Sputum Induction in Severe Asthma by a Standardized Protocol
Predictors of Excessive Bronchoconstriction

ANNEKE ten BRINKE, CINDY de LANGE, AEILKO H. ZWINDERMAN, KLAUS F. RABE, PETER J. STERK, and ELISABETH H. BEL

Departments of Pulmonary Diseases and Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands

Sputum induction is a noninvasive method to evaluate airway inflammation. We investigated whether it can be safely and successfully performed in patients with severe, difficult-to-control asthma, and whether the patients at risk can be identified. Ninety-three severe asthmatics were included, all symptomatic despite inhaled corticosteroids (> 1,600 μg/d) and long-acting β2-agonists > 1 yr. Patients with a postbronchodilator FEV1 < 1 L and < 50% predicted were excluded from participation. Sputum induction was performed according to a strict protocol, using 0.9%, 3.0%, and 4.5% NaCl inhalation. In 74% (CI: 64 to 83%) of patients an adequate sputum sample could be obtained. Twenty-two percent (CI: 14 to 33%) developed excessive bronchoconstriction (decrease in FEV1 > 15% from baseline) despite the continuing use of long-acting bronchodilators and pretreatment with 400 μg salbutamol. The decrease in FEV1 was associated with increased use of rescue short-acting β2-agonists in the previous 2 d (r = 0.51, p = 0.002), lower postbronchodilator FEV1 (r = −0.31, p = 0.004), and lower provocative concentration of histamine causing a 20% reduction in FEV1 (PC20) (r = −0.52, p < 0.001). Recent use of short-acting β2-agonist increased the risk for excessive bronchoconstriction 10.2-fold (CI: 1.2 to 109.8). In conclusion, sputum induction can be safely and successfully performed in patients with severe, difficult-to-control asthma if a standardized protocol is used. However, severe bronchoconstriction may occur despite regular use of long-acting β2-agonist and pretreatment with salbutamol 400 μg. In particular, patients who have used additional short-acting β2-agonists as rescue medication during the days preceding the induction are at high risk.

Keywords: asthma; bronchoconstriction; safety; saline solution; hypertonic; sputum

Analysis of induced sputum is widely used in the evaluation of airway inflammation in mild to moderate asthma (1). This relatively noninvasive method could also be of great value in patients with severe asthma, in whom the use of more invasive techniques such as fiberoptic bronchoscopy may be associated with significant adverse effects (2, 3). Sputum induction involves the inhalation of hypertonic or isotonic saline aerosols, which is known to lead to acute airway narrowing in some patients with asthma (4–6). Pretreatment with a bronchodilator before the procedure is, therefore, recommended (7, 8).

Although the safety and feasibility of sputum induction in mild to moderate stable asthma has been well documented (1, 9, 10), data in patients with severe and difficult-to-control asthma are limited (11). In particular in patients on regular long-acting β2-agonists, in whom tolerance to the bronchoprotective effects of these drugs may develop (12), safety data are scarce. In addition, objective predictors of excessive airway narrowing have not yet been established, although some studies suggest that the level of baseline airflow limitation (10, 13), airway hyperresponsiveness (10, 13), and recent overuse of short-acting β2-agonists (14, 15) may have some predictive value.

The aim of the present study was to evaluate the safety and efficacy of sputum induction according to a strict protocol in patients with severe asthma, who used long-acting β2-agonists and high-dose inhaled or oral corticosteroids on a regular basis. We postulated that the reduction in forced expiratory volume in one second (FEV1) caused by the induction procedure could be predicted by disease severity or markers of (airways) inflammation. To that end, we examined the association between the fall in FEV1 due to the induction procedure and the recent use of short-acting bronchodilators, postbronchodilator FEV1, reversibility in FEV1, airway responsiveness to histamine, level of nitric oxide (NO) in exhaled air, and peripheral blood eosinophil count.

METHODS

Patients

Ninety-three nonsmoking patients with severe bronchial asthma (age 18 to 75 yr) were recruited from 10 different hospitals. They had a history of episodic dyspnea and wheezing, a documented reversibility in FEV1 of > 12% predicted (16) or airway hyperresponsiveness to inhaled histamine. All patients used regular treatment with high doses of inhaled corticosteroids (dose ≥ 1,600 μg/d beclometasone or equivalent) and long-acting β2-agonists for more than 1 yr. They were all symptomatic and had received at least one course of oral corticosteroids during the past year for severe exacerbations or were on maintenance therapy with oral prednisone ≥ 5 mg/d. Current smokers and ex-smokers with a smoking history > 10 pack-years were excluded, as were the patients with a respiratory tract infection within the 2 wk preceding the study. The study was approved by the Hospital Medical Ethics Committee, and all patients gave informed consent.

