

Original Article

Diagnostic Accuracy of Inflammatory Markers for Diagnosing Occupational Asthma

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What is already known about this topic? The lack of airway hyperresponsiveness makes the diagnosis of occupational asthma (OA) very unlikely in workers still exposed to the offending agent. Sputum eosinophil count is an effective aid to the diagnosis of OA.

What does this article add to our knowledge? In contrast to its utility in the management of asthma, blood eosinophil counts do not appear to be a reliable surrogate marker of airway inflammation to be used in the investigation of OA.

How does this study impact current management guidelines? Performing both sputum induction and methacholine challenge before and after exposure to the offending agent may represent an effective alternative to a specific inhalation challenge for diagnosing OA when this test is unavailable.

BACKGROUND: The assessment of airway responsiveness and inflammation is key to the investigation of occupational asthma (OA).

OBJECTIVE: We sought to assess and compare the diagnostic accuracies of the blood and sputum eosinophil counts and the methacholine challenge for the diagnosis of OA.

METHODS: We conducted a retrospective study assessing 618 patients who underwent specific inhalation challenges (SICs) for symptoms suggestive of OA between 2000 and 2015. A sputum induction and a methacholine challenge were performed before and after SICs. Blood samples were collected in all subjects before the SICs and in 100 subjects before and after SICs. The diagnostic accuracies of blood and sputum eosinophil counts and methacholine challenge were calculated for diagnosing OA. **RESULTS:** The change in blood eosinophil count failed to differentiate workers with positive and negative SICs. The change in sputum eosinophil counts induced by the exposure to

the offending agent had the highest diagnostic accuracy (receiver operating characteristic area under the curve: 86% [95% confidence interval: 0.8-0.9, $P < .001$]) for diagnosing OA compared with changes in concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second (PC_{20}) and blood eosinophils. Combining a 2-fold or greater decrease in PC_{20} or a 3% or greater increase in sputum eosinophil count achieved a sensitivity of 84% and a specificity of 74% with a negative predictive value of 91% for the diagnosis of OA.

CONCLUSIONS: Blood eosinophil counts do not appear to be an effective aid for diagnosing OA. The performance of both sputum cell count analysis and a methacholine challenge before and after exposure to the offending agent may represent an effective alternative in diagnosing OA when SICs are unavailable. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;■:■-■)

Key words: Occupational asthma; Blood eosinophil count; Sputum eosinophil count; Airway responsiveness

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Occupational asthma has been defined as a type of asthma caused by conditions attributable to the workplace and not to causes outside the workplace.¹ Occupational asthma can be caused by the sensitization to a specific agent at work ("allergic occupational asthma" or "sensitizer-induced occupational asthma")—hereafter referred to as occupational asthma (OA)—or by the acute or chronic inhalation of high to moderate concentration of irritant agents ("irritant-induced asthma").² The diagnosis of allergic OA requires a comprehensive investigation involving the performance of different and complementary tests. Assessing airway responsiveness and airway inflammation is key to the investigation of OA. The lack of airway hyperresponsiveness makes the diagnosis of OA very unlikely in workers who are still exposed to the offending agent.³ Sputum eosinophil counts have been shown as an effective aid to the investigation of OA during specific inhalation challenges

Abbreviations used

AUC- Area under the curve
 CI- Confidence interval
 HMW- High-molecular weight agent
 ICS- Inhaled corticosteroids
 IQR- Interquartile ranges
 LMW- Low-molecular weight agent
 OA- Occupational asthma
 PC₂₀- Concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second
 ROC- Receiver operating characteristic
 SIC- Specific inhalation challenge

