

Original article

Sputum eosinophilia: an early marker of bronchial response to occupational agents

Background: False-negative responses to specific inhalation challenge (SIC) with occupational agents may occur. We explored whether assessing changes in sputum cell counts would help improve the identification of bronchial reactivity to occupational agents during SICs.

Methods: The predictive value of the changes in sputum cell counts after a negative FEV₁ response to a first challenge exposure to an occupational agent was determined using the changes in airway calibre observed during repeated challenges as the 'gold standard'. The study included 68 subjects investigated for work-related asthma in a tertiary centre. After a control day, the subjects were challenged with the suspected occupational agent(s) for up to 2 h. All subjects who did not show an asthmatic reaction were re-challenged on the following day. Additional challenges were proposed to those who demonstrated a $\geq 2\%$ increase in sputum eosinophils or an increase in nonspecific bronchial hyper-responsiveness to histamine after the second challenge day.

Results: Six of the 35 subjects without changes in FEV₁ on the first challenge developed an asthmatic reaction on subsequent challenges. ROC analysis revealed that a $> 3\%$ increase in sputum eosinophils at the end of the first challenge day was the most accurate parameter for predicting the development of an asthmatic response on subsequent challenges with a sensitivity of 67% and a specificity of 97%.

Conclusions: An increase in sputum eosinophils is an early marker of specific bronchial reactivity to occupational agents, which may help to identify subjects who will develop an asthmatic reaction only after repeated exposure.

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There is accumulating evidence that work-related asthma is a prevalent disorder (1), although it is often difficult to differentiate immunologically-mediated occupational asthma (OA) from work-exacerbated asthma in a clinical practice (2). Under- and over-diagnosis of these conditions can both lead to substantial and avoidable adverse consequences for workers, employers, health-care insurance organizations and society (3). As a result, the clinical diagnosis of OA should be established with the highest level of confidence. The clinical history, immunological tests and serial assessments of peak expiratory flow and nonspecific bronchial hyperresponsiveness (NSBR) at and off work can assist clinicians in a stepwise diagnostic approach, although specific inhalation challenges (SIC) with the suspected occupational agent is still acknowledged as the most reliable method for establishing the diagnosis of OA (4, 5). However, even SICs may result in false-negative responses, especially when specific bronchial reactivity to the sensitizing agent has declined after cessation of workplace exposure

(6, 7). A postchallenge increase in the level of NSBR has been proposed as an earlier and more sensitive marker of bronchial responsiveness to occupational agents than changes in airway calibre (8, 9). In recent years, airway inflammation, a key feature of asthma and OA, has been increasingly investigated through induced sputum examination, because the technique is noninvasive and can be performed serially over time (10). Sputum studies have shown that exposure to occupational agents at work (11, 12) and in the laboratory (13–16) can induce an eosinophilic and less consistently, a neutrophilic inflammation of the airways in sensitized subjects. It has also been shown that an increase in sputum eosinophils may precede functional changes after repeated exposures to low doses of occupational agents in sensitized subjects (17).

The aim of this study was to examine whether assessment of the changes in sputum cell counts might be useful for minimizing false-negative results of SIC with occupational agents.

Methods

Study design

Consecutive subjects investigated for a work-related worsening of asthma symptoms in a tertiary centre over a two-year period were included in this study. The subjects were administered a detailed medical and occupational questionnaire (18), skin-prick tests with a battery of common inhalant allergens and workplace allergens if available, and SICs with the agent(s) suspected of causing work-related symptoms. Sputum induction and measurement of NSBR were performed 6–8 h after the end of each control and active challenge. The procedures used in the study were approved by the Hospital's Ethics Committee.

The subjects underwent SICs according to an algorithm summarized in Fig. 1. After a control day, the subjects were challenged with the incriminated occupational agent (challenge day 1) for gradually increasing periods of time until a significant ($\geq 20\%$) fall in FEV_1 occurred or a cumulative exposure of 2 h was completed. All subjects who did not show a $\geq 20\%$ decline in FEV_1 during challenge day 1 underwent a second challenge for a maximum of 2 h on the following day (i.e. challenge day 2). Additional challenges with occupational agents were proposed only to those subjects who did not demonstrate $\geq 20\%$ fall in FEV_1 during challenge day 2, but who showed a ≥ 3 -fold decrease in the provocative concentration of histamine causing a 20% fall in FEV_1 (PC_{20}) (8) or an increase in sputum eosinophils of 2% points or more as compared with the control day value, since the latter cut-off value exceeds the spontaneous variability in asthmatic subjects (15) and has been proposed as a clinically significant threshold value (12).

