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Frequency and determinants of exaggerated bronchoconstriction during shortened methacholine challenge tests in epidemiological and clinical set-ups

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Frequency and determinants of exaggerated bronchoconstriction during shortened methacholine challenge tests in epidemiological and clinical setups. S. Troyanov, J-L. Malo, A. Cartier, D. Gautrin. ©ERS Journals Ltd 2000.

ABSTRACT: The European Respiratory Society guidelines on bronchial provocation testing have proposed time-saving procedures, which may lead to unwanted exaggerated responses. The frequency and determinants of exaggerated bronchoconstriction in response to methacholine inhalation testing in clinical and epidemiological settings have not been assessed.

The authors evaluated: 1) the prevalence of exaggerated bronchoconstriction, 2) its relation to time-saving measures (starting methacholine concentration and skipping concentrations); and 3) associations between such reactions and risk factors, respiratory symptoms and/or lung function parameters. Clinical (n=408) and epidemiological (n=711) groups were included. Exaggerated bronchoconstriction was defined as either a fall ≥20% following saline or a ≥30% fall in forced expiratory volume in one second (FEV_1) after methacholine inhalations. Cases were compared with two groups of subjects: 1) with measurable bronchial responsiveness (MBR); and 2) without MBR.

In the epidemiological group, 84 subjects (12%) presented exaggerated bronchoconstriction. Skipped concentrations accounted for an exaggerated bronchoconstriction in 18 of these. In the clinical group, 41 subjects (10%) experienced exaggerated reactions. Skipped concentrations accounted for an exaggerated bronchoconstriction in five of these. The provocative concentration of methacholine causing a 20% fall in FEV_1 values were marginally lower in subjects with exaggerated bronchoconstriction. Questionnaire analysis in epidemiological subjects showed some symptoms of asthma and rhinitis to be more prevalent in cases than in subjects without MBR.

In conclusion, methacholine inhalation tests with time-saving measures result in a 10% risk of exaggerated bronchoconstriction as defined in this study and bronchial responsiveness is more prominent in subjects with exaggerated bronchoconstriction. *Eur Respir J 2000; 16: 9–14.*

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Inhalation challenges with pharmacological agents (acetylcholine derivatives, histamine) were first used in humans in the 1940s [1–3]. They were initially proposed to assess nonspecific bronchial responsiveness for clinical purposes. Efforts of standardization were made in the 1970s [4–6]. Inhalation tests have been used more recently in epidemiological settings [7, 8]. Experienced technicians perform this procedure without onsite physician attendance.

To reduce the duration of the test, the initially proposed protocols for inhalation have been modified [9, 10]. Now, the starting concentration can be >0.03 $mg \cdot mL^{-1}$ and concentrations can be skipped using information about current medication and baseline forced expiratory volume in one second (FEV_1). A special committee of the European Respiratory Society (ERS) has issued statements calling for superior and safer guidelines for the testing procedure [11].

Although methacholine inhalation tests appear safe and no written report of persisting deterioration of asthma or deaths has surfaced, the frequency and factors that condition exaggerated bronchoconstriction, which can occur with such procedures, have not, to the authors' knowledge, been reported. The purpose of this study was therefore to assess the prevalence of exaggerated bronchoconstriction to methacholine in epidemiological and clinical set-ups in an effort to validate the ERS shortening recommendations [11]. The authors also wanted to determine whether suspected respiratory symptoms and/or functional parameters were associated with such reactions by comparing subjects with exaggerated bronchoconstriction with a comparison group without.

Subjects and methods

Study design

To evaluate the risk of bronchial challenge testing in different settings, an epidemiological group from a prospective epidemiological study performed in a population of

young adults and a clinical group of subjects referred for methacholine testing in a tertiary care hospital, were studied. Subjects in both groups underwent spirometry before assessment of bronchial responsiveness to methacholine. The epidemiological group also answered a questionnaire on symptoms and health status before bronchial challenge test. The decision to use an epidemiological group for this study was made *a posteriori* with respect to the gathering of the data while it was prospective for the clinical group.

