponsiveness
OEB- Nccupational eosinophilic bronchitis
$PC/PD_{15\%-20\%}$- Provocative concentration or dose of the pharmacological agent inducing a 15% or 20% fall in FEV_1
SIC- Specific inhalation challenge

symptoms to identify isolated increases in sputum eosinophils that are consistent with a diagnosis of OEB. **(J Allergy Clin Immunol Pract 2021;9:937-44)**

Key words: *Asthma; Eosinophilic bronchitis; Occupational diseases*

INTRODUCTION

Nonasthmatic eosinophilic bronchitis (NAEB), a well-documented cause of chronic cough, is characterized by sputum eosinophilia without evidence of variable airflow obstruction and nonspecific bronchial hyperresponsiveness (NSBH).[1,2] A similar condition caused by various high-molecular-weight (HMW) and low-molecular-weight (LMW) workplace-sensitizing agents has been described, but only as anecdotal case reports[3-12] (see Tables E1 and E2 in this article's Online Repository at www.jaci-inpractice.org).

The proposed objective criteria for identifying occupational eosinophilic bronchitis (OEB) were (1) a significant (>3%) increase in sputum eosinophil count related to exposure to the offending agent either at work or after a specific inhalation challenge (SIC) in the laboratory and (2) the absence of variable airway obstruction or NSBH on exposure to the offending agent.[5] However, the assessment of sputum eosniophils is not widely available in clinical practice, because sputum induction and processing are time consuming and require technical expertise. In addition, sputum induction is unsuccessful in a substantial fraction (~20%) of the subjects. In contrast, the measurement of fractional exhaled nitric oxide (FENO) as a surrogate marker for eosinophilic airway inflammation is simple, fast, and feasible in almost all patients.[13,14] Although the accuracy of FENO measurement in identifying NAEB has been determined,[15] there is only scarce information on the usefulness of FENO in the clinical investigation of OEB from a few case reports[10-12] (see Table E2).

The aim of this retrospective study was to further delineate the clinical phenotype of subjects who demonstrated an increase in sputum eosinophils without physiological evidence of asthma during an SIC with occupational agents. We also sought to evaluate the validity of FENO measurements in identifying OEB during an SIC.

METHODS

Study design

This retrospective, observational study was conducted among subjects who completed an SIC procedure in 6 of the 20 centers participating in the European network for the PHenotyping of OCcupational ASthma.[16,17] These 6 centers were selected because the assessment of induced sputum was routinely performed before and after an SIC.

Populations

For the purpose of this study, OEB was defined *a priori* by the following criteria[5]: (1) absence of variable airflow obstruction and NSBH both before and after challenge exposure to the suspected workplace agent and (2) a postchallenge increase of 3% or more in sputum eosinophils compared with the baseline value. To identify the subjects who fulfilled these criteria, the following selection process was applied (Figure 1). Over the period from January 2006 to December 2018, a total of 371 subjects with a negative SIC (ie, fall in FEV_1 <15% and <2-fold increase in the postchallenge level of NSBH compared with the baseline value)[18] and available sputum eosinophil count both before and after the SIC procedure were reported. One hundred twelve of these subjects were excluded because of the presence of baseline NSBH, which did allow them to meet the criteria for OEB.[5] This process resulted in 259 subjects with a negative SIC in terms of the changes in FEV_1 and who also failed to show any evidence of NSBH both before and after the SIC procedure. Among these 259 subjects, those who failed to demonstrate sputum eosinophilia were considered as "negative SIC controls" (n = 226).

During the same period, 203 subjects with a positive SIC were identified. Those with a positive SIC response but without a baseline NSBH were regarded as "positive SIC controls" for this analysis (n = 30).

Ethics

Approval by the local institutional review board was obtained in each center.[16,17] The central database at the Strasbourg University was approved by 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*.

Data collection

The data collection process used by the European network for the PHenotyping of OCcupational ASthma has previously been described.[16,17] Briefly, anonymized information on demographic, clinical, occupational, and physiological characteristics of the subjects at the time of the diagnostic evaluation was entered in a standardized Excel spreadsheet in each participating center. These local databases were then checked by 3 investigators (O.V., C.R., and J.D.), pooled together, and centralized at the Strasbourg University.

Demographic and clinical characteristics

Information was gathered on the following: (1) job and suspected offending agent; (2) demographic and clinical characteristics; (3) nature and timing of work-related respiratory symptoms in relation with work exposure; and (4) coexisting disorders.

Lung function assessments

The database collected the baseline prebronchodilator forced vital capacity (FVC) and FEV_1 values measured at the time of the SIC procedure before challenge exposure to the causal agent. The level of