Specific inhalation challenges

SICs were completed in 5-cubic-meter challenge rooms according to international guidelines (19) and a previously described protocol (4). Medications were withdrawn according to recommendations (19). During the first day, the subjects were exposed to a control product (e.g. lactose powder, pine dust, diluent usually mixed with the tested compound) for 30 min to ensure that FEV_1 fluctuations were $\leq 12\%$ of baseline value. Spirometry (20, 21) was obtained before exposure and reassessed every 15 min for the first hour, every 30 min for the second hour and then hourly for a total of 6 h. At the end of the control day, the baseline level of NSBR was established using the method described by Cockcroft et al. (19). After the histamine bronchoprovocation, the subjects were administered an inhaled bronchodilator (salbutamol 400 μ g) and sputum was induced.

On the following days, the subjects were challenged with the occupational agent(s) suspected of causing their work-related symptoms, based upon the clinical history and an inspection of the workplace by hygienists from the Worker's Compensation Board (*Fonds des Maladies Professionnelles*). Challenge exposures to occupational agents were produced in different ways (i.e. powder, aerosol, gas or fume) in order to reproduce, as close as possible, the conditions of exposure at the workplace (4). During active challenge days, the FEV_1 was measured for at least 6 h postchallenge at the same intervals as on the control days. The level of NSBR was reassessed 6–8 h after the end of exposure if the FEV_1 was within 10% of the preexposure value. Peak expiratory flow was monitored hourly during the day and evening, as well as at night whenever required.

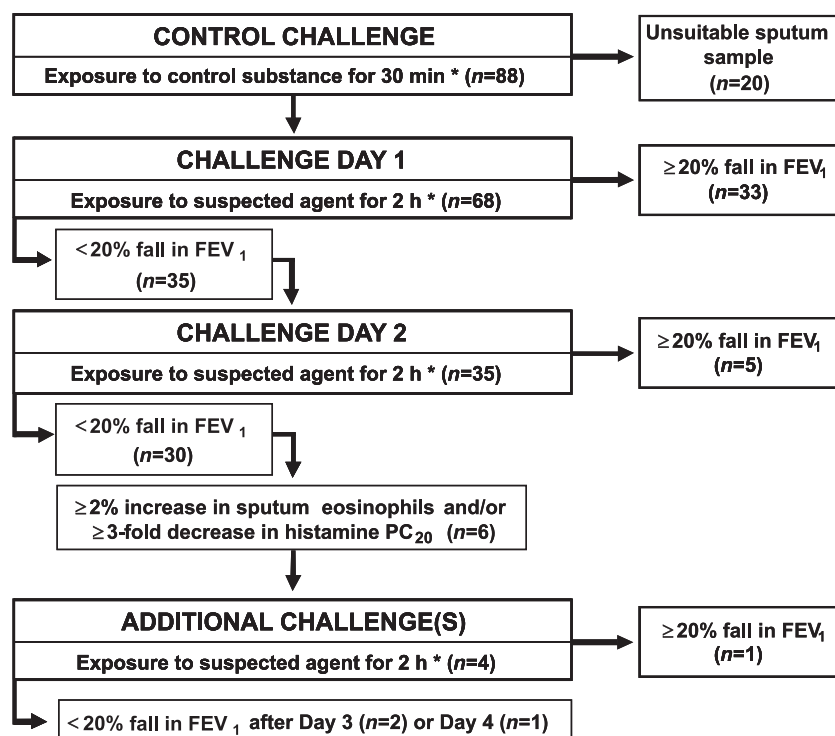


Figure 1. A schematic diagram summarizing the algorithm and the results of specific inhalation challenges. *Measurement of nonspecific bronchial responsiveness to histamine and sputum induction were performed 6–8 h after the end of exposures.

An SIC result was considered positive when a sustained $\geq 20\%$ fall in FEV₁ was recorded on two consecutive assessments. Subjects with a negative SIC were asked to record their peak expiratory flows every 2 h for 2–4 weeks at work, and to return to the laboratory for an assessment of the NSBR and sputum induction.