Subjects

Two groups of subjects were studied: 1) epidemiological group. The epidemiological group comprised 769 subjects participating in an epidemiological survey to assess the prevalence and incidence of immunological sensitization, bronchial responsiveness and respiratory symptoms in contact with high-molecular-weight occupational allergens [12]. They were students entering study programmes in animal health, pastry-making or dental-hygiene technology in 14 different schools. Spirometry, bronchial provocation test and questionnaire were obtained between September 1993 and December 1995. The participants gave written consent; 2) clinical group. The clinical group comprised 408 consecutive subjects assessed at the pulmonary function laboratory of Sacré-Coeur hospital in Montreal (Canada) and referred by general practitioners or specialists. Spirometry, methacholine inhalation test and medication history were obtained between March 1998 and March 1999. None of the subjects included in the epidemiological or clinical groups had ever undergone a methacholine inhalation test.

Study measures

Subjects in both groups had spirometry (FEV_1, forced vital capacity (FVC)) assessed according to proposed criteria [13]. Methacholine inhalation tests were performed using a Wright′s nebulizer (Roxon, medi-tech, Etée, Montreal, Quebec, Canada; output=0.14 $mL \cdot min^{-1}$) at tidal volume for 2 min according to guidelines from the ERS [11]. Each subject inhaled saline solution (0.9%) followed by doubling doses of methacholine until a 20% fall in FEV_1 occurred or all concentrations up to a maximum of 32 $mg \cdot mL^{-1}$ had been used. For subjects in the clinical cohort and evaluated for occupational asthma, the test was pursued to a maximum of 128 $mg \cdot mL^{-1}$.

Criteria for setting the starting concentration of methacholine in the clinical group were chosen along recommendations of the ERS [11]: 1) baseline FEV_1 of $\geq$1.5 L; 2) if FEV_1 fell by >20% following saline inhalation, methacholine inhalation was not carried out; 3) if FEV_1 was <70% of the predicted value or FEV_1 fell by 10–20% following saline inhalation, the starting dose of methacholine was 0.03 $mg \cdot mL^{-1}$ for subjects taking corticosteroids (any form) and 0.125 $mg \cdot mL^{-1}$ for other subjects; 4) if FEV_1 was >70% pred and FEV_1 fell by <10% following saline inhalation, the starting dose of methacholine was 0.125 $mg \cdot mL^{-1}$ for subjects taking corticosteroids (any form), 0.25 $mg \cdot mL^{-1}$ those taking daily bronchodilators, 1 $mg \cdot mL^{-1}$ for those taking bronchodilators occasionally and 2 $mg \cdot mL^{-1}$ for others. If the total fall in FEV_1 was <7% after a concentration, the next doubling concentration was skipped and the higher dose nebulized.

The procedure for performing the methacholine test in the epidemiological cohort was slightly modified from the ERS guidelines [11] stated in the previous paragraph. The minimal starting FEV_1 was 2 L to take into account the absence of an onsite physician. The starting concentration of methacholine was chosen as follows: 1) if FEV_1 fell by >10% following saline inhalation, the test was cancelled; 2) if the subject reported respiratory symptoms, the starting concentration was 0.5 $mg \cdot mL^{-1}$; 3) or they had an FEV_1 <80% pred, the starting concentration of methacholine was 0.03 $mg \cdot mL^{-1}$. Otherwise, the starting methacholine concentration was 2 $mg \cdot mL^{-1}$. If the total fall in FEV_1 was <5% after a concentration, the next doubling concentration was skipped and the higher dose nebulized. In addition to the ERS guidelines, when the starting FEV_1 was <80% pred, no skipped concentrations were allowed.

Significant bronchoconstriction was defined as a $\geq$20% fall in FEV_1 following the saline inhalation or a $\geq$30% fall at the last dose of methacholine inhaled (case definition). Subjects with a fall in FEV_1 of $\geq$10% at the end of the test were administered salbutamol with a metered-dose inhaler at a dose of 200 μg, once or twice in the event of the absence of a satisfactory recovery in FEV_1 (FEV_1 <90% baseline value). The concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) was obtained from individual dose-response curves drawn on a semi-logarithmic scale using non-cumulative doses. Logarithmic transformation of PC_{20} was used for statistical analysis. Reference values for FEV_1 and FEV_1/FVC were obtained from KNUDSON *et al.* [14].

