Changes in Exhaled Nitric Oxide and Breath pH during Fluticasone Wean in Asthma

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Key Words

Acidopnea • Asthma • Breath condensate pH •
Corticosteroids • Exhaled nitric oxide • Nitrosopnea

Abstract

Background: Inhaled corticosteroid (ICS) therapy improves asthma outcome. Both the anti-inflammatory efficacy and toxicities of ICS therapy are dose dependent. Therefore, there is interest in monitoring airway inflammation during ICS dose adjustments. **Objective:** Fraction of expired nitric oxide (FENO) and exhaled breath condensate (EBC) pH were studied as noninvasive, corticosteroid-responsive markers of airway inflammation. **Methods:** We prospectively studied the effect of stepwise ICS wean on FENO and EBC pH over 6 months in otherwise healthy adults with moderate persistent asthma. **Results:** Eighteen subjects completed the initial dose titration and 13 completed the protocol. Of these, 7 weaned off ICS completely and 6 had exacerbations. FENO rose significantly with ICS withdrawal, though there was heterogeneity in the starting level and the degree of rise. EBC pH was collected at home in all subjects and fell more in subjects who had an exacerbation than in those who did not. The decrease in pH was associated with hazard of exacerbation. **Conclusion:** FENO can be a patient-specific index

of airway inflammation during ICS dose titration; change in EBC pH is one home marker that might possibly be used during ICS dose titration. However, additional studies are required.

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Introduction

Inhaled corticosteroid (ICS) therapy improves the outcome for patients with asthma [1–3]. Both the anti-inflammatory efficacy and toxicities of ICS therapy are dose dependent [3, 4]. Therefore, there is broad agreement that individual dosing should be maintained at the minimum required to achieve good control of asthmatic airway inflammation [1–4]. However, controversy remains regarding how best to (1) measure the adequacy of this control, and (2) predict, in a clinic visit, whether dosing is adequate to maintain control. In this regard, it is of interest that many asthmatic patients with normal spi-

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Table 1. Exacerbation criteria

1	Six or more puffs of albuterol per day
2	Increase in daily asthma symptom score of 1 or more consecutive days ¹
3	Drop in peak flow or FEV ₁ 20%
4	Waking up at night more than 2 times requiring albuterol
5	Worsening symptoms of asthma or dyspnea, and flu or virus
6	Need for systemic corticosteroids

¹ Asthma control questions modified for daily use [13].

rometry have ongoing airway inflammation and are at risk for exacerbations [3, 5]. Therefore, spirometry alone may not be the ideal method for adjusting the dose of anti-inflammatory medications such as ICS.

Because of the risk, inconvenience and expense of routine bronchoscopic or induced sputum analysis in the clinic setting, recent interest has focused on the development of simple, noninvasive tests for airway inflammation in patients with asthma. Two measurements have received particular attention: the fraction of expired nitric oxide (FENO) [6–9] and exhaled breath condensate (EBC) pH [10–12]. FENO is high in patients with allergic asthma and generally decreases with ICS therapy; it is likely to be a useful adjunct to symptoms analysis in ICS dose titration [6–8, 13, 14]. Breath condensate pH is low during acute asthma exacerbations and increases with corticosteroid dosing [10]. However, the effect of systematic dose weaning on FENO and EBC pH is incompletely understood. Therefore, we studied FENO and EBC pH longitudinally in subjects with moderate, persistent asthma during a fluticasone dose reduction protocol.

Materials and Methods

Subjects

Subjects with moderate, persistent asthma according to NHLBI EP2 guidelines [3] who were 18–40 years old were recruited by public advertisement. Subjects were excluded who had (1) any comorbidity other than atopic disease; (2) a smoking history; (3) an adverse reaction to fluticasone; (4) a requirement for more than 500 µg/day of fluticasone or an equivalent; (5) a forced expiratory volume in 1 s (FEV₁) <40%; (6) a room air oxygen saturation of <92%; (7) any evidence of an intercurrent illness and/or exacerbation; (8) an acute asthma exacerbation (table 1) at the time of enrollment; (9) pneumonia within the past year; (10) pregnancy, or (11) a requirement for nitrovasodilator or diuretic therapy of any kind. Recruitment was done primarily in September and October; the majority of the participants in this 6-month study were college students.

