Airway Responsiveness to Adenosine 5’-Monophosphate and Exhaled Nitric Oxide Measurements*

Predictive Value as Markers for Reducing the Dose of Inhaled Corticosteroids in Asthmatic Subjects

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Objectives: To investigate the utility of the determination of airway responsiveness to inhaled adenosine 5’-monophosphate (AMP) and exhaled nitric oxide (ENO) levels as markers for safely reducing the dose of inhaled corticosteroids (ICS) in patients with asthma well controlled with a moderately high ICS dose.

Methods: A total of 37 patients with asthma well controlled for at least 3 months by treatment with a moderately high ICS dose (beclomethasone dipropionate, 500 to 1,000 mg or equivalent daily) were included in the study. Patients were treated for a 2-week run-in (baseline) period with their usual dose of ICS. For the next 12 weeks, patients were treated with ICS at half the previous dose, maintaining the same inhalation device. At the end of the baseline period and after 2 weeks, 8 weeks, and 12 weeks of treatment with a reduced dose of ICS, measurements were made in the following order: ENO, spirometry, and AMP challenge. Furthermore, patients completed a diary twice daily recording peak expiratory flow, daytime and nighttime symptoms, and use of rescue albuterol.

Results: Ten patients had an asthma exacerbation. Using a Kaplan-Meier survival analysis, the significant predictors of a failure of ICS reduction were having both bronchoconstriction in response to AMP and ENO levels > 15 parts per billion (ppb) at baseline (p = 0.006), as well as having both bronchoconstriction in response to AMP and ENO levels ≥ 20 ppb at baseline (p = 0.033). Having a decrease in the provocative concentration of AMP causing a 20% fall in FEV₁ of at least one doubling concentration 2 weeks after the dose of ICS was halved was a borderline significant predictor for failure of ICS reduction (p = 0.062).

Conclusion: These observations suggest that in asthmatic patients well controlled with ICS, the determination of AMP responsiveness and ENO levels may be useful to identifying those subjects whose condition will or will not deteriorate when the dose of ICS is reduced.

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Key words: adenosine 5’-monophosphate; airway responsiveness; inhaled corticosteroids; nitric oxide

Abbreviations: AMP = adenosine 5’-monophosphate; CI = confidence interval; ENO = exhaled nitric oxide; ICS = inhaled corticosteroids; OR = odds ratio; PC₂₀ = provocative concentration of adenosine 5’-monophosphate causing a 20% fall in FEV₁; PEF = peak expiratory flow; ppb = parts per billion

Current therapy for asthma is aimed at suppressing the inflammatory process in the wall of the airways, and the use of inhaled corticosteroids (ICS) is the basis for effective long-term control in all but the most intermittent cases.¹ Current asthma guidelines¹,² emphasize the importance of gaining prompt control of asthmatic symptoms, recommending that patients with persistent asthma should initially receive a moderately high dose of ICS. Once control is achieved, the dose of ICS should be reduced to the minimal level required to maintain disease control. The strongest motivation for “stepping-down” therapy is to minimize adverse effects associated with prolonged usage of high dosages of ICS.³ Current guidelines¹,² suggest that reduction in ICS should be...
Airway inflammation plays a central role in the pathogenesis of asthma and is associated with an increase in airway responsiveness to various spasmodens. Clinically and for research purposes, airway responsiveness is measured by bronchial challenge, usually with methacholine or histamine, however, in the past decade, adenosine 5’-monophosphate (AMP) has been introduced as a bronchoconstrictive stimulus. Whereas histamine and methacholine act by a direct effect on the relevant receptors on airway smooth muscle stimulating airway muscle concentration directly, AMP-induced bronchoconstriction occurs predominantly indirectly by stimulation of A2-adenine nucleotide receptors on mast cells that facilitate the release of proinflammatory mediators (histamine and leukotrienes) with subsequent smooth-muscle contraction. It has been suggested in several studies that the bronchial response to AMP is more closely associated with airway inflammation than the response to direct bronchoconstrictors such as histamine or methacholine.

Exhaled nitric oxide (ENO) levels are known to be increased in patients with asthma. Nitric oxide is a messenger molecule generated from L-arginine by the action of several distinct enzymes called nitric oxide synthases. It has been suggested that the increased ENO levels found in asthmatic subjects result from an increase in the expression of inducible nitric oxide synthase in the respiratory tract induced by the action of proinflammatory cytokines. In addition, ENO levels are correlated with eosinophils in induced sputum. Therefore, ENO has been proposed as a noninvasive marker of airway inflammation in asthma.