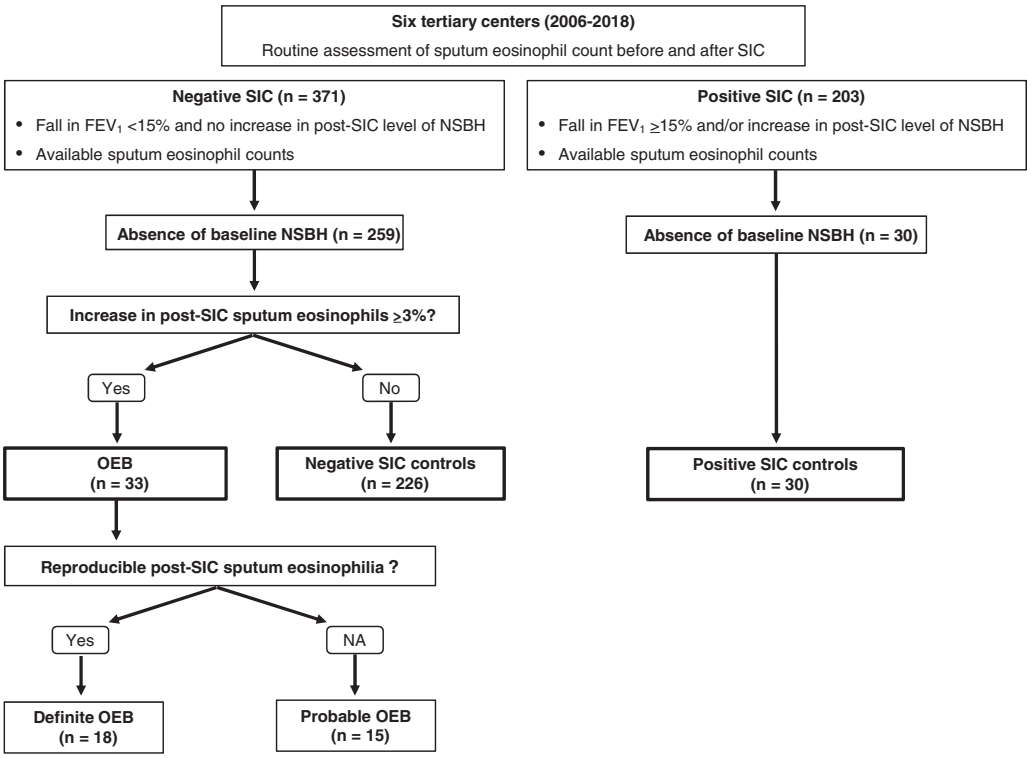

FIGURE 1. Flowchart of the study. *NA*, Not available.

NSBH at baseline and 24 hours after challenge exposure was recorded and expressed as the concentration or dose of the pharmacological agent (histamine or methacholine) inducing a 15% or 20% fall in FEV_1 ($PC/PD_{15\%-20\%}$) according to the bronchoprovocation method used in each center (see Table E3 in this article's Online Repository at www.jaci-inpractice.org). The absence of NSBH was defined on the basais of available recommendations[19-21] or using a consensus Delphi approach among investigators.[16] The absence of a significant increase in postchallenge NSBH was defined by a less than 2-fold decrease in the $PC/PD_{15\%-20\%}$ value measured 24 hours after the challenge exposure as compared with the baseline value.[18,22,23]

Specific inhalation challenge

The methodology of SIC conformed to international recommendations in terms of safety precautions, "placebo" challenge on a separate day, and duration of functional monitoring (ie, at least 6 hours).[18,19] A negative SIC result was defined by a less than 15% fall in FEV_1 at any time during the postchallenge monitoring and a less than 2-fold increase in the postchallenge $PC/PD_{15\%-20\%}$ value.

Sputum eosinophils

Sputum eosinophil counts collected at baseline and 24 hours after challenge exposure were expressed as a percentage of total cell count. Detailed information on the methodology used for sputum induction and processing is available in Appendix E2 of this article's Online Repository at www.jaci-inpractice.org. A postchallenge increase of 3% or more in sputum eosinophil count[24,25] was considered significant. The participating investigators completed an additional questionnaire to evaluate whether the observed increase in sputum eosinophil count and absence of significant change in FEV_1 was confirmed on repeated challenge exposure to the suspected occupational agent.[5] The subjects with a reproducible increase in sputum eosinophils were considered as having "definite OEB," whereas the others were categorized as "probable OEB" (Figure 1).

Fractional exhaled nitric oxide

The F_{ENO} level was measured at baseline and 24 hours post-SIC in 5 of the 6 centers according to the European Respiratory Society and the American Thoracic Society recommendations.[26]

Data analysis

Continuous measures were summarized by medians and interquartile ranges and categorical variables by their frequencies and proportions. The comparison between groups of subjects was made using the Fisher exact or χ^2 test for categorical variables and nonparametric tests for numerical variables (R software 3.4.1; www.r-project.org). Receiver-operating characteristics analysis was conducted to determine the accuracy of the postchallenge increase in F_{ENO} (the post-SIC value minus the baseline value) in predicting the development of an isolated increase in sputum eosinophils (ie, OEB) among subjects who showed a negative SIC result in terms of functional parameters and had available F_{ENO} measurements ($n = 109$). Sensitivity, specificity, positive and negative predictive values, and Youden index (ie, sensitivity + specificity − 1) were calculated for the following cutoff values: (1) the "optimal" cutoff identified as the change in F_{ENO} that yielded the highest Youden index and (2) the value that provided a specificity greater than 95%. A postchallenge increase of more than 17.5 ppb in F_{ENO} was also used because this threshold value has been previously found to provide a high specificity (90%) in predicting the occurrence of an asthmatic reaction during SICs with various occupational agents.[27] *P* values less than .05 were considered significant.

TABLE I. Clinical characteristics and markers of airway inflammation in subjects with OEB compared with negative and positive controls

