

Changes in sputum cell counts after exposure to occupational agents: What do they mean?

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Background: Exposure to occupational agents can induce eosinophilic inflammation in subjects with occupational asthma (OA). It might also induce nonspecific changes in airway inflammation in subjects without OA.

Objectives: We sought to investigate the changes in airway inflammation induced by exposure to occupational agents in subjects with and without OA and to determine which changes in sputum eosinophil numbers and bronchial responsiveness to methacholine should be regarded as clinically significant for predicting a 20% fall in FEV₁.

Methods: We performed specific inhalation challenges (SICs) in 3 groups of subjects: subjects reporting a history consistent with OA with a positive SIC response (n = 17); subjects reporting a history consistent with OA with a negative SIC response (n = 14); and asthmatic subjects without any history of OA (n = 10). Induced sputum and methacholine challenges were performed at the end of the control day and again at the end of the last day of exposure; the last day of exposure was always performed in the laboratory.

Results: There was an increase in median sputum eosinophil and neutrophil numbers in subjects with positive SIC responses. Cell counts remained unchanged after exposure in asthmatic subjects without OA. A combination of a greater than 0.26 10⁶/mL increase in sputum eosinophil numbers and a decrease in the concentration of methacholine inducing a 20% fall in FEV₁ of at least 1.8-fold compared with baseline values predicted a 20% fall in FEV₁ in 96% (95% CI, 70%-99%) of patients.

Conclusion: Exposure to occupational agents per se does not induce airway inflammation. Changes in both sputum eosinophil counts and methacholine responsiveness are satisfactory predictors of a significant bronchial responsiveness to occupational agents. (*J Allergy Clin Immunol* 2001;107:1063-8.)

Key words: Occupational asthma, induced sputum, eosinophils, airway hyperresponsiveness

Abbreviations used

HMW:	High molecular weight
LMW:	Low molecular weight
OA:	Occupational asthma
PC ₂₀ :	Concentration of methacholine inducing a 20% fall in FEV ₁
ROC:	Receiver-operating characteristic
SIC:	Specific inhalation challenge
TDI:	Toluene diisocyanate

The pathologic features of occupational asthma (OA) are similar to those of atopic asthma.¹ Exposure to occupational agents can induce eosinophilic inflammation in sensitized subjects.^{2,3} Workers with OA have higher eosinophil counts when at work than workers without OA. This eosinophilia resolves after 3 weeks away from work.⁴ Airway inflammation in OA has been studied mostly in subjects exposed to low-molecular-weight (LMW) agents, which are recognized to induce predominantly late asthmatic reactions.^{3,5-8} An increased number of eosinophils in the airways is often associated with the development of late allergic responses and is not reported as being evoked by immediate asthmatic reactions.^{9,10} However, the nature of inflammatory responses to high-molecular-weight (HMW) agents, which mostly induce isolated immediate reactions, has not been carefully studied to our knowledge.

Obata et al³ have reported an increased number of sputum eosinophils after exposure to red cedar during specific inhalation challenge (SIC) in workers who reported a history suggestive of OA but who had a negative inhalation challenge response. Airway inflammation can be assessed by using bronchoalveolar lavage, bronchial biopsy, or induced sputum tests. Induced sputum tests are being used increasingly in research in the investigation of asthma and OA because of their noninvasive nature. Before using induced sputum in the routine investigation of OA, it is essential to ensure that an increase in eosinophilic airway inflammation cannot be induced nonspecifically by exposure to irritant levels of occupational agents. Indeed, some occupational agents, such as isocyanates, can be irritants at high concentrations; they can even induce reactive airway dysfunction syndrome at high concentrations without sensitization to the agent.¹¹ It is also important to determine the magnitude of

From the Department of Chest Medicine, Hôpital du Sacré-Coeur, Montreal. Supported by the Asthma Society of Canada and Sainte-Jeanne d'Arc Foundation. C. Lemière and J. L. Malo are scholars of the Fonds de la recherche en santé du Québec.

Received for publication December 27, 2000; revised February 14, 2001; accepted for publication February 23, 2001.