The bronchial response to AMP improves to a longer extent after therapy with ICS in patients with asthma than the response to methacholine. More importantly, the ICS-induced improvement of AMP responsiveness is also more closely related to the concomitant reduction in airway inflammation than the methacholine responsiveness. Furthermore, ENO levels are known to be increased in asthma during an exacerbation, to decrease with ICS therapy, and to rise as the dose of ICS is reduced. These findings suggest that the determination of AMP responsiveness and ENO levels might be helpful in identifying patients likely to tolerate a “step-down” in therapy with ICS. The purpose of the current study was to evaluate the utility of the determination of airway responsiveness to inhaled AMP and ENO levels as markers for safely reducing the dose of ICS in patients with asthma well controlled for at least 3 months with a moderately high ICS dose.

**Materials and Methods**

**Subjects**

Patients 18 to 60 years old with a previous history of asthma and who had been treated with an ICS for at least 6 months were enrolled in the study. Asthma was diagnosed by the presence of symptoms of wheeze, breathlessness, or cough plus methacholine airway hyperresponsiveness with a provocative concentration of AMP causing a 20% fall in FEV1 (PC20) of < 8 mg/mL if the FEV1/FVC was > 70%, or an improvement of the FEV1 from predicted of ≥ 15% after 200 μg of inhaled albuterol if the FEV1/FVC was < 70%. Subjects with stable asthma requiring ICS at medium to high doses (beclomethasone, 500 to 1,000 μg) to maintain asthma control were recruited. In the 3 months before the study, patients had asthma symptoms no more than twice a week and did not wake at night because of asthma. They had no changes in their dose of ICS in the last 6 months, and FEV1 at baseline had to be > 80% of predicted. All patients were nonsmokers, and none had a history of chronic bronchitis, emphysema, or respiratory tract infections during the 4 weeks before the study. Current smokers, pregnant women, patients with only seasonal symptoms and skin sensitization to pollen allergens, and patients with significant renal, hepatic, or cardiovascular disease were specifically excluded. The study protocol was approved by the local ethics committee, and written informed consent was obtained from all participants.

**Study Design**

This prospective study was performed in a single-blind manner. The study duration was 14 weeks, and it consisted of a run-in (baseline) period (2 weeks) during which patients continued to receive the same doses as previously of ICS, and a dose reduction period (12 weeks), in which the current ICS dose was halved. At the screening visit, patients completed a questionnaire about their asthma history and current medications. Additionally, spirometry was performed. During the run-in period, all subjects were treated with their usual dose of ICS, in order to demonstrate stability with the habitual dose of ICS. During this period, patients measured peak expiratory flow (PEF) in the morning.
and in the evening. In addition, asthma symptoms during the day and during the night, and use of rescue albuterol were recorded in diaries. A prerequisite for study enrollment was a stable clinical condition, defined as follows: (1) no more than four doses of as-needed albuterol, (2) ≤ 4 days with PEF variability ≥ 20%, and (3) mean daytime and nighttime symptom scores < 1.

For the next 12 weeks, eligible patients were treated with ICS at half their previous dose, maintaining the same inhalation device. Patients completed a diary twice daily recording PEF, daytime and nighttime symptoms, and use of rescue albuterol. At the end of the run-in period and after 2 weeks, 8 weeks, and 12 weeks of treatment with a reduced dose of ICS, measurements were made in the following order: ENO, spirometry, and AMP challenge. Albuterol metered-dose inhaler and antihistamines (ie, cetirizine and loratadine) were used on an as-needed basis to control pulmonary or nasal symptoms, respectively. Patients were asked not to take albuterol for at least 6 h, ICS for at least 12 h, and antihistamines for at least 72 h before each visit. The follow-up visits were performed by personnel blinded to the results of the AMP challenge and ENO determination.

The study was suspended for individual patients whenever exacerbations occurred, or at week 14 if there had been no exacerbations. An exacerbation was defined as one of the following criteria: (1) a decrease in morning PEF of > 20% on at least 2 consecutive days as compared with the mean of the last 7 days of the run-in period, (2) awakening on ≥ 3 nights per week, or (3) bronchodilator use of one to two times per day for at least 4 consecutive days. Patients with an exacerbation went to the laboratory as soon as possible within the next 24 h for the same investigations as used regularly in the study. If there was an FEV1 of < 70% predicted, the AMP challenge test was not to be performed.