Characteristic	OEB (n = 33)	Negative SIC controls (n = 226)	*P* value vs OEB	Positive SIC controls (n = 30)	*P* value vs OEB
Age (y)*	46 (41 to 59)	44 (35 to 52)	.120	40 (35 to 52)	.090
Sex: male	23 (70)	134 (59)	.340	18 (60)	.440
Body mass index (kg/m^2)*	27 (26 to 31)	27 (24 to 30)	.390	27 (25 to 32)	.900
Current/ex/never smoker	9 (27)/13 (39)/11 (33)	40 (18)/71 (31)/115 (51)	.150	8 (27)/6 (20)/16 (53)	.170
Atopy†	15 (46)	99 of 222 (45)	>.999	15 (50)	.800
Type of agent, LMW agent	24 (73)	151 (67)	.560	7 (23)	**<.001**
Duration of exposure before symptom onset (mo)*	84 (24 to 170)	120.0 (35 to 228)	.270	132 (77 to 240)	**.040**
Duration of symptoms at work (mo)*	24 (12 to 48)	36 (14 to 84)	.200	55 (24 to 90)	**.030**
Interval of time since last work exposure (mo)*	6 (0.1 to 10)	5 (0 to 13)	.690	8 (0.1 to 20)	.460
Work-related respiratory symptoms					
Cough	26 of 33 (79)	185 of 224 (83)	.630	26 of 30 (87)	.510
Isolated cough	6 of 29 (21)	12 of 220 (5)	**.003**	0 of 30	**.010**
Sputum production	9 of 29 (31)	67 of 221 (30)	>.999	5 of 30 (17)	.230
Wheezing	8 of 30 (27)	80 of 221 (36)	.420	20 of 30 (67)	**.004**
Chest tightness	11 of 32 (34)	50 of 221 (23)	.180	9 of 30 (30)	.790
Shortness of breath	27 of 33 (82)	194 of 224 (87)	.430	30 of 30 (100)	**.030**
Work-related rhinitis	16 of 33 (48)	124 of 226 (55)	.580	26 of 30 (87)	**.002**
Medication					
No treatment	18 of 31 (58)	59 of 200 (30)	**.020**	4 of 30 (13)	**.001**
Short-acting β_2-agonist	10 of 32 (31)	100 of 210 (48)	.090	24 of 30 (80)	**<.001**
Inhaled corticosteroids	12 of 31 (39)	116 of 210 (55)	.120	23 of 30 (77)	**.004**
Coexisting conditions					
Chronic rhinosinusitis	1 (3)	16 (7)	.710	2 (6.7)	.600
Gastroesophageal reflux	2 (6)	23 (10)	.750	NA	NA
Baseline spirometry					
FVC (% predicted)*	99 (90 to 109)	104 (95 to 115)	.160	102 (93 to 116)	.350
FEV_1 (% predicted)*	94 (86 to 108)	101 (91 to 109)	.160	96 (89 to 108)	.370
FEV_1/FVC (%)*	79 (74 to 83)	79 (75 to 84)	.320	82 (79 to 89)	.160
Duration of SIC exposure (min)*	90 (60 to 120)	60 (30 to 90)	.050	48 (30 to 60)	**<.001**
Cough during challenge exposure	25 of 32 (78)	49 of 204 (20)	**<.001**	NA	NA
Blood eosinophils	(n = 28)	(n = 192)		(n = 28)	
Cells/μL*	233 (200 to 304)	200 (100 to 290)	**.010**	200 (200 to 400)	.800
>300/μL	10 (36)	47 (24.5)	.250	13 (46)	.590
Baseline sputum eosinophils					
%*	2 (0 to 4)	2 (1 to 2)	.180	1 (1 to 3)	.810
≥3%	10 (30)	48 (21.2)	.270	9 (30)	>.999
Postchallenge sputum eosinophils					
%*	7 (5 to 12)	1 (0 to 2)	**<.001**	6 (3 to 15)	.330
Change from baseline (%)*	5 (4 to 8)	0 (−1 to 0.5)	**<.001**	4 (2 to 12)	.360
Baseline sputum neutrophils					
(%)*	38 (27 to 68)	48 (29 to 67)	.520	54 (50 to 73)	**.001**
Postchallenge sputum neutrophils					
(%)*	54 (35 to 61)	53 (32 to 71)	.450	46 (41 to 55)	.990
Baseline F_{ENO}	(n = 21)	(n = 89)		(n = 7)	
ppb*	14 (11 to 28)	15 (10 to 21)	.470	13 (10 to 22)	.520
Postchallenge F_{ENO}	(n = 21)	(n = 88)		(n = 7)	
ppb*	24 (16 to 50)	16 (12 to 21)	**.004**	45 (25 to 72)	.120
Change from baseline (ppb)*	4 (1 to 16)	1 (−1 to 4)	**.020**	29 (13 to 40)	**.010**
Change from baseline ≥17.5 ppb	5 (24)	3 (3)	**.006**	4 (57)	.170

NA, Not available.

Data are presented as n (% of available data) unless otherwise specified. Values in boldface are statistically significant ($P < .05$).

*Median value with interquartile range within parentheses.

†Atopy defined by the presence of ≥1 positive skin prick test result to common allergens.

TABLE II. Causal agents of OEB

Causal agent	No. of subjects	Job/industry	Skin prick testing*	Specific IgE*
LMW agents (n = 24)				
Isocyanates				
Methylene diphenyl diisocyanate (MDI)	10	Development of thermoplastic elastomers; mixing of PU components; manufacture of coatings; plastic industry maintenance; PU resin molding; automotive parts manufacture; use of PU glues for windows manufacture or plastic sheets lamination; PU coating in printing process	Negative 8/8	Negative 8/8
Hexamethylene diisocyanate (HDI)	1	Glass painter	Negative 1/1	Negative 1/1
Mixture of isocyanates	2	Luggage manufacture (PU glue); foam rubber production	ND	Positive 1/2
Persulfate salts	2	Hairdressers	Negative 2/2	ND
Disinfectant containing QAC	1	Nurse	ND	ND
Disinfectant containing QAC and glutaraldehyde	1	Nurse	ND	ND
Chloramine-T (*N*-chloro 4-methylbenzenesulfonamide)	1	Chloramine-T powder conditioner	ND	Negative 1/1
Methylchloroisothiazolinone	1	Offset printing	ND	ND
Paraphenylendiamine	1	Hairdresser	Negative 1/1	ND
Styrene	1	Resin lamination worker	ND	ND
Metals (chromium and cobalt)	1	Electonic industry assembler	Negative 1/1	ND
Metal working fluid	1	Blade grinder	ND	ND
Stainless steel welding	1	Welder	ND	ND
HMW agents (n = 9)				
Flour (wheat, rye)	7	Bakers	Positive 7/7	Positive 7/7
Latex	1	Hospital maintenance technician	Negative 1/1	Positive 1/1
Mold (*Penicillium notatum*)	1	Nurse (damp building)	Positive 1/1	Positive 1/1

ND, Not done; *PU*, polyurethane; *QAC*, quaternary ammonium compound (dimethyldidecyl ammonium chloride).
*Expressed as the number of positive or negative test results among those performed.

RESULTS

The demographic, clinical, and functional characteristics of the subjects as well as the results of airway inflammatory markers measurements are summarized in Table I. An isolated increase in postchallenge sputum eosinophils was documented in 33 of the 259 subjects (13%) with a negative SIC who failed to demonstrate NSBH at both baseline and postchallenge assessments. Thus, the prevalence of OEB was 6% (33 of 574) among the whole cohort of subjects evaluated for work-related asthma symptoms and 14% (33 of 236) among those with a work-related respiratory condition confirmed by the SIC procedure during the study period, including occupational asthma and OEB.

The post-SIC increase in sputum eosinophils was confirmed on a repeated challenge in 18 subjects who completed a rechallenge procedure to verify that sputum eosinophilia was reproducible and that the additional challenge did not induce a fall in FEV_1. The clinical and functional charateristics of these 18 subjects with "definite OEB" did not differ from those with "probable OEB" (data not detailed).

Most causal agents involved in the 33 subjects categorized as OEB were LMW compounds (n = 24), with isocyanates accounting for half (n = 13) of these agents (Table II). HMW agents included predominantly wheat and rye flour (n = 7).