In the case of the epidemiological group, a questionnaire derived from the standardized questionnaire of the International Union against Tuberculosis and Lung Disease [15] was administered by an experienced nurse. Information was obtained on respiratory symptoms (wheezing, shortness of breath, nocturnal cough, expectoration, and chest tightness), morning nasal obstruction and rhinitis. These symptoms were assessed in relation to cold air exposure, exercise or strong efforts, seasons, night-time, pets and environmental pollutants or allergens (dust, smoke, odours, feathers, trees, grass, flowers). History of physician-based diagnosis of asthma, chronic pulmonary obstructive disease, eczema, hay fever and urticaria as well as information on current respiratory medication and tobacco use, were obtained.

Statistical analysis

Descriptive analysis was used to show frequency and proportions of exaggerated bronchoconstriction with respect to the number of subjects in each group. To evaluate determinants of adverse reaction to bronchial challenge tests, cases were compared with two groups of subjects: 1) subjects with measurable bronchial responsiveness (MBR) ($PC_{20} \leq 32$ $mg \cdot mL^{-1}$); 2) subjects without MBR ($PC_{20} > 32$ $mg \cdot mL^{-1}$); Indeed, it was suspected that the fact of having MBR would be determinant, *per se*, of increased risk of exaggerated bronchoconstriction. Pairing was done according to sex and age (±5 yrs), and randomly within sex and age categories. In the epidemiological cohort, the school

attended by the students was an additional matching variable. Analysis of variance was used to compare means between groups with *post-hoc* analysis using the least significant difference method for significant results. FEV_1, FVC and FEV_1/FVC were compared (as percentages of their predicted value). The number of inhaled concentrations of methacholine, FEV_1 after bronchodilator (expressed as a percentage of the predicted FEV_1) and the number of bronchodilator inhalations administered were compared between groups. Chi-squared analysis was used to compare the frequency of subjects between groups with: 1) baseline FEV_1 variability >5% (defined as maximal difference between all trials of FEV_1 expressed as a percentage of the best baseline FEV_1; 2) baseline FEV_1 reproducibility criteria achieved with ≥4 trials; and 3) skipped methacholine concentrations. In the epidemiological group, Chi-squared values and odd ratios (OR) ±95% confidence intervals (CI) were estimated from contingency tables for each variable in the questionnaire in order to test the hypothesis of no difference in the frequency of positive answers between groups. Multivariate logistic regression analysis with a backward stepwise method was used to determine the relation between the variables meeting a $p \leq 0.05$ value criterion in Chi-square analysis on the one hand and an exaggerated reaction on the other hand. Unpaired t-test analysis was used to compare log PC_{20} and the percentage drop of FEV_1 before the last concentration between cases and the subjects with MBR.

Analyses were performed with SPSS statistical software package version 9.1 (SPSS Inc., Chicago, IL, USA). All statistical tests were two-sided and a p-value of ≤0.05 was considered significant.

Results

Spirometry and bronchial provocation tests

The baseline characteristics of the epidemiological cohort of students has been described previously [12]. The majority of subjects, 711 (92%) agreed to a methacholine inhalation test. Their mean±SD age was 22.0±6.9 yrs and 85% were females. Thirteen subjects (2%) were on inhaled steroids, one on sodium cromoglycate and two took oral steroids. The median starting dose for bronchial provocation test was 2.0 $mg \cdot mL^{-1}$ of methacholine (522 subjects or 73%); 60 subjects (8%) started at 0.03 $mg \cdot mL^{-1}$ and 129 (18%) at 0.5 $mg \cdot mL^{-1}$. Results of methacholine inhalation tests are shown in table 1. The proportion of exaggerated bronchospastic reactions was 11.8% (84/711). Nine out of 711 subjects (1.3%) had a ≥20% FEV_1 drop after saline inhalation. No exaggerated bronchospastic reactions occurred at the first methacholine inhalation. A total of 623 subjects had skipped concentrations; 2.9% of these (18/623) had a ≥30% FEV_1 drop following omission of a concentration. All subjects had a return of FEV_1 to at least 90% baseline after 200 μg of salbutamol administered once or twice (380/711 subjects had at least one bronchodilator administration). In the only case in whom the FEV_1 value did not return to ≥90% of baseline, spirometry was not reproducible after bronchodilator administration.