Asthma Symptoms

Asthma symptoms were rated by the patient in the morning on arising and in the evening before retiring, with scores ranging from 0 (no symptoms) to 3 (maximal symptoms, severe impairment of daytime activities/no sleep during nighttime).

Albuterol Requirement

The number of nighttime albuterol inhalations for the preceding night was recorded in diary format in the morning on arising, and the number of daytime inhalations was recorded in the evening before retiring.

Pulmonary Function

Lung function (flow-volume curves) was measured using a calibrated pneumotachograph (Jaeger MasterScope; Erich Jaeger GmbH; Würzburg, Germany) according to standardized guidelines.28 Baseline FEV1 and FVC levels were measured until three reproducible recordings differing by < 5% were obtained. Maneuvers were accepted as technically satisfactory if the back-extrapolated volume was < 150 mL or 5% of FVC, and if the expiratory time was at least 6 s. The highest values were used for analyses. Reference values were those of the European Community for Coal and Steel.27

Peak Flow Recording

The PEF rate was measured at home each day in the morning on arising and in the evening before retiring, using a mini-Wright peak flowmeter (Clement Clarke International, London, UK) during the whole study period. Each measurement consisted of three attempts, and the highest value was recorded. Patients were instructed to measure PEF before using albuterol as needed.

AMP Challenge

Airway responsiveness to AMP was assessed using a standardized dosimetric method, as described in detail previously.29 AMP (Sigma Chemical; St. Louis, MO) was dissolved freshly in 0.9% saline solution to produce a doubling concentration range of 0.39 to 400 mg/mL. Each solution was administered from a jet nebulizer attached to a breath-activated dosimeter (model MB3; Mefar; Brescia, Italy) at a nebulization time of 1 s with a pause time of 6 s. The nebulizer delivers particles with an aerodynamic mass median diameter of 3.5 to 4.0 μm at an output of 10 μL per breath. Patients inhaled the aerosolized AMP solutions in five inhalations from functional residual capacity to total lung capacity through a mouthpiece with the patient’s nose clipped. Normal saline solution was inhaled initially, followed by five breaths of doubling concentrations of AMP at 2- to 3-min intervals. Single measurements of FEV1 were made 60 to 90 s after the inhalation of each concentration, unless the forced expiratory maneuver was judged to be technically unsatisfactory. The test was interrupted when a fall in FEV1 of at least 20% from the post-saline solution value was recorded or the maximum concentration had been administered. The PC20 was calculated using a formula given by Cockcroft et al.29

ENO Measurement Technique

ENO concentration was measured on-line by the restricted-breath analysis according to the recommendations of the American Thoracic Society,30 using a chemiluminescence analyzer (NIOS; Aerocrine, Solna, Sweden), as described in detail previously.28,31 Briefly, the patient was instructed to inhale through the mouth to total lung capacity and immediately exhale through the mouthpiece against a fixed resistance. Patients inhaled ambient air that was passed through a filter to reduce inhaled nitric oxide concentrations to < 5 parts per billion (ppb). Maintenance of a constant flow was achieved using a visual feedback display on the computer screen. A flow rate of 45 mL/s was used. An exhalation of 10 s with a plateau of at least 3 s in duration was required to calculate the nitric oxide plateau. Patients repeated the maneuver until three acceptable tests were performed, and the average of the three plateau values was recorded.

Statistical Analysis

Data were analyzed with standard statistical software packages (SPSS, version 10.0 for Windows; SPSS, Chicago, IL; and InStat for Windows, version 3.00; GraphPad Software; San Diego, CA). Variability in PEF was calculated with the following formula: (maximum PEF − minimum PEF)/mean PEF of the day × 100. All PC20 and ENO values were log-transformed before analysis and presented as geometric means with 95% confidence intervals (CIs). All other numerical variables are reported as arithmetic means with 95% CI. Comparisons of the baseline characteristics between groups were performed by unpaired t tests for continuous data and by Fisher exact tests for categorical data. Possible predictors for failure of ICS reduction were determined using log-rank test. Kaplan-Meier survival curves were used to demonstrate the probability of failure of ICS reduction between subjects with increased and those with normal responsiveness to AMP, and between patients with normal ENO concentrations and those with increased ENO. For PC20, the cut-off points evaluated were 400 mg/mL at baseline and one doubling concentration increase over the baseline value 2 weeks after the dose of ICS was halved.
Specific ENO cut-off points evaluated included 15 ppb, 20 ppb, and 30 ppb at baseline, and 10 ppb increase over the baseline value 2 weeks after the dose of ICS was halved. Logistic parameters were estimated using maximum likelihood estimation and evaluated using the likelihood ratio test. Odds ratios (ORs) with a CI > 1 indicate a significantly increased risk for failure of ICS reduction. Correlations were assessed using the Pearson correlation coefficient; p values are two sided, and values < 0.05 were considered statistically significant.