The proportion of subjects who reported work-related cough did not differ between OEB and control groups (Table I). However, the subjects who fulfilled the objective criteria for OEB reported significantly more often (21%) an isolated cough at work compared with the negative (5%) and positive (0%) SIC controls (Table I). The SIC exposure to the suspected occupational agents elicited cough in a higher proportion of subjects categorized as OEB (25 of 32; 78%) compared with those who showed a negative SIC (49 of 204; 20%).

In comparison with positive SIC controls, the subjects with OEB experienced less frequently work-related wheezing and rhinitis, were less often treated with asthma medications, and reported a shorter duration of symptoms at work.

Among subjects with available FENO measurements, those who demonstrated an isolated increase in sputum eosinophils (n = 21) showed a slightly greater median (interquartile range) increase in postchallenge FENO (4 ppb; 1-16) than the negative SIC controls (n = 88; 1 ppb; −1 to 4; $P = .020$), but this increase was less marked than that observed in the positive SIC controls (29 ppb; 13-40; $P = .010$). The receiver-operating characteristic curve assessing the association of changes in FENO before and after challenge exposure and the development of an isolated increase in sputum eosinophils among 109 subjects with a negative SIC is illustrated in Figure E1 in this article's Online Repository at www.jaci-inpractice.org. The sensitivity, specificity, and predictive values of different cutoff values for the FENO changes in identifying OEB are summarized in Table III. The

TABLE III. Sensitivty and specificity of the postchallenge increase in FENO in identifying an isolated increase in sputum eosinophils

Threshold value of post-SIC change in FENO	Sensitivity	Specificity	Positive predictive value	Negative predictive value
>8 ppb ("optimal" cutoff)*	43 (24-67)	90 (83-95)	50 (30-70)	87 (83-92)
>14 ppb (cutoff value providing a specificity >95%)*	33 (14-52)	96 (92-100)	70 (43-100)	86 (82-90)
>17.5 ppb†	24 (9-43)	97 (92-100)	82 (29-100)	84 (81-88)

Data are expressed as % with 95% CI between parentheses.
*Threshold values derived from the receiver-operating characteristics curve assessing the effectiveness of postchallenge changes in FENO in predicting the development of an isolated increase in sputum eosinophils among subjects with a negative SIC (area under the curve, 0.65; 95% CI, 0.51-0.82).
†Threshold value that provided a specificity of 90% and a sensitivity of 45% in predicting the development of an asthmatic reaction in a previously published cohort of subjects who completed an SIC with various agents.[27]

sensitivity of the post-SIC increase in FENO was low and decreased from 43% to 24% by increasing the cutoff value from 8 ppb to 17.5 ppb, whereas the specificity increased from 90% to 97%.

DISCUSSION

To our knowledge, this study is the first attempt in identifying the characteristics of subjects who demonstrate an isolated increase in sputum eosinophils after challenge exposure to occupational agents in a large cohort of subjects who completed an SIC procedure. There is currently scarce information on the prevalence of OEB among patients evaluated for work-related asthma symptoms. In 2 small series of workers who completed an SIC procedure for the investigation of work-related asthma symptoms, a diagnosis of OEB was demonstrated through SIC in 3.3% of 30 health care workers exposed to latex[4] and 4.7% of 21 bakers.[5] In a cross-sectional survey of 42 mushroom workers with work-related cough, 7.1% received a diagnosis of eosinophilic bronchitis although the exposure-relatedness of sputum eosinophilia was not evaluated.[28] In our cohort, OEB was documented through an isolated post-SIC increase in sputum eosinophils in 13% of the 259 subjects who failed to demonstrate either variable airflow obstruction or NSBH after challenge exposure to the suspected occupational agent.

The findings of this cohort challenge the current perception that isolated cough is a cardinal feature of OEB.[5] The subjects categorized as OEB in this cohort experienced significantly more often an isolated cough at work than the negative and positive SIC controls, although this symptom was present in only 21% of them. However, this finding is consistent with previously published cases of OEB, indicating that an isolated cough is not a constant feature. In these previous reports, 5 of 12 (42%) subjects reported cough associated with other asthma symptoms (see Table E2). However, subjects with an isolated eosinophilic response reported less frequently wheezing at work and a shorter duration of symptoms before the diagnostic evaluation as compared with those with a positive SIC. These clinical features may suggest that OEB is an early "preclinical" stage of occupational asthma. The relationship between NAEB and nonoccupational asthma remains uncertain. Although both conditions are characterized by eosinophilic airway inflammation, follow-up studies indicated that the outcome of NAEB is most often characterized by recurrent episodes of cough and persistent sputum eosinophilia, whereas typical asthma with variable airway obstruction and/or NSBH develop in less than 10% of affected subjects.[29-31] There have been anecdotal reports of a beneficial effect of inhaled corticosteroids or removal from exposure in OEB, but only over short-term periods (see Table E2). The outcome of OEB could not be further delineated in our cohort because follow-up data were not available. Prospective multicenter studies are required to explore the long-term outcome of OEB and the effects of pharmacological and environmental interventions.

The agents involved in OEB in this cohort (Table II) as well as in previous reports (see Table E1) have also been identified as causing sensitizer-induced occupational asthma. However, some of the LMW agents in this cohort have not previously been documented as causing OEB, including quaternary ammonium compounds, methylchloroisothiazolinone, and paraphenylendiamine. Remarkably, *Penicillium notatum*, a ubiquitous allergen, was involved in the development of OEB in a nurse working in a damp building. The role of nonoccupational inhalant allergens has also been occasionally documented in NAEB.[32,33] These observations suggest that the involvement of environmental allergens in the development of NAEB should be further investigated.

The measurement of FENO has been proposed as a simple tool for diagnosing NAEB. Among individuals with chronic cough, FENO showed a moderate diagnostic accuracy with an estimated sensitivity of 72% and a specificity of 83% in identifying nonoccupational eosinophilic bronchitis.[15] So far, only 3 case reports have described subjects diagnosed as having OEB on the basis of an increase in FENO after SIC or workplace exposure (see Table E2). Our findings indicate that the assessment of FENO should not be regarded as a reliable alternative to sputum analysis in identifying subjects with OEB because of the low sensitivity of the test in this setting, although the specificity was high. These findings further highlight the discordances between sputum eosinophil counts and FENO that have already been documented in nonoccupational[34] and occupational[35] asthma.

