

ORIGINAL ARTICLE

Association of bronchial reactivity to occupational agents with methacholine reactivity, sputum cells and immunoglobulin E-mediated reactivity

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Summary

Background Bronchial responsiveness and IgE-mediated reactivity are associated with specific bronchial reactivity to allergens.

Objective Our aim was to examine whether airway inflammation also plays a role.

Methods Retrospective analysis of all subjects who underwent specific inhalation challenges in the investigation of occupational asthma (OA) since 2000. Responsiveness to methacholine (PC₂₀) and levels of eosinophils and neutrophils in induced sputum on the control day were associated with the presence of OA (positive-specific inhalation challenge). In a sample of subjects exposed to wheat flour, we also examined the role of specific IgE-mediated reactivity (skin reactivity, specific IgE).

Results PC₂₀ level was significantly more often normal in subjects with OA (35 of 129, 27% instances) by comparison with non-OA (15 of 189, 8% instances), but the positive predictive value of responsiveness to methacholine for OA was low (35%). Coupling information on the level of eosinophils to responsiveness to methacholine increased positive predictive values for OA from 39% to 69% depending on the thresholds used. The best balance of positive (69%) and negative (60%) predictive values was obtained in the case of normal PC₂₀ and eosinophils $\geq 3\%$. In a multivariate analysis carried out in 34 subjects exposed to wheat flour, responsiveness to methacholine, sputum eosinophils, skin wheal size and levels of specific IgE were all significantly associated with OA to wheat flour.

Conclusion and Clinical Relevance Information on the level of sputum eosinophils in addition to PC₂₀ provides a better association with OA vs. non-OA when PC₂₀ is normal. Levels of sputum eosinophils in addition to PC₂₀ and IgE-mediated reactivity increase the likelihood of OA due to wheat flour.

Keywords asthma, occupational asthma, specific and non-specific bronchial reactivity

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Introduction

Specific inhalation challenges are used in the diagnosis of occupational asthma (OA). Challenges involve exposing workers to the suspected occupational agent in a dose-progressive manner [1]. Although the safety of the test has been improved by using innovative equipment [2] that has recently been updated [3], prevention of excessive asthmatic reactions is an ongoing concern.

The occurrence of asthmatic reactions to an inhalant sensitizer is conditioned by the status of non-specific bronchial reactivity and immunological reactivity. This was initially proposed in the 1950s by the French physi-

ologist and pneumologist Robert Tiffeneau [4, 5]. A more quantitative prediction formula that considers the diameter of skin prick test and non-specific reactivity was proposed by Hargreave and Cockcroft in the late 1970s [6] and 1980s [7]. Studies have shown that the concomitant effect of immunologic reactivity and non-specific bronchial responsiveness is also relevant in the case of OA with regard to occupational allergens [8–10]. One study failed to show a significant association between skin reactivity and specific bronchial responsiveness to wheat flour, although there was a trend [11]. The status of immunological reactivity seems even more important than the status of non-specific bronchial responsiveness [10] as

also suggested by Lemi re and co-workers, who were able to elicit immediate bronchial reactions in subjects with persistent IgE-mediated reactivity to an occupational agent but normal bronchial responsiveness to methacholine [12].

Airway inflammation, eosinophilic and also neutrophilic, is a key feature of asthma. The development of non-invasive monitoring tools of airway inflammation by induced sputum and exhaled NO has greatly facilitated assessment. Monitoring airway inflammation has been proposed in the management of asthma [13–16].

It can be hypothesized that the presence of airway inflammation may facilitate the induction of an asthmatic reaction by an allergen. The increased number of inflammatory cells and concentrations of mediators can indeed enhance the allergenic response and contribute to non-specific bronchial hyperresponsiveness [17]. By using a database of subjects investigated for OA in our centre, our aim was to examine the association of airway inflammation and non-specific bronchial hyperresponsiveness with specific bronchial reactivity to an occupational agent. In addition, in a subgroup of subjects with OA due to wheat flour, we examined the combined effect of IgE specific reactivity.