Results

Forty-seven patients were initially selected, but data from only 37 patients were analyzed. Four patients did not strictly fulfill entry criteria, two patients declined the previous acceptance of participation, two patients were unavailable for follow-up, and in two patients an error was made in performing the AMP challenge. Table 1 shows baseline characteristics of the 37 patients studied; 19 of the 37 patients had bronchoconstriction in response to AMP.

Twenty-seven patients did not have exacerbations when the dose of ICS was halved, whereas 10 patients had an asthma exacerbation: 2 patients after 3 weeks, 3 patients after 4 weeks, 1 patient after 6 weeks, 1 patient after 8 weeks, 2 patients after 9 weeks, and 1 patient after 11 weeks of treatment with a reduced dose of ICS. Six patients had exacerbations reflected by nocturnal awakening, four patients had exacerbations reflected by a decrease in morning PEF of > 20%, and five patients had exacerbations reflected by an increased use of inhaled albuterol. Five patients fulfilled two or more criteria at the time of the exacerbation. None had severe exacerbations that required hospital admission. PC_{20} at the time of exacerbations was not determined in two patients for safety reasons.

There were no significant differences in clinical characteristics, pulmonary function, or ENO levels at baseline between the groups that did and did not have exacerbations (Table 2). A higher responsiveness to inhaled AMP was found in patients with exacerbations, but this difference was of marginal significance (p = 0.07).

Predictive Power Using Baseline Data Before Steroid Dose Reduction

Having both bronchoconstriction in response to AMP and increased ENO levels (cut-off points, 15 ppb or 20 ppb) was a significant predictor for failure in ICS reduction (Fig 1). By contrast, having either bronchoconstriction in response to AMP or increased ENO levels alone was not a predictor for failure of ICS reduction. The ORs for failure of ICS reduction (Table 3) were 8.17 (95% CI, 1.60 to 41.64; p < 0.05) for the presence of both AMP bronchoconstriction and increased ENO levels at a cut-off point of 15 ppb, and 5.25 (95% CI, 1.11 to 24.91; p < 0.05) for the presence of both AMP bronchoconstriction and increased ENO levels at a cut-off point of 20 ppb.

Predictive Power Using the Modifications After 2 Weeks of ICS Dose Reduction

Figure 2 shows the survival curve using the modifications of PC_{20} and ENO values 2 weeks after the dose of ICS was halved. Having a decrease in PC_{20} (cut-off point, one doubling concentration) was a borderline significant predictor for failure of ICS reduction (p = 0.062), whereas having an increase in ENO levels (cut-off point, 10 ppb) was not a predictor for failure of ICS reduction. Only three patients showed both a decrease in PC_{20} values greater than or equal to one doubling concentration and an increase in ENO ≥ 10 ppb.

Correlations

Correlations between the changes from baseline in PC_{20} that occurred during an exacerbation (group with exacerbation) or at the end of study period (group without exacerbation), and the changes in symptoms, albuterol use, lung function, and ENO levels are presented in Table 4. Data from both patient groups were pooled for analysis of correlation. There were significant correlations between the changes in PC_{20} values with the changes in daytime symptom scores (r = 0.41, p = 0.02) and amount of albuterol use (r = 0.43,
p = 0.009), but no significant correlation was found between the changes in PC_{20} and the changes in either ENO levels, nighttime symptom scores, or pulmonary function. Furthermore, the changes in ENO values were not significantly related with the changes in either clinical manifestations or pulmonary function.

**Discussion**

The results of the present study suggest that, in asthmatic patients with their disease already stabilized and well controlled by use of ICS, measurements of AMP responsiveness and ENO levels can be used to predict the success or failure of reduction in ICS dose. At baseline, having both ENO levels of at least 15 ppb or 20 ppb and bronchoconstriction in response to AMP was a clear predictor for failure of ICS reduction, whereas having either AMP bronchoconstriction or increased ENO levels alone was not a predictor. Furthermore, a doubling concentration decrease in PC_{20}, 2 weeks after the dose of ICS was halved seems to have some predictive value for failure in ICS reduction, whereas the increase in ENO levels did not have predictive value for exacerbations following ICS reduction.