Sputum examination

Sputum was induced 6–8 h after the end of each control and challenge exposures by inhaling increasing concentrations (3%, 4% and 5%) of hypertonic saline generated by a Fisoneb ultrasonic nebulizer (Fisoneb, Karapharm Lab., Marseille, France) for periods of 7 min and processed within 2 h (22). Total cell count, cell viability and squamous cell contamination were assessed using the trypan blue exclusion method in a Burkner haemocytometer. The sample was considered adequate for analysis when there were fewer than 20% squamous cells and viability was more than 40%. Differential cell counts were determined by counting 400 nucleated nonsquamous cells per slide on cytospin preparations (Shandon 2 cytocentrifuge; Thermo Fisher Scientific Inc., Waltham, MA, USA) stained with May–Grünwald–Giemsa. The results were expressed as a percentage of total nonsquamous cells and as the absolute number of cells in millions per ml of sputum.

Analysis

Data are presented as the median value with 25th and 75th percentiles unless otherwise specified. Comparison between subjects was made using the chi-squared test, Fisher exact test or Wilcoxon rank-sum test as appropriate. The Wilcoxon signed-rank test was used for comparing variables before and after SIC in the same subjects. The analysis was performed using the SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA). A receiver-operating characteristic (ROC) analysis was conducted in order to determine the ability for changes in sputum cell counts after the first active challenge to predict the development of an asthmatic reaction on subsequent challenges in subjects changes in FEV₁ $< 20\%$ during the challenge day 1 (MedCalc 9.4.2.0 software, Mariakerke, Belgium). ROC analysis was also performed to evaluate the predictive value of the change in histamine PC₂₀ values and the maximum fall in FEV₁ on challenge day 1. All tests were two-tailed. A *P*-value < 0.05 was considered significant.

Results

Twenty of 88 subjects evaluated for work-related asthma symptoms during the study period were excluded from the analysis because of inappropriate sputum samples. There were no significant differences between subjects included or excluded from analysis in terms of baseline clinical features and response to SICs (Table 1). SICs were positive in eight of these 20 excluded patients, including five who showed an asthmatic response only after repeated challenge. The 39 eligible subjects with a positive SIC (either after challenge day 1 or after subsequent challenges) showed a slightly lower baseline histamine PC₂₀ value (13.0 [2.3–26.7] mg/ml) (Table 1) and a higher sputum eosinophil percentage (1.4 [0.5–5.0]%) (Table 3) than those with a negative SIC (28.0

[6.0–32.0] mg/ml, *P* = 0.049 and 0.5 [0.0–1.5]%, *P* = 0.043, respectively).

Results of SICs

The first challenge exposure to occupational agents (challenge day 1) resulted in a $\geq 20\%$ fall in FEV₁ in 33 of the 68 (48%) subjects (Fig. 1). Among the 35 subjects who did not demonstrate significant changes in airway calibre during challenge day 1, five (14.3%) developed an asthmatic reaction during challenge day 2 (Subjects nos. 1–5 in Table 2). Of the 30 subjects without changes in FEV₁ during challenge day 2, five subjects (Nos. 6–10) showed a postchallenge increase in sputum eosinophils $\geq 2\%$ as compared with the control day value. Four of these five subjects agreed to perform a third challenge day with the suspected agents. Subject no. 6 developed an asthmatic reaction to hexamethylene diisocyanate while his postchallenge histamine PC₂₀ value remained in the nonasthmatic range. Subjects no. 7 and 8 demonstrated a slight, but persistent increase in sputum eosinophils after exposure to chloramine-T powder (Subject no. 7) or latex gloves (Subject no. 8) during challenge day 3, although there was no decline in either the FEV₁, or in the PC₂₀ value. A fourth challenge with chloramine-T did not elicit significant changes in functional parameters in Subject no. 7, while Subject no. 8 did not agree to perform a fourth challenge with latex. In Subject no. 9, sputum eosinophilia was no longer documented after exposure to flour during challenge day 3. Subject no. 10 did not agree to perform a third challenge.