Material and methods

Patients

We examined data concerning specific inhalation challenges with occupational agents of 665 subjects investigated from 2000 onwards; at the time, induced sputum was routinely incorporated in the assessment.

Procedures

Briefly, as described elsewhere [1, 18], subjects investigated for possible OA first undergo a day of testing in which they are exposed to a control product. Spirometry is measured before and serially for 7–8 h after exposure [19], with assessment at the end of the day of responsiveness to methacholine [20, 21] and evaluation of inflammatory cells in induced sputum with aerosols of hypertonic saline (3%, 4% and 5%) each for 7 min according to a standardized methodology [22]. Over the next few days, subjects are exposed to the suspected occupational agents in the hospital laboratory, generated as a dry or wet aerosol or as a vapour, either by the 'realistic way' in a cubicle or using closed-circuit equipment with direct assessment and control of concentrations. In 97 instances, testing was carried out at the workplace because exposure could not be reproduced satisfactorily in the hospital laboratory. A final diagnosis of OA, asthma or neither OA nor asthma is made. The diagnosis of OA is retained if there are significant changes in forced expiratory volume in 1 s (FEV_1) ($\geq 20\%$) after exposure to the suspected agent in

the absence of significant changes in FEV_1 ($< 10\%$) on the control day. If changes in FEV_1 from 10% to 20% were observed on exposure to the suspected agent, the duration of exposure was prolonged on the following day to exclude OA. Subjects for whom the diagnosis of OA is excluded as a result of negative challenges are further assessed by serial monitoring of peak expiratory flows on return to work.

Skin testing was carried out by the prick method [23] using a battery of 15 ubiquitous commercial common aeroallergens. Atopy was defined as at least one immediate reaction [23]. The wheat extract that was used was obtained from Omega (Montreal, Canada) at 1 : 10 concentration.

Analysis of results

The information obtained included demographic, clinical and functional variables: atopic status, spirometry [FEV_1 , FEV_1 /forced vital capacity (FVC)], provocative concentration of methacholine causing a fall of 20% in FEV_1 (PC_{20}), percentage of eosinophils and neutrophils in induced sputum; in the case of eosinophils, cut-off thresholds of 1%, 2% and 3% were selected, the upper limit of 2 SD of the mean value of the distribution in normal subjects having been described as 2.2% [24]. A separate analysis was performed for those taking and not taking inhaled steroids. In a group of 34 subjects investigated for possible OA to wheat flour, we also examined the results of immediate skin reactivity and specific IgE to this allergen. Specific IgE to wheat flour was assessed by a modified ELISA. Briefly, the wheat flour extract was dissolved in a carbonate buffer (100 μ g/mL) and coated in a 96-well plate overnight at 4 °C. After the washing and blocking steps, sera (1 : 2 dilution in blocking buffer) were incubated for 2 h at room temperature. The levels of specific IgE having bound to the coated wheat flour proteins were determined by adding a biotin mouse anti-human IgE (BD Pharmingen, Mississauga, ON, Canada) (dilution 1 : 250) followed by incubation with streptavidin-HRP (R&D Systems, Minneapolis, MN, USA) (dilution 1 : 200). Stabilized TBM substrate (Calbiochem, San Diego, CA, USA) was then added to coated wells for 20 min and the reaction stopped with a H_2SO_4 solution (2 N). The plate was read at 450 nm, and specific optical densities (OD) were calculated by subtracting the background value.

Statistical analysis was carried out by examining information derived from 2 $\times$ 2 tables. In the group of subjects with possible OA to wheat flour, a logistic regression was used in examining the probability of OA from the status of immunological reactivity to flour (skin test wheal diameter graded from 0 – negative to 4 – highly positive and levels of specific IgE to flour), sputum eosinophil levels ($\geq 1\%$ or $\geq 3\%$ vs. lower) and PC_{20} level (≤ 16 mg/mL vs. higher).

The ethics committee of Sacré-Coeur Hospital examined and accepted the protocol. No consent form was required because data were examined in an anonymous way. Subjects were not identified by names but by coding.