In the clinical group, 408 consecutive subjects were assessed. The mean age was 46.5±14.8 yrs and 51% were female (206/408). Forty-six per cent of subjects were taking corticosteroids (inhaled in 182 cases (45%) and oral in four cases (1%)). The median starting dose for bronchial provocation test was 0.125 $mg \cdot mL^{-1}$; 52 subjects (13%) started at 0.03 $mg \cdot mL^{-1}$, 61 (15%) at 1 $mg \cdot mL^{-1}$ and 118 (29%) at 2 $mg \cdot mL^{-1}$. Results of methacholine inhalation tests are shown in table 1. The proportion of exaggerated bronchospastic reaction was 10.0% (41/408). One subject had a ≥20% FEV_1 drop to saline inhalation. No exaggerated bronchospastic reactions occurred at the first methacholine inhalation. A total of 346 subjects had skipped concentrations, 1.4% of these (5/346) had a ≥30% FEV_1 drop following omission of a concentration. All subjects who required the administration of a bronchodilator had a return to 90% baseline FEV_1 after 200 μg of salbutamol administered once in 276/335 or 82% of instances for whom it was required or twice (59 cases).

Table 1. – Bronchial challenge to methacholine and frequency of exaggerated reactions in the epidemiological and clinical groups

	Epidemiological group (n=711)	Clinical group (n=408)
PC_{20} ≤32 $mg \cdot mL^{-1}$	248 (34.9)	213 (52.2)
FEV_1 fall ≥10% after saline	26 (3.7)	12 (2.9)
FEV_1 fall ≥20% after saline	9 (1.3)	1 (0.25)
FEV_1 fall after methacholine inhalation		
30–39%	50	30
40–49%	12	8
≥50%	13	2
Total	75 (10.5)	40 (9.8)
Frequency of exaggerated reactions	84 (11.8)	41 (10.0)

Data are number of subjects with percentages in parentheses.

In the epidemiological group, 72 of the 84 subjects with exaggerated bronchospastic reactions could be paired for the case-control analysis of determinants of adverse reactions (table 2). FEV_1 and FVC prebronchodilator, and FEV_1 after bronchodilator were significantly lower in cases (group I) and in those with MBR (group II) by comparison with subjects without MBR (group III). Only two subjects, the two in group II, had an FEV_1 value <80% pred. All subjects without MBR (group III) had at least one skipped concentration of methacholine in comparison with 72.3% of subjects with MBR (group II) and 80% of cases (group I) who had two or more doses of methacholine administered. The percentage fall in FEV_1 before the last concentration administered was similar in group I and II (11±5% *versus* 10±5%, p=0.15). PC_{20} was marginally lower in group I than in group II (p=0.06). There were no other significant differences between these two groups.

In the clinical group, all cases were paired for case-control analysis of determinants of adverse reactions (table 3). FEV_1 and FEV_1/FVC values (% pred) were significantly lower in cases (group I) and in those with MBR (group II) than in subjects without MBR (group III). All subjects without MBR (group III) had at least one skipped concentration of methacholine as compared with 72.5% of cases (group I) and 82.5% of subjects with MBR (group II), who had two or more doses of methacholine

Table 2. – Baseline characteristics and bronchial challenge to methacholine in the epidemiological group