Pulmonary Function and NO in Exhaled Air

FEV1 measurements were performed according to standard lung function technique (16), using a dry rolling-seal spirometer (Morgan Spiroflow, Morgan, UK) and predicted values were obtained from Quanjer and coworkers (16). Data on airway responsiveness and reversibility (if available) were taken from measurements performed during the preceding 6 mo. Airway responsiveness to histamine was measured using the standardized tidal breathing method (17), and expressed as the provocative concentration causing a 20% reduction in FEV1 (PC20). Reversibility in FEV1 was measured 30 min after administration of 400 μg salbutamol and 80 μg ipratropium bromide (18), and was expressed as (FEV1 post – FEV1 baseline)/FEV1 predicted (16). A decrease in FEV1 after sputum induction of > 15% from postbronchodilator baseline was defined as excessive bronchoconstriction. Exhaled NO measurements were performed according to present rec-

(Received in original form September 22, 2000 and in revised form April 27, 2001)

Supported by the Netherlands Asthma Foundation (Grant 97.24).

Correspondence and requests for reprints should be addressed to A. ten Brinke, Department of Pulmonary Diseases, C3-P, Leiden University Medical Center, P.O. Box 9600, NL-2300 RC Leiden, The Netherlands. E-mail: a.ten_brinke@lumc.nl

Am J Respir Crit Care Med Vol 164, pp 749–753, 2001

Internet address: www.atsjournals.org
Sputum Induction and Processing

Patients were allowed to continue their own antiasthma medication before sputum induction and all procedures were conducted in the morning. Patients were additionally pretreated with 400 μg of salbutamol using a metered-dose inhaler with a spacer device. Patients with a postbronchodilator FEV\textsubscript{1} < 50% of predicted and < 1.0 L were excluded from the induction procedure (11, 21) and were asked to expectorate sputum spontaneously. In all other patients sputum was induced according to a strict protocol modified from a previously validated method (22), including adjustments for safety reasons. Table 1 shows the outline of this protocol in detail. Saline solutions were nebulized by an ultrasonic nebulizer (Ultraneb 2000; DeVilbiss, Somerset, PA) with an output of 2.5 ml/min used. After assessment of post-salbutamol baseline lung function, the patients started the procedure with the inhalation of 0.9% NaCl, for two subsequent try-out periods of 1 min. Depending on baseline FEV\textsubscript{1} values, the induced decline in FEV\textsubscript{1}, and the accompanying symptoms (Table 1), isotonic saline only, or increasing saline concentrations of 0.9%, 3.0%, and 4.5% were used. Spirometry was repeated at 2.5 min and at the end of each inhalation period of 5 min, or earlier in the event of troublesome symptoms. The procedure was discontinued whenever predetermined criteria for increased bronchoconstriction were reached (Table 1). Subjects were asked to rinse their mouth with tap water after each inhalation period and were encouraged to cough and expectorate sputum if present in a clean plastic container. Whole sputum samples were processed according to a validated protocol (22).

Statistical Analysis

Non-normally distributed parameters were log-transformed before statistical analysis. In the analysis PC\textsubscript{20} histamine was censored to 8.0 mg/ml, if a decrease in FEV\textsubscript{1} of > 20% was not reached at the highest dose given (8 mg/ml). Differences between subgroups were investigated by unpaired Student’s t tests, Wilcoxon rank tests, or chi-square analyses, whenever appropriate. Spearman rank correlation coefficients (r\textsubscript{s}) were used to analyze the relationship between maximal decrease in FEV\textsubscript{1} and possible predictors of this fall.

The following contrasts in potential predictors were considered for patients with and without excessive bronchoconstriction (defined as a fall in FEV\textsubscript{1} > 15% from baseline): > 1 puff of 200 μg salbutamol equivalent versus 0 puffs during the 2 d preceding the study; postbronchodilator baseline FEV\textsubscript{1} < 75% of predicted versus > 75% of predicted; and PC\textsubscript{20} histamine ≤ 1.0 mg/ml versus > 1.0 mg/ml. Adjusted odds ratios (OR) for patients with excessive bronchoconstriction versus those without as the reference group were obtained by multiple logistic regression analyses with the aforementioned potential predictors as independent parameters forced into the model. All analyses were performed using the Statistical Package of the Social Sciences (SPSS for Windows, release 9.0; SPSS Inc., Chicago, IL). p Values less than 0.05 were considered statistically significant.