(SICs)^{4,5} because exposure to the offending agent induces an increase in sputum eosinophil counts in the majority of workers with OA.⁶ Furthermore, changes in airway inflammation precede changes in airway caliber after exposure to the offending agent.⁷ However, sputum induction and processing remains a cumbersome procedure that is not widely available. In contrast, blood eosinophil counts are not only widely available but also easy to perform. They have been shown to be reasonably well correlated with sputum eosinophil counts.^{8,9} Blood eosinophil counts have been increasingly used as a surrogate marker of airway inflammation over the past 10 years. Blood eosinophil counts can identify eosinophilic phenotypes of asthma quite accurately when blood eosinophil counts are higher than $0.45 \times 10^9/L$.¹⁰ Blood eosinophil counts higher than $0.15 \times 10^9/L$ seem to predict a good clinical response to some of the new biological agents.¹¹ However, to our knowledge, the diagnostic accuracy of blood eosinophil counts has never been assessed and compared with the current tests used in the investigation of OA.

The aim of this study was to assess and compare the respective diagnostic accuracies of blood eosinophil counts, sputum eosinophil counts, and airway responsiveness to methacholine for diagnosing OA using an SIC as the standard of reference.

MATERIALS AND METHODS

Definitions

OA was defined as a positive SIC in the laboratory or at the workplace.

Non-OA was defined as a diagnosis of asthma based on a reversible airflow limitation or a concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second (PC₂₀) equal to or lower than 16 with a history suggestive of asthma with negative SICs.

No functional evidence of asthma referred to subjects without airflow limitation and with PC₂₀ > 16 mg/mL.

Subjects

This retrospective study was conducted using a database that includes 618 subjects investigated at the Hôpital du Sacré-Coeur de Montréal (Montreal, Quebec, Canada) where they had undergone SICs between 2000 and 2015 for symptoms suggestive of OA. The Hôpital du Sacré-Coeur de Montréal is a tertiary care center, which is one of the 2 referral centers for OA in the province of Quebec. A sputum induction and a methacholine inhalation challenge were performed before and after the SICs. Blood samples were collected in all subjects before the SICs and in a subgroup of subjects before and after the SICs. The study was approved by the Research Ethics Committee of Sacré-Coeur Hospital.

Procedures

- Spirometry was assessed in accordance with the standards of the American Thoracic Society.¹² A methacholine inhalation challenge was performed as previously described with a Wright nebulizer (output 0.14 mL/min) at tidal volume breathing for 2 minutes.¹³
- Sputum was induced using inhalations of increasing concentrations (3%, 4%, 5%) of hypertonic saline and processed as previously described.¹⁴
- Blood was drawn in every worker before the investigation to obtain a blood cell count. The procedure was repeated only in subjects who were likely to have a positive SIC response.
- Subjects underwent skin prick tests with 14 common inhalants. Atopy was defined by the presence of at least one positive skin test with a wheal diameter ≥ 3 mm.
- SICs: As previously described, SICs were performed in the laboratory or at the workplace.¹⁵ On the first day, the subjects were either not exposed to any substance or exposed to a sham substance (eg, lactose or thinner) for 30 minutes to ensure that their asthma was stable. On subsequent days, the subjects were progressively exposed to the occupational agent suspected of causing their asthma-like symptoms. When the SIC was negative in the laboratory or when the workplace exposure could not be reproduced in the laboratory, the subjects were returned to their workplace under the supervision of a respiratory technologist, who assessed their respiratory function hourly over 7 hours on 2 consecutive days. SICs were considered positive if there was a fall in forced expiratory volume in 1 second of 20% or greater after exposure. The diagnosis of OA was retained if an SIC was positive. Methacholine challenge and sputum induction were performed at the end of the control day and at the end of the last day of exposure. Long-acting β_2 -agonists were interrupted 72 hours before the challenge. Inhaled corticosteroids (ICS) were continued during the challenge, but the total daily dose was administered 12 hours before the challenge or in the evenings of the challenge days.