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0091-6749/2001 \$35.00 + 0 1/86/115486

doi:10.1067/mai.2001.115486

TABLE I. Characteristics of the subjects studied

	Workers with positive SIC responses	Workers with negative SIC responses	Asthmatic responses without OA
No.	17	14	10
Sex (M)	14	14	2
Age (y)	43.2 $\pm$ 13.7	40.8 $\pm$ 12.0	33.8 $\pm$ 14.4
Patients with atopy	12	10	9
Smoking habits	11 S, 6 NS	7 S, 7 NS	5 S, 5 NS
Agents	HMW, 9; LMW, 8	HMW, 2; LMW, 12	HMW, 5; LMW, 5
Duration of exposure before onset of symptoms (y)	6.3 $\pm$ 9.4	8.1 $\pm$ 12.5	NA
Duration of exposure after onset of symptoms (y)	4.7 $\pm$ 6.5	1.7 $\pm$ 1.2	NA
Duration of asthma (y)	4.7 $\pm$ 6.5	1.7 $\pm$ 1.2	12.8 $\pm$ 10.4*
FEV ₁ (% predicted)	94.4 $\pm$ 14.1	93.4 $\pm$ 18.5	96.1 $\pm$ 16.1
PC ₂₀ (mg/mL)	6.0 $\pm$ 5.0	19.4 $\pm$ 16.6	0.65 $\pm$ 0.4*

PC₂₀ is expressed as geometric mean $\pm$ SD.

S, Smokers; NS, nonsmokers.

* $P \leq .01$.

changes in sputum eosinophils that should be considered clinically significant.

The aim of this study was to investigate the changes in sputum cell counts after exposure to occupational agents in subjects with positive inhalation challenges, subjects with negative inhalation challenges, and asthmatic control subjects without OA and determine which changes in sputum eosinophil numbers and airway responsiveness should be regarded as clinically significant. We also wished to characterize the changes in sputum cell counts after immediate and late asthmatic reactions.

METHODS

Subjects

We included willing, adult subjects who were referred to our center for possible OA over a 2-year period, as well as subjects who had a diagnosis of asthma according to the "Guidelines for the Diagnosis and Management of Asthma,"¹² with a concentration of methacholine inducing a 20% fall in FEV₁ (PC₂₀) of less than 8 mg/mL and who were able to produce sputum. All subjects underwent specific inhalation challenges to occupational agents. The study was approved by the Research Ethics Committee of Sacre-Coeur Hospital, and all subjects gave written informed consent.

SICs

SICs were performed as previously described.¹³ On the control day, spirometry was performed by using a Collins spirometer (W.E. Collins Inc, Braintree, Mass). FEV₁ was measured every 10 minutes for the first hour, every 30 minutes for the second hour, and then hourly for a total of 7 hours to ensure that there was no significant fluctuation. On subsequent days, the subjects were exposed by using a closed-circuit apparatus,^{14,15} allowing an accurate generation of the concentration of particles (flour) or isocyanates ($n = 9$) or, in a realistic way, reproducing as closely as possible the conditions encountered in the workplace ($n = 22$). The duration of exposure to the offending agent was variable and lasted until the occurrence of an asthmatic reaction (20 seconds in the case of the shortest exposure) for up to 2 hours. FEV₁ was measured at the same intervals as on the control day. An SIC response was considered positive if there was a fall in FEV₁ of 20% or greater. In an exposure chamber 5 asthmatic subjects without OA were exposed to flour and 5 to cyanoacrylate, a commonly used glue, in a realistic way for 2 hours. We chose these agents because people are likely to be exposed to

them in their daily lives. Methacholine challenge¹⁶ and sputum induction¹⁷ were performed at the end of the control day and at the end of the last day of exposure, as previously described.

Skin testing

Skin tests to occupational agents (flour, animals, or latex) or to common inhalants were performed with commercial extracts (Omega, Montreal, Quebec, Canada). A positive reaction was defined as a wheal diameter of 3 mm or larger in the absence of reaction to a control diluent and with a positive reaction to histamine phosphate (1 mg/mL). A subject was considered atopic if at least one skin prick test response to common inhalants was positive.

Sputum induction and processing

Sputum induction was performed after each methacholine challenge. Subjects inhaled 200 μ g of salbutamol and, subsequently, aerosols of hypertonic saline at increasing concentrations (3%, 4%, and 5%) generated by a Fisoneb ultrasonic nebulizer (Canadian Medical Products Ltd, Markham, Ontario).¹⁷ Subjects inhaled each of the concentrations of saline for 7 minutes. They were instructed to cough into a sterile container, and the expectorate was processed within 2 hours.