**TABLE 1. PROTOCOL FOR MONITORING FEV\textsubscript{1} DURING SPUTUM INDUCTION**

<table>
<thead>
<tr>
<th>Postbronchodilator* FEV\textsubscript{1} &lt; 50% pred and &lt; 1.0 L</th>
<th>No sputum induction</th>
<th>Postbronchodilator* FEV\textsubscript{1} &lt; 50% pred or &lt; 1.5 L and &gt; 1.0 L</th>
<th>2 min try-out 0.9% NaCl, followed by 3 × 5 min 0.9% NaCl</th>
<th>If fall in FEV\textsubscript{1} &lt; 10% from baseline (post-salbutamol), continue sputum induction</th>
<th>If fall in FEV\textsubscript{1} &gt; 10% from baseline or troublesome symptoms, stop sputum induction</th>
<th>Postbronchodilator* FEV\textsubscript{1} &lt; 50% pred and &gt; 1.5 L</th>
<th>2 min try-out 0.9%, followed by 3 × 5 min 0.9, 3.0, or 4.5% NaCl</th>
<th>If fall in FEV\textsubscript{1} &lt; 10% from baseline (post-salbutamol), continue induction, use higher concentration</th>
<th>If fall in FEV\textsubscript{1} &gt; 10% but &lt; 15% from baseline, continue sputum induction, use same concentration</th>
<th>If fall in FEV\textsubscript{1} &gt; 15% from baseline or troublesome symptoms, stop sputum induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Postbronchodilator: after pretreatment with salbutamol 400 μg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS**

Patients and Sputum Induction

Ninety-three patients with severe asthma (71% females) participated in the study. They had a mean (± SD) age of 47.8 ± 12.8 yr and a median (range) asthma duration of 23.5 (2 to 63) yr. All patients used inhaled corticosteroids (range 1.600 to 6,400 μg/d) and long-acting β\textsubscript{2}-agonists, and 31% needed maintenance therapy with oral prednisone (range 5 to 40 mg/d). Seventeen patients used theophylline, 50 patients were taking anticholinergics, and another 17 patients used daily antihistamines. Data on reversibility and airway responsiveness could be obtained in 98% and 54% of the patients, respectively. The median (range) of reversibility in FEV\textsubscript{1} after inhalation of salbutamol and ipratropium bromide was 9.9% (1.5 to 35.9%) of predicted, of PC\textsubscript{20} histamine 1.41 (0.02 to 8.0) mg/ml, and of the concentration of NO in exhaled air 10.0 (1 to 202) parts per billion (ppb). Eight of the 93 patients were excluded from the induction procedure because of a postbronchodilator FEV\textsubscript{1} < 50% or < 1.0 L (Table 1). Four of these eight patients, however, were able to produce an adequate sputum sample spontaneously.

Of the remaining 85 patients, 13 had sputum induction with isotonic saline alone, and 72 with increasing concentrations of NaCl, as stated in our protocol. Thirty-five percent of all patients completed the whole induction period. However, the majority of them had to stop earlier, owing to a fall in FEV\textsubscript{1} or symptoms (Table 2). Nevertheless, despite a frequent premature end of the induction procedure, in 74% (95% confidence interval [CI]:64 to 83%) of these patients with severe asthma an adequate sputum sample could be obtained. Sputum differential cell counts are presented in Figure 1. The viability of the cells was satisfactory (mean 69%, SD 19%) and the mean (± SD) percent of squamous cells was 31 ± 26%, both comparable to previous studies using slightly different induction protocols, but similar processing procedures, in patients with less severe asthma (13, 22). There was no difference in any of the sputum differential cell counts between patients who completed the whole induction procedure and those who had to terminate the procedure prematurely because of side effects (p > 0.1).