Statistical analysis

Descriptive statistics were used to summarize clinical and demographic characteristics of the sample. Results were expressed as means and standard deviation, except for data with an abnormal distribution, which were expressed as median and interquartile ranges (IQR) (which are the difference between the 75th and 25th percentiles). Correlations between blood, sputum eosinophil count, and methacholine PC₂₀ were examined by using a Spearman rank-order test. Changes in blood and sputum eosinophil counts after exposure to the offending agent were expressed as differences, whereas changes in PC₂₀ were expressed as ratios (PC₂₀ before exposure/PC₂₀ after exposure to the offending agent). Receiver operating characteristic (ROC) curves were built to calculate the respective diagnosis accuracies of blood and sputum eosinophil counts and PC₂₀ methacholine for differentiating subjects with positive and negative SIC. ROC curves give information on the discriminatory ability of a test. The closer the apex of the curve is to the upper-left corner, the better the discriminatory property of the test will be. This ability is also measured quantitatively by the area under the curve (AUC). Although an AUC value of greater than 96% indicates excellent discriminatory ability, a value of 50% indicates no discriminative value. $AUC \leq 75\%$ are usually not clinically useful.¹⁶

TABLE I. Characteristics of the subjects

	Non-OA (n = 229)	OA (n = 152)	No functional evidence of asthma (n = 237)
Sex, M, n (%)	144 (62.9)	104 (68.4)	138 (58.2)
Age, y	52.8 ± 11.0	50.0 ± 11.3	50.2 ± 10.9
BMI, kg/m ²	28.9 ± 6.3	28.3 ± 5.4	27.9 ± 6.1
Atopy, n (%)	157 (68.6%)	123 (80.9%)	154 (65.0%)
Asthma history, n (%)	50 (21.8%)	19 (12.5%)	18 (7.6%)
Smoking habits, ExS/CS/NS, n (%)	84 (36.7%)/64 (27.9%)/79 (34.5%)	59 (38.8%)/37 (24.3%)/56 (36.8%)	72 (30.4%)/55 (23.2%)/108 (45.6%)
Pack-years	19.8 ± 15.2	16.2 ± 14.9	14.1 ± 13.2
Subjects taking ICS, n (%)	150 (65.5%)	86 (56.6%)	65 (27.4%)
Duration of exposure, y	11.5 ± 10.9	10.1 ± 9.6	10.4 ± 10.4
Occupational agents, LMW/HMW, n (%)	168 (73.4%)/53 (23.1%)	81 (53.3%)/68 (44.7%)	182 (76.8%)/48 (20.2%)
FEV ₁ , % predicted	86.5 ± 16.2	90.7 ± 17.4	100.6 ± 14.4
PC ₂₀ baseline, GM (range), mg/mL	3.3 (128.0)	4.6 (127.9)	73.7 (127.3)
PC ₂₀ after SIC, GM (range), mg/mL	3.6 (128.0)	2.4 (128.0)	78.0 (127.3)
Sputum eos baseline, % median (IQR)	1.0 (2.3)	1.0 (3.7)	0.3 (1.0)
Sputum eos after SIC, % median (IQR)	0.8 (2.0)	6.2 (17.4)	0.3 (1.4)
Blood eos baseline, median (IQR), 10 ⁹ /L	0.2 (0.2)	0.2 (0.3)	0.1 (0.2)

BMI, Body mass index; CS, current smoker; eos, eosinophils; ExS, ex-smoker; FEV₁, forced expiratory volume in 1 s; GM, geometric mean; HMW, high-molecular weight agent; ICS, inhaled corticosteroids; IQR, interquartile range; LMW, low-molecular weight agent; M, male; NS, nonsmoker; OA, occupational asthma; PC₂₀, concentration of methacholine inducing a 20% fall in FEV₁; SIC, specific inhalation challenge.

Data given as mean ± SD unless otherwise noted.