Sputum examination was performed as previously described.¹⁸ Briefly, sputum was selected from the expectorate, and a weighed aliquot was dispersed with 4 volumes of freshly prepared dithiothreitol (Sputalysin 10%; Calbiochem Corp, San Diego, Calif). The mixture was briefly vortically spun, and after being rocked at room temperature for 15 minutes, 4 volumes of Dulbecco's PBS (Gibco Diagnostics, Tucson, Ariz) were added, after which the dispersed sample was filtered through a 48- μ m nylon mesh. Total cell count and cell viability of the filtrate were measured by using trypan blue exclusion, and cytopsin preparations were made (Cytopsin 3 cyto-centrifuge; Shandon Southern Instruments, Sewickley, Pa). One cytopsin preparation was dried and Wright-stained for a differential cell count of 400 cells, excluding squamous cells.

Data analysis

Results were expressed as means and SD, except for data with nonnormal distribution, which were expressed as medians and interquartile ranges. The paired t test and the Wilcoxon signed-rank test were used to compare PC₂₀ and cell counts before and after SICs. The Mann-Whitney test was used to compare cell counts between groups. Correlations between sputum eosinophils, PC₂₀, and maximum fall in FEV₁ were examined by using a Spearman rank-order test. To determine the cutoff of changes in sputum

TABLE II. Total and differential cell counts before and after exposure to occupational agents

	Workers with positive SIC responses		Workers with negative SIC responses		Asthmatic subjects without OA	
	Baseline	After exposure	Baseline	After exposure	Baseline	After exposure
TCC (10 ⁶ /mL)	2.0 (1.5)	6.5 (11.2)*	2.7 (5.5)	3.5 (2.7)	2.2 (1.9)	1.9 (1.1)
Eosinophils (%)	0.5 (2.0)	9.0 (18.5)*	1.0 (1.1)	2.5 (10.7)*	2.6 (4.1)	2.0 (34.0)
Eosinophils (10 ⁶ /mL)	0.02 (0.04)	0.8 (0.9)*	0.04 (0.06)	0.09 (0.2)*	0.04 (0.1)	0.03 (0.05)
Neutrophils (%)	29.5 (41.9)	58.0 (30.0)	41.3 (58.9)	51.0 (41.7)	26.0 (12.0)	32.6 (36.0)
Neutrophils (10 ⁶ /mL)	0.5 (0.9)	3.1 (5.4)*	0.7 (3.6)	1.9 (1.9)	0.4 (0.8)	0.5 (0.8)
Macrophages (%)	68.2 (44.4)	27.0 (27.6)*	54.0 (42.4)	42.5 (30.3)	64.4 (21.4)	58.8 (37.4)
Macrophages (10 ⁶ /mL)	1.0 (1.3)	1.4 (3.2)	1.3 (1.5)	1.1 (1.4)	1.3 (0.8)	0.9 (1.3)
Lymphocytes (%)	0.7 (1.0)	0.2 (0.9)	0.4 (1.0)	1.0 (1.5)	1.6 (2.1)	0.9 (2.2)
Lymphocytes (10 ⁶ /mL)	0.01 (0.02)	0.01 (0.04)	0.008 (0.02)	0.02 (0.07)	0.03 (0.04)	0.009 (0.05)

Results are expressed as median (interquartile range).

TCC, Total cell count.

* $P < .05$ between baseline values and values after exposure to occupational agents.

eosinophil numbers and airway responsiveness, which were the most discriminating for predicting a 20% fall in FEV₁ after exposure to sensitizers, we built a receiver-operating characteristic (ROC) curve. We performed logistic regression with maximum percentage fall in FEV₁ after exposure to occupational agents as the dependent variable to examine the relationship between maximum fall in FEV₁ after exposure to the occupational agent and changes in sputum eosinophil numbers and airway responsiveness. Maximum percentage fall in FEV₁ was expressed as a binary variable and was coded 0 if the fall was below 20% and 1 if the fall was above 20%. Changes in sputum eosinophil numbers and airway responsiveness were also expressed as binary variables and coded 0 if the changes were below the cutoffs determined according to the ROC curves and 1 if the changes were above those cutoffs. We also calculated the probability and the 95% CI of a 20% fall in FEV₁ for each combination of changes in eosinophils and airway responsiveness: both below the cutoff; both above the cutoff; and changes in either eosinophils or PC₂₀ above the cutoff. Significance was accepted at a level of 95%. The analysis was performed by using the statistical package SPSS 9.0, SAS for logistic regression, and Rockit 0.9B (developed by Charles Metz University of Chicago) for ROC curves.