In 27% (10 of 37 patients) in the current study, an exacerbation of asthma was detected after reduction of ICS; therefore, from these results it is evident that in most patients with asthma with disease well controlled with a moderately high ICS dose, the dose of the drug can be reduced without clinical deterioration. In contrast, previous studies reported that reducing the dose of ICS produced a relapse of asthma in 47 to 78% of patients. Differences in results might be explained, at least in part, by differences in patient characteristics. The overall asthma control in our patients was probably better at study entry than that in other studies, and this was undoubtedly due to our strict inclusion criteria. For example, these differences in patient characteristics are reflected in the as-needed β_{2}-agonist consumption at baseline, which was between zero inhalations and four inhalations per day in the study of Price et al., but only between zero inhalations and two inhalations per week in this study. The reason for selecting patients with stable asthma in good control is that this group would most likely be considered for ICS reduction. Indeed, Juniper et al. examined the effect of ICS reduction on airway responsiveness to methacholine after 1 year of ICS therapy; although a 50% reduction in ICS dose was not associated with a change in airway responsiveness over a 3-month period, five subjects (35%) experienced a deterioration in symptoms and a decrease in FEV_{1}. Another study observed that the long-term remission during a 2-year treatment period with high doses of budesonide was maintained for well more than 1 year in patients treated with a dose that was reduced to one third of the previous dosage; however, four patients (21%) deteriorated after reduction of inhaled budesonide. More recently, Prieto et al. reported that in most patients with asthma well controlled with a moderately high dose of budesonide (800 μg/d), a 75% reduction in ICS dose is possible without loss of asthma control; however, 11 subjects (24%) deterio-

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**Table 2—Clinical Characteristics, Pulmonary Function, AMP Responsiveness, and ENO at Baseline in Subjects With and Without Exacerbation**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exacerbation</th>
<th>No Exacerbation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>10</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Age, yr (23.3–39.1)</td>
<td>31.2</td>
<td>32.6 (95% CI)</td>
<td>0.72</td>
</tr>
<tr>
<td>Male/female gender</td>
<td>1/9</td>
<td>10/17</td>
<td>0.22</td>
</tr>
<tr>
<td>Ex-smokers/nonsmokers</td>
<td>1/9</td>
<td>9/18</td>
<td>0.23</td>
</tr>
<tr>
<td>Duration of asthma, yr</td>
<td>17.5 (12.1–22.9)</td>
<td>16.6 (13.0–20.2)</td>
<td>0.79</td>
</tr>
<tr>
<td>Skin test result positive</td>
<td>8</td>
<td>22</td>
<td>0.90</td>
</tr>
<tr>
<td>Duration of ICS use, mo</td>
<td>22.8 (7.5–38.1)</td>
<td>29.4 (18.4–40.4)</td>
<td>0.50</td>
</tr>
<tr>
<td>ICS dose, μg/d (beclomethasone equivalent)</td>
<td>630 (465–795)</td>
<td>615 (473–757)</td>
<td>0.90</td>
</tr>
<tr>
<td>FEVi % predicted</td>
<td>96.3 (89.0–103.6)</td>
<td>101.1 (95.4–106.9)</td>
<td>0.34</td>
</tr>
<tr>
<td>FEF_{1}/FVC, %</td>
<td>77.7 (72.5–82.9)</td>
<td>81.8 (79.0–84.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>Morning PEF, L/min</td>
<td>425 (391–458)</td>
<td>392 (355–430)</td>
<td>0.20</td>
</tr>
<tr>
<td>Evening PEF, L/min</td>
<td>440 (402–477)</td>
<td>413 (345–492)</td>
<td>0.47</td>
</tr>
<tr>
<td>Daytime symptom score</td>
<td>0.04 (0.00–0.08)</td>
<td>0.06 (0.00–0.12)</td>
<td>0.52</td>
</tr>
<tr>
<td>Nighttime symptom score</td>
<td>0.04 (0.00–0.09)</td>
<td>0.01 (0.00–0.03)</td>
<td>0.31</td>
</tr>
<tr>
<td>Albuterol, puffs/d</td>
<td>0.08 (0.00–0.18)</td>
<td>0.03 (0.00–0.06)</td>
<td>0.25</td>
</tr>
<tr>
<td>PC_{20}, mg/mL</td>
<td>63.1 (18.2–218.8)</td>
<td>199.5 (128.8–309.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>ENO, ppb</td>
<td>25.7 (15.8–42.6)</td>
<td>24.5 (18.2–33.1)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

*Data are presented as No. or mean (95% CI) unless otherwise indicated.
†Geometric mean (95% CI).
rated after reduction of ICS. These findings are consistent with the present results.