TABLE II. Characteristics of subjects (n = 100) who had a blood sample before and after the SICs

	Non-OA (n = 16)	OA (n = 71)	No functional evidence of asthma (n = 13)
Sex, M, n (%)	9 (56.3)	53 (74.6)	7 (53.8)
Age, y	54.4 ± 10.4	48.3 ± 10.9	57.8 ± 10.9
BMI, kg/m ²	31.3 ± 6.6	28.7 ± 5.1	28.7 ± 5.6
Atopy, n (%)	12 (75.0)	63 (88.7)	9 (69.2)
Asthma history, n (%)	4 (25.0)	6 (8.5)	1 (7.7)
Smoking habits, ExS/CS/NS, n (%)	4 (25.0)/2 (12.5)/10 (62.5)	26 (36.6)/16 (22.5)/29 (40.8)	3 (23.1)/4 (30.8)/6 (46.2)
Pack-years	22.6 ± 9.2	14.7 ± 11.7	16.9 ± 17.9
Subjects taking ICS, n (%)	12 (75.0)	33 (46.5)	1 (7.7)
Duration of exposure, y	13.6 ± 11.4	9.7 ± 9.1	19.5 ± 15.1
Molecular weight, LMW/HMW, n (%)	11 (68.8)/2 (12.5)	35 (49.3)/35 (49.3)	8 (61.5)/3 (23.1)
PC ₂₀ baseline, GM (range), mg/mL	2.9 (127.9)	4.6 (127.9)	90.7 (91.0)
PC ₂₀ after SIC, GM (range), mg/mL	3.2 (100.8)	1.9 (128.0)	63.1 (120.0)
FEV ₁ , % predicted	87.2 ± 17.3	92.4 ± 15.0	103.4 ± 12.3
Sputum eos baseline, median (IQR), %	0.3 (2.2)	1.0 (2.5)	0.3 (1.3)
Sputum eos after SIC, median (IQR), %	1.0 (2.2)	8.0 (20.5)	0.3 (1.9)
Blood eos baseline, median (IQR), 10 ⁹ /L	0.2 (0.3)	0.2 (0.3)	0.07 (0.1)
Blood eos after SIC, median (IQR), 10 ⁹ /L	0.16 (0.4)	0.3 (0.3)	0.09 (0.08)

BMI, Body mass index; CS, current smoker; eos, eosinophil counts; ExS, ex-smoker; FEV₁, forced expiratory volume in 1 second; GM, geometric mean; HMW, high-molecular weight agent; ICS, inhaled corticosteroids; IQR, interquartile range; LMW, low-molecular weight agent; M, male; NS, nonsmoker; OA, occupational asthma; PC₂₀, concentration of methacholine inducing a 20% fall in FEV₁; SIC, specific inhalation challenge.

Data given as mean ± SD unless otherwise noted.

The statistical analysis was performed using SPSS version 22.0 (IBM Corp., Armonk, NY).

RESULTS

Characteristics of the subjects

Six-hundred and eighteen subjects were investigated. Of them, 152 subjects were diagnosed with OA, 229 had non-OA, and 237

did not have clinical or functional evidence of asthma. Among the subjects with OA, 140 underwent SIC only in the laboratory, 13 at the workplace, and 22 both in the laboratory and at the workplace. Among the subjects with OA, 81 were exposed to low-molecular weight (LMW) agents, whereas 68 to high-molecular weight (HMW) agents. The sensitizers that caused OA are presented in Table E1 (available in this article's Online Repository at www.jaci-inpractice.org). There was no statistical difference between the