	Cases (group I) (n=72)	Subjects with MBR (group II) PC_{20} ≤32 mg·mL^{-1} (n=72)	Subjects without MBR (group III) PC_{20} >32 mg·mL^{-1} (n=72)	p-value#
Age yrs	20.2±4.4	20.3±4.5	20.2±4.4	ns
FEV_1 % pred	100±8	100±11	105±9	0.002, I<III (0.004)
FVC % pred	101±11	101±10	106±10	0.02, I<III (0.01)
FEV_1/FVC % pred	100±7	99±6	101±6	NS
Baseline FEV_1 variability >5%* n, %	28 (39)	20 (28)	22 (31)	NS
Four or more trials necessary for reproducibility of FEV_1 n, %	26 (36)	19 (26)	20 (28)	NS
FEV_1 change after saline %	-6 (5– -35)	-3 (1– -9)	3 (-35–12)	
FEV_1 total fall %	37 (20–72)	24 (20–29)	6 (-6–18)	
FEV_1 following BDT % baseline FEV_1	96±4	97±5	100±4**	0.04, I<III (0.01)
Number of methacholine concentrations	3.7 (2–7)	3.9 (3–8)	3.7 (1–8)	NS
Skipped methacholine concentrations*	47/65 (72)	56/70 (80)	72/72 (100)	I<III, (0.001)
PC_{20} mg·mL^{-1}	5.8 (0.04–27.1)	8.5 (0.02–32)	–	0.06
Number of post-test bronchodilator inhalations	1.4 (1–2)	1.1 (1–2)	1.1 (1–2)**	NS

Data are mean±SD or range of values, otherwise n with percentages; MBR: measurable bronchial responsiveness; PC_{20}: provocative concentration causing a 20% fall in forced expiratory volume in one second; NS: nonsignificant (p>0.1); FVC: forced vital capacity; BDT: bronchodilator. Cases were defined as a fall in FEV_1 ≥20% after saline inhalation or as a fall in FEV_1 ≥30% after methacholine inhalation. *: number of subjects/total number of subjects with interpretable data; seven subjects in group I had a significant fall after diluant; two subjects in group II had only one methacholine concentration to which they reacted; **: only 13 subjects received BDT; #: obtained from the overall comparison of the three groups.

administered. The percentage fall in FEV_1 before the last concentration administered was similar in groups I and II (12±5% *versus* 13±4%, p=0.76). PC_{20} was significantly lower in group I than in group II. The number of subjects taking inhaled corticosteroids was similar among the three groups.

Respiratory symptoms in the epidemiological group

For each questionnaire study variable, there were no statistically significant differences between the proportion of positive answers between group I and group II. In the comparison of groups I to group III, cases complained more often of wheezing, shortness of breath, nocturnal cough, morning nasal obstruction and rhinitis (table 4). They presented respiratory symptoms more often upon strong efforts, exposure to cold air and to dust, animals or feathers. Physician-based diagnosis of hay fever was more prevalent among cases. Nocturnal cough and respiratory symptoms to dust, animals or feathers were also significant determinants, but could not be integrated in the multivariate model because no subjects in the comparison group presented those symptoms. Prevalence factors studied, such as sputum production, chest tightness, tobacco use and symptoms in relation to night-time, seasons, smoke, odours, trees, grass or flowers, were not significantly different in cases (group I) than in subjects without MBR (group III) (not shown). Prevalence of physician-based diagnosis of asthma, chronic obstructive pulmonary disease, urticaria or eczema, and current use of respiratory medication was similar among these two groups. Stepwise multivariate analysis of the effect of these clinical parameters on exaggerated bronchoconstriction showed respiratory symptoms upon strong efforts (OR=2.9, 95% CI= 1.4–6.1, p=0.004) and symptoms of rhinitis (OR=2.3, 95% CI=1.1–4.6, p=0.02) to be the most important determinants separating cases (Group I) from those without MBR (Group III).

Discussion

This study shows that methacholine inhalation tests using time-saving measures and following the ERS guidelines [11] result in nearly 10% of exaggerated bronchoconstriction in epidemiological and clinical settings. Bronchial responsiveness is more prominent in subjects with exaggerated bronchoconstriction.