Results

Table 1 lists the baseline characteristics of the subjects. The sample was made of approximately two-thirds of men with a mean age of 41.6 years. The median duration of exposure with symptoms was 1.7 years and the median

Table 1. Baseline characteristics of subjects ($n = 519$)

Sex (M/F)	334 (64.4%)/185 (35.6%)
Age at diagnosis (years)*	41.6 ± 11.1
Duration of exposure with symptoms (years)**	1.7 (0.8, 4.6)
Delay between cessation of exposure and testing (days)**	76 (1, 173)
Responsible agent	
High molecular weight	136 (26.2%)
Flour	42 (8.1%)
Cereals	8 (1.5%)
Various proteins	37 (7.1%)
Low-molecular weight	355 (68.4%)
Isocyanates	98 (18.9%)
Wood dust	37 (7.1%)
Metals	29 (5.6%)
Resins and glues	35 (6.7%)
Unknown***	28 (5.4%)
Atopy (present/absent/unknown)	358/129/32 (69%/24.9%/6.2%)
Smoking habits (smokers/ ex-smokers/non-smokers/unknown)	147/172/190/10 (28.3%/33.1%/36.6%/2.0%)
Treatment with inhaled steroids (yes/no/unknown)	250/258/11 (48.3%/49.7%/2.1%)
Final diagnosis	
Occupational asthma	129 (24.9%)
Non-occupational asthma	187 (36.0%)
No occupational asthma, no asthma	203 (39.1%)
FEV ₁ (% pred)*	93.5 ± 16.3
n with values < 80% pred	98 (18.9%)
FEV ₁ /forced vital capacity (%)*	77.3 ± 8.8
n with values < 70%	92 (17.7%)
PC ₂₀ ≤ 16 mg/mL	285 (54.9%)
Induced sputum	
Eosinophils ≥ 1%	191 (36.8%)
Eosinophils ≥ 2%	130 (25.0%)
Eosinophils ≥ 3%	90 (17.3%)
Neutrophils ≥ 60%	141 (27.2%)

Number with % in parentheses.

*Mean ± SD.

**Median value, 25% and 75% interquartiles in parentheses;

***In 97 subjects, exposure to the potential occupational agent was carried out at the workplace, and in 28 instances, the nature of the product could not be determined.

delay between cessation of exposure and testing was 76 days. The agents that were probably responsible were more often of the low-molecular weight type. Atopy was often present. About half of the subjects were treated with inhaled corticosteroids. Not shown in Table 1, the percentages of subjects taking inhaled steroids were similar in subjects with a confirmed diagnosis of OA (60.5%) and in subjects with non-OA (66.8%). A confirmed diagnosis of OA was made in nearly one-quarter of the subjects (63% or 48.9% showing isolated immediate reactions, 31% or 24.1% an isolated late reaction, 16% or 12.4% dual reactions and 19% or 14.6% atypical reactions) and 36% had a diagnosis of non-OA. For the remaining subjects, another diagnosis (rhinitis, hyperventilation, etc.) was made. Nearly 20% of subjects had significant airway obstruction and bronchial hyper-responsiveness was documented in 54.9% of subjects at the time of investigation. The presence of an increased number of eosinophils in induced sputum was frequent, from 36.8% to 17.3% depending on the thresholds that were selected, that is 1%, 2% or 3%. The percentages of eosinophils were not significantly different in subjects taking inhaled steroids (median: 0.8%, 25–75% interquartiles: 0–0.2%) and subjects not on inhaled steroids (median: 0.7, 25–75% interquartiles: 0–2%). The proportions of subjects with eosinophil levels ≥ 1%, 2% and 3% were not different in the groups with (51%, 52% and 54%, respectively) and without inhaled steroids. Not shown in Table 1, 146 subjects, in addition to the 519 (22% of a total of 655 subjects), were not included in the analysis because they were not able to produce reliable samples of induced sputum. The percentages of subjects with, respectively, OA, non-OA and other diagnoses in these 146 subjects were 24.1%, 32.8% and 43.1%, comparable with the percentages that prevailed for the group of 519 subjects.