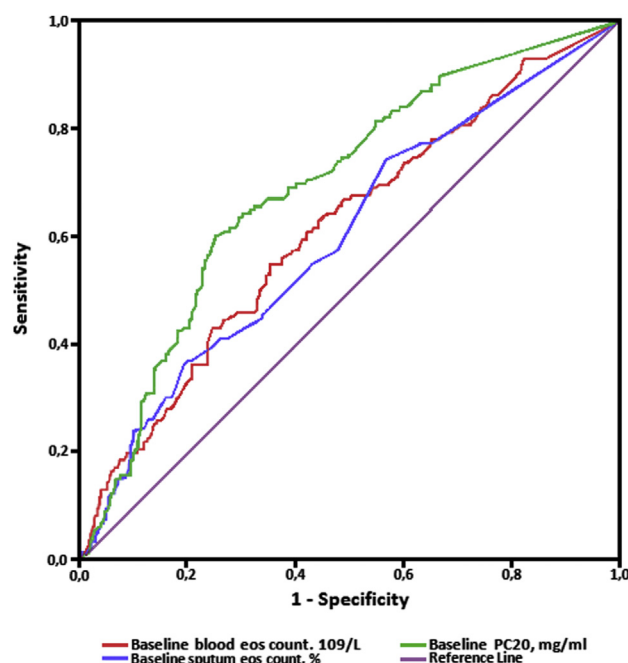


FIGURE 1. Receiver operating characteristic curves evaluating the effectiveness of PC_{20} , blood eosinophilia, and sputum eosinophilia before SICs for diagnosing occupational asthma ($n = 591$). PC_{20} , Concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second; SIC , specific inhalation challenge.

number of positive SICs between the asthmatic subjects treated with ICS (36.8%) and those who were not treated with ICS (45.5%; $P = .09$). The clinical and demographic characteristics of the subjects are presented in Table I.

The increase in sputum eosinophil counts after exposure to the offending agent was higher in subjects with OA (median [IQR]: 4.5% [13.5%]) than in subjects without OA ($P < .001$). The change in PC_{20} observed in subjects with OA (geometric mean [range]: 2.2 [144.9]) was higher than in subjects without OA ($P < .001$) (Table I).

Among the 100 subjects in whom a blood sample was drawn before and after the SICs, the change in blood eosinophil counts before and after SICs was similar in subjects with and without OA ($P = .462$) (Table II).

Correlations between blood and sputum eosinophil counts and PC_{20} methacholine

The correlations between blood and sputum eosinophil counts ($r_s = 0.381$, $P < .001$), PC_{20} and sputum eosinophil counts ($r_s = -0.235$, $P < .001$), and between PC_{20} and blood eosinophil counts performed before on the control day ($r_s = -0.171$, $P < .001$) were statistically significant, but weak.

There was a statistically significant but weak correlation between the changes in sputum eosinophil counts and PC_{20} performed before and after exposure to the offending agent ($r_s = 0.235$, $P < .001$), whereas we did not find any statistically significant correlation between changes in blood and sputum eosinophil counts performed before and after exposure to the offending agent ($r_s = 0.113$, $P = .282$)

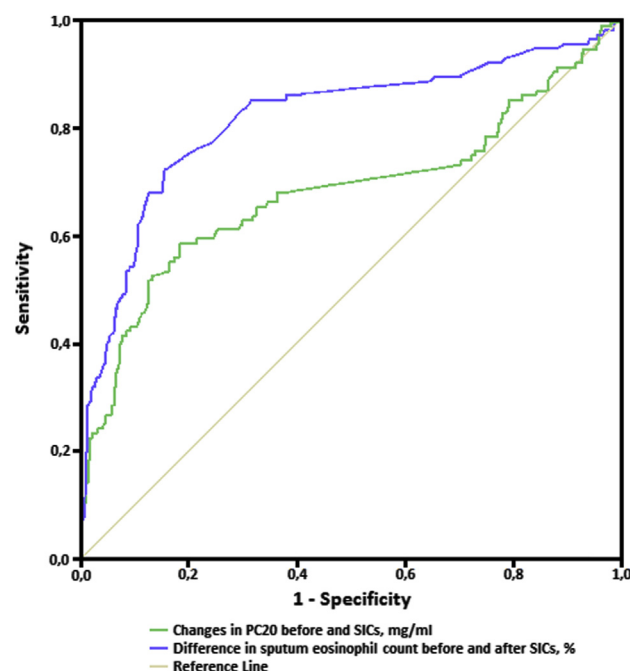


FIGURE 2. Receiver operating characteristic curves evaluating the effectiveness of the change in PC_{20} and sputum eosinophilia after the SICs for diagnosing occupational asthma ($n = 477$). PC_{20} , Concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second; SIC , specific inhalation challenge.

and between the changes in blood eosinophil counts and in PC_{20} performed before and after exposure ($r_s = 0.006$, $P = .955$).