Fraction of FENO Measurement

FENO was measured once per month, using an exhaled NO (eNO) monitoring system NIOX (Aerocrine, Solna, Sweden) via chemiluminescence according to ATS guidelines [6].

EBC Collection and Analysis

EBC was collected at home for 10 min using RTube (Respiratory Research, Inc., Charlottesville, Va., USA) twice a week; samples were stored at –4°C in home freezers and brought to the laboratory once per month for pH analysis as previously described [10–12, 15, 16]. Condensate pH stability was measured in this setting and shown to be stable and highly repeatable (coefficient of variation <5%, for both asthmatic and normal subjects) in several studies [10, 15, 17].

Spirometry

Spirometry was performed (Collins Eagle, Braintree, Mass., USA) according to ATS guidelines [18].

Study Design

Subjects underwent an initial assessment, including history, physical examination, spirometry, FENO measurement and EBC pH measurement. Patients not on fluticasone were changed to fluticasone 220 µg/inhalation, 1 inhalation twice a day. Except for the change to uniform ICS dosing, all other asthma medications were left unchanged. After a 4-week run-in on fluticasone 220 µg twice daily, subjects underwent repeat FENO and EBC pH testing as well as spirometry. Subjects then underwent a step-wise dose reduction according to the following protocol: 220 µg once a day for a month, 110 µg once a day for a month, 44 µg a day for a month, and 0 µg per day for 2 months. During this titration, spirometry, FENO and EBC pH were measured at monthly intervals. EBC pH was performed at home twice a week. Patients were instructed to call the coordinator and/or study physician within 24 h of the development of new symptoms (cough, congestion, dyspnea, chest pain, exercise intolerance, increased rescue medication use or other concerns). Peak expiratory flow recordings and daily albuterol use were recorded at home. Patients were instructed to perform daily measurements of EBC pH at home if they experienced an increase in asthma symptoms or albuterol use, or an upper respiratory infection, for 5 consecutive days. Subjects who met specific criteria (table 1) were considered to have had an exacerbation and were withdrawn from the titration protocol. They were treated according to the judgment of the study physician. This study was reviewed and approved by the University of Virginia Human Investigation Committee (Institutional Review Board).

Statistical Analysis

Plots of raw means were first provided to show the longitudinal pattern of the EBC pH, FENO and FEV₁ values. We then calculated the mean difference of EBC pH, FENO and FEV₁ levels between 2 adjacent dose levels. The *t* test and nonparametric Wilcoxon signed rank test were used to test if the mean differences were departed from zero, with Bonferroni adjustment for multiple comparisons. Mixed models (SAS Proc Mixed) were employed to find the relationship between the repeated measures of EBC pH, FENO and FEV₁, respectively, and dosage, taking into account the correlation between measurements at different time points on the same subject. For each of these 3 measures, we also used Cox's

proportional hazards models [19] to assess the effect of these risk factors on the exacerbation hazard: (1) the difference between the last observed measurement and the baseline measurement, and (2) the last observed value. The statistical analyses were carried out in SAS 9.1 (SAS Institute Inc., Cary, N.C., USA).

Results

Subjects

Twenty-one subjects (mean age 29.7 years, range 18–40, 3 males/18 females) participated in this study (table 2). Three subjects were unable to begin titrating their dose because of missing the first follow-up visit and were excluded from analysis. An additional 5 subjects were unable to complete the full 6-month protocol because of leaving the area. Seven subjects completed the full titration. Six subjects had exacerbations requiring that the titration be stopped, though 2 of these were able to wean to 44 µg before being stopped. One of these 6 exacerbations was associated with the onset of pregnancy. All 18 subjects who began the titration tolerated being weaned from 220 µg of fluticasone twice daily (440 µg a day) to 220 µg once daily.

Overall Effects of Fluticasone Withdrawal

We first calculated the difference in outcomes between each 2 adjacent dose levels to determine whether the mean (median) difference departed from zero. For FENO, the only significant difference was between 110 and 44 µg (mean difference 12.7 ppb; $p = 0.002$ for Wilcoxon signed rank test), which remained significant after Bonferroni adjustment for multiple comparisons. We found that pairwise changes in EBC pH were not significant between any 2 adjacent dose levels. FEV₁ decreased by 2.4% between dose 440 to 220 µg ($p = 0.02$ for Wilcoxon signed rank test), but this was no longer significant after Bonferroni adjustment.