As shown in Table 2, nearly half (65/129) of subjects with OA had levels of eosinophils < 1% and 35/129 (27%), normal bronchial responsiveness on the control day. Proportionally fewer subjects with OA had abnormal PC₂₀ at the time of investigation (RR = 0.47). The positive predictive values of levels of eosinophils in identifying cases of OA vs. non-OA were 43.8% (threshold at 1%) and 49.3% (threshold at 3%), higher than the value of 35.4% by using the PC₂₀ threshold of 16 mg/mL. The negative predictive value was also lower for PC₂₀ than for sputum eosinophils. However, by comparing OA with all the other subjects (non-OA + other diagnoses) (bottom section of Table 2), the positive predictive values were low and comparable by using sputum eosinophils or PC₂₀ whereas the negative predictive values were much higher whatever the means. In the analysis of results shown in Table 2, the positive and negative predictive values were comparable by separating those not taking from those taking inhaled steroids. The levels of sputum neutrophils were not

Table 2. Results of univariate analysis

	Sputum eosinophils		Threshold at 3%		PC ₂₀	
	Threshold at 1% ≥ 1%	< 1%	≥ 3%	< 3%	Threshold at 16 mg/mL ≤ 16 mg/mL	> 16 mg/mL
Occupational asthma	64	65	37	92	94	35
Non-occupational asthma	82	105	38	149	172	15
Positive predictive value	43.8% (46.1%, 40.5%)		49.3% (52.9%, 45%)		35.4% (34.8%, 35.2%)	
Negative predictive value	58.3% (56.2%, 63.8%)		61.6% (58.2%, 63.9%)		30.0% (22.2%, 40.9%)	
RR	1.10 (0.91–1.32)		1.28 (0.97–1.70)		0.47 (0.30–0.71)	
OR	1.25 (0.80–1.96)		1.57 (0.93–2.65)		0.24 (0.12–0.45)	
Occupational asthma	64	65	37	92	94	35
Non-occupational asthma and other diagnosis	127	263	53	337	191	199
Positive predictive value	33.5% (31.9%, 34.7%)		41.1% (42.8%, 40.0%)		33% (31.2%, 33.5%)	
Negative predictive value	80.2% (87.1%, 72.7%)		81.6% (84.7%, 72.1%)		85.0% (87.0%, 80%)	
RR	1.20 (1.07–1.35)		2.54 (1.57–4.11)		1.27 (1.15–1.40)	
OR	2.03 (1.35–3.05)		2.53 (1.57–4.08)		2.81 (1.82–4.35)	

Number of subjects listed in each category; positive and negative predictive values are given for the whole group and by distinction for those, respectively, not taking and taking inhaled steroids in parentheses.

OR, odds ratio; RR, relative risk. 95% Confidence Interval of RR and OR in parentheses.

significantly associated with the final diagnosis (not shown in Table 2).

Table 3 presents results of a bivariate analysis that was examined by stratifying according to the levels of eosinophils and PC₂₀. By using eosinophil thresholds ≥ 1% and 3% combined with a PC₂₀ value > 16 mg/mL, it can first be seen that the ratios of positive and negative predictive values were more satisfactory than by using PC₂₀ or eosinophil levels alone (Table 2), with significant RR and OR values. Moreover, positive predictive values were higher in subjects not taking inhaled steroids (first figures listed in parentheses) with virtually unchanged negative predictive values. Subjects with OA were more likely to have normal PC₂₀ results than subjects with non-OA, but more likely to show increased sputum eosinophils than subjects with non-OA.

Table 4 shows that 84.3% of all subjects with normal levels of PC₂₀ (> 16 mg/mL) and eosinophils (< 1%) had a final diagnosis that was neither OA nor non-OA. On the other hand, a large proportion (60.2%) of all subjects with bronchial hyperresponsiveness but normal eosinophil levels were affected with non-OA. All subjects except one with bronchial hyperresponsiveness and sputum eosinophilia ≥ 1% suffered from either OA (38.0%) or non-OA (61.1%).