Diagnostic accuracy of blood and sputum eosinophil count, and methacholine PC_{20} measured on the control day (before exposure to the offending agent)

The diagnostic accuracy of methacholine PC_{20} , blood, and sputum eosinophil counts before exposure to the offending agent to predict positive and negative SICs, described as AUC ROC, was respectively 69% (95% confidence interval [CI]: 0.6-0.7, $P < .001$), 61% (95% CI: 0.6-0.7, $P < .001$), and 61% (95% CI: 0.6-0.7, $P < .001$) (Figure 1).

Diagnostic accuracy of changes in blood and sputum eosinophil count, and methacholine PC_{20} measured before and after SICs

The diagnostic accuracy of the change in PC_{20} and in sputum eosinophil counts before and after exposure to the offending agent to differentiate positive and negative SICs, described as AUC ROC, was respectively 69% (95% CI [0.6-0.8], $P < .001$) and 82% (95% CI [0.8-0.9], $P < .001$) in the entire population (Figure 2). We also assessed the diagnostic accuracies of the change in PC_{20} and in sputum eosinophil counts before and after exposure to either HMW or LMW agents to differentiate positive and negative SICs. The results were similar for both types of agents. For LMW agents, the AUC ROC of sputum eosinophils and PC_{20} methacholine were respectively 80% (95% CI: 0.7-0.9) and 67% (95% CI: 0.57-0.8), and for HMW agents, the ROC AUC of sputum eosinophils and PC_{20}

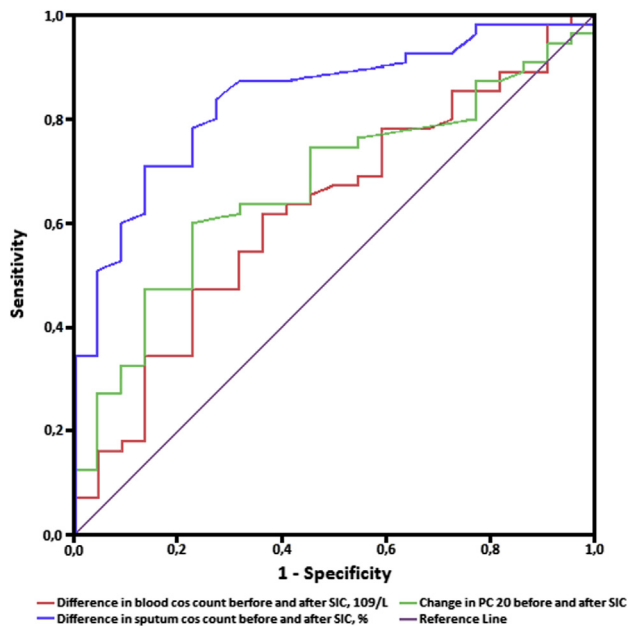


FIGURE 3. Receiver operating characteristic curves evaluating the effectiveness of the change in PC_{20} , blood eosinophilia, and sputum eosinophilia after the SICs for diagnosing occupational asthma ($n = 77$). PC_{20} , Concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second; SIC , specific inhalation challenge.

methacholine were respectively 86% (95% CI: 0.8-0.9) and 70% (95% CI: 0.6-0.8).

The same analysis was performed in the 100 subjects in whom blood and sputum cell counts as well as methacholine challenge were obtained before and after SIC. The change in blood eosinophil counts between control day and after exposure was unable to differentiate subjects with positive and negative SICs (ROC AUC = 64%, 95% CI: 0.5-0.8, $P = .06$). In contrast, in these subjects, the diagnostic accuracies of the changes in methacholine PC_{20} and sputum eosinophil counts for differentiating subjects with positive and negative SICs were respectively 69% (95% CI: 0.6-0.8, $P = .010$) and 86% (95% CI: 0.8-0.9, $P < .001$) (Figure 3).