Since proposals for standardizing inhalation challenge tests with pharmacological agents have been issued [5, 6], time-saving measures to carry on testing have been proposed. Initially, the proposal for the concentration of the first aerosol of methacholine was to administer 0.03 mg·mL^{-1} and all doubling concentrations until a 20% FEV_1 drop was obtained regardless of the clinical state of the subject and the percentage fall in FEV_1 at the previous administered concentration. Accordingly, a subject with normal spirometry and no responsiveness to methacholine would be administered 11 nebulizations of methacholine (from 0.03 mg·mL^{-1} to 32 mg·mL^{-1}). It was proposed that the test procedure be shortened by enabling different starting concentrations as a function of clinical status and allowing skipped concentrations when the previous inhalation caused no significant fall in FEV_1. The first proposal for a shortened methodology was made by YAN *et al*. [9] followed by Canadian guidelines [10].The ERS guidelines for bronchoprovocation also proposed a shortened protocol, which was, with minor modifications, used in the current study [11]. These measures allow an economy of time, especially in epidemiological studies outside the hospital where the prevalence of normal subjects and of normal bronchial responsiveness is higher. However, it

Table 3. – Baseline characteristics and bronchoprovocation tests to methacholine in the clinical group

	Cases (group I) (n=41)	Subjects with MBR (group II) PC_{20} ≤32 mg·mL^{-1} (n=41)	Subjects without MBR (group III) PC_{20} >32 mg·mL^{-1} (n=41)	p-value[#]
Age yrs	42±14	42±13	43±14	NS
FEV_1 % pred	87±15	91±15	103±15	<0.001, I<III (<0.001)
FVC % pred	99±17	101±16	106±20	NS
FEV_1/FVC % pred	89±13	91±9	99±8	<0.001, I<III (<0.001)
Baseline FEV_1 variability >5%* n, %	14/41 (34)	13/41 (32)	14/41 (34)	NS
Four or more trials necessary for reproducibility of FEV_1 n, %	15/41 (37)	12/41 (29)	11/41 (27)	NS
FEV_1 change after saline %	-5 (2– -28)	-2 (6– -11)	-3 (1–7)	
FEV_1 total fall %	36 (28–52)	23 (20–29)	8 (0–19)	
FEV_1 following BDT % baseline FEV_1	98±6	97±7	95±4*	NS
Number of methacholine concentrations	4.0 (2–8)	4.2 (3–7)	4.4 (1–7)	
Skipped methacholine concentrations*	29/40	33/40	41/41	I<III (<0.001)
PC_{20} mg·mL^{-1}	2.1 (0.3–20.1)	5.8 (0.1–32.1)	-	
Number of post-test bronchodilator inhalations	1.2 (1–2)	1.2 (1–2)	1.2 (1–2)**	
Inhaled corticosteroids	16/41	10/41	17/41	NS

Data are mean±SD or range of values, otherwise n with percentage; MBR: measurable bronchial responsiveness; PC_{20}: provocative concentration causing a 20% fall in forced expiratory volume in one second; NS: nonsignificant (p>0.1); FVC: forced vital capacity; BDT: bronchodilator. Cases were as a fall in FEV_1 ≥20% after saline inhalation or as a fall in FEV_1 ≥30% after methacholine inhalation. *: number of subjects/total number of subjects with interpretable data; one subject in group I reacted to diluant; one subject in group II had only one methacholine concentration to which they reacted; **: only 13 subjects received BDT; [#]: obtained from the overall comparison of the three groups.

has to be ensured that these modifications result in safe tests.