Limitations of the study

The strength of this study was the multicenter design of the study, which allowed for gathering a large cohort of patients evaluated by SIC and induced sputum for work-related asthma symptoms. However, several limitations deserve further consideration. A major limitation of this retrospective study is that we were not able to confirm in all subjects that the increase in sputum eosinophils was reproducible and that a longer duration of challenge exposure in the laboratory or at work would have elicited an asthmatic reaction in the subjects categorized as OEB.[24] None of our subjects had been investigated through the analysis of induced sputum collected after a prolonged exposure at work. Nevertheless, the post-SIC increase in sputum eosinophils was documented as being reproducible on repeated challenge in 18 of 33 subjects, and the clinical and functional

charateristics of these subjects with "definite OEB" did not differ from those with "probable OEB." The duration of challenge exposure to the suspected occupational agents during the SIC was longer in subjects with OEB than in those with a negative SIC (Table I), suggesting that the duration of exposure did not account for the absence of changes in FEV_1 in subjects categorized as OEB. This longer duration of exposure probably resulted from the high prevalence of cough elicited during the challenges in subjects with OEB. Indeed, the duration of challenge exposure is usually prolonged when the patients experience respiratory symptoms that may suggest an impending asthmatic reaction.

An influx of eosinophils has been documented after nasal allergen provocation challenge in sputum samples and bronchial biopsies in subjects with nonoccupational allergic rhinitis without NSBH.[36,37] However, it is highly unlikely that work-related rhinitis by itself would explain the isolated increase in sputum eosinophils in subjects who met the criteria for OEB because the prevalence of work-related rhinitis was similar in the subjects classified as OEB (48%) compared with the control subjects with a negative SIC without sputum eosinophilia (55%). Of note, subjects with OEB experienced significantly less frequently work-related rhinitis symptoms and they were more often exposed to LMW agents, compared with those with a positive SIC. This finding is consistent with the lower prevalence of occupational rhinitis among subjects with occupational asthma caused by LMW agents compared with HMW agents.[38]

Another limitation of this retrospective multicenter study resulted from the use of different—though validated—methods for assessing NSBH, sputum cells, and FENO. However, these between-center differences are unlikely to have affected the findings because the interpretation of these methods was standardized for the whole cohort (see Appendix E2 and Table E3 in this article's Online Repository at www.jaci-inpractice.org) and the results of the measurements were compared before and after the SIC using the same method in each participating center.

CONCLUSIONS

This study suggests that a substantial fraction of subjects who fail to demonstrate any functional evidence of asthma during SIC with workplace agents may develop an isolated sputum eosinophilia consistent with a diagnosis of OEB. The findings reinforce the importance of monitoring airway inflammation by means of induced sputum in the investigation of work-related asthma.

Acknowledgment

The authors thank James Hatch for reviewing the manuscript.

REFERENCES

1. Brightling CE. Chronic cough due to nonasthmatic eosinophilic bronchitis: ACCP evidence-based clinical practice guidelines. Chest 2006;129:116S-21S.
2. Diver S, Russell RJ, Brightling CE. Cough and eosinophilia. J Allergy Clin Immunol Pract 2019;7:1740-7.
3. Lemiere C, Efthimiadis A, Hargreave FE. Occupational eosinophilic bronchitis without asthma: an unknown occupational airway disease. J Allergy Clin Immunol 1997;100:852-3.
4. Quirce S, Fernandez-Nieto M, de Miguel J, Sastre J. Chronic cough due to latex-induced eosinophilic bronchitis. J Allergy Clin Immunol 2001;108:143.
5. Quirce S. Eosinophilic bronchitis in the workplace. Curr Opin Allergy Clin Immunol 2004;4:87-91.
6. Yacoub MR, Malo JL, Labrecque M, Cartier A, Lemiere C. Occupational eosinophilic bronchitis. Allergy 2005;60:1542-4.
7. Krakowiak AM, Dudek W, Ruta U, Palczynski C. Occupational eosinophilic bronchitis without asthma due to chloramine exposure. Occup Med (Lond) 2005;55:396-8.
8. Di Stefano F, Di Giampaolo L, Verna N, Di Gioacchino M. Occupational eosinophilic bronchitis in a foundry worker exposed to isocyanate and a baker exposed to flour. Thorax 2007;62:368-70.
9. Barranco P, Fernandez-Nieto M, del Pozo V, Sastre B, Larco JI, Quirce S. Nonasthmatic eosinophilic bronchitis in a baker caused by fungal alpha-amylase and wheat flour. J Investig Allergol Clin Immunol 2008;18:494-5.
10. Pala G, Pignatti P, Moscato G. The use of fractional exhaled nitric oxide in investigation of work-related cough in a hairdresser. Am J Ind Med 2011;54:565-8.
11. Arochena L, Fernandez-Nieto M, Aguado E, Garcia del Potro M, Sastre J. Eosinophilic bronchitis caused by styrene. J Investig Allergol Clin Immunol 2014;24:68-9.
12. Wiggans RE, Barber CM. Metalworking fluids: a new cause of occupational non-asthmatic eosinophilic bronchitis. Thorax 2017;72:579-80.
13. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 2011;184:602-15.
14. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. Respir Med 2014;108:830-41.
15. Song WJ, Kim HJ, Shim JS, Won HK, Kang SY, Sohn KH, et al. Diagnostic accuracy of fractional exhaled nitric oxide measurement in predicting cough-variant asthma and eosinophilic bronchitis in adults with chronic cough: a systematic review and meta-analysis. J Allergy Clin Immunol 2017;140:701-9.
16. Vandenplas O, Godet J, Hurdubaea L, Rifflart C, Suojalehto H, Wiszniewska M, et al. Are high- and low-molecular-weight sensitizing agents associated with different clinical phenotypes of occupational asthma? Allergy 2019;74:261-72.
17. Vandenplas O, Godet J, Hurdubaea L, Rifflart C, Suojalehto H, Walusiak-Skorupa J, et al. Severe occupational asthma: insights from a multicenter European cohort. J Allergy Clin Immunol Pract 2019;7:2309-2318.e4
18. Vandenplas O, Suojalehto H, Aasen TB, Baur X, Burge PS, de Blay F, et al. Specific inhalation challenge in the diagnosis of occupational asthma: consensus statement. Eur Respir J 2014;43:1573-87.
19. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:53-83.
20. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 2000;161:309-29.
21. Sovijarvi AR, Malmberg LP, Reinikainen K, Rytila P, Poppius H. A rapid dosimetric method with controlled tidal breathing for histamine challenge: repeatability and distribution of bronchial reactivity in a clinical material. Chest 1993;104:164-70.
22. Vandenplas O, Delwiche JP, Jamart J, Van de Weyer R. Increase in non-specific bronchial hyperresponsiveness as an early marker of bronchial response to occupational agents during specific inhalation challenges. Thorax 1996;51:472-8.
23. Sastre J, Fernandez-Nieto M, Novalbos A, De Las Heras M, Cuesta J, Quirce S. Need for monitoring nonspecific bronchial hyperresponsiveness before and after isocyanate inhalation challenge. Chest 2003;123:1276-9.
24. Vandenplas O, D'Alpaos V, Heymans J, Jamart J, Thimpont J, Huaux F, et al. Sputum eosinophilia: an early marker of bronchial response to occupational agents. Allergy 2009;64:754-61.
25. Lemiere C, D'Alpaos V, Chaboillez S, Cesar M, Wattiez M, Chiry S, et al. Investigation of occupational asthma: sputum cell counts or exhaled nitric oxide? Chest 2010;137:617-22.
26. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30.
27. Lemiere C, NGuyen S, Sava F, D'Alpaos V, Huaux F, Vandenplas O. Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents. J Allergy Clin Immunol 2014;134:1063-7.