RESULTS

Forty-one subjects were included: 17 with positive SIC responses; 14 with negative SIC responses; and 10 with moderate personal asthma but without OA.

At the time of the study, only 4 of the 14 subjects with negative SIC responses were still exposed to the suspected work-related agent and were complaining of symptoms at the workplace. The other 9 patients had been removed from exposure for 0.3 ± 0.3 years, whereas subjects with positive SIC responses had not been exposed for 0.5 ± 0.8 years, which is not statistically different. Among the 17 workers who had a positive SIC response, 8 were exposed to LMW agents and 9 to HMW agents. Ten experienced an immediate asthmatic reaction, and of these, 8 were exposed to HMW agents (flour, 4; barley, 1; oat, 1; cat, 1; and guinea pig, 1) and 2 to LMW agents (toluene diisocyanate [TDI] and phenylmethylenediisocyanate). Seven experienced an early late asthmatic reaction, and of these, 6 were exposed to LMW agents (2 to hexamethylene diisocyanate, 2 to TDI, 1 to phenylmethylenediisocyanate, and 1 to cyanoacrylates), and 1 was

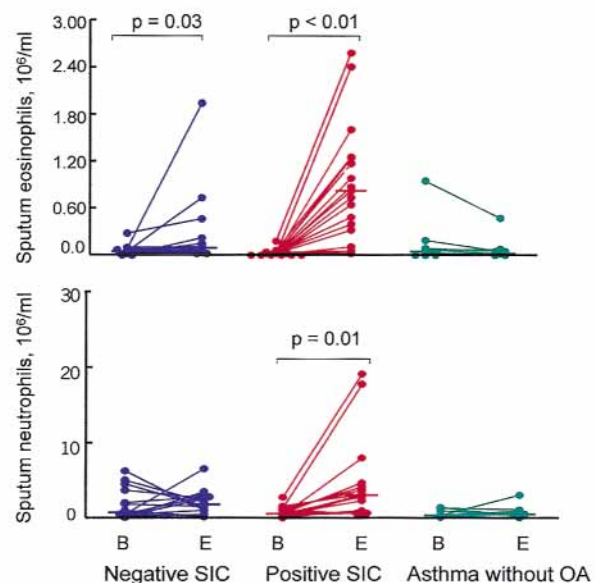


FIG 1. Changes in absolute sputum eosinophil and neutrophil counts after exposure to occupational agents in subjects with history suggestive of OA with negative (blue lines) and positive SIC responses (red lines) and asthmatic subjects without OA (green lines). Medians are represented by horizontal lines.

exposed to HMW agents (flour). A total of 7 subjects were exposed to isocyanates. The characteristics of the subjects are presented in Table I.

Sputum cell counts

Results are reported in Table II. Subjects with positive SIC responses had a significant increase in sputum eosinophil (median [interquartile range (IQR)]: $9.0 [18.5] \times 10^6/\text{mL}$) and neutrophil (median [IQR]: $3.1 [5.4] \times 10^6/\text{mL}$) numbers after exposure to the occupational agents compared with baseline numbers (median [IQR]: $0.02 [0.04] \times 10^6/\text{mL}$, $P < .01$; and $0.5 [0.9] \times 10^6/\text{mL}$; $P = .01$, respectively; Fig 1). There was a significant decrease in the percentage of macrophages after exposure compared with that on the control day ($P = .04$), but the absolute count remained unchanged. There was also a significant increase