Next, we fitted repeated measures models to examine whether there was an overall trend during dose titration. The covariance structure was taken to be autoregressive to adjust for the intrasubject correlation. As shown in figure 1, there was a linearly increasing pattern for FENO associated with dose titration ($p = 0.01$). We also discovered a significant linearly decreasing pattern ($p = 0.03$) for FEV₁ during dose titration. For EBC pH, no significant trend existed during titration. We included analysis of age in all mixed models. We found that age was a significant predictor for FEV₁ ($p = 0.001$): each year increase in age reduced the FEV₁ by 1.1%.

Table 2. Subject characteristics

Mean age, years	29.7 ± 7.4
Mean FEV ₁ at baseline, %	93 ± 12
Mean eNO at baseline, ppb	23.6 ± 11.0
Mean EBC pH at baseline, pH units	7.8 ± 0.6

We also did separate plots for those who experienced exacerbation and those who did not. Please see figure 2 for FENO, EBC pH and FEV₁, respectively. It appears that pH values of the exacerbation group are always lower than those of the nonexacerbation group. However, due to small sample size, such comparison is not significant at any dose level.

Anticipation of Exacerbations

FEV₁, FENO and EBC pH – and between-test change in (Δ) FEV₁, ΔeNO and ΔEBC pH – were studied for their ability to identify and predict exacerbations. The fall in EBC pH was greater in the 6 subjects who had an exacerbation (mean – 0.58, SD 0.70) than in the 7 who did not (mean +0.16, SD 0.13) with a p value of 0.05. We also observed that the last measured pH value in the exacerbation group (mean 7.2, SD 0.90) was slightly lower than in the nonexacerbation group (mean 8.0, SD 0.10) with $p = 0.08$. Other parameters did not differ.

We used a Cox’s proportional hazards model to assess the effect of FEV₁, FENO and EBC pH values on the hazard of exacerbation. The response is the time (in months) to exacerbation, loss of follow-up or end of study (if the patient completed the full titration). Patients lost to follow-up were considered censored at the last follow-up time. Patients completing the full titration were considered censored at the end of the study. Therefore, all 18 subjects were included in this analysis. To handle ties in failure times, we adopted the ‘exact’ adjustment in SAS Proc PHREG. In all subsequent models, we adjust for patient age.

We first considered the drop in EBC pH value at the last observation time from that at baseline as a time-dependent covariate in the Cox’s model for the hazard of exacerbation. By this, we can test if a subject with a larger decrease (from study onset) in pH was more likely to experience exacerbation. We found that a 0.1-unit larger drop in pH level was associated with a 25% higher hazard ratio ($p = 0.02$). Second, we included the last observed pH value before exacerbation (or censoring) as a time-dependent covariate in the model for exacerbation hazard. It appears that exacerbation was preceded by a lower pH

Fig. 1. Time courses of eNO (a), EBC pH (b) and FEV₁ (c) values during titration: raw means with 95% confidence intervals.

value: 0.1 unit lower in pH level was associated with a 19% increase in hazard ratio ($p = 0.02$).

We performed a similar analysis for FEV₁ and FENO. None of these time-dependent predictors were significant for the exacerbation hazard.

To determine the effect of the initial EBC pH on exacerbation hazard, we used a Cox model with the baseline pH level as the covariate. The result shows that 0.1 unit lower in the baseline pH level was associated with a 32% increase in the hazard of exacerbation. Finally, we also included as a time-dependent predictor the difference (Δ EBC pH, Δ FEV₁, Δ eNO) between the 2 most recent adjacent months to investigate if a large recent change is associated with the hazard of exacerbation. However, none of the results were significant.

Neither FENO nor EBC pH values varied significantly with albuterol use.

Discussion

The mainstay of therapy for patients with moderate and severe asthma is the use of anti-inflammatory medications. However, airway inflammation is difficult to measure in the clinical setting, and judgments about ICS use and dosage are made based on reported symptoms, physical examination and spirometry, indices that are insensitive to markers of inflammation [1–5]. Recently, several studies have suggested that high levels of FENO ('nitrosopnea') and low breath condensate pH ('acidopnea') are associated with allergic asthma and with asthma exacerbations, respectively [7, 10, 13, 20–22]. FENO has been used in conjunction with symptoms and markers of air-flow obstruction to guide ICS dosing in adults and children with asthma [7, 8, 23]. Here, we measured FENO and EBC pH during a regimented, prospective ICS withdrawal protocol in adults with moderate persistent asthma,