Overall, among the 35 subjects who did not show significant changes in FEV₁ after the first challenge with occupational agents (i.e. challenge day 1), six [17%, 95% confidence interval (CI): 7–34%] subjects developed an asthmatic reaction during subsequent challenge tests, including five subjects on challenge day 2 and one subject on challenge day 3. Although their small number precluded a reliable statistical comparison, these six subjects who developed an asthmatic reaction after repeated SICs did not differ from those who responded to the first challenge in terms of demographic and clinical characteristics (Table 1). They showed a slightly higher baseline histamine PC₂₀ value [22.3 (14.2–26.9) mg/ml] as compared with the subjects who developed a positive response on the challenge day 1 [8.8 (1.8–27.4) mg/ml], although the difference did not reach statistical significance (*P* = 0.23). None of the 29 subjects with a negative FEV₁ response to SICs had a > 3 -fold drop in histamine PC₂₀ value at the end of challenge day 2. Among the 17 of 29 subjects with a final negative SIC result who agreed or were allowed to perform additional tests at the workplace, the absence of OA was further substantiated by a negative peak expiratory flow monitoring alone (*n* = 10), measurement of histamine PC₂₀ at work alone (*n* = 5) and assessment of histamine PC₂₀ combined with sputum

Table 1. Demographic and clinical characteristics of the subjects

	Subjects with a positive SIC (<i>n</i> = 39)		Subjects with a negative SIC (<i>n</i> = 29)	Subjects excluded from analysis (<i>n</i> = 20)
	On the first challenge day (<i>n</i> = 33)	On a subsequent challenge day (<i>n</i> = 6)		
Gender (male), <i>n</i> (%)	20 (61)	6 (100)	19 (66)	12 (60)
Age	40 (30–47)	38 (36–46)	40 (32–48)	40 (31–47)
Current/ex-smokers, <i>n</i> (%)	7 (21)/5 (15)	1 (17)/1 (17)	7 (24)/9 (31)	2 (10)/1 (5)
Atopy, <i>n</i> (%)	20 (61)	3 (50)	14 (48)	9 (45)
Preexisting asthma, <i>n</i> (%)	8 (24)	1 (17)	6 (21)	3 (15)
Duration of asthma at work, months	35.0 (17.0–83.5)	25.0 (5.0–54.0)	26.0 (16.0–50.5)	46 (16–74)
Time elapsed since last work exposure, months	3.0 (0.1–15.0)	4.2 (0.8–11.8)	3.0 (0.1–23.0)	8.0 (0.1–16.0)
Work-related rhinitis, <i>n</i> (%)	27 (82)	5 (83)	22 (76)	15 (75)
Medication, <i>n</i> (%):				
Short-acting beta2-agonist	14 (42)	4 (67)	12 (41)	7 (35)
Long-acting beta2-agonist	18 (54)	2 (33)	10 (34)	8 (40)
Inhaled corticosteroid	19 (58)	3 (50)	15 (52)	11 (55)
FEV ₁ (%) predictive	92 (78–96)	96 (88–110)	85 (74–98)	92 (78–98)
FEV ₁ /FVC (%) predictive	95 (88–101)	98 (89–103)	97 (88–100)	93 (89–99)
Type of occupational agents, <i>n</i> (%)				
Low molecular weight	10 (30)	3 (50)	17 (59)	12 (60)
High molecular weight	22 (67)	3 (50)	12 (41)	8 (40)
Suspected agents, <i>n</i>				
Flour	13	2	8	4
Latex	6	0	4	3
Woods	1	1	4	1
Isocyanates	2	2	1	1
Quaternary ammoniums	4	0	1	1
Persulfate salts	0	0	3	0
Enzymes	2	1	0	0
Miscellaneous agents	5	0	8	10
Type of asthmatic reaction, <i>n</i> (%)				
Immediate	22 (67)	5 (83)	—	3 (15)
Dual	5 (15)	0	—	2 (10)
Late	1 (3)	1 (17)	—	1 (5)
Atypical	5 (15)	0	—	2 (10)
Prechallenge PC ₂₀ mg/ml	8.8 (1.8–27.4)	22.3 (14.2–26.9)	28.0 (6.0–32.0)	19.6 (2.9–32.0)† 28.1 (3.8–32)‡
Postchallenge PC ₂₀ mg/ml	6.2 (1.6–16.0)*	14.4 (7.3–23.3)	20.5 (5.9–32.0)	2.0 (0.7–6.1)† 29.0 (4.0–32.0)‡

Data are presented as median value with 25th–75th percentiles in parentheses, unless otherwise specified. FEV₁, forced expiratory volume in one-second; FVC, forced vital capacity; PC₂₀, provocative concentration of histamine causing a 20% fall in FEV₁; SIC, specific inhalation challenge.