It is important to point out that some aspects of the study design were chosen to closely emulate what might happen in the normal clinical setting when a reduction in the dose of ICS is attempted, including the single-blind design and the use of different ICS products and doses. One could argue that some patients may be prone to report more severe clinical manifestations because they were informed that the ICS dose was reduced. However, this effect would be independent of the response to inhaled AMP or ENO levels and, therefore, should not have biased the study results. Furthermore, although our study was not done in a double-blind manner, the identification of exacerbations was performed by personnel blinded to the results of the AMP challenge and ENO determination.

From our results, it seems that reducing the dose of ICS is possible in many asthmatic patients in a stable phase of the disease; however, despite recommendations about the need for stepwise reduction in ICS therapy as part of long-term management, algorithms about how to do this are currently lacking. A primary goal of this investigation was to evaluate the utility of the determination of AMP responsiveness and ENO levels as markers for safely reducing the dose of ICS in patients with asthma well controlled with a moderately high dose of ICS. Our study indicates that having both bronchoconstriction in response to AMP and ENO levels of at least 15 ppb at baseline is a predictor for failure of ICS reduction.

To the best of our knowledge, there are no studies that have investigated the utility of the determination of AMP responsiveness as a marker for safety reducing the dose of ICS. At baseline, having bronchoconstriction in response to AMP was not a significant predictor for failure of ICS reduction. Our findings are supported by Leuppi and coworkers, who demonstrated that having airway hyperresponsiveness to mannitol (an indirect bronchoconstrictor) was not a significant predictor for failure of ICS reduction. By contrast, having airway hyperresponsiveness to both histamine (a direct bronchoconstrictor) and mannitol was a clear predictor for failure of ICS reduction. In the present study, the responsiveness to a direct bronchoconstrictor agent was not determined, and so the relevance of this factor in our patients cannot be ascertained; however, the results of our study suggest that a decrease in \( \text{PC}_{20} \) of at least one doubling concentration 2 weeks after the dose of ICS was halved is a predictor of borderline significance for failure of ICS reduction. Further-

Table 3—ORs for Failure of ICS Reduction

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{PC}_{20} \leq 400 \text{ mg/mL} )</td>
<td>2.92</td>
<td>0.62–13.76</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>ENO ( \geq 15 \text{ ppb} )</td>
<td>1.68</td>
<td>0.29–9.75</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>ENO ( \geq 20 \text{ ppb} )</td>
<td>1.20</td>
<td>0.27–5.25</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>ENO ( \geq 30 \text{ ppb} )</td>
<td>1.13</td>
<td>0.26–5.02</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>( \text{PC}_{20} \leq 400 \text{ mg/mL} ) plus ENO ( \geq 15 \text{ ppb} )</td>
<td>8.17</td>
<td>1.60–41.64</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>( \text{PC}_{20} \leq 400 \text{ mg/mL} ) plus ENO ( \geq 20 \text{ ppb} )</td>
<td>5.25</td>
<td>1.11–24.92</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>( \text{PC}_{20} \leq 400 \text{ mg/mL} ) plus ENO ( \geq 30 \text{ ppb} )</td>
<td>1.89</td>
<td>0.36–9.97</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Changes from baseline*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta \text{PC}_{20} \geq 1 ) doubling concentration</td>
<td>3.50</td>
<td>0.75–16.27</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>( \Delta \text{ENO} \geq 10 \text{ ppb} )</td>
<td>1.89</td>
<td>0.36–9.97</td>
<td>&gt; 0.05</td>
</tr>
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</table>

*Changes from the run-in period to the visit performed 2 weeks after the reduction of ICS.
more, when measured longitudinally, the changes in PC20, but not the changes in ENO, correlated significantly with changes in either symptoms or albuterol use. These findings provide additional support for the use of AMP measurements as a tool in the assessment of asthma control. Thus, although additional studies involving a larger number of patients are necessary to confirm the utility of the changes in PC20 for predicting failure of ICS reduction, our findings suggest that changes in PC20 following the reduction of ICS may potentially provide information more useful for predicting loss of control than did single measurements at baseline.