In a sample of 34 subjects exposed to wheat flour, we assessed specific IgE antibodies. As shown in Fig. 1, in these subjects there was a satisfactory correlation between results of skin prick tests and specific IgE antibodies to wheat flour. Results of the logistic regressions are shown in Table 5. In all these regressions, wheat weal size and specific IgE values as well as PC₂₀ ≤ 16 mg/mL were almost always significantly associated with the presence of OA. Also, by introducing in a single regression levels of

eosinophils ≥ 1%, wheat IgE value and wheat skin weal size as well as PC₂₀, all these factors were shown to be significantly associated with the presence of OA.

Discussion

This study shows that the level of eosinophils provides slightly higher positive predictive values than PC₂₀ for the likelihood of presenting OA by comparison with non-OA. Moreover, by comparison with subjects with non-OA, subjects with OA are more likely to react to an occupational agent and have a diagnosis of OA if PC₂₀ is normal and the level of sputum eosinophils is increased on the control day preceding exposure to the relevant occupational agent. Normal value for the level of PC₂₀ and sputum eosinophil on the control day can be related to the fact that, at the time of starting investigation, subjects had not been exposed at work for a median period of 76 days. Stopping exposure to the relevant occupational agent results in improvement in asthma [25]. It is interesting to note that, by comparison with bronchial responsiveness to methacholine, proportionally more subjects still showed eosinophilic inflammation. Levels of sputum eosinophils generally retrocede towards normal levels shortly after stopping exposure to the agent causing OA [26]. However, they can persist for many years [27].

More importantly, this study shows that the addition of information on the PC₂₀ level and sputum eosinophils results in a higher positive predictive value by comparing subjects with OA and non-OA when PC₂₀ is normal, this being more pronounced by examining subjects not taking inhaled steroids. Examination of non-specific bronchial responsiveness and induced sputum play key

Table 3. Association of responsiveness to methacholine and sputum eosinophils in combination with final diagnosis

	Eosinophils $\geq 1\%$ and PC ₂₀ ≤ 16	Others	Eosinophils $\geq 3\%$ and PC ₂₀ ≤ 16	Others	Eosinophils $\geq 1\%$ and PC ₂₀ > 16	Others	Eosinophils $\geq 3\%$ and PC ₂₀ > 16	Others
Occupational asthma	48	81	28	101	16	113	9	120
Non-occupational asthma	74	113	34	153	8	179	4	183
Positive predictive value	39.3% (36.7%, 39.4%)		45.2% (42.3%, 45.7%)		61.1% (75.0%, 50.0%)		69.2% (87.5%, 40%)	
Negative predictive value	58.0% (48.4%, 62.9%)		60.2% (54.0%, 63.8%)		66.6% (59.7%, 62.6%)		60.2% (58.0%, 62.1%)	
RR	0.94 (0.71–1.24)		1.13 (0.8–1.56)		1.71 (1.25–2.36)		1.74 (1.18–2.56)	
OR	0.90 (0.56–1.42)		1.23 (0.71–2.18)		3.15 (1.30–7.6)		3.43 (1.03–11.39)	
Occupational asthma	48	81	28	101	16	113	9	120
Non-occupational asthma	75	315	34	356	52	338	19	371
and other diagnosis								
Positive predictive value	41.6% (36.0%, 39.4%)		45.1% (42.3%, 45.7%)		23.5% (27.2%, 19.0%)		32.1% (43.7%, 20.0%)	
Negative predictive value	78.3% (84.1%, 73.7%)		77.8% (82.7%, 72.5%)		74.8% (81.7%, 68.9%)		75.5% (81.8%, 69%)	
RR	1.90 (1.42–2.55)		2.03 (1.47–2.81)		0.94 (0.59–1.48)		1.31 (0.75–2.30)	
OR	2.48 (1.60–3.84)		2.89 (1.67–5.0)		0.92 (0.50–1.67)		1.46 (0.64–3.32)	

Number of subjects listed in each category; positive and negative predictive values are given for the whole group and by distinction for those, respectively, not taking and taking inhaled steroids in parentheses.