**P* < 0.01 when compared with prechallenge values.

†Subjects with a positive SIC (*n* = 8), *P* = 0.43 when comparing pre and postchallenge PC₂₀ values.

‡Subjects with a negative SIC (*n* = 12).

cytology during workplace exposure (*n* = 2). Table 3 summarizes the changes in sputum cell counts between the control day and the last active challenge day in all subjects.

Receiver-operating characteristic curves

The ROC analysis of sputum and functional changes after challenge day 1 in subjects with a negative FEV₁ response is detailed in Table 4. An increase in the percentage of sputum eosinophils was the most discriminating variable for predicting the occurrence of a ≥ 20% fall in FEV₁ during subsequent challenges.

The area under the curve for the difference between the postchallenge day 1 and the control day values was 0.81 (95% CI: 0.64–0.92) for the percentage of sputum eosinophils and 0.71 (95% CI: 0.53–0.85) for the absolute number of eosinophils (Fig. 2). An increase of at least 3% in sputum eosinophils achieved the most satisfying trade-off between sensitivity (67%, 95% CI: 23%–95%) and specificity (97%, 95% CI: 82%–99%) for identifying the subsequent development of an asthmatic response. The area under the curve for the changes in sputum neutrophils was also satisfactory (Table 4) However, only decreases in the percentage of sputum neutrophils were predictive for a subsequent

Table 2. Detailed results of sequential SICs in selected subjects

Subject no.	Agent	Specific IgE antibodies*	Control day		Challenge day 1		Challenge day 2			Additional challenge(s)		
			PC ₂₀ (mg/ml)	Eosinos (%)	PC ₂₀ (mg/ml)	Eosinos (%)	Reaction (% fall in FEV ₁)	PC ₂₀ (mg/ml)	Eosinos (%)	Reaction (% fall in FEV ₁)	PC ₂₀ (mg/ml)	Eosinos (%)
Subjects with a positive response to challenge day 2 or additional challenge(s)												
1	Isocyanate	–	20.6	0.5	5.3	9.0	L (–23)	ND	13.2	ND	ND	ND
2	Flour	+	25.6	10.0	21.3	40.5	/ (–27)	14.7	64.0	ND	ND	ND
3	Wood	NA	24	1.0	> 32	6.9	/ (–31)	> 32	20.0	ND	ND	ND
4	Papain	+	2.0	2.0	1.8	1.0	/ (–24)	3.4	13.0	ND	ND	ND
5	Flour	+	18.3	1.4	> 32	3.3	/ (–25)	11.3	60.9	ND	ND	ND
6	Isocyanate	–	> 32	2.6	> 32	7.0	–	> 32	12.2	/ (–24)†	14.4	27.5
Subjects with a ≥2% increase in sputum eosinophils after challenge day 2, but a negative SIC response												
7	Chloramine-T	–	26	0	> 32	3.0	–	> 32	2.8	–‡	> 32	4.2
8	Latex	+	> 32	0	27.0	3.0	–	22.2	6.0	–†	19.5	3.5
9	Flour	–	32.0	0.5	ND	3.0	–	14.3	3.5	–†	13.0	0.5
10	Latex	+	> 32	0	> 32	2.0	–	> 32	7.0	ND	ND	ND

L, immediate reaction; L, (isolated) late reaction; FEV₁, forced expiratory volume in one second, PC₂₀, provocative concentration of histamine causing a 20% fall in FEV₁; Eosinos, sputum eosinophils expressed as % of total nonsquamous cells; ND, not done; NA, not available, SIC, specific inhalation challenge.

*Specific IgE antibodies assessed using either the ImmunoCAP method (Phadia Diagnostics, Uppsala, Sweden) alone for isocyanates and chloramine-T or both skin-prick tests and the ImmunoCAP method for flour, latex and papain.

†Results of challenge day 3.