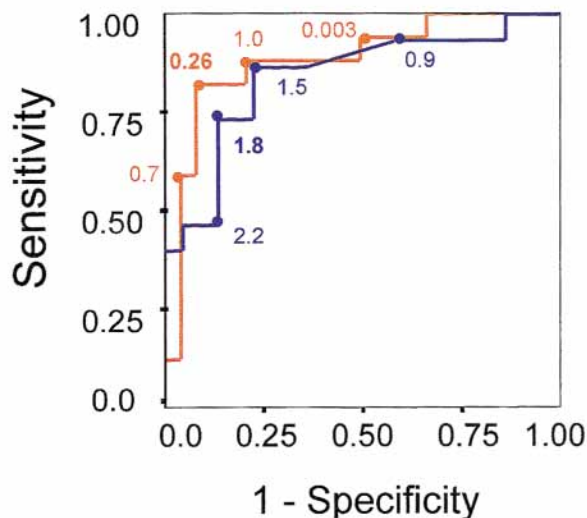


FIG 2. ROC curves. Blue dots represent the ratio of PC₂₀ between exposure day and control day; orange dots represent the difference in sputum eosinophil numbers between exposure day and control day. The most relevant cutoffs are in bold.

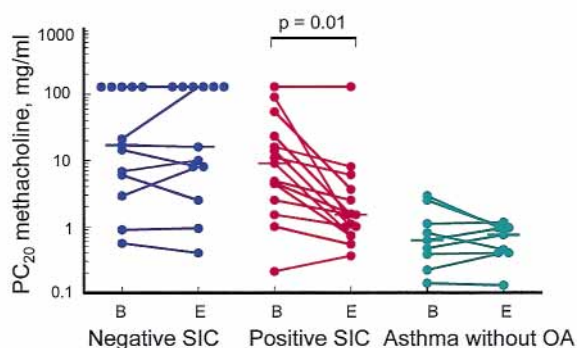


FIG 3. Changes in methacholine PC₂₀ after exposure to occupational agents in subjects with history suggestive of OA with negative (blue lines) and positive (red lines) SIC responses and asthmatic subjects without OA (green lines). Medians are represented by horizontal lines.

in sputum eosinophil numbers and percentage among subjects with negative SIC responses, although it was less prominent than among workers with positive SIC responses. There was no change in neutrophil numbers after exposure in workers with negative SIC responses.

A clinically significant increase in sputum eosinophil numbers occurred in 2 of 14 subjects with negative SIC responses, in 13 of 17 subjects with positive SIC responses, and in none of the subjects without OA. We considered an increase in sputum eosinophil numbers to be clinically significant if it measured at least 0.26×10^6 eosinophils/mL after exposure compared with baseline values. We set this cutoff by using ROC curves that are detailed in the following section. Total and differential cell counts remained unchanged after exposure to occu-

pational agents in asthmatic subjects not sensitized to the occupational agents.

Difference in cell counts between immediate and late asthmatic reaction

Percentage and absolute numbers of eosinophils were higher on the day of asthmatic reaction after both immediate (median [IQR]: 14.5% [21.3%], $1.0 [1.5] \times 10^6/\text{mL}$) and late (median [IQR]: 7.5% [8.0%], $0.8 [0.5] \times 10^6/\text{mL}$) asthmatic reactions than values on the control day (median [IQR]: 0.50% [2.0%], $0.02 [0.04] \times 10^6/\text{mL}$; and 0.4% [2.1%], $0.007 [0.04] \times 10^6/\text{mL}$, respectively), but these increases were not greater after late asthmatic reactions than after immediate reactions ($P = .5$ and $P = .8$, respectively). The increase in neutrophil numbers was similar after immediate (median [IQR]: $1.9 [3.8] \times 10^6/\text{mL}$) and late (median [IQR]: $2.8 [7.6] \times 10^6/\text{mL}$) asthmatic reactions. We did not find any significant difference in sputum cell composition after occurrence of isolated asthmatic reactions compared with late asthmatic reactions.

ROC curves

Using ROC curves, we calculated a cutoff for predicting a 20% fall in FEV₁ for the most relevant changes in sputum eosinophil number and airway responsiveness compared with baseline values (Fig 2). The area under the curve for difference in sputum eosinophil numbers (0.88 [0.06]) and ratio between PC₂₀ before and after challenge (0.84 ± 0.07) were equally sensitive and specific for predicting a 20% fall in FEV₁ ($P = .3$). The best cutoff for predicting a 20% fall in FEV₁ was an increase of at least $0.26 \times 10^6/\text{mL}$ in the number of sputum eosinophils compared with baseline values (sensitivity, 82%; 1 – specificity, 8.3%) and at least a 1.8-fold decrease in PC₂₀ compared with baseline values (sensitivity, 73%; 1 – specificity, 14%).