OR, odds ratio; RR, relative risk. 95% Confidence Interval of RR and OR in parentheses.

roles in the investigation of OA [26]. We show that both factors contribute to the likelihood of presenting asthmatic reactions on exposure to an agent that may cause asthma. Non-specific bronchial responsiveness has been related to various components of the inflammatory and remodelling aspects of asthma. It is interesting to note that eosinophilic inflammation plays a role, as initially proposed by Bousquet et al. [17]. The presence of airway inflammation makes the asthmatic reaction on exposure to an allergen more likely to occur because cells and mediators are present, activated and ready to intervene. Neutrophilic infiltration has been recognized as a feature of severe asthma [28]. Neutrophils seem to play a role in OA due to low-molecular-weight agents [29] and especially polyisocyanates [30]. However, in this study we were unable to find significant associations between neutrophil levels in induced sputum and specific bronchial reactivity.

Tiffeneau originally suspected that the status of immunological reactivity and non-specific bronchial responsiveness both played a role in susceptibility to developing an asthmatic reaction on exposure to a relevant allergen [4]. This was confirmed for ubiquitous inhalants [6, 7] and occupational agents [8–10]. We generated a multivariate model to find out whether eosinophilic level also contributes to this susceptibility in a sample of 34 subjects exposed to wheat flour. We found this to be the case in addition to non-specific bronchial responsiveness and immunological reactivity. Interestingly, both skin test weal diameter and levels of specific IgE seem to be involved. Like others, we found a significant relationship between the size of the weal diameter to wheat and the levels of specific IgE, although this association is far from being perfect [31] even in the more specific case of wheat flour solutions [32]. It is therefore likely that a positive reaction using an *in vitro* or *in vivo* method may reflect different aspects of IgE-mediated immunological reactivity. Finally, in the studies assessing the relationship between methacholine reactivity, skin sensitivity and outcome of specific inhalation challenge [6, 7], the skin end-point titration with the allergen is commonly used. This is considered to be a more precise index of skin reactivity than the weal size with a single allergen concentration. Our specific challenges were not carried out by using soluble preparations of wheat, but rather the dry particles, which precluded using the skin end-point titration. Even with a less precise index, we were nevertheless able to show that the result of prick skin test plays a role in determining specific airway reactivity besides specific IgE levels, eosinophil level and PC₂₀.

Although our analysis was retrospective, it is based on a large number of subjects who were investigated prospectively using a standardized procedure. In our study, we were able to retain 78% (519/665) of subjects who were assessed in our centre from 2000 onwards, the remainder

Table 4. Levels of bronchial responsiveness and induced sputum according to final diagnosis

	Occupational asthma	Non-occupational asthma	Other diagnoses	Total
Threshold of eosinophils at 1%				
PC ₂₀ > 16 mg/mL and eosinophils < 1%	19 (11.5%)	7 (4.2%)	140 (84.3%)	166
Not on inhaled steroids	9 (7.6%)	2 (1.1%)	107 (90.7%)	118
On inhaled steroids	9 (20.5%)	5 (11.4%)	30 (68.2%)	44
PC ₂₀ ≤ 16 mg/mL and eosinophils < 1%	46 (28.6%)	97 (60.2%)	18 (11.2%)	161
Not on inhaled steroids	12 (26.1%)	25 (54.3%)	9 (19.6%)	46
On inhaled steroids	34 (29.8%)	71 (62.3%)	9 (7.9%)	114
Eosinophils ≥ 1% and PC ₂₀ > 16 mg/mL	16 (24.0%)	8 (12.1%)	42 (63.6%)	66
Not on inhaled steroids	12 (27.3%)	4 (9.1%)	28 (63.6%)	44
On inhaled steroids	4 (19.0%)	4 (19.0%)	13 (61.9%)	21
PC ₂₀ ≤ 16 mg/mL and eosinophils ≥ 1%	46 (38.0%)	74 (61.1%)	1 (0.9%)	121
Not on inhaled steroids	18 (36.0%)	31 (62.0%)	1 (2%)	50
On inhaled steroids	28 (39.4%)	43 (60.6%)	0 (0%)	71
Threshold of eosinophils at 3%				
PC ₂₀ > 16 mg/mL and eosinophils < 3%	26 (12.6%)	11 (5.3%)	169 (82.0%)	206
Not on inhaled steroids	14 (9.6%)	5 (3.4%)	127 (87.0%)	146
On inhaled steroids	11 (20%)	6 (10.9%)	38 (69.1%)	55
PC ₂₀ ≤ 16 mg/mL and eosinophils < 3%	66 (29.6%)	138 (61.9%)	19 (8.5%)	223
Not on inhaled steroids	19 (27.1%)	41 (58.5%)	10 (14.2%)	70
On inhaled steroids	46 (30.6%)	95 (63.3%)	9 (6.0%)	150
Eosinophils ≥ 3% and PC ₂₀ > 16 mg/mL	9 (32.3%)	4 (14.3%)	15 (53.5%)	28
Not on inhaled steroids	7 (43.8%)	1 (6.2%)	8 (50.0%)	16
On inhaled steroids	2 (20.0%)	3 (30.0%)	5 (50.0%)	10
PC ₂₀ ≤ 16 mg/mL and eosinophils ≥ 3%	28 (45.2%)	34 (54.8%)	0 (0%)	62
Not on inhaled steroids	11 (42.3%)	15 (57.7%)	0 (0%)	26
On inhaled steroids	16 (45.2%)	19 (54.8%)	0 (0%)	35