‡Results of challenge day 4.

response. Changes in the level of nonspecific bronchial hyperresponsiveness yielded a poor discriminating value with an area under the curve for the ratio between histamine PC₂₀ at baseline and after challenge day 1 of 0.54 (95% CI: 0.36–0.71). In this sample of subjects evaluated for work-related asthma symptoms in a tertiary centre, the prevalence of positive SIC response was 39/68 (57%), the positive predictive value of an >3% increase in postchallenge day 1 sputum eosinophils for the development of a positive response to repeated challenges was 75%, and the negative predictive value was 93%.

Discussion

This study showed that an increase in sputum eosinophils may precede functional (FEV₁ and NSBR) changes after exposure to occupational sensitizers. These results further support the concept that sputum eosinophilia is an earlier marker of specific bronchial reactivity to occupational agents than changes in airway calibre. The findings confirm and expand those reported by Lemiere and co-workers who demonstrated that an increase in sputum eosinophils and interleukin-5 preceded the occurrence of changes in FEV₁ and methacholine PC₂₀ after repeated

Table 3. Changes in sputum cell counts between control day and last active challenge

	Positive SIC (n = 39)		
	First challenge day (n = 33)	Subsequent challenge day (n = 6)	Negative SIC (n = 29)
<i>Prechallenge sputum cell counts</i>			
TCC, ×10 ⁶ /ml	1.22 (0.82–2.46)	1.80 (0.94–3.66)	1.24 (0.92–3.44)
Eosinophils (%)	1.0 (0.4–5.5)	1.7 (0.9–4.4)	0.5 (0.0–1.5)§
Eosinophils × 10 ⁶ /ml	0.02 (0.00–0.08)	0.04 (0.02–0.15)	0.01 (0.00–0.02)
Neutrophils (%)	63.0 (51.2–77.5)	80.2 (62.2–91.5)	71.0 (45.0–78.2)
Neutrophils × 10 ⁶ /ml	0.92 (0.40–1.98)	1.51 (0.58–3.26)	0.84 (0.50–2.54)
<i>Postchallenge sputum cell counts</i>			
TCC, × 10 ⁶ /ml	2.50 (1.70–5.28)*	2.21 (1.66–2.98)	2.24 (1.55–5.09)
Eosinophils (%)	10.0 (6.0–21.5)*	23.8 (13.2–61.7)‡	1.0 (0.4–2.2)
Eosinophils × 10 ⁶ /ml	0.27 (0.11–0.69)*	0.53 (0.22–1.76)‡	0.03 (0.00–0.06)
Neutrophils (%)	70.5 (53.4–84.5)	51.0 (29.4–75.0)	82.8 (68.8–90.0)*
Neutrophils × 10 ⁶ /ml	1.72 (0.90–3.02)†	0.88 (0.75–1.62)	1.86 (1.24–4.24)†

Data are presented as median value with 25th–75th percentiles in parentheses, unless otherwise specified. SIC, specific inhalation challenge, TCC, total cell count.

*P ≤ 0.001 when compared with prechallenge values.

†P ≤ 0.01 when compared with prechallenge values.

‡P < 0.05 when compared with prechallenge values.

§P = 0.046 when compared with baseline values in all subjects with a positive SIC.

Table 4. ROC curve analysis of the changes in sputum and functional parameters recorded after the first challenge day

	Cut-off value*	Area under the curve	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Sputum eosinophils (%)	Increase > 3	0.81 (0.64–0.92)	67 (23–95)	97 (82–99)	80 (28–99)	93 (78–99)
Sputum eosinophils $\times 10^6/\text{ml}$	Increase > 0.06	0.71 (0.53–0.85)	67 (23–95)	86 (68–96)	50 (16–84)	92.6 (76–99)
Sputum neutrophils (%)	Increase ≤ 4	0.74 (0.56–0.87)	83 (36–97)	62 (42–79)	31.3 (11–59)	94.7 (74–99)
Sputum neutrophils $\times 10^6/\text{ml}$	Decrease ≥ 0.5	0.62 (0.44–0.78)	50 (12–88)	83 (64–94)	37.5 (8–76)	89 (71–98)
Maximum fall in FEV ₁ †	> 8	0.59 (0.41–0.75)	50 (12–88)	76 (56–90)	30.0 (7–65)	88 (69–98)
Changes in histamine PC ₂₀ ‡	> 1.33	0.53 (0.36–0.71)	33 (5–77)	93 (76–99)	50.0 (7–93)	86.7 (70–96)

95% confidence intervals are presented between parentheses; PC₂₀, provocative concentration of histamine causing a 20% fall in FEV₁; ROC, receiver-operating characteristic.