Induced Bronchoconstriction

The patients who started the sputum induction procedure showed a wide range in postbronchodilator baseline FEV\textsubscript{1} values, varying from normal lung function to very severe airflow limitation (Table 3). Various changes in FEV\textsubscript{1} resulting from the saline inhalation were observed in these patients, with 91% of the patients showing a decrease in FEV\textsubscript{1}, up to 2.08 L or 45% from postbronchodilator baseline (Figure 2). Nineteen patients (22%; CI: 14 to 33%) had a decrease of > 15%, three who inhaled 0.9% NaCl only, and 16 who continued with hypertonic saline inhalation.

All subjects who had a decrease in FEV\textsubscript{1} recovered to more than 95% of postbronchodilator baseline values within 30 min after additional inhalation of 400 μg of salbutamol or 80 μg of ipratropium bromide, or both, with the exception of one patient with a fall in FEV\textsubscript{1} of 45% from postbronchodilator baseline. After administration of salbutamol and ipratropium bromide he quickly recovered to values of approximately 25% from baseline. Only after repeated nebulization with the same bronchodilators he reached baseline value again after about 1 h.

Factors Associated with Excessive Bronchoconstriction

In Table 4 the patients with and without excessive bronchoconstriction during sputum induction are compared. The pa-
patients with a decline in $FEV_1 > 15\%$ from baseline values used significantly more short-acting $\beta_2$-agonists in the 2 d before the sputum induction (median 6 puffs) compared with those without excessive bronchoconstriction (median 0 puffs). Other demographic, clinical, physiologic, and inflammatory characteristics were not significantly different between the groups.

The maximal decrease in $FEV_1$ (percent of baseline) during sputum induction was significantly related to an increased number of puffs of short-acting $\beta_2$-agonist used in the 2 d preceding the induction ($r_s = -0.51, p = 0.002$), to lower postbronchodilator baseline $FEV_1$ (percentage of predicted) ($r_s = 0.31, p = 0.004$), and to decreased PC$_{20}$ histamine ($r_s = 0.52, p < 0.001$). Also a higher sputum eosinophil percentage was significantly related ($r_s = -0.32, p = 0.01$) to an enhanced decline in $FEV_1$, but this can obviously not be used as a predictor. No significant relationship was found between fall in $FEV_1$ and bronchodilator reversibility, exhaled NO, or eosinophils in peripheral blood ($p > 0.2$). In the multivariate logistic regression analysis the patients who used 1 or more puffs of a short-acting $\beta_2$-agonist as rescue medication in the 2 d before the study had a 10.2-fold (CI: 1.2 to 109.8) increased risk for a reduction in $FEV_1$ of more than 15% from baseline value as compared with the severe asthma patients who used their long-acting $\beta_2$-agonist without additional rescue short-acting bronchodilators. PC$_{20}$ histamine $< 1.0$ mg/ml (adjusted OR = 1.6, CI: 0.4 to 7.1) and baseline $FEV_1 < 75\%$ of predicted (adjusted OR = 1.8, CI: 0.6 to 4.9) did not appear to be independent predictors.

**DISCUSSION**

The present study shows that sputum induction in patients with severe asthma can be performed safely when using a strict protocol under supervision of an experienced physician, and that an adequate sputum sample can be obtained in 74% of patients. Despite the continuing use of long-acting $\beta_2$-agonists and pretreatment with a short-acting bronchodilator, 22% of the patients in the present study developed excessive bronchoconstriction after isotonic or hypertonic saline inhalation, or both. The decrease in $FEV_1$ was related to an increased number of puffs of short-acting $\beta_2$-agonists during the 2 d preceding the sputum induction, to a lower baseline postbronchodilator $FEV_1$, and to a lower PC$_{20}$ histamine, but not to reversibility in $FEV_1$, concentrations of exhaled NO, or blood eosinophil count. The only independent and strong predictor of excessive saline-induced bronchoconstriction was the recent use of short-acting $\beta_2$-agonists as rescue medication, probably indicating more unstable disease.

This is the first prospective study addressing the safety and success rate of sputum induction in severe asthma according to a standardized protocol in a patient group large enough to explore potential predictors of excessive bronchoconstriction. Excessive bronchoconstriction after sputum induction was observed in some of our patients as has been reported in several studies in mild and moderate asthma (10, 21, 23). One study did not report excessive saline-induced airway narrowing in patients with severe asthma, probably because the sputum induction procedure was discontinued whenever an adequate sputum sample was obtained (11). In our study we tried as much as possible to complete the 17-min induction procedure, because it has been shown that the duration of saline inhalation influences the cellular constituents of induced sputum (24). By using this modified sputum induction protocol for severe asthma, we obtained adequate sputum samples in 74% of the patients, a success rate comparable to other studies using hypertonic saline in patients with varying severity of asthma (11, 21).