28. Tanaka H, Saikai T, Sugawara H, Takeya I, Tsunematsu K, Matsuura A, et al. Workplace-related chronic cough on a mushroom farm. Chest 2002;122:1080-5.
29. Berry MA, Hargadon B, McKenna S, Shaw D, Green RH, Brightling CE, et al. Observational study of the natural history of eosinophilic bronchitis. Clin Exp Allergy 2005;35:598-601.
30. Park SW, Lee YM, Jang AS, Lee JH, Hwangbo Y, Kim DJ, et al. Development of chronic airway obstruction in patients with eosinophilic bronchitis: a prospective follow-up study. Chest 2004;125:1998-2004.
31. Lai K, Liu B, Xu D, Han L, Lin L, Xi Y, et al. Will nonasthmatic eosinophilic bronchitis develop into chronic airway obstruction? A prospective, observational study. Chest 2015;148:887-94.
32. Ogawa H, Fujimura M, Amaike S, Nishiura Y, Nakagawa-Yoshida K, Suga M, et al. Seasonal chronic cough with sputum eosinophilia caused by Trichosporon cutaneum (*Trichosporon asahii*). Int Arch Allergy Immunol 1998;116:162-5.
33. Bobolea I, Barranco P, Sastre B, Fernandez-Nieto M, del Pozo V, Quirce S. Seasonal eosinophilic bronchitis due to allergy to *Cupressus arizonica* pollen. Ann Allergy Asthma Immunol 2011;106:448-9.
34. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. Lancet Respir Med 2015;3:290-300.
35. Beretta C, Rifflart C, Evrard G, Jamart J, Thimpont J, Vandenplas O. Assessment of eosinophilic airway inflammation as a contribution to the diagnosis of occupational asthma. Allergy 2018;73:206-13.
36. Braunstahl GJ, Overbeek SE, Kleinjan A, Prins JB, Hoogsteden HC, Fokkens WJ. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. J Allergy Clin Immunol 2001;107:469-76.
37. Wang W, Xian M, Xie Y, Zheng J, Li J. Aggravation of airway inflammation and hyper-responsiveness following nasal challenge with *Dermatophagoides pteronyssinus* in perennial allergic rhinitis without symptoms of asthma. Allergy 2016;71:378-86.
38. Vandenplas O, Hox V, Bernstein D. Occupational rhinitis. J Allergy Clin Immunol Pract 2020;8:3311-21.

ONLINE REPOSITORY

APPENDIX E1

List of European network for the PHenotyping of OCcupational ASthma investigators:

- Olivier Vandenplas and Catherine Rifflart (Department of Chest Medicine, Centre Hospitalier Universitaire UCL Namur, Université Catholique de Louvain, Yvoir, Belgium)
- Pavlina Klusackova (Department of Occupational Medicine, General University Hospital, 1st Faculty of Medicine, Charles University, Prague, Czech Republic)
- David Sherson (Department of Pulmonary Medicine and Occupational Medicine, Odense University Hospital, Odense, Denmark)
- Hille Suojalehto, Katri Suuronen, Irmeli Lindström, and Pirjo Hölttä (Occcupational Health, Finnish Institute of Occupational Health, Helsinki, Finland)
- Paula Kauppi (Department of Allergy, Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland)
- Frédéric de Blay, Laura Hurdubaea, and Nicolas Migueres (Division of Asthma and Allergy, Department of Chest Diseases, University Hospital of Strasbourg, Fédération de Médecine translationnelle, Strasbourg University, Strasbourg, France)
- Rolf Merget and Vera van Kampen (Institute for Prevention and Occupational Medicine of the German Social Accident Insurance [IPA], Ruhr University, Bochum, Germany)
- Alexandra M. Preisser and Volker Harth (Institute for Occupational and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany)
- Piero Maestrelli and Paola Mason (Department of Cardiac-Thoracic-Vascular Sciences and Public Health, University of Padova, Padova, Italy)
- Gianna Moscato and Patrizia Pignatti (Department of Public Health, Experimental and Forensic Medicine, Specialization School in Occupational Medicine, University of Pavia, Pavia, Italy)
- Pierluigi Paggiaro and Donatella Talini (Cardio-Thoracic and Vascular Department, University of Pisa, Pisa, Italy)
- Marco dell'Omo and Ilenia Foletti (Department of Medicine, Section of Occupational Medicine, Respiratory Diseases and Occupational and Environmental Toxicology, University of Perugia, Italy)
- Cecilie Svanes and Jorunn Kirkeleit (Department of Occupational Medicine, Haukeland University Hospital, Bergen, Norway)
- Jolanta Walusiak-Skorupa, Marta Wiszniewska, and Agnieszka Lipińska-Ojrzanowska (Department of Occupational Diseases and Environmental Health, Nofer Institute of Occupational Medicine, Lodz, Poland)
- Xavier Munoz and Christian Romero-Mesones (Servei Pneumologia, Hospital Vall d'Hebron, Universitat Autonoma de Barcelona and CIBER de Enfermedades Respiratorias [CIBERES], Barcelona, Spain)
- Joaquin Sastre and Mar Fernandez-Nieto (Department of Allergy, Fundacion Jimenez Dıaz, Universidad Autonoma de Madrid and CIBER de Enfermedades Respiratorias [CIBERES], Madrid, Spain)
- Santiago Quirce and David Loli (Department of Allergy, La Paz University Hospital, IdiPAZ and CIBER de Enfermedades Respiratorias [CIBERES], Madrid, Spain)
- Paul Cullinan and Julie Cannon (Department of Occupational and Environmental Medicine, Royal Brompton Hospital and Imperial College [NHLI], Royal Brompton and Harefield NHS Foundation Trust, London, UK)
- Sherwood Burge and Vicky Moore (Occupational Lung Disease Unit, Birmingham Heartlands Hospital, Birmingham, UK)
- Jennifer Hoyle (Department of Respiratory Medicine, North Manchester General Hospital, Manchester, UK)