Fig. 2. Mean eNO (a), EBC pH (b) and FEV₁ (c) trajectories for patients with and without exacerbation, with 95% confidence intervals. The fall in EBC pH was greater in the 6 subjects who had an exacerbation (mean -0.58 , SD 0.70) than in the 7 who did not (mean $+0.16$, SD 0.13), with a p value of 0.05 . The last observed pH value in the exacerbation group (mean 7.2 , SD 0.90) is smaller than that in the nonexacerbation group (mean 8.0 , SD 0.10), with $p = 0.08$. Other parameters did not differ. Individual pH (d) and FENO (e) values are presented for patients who had an exacerbation.

and we studied whether these parameters could be used to anticipate the success or failure of a dose decrease.

As expected, individual FENO levels generally rose with ICS withdrawal. Therefore, our data are consistent with previous studies in suggesting that an increase in

FENO may be one indicator to follow to determine the extent to which an ICS dose decrease is permissible for a worrisome increase in asthmatic airway inflammation [7, 8, 23, 24]. There could have been a survivor effect, such that those subjects who tolerated withdrawal had an over-

all lower FENO, attenuating the overall population mean increase observed with titration because those who 'survived' to lower the dose had lower FENO values throughout.

Previous studies have suggested that EBC pH (1) is low in acute, viral respiratory-associated asthma exacerbations [9]; (2) falls during acute viral respiratory infections [25]; (3) increases with systemic corticosteroid therapy [9], and (4) could be affected by gastroesophageal reflux, which can also affect EBC pH [26, 27]. In the current study, we found that EBC can be readily collected and stored at home, and that a change in EBC pH was observed in those subjects that failed to wean completely from ICS, but not in those who did not. Thus, EBC pH could possibly prove to be a home assay that is useful as one indicator that an ICS wean will be unsuccessful. We also found that the initial pH level predicted failure to wean. However, additional studies will be required. There was a good deal of intersubject variability among subjects who had an exacerbation, likely reflecting the heterogeneity of determinants of asthma exacerbation reflected in airway pH, ranging from response to viral infection to gastroesophageal reflux [11, 17, 28]. The principle difference with previous studies of pH in asthma exacerbations is that previous studies, in cross-section only, looked at exacerbations severe enough to require emergency department visits and/or hospitalization [10].

Several individual patients were of interest. Two of our subjects with moderate, persistent asthma had persistently low EBC pH. We have also seen this in control subjects [10, 16]. Careful review did not reveal any distinguishing characteristics of these patients. Sustained acidopnea unrelated to asthma exacerbation has been observed in active tuberculosis [27], human rhinovirus infection [25], and chronic bronchitis and cystic fibrosis [12, 27], suggesting that chronic airway infection could explain persistently low EBC pH; it may also be associated with gastroesophageal reflux [17]. We do not have evidence for a sustained airway infection or reflux in these 2 subjects. In a third subject, EBC pH fell, and the patient had an exacerbation, early in pregnancy. Pregnancy-associated worsening of asthma is well described [29, 30], but poorly understood; it may be of interest to study EBC pH in pregnancy-associated asthma in a larger cohort in the future.

Taken together, our data confirm previous observations that there is not one parameter that should be used in isolation to make ICS dosing adjustments in subjects with asthma [28, 31]. Even in the relatively homogeneous population of nonsmoking, otherwise healthy young

adults defined as having moderate persistent asthma by NHLBI guidelines, the biomarker and clinical responses to decreasing ICS dose were not uniform. This limitation of each individual test is likely to reflect the complexity of biological determinants for each value in humans. It may reflect the complex determinants of asthma other than airway inflammation, including, for example, peripheral autonomic effects and S-nitrosothiol biochemistry [32–34]. However, there were trends toward prediction, and this negative study should be interpreted with caution in the context of the relatively small sample studied.

In summary, we have shown in a homogeneous population of patients with moderate asthma that FENO increases early in the course of ICS withdrawal and that a decrease in EBC pH may predict failure to wean. However, our findings suggest that neither FENO nor EBC pH should be used in isolation as a tool to quantitate the appropriateness and success of ICS weaning in this population. These observations confirm the complexity of asthma and the importance of integrating diverse data – from the history and physical exam, measures of airflow obstruction and measures of inflammation – into making clinical decisions about ICS dosing.

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