*Cut-off value derived from ROC analysis.

†Maximum fall FEV₁ recorded during challenge day 1, expressed as % from baseline.

‡Expressed as the ratio of control day PC₂₀/first challenge day PC₂₀ values.

challenges with low doses of occupational agents in patients with ascertained OA (17).

We investigated the usefulness of adding sputum cell counts to functional parameters during SICs in a 'real-life' diagnostic process. Hence, the findings may have direct implications in the clinical investigation of OA through inhalation challenges in the laboratory. Our data further outlines that an SIC involving a short period of exposure to the suspected occupational agent (usually 30–60 min) is not sensitive enough for accurately excluding the diagnosis of OA. A substantial proportion [6 of 35, 17% (95% CI: 7–34%)] of subjects with work-related

asthma required repeated challenge exposures before demonstrating a positive asthmatic response to occupational agents. The ROC analysis revealed that an increase in sputum eosinophils greater than 3% after a two-hour challenge exposure to occupational agents achieved the optimum trade-off between sensitivity (67%, 95% CI: 33%–95%) and specificity (97%, 95% CI: 82%–99%) for predicting the development of an asthmatic response on subsequent challenge(s) (Fig. 2). These results should, however, be prospectively validated in a larger sample of patients undergoing SICs. Determining cut-off values from ROC curves implies that false-positive and false-negative results are regarded as having similar clinical impacts. In clinical practice, the cut-off value for the postchallenge increase in sputum eosinophils should be lowered if the test is aimed at improving the sensitivity of SICs in order to prevent false negative results, which seems most desirable in the field of OA.

In this study, an increase in sputum eosinophils appeared to be a more accurate predictor of bronchial responses to occupational agents than changes in histamine PC₂₀ values, although changes in postchallenge NSBR have been previously proposed as early indices of bronchial reactivity to these agents (8, 9). Such discordances between changes in sputum eosinophil counts and NSBR are consistent with previous studies showing that airway eosinophilia can be dissociated from airway responsiveness and airflow obstruction in asthma (23, 24). Although an increase in sputum neutrophils has been described after SICs, especially with low-molecular-weight chemicals (16), we found that only decreases in % neutrophils after a first challenge were predictive for the development of an asthmatic reaction on repeated challenge(s), which is likely to result from a mirror effect of the changes in eosinophils. Noticeably, we observed an increase in the absolute number of sputum neutrophils after both negative and positive SICs (Table 3), similar to what has been reported in a previous study comparing sputum cells at work and away from work (12).

Several potential limitations of this study should be carefully considered. Subjects with a negative FEV₁ response after two active challenge days were not systematically rechallenged on subsequent days. Accordingly,

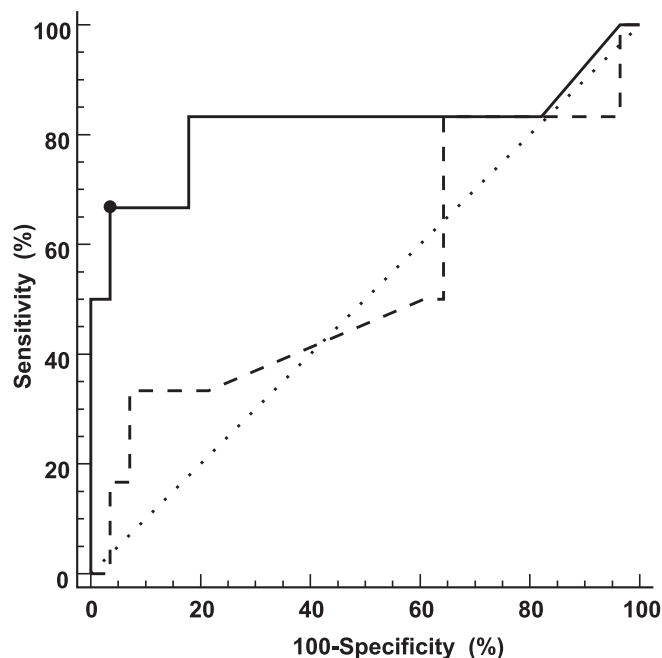


Figure 2. Receiver-operating characteristic (ROC) plots for the differences in the percentage of sputum eosinophils between challenge day 1 and the control day for predicting the development of an asthmatic reaction during subsequent challenges. The most accurate cut-off value (i.e. a > 3% increase) is bolded. ROC curve for the ratio of control day PC₂₀/first challenge day PC₂₀ values is also illustrated (dotted lines).