It has been suggested in the literature that changes in ENO measured over time have higher predictive value for exacerbations following ICS reduction than do single measurements.37 In a study involving asthmatic patients receiving high-dose ICS, Tamaoki and associates38 reported that ENO was increased by 6 weeks after the initial doses were halved, in association with a loss of asthma control. Furthermore, when measured longitudinally, the changes in ENO correlated significantly not only with changes in airway responsiveness to indirect bronchoconstrictor agents (hypertonic saline solution), but also with changes in lung function and asthma symptoms.36,37 We were unable to confirm these observations. In our study, the changes in ENO levels after 2 weeks of treatment with a reduced dose of ICS did not have predictive value for exacerbations following ICS reduction. Indeed, no correlation was found between the changes in ENO and either changes in AMP responsiveness, lung function, albuterol use, or asthma symptoms. The reasons for such discrepancies might be related, at least in part, to important differences in study design and ENO measurement technique. In the study by Jatakanon et al36 the dose of ICS was reduced by 75%, whereas it was reduced by 50% in the present study. In addition, in the study by Jones et al,37 an exacerbation of asthma was induced by withdrawing patients completely from greater asthma severity irrespective of steroid use. In contrast, several studies34,36 have demonstrated that a single baseline assessment of ENO had a low power to predict asthma deterioration during the reduction of ICS treatment. Although our results confirm that a single baseline assessment of ENO is not useful to predict the evolution of asthma following the reduction of ICS dose, they clearly suggest that the concomitant determination of ENO and PC20 at baseline provides a relevant information to predict the success or failure of ICS reduction.

Table 4—Correlations Between the Changes in PC20 or ENO Levels and the Changes in Clinical Manifestations and Pulmonary Function

<table>
<thead>
<tr>
<th>Variables</th>
<th>PC20 Correlation</th>
<th>PC20 p Value</th>
<th>ENO Correlation</th>
<th>ENO p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime symptom score</td>
<td>-0.41</td>
<td>0.02</td>
<td>0.04</td>
<td>0.77</td>
</tr>
<tr>
<td>Nighttime symptom score</td>
<td>-0.30</td>
<td>0.08</td>
<td>0.05</td>
<td>0.77</td>
</tr>
<tr>
<td>Albuterol use</td>
<td>-0.43</td>
<td>0.009</td>
<td>0.11</td>
<td>0.91</td>
</tr>
<tr>
<td>FEV1</td>
<td>0.16</td>
<td>0.36</td>
<td>0.13</td>
<td>0.92</td>
</tr>
<tr>
<td>Morning PEF</td>
<td>0.02</td>
<td>0.97</td>
<td>-0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>Evening PEF</td>
<td>-0.17</td>
<td>0.33</td>
<td>-0.05</td>
<td>0.98</td>
</tr>
<tr>
<td>ENO</td>
<td>-0.03</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Kaplan-Meier survival curve based on the changes from the run-in period to the visit performed 2 weeks after the reduction of ICS dose of either PC20 at a cut-off point of one doubling concentration (top, A) or ENO levels at a cut-off point of 10 ppb (bottom, B). The dashed line represents the group with positive result, and the continuous line represents the group with negative result to each determination.
inhaled steroids. Furthermore, the ENO levels measured in our study tend to be somewhat higher than those measured in other reports. The reasons for such discrepancies might be related to important differences in methodology. It has been shown that there is a marked flow dependence of ENO values, with lower values measured at high flow rates and vice versa. We used an expiratory flow of 45 mL/s, which is lower than the flow used in other studies.

In conclusion, it seems that in most patients with asthma well controlled with a moderately high dose of ICS, control of the disease can be maintained for at least 3 months with a low dose of the drug; therefore, once control of asthma is sustained for several months with a moderately high dose of ICS, it is justified to consider a reduction in therapy. The presence of both AMP bronchoconstriction and ENO levels of at least 15 ppb at baseline is a clear predictor for failure of ICS reduction. We would recommended, therefore, having information on both AMP responsiveness and ENO levels at baseline as a means to identifying those asthmatic patients whose condition will or will not deteriorate when the dose of ICS is reduced.

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