Sensitivities, specificities, and predictive values of sputum and blood eosinophil counts and PC_{20} associated with a diagnosis of OA

Table III summarizes the sensitivities, specificities, and predictive values of sputum and blood eosinophil counts and PC_{20} separately or in combination for diagnosing OA.

The combination of a 2-fold or greater decrease in PC_{20} and a $\geq 3\%$ increase in sputum eosinophil counts achieved a sensitivity of 24%, a specificity of 97%, and a negative predictive value of 80%, whereas a 2-fold or greater decrease in PC_{20} or a $\geq 3\%$ increase in sputum eosinophil counts achieved a sensitivity of

84% and a specificity of 74% with a negative predictive value of 91% (Table III).

DISCUSSION

This study showed that in contrast to a methacholine challenge and sputum eosinophil counts, blood eosinophil counts do not seem to be an effective aid in diagnosing OA.

Blood eosinophil counts have been shown to be the biomarker the most closely associated with sputum eosinophils compared with fractional concentration of exhaled nitric oxide and periotin.⁹ We found only a weak correlation between blood and sputum eosinophil counts in our population. This correlation appears slightly lower than those found in studies that included only subjects with severe asthma treated by ICS,^{10,17} but similar to the studies that included subjects with mild-to-severe asthma.¹⁸

We also found a weak correlation between baseline PC_{20} and sputum eosinophils and between changes in PC_{20} and sputum eosinophils after exposure to the offending agent, as previously described.^{19,20} These results confirm that airway responsiveness is likely to be more dependent on the structural changes of the airway rather than on airway inflammation.²¹

Blood and sputum cell counts collected before the performance of SICs were less effective than a methacholine challenge to differentiate workers with a positive and negative SIC. Our study showed that a $PC_{20} \leq 16$ mg/mL before the SICs achieved an 86% negative predictive value for the diagnosis of OA. This is consistent with our previous findings showing respectively 95% and 82% negative predictive values of methacholine challenge for diagnosing OA depending on whether or not the subjects were working at the time of the investigation.³

In this study, an increase in sputum eosinophil counts induced by the exposure to the offending agent was more accurate to reflect a positive SIC than an increase in airway responsiveness. These results are consistent with previous studies, which reported that a change in sputum eosinophil counts after SICs achieved a specificity of 81% to 97% for the diagnosis of OA depending on the sputum eosinophils threshold (2% vs 3%).^{5,22} The combination of both tests may be helpful in clinical practice to predict the diagnosis of OA in centers with no access to SICs. The combination of a 3% or greater change in sputum eosinophil count and a 2-fold or greater decrease in PC_{20} between baseline and after exposure achieved a much higher sensitivity and a similar specificity for diagnosing OA than one of these 2 tests used alone, whereas both a 3% or greater change in sputum eosinophilia and a 2-fold or greater decrease in PC_{20} between baseline and after exposure achieved a high specificity (97%), but a very low sensitivity for the diagnosis of OA. In contrast, the change in blood eosinophil counts was unable to differentiate subjects with positive and negative SICs.

The main limitation of SICs is the accessibility to those tests. Performing a methacholine inhalation challenge and a sputum induction before and after a period at work and away from work could be an interesting alternative to the performance of SICs in centers that do have access to those

TABLE III. Sensitivities, specificities predictive values, and likelihood ratio of sputum eosinophil counts, blood eosinophil counts, and PC₂₀ associated with a diagnosis of OA in the whole population (n = 618)