Prediction of occurrence of a 20% fall in FEV₁ by looking at changes in sputum eosinophil numbers and changes in PC₂₀

There was a correlation between the maximum fall in FEV₁ and the changes in sputum eosinophils ($\rho = 0.5$, $P < .001$) and neutrophils ($\rho = 0.6$, $P < .001$) on one hand and the changes in PC₂₀ ($\rho = -0.6$, $P < .001$) on the other hand. There was a weak negative correlation between changes in the number of sputum eosinophils and neutrophils and changes in airway responsiveness ($\rho = -0.4$, $P = .03$ and $\rho = -0.4$, $P = .01$, respectively). There was a significant decrease in PC₂₀ (1.9 ± 0.22 mg/mL) after exposure to occupational agents compared with baseline values (6.0 ± 5.0 mg/mL, $P = .01$) in subjects with positive SIC responses only (Fig 3).

Table III summarizes the results of multiple logistic regression analyses of the potential determinants of FEV₁ fall after exposure to occupational agents. We found that changes in sputum eosinophils greater than $0.26 \times 10^6/\text{mL}$ and changes in airway responsiveness greater than 1.8-fold explain some of the variation in the maxi-

mum FEV₁ fall. However, the relatively low number of subjects in each group results in a wide range in the CIs for the odds ratios. We could not calculate an interaction between changes in sputum eosinophils and changes in PC₂₀ because of the small number of subjects with a fall in FEV₁ and no changes in number of sputum eosinophils and PC₂₀. A concomitant change in PC₂₀ and eosinophils gives a 96% (95% CI, 70%-99%) probability of at least a 20% fall in FEV₁, no change in both parameters gives a probability of occurrence of 20% fall in FEV₁ of 4% (95% CI, 0.5%-26%), and a change in sputum eosinophils of at least 0.26 × 10⁶/mL with a decrease in PC₂₀ of less than 1.8-fold gives a probability of occurrence of 20% fall in FEV₁ of 66% (95% CI, 26%-91%), whereas a decrease of at least 1.8-fold in PC₂₀ without change in sputum eosinophil numbers gives a probability of occurrence of 20% fall in FEV₁ of 38% (95% CI, 12%-74%).

DISCUSSION

This study shows that both sputum eosinophils and neutrophils are increased in most of the subjects after exposure to LMW or HMW agents, in whom a 20% fall in FEV₁ was observed. These findings are consistent with previously published studies showing an increase of eosinophils and neutrophils after asthmatic reactions induced by occupational agents.^{2,3,6,19-21} However, those studies investigated OA caused by LMW agents and OA caused by grain dust, the pathophysiology of which is probably more complex than that of OA caused by other HMW agents, such as flour. This is the first time, to our knowledge, that an increase in both eosinophil and neutrophil numbers is demonstrated after exposure to HMW agents inducing isolated immediate reactions. We did not find a more prominent eosinophilic inflammation after late rather than immediate asthmatic reactions. These findings are consistent with previously made observations in atopic asthmatic subjects in whom both eosinophil and neutrophil numbers were increased after the occurrence of both immediate and late asthmatic reactions induced by challenges to common inhalants.²²

We also found that 2 workers with negative challenge responses had a significant increase in sputum eosinophil numbers after exposure to occupational agents. One of these 2 subjects clearly had occupational rhinitis, and it is known that subjects with rhinitis also show airway inflammation and remodeling.^{23,24} In such subjects the exposure to occupational agents might also induce an increase in eosinophil numbers. Eosinophilic bronchitis is typically seen in asthma when asthma is defined physiologically as variable airflow obstruction. However, eosinophilic bronchitis can occur without the physiologic abnormalities of asthma, as described by Gibson et al.²⁵ Eosinophilic bronchitis without asthma can also appear after exposure to occupational agents, such as acrylates.²⁶ Exposure to other occupational agents may also induce the same condition in the absence of asthma. Increased sputum eosinophil numbers could also be explained by the occurrence of false-negative challenges in some workers. Indeed, we might