APPENDIX E2

Induced sputum analysis

The 6 participating centers completed a detailed questionnaire pertaining to the method used for the induction and analysis of sputum samples.[E1] Sputum was induced through different methods on the basis of inhalation of nebulized isotonic saline (n = 1) or a single concentration of hypertonic solution (ie, 3% [n = 1]) or increasing concentrations from 0.9% or 3% to 4% and 5% (n = 4) of hypertonic solutions for a total maximum duration of 15 to 40 minutes[E2] because it has been shown that differential sputum cell counts are not significantly affected by using different saline concentrations.[E3,E4]

Two methods were used for processing the sputum samples: (1) selecting viscid portions from the expectorate (2 centers)[E5] or (2) using the whole expectorate (4 centers).[E6] There is conflicting information as to whether differential cell counts differ between these 2 methods,[E2] but it is unlikely this has had any effect on the comparison of sputum cell counts before and after the SIC in the same individuals. Homogenization of the sample was achieved by adding dithiothreitol (0.1%) in all centers. All centers applied quality criteria based on the cell viability (ie, at least 40%) and the level of contamination by squamous cells.[E2] The accepted squamous cell contamination was less than 20% in 3 centers, less than 30% in 1 center, and less than 50% in 2 centers. The differential cell count was determined by counting a minimum of 400 nonsquamous cells. The data were collected as the percentage of eosinophils and neutrophils relative to the total number of nonsquamous cells. A blind assessment of the between-center repeatability of the cell-count reading was not performed.

TABLE E1. Agents causing OEB in published case reports

Agent	No. of subjects (n = 12)	Reference
HMW agents		
Alpha-amylase	1*	Barranco et al,[E7] 2008
Lysozyme	1 (4.7%)†	Quirce,[E8] 2004
Natural rubber latex gloves	1 (3.3%)‡	Quirce et al,[E9] 2001
Wheat flour	1	Di Stefano et al,[E10] 2007
	1*	Barranco et al,[E7] 2008
LMW agents		
Acrylate compounds	1	Lemiere et al,[E11] 1997
Ammonium persulfate	1	Pala et al,[E12] 2011
Chloramine-T (*N*-chloro-*p*-toluene sulphonamide)	1	Krakowiak et al,[E13] 2005
Formaldehyde	1	Yacoub et al,[E14] 2005
Isocyanate (methylene diphenyl isocyanate)	1	Di Stefano et al,[E10] 2007
Metal working fluid	1	Wiggans and Barber,[E15] 2017
Stainless steel welding fumes	1	Yacoub et al,[E14] 2005
Styrene	1	Arochena et al,[E16] 2014

The case reported by Kobayashi[E17] was not included in this review because the text is available only in Japanese.
*One subject developed OEB caused by both wheat flour and alpha-amylase.[E7]
†Estimated prevalence of 4.7% among a group of 21 bakers with work-related symptoms.
‡Estimated prevalence of 3.3% among 30 health care workers with work-related symptoms.

TABLE E2. Characteristics of subjects described in published case reports of OEB

Characteristic		No. of subjects with available data (references)
Age (y)	49 (40-52)	12
Sex (female/male)	6/6	12
Smoking habits (never/ex/current)	7/3/2	12
Atopy*	1	6
Total IgE (kU_A/L)†	92 (54-132)	7
Blood eosinophils (cells/μL)†	200 (130-340)	5
Duration of exposure before onset of symptoms (mo)†	102 (86-126)	12
Duration of symptoms at work (mo)†	36 (22-54)	11
Interval between last work exposure and evaluation (mo)	2.6 (1.3-3.0)	7
Duration of challenge exposure during SIC (min)†	30 (30-60)	9
Work-related symptoms		
Cough	12	12
Isolated cough	5	E9,E10,E12,E13
Isolated cough with sputum	2	E7,E15
Cough with other asthma-like symptoms	5	E8,E11,E14,E16
Rhinitis	3	12
Inhaled corticosteroids	5	11
Inhaled short-acting β_2-agonist	2	11
FEV_1 (% predicted)	103 (94-106)	7
FEV1/FVC (%)	78 (78-85)	7
Exposure-related changes in sputum eosinophils	10	10
At/off work only‡	1	E16
Pre/post-SIC only	3	E8,E9,E13
At/off work and pre/post-SIC	6	E7,E10,E11,E14
Exposure-related changes in FENO	3	3
At/off work	2	E15,E16
Pre/post-SIC	1	E12
Absence of work-related changes in peak expiratory flow rates	7	7
Absence of exposure-related changes in NSBH		
At/off work	2	2
Pre/post-SIC	11	11
Outcome		
Resolution of symptoms after ICS for 1-3 mo	3	E7,E9,E10
Resolution of symptoms after removal	1	E10
Resolution of symptoms with ICS and removal	1	E13
Resolution of symptoms with ICS and reduced exposure	1	E15

ICS, Inhaled corticosteroid.
Data are presented as n (%) of available values unless otherwise specified.
*Atopy defined by the presence of ≥1 positive skin prick test result to common allergens.
†Median value with interquartile range within parentheses.
‡No changes in sputum eosinophils and FENO during SIC.