In the present study, the saline inhalation resulted in exaggerated reactions in <1% of all subjects (10/1,119). Modification in the starting concentration was never immediately followed by any exaggerated reaction. Also, skipping methacholine concentrations resulted in exaggerated reactions in 2.3% of subjects (23/969 with skipped concentrations, 18 in the epidemiological group and five in the clinical group). Skipping methacholine concentrations accounted for only a small proportion of the exaggerated reactions (23/125 or 18.4% of all cases). Such results support the safety of shortened methacholine challenge tests in avoiding exaggerated bronchoconstriction. These findings are similar to those published by KREMER *et al.* [16]. Exaggerated bronchoconstriction was defined as a ≥40% fall in FEV_1. Modifying the starting concentration and allowing skipped concentrations resulted in a small number of exaggerated reactions (five subjects out of 697 or 0.7%). In the present study, the overall proportion of occurrences of fall in FEV_1 ≥40% after methacholine, excluding those after saline inhalation, was 35/1,119 or 3.1%.

This study showed that the frequency of exaggerated bronchospastic response was similar in the epidemiological and clinical cohorts. The protocol used was slightly different however. On the one hand, subjects in the clinical cohort were clinically more at risk of showing hyperresponsiveness to methacholine, having been referred by

Table 4. – Comparison of symptoms and health status in the epidemiological sample by univariate analysis

	Number of cases (out of 72)	Number of comparison subjects (out of 72)	OR	95% CI	p-value
Factors					
Wheezing	20	7	3.6	1.4–9.1	0.01
Shortness of breath	24	9	3.5	1.5–8.2	0.005
Nocturnal cough	9	0	*	*	0.003
Symptoms of allergic rhinitis	42	26	2.5	1.3–4.8	0.01
Nasal obstruction in the morning	11	3	4.1	1.1–15.6	0.05
Respiratory symptoms upon strong effects	34	16	3.1	1.5–6.5	0.003
Respiratory symptoms upon cold exposure	24	12	2.5	1.1–5.5	0.03
Respiratory symptoms on contact with dust, animals or feathers	9	0	*	*	0.003
Diagnosis of asthma	13	6	2.4	0.9–6.8	0.14
Current respiratory medication	11	4	3.1	0.9–10.1	0.10
Diagnosis of hay fever	11	3	4.1	1.1–15.6	0.05

Cases *versus* subjects with a provocative concentration causing a 20% fall in forced expiratory volume in one second >32 mg·mL^{-1}. 2×2 contingency tables were used to generate odds ratio (OR) and 95% confidence interval (95% CI); p-value in Chi-squared analysis. *: no comparison subjects had symptoms, therefore no OR could be derived.

physicians because they presented respiratory symptoms. However, 46% of subjects were treated with corticosteroids, which may have reduced bronchial hyperresponsiveness. Also, since subjects in the clinical cohort were taking medications and had lower baseline FEV_1, they started the provocation test with a slightly lower methacholine concentration than did those in the epidemiological cohort; a lower starting concentration in comparison to the epidemiological cohort may also have protected them. On the other hand, subjects in the epidemiological cohort may also be at risk of exaggerated reactions as the group may contain individuals who are not aware of possible symptoms and are rarely on inhaled steroids (this proportion was only 2% in the present cohort).

Exaggerated reactions were not an artefact of the methodology since the FEV_1 drop before the last concentration administered was similar in subjects presenting an exaggerated reaction to that of subjects without such a reaction (11% and 10%, respectively in the clinical cohort). One might have reasonably hypothesized that subjects who experienced a fall in FEV_1 $\geq$30% were those who, at the previous dose, had a fall in FEV_1 close to but not reaching 20%.

PRATTER *et al*. [17] showed that FEV_1 changes were readily reversible in all instances of 62 consecutive positive methacholine tests, including 82% who were administered only two puffs of salbutamol, the remaining subjects receiving one extra puff for recovery. All of the current subjects (in one subject, the spirometry after bronchodilator was not reproducible) also had a return of FEV_1 to $\geq$90% baseline after two puffs of salbutamol.

In summary, methacholine inhalation tests using the abbreviated procedure suggested by the European Respiratory Society result in a frequency of exaggerated bronchoconstriction of ~10%. Some respiratory symptoms are associated with exaggerated reactions. Subjects with measurable bronchial responsiveness and exaggerated reactions did not differ from those with measurable bronchial responsiveness but without exaggerated reactions except for a slightly more pronounced bronchial hyperresponsiveness.

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