Number of subjects with percentages of the total in parentheses.

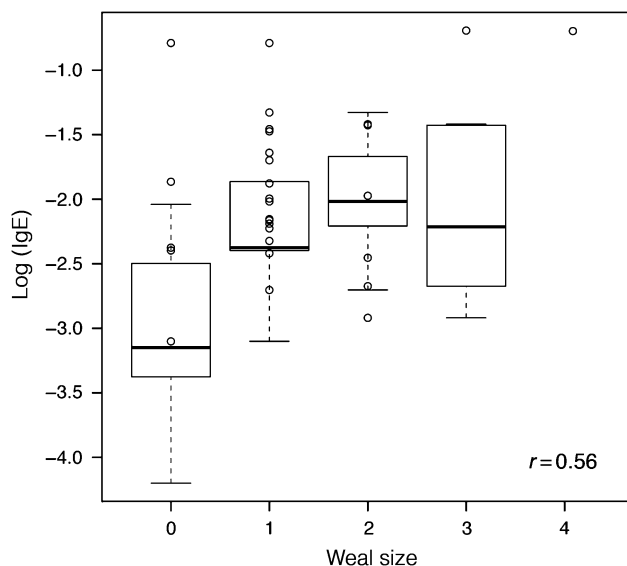


Fig. 1. Relationship between weal size of the immediate skin reaction and specific IgE values (log) to wheat. Weal size graded as follows : 0 : 0 mm; 1 : 1–2 mm; 2 : 3–5 mm; 3 : 6–9 mm; 4 : 10 mm or more. Significant *r* value of the correlation given on the right end side.

being subjects who were unable to provide valuable and interpretable sputum on the control day of testing. This sample represents the largest series of subjects ever

described, previous contributions [9–11] including fewer subjects and no information on induced sputum. We showed that the proportions of subjects with OA, non-OA and other diagnoses in the 116 subjects who were unable to produce interpretable sputum were similar to the group of 519 subjects. Although a proportion of subjects are unable to produce satisfactory samples of sputum, this assessment seems more valuable than exhaled NO in the assessment of airway inflammation, as recently documented in subjects investigated for OA [33].

Approximately, half of our subjects were on inhaled steroids at the time of testing. Our study represents a 'real life' situation in which subjects are referred for possible OA, some taking inhaled steroids and others not. One of the most important requirements in the interpretation of specific inhalation challenges is in establishing that airway calibre is stable on a control day. Stopping inhaled steroids often results in unstable asthma, which makes specific inhalation challenges not interpretable. Inhaled steroids do not affect the magnitude of immediate asthmatic reactions, which occurred in nearly 50% of our subjects with OA. Although they can diminish the magnitude of late reactions, which were documented in 31% of subjects with OA, they rarely entirely suppress such reactions. If changes in FEV₁ from 10% to 20% were observed in a subject, the duration of exposure was

Table 5. Multivariate analysis for the predictive value of the presence of occupational asthma