In this group of severe asthma patients the saline-induced decrease in $FEV_1$ was related to a lower baseline $FEV_1$ and a lower PC$_{20}$ histamine, which confirms the findings of a retrospective study in mild to moderate asthma (10). In addition, in the present study, increased airway narrowing was associated with the recent use of short-acting $\beta_2$-agonists, which has already been suggested as potential predictor of excessive airway narrowing in patients with exacerbations of asthma (15). When analyzing these three factors in one model, the only independent and strong predictor of excessive airway narrowing appeared to be the use of rescue short-acting $\beta_2$-agonists in the 2 d before the induction procedure.

The results of this study do not seem to be influenced by patient selection or methods used. We induced sputum according to a strictly standardized protocol in a large, well-defined group of patients, with a wide range in level of airways obstruction, representing the population of patients with severe asthma who visit chest physicians in general hospitals. Data on

<table>
<thead>
<tr>
<th>TABLE 2. FEASIBILITY OF SPUTUM INDUCTION PROCEDURE IN SEVERE ASTHMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postbronchodilator FEV$_1$ ≤ 1.5 L or ≤ 50% pred</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Postbronchodilator FEV$_1$ &gt; 1.5 L and &gt; 50% pred</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

**TABLE 3. LUNG FUNCTION BEFORE AND DURING SPUTUM INDUCTION**

<table>
<thead>
<tr>
<th>Postbronchodilator FEV$_1$ before sputum induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>liter</td>
</tr>
</tbody>
</table>

| % predicted | 78.0 | 27 to 133 |

<table>
<thead>
<tr>
<th>Maximal fall in FEV$_1$, from pb baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>in ml</td>
</tr>
<tr>
<td>in % of baseline</td>
</tr>
<tr>
<td>in % of predicted</td>
</tr>
</tbody>
</table>

*Definition of abbreviation: pb = postbronchodilator.*
airway responsiveness were not available in all patients, owing to a low prebronchodilator FEV₁ or because the patients were incapable of discontinuing their bronchodilators without having severe symptoms. However, we do not think this has significantly influenced the results, because there were no differences between the patients who were able or unable to perform these tests with respect to the efficacy of the sputum induction procedure and the saline-induced fall in FEV₁ (data not shown). In our protocol we used a reduction in FEV₁ of more than 15% or even 10% from baseline as criterion for discontinuation (Table 1). Although several other studies allowed a fall up to 20% from baseline (9, 21, 25), we decided upon this criterion for safety reasons, in particular in view of the low postbronchodilator FEV₁ values in some of the patients. Because we were successful in obtaining adequate sputum samples in the majority of our patients, without serious adverse effects, we consider these stop criteria to be justified and safe.

Recent use of a short-acting β₂-agonist appeared to be a strong predictor of excessive bronchoconstriction in our patients with severe asthma. This could be explained by more unstable or more severe disease in patients who need short-acting β₂-agonists in addition to their regular long-acting β₂-agonists. The observation that patients with the most severe airway narrowing were the ones with the lowest postbronchodilator baseline lung function, lowest PC₂₀ histamine, and highest sputum eosinophil percentages, fits in with this assumption. An alternative explanation could be that the increased airway narrowing during sputum induction in patients with overuse of short-acting β₂-agonists (15) is the result of a tolerance effect (26). In the present study all patients were using long-acting β₂-agonists for more than 1 yr, and were therefore likely to have developed tolerance to the bronchoprotective effects of these drugs (12, 27). An independent effect of short-acting β₂-agonists on the degree of saline-induced bronchoconstriction in these patients is remarkable, and is in agreement with the impression that prolonged use of short-acting, but not long-acting β₂-agonists might have a deteriorating effect on asthma severity (28).

The results of the study indicate that the present protocol can be recommended for sputum induction in patients with severe, difficult-to-control asthma provided that the postbronchodilator FEV₁ is at least 1 L or 50% of the predicted value. Before sputum induction, however, patients should be asked about their need for short-acting β₂-agonists during the last 2 d. A negative answer is likely to predict an uncomplicated procedure, although careful monitoring is still needed. However, in case of a positive answer, one should bear in mind that the patient will have a 10-fold increased risk for excessive bronchoconstriction during the sputum induction.