we cannot formally exclude that some subjects might have reacted if all of them, including those without sputum eosinophilia, had been further challenged. Because of practical constraints, we proposed additional challenges only to subjects who showed an increase in NSBR (8) or an increase in sputum eosinophils at the end of the second challenge. An increase in sputum eosinophils $\geq 2\%$ from baseline was selected *a priori*, because this 'cut-off' value has been considered as being clinically significant in previous studies (12, 15). The occurrence of false-negative results seems, however, unlikely since all subjects were challenged with the suspected agent(s) for prolonged periods (at least 4 h). It is also unlikely that asthmatic responses and sputum eosinophilia might have been inhibited by inhaled corticosteroids (12, 25), since the proportion of subjects treated with corticosteroids was similar in subjects with negative and positive SICs. Finally, some subjects may not have been challenged with the agent that actually caused asthma at work. SICs were carried out in a realistic way, aimed at mimicking workplace exposure as close as possible. Sixty-nine percentage of subjects with a negative SIC were challenged with multiple agents to which they were exposed at work. The possibility of OA could be further ruled out by the assessment of peak expiratory flows, NSBR and/or sputum cytology after resuming work in 17 subjects. These procedures could not be systematically executed after negative SICs since most of the subjects had resigned their jobs and were not allowed to be reexposed at work or they were not interested in performing further tests. The major practical limitation of sputum cell assessment results from the failure to obtain suitable samples in a substantial proportion of the subjects (23%), which is similar to figures reported in previous studies (26, 27). Therefore, there is a need for further evaluating the diagnostic value of changes in the levels of exhaled nitric oxide during SICs, since the technique is less time-consuming and would be more easily available (28).

The persistent increase of sputum eosinophils in the four subjects with work-related respiratory symptoms, but with negative SIC response may have been related to eosinophilic bronchitis and/or occupational rhinitis (Table 2). Eosinophilic bronchitis is characterized by asthma-like symptoms, mainly chronic cough, and sputum eosinophilia ($> 2.5\%$) in the absence of variable

airflow obstruction and NSBR. Three of these four subjects showed a slight, but persistent increase in sputum eosinophils after repeated challenge exposures to chloramine-T (Subject no. 7) or latex (Subjects nos. 8 and 10) without significant changes neither in FEV₁ nor in histamine PC₂₀. Subjects nos. 8 and 10 demonstrated IgE-mediated sensitization to latex, while specific IgE antibodies to chloramine-T could not be detected in Subject no. 7. Noticeably, these two agents have been previously documented as causing occupational eosinophilic bronchitis (29, 30). Three of the four subjects (Subjects nos. 7, 8 and 10) also reported work-related symptoms of rhinitis. An increase in sputum eosinophils has been documented after exposure to common inhalant allergens in nonasthmatic patients with allergic rhinitis (31). These findings underline the relevance of assessing airway inflammation by means of induced sputum in the investigation of work-related respiratory symptoms.

Despite its inherent limitations, the present study shows that an increase in sputum eosinophils is useful in identifying subjects in whom specific bronchial reactivity to occupational agents will become demonstrable only after repeated challenges. The addition of an induced sputum examination to the functional tests might therefore improve the diagnostic sensitivity of SICs. In the absence of changes in functional parameters, a significant increase in postchallenge sputum eosinophilia suggests that further challenge exposures in the laboratory and/or at the workplace are needed before excluding the diagnosis of OA. This approach should enhance the degree of confidence with which the diagnosis OA can be excluded after SICs. Further research is needed to determine whether a postchallenge increase in sputum eosinophils could be considered as reflecting a significant bronchial response when changes in spirometry do not fulfil the recommended criteria, which would allow reducing for the number of challenges and the cost of the procedure (32).

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