	P-value	OR	95% CI
Threshold of eosinophils at 1%			
PC ₂₀ ≤ 16 mg/mL	0.06	3.95	0.95–16.33
Eosinophils ≥ 1%	0.29	2.24	0.50–9.99
PC ₂₀ ≤ 16 mg/mL	0.07	7.35	0.85–63.3
Eosinophils ≥ 1%	0.04	22.49	1.09–463.8
Wheat weal size	0.01	7.44	1.51–35.5
PC ₂₀ ≤ 16 mg/mL	0.01	13.65	1.71–109.31
Eosinophils ≥ 1%	0.26	2.89	0.46–18.24
Wheat IgE value	0.02	1.02	1.00–1.03
PC ₂₀ ≤ 16 mg/mL	0.01	15.38	1.71–138.7
Eosinophils ≥ 1%	0.36	2.34	0.38–14.25
Log (wheat IgE value)	0.005	10.76	2.09–55.50
PC ₂₀ ≤ 16 mg/mL	0.05	25.89	1.06–631.94
Eosinophils ≥ 1%	0.06	144.6	0.79–264.67
Wheat IgE value	0.05	1.02	1.00–1.03
Wheat weal size	0.05	13.0	0.95–177.27
Threshold of eosinophils at 3%			
PC ₂₀ ≤ 16 mg/mL	0.07	3.82	0.91–15.98
Eosinophils ≥ 3%	0.54	1.70	0.26–11.87
PC ₂₀ ≤ 16 mg/mL	0.08	5.23	0.79–34.8
Eosinophils ≥ 3%	0.18	6.7	0.42–104.8
Wheat weal size	0.01	4.14	1.34–12.76
PC ₂₀ ≤ 16 mg/mL	0.02	9.34	1.33–66.16
Eosinophils ≥ 3%	0.77	1.36	0.17–10.56
Wheat IgE value	0.03	– *	– *
PC ₂₀ ≤ 16 mg/mL	0.02	10.38	1.41–76.6
Eosinophils ≥ 3%	0.82	1.26	0.15–10.0
Log (wheat IgE value)	0.008	9.77	1.79–53.4
PC ₂₀ ≤ 16 mg/mL	0.04	9.77	1.05–90.6
Eosinophils ≥ 3%	0.19	6.89	0.38–125.3
Wheat IgE value	0.10	– *	– *
Wheat weal size	0.06	3.54	0.96–13.03

*Value could not be calculated, the denominator being zero.

OR, odds ratio; 95% CI, 95% confidence interval.

prolonged on the following day to exclude OA. Furthermore, our investigation of subjects referred for OA also includes monitoring of peak expiratory flows. Whenever return to work suggests OA according to peak expiratory flows, specific inhalation challenges are repeated to exclude OA. The percentages of subjects taking inhaled steroids in those with OA and non-OA were similar (60.5% and 66.8%, respectively) as well as the percentages of eosinophils in those taking or not taking inhaled steroids. In the analysis of our results, selection of a positive threshold for PC₂₀ at the upper limit (≤ 16 mg/mL) and eosinophils at a relatively low value of 1% was intended to counterbalance the possible 'negative' effect that inhaled steroids may have had on these results. The positive predictive and negative values of thresholds of eosinophils at 1% and 3% as well as of PC₂₀, analysed separately, were comparable in those not taking and taking inhaled steroids (Table 2). However, by combining levels of eosinophils and PC₂₀ values (Table 3), having eosinophil levels ≥ 1% and ≥ 3% and a PC₂₀ > 16 mg/mL resulted in higher positive predictive values in subjects not taking inhaled steroids, which shows that inhaled steroids had probably exerted some effects in this group.

Our study has practical implications and clinical relevance. Specific inhalation challenges in the laboratory or the workplace are regarded as the best tool for confirming OA. One of the difficulties in the procedure of these tests is the risk of a potentially great asthmatic reaction. Equipment designed to reduce this risk has been developed in recent years [2]. Information on baseline immunological, functional and inflammatory bronchial status at the time of starting exposure, as advocated in this work, should also contribute to reducing risks and promoting the use of such testing.

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