In conclusion, sputum induction can be performed safely and successfully in patients with severe asthma receiving regular long-acting β₂-agonists and high doses of inhaled or oral corticosteroids, provided that a strictly standardized protocol is being used. Severe bronchoconstriction may occur in patients with low baseline FEV₁ values and marked airway hyperresponsiveness. In particular, patients who have used any rescue short-acting β₂-agonist on the 2 d preceding the induction procedure (in addition to their long-acting bronchodilator therapy) seem to be at increased risk for excessive bronchoconstriction.

Acknowledgment: The authors thank M. C. Timmers and H. van der Veen for technical assistance, and the chest physicians of the participating hospitals for their cooperation (P. I. van Spiegel, G. Visschers, Slotervaart Hospital, Amsterdam; A. H. M. van der Heijden, Rode Kruis Hospital, Beverwijk; B. J. M. Pannekoek, Reinier de Graaf Gasthuis, Delft; H. H. Berendsen, K. W. van Kralingen, Bronovo Hospital, Den Haag; H. G. M. Heijerman, A. C. Roldaan, Leyenburg Hospital, Den Haag; A. H. M. van der Heijden, Spaarne Hospital, Heemstede; H. C. J. van Klink, Diaconessenhuis, Leiden; C. R. Apap, St. Antoniushove, Leidschendam; and A. Rudolphus, K. Y. Tan, St. Franciscus Gasthuis, Rotterdam).

**TABLE 4. COMPARISON OF SEVERELY ASTHMATIC PATIENTS WITH AND WITHOUT EXCESSIVE BRONCHOCONSTRICTION DURING SPUTUM INDUCTION**

<table>
<thead>
<tr>
<th></th>
<th>≤ 15% Fall in FEV₁ from pb Baseline (n = 66)</th>
<th>&gt; 15% Fall in FEV₁ from pb Baseline (n = 19)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr*</td>
<td>48.5 ± 11.9</td>
<td>43.5 ± 14.2</td>
<td>0.13</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>18/48</td>
<td>6/13</td>
<td>0.71</td>
</tr>
<tr>
<td>Age at onset asthma, yr†</td>
<td>14.5 (0.5–56)</td>
<td>11.0 (0.5–60)</td>
<td>0.31</td>
</tr>
<tr>
<td>Asthma duration, yr†</td>
<td>18.5 (2–63)</td>
<td>29 (3–50)</td>
<td>0.99</td>
</tr>
<tr>
<td>Maintenance oral steroids, %</td>
<td>27.3</td>
<td>31.6</td>
<td>0.71</td>
</tr>
<tr>
<td>Exercise tolerance, WHO class†</td>
<td>2 (0–3)</td>
<td>2 (0–3)</td>
<td>0.95</td>
</tr>
<tr>
<td>Nocturnal symptoms, nights/wk†</td>
<td>2 (0–5)</td>
<td>1 (0–5)</td>
<td>0.97</td>
</tr>
<tr>
<td>SA β₂-agonist, puffs†</td>
<td>0 (0–16)</td>
<td>6 (0–18)</td>
<td>0.02</td>
</tr>
<tr>
<td>Positive RAST, %</td>
<td>62.1</td>
<td>66.7</td>
<td>0.72</td>
</tr>
<tr>
<td>PC₂₀ histamine, mg/ml²</td>
<td>1.19 ± 2.5</td>
<td>0.37 ± 3.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Reversibility FEV₁, % pred†</td>
<td>9.5 (2–29)</td>
<td>10.4 (3–36)</td>
<td>0.56</td>
</tr>
<tr>
<td>pb FEV₁, % pred*</td>
<td>78.4 ± 22.1</td>
<td>74.7 ± 19.0</td>
<td>0.52</td>
</tr>
<tr>
<td>Exhaled NO, ppb²</td>
<td>10.0 (1–124)</td>
<td>11.0 (1–62)</td>
<td>0.74</td>
</tr>
<tr>
<td>Blood eosinophils, 10³/L³</td>
<td>0.22 (0.02–1.04)</td>
<td>0.16 (0.05–0.52)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

**Definition of abbreviations:** pb = postbronchodilator; SA = short-acting, total number of puffs of salbutamol 200 µg equivalent used in the 2 d preceding the study; WHO = World Health Organization.

* mean ± SD
† median (range).
² geometric mean ± SD (in doubling doses).
References