TABLE III. Logistic regression

Covariates	Odds ratio	95% CI
Changes in sputum eosinophil numbers	45.7	4.1-508.6
Changes in airway responsiveness	14.6	1.4-155.7

The dependent variable is the fall in FEV₁ after exposure to occupational agents (above or below 20%). Changes in sputum eosinophil numbers are scored as 0 if changes are 0.26 × 10⁶/mL or less and 1 if greater than 0.26 × 10⁶/mL. Changes in airway responsiveness are scored as 0 if changes in PC₂₀ are 1.8-fold or less of the baseline value and 1 if changes in PC₂₀ are greater than 1.8-fold of the baseline value. Hosmer-Lemeshow goodness of fit: χ^2 , 3.14; P = .2.

have missed the diagnosis of OA in these subjects because we did not expose them long enough. Increased duration of exposure might have induced an asthmatic reaction in those subjects who already had an eosinophilic inflammation because it has been previously demonstrated that an increase in sputum eosinophil numbers precedes the occurrence of asthma exacerbations.^{27,28}

At the time of the study, only 4 of the 14 workers with negative SIC responses were still exposed to the offending agent at their workplace. However, it is unlikely that the SIC responses were falsely negative because the workers had been removed from exposure before the challenge. Indeed, we previously showed that the majority of workers who had a diagnosis of OA in the past and who became asymptomatic after removal from exposure had asthmatic reactions almost immediately on re-exposure to the relevant agent.²⁹ Hence we believe that the vast majority of the workers with negative SIC responses did not have OA.

It is unlikely that the increase in eosinophil numbers compared with baseline values is due to a nonspecific effect of occupational agents. We did not find any change in sputum cell composition after exposure to occupational agents (flour and acrylates) in asthmatic subjects not sensitized to those agents. We exposed asthmatic subjects with moderate airway responsiveness to the maximal dose we usually used for subjects with a history suggestive of OA. If exposure to occupational agents induced a nonspecific inflammatory effect, we are confident that it would have been detected. We did not expose these subjects to isocyanates; however, a previous study showed no change in sputum eosinophil numbers after exposure to TDI in subjects without OA.²

Although there was a significant increase in sputum neutrophil numbers after positive SIC responses, this increase seems more variable. Sputum neutrophil numbers have been shown to increase after exposure to isocyanates. It might be possible that this increase be more related to the type of agent than the increase in sputum eosinophil numbers, which occurred in almost all subjects with positive SIC responses.

We found that an increase of 0.26 × 10⁶/mL in sputum eosinophil numbers and a 1.8-fold decrease in PC₂₀ were the most discriminating cutoffs for predicting the occurrence of a 20% fall in FEV₁. Normal values of sputum eosinophils have been established at 0.4% ± 0.9%³⁰; however, to our knowledge, this is the first time that an effort to determine what increase in sputum eosinophil

numbers should be regarded as clinically significant has been made. Changes in PC₂₀ methacholine values before and after SICs or between periods at work and away from work are usually considered clinically significant when they exceed 2-fold dilutions of methacholine.^{31,32} These cutoffs have been chosen because the variability of PC₂₀ performed on the same subjects on 2 occasions did not exceed 2-fold dilutions. The cutoff of 1.8-fold dilutions that we established in our study with ROC curves is thus very close to the cutoff reported in the literature.

These findings give a hint for interpretation of changes in eosinophils and PC₂₀ for future studies to be performed in the workplace. Indeed, workers in whom an increase of at least $0.26 \times 10^6/\text{mL}$ sputum eosinophils is combined with a decrease in PC₂₀ of at least 1.8-fold when at work compared with periods away from work are likely to have a 20% fall in FEV₁ when exposed to the relevant agent; in other words, they are likely to have OA. Maximum fall in FEV₁ is partly explained by both changes in sputum eosinophil numbers and changes in PC₂₀. Because there is evidence that eosinophilic inflammation and airway responsiveness are not closely linked,^{33,34} these parameters are likely to reflect different and complementary components of the asthmatic disease. Changes in both sputum eosinophil numbers and airway responsiveness are satisfactory predictors of specific bronchial responsiveness to occupational agents. Therefore the findings of the current study emphasize the need to evaluate both airway responsiveness and airway inflammation in the investigation of OA.

We thank Dr Lucie Blais and Lori Schubert for reviewing the manuscript.

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