	Sensitivity	Specificity	PPV	NPV	Likelihood ratio
Before SICs					
Sputum eos $\geq 3\%$	0.31	0.85	0.41	0.79	2.1
Blood eos $\geq 0.30 \times 10^9$ cells/mL	0.35	0.79	0.36	0.79	1.7
PC ₂₀ ≤ 16 mg/mL	0.71	0.57	0.35	0.86	1.7
Sputum eos $\geq 3\%$ or PC ₂₀ ≤ 16 mg/mL	0.79	0.48	0.33	0.87	1.5
Sputum eos $\geq 3\%$ and PC ₂₀ ≤ 16 mg/mL	0.23	0.91	0.46	0.78	2.6
Sputum eos $\geq 3\%$ or blood eos $\geq 0.30 \times 10^9$ /L	0.66	0.74	0.46	0.87	2.5
Sputum eos $\geq 3\%$ and blood eos $\geq 0.30 \times 10^9$ /L	0.17	0.92	0.41	0.77	2.1
PC ₂₀ ≤ 16 mg/mL or blood eos $\geq 0.30 \times 10^9$ /L	0.81	0.49	0.34	0.89	1.6
PC ₂₀ ≤ 16 mg/mL and blood eos $\geq 0.30 \times 10^9$ /L	0.25	0.87	0.40	0.78	1.9
Changes after SICs					
Increase in sputum eos $\geq 3\%$	0.57	0.90	0.68	0.85	5.7
2-fold decrease or greater in PC ₂₀	0.52	0.85	0.50	0.86	3.5
Increase in sputum eos $\geq 3\%$ and 2-fold decrease or greater in PC ₂₀	0.24	0.97	0.76	0.80	8
Increase in sputum eos $\geq 3\%$ or 2-fold decrease or greater in PC ₂₀	0.84	0.74	0.59	0.91	3.2

eos, Eosinophil counts; NPV, negative predictive value; OA, occupational asthma; PC₂₀, concentration of methacholine inducing a 20% fall in FEV₁; PPV, positive predictive value; SIC, specific inhalation challenge.

tests. However, this hypothesis will need to be tested prospectively.

Our study has some limitations. Because of the retrospective nature of the study, the subjects in whom a blood sample was collected before and after the SICs were mostly those with a positive SIC response. This selection bias may have underestimated the diagnostic accuracy of blood eosinophils. However, the impact of this selection bias is likely to be limited as we found very similar diagnostic accuracies in the sputum eosinophil count and the methacholine challenge in the entire population compared with the group of 100 subjects who underwent blood cell count before and after SIC. This study has been performed in a tertiary center where the pretest probability of the tests is likely to be higher than in a general population. Those results would need to be confirmed by a prospective study. However, considering the prevalence of the disease, a prospective study including a large sample size will be difficult to perform in primary care. Almost half of the subjects investigated were taking ICS at the time of the investigation. Although ICS can blunt the asthmatic response to allergen exposure, the proportion of positive SICs was not statistically different between subjects treated with and without ICS. The strength of our study is to present data on a large sample of subjects with OA in whom the diagnosis was made objectively using SICs.

In conclusion, blood eosinophil counts do not appear to be a reliable surrogate marker of airway inflammation to be used in the investigation of OA. Although the assessment of airway responsiveness before exposure to the offending agent is the most useful test to discard a diagnosis of OA, the change in sputum eosinophil counts after exposure to the occupational agent appears to have the best diagnostic accuracy compared with changes in methacholine challenge or blood cell count to reflect a diagnosis of OA. Furthermore, assessing both sputum eosinophil counts and airway responsiveness before and after exposure to the

occupational agent improves the negative predictive value for OA compared with single markers.

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TABLE E1. Occupational agents identified in subjects with occupational asthma

Occupational agents	Number of subjects, n (%)
High-molecular weight agents	
Flour	37 (24.3)
Proteins (various)	9 (5.9)
Animals	9 (4.6)
Latex	4 (2.6)
Cereals	4 (2.6)
Sea food	1 (0.7)
Chicken	1 (0.7)
Multiple agents	3 (1.9)
Low-molecular weight agents	
Isocyanates	40 (26.3)
Resins, glues	11 (7.2)
Wood dust	11 (7.2)
Chemicals (various)	7 (4.6)
Metals	4 (2.6)
Persulfates	1 (0.7)
Multiple agents	7 (4.6)
Unknown	3 (1.9)
Total	152 (100)