TABLE E3. Methods used for measuring the level of nonspecific bronchial hyperresponsiveness

Method and pharmacological agent (reference)	Threshold values for defining the absence of NSBH
Tidal breath method with histamine[E18,E19]	$PC_{20} > 16$ mg/mL
Tidal breath method with methacholine[E18,E19]	$PC_{20} > 16$ mg/mL
Five-breath dosimeter method with methacholine[E18,E19]	$PD_{20} > 1.5$ mg
$PC_{20} > 16$ mg/mL	
Rapid dosimeter method with histamine[E20]	$PD_{15} > 1.6$ mg
Reservoir bag dosimeter method with methacholine	PD_{20} or PD_{100} sRt > 0.3 mg

PD_{100} sRt, Provocative concentration of pharmacological agent inducing a doubling of specific airway resistance (sRt).

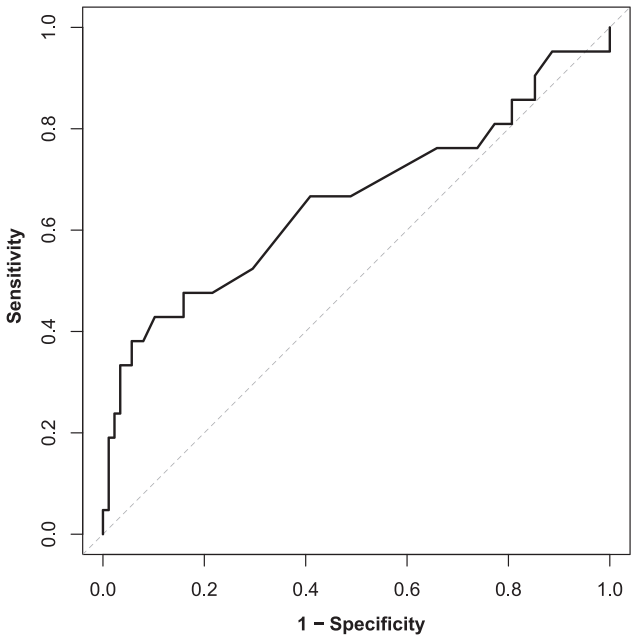

FIGURE E1. Receiver-operating characteristic curve assessing the effectiveness of postchallenge changes in FENO concentration in identifying an increase in sputum eosinophils among subjects with a negative SIC.

REFERENCES

E1. Vandenplas O, Godet J, Hurdubaea L, Rifflart C, Suojalehto H, Wiszniewska M, et al. Are high- and low-molecular-weight sensitizing agents associated with different clinical phenotypes of occupational asthma? Allergy 2019;74:261-72.

E2. Efthimiadis A, Spanevello A, Hamid Q, Kelly MM, Linden M, Louis R, et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. Eur Respir J Suppl 2002;37:19s-23s.

E3. Popov TA, Pizzichini MM, Pizzichini E, Kolendowicz R, Punthakee Z, Dolovich J, et al. Some technical factors influencing the induction of sputum for cell analysis. Eur Respir J 1995;8:559-65.

E4. Bacci E, Cianchetti S, Paggiaro PL, Carnevali S, Bancalari L, Dente FL, et al. Comparison between hypertonic and isotonic saline-induced sputum in the evaluation of airway inflammation in subjects with moderate asthma. Clin Exp Allergy 1996;26:1395-400.

E5. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992;47:25-9.

E6. Fahy JV, Liu J, Wong H, Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. Am Rev Respir Dis 1993;147:1126-31.

E7. Barranco P, Fernandez-Nieto M, del Pozo V, Sastre B, Larco JI, Quirce S. Nonasthmatic eosinophilic bronchitis in a baker caused by fungal alpha-amylase and wheat flour. J Investig Allergol Clin Immunol 2008;18:494-5.

E8. Quirce S. Eosinophilic bronchitis in the workplace. Curr Opin Allergy Clin Immunol 2004;4:87-91.

E9. Quirce S, Fernandez-Nieto M, de Miguel J, Sastre J. Chronic cough due to latex-induced eosinophilic bronchitis. J Allergy Clin Immunol 2001;108:143.

E10. Di Stefano F, Di Giampaolo L, Verna N, Di Gioacchino M. Occupational eosinophilic bronchitis in a foundry worker exposed to isocyanate and a baker exposed to flour. Thorax 2007;62:368-70.

E11. Lemiere C, Efthimiadis A, Hargreave FE. Occupational eosinophilic bronchitis without asthma: an unknown occupational airway disease. J Allergy Clin Immunol 1997;100:852-3.

E12. Pala G, Pignatti P, Moscato G. The use of fractional exhaled nitric oxide in investigation of work-related cough in a hairdresser. Am J Ind Med 2011;54:565-8.

E13. Krakowiak AM, Dudek W, Ruta U, Palczynski C. Occupational eosinophilic bronchitis without asthma due to chloramine exposure. Occup Med (Lond) 2005;55:396-8.

E14. Yacoub MR, Malo JL, Labrecque M, Cartier A, Lemiere C. Occupational eosinophilic bronchitis. Allergy 2005;60:1542-4.

E15. Wiggans RE, Barber CM. Metalworking fluids: a new cause of occupational non-asthmatic eosinophilic bronchitis. Thorax 2017;72:579-80.

E16. Arochena L, Fernandez-Nieto M, Aguado E, Garcia del Potro M, Sastre J. Eosinophilic bronchitis caused by styrene. J Investig Allergol Clin Immunol 2014;24:68-9.

E17. Kobayashi O. A case of eosinophilic bronchitis due to epoxy resin system hardener, methle endo methylene tetrahydro phthalic anhydride. Arerugi 1994;43:660-2.

E18. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:53-83.

E19. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 2000;161:309-29.

E20. Sovijarvi AR, Malmberg LP, Reinikainen K, Rytila P, Poppius H. A rapid dosimetric method with controlled tidal breathing for histamine challenge: repeatability and distribution of bronchial reactivity in a clinical material. Chest 1993